Book of Abstracts

The Seventh Multidisciplinary Conference on Drug Research

Book of Abstracts: The Seventh Multidisciplinary Conference on Drug Research

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Programme

Sunday, 9 May

REGISTRATION

Registration from 15:00 to 22:00
Sunday afternoon, 9 May, 15:00
Hotel Hyrny - Secretariat VII MKNOL

Supper

From 18:00 to 20:00
Sunday evening, 9 May, 18:00
Hyrny Hotel

Monday, 10 May

Breakfast
Monday morning, 10 May, 7:30

Session I
Monday morning, 10 May, 9:00
Conference room
Chair: Osman Achmatowicz, Zbigniew Kałuża

9:15 Invited oral

High throughput calcium screens for identification of drug targets and drug discovery in neurodegenerative diseases

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9:45 Invited oral

Olefin metathesis as an emerging tool in the synthesis of natural and bioactive compounds

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Olefin metathesis reactions represent an attractive and powerful transformation for the formation of new carbon--carbon double bonds. This area is now quite familiar to most R&D chemists as numerous catalysts are available that enable a plethora of olefin metathesis reactions. Now, after development of new more efficient olefin metathesis catalysts, the number of pharmaceutical companies considering the use of this methodology in in drug production is increasing [1,2].

Our research group is focusing on developing of new olefin metathesis catalysts [3]. Some of our catalysts have found successful applications in the synthesis of a number natural and biologically active products [3]. In particular, the application of metathesis in the synthesis of BILN 2061, the first reported hepatitis C virus (HCV) NS3 protease inhibitor to have shown an antiviral effect in infected humans, will be presented [4].

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10:15 Oral

Alkyl-nitroquipazine derivatives as serotonin transporter and 5-HT$_{1A}$ receptor ligands

Zdzislaw Chilmonczyk[1,7], Małgorzata Jarończyk[1], Karol Wołosewicz[2], Ingebrigt Sylte[6], Mari Gabrielsen[6], Gabriel Nowak[4], Aleksander P. Mazurek[1,3], Andrzej J. Bojarski[4], Jerzy Kossakowski[5]

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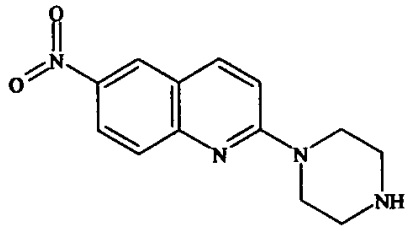
Inhibition of serotonin transporter (SERT) results in increased concentration of serotonin in the synaptic cleft. Drugs selectively inhibiting SERT have been used in many psychiatric disorders including depression, anxiety and obsessive compulsive disorder. 6-Nitroquipazine (**1**) is a potent SERT inhibitor with an affinity constant K_i = 0.17 nM [1] having higher affinity than clinically used inhibitors such as fluoxetine, fluvoxamine, paroxetine or sertraline [2].



1

Here we report the synthesis and pharmacological evaluation of a series of alkyl-nitroquipazine analogues as mixed 5-HT_{1A} receptor/ SERT ligands. The compounds exhibited diversified 5-HT_{1A} receptor and SERT affinity with saw-like affinity-alkyl chain length relationship. Some of them affected SERT functioning *in vitro* and *in vivo*.

This study was partly supported by a grant PNRF-103-AI-1/07 from Norway through the Norwegian Financial Mechanism.

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10:35 Oral

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

Michał Fedoryński[1], Barbara Kąkol[2]

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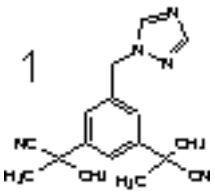
Anastrozole **1** is an anti-tumor drug for the treatment of breast cancer.

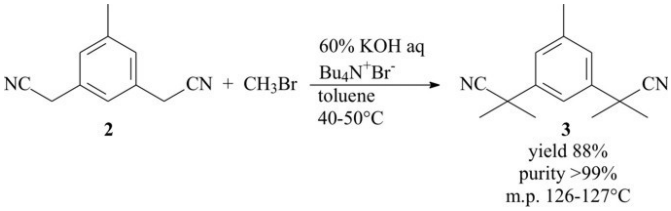
A key step in synthesis of **1** is exhaustive methylation of 3,5-bis(cyanomethyl)toluene **2**, whose elegant synthesis from commercially available 5-methylisophthalic acid was described recently[1].



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 PTC (phase transfer catalysis, 50%NaOH in the presence of a catalyst - benzyltriethylammonium chloride) for this purpose was patented[3], however purity of product obtained precluded its use for synthesis of **1**[4]. This result is not surprising, because it is well known, that introduction of the second alkyl group to the 2-arylalkane nitriles under PTC conditions proceeds with difficulty.

We found recently, that PTC alkylation of phenylacetonitrile derivatives carried out in the presence of 60-75% aqueous KOH, instead of the typical 50% NaOH, provide substantial improvements in the overall yield and purity of products[5,6].

Application of such system to methylation of **2** (methyl bromide as an alkylating agent, 60% KOH aqueous solution as a base in the presence of 1 molar percent of tetrabutylammonium bromide, toluene as a solvent) resulted in formation of tetramethylated product **3** in high isolated yield 88% and purity exceeding 99% after crystallization from ethanol.



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Coffee break

Monday morning, 10 May, 10:55
Hyrny Hotel - Patio and Garden

Session II

Monday morning, 10 May, 11:30
Conference room
Chair: Elżbieta Anuszewska, Wiesław Szeja

11:30 Invited oral

Synthetic Technologies of Pharmaceutical Substances

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Pharmaceutical substances represent nowadays one the most advanced and complicated technological products. In this lecture an attempt will be made to outline the specific features that differ these substances from all other synthetic compounds. The most common convergent approach to the synthesis of pharmaceutical substances will be discussed on a number of examples from several chemical classes. The convergent synthesis allows not only for the preparation of a series of structurally related known and new analogs from the same advanced intermediates, but also provides a way to construct the target molecule at very last stages of synthesis. This way a more efficient separation might be obtained of final product from the substrates that opens a way to the synthetic product of pharmaceutical quality. Selection of a synthetic route for pharmaceutical substance is based on several criteria, typical for all chemical compounds, like the efficiency of chemical synthesis (yields and number of steps, extreme conditions etc.) but also on several other specific factors. One of these is the selection of synthetic methods that not only do not infringe the intellectual property rights of other researchers but also that can be claimed by new patent applications. Other important criteria include the commercial availability of raw materials of reliable quality and the feasibility to purify the synthetic product to the pharmaceutical grade. In order to turn the synthetic method into a technology it is crucial to acquire initially a sufficient understanding of the reaction to define the critical parameters of the process and the design space. Chemical and economical optimization of these parameters by statistical methods, like Fractional Factorial Design, often results in much higher yields of the process, less extreme conditions and more efficient use of materials, reaction volume, timing and equipment. In a pharmaceutical synthesis it is important not only to synthesize the final product of the top purity but also obtain the material that is suitable for the further pharmaceutical formulation. Therefore what also matters are the physicochemical, solid state and surface properties of the product, like the right polymorphic form and the convenient particle size and shape distribution. In the summary, the pharmaceutical technology might be considered as a validated chemical process with identified and optimized critical parameters. The process that is fully controlled by validated analytical methods that are used to define the quality also of raw materials and advanced intermediates, in order to prepare repeatedly the final product of pharmaceutical grade, suitable for further pharmaceutical formulation.

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12:00 Oral

Design and synthesis of hybrid structures of pyrrolidin-2-one and arylalkylpiperazine as compounds with potential cardiovascular activity*

Katarzyna Kulig

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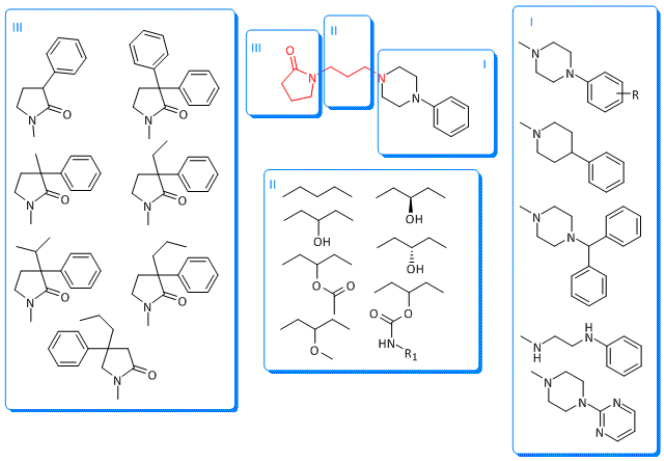
For much of the past century, drug discovery relied largely on the use of animal models of disease as the first-line screens for testing the compounds produced by medicinal chemists. When compounds were inactive, it was unclear whether this was because they no longer interacted with a molecular target or simply whether they had failed to reach the site of action. Inexorably, the drug discovery paradigm shifted toward a reductionist "one-target, one-disease" approach that continues to dominate the pharmaceutical industry today. There is an increasing readiness to challenge the current paradigm and to consider developing agents that modulate multiple targets simultaneously (polypharmacology), with the aim of enhancing efficacy or improving safety relative to drugs that address only a single target.

In search of our study a series of hybrid structures of pyrrolidin-2-one and arylalkylpiperazine was design. It was assumed arylalkylpiperazine fragment of molecule determine affinity of molecules obtained towards a-adrenoceptor (a-AR), while pyrrolidin-2-one may effect on its selectivity towards a-AR subtypes.

The obtained compounds were tested for their affinity for both a_1- and a_2-AR as well as for their *in vitro* activity in adrenaline induced arrhythmia and hypertension in rats.



*This study is a subject of habilitation thesis entilted „Stuktury hybrydowe pirolidyn-2-onów i aryloalkilopiperazyn o potencjalnej aktywności biologicznej/krążeniowej"; Wydawnictwo Medycyna Praktyczna, Kraków 2009.

12:20 Oral

Traceless chiral coupling reagent. A new concept for synthesis of optically active products from racemic carboxylic acids.

Beata Kolesińska, Katarzyna Kasperowicz-Frankowska, Justyna Frączyk, Zbigniew J. Kamiński

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The advances in stereoselective bioanalysis led to a new awareness of the importance of stereoselective pharmacodynamics and pharmacokinetics, enabling the differentiation of the relative contributions

of enantiomers to overall drug action. This results in formal obligation to recognize the occurrence of chirality in new drugs, attempt to separate the stereoisomers, assess the contribution of the various stereoisomers to the activity of interest and make a rational selection of the stereoisomeric form that is proposed for marketing. In order to facilitate this studies in case of syntheses involving condensation of chiral carboxylic acid we developed the concept of a traceless chiral coupling reagent useful for enantioselective synthesis of optically active products (also peptides) from racemic carboxylic acids (amino acids). According to the concept coupling reagents consists of two fragments with chiral auxiliary responsible for enantioselectivity expelled just during activation of carboxylic group. The reagent were prepared by the treatment of chiral ammonium tetrafluoroborate (**1**) with 2-chloro-4,6-dimethoxy-1,3,5-triazine (**2**) in the presence of sodium bicarbonate yielding stable N-(4,6-dimethoxy-1,3,5-triazin-2-yl)ammonium tetrafluoroborate (**3**) in high yield [1a]. Chiral coupling reagents **3** were found stable at room temperature and in a broad range of solvents yielded in high yield acylated products (amides, esters and peptides) [1b] with fully predictable configuration and ee (dr) ranging from 95/5 to 60/40 under reaction conditions identical as optimized for its achiral equivalent.

Acknowledgement: This work was supported by MSHE Grants **6/PMPP/U/30-09.08/E-370/2009** and N N204 **326737**.

Literature:

[1] (a) B. Kolesińska, Z.J. Kamiński, *Org. Lett*., 11 (3), 765-768 (2009). (b) B. Kolesińska, K. Kasperowicz, M. Sochacki, A. Mazur, S. Jankowski, Z.J. Kamiński. *Tetrahedron Lett*. 51, 20–22 (2010).

12:40 Oral

An old drug with new perspectives: How stereochemistry of fenoterol and fenoterol derivatives influences the β-adrenergic binding and the G-protein coupling selectivity.

Krzysztof Jóźwiak[1], Anita Płazińska[1], Lawrence Toll[3], Anthony Woo[2], Rui-Ping Xiao[2], Irving W. Wainer[2]

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The β_2 adrenergic receptor (β2-AR) has emerged as a model system for studying the ligand recognition process and the mechanism of GPCR activation (c.f. [1]). Fenoterol, a β2-AR selective agonist is used in therapy as a racemic mixture of (R,R)- and (S,S)- isomers (Fig.1.). In the current project we studied the four stereoisomers of fenoterol and several derivatives modified on the aminoalkyl tail. Radioligand binding studies determined that stereochemistry greatly influences the binding affinity with relative order uniform for all studied derivatives: (R,R)>(R,S)>(S,R)>(S,S) [2]. Control experiments on binding affinity towards the β1-AR show that β2-AR vs. β1-AR selectivity is the greatest for (R,R)- configuration of the ligand, and in case of some new derivatives this selectivity reaches exceptionally high value (e.g., (R,R)-4-methoxy-1-naphtylfenoterol ($\beta1\text{-AR})/K_i(\beta1\text{-AR})=573$) [3]. Functional activities of the compounds were determined using induced cAMP accumulation and cardiomyocyte contractility and the results confirmed that the (R,R)-configuration of fenoterol based derivatives is the most effective.

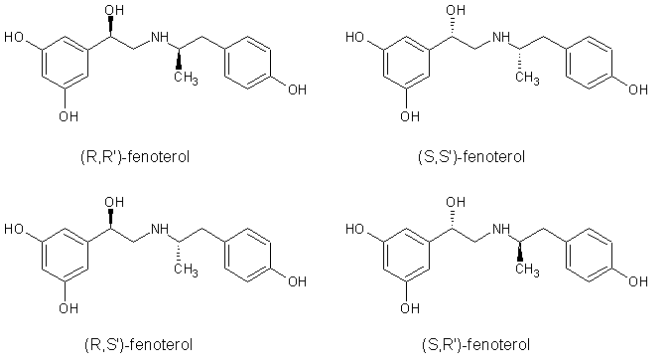

Figure 1. Stereochemistry of fenoterol.

Subsequent Van't Hoff analysis of fenoterol isomers shows very different thermodynamic characteristic of binding depending on the stereoconfiguration of the molecule. The binding of (S,S)- and (S,R)- isomers is almost entirely enthalpy controlled whereas the binding of (R,R)- and (R,S)- fenoterol is purely entropy driven [4]. The latter observation contradicts the "enthalpy-entropy discrimination" paradigm commonly accepted for β-adrenergic receptors [5] according to which the binding of full agonist is controlled by the combination of enthalpy and entropy while binding of antagonists is mainly entropy controlled.

The stereochemistry of the fenoterol molecule also affects the coupling of the AR to the Gs and Gi proteins. In cardiomyocyte studies, the addition of pertussis toxin had no effect on the activity of (R,R)-fenoterol indicating that the compound selectively activates Gs protein signaling upon binding to the receptor [6]. When (S,R)-fenoterol was used as the agonist, the addition of pertussis toxin significantly reduced cardiomyocyte contractility indicating that the compound activates both Gi and Gs protein [6]. The same pattern of stereo-discrimination was observed with the 4-methoxyfenoterol derivatives [6].

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 β2-receptor as evidenced by the Gs/Gi selectivity observation. It opens new perspectives for developing potent and highly selective β2-AR agonists. For example (R,R)-methoxyfenoterol is currently clinically tested for treatment of congestive heart failure, some other derivatives appears as very effective inhibitors of tumor cells mitogenesis in *in vitro* studies.

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13:00 Oral

N-alkyl derivatives of nystatin with improved selective toxicity

Sławomir Milewski, Joanna Boros, Natalia Salewska, Maria J. Milewska, Edward Borowski

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Nystatin, a polyene macrolide antibiotic produced by S*treptomyces noursei* is used as an antifungal drug for the treatment of broad spectrum of superficial, oral and gastrointestinal fungal diseases. Its possible application in chemotherapy of disseminated fungal infections is precluded, due to the high mammalian toxicity, including a hemolytic effect.

Research efforts at our laboratory are aimed at the improvement of the chemotherapeutic index of polyene macrolide antibiotics by their rational chemical modification. We previously showed that mammalian toxicity of amphotericin B could be strongly reduced due to the chemical modification of the amino group of mycosamine.[1,2] The same rationale was now applied for the construction of *N*-alkyl derivatives of nystatin. Novel compounds were synthesized upon Michaelis addition of *N*-substituted maleimides to nystatin under mild conditions The new nystatin derivatives were tested for antifungal *in vitro* activity, hemolytic activity and induction of potassium leakage from fungal and mammalian cells. Some of the novel compounds demonstrated substantial reduction of mammalian toxicity in comparison with nystatin, while their antifungal efficacy was only slightly diminished. Multidrug-resistant clinical *Candida albicans* strains demonstrated similar susceptibility to novel *N*-alkyl derivatives of nystatin as their drug-sensitive counterparts.

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Dinner

Monday afternoon, 10 May, 13:20
Hyrny Hotel - Canteen

Session III

Monday afternoon, 10 May, 15:20
Conference room
Chair: Zofia Lipkowska, Andrzej Kutner

15:20 Invited oral

Particles for controlled drug delivery

Tomasz Ciach

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The number on newly registered pharmaceutical active entities is slowly decreasing over the last two decades, despite the constant increase of spending on the research. On the other hand the societies are getting older and the demand for new efficient and convenient therapies is growing. One way to solve this problem is to make a better use of existing, already know substances. It can be done by the development of new encapsulation and delivery methods for active substances. Encapsulation of a drug can efficiently modify its pharmacokinetic and can deliver the drug molecules to the desired area, and release it in the desired manner. To achieve that, the drugs are encapsulated in biodegradable or biocompatible polymers, sometimes equipped in targeting molecules. The majority of particles, used for controlled release of medicine are obtained by wet chemistry techniques, mostly by different types of emulsification and emulsion polymerisation processes. This offers many advantages like high volumetric production rates and simplicity of the process. The wet route is however not capable to fulfil all requirements with respect to different types of polymers and surface chemistries. Also the efficiency of encapsulation of the active compound is not always high in the wet methods. An alternative way to the wet route for the production of drug containing particles is the aerosol route. This group of techniques is also a natural choice for drug delivery via inhalation.

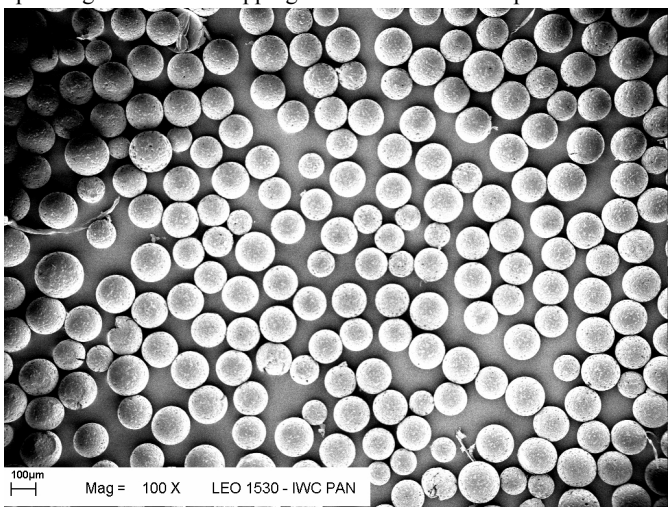
This paper shows a possibility of applying of the Electro Hydro Dynamic Atomisation (EHDA) for the production of biodegradable particles for controlled drug delivery. EHDA or electrospraying is a versatile and gentle method to atomise liquids. It allows the production of particles with a narrow size distribution with an easy way to tune to a desired size. Particles with a wide variety of chemical compositions can be made this way. As a precursor for particles virtually each compound that dissolves in a suitable liquid can be used. Generally EHDA refers to a process, where a liquid jet breaks up into droplets under the influence of electrical forces. Depending on the strength of the electric stresses in the liquid surface relative to the surface tension different spraying modes will be obtained. For the production of pharmaceutical micro particles the so called cone-jet mode seems to be the most promising. In this mode a liquid is pumped through a nozzle at a low flow rate. An electric field of sufficient strength is applied over the nozzle and some counter electrode and the droplet at the nozzle is transformed into a conical shape, from the apex of which a jet emerges. This liquid jet breaks up into small droplets due to hydrodynamic instabilities. After solvent evaporation droplets forms mono dispersed particles sized

from nanometers up to tens of micrometers. Standard deviation of particle sizes is usually in the range of 1.15 – 1.2. EHDA method of particles productions is very gentle so even vulnerable peptides survive the atomisation process. Diameter of particles obtained by EHDA can vary from tens of nanometrs to hundreds of micrometers. Smallest particles can be applied in targeted drug delivery for cancer treatment while biggest as long term slow release particles for intramuscular delivery. Smallest are able to carry the drug for few hours only, but can accumulate in the cancer area. Biggest degrade slowly and can release the drug for 4-8 weeks. Particles of about 100 micrometer diameter made of hydrogell of heavy chelating properties can serve as biodegradable radioactive isotope container for local cancer treatment.

Typical EHDA setup is presented in the picture below.

Spraying nozzle is supplied with the particles precursor solution and connected to the high voltage power supply. Below the nozle a stabilising ring connected to intermidiate voltage is placed. Particles are collected on the grounded metal plate below.

Example of drug containing particles obtained in the EHDA setup operating in the microdripping mode is shown in the picture below.



Presented particles containes Risperidon which is released, after immersion in water, for about 8 weeks.

15:50 Oral

Cyclic Enaminones as Building Blocks in the Synthesis of Piperidine-Containing Natural Products and Biologically Active Compounds

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Cyclic enaminoketones and esters are gaining increased interest, particularly dihydropyridones, which are known as important intermediates for the synthesis of piperidine-containing natural products.[1] Enaminones are versatile synthetic intermediates that combine the ambident nucleophilicity of enamines with the ambident electrophilicity of enones. Enaminoketones have proven to be versatile building blocks for the synthesis of various heterocycles such as pyridine, pyrimidine and pyrrole derivatives. Enaminone systems have “enone” character, and may act as acceptors in both 1,2 and 1,4-additions. In this way the enaminone serves as a scaffold for annulation, and can gain access to systems such as indolizidines, quinolizidines, perhydroindoles and benzoquinolizidine, all of which are common motifs in alkaloid structures. Enaminones are frequently employed as building blocks for the preparation of a variety bicyclic compounds and have been also recognized as potential anticonvulsant compounds.

(1) A structure search of the piperidine ring using the electronic version of the Drug Data Report (MDL Drug Data Report) revealed over 12 000 discrete piperidine entities that have been mentioned in clinical and preclinical studies.

16:10 Oral

An Approach for Application of Synthetic Peptides as Markers of Atherosclerotic Diseases

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Autoimmune or atherosclerotic diseases are resulted by multifunctional interactions including genetic predisposition, environmental circumstances, but also chronic bacterial or viral infections. One of essential factor is molecular mimicry of between human proteins and bacterial antigens observed in infections with *P. mirabilis*, *H. pylori*, *Chlamydia* and many others. In recent years, some papers have suggested that *H. pylori* plays a role in various extragastric diseases, for example, ischemic heart disease, idiopathic thrombocytopenic purpura, iron-deficiency anemia, and atherosclerosis. Since bacterial urease has been suggested to be a immunodominant antigen detected in infected persons, we considered that antibodies against this enzyme might be important in quest for the new marker of coronary heart diseases as well as *Helicobacter*-induced gastritis.

Peptide sequences (8-19-mer) characteristic for ureases of *H. pylori*, *Proteus sp*, *Vibriosp*, *Staphylococus*, *C. ensiformis* were synthesized on solid phase using 2-chlorotrityl resin and triazine based coupling reagent (DMT/NMM/BF_4) [1].

The study population consisted of 40 healthy blood donors and 30 atherosclerotic patients. We observed statistically significantly

stronger reactions of BK-61A peptide with *H. pylori*-infected atherosclerotic patients than with *H. pylori*-infected healthy blood donors. One can suggest that *H. pylori* urease generated specific human antibodies which, by molecular mimicry reacted with native human tissue peptides. This may have started inflammatory processes, such as complement activation *via* the classical pathway and macrophage and neutrophil activation. Amino acid sequence similarity was analyzed by the Basic Local Alignment Search Tool (Blast), which showed that the *H. pylori* urease fragment SIKEDVQF demonstrates as much as 75% similarity to several human proteins.

[1] Michał Arabski, Iwona Konieczna, Dariusz Sołowiej, Alicja Rogoń, Beata Kolesińska,

Zbigniew J. Kamiński, Wiesław Kaca, *Clinical Biochemistry*, **43** (2010) 115–123.

16:30 Oral

Multi-target-directed ligands in design and discovery of potential drugs for Alzheimer's disease

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Among the various drug discovery methods, a very promising modern approach relies on designing multiple ligands (DMLs). This methodology has been developed especially for drugs aimed at treatment of disorders with complex pathological mechanisms. One such disorder is Alzheimer's disease (AD), currently the most common multi-factorial neurodegenerative disease. The physiopathology of AD is complex and still eludes full understanding. Severe neuron and synapse loss, formation of intracellular fibrils of polymerized tau protein and extracellular β-amyloid (Aβ) deposits are prominent in AD. In addition, there is some evidence pointing to the role of oxidative stress, metal ion disregulation, inflammation and cell cycle regulatory failure in AD pathogenesis. There are many attractive targets for the development of anti-AD drugs, and the multi-factor nature of this disease requires multi-target-directed compounds, which can be beneficial for AD treatment. Successful outcomes of applying the multi-target-directed ligand methodology will be presented. These include examples of new compounds obtained via combination of structurally active moieties interacting with different targets. Our study concerned on the synthesis and investigation of new hybrid molecules bearing two moieties: 1-benzylpiperidino-4-amino group and heterocyclic rings linked by alkyl chain as potential AChE dual binding site and (butyrylcholinesterase) BuChE inhibitors. Cholinesterases exert secondary functions among which the mediating the processing and deposition of Aβ peptide seems to be crucial for the development of the AD. Identification of the peripheral anionic binding site of AChE as a fragment responsible for binding with Aβ and resulting fibrillogenesis caused the interest in synthesis of dual binding site AChE inhibitors.

Coffee beak

Monday afternoon, 10 May, 16:50
Hyrny Hotel- Patio and Garden

Poster Session I (Presentation of posters with odd numbers)

Monday afternoon, 10 May, 17:20
Hyrny Hotel

17:20 Poster 1

Modified SDS micelles for amino acid separation by MEKC. Application for amino acid profiling in formulations for parental use.

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Amino acids are widely used in medicine, particularly in products for clinical, parenteral and enteral nutrition. The products usually contain a mixture of α-amino acids and derivatives with a composition developed according to the type of primary disease and patient age. Parenteral nutrition is applied to patients in poor conditions when feeding throughout the digestive tract is inappropriate or impossible. Thus the quality and correctness of the formulations is extremely important issue of these products.

The quality control of the products above all requires amino acid analysis. There are a lot of chromatographic methods reported for the purpose with the most popular ion-exchange chromatography in pH-gradient elution with post-column ninhydrin derivatization. The separations usually require a multistep-gradient elution with long conditioning and analysis time. Additionally a few amino acids like tryptophan, cysteine, acetyltyrosine and acetylcysteine are assayed using disparate methods. Capillary electrophoresis is a rapidly growing analytical technique in recent years, especially useful for analyzing complex samples. It offers advantages of high separation efficiency and throughput that contributes to single-step analysis and lowering the costs.

The present study proposes a new method for amino acids determination that can be applied for amino acid profiling in solutions for parenteral nutriton. The MEKC method based on mixed micellar system was developed for the separation of 6-amino- quinolyl-N-hydroxysuccinimidyl carbamate (AQC) derivatized amino acids. Background electrolyte was based on Tris-borate buffer of high alkaline pH. Sodium dodecyl sulphate micelles were modified by use of 1,2-hexanodiol as a co-surfactant. The effect of the modifier on amino acid migration was studied with respect to hydrophobicity of analytes . The modifier appeared suitable to improve the separation of AQC tagged amino acids without adverse effect on buffer ionic strength or EOF velocity. The method was successfully validated and applied for amino acid profiling in medicinal preparations for parenteral nutrition. The results obtained were compared with a reference chromatographic method

17:20 Poster 3

Antitumor triazoloacridinone C-1305 as a potent FLT3 tyrosine kinase inhibitor in human leukemia MV4;11 cells.

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Constitutively active internal tandem duplication (ITD) in the juxtamembrane domain of Fms-like tyrosine kinase 3 (FLT3) is the most common molecular defect associated with acute myleoid leukemia. Its presence corresponds to a poor outcome in patients with acute myleoid leukemia who receive conventional chemotherapy. Therefore, FLT3-ITD has been considered to be an attractive molecular target for a novel therapeutic modality. Although several kinds of FLT3 inhibitors have been established *in vitro*, their activities as single agents have not been satisfactory in clinical studies. These observations encouraged us to seek for other classes of FLT3 inhibitors.

Recent studies showed that antitumor imidazoacridinone C-1311, synthesized in our Department and selected for phase II clinical trials, is highly potent inhibitor of certain receptor tyrosine kinase (RTKs), with nanomolar potency against FLT3 kinase.

The aim of this work was to investigate whether our next acridinone derivative, triazoloacridinone C-1305, a structural analog of C-1311, exhibited inhibitory activity towards FLT3 kinase *in vitro.* The antitumor C-1305 is DNA-damaging agent selected for extended preclinical trials.

Here, we tested C-1305 on two human leukemic cell lines with contrasting FLT3 status; MV4;11 cells with internal tandem duplications (ITD) mutation in the FLT3 receptor versus RS4;11 cells expressing wild type FLT3. Treatment of FLT3-ITD-positive cells, MV4;11 with C-1305 for 72 h suppressed cells' proliferation with an EC_{50} concentration of the drug 0,2 mM. In contrast, significantly higher concentration of the drug (EC_{50} 1,8 mM) was required to inhibit the growth of RS4;11 FLT3-WT cells. The direct effect of C-1305 on FLT3 receptor activation in MV4;11 cells was determined by analysis of its phosphorylation status. C-1305 reduced FLT3 phosphorylation in a time-dependent manner but at concentrations higher than EC_{50} value (between 5 and 10 μM). To determined whether the cytotoxic effect of C-1305 on MV4;11 FLT3-ITD-positive cells was due to the induction of apoptosis, we conducted annexin V binding assay as well as analysis of sub-G1 DNA fraction using flow cytometry. In both tests, the number of apoptotic cells increased after treatment with C-1305 in a time– and dose-dependent manner. The morphological examination of MV4;11 cells further confirmed the presence of apoptotic cells following C-1305 treatment, as evidenced by chromatin condensation and formation of apoptotic bodies. Importantly, apoptotic cell death was observed at concentrations of C-1305 required to block FLT3 phosphorylation suggesting that inhibition of FLT3 kinase by C-1305 may account for its cytotoxic activity in MV4;11 cells.

The overall results suggest that C-1305 is new inhibitor of FLT3 kinase *in vitro* which exerts potent activity towards acute leukemia cells harboring activating mutations of FLT3 tyrosine kinase.

17:20 Poster 5

Inhibition of DNA topoisomerases I and II by G3 PAMAM-NH_2 dendrimer - modified digoxin and proscillaridin A conjugates in a cell free system

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One specific way to overcome the side effects of cancer chemotherapy and to achieve improved therapeutic effects in the treatment of cancer is to develop drug delivery systems that enhance tumor cytotoxicity and cellular entry. Dendrimers, due to their controllable size and monodispersity, can act as excellent carriers for a wide range of molecules, which can be encapsulated in the interior of the dendrimer or interact with the dendrimer's terminal groups. Since dendrimers are synthesized from branched monomer units in a stepwise manner, it is possible to conduct a precise control on molecule size, shape, dimension, density, polarity, and solubility by choosing different branching units and surface functional groups. Cardiotonic steroids have long been and continue to be used in the treatment of congestive heart failure as positive inotropic agents. Epidemiological studies had shown that digitalis has also anti-cancer effects. Over the last 10 years, interest in developing cardiotonic steroids as anticancer agents has grown progressively. The studies on the structure–activity relationship revealed that lactone in position 17β is crucial for the cardiotoxicity of digoxin and proscillaridin A. Therefore, we synthesized two compounds Dig and Prosc, derivatives of these glycosides containing the carboxylic group instead of the lactone moiety. In the present study Dig and Prosc were conjugated to G3 PAMAM dendrimers (with 32 primary amino groups on surface) via amide linkage. Our previous experimental studies have demonstrated that these compounds treatment prevented the growth and decreased the number of viable cells in both estrogen-dependent MCF-7 and estrogen-independent MDA-MB-231 breast cancer cells. To test whether cytotoxic properties were related to DNA-binding and topoisomerases action, these conjugates were evaluated in a cell-free system. While the parent drug - Dig inhibited only topoisomerase II at concentration 100 nM, PAMAM-Dig conjugate inhibited both topoisomerase II at lower 30 nM concentration and additionally – inhibited topoisomerase I at the same 30 nM concentration. Prosc – as a parent drug was a potent poison of topoisomerase I and II at 100 nM and 30 nM, respectively, whereas PAMAM-Prosc inhibited either topoisomerase I and II at lower concentration of 30 nM. These studies suggest that the conjugation of modified glycosides Dig and Prosc with G3 PAMAM-NH_2 significantly improved the ability of parent compounds to inhibition of DNA topoisomerases. It seems to be very important, because poisoning of topoisomerases is fre-

quently associated with apoptosis and the level of topoisomerases in cancer cells are generally higher than in normal ones. Therefore, drugs able to interact with both DNA topoisomerase types may show selectivity for cancer cells.

17:20 Poster 7

The development of a GC method for analysis of residual acetic acid, triethylamine and N,N-dimethylformamide in hydrochloride salts of pharmaceutical substances

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Methods for determination of acetic acid, triethylamine and N,N-dimethylformamide as residual solventsin an API hydrochloride by GC method have been developed.

The main issue of the study was to find a solvent, which would guarantee dissolution of hydrochloride of pharmaceutical substances on the one hand, and allow for quantitative determination of acetic acid, triethylamine and N,N-dimethylformamide on the other. The tested substance was insoluble in solvents usually applied for GC analyses as: DMA, DMSO and DMI. After testing a few polar solvents, a mixture: 20% v/v of 25% aq NH_4OH in methanol was used.

The residual solvents were determined by direct injection gas chromatography with the use of flame-ionization detector and HP-PlotQ column (30 m long, 0,32 mm ID, 20 μm stationary phase).

Observed peak areas in the chromatograms of the standard working solutions (specification levels of analytes) were smaller than the corresponding peak areas in the chromatograms of the spiked tested substance sample with analytes solution at the same specification levels. That's why in the GC method the spiked sample with analyzed residual solvents at the specification levels must be used as standard solution.

According to the European Agency for the Evaluation of Medical Products it is considered that the amount of said solvents in pharmaceutical product must not exceed: acetic acid 5 000 ppm and N,N-dimethylformamide 880 ppm. For triethylamine specification limit has been established at the level NMT 0.15% (according to ICH Harmonised Tripartite Guideline – known impurity).

The method was validated and the validation included: selectivity, specificity, system precision, method precision, intermediate precision, accuracy (recovery), linearity, limits of detection and quantitation, robustness.

17:20 Poster 9

Antitumor activity of carrier-methotrexate conjugates.

Janusz Boratyński[1,2], Mohamed S. Omar[1], Dmitry Nevozhay[1], Urszula Kańska[1], Tomasz Goszczyński[1], Jarosław Ciekot[2], Joanna Wietrzyk[1]

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Background:

Methotrexate is one of the most widely used drugs in the treatment of malignancies for several decades. However, its low plasma half-life, toxicity to normal proliferating cells and other limitations impel scientists to search for improved forms of methotrexate. Conjugation of the drug with macromolecular carriers is one of the strategies frequently applied to improve therapeutic properties of anticancer drugs. We developed several conjugates of methotrexate and raltitrexed with different carriers, namely with fibrinogen, albumin, glycated proteins, dextrans and mannan. Conjugates were synthesized in a reaction of active esters or anhydrides of the drugs with amino or hydroxyl groups of carriers. Conjugates were studied both *in vitro* and *in vivo*. The majority of our carrier-methotrexate conjugates revealed improved antitumor activity *in vivo* against P388 leukemia as compared to original methotrexate. Conjugates of raltitrexed with different carriers were not active in therapy of experimental P388 leukemia.

Methods:

Glycation – lyophilizates containing mixture of glucose or fructose with bovine fibrinogen were heated at 65ºC for 30 min. Conjugates of glycated fibrinogen-MTX were obtained in the reaction with methotrexate anhydride. $B_6D_2F_1$ mice bearing P388 leukemia were i.p. injected once with free MTX or one of the fibrinogen-methotrexate conjugates at a dose of 40 mg/kg.

Results:

Up to 30% of all amino groups in fibrinogen carrier were substituted by "carbohydrate" and 25-45% by methotrexate. Depending on the conjugates used, there were 12-62% of mice survived more than two months after administration of conjugates, while the untreated mice died within two weeks. However, in most groups, some mice died of toxic effects of the conjugates.

Conclusion: Methotrexate substituted fibrinogen is very effective in experimental tumor treatment.

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17:20 Poster 11

Tricyclic derivatives of theophylline as potential ligands of CNS

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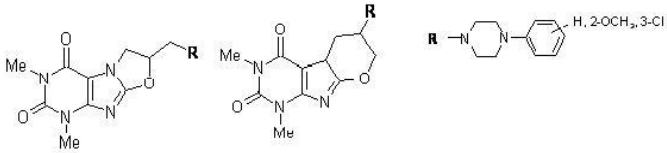
The multidirectional profile of pharmacological activity of methylxanthines and their biochemical mechanism of action is the reason for the development of the research in this group. Tricyclic theophylline derivatives, annelated six or seven membered heterocyclic ring at 7,8-position of theophylline generally demonstrated a different profile of its central nervous system activity, in comparison to the reference compound (theophylline). The pharmacological evaluation of a series of tricyclic theophylline derivatives with a pyrimido- or diazepino-moiety demonstrated sedative, hypothermizing and neuroleptic-like effects on the CNS [1, 2]. Derivatives of imidazo- and pyrimido[2,1-f]theophylline with various LCAPs moiety showed high or very high 5-HT_{1A} receptor affinity and diversified pharmacological profile. Preclinical studies indicated that 8-[3-(N4-phenyl)-piperazin-N1-yl-propyl]-1,3-dimethyl-(1H,8H)-imidazo[2,1-f]purine-2,4-dione exerts anxiolytic-like activity in the four-plate test in mice; however its effect was weaker, than that produced by Diazepam. This compound and 8-[3-(N4-20- metoxyphenyl)-piperazin-N1-yl-propyl]-1,3-dimethyl- (1H,8H)-imidazo-[2,1-f]purine-2,4-dione behaved like antidepressants in the forced swimming test in mice; and their activity in that model was comparable with the effect of Imipramine [3]. Derivatives of oxazolo- and oxazino-purinediones as bioisosteric analogs of 8-styryl xanthines, showed mainly affinity for adenosine A_{2A} and some of them showed anticonvulsant activity in MES and scMet tests [4].

As a continuation of our studies, in this course we decided to designed, synthesized and preliminary evaluated on pharmacological studies, derivatives of oxazolo- and oxazino[2,3-f]theophyllines with various arylpiperazinyl moiety. These compounds were synthesized by cyclization of 8-bromotheophylline with various oxiranes, in presence of a catalytic amount of pirydine.



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17:20 Poster 13

Evaluation of proliferation and cellular death of A375 cell line in the presence of HDACs inhibitors

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Inhibitors of histone deacetylases (HDACs) are a group of compounds displaying clear anticancer activity. They have been shown to inhibit proliferation, induce apoptosis and augment differentiation in a variety of tumour cells in vitro. Valproic acid (VPA), a well known antiepileptic drug, has been shown to inhibit HDACs in some transformed cell lines. Several studies confirmed the influence of VPA on proliferation, apoptosis and differentiation processes in malignant cells. Besides, its antitumor properties include the inhibition of angiogenesis and metastasis. In melanoma cells VPA induced the cell cycle arrest in G1 phase as well as apoptosis. This effect was associated with up-regulation of P16 protein – a cell cycle inhibitor. Generally, VPA is considered as a candidate drug useful both in the chemotherapy of advanced neoplasias and chemoprevention or control of residual minimal disease. Clinical trials included phase I/II study of the therapy with VPA combined standard chemoimmunotherapy of patients suffering from advanced stage melanoma.

Butyric acid, a four-carbon fatty acid, is a well known inhibitor of histone deacetylases. It is formed in the human colon as a result of anaerobic bacterial fermentation of dietary fiber. It is believed that butyrate plays an important chemopreventive role in colorectal carcinogenesis.

The aim of our study was to compare the influence of VPA and sodium butyrate (NaB) on morphology, growth rate and apoptosis in human melanoma cell line A375. Both compounds were used at concentrations ranging from 0.1 to 10 mM. Cell proliferation was measured using sulforhodamine B, a protein-binding dye. Apoptosis was characterized morphologically by acridine orange staining of detached cells to visualize the condensed chromatin and fragmented nuclei of apoptotic cells. Moreover, caspase-3 activity was determined for reliable quantitative evaluation of apoptosis.

Inhibition of cell growth was found in cells treated with both compounds at the concentration of 1 mM. Incubation of cells with tested compounds at concentrations of 3mM and 10mM resulted in considerable changes in cell morphology. Numerous cells detached from the tissue culture dishes and floated in the medium. Examination of these cells by acridine orange staining revealed the typical morphological characteristics of apoptosis such as condensed chromatin and fragmented nuclei. The manner of action of examined compounds (VPA and NaB) was similar.

17:20 Poster 15

Research on isolation and identification of cyclolinopeptides from waste products of the flax linseed (Linum usitatissimum L.)

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Flax is one of the oldest crop plants. Various parts of the plant have been used to make fabric, dye or paper. Flax is also known for its beneficial effect on health. The linseed supplies about 8% mucilages, from 30 to 40% of oil, 25% of proteins, glucosides, sterols, enzymes, mineral salts and other compounds.

Rich composition of the bioactive compounds included in flax, especially proteins like cyclolinopeptides (cyclolinopeptide A, naturally existing immunomodulatory peptide) induced us to interest in this topic. The cyclolinopeptides are included not only in the linseed, but also in the scrap material, roots, oilcakes and chaff.

The purpose of the work was isolated fraction of cyclolinopeptides from oilcakes and chaffs. The individual cyclolinopeptide were separated with chromatographic method and identified by spectroscopic methods.

To solvent extraction method various organic solvents (hexane, cyclohexane and toluene) have been used for examinations. The process of the extraction was carried out periodically with solvents in the room temperature and in their boiling point.

The percentage content of cyclolinopeptide A in individual fractions was established by the HPLC method.

The method of distinguishing the cyclolinopeptide CLA from linseed cakes was drawn up. The first step was the solvent extraction in the room temperature, and then the threefold extraction in the boiling point. The preliminary extraction was applied to removed oil remains from oilcakes.

Cyclolinopeptide A was obtained with high productivity about 0.02 % (in converting into the plant material) the content of cyclolinopeptide A in plant material amount 0.66 %. Moreover, the LC-MS/MS method confirmed that the extract from linseed cakes contains cyclolinopeptide A and comparable amounts of cyclolinopeptide E.

17:20 Poster 17

Baicalin inhibits free radicals processes caused by chromium and cisplatin.

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In the presented study the antioxidant activity of flavonoid from Scutellaria baicalensis – baicalin on lipid peroxidation (TBARS) caused by ions of Cr(III) and Cr(VI) was evaluated. Also the scavenging ability of baicalin towards hydroxyl radical ($^{\cdot}$OH) generated by chromium ions was investigated. Moreover the potential activity of baicalin in lowering the enhancement free radicals processes caused by cytotoxic drug -cisplatin was evaluated.

The chromium is essential in the organism e.g. for the correct metabolism of the glucose (an ingredient of the glucose tolerance factor - GTF), but it is also a toxic metal. The toxicity of the chromium depends closely on its valence and it is connected substantially with oxidative properties. Cr(VI) is classified by IARC to carcinogens for people. The mechanism of Cr(VI)-induced toxicity and carcinogenesis is not well understood, although recent study suggest the involvement of Cr(VI) in free radical formation. Similarly toxic effects of cisplatin, mainly ototoxicity, nephrotoxicity or the hepatotoxicity could be connected with ROS (reactive oxygen species) generation.

Scutellaria baicalensis is the Chinese plant rich in flavonoids, especially baicalin, baicalein and wogonosid . The extract received from this plant (Antoxid) showed to be active radicals scavenger, also inhibiting the lipid peroxidation caused by the chromium compounds. It was interesting to know whether baicalin abundantly present in Antoxid can be responsible for this antioxidative effect.

The research was performed on *in vitro* mitochondrial model from human placenta and erythrocytes from fresh blood. Baicalin at the concentrations from 10 μmol to 200 μmol showed the antioxidative effect by the decrease TBARS level in the erythrocytes ($p<0,05$). At concentrations from 20 μmol to 200 μmol decreased the content of hydroxyl radicals in the mitochondrial suspension ($p<0,05$). Moreover the experiment demonstrated the baicalin effectiveness in the inhibiting lipid peroxidation and hydroxyl radical formation enhanced with Cr (III) and Cr (VI) ions and cisplatin. It seems probable that Antoxid beneficial effect shown earlier is a consequence of the high content of baicalin.

17:20 Poster 19

Development of dendrimeric somatostatin analogues

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Low molecular weight dendrimeric peptides are very prospective compounds. Their terminal functional groups can be functionalized towards specific medical purpose. Cancer disease is in many countries primary cause of deaths. However, number of available therapies is still limited. In this field somatostatin analogues constitute new promising class of molecules targeting new type of receptor.

Somatostatin (SST) is a peptide that was originally characterized as a physiological inhibitor of growth hormone. In addition, SST has another multiple functions. It regulates endocrine and exocrine secretion, possesses antiproliferative properties and acts as a neuro-

transmitter/neuromodulator. These diverse physiological effects are mediated by a family of G-protein-coupled receptors, called somatostatin receptors sst_1-sst_5. Presently, there are several cyclic synthetic somatostatin analogues (eg octreotide, lanreotide), clinically used for cancer therapy and gastrointestinal disorders, that primarily ineract with receptors sst_2.

Our goal is to use peptide dendrimers to design molecules with somatostatin-like biological activity. For this purpose, restricted somatostatin sequences have been attached to dendrimer branches in order to mimic topography of active structure(s) responsible for interactions with somatostatin receptors.

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Salivary aldehyde dehydrogenase activity in healthy subject's group - influence of drugs intake, preliminary research.

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The salivary aldehyde dehydrogenase (ALDH3A1) oxidize mainly aromatic and long chain aliphatic aldehydes. ALDH3A1 protects organisms from aldehydes originating from food and air pollution, and can also be an important factor in chemical carcinogenesis prevention. The salivary ALDH3A1 in most examined samples is > 60% inactive as a result of sulphhydryl groups oxidation in the active site. Our previous studies showed that ALDH3A1 activity (total activity and inactivation degree) is strongly variable intra-individually during the day time and within healthy population. Causes of this high variability are still being examined.

Saliva samples were collected directly to test tubes with 50mM pyrophosphate buffer (pH 8.1) with 0.5 mM EDTA and 1mM GSH. The activity was measured in the presents of 1mM GSH, which prevent futher oxidation of enzyme and in the presents of 0.5 mM DTT, the thiol which reactivate oxidated enzyme (total activity). The ALDH inactivation degree was calculated using formula:

$$I = \left(1 - \frac{V_{GSH}}{V_{DTT}}\right) \cdot 100\%$$

where V_{GSH} and V_{DTT} are reaction rates determined in the presence of 1 mM GSH and 0.5 mM DTT, respectively.

The aim of this study was to describe influence of drugs intake on salivary aldehyde dehydrogenase activity. Hierarchical clustering divided two dimensional data (total ALDH activity, inactivation degree) derived from a group of 116 subjects into four groups. Groups were analyzed by a correspondence analysis. Both total ALDH3A1 activity and an inactivation degree vary in healthy subjects depending on a cigarette smoking, alcohol and coffee consumption as well as an age and a diet. The preliminary study showed that hypotensive, antinflamatory and hormonal contraceptive drugs also affect salivary ALDH activity. These investigations are important and may be useful for food safety and nutrition research.

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Synthesis of embutramide in mild conditions

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Embutramide is a potent sedative drug. It was developed as a general anesthetic agent, but was found to be too dangerous for assumed purpose. It was used in veterinary medicine for euthanasia of a range of different animals, mainly small animals kept as pets but also large farm animals.

In our studies of three-step synthesis of Embutramide we have selected Phase-Transfer conditions[1] for alkylation of 1 in a first step and for catalytic reduction of **2** using sodium borohydride, nickel or cobalt salts[2] (Figure 1). Contrary to well known method of synthesis of amine **3**[3], used reagents provided mild conditions of reduction process under atmospheric pressure. The optimization of alkylation process of 1 will be presented in this article. The best results were obtained when 60% solution of KOH as a base and TBAB as a catalyst were used. Under these conditions the excellent yield and purity of the product was obtained and the product can be used for the next step without any purification. In the second step nitrile **2** was reduced to amine **3** by excess sodium borohydride, cobalt (II) chloride or nickel (II) chloride, in methanol. The amine **3** of good yield and very good purity was obtained (impurities were significantly limited).

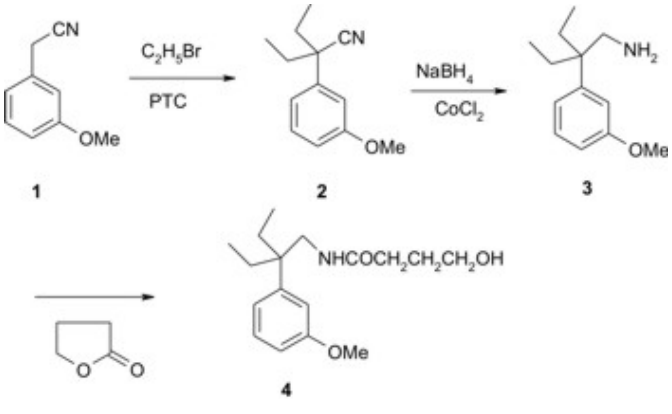

Fig 1 Synthesis of Embutramide 4

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17:20 Poster 25

The possible role of HSP60 in synergistic action of anthracyclines and sulindac in HeLa cells

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Background. As was observed in the earlier studies doxorubicin (DOX) induced apoptosis in HeLa cells and that effect was potentiated significantly by sulindac (SUL). **Aim.** The aim of the current work was to study: - the effects od DOX and SUL on *HSP60, HSF1* and HSP60 expression; - the influence of DOX and SUL on HSP60 translocation.

Methods. Expression of *HSP60* and *HSF1* was determined with QRT-PCR; the *HSP60* and *HSF1* expression and localization of HSP60 was evaluated with Western blot. The 24-hr cultures were co-incubated with DOX-1 micromole and/or SUL-50 micromoles.

Results. The significant induction of *HSF1* and *HSP60* mRNA level was observed after exposure of the cells to DOX-1 micromole. SUL-50 micromoles alone caused moderate increase in mRNA level. The significant decrease in expression of *HSF1* and *HSP60* was noted after DOX-1 micromole and SUL-50 micromoles simultaneous treatment. HSP60 appeared in the higher levels in cytosol than in mitochondria but no intracellular translocation was noted.

Conclusions: - the effects of HSP60 and *HSF1* evoked in the cells depend on the inducer; - proapoptotic action of DOX+SUL may correlate to the increased expression of *HSF1* and *HSP60; -HSP60* mRNA level and the regulation of that protein expression depend on the apoptotic inducer; - the role of HSP60 in apoptosis expressed in potential shift between mitochondria and cytosol is determined by the apoptotic inducer and the cell type.

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Influence of troglitazone, sodium butyrate and 5-aminosalicylic acid on the chemokine ENA-78/CXCL5 secretion in the intestinal subepithelial myofibroblasts

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Chronic mucosal inflammation is a hallmark of inflammatory bowel disease (IBD). Concentrations of proinflammatory cytokines are noticeably increased in the intestinal mucosa of IBD patients. It has been demonstrated that nuclear factor kappaB (NFkappaB) plays a central role in the regulation of inflammatory signaling pathways in the colon tissue. NFkappaB activates expression of many genes associated with immune function in the gut, e.g. IL-8, IL-6, TNF-α, GM-CSF, and ENA-78/CXCL5 [1]. IL-8 and ENA-78/CXCL5 play complementary and sequential roles in neutrophil recruitment in ulcerative colitis [2]. Fundamental biological processes such as cell motility, proliferation, differentiation, apoptosis, morphogenesis, tissue repair, inflammation, and the immune response in the gut tissue are controlled by myofibroblasts located in the lamina propria under the epithelial cell layer [3].

The present study was aimed at evaluating ENA-78/CXCL5 secretion by human normal colon myofibroblasts CCD-18Co treated with troglitazone (Tro), 5-aminosalicylic acid (5-ASA), sodium butyrate (NaB), and NFκB inhibitor BAY 11–7082.

ENA-78/CXCL5 secretion by TNF-α – stimulated myofibroblasts was evaluated using the commercially available enzyme-linked immunosorbent assay (ELISA) kit. Viability of CCD-18Co cells treated with the chemicals was measured using the XTT tetrazolium salt based assay.

The results showed that 5-ASA, NaB, and BAY 11–7082 decreased ENA-78/CXCL5 secretion in a dose-dependent manner ,while troglitazone (10 - 30 μM) increased secretion of this chemokine by myofibroblasts. All the tested compounds decreased viability of the cells.

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The Pharmacopoeia in preparation and assessment of pharmaceutical, chemical and biological documentation of medicinal products

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Quality of medicinal product is assessed in the authorization process on the basis of chemical, pharmaceutical and biological documentation attached to the application for granting Marketing Authorization. This documentation forms Module 3 of registration dossier in the format of Common Technical Document - CTD. CTD indicates only the presentation and format of the data and documentation which should be attached to the application form. The testing procedures documenting the quality of active substance(s), excipient(s), pharmaceutical form and packaging are specified by the European Pharmacopoeia or its Polish version (Polish Pharmacopoeia) and the relevant guidelines. The guidelines are listed in annex to module 3. They are available on the EMA website: http://www.ema.europa.eu/htms/human/humanguidelines/quality.htm

Active substance(s) and excipient(s) which have no individual monographs of pharmacopoeia should be compliant to the requirements of the general monograph “Substances for pharmaceutical use”.

In case where the above mentioned monographs are described neither in the European Pharmacopoeia nor in the pharmacopoeia of a Member State, compliance with the monograph of a third country pharmacopoeia can be accepted. In such cases, the applicant shall submit a copy of the monograph accompanied by the validation of the analytical procedures contained in the monograph and by a translation where appropriate.

The quality standards represented by monographs are valid only where the articles in question are produced within the framework of a suitable quality system.

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The European Pharmacopoeia in the Polish Pharmacopoeia

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Correct quality of medicinal products is connected with full filing all the criteria of quality of: identification, purity, content of active substance and functionality which are included in suitable documents, e.g. in pharmacopoeial monographs. General and individual monographs compose the Pharmacopoeia, however the national and international legislation makes them obligatory standards at a given territory.

The European Pharmacopoeia is obligatory in member states of *Convention on the Elaboration of a European Pharmacopoeia* replacing any national monograph on the same subject. The Polish version of European Pharmacopoeia is published as Polish Pharmacopoeia VII/VIII edition by The Office for Registration of Medicinal Products, Medical Devices and Biocidal Products. The implementation of European Pharmacopoeia requirements into Polish Pharmacopoeia changes existing rules of Polish Pharmacopoeia applications.

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Direct enantiomeric resolution of tamsulosin, clinically used chiral O-phenylethanolamine derivative with α_1-blocking activity

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Tamsulosin is an α_1-adrenoreceptor antagonist for the treatment of symptomatic benign prostatic hyperplasia. It was one of the first subtype-selective α_1-adrenoceptor antagonist with specificity for prostatic α_1-adrenoceptors to become available for the treatment of patients with symptomatic benign prostatic hyperplasia (BPH). Benign prostatic hyperplasia is a common disease affecting elderly men (about 50%), and leads to a variety of urological symptoms. Urinary flow is influenced by many factors, particularly by prostatic and urethal smooth muscle tone, which is mainly controlled by α_1-adrenoceptors. The medical treatment of BPH is amongst others directed towards the use of agents that block those receptors. It has been reported that in rabbit lower urinary tract and prostate preparations (R)-enancjomer of tamsulosin was 50-141 times more potent than (S)-enantiomer [1].

Here we report 3 methods for direct chromatographic determination of (R)- and (S)-tamsulosin on two carbohydrate - amylose tris(3,5-dimethylphenylcarbamate) and tris-(S)-1-phenylethyl- carbamate - and on β-cyclodextrine bound chiral stationary phases ($R_S \geq 2.69$). Employed mobile phases were hexane-ethanol-ethanolamine (80:20:0.2) for amylose tris(3,5-dimethylphenylcarbamate), hexane-ethanol-diethylamine (75:25:0,025) for amylase tris-(S)-1-phenylethylcarbamate and phosphate buffer (pH 7.0)-acetonitrile (65:35) for cyclodextrine stationary phase. The elution order of enantiomers was (S) before (R) on carbohydrate and (R) before (S) on cyclodextrine stationary phases. It should be noted that on cellulose tris(3,5-dimethylphenylcarbamate) only poor enantioresolution hase been obtained (R_S between 0.57 and 0.93). That would suggest that helical amylose structure should be important for the enantiodifferentiation [3].

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Improvement of the Phase-Transfer Catalysis (PTC) method for synthesis of L-proline derivatives

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The objective of the research work is to show a simple method of synthesis of 4-cyclohexyl-S-proline as an intermediate product in the preparation of Fosinopril - prodrug used in the treatment of hypertension and acute or chronic congestive heart failure.

Our three-steps method includes simple, commonly known reactions such as aldol reaction, Michael reaction, hydrogen reduction.

In the first stage of the suggested synthesis we used aldol condensation. That powerful method is used to form carbon–carbon bonds between clohexane carboxaldehyde condensed with molecule containing an acidic hydrogen atom.

In the second step of the 4-cyclohexyl-S-proline synthesis the Michael reaction was used. In which consist of the nucleophilic addition of a carboanion to an α, β unsaturated carbonyl compound and formation of C-C bonds. The resulting product contains one asymmetric carbon atom. In an extension of those studies, we report amplification of our qualitative reports by determining the enantiomeric excess after second and third step of reaction.

In the synthesis the Phase Transfer Catalysis (PTC) method was applied. Moreover, the reaction was carried out with the primary and secondary amines as catalyst. The final product was collected and purified by repeated column chromatography on silica gel. The structures were confirmed by spectroscopic methods: UV-vis, IR, ^{1}H NMR.

The presented method of preparation of 4-cyclohexyl-S-proline, as an intermediate in the synthesis of Fosinopril, is highly effective and competitive with other methods presented in the literature.

That work is a part of a research project No. N N209 237336 financed by the Ministry of Science and Higher Education.

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Synthesis and physicochemical properties of cationic chitosan derivative for heparin complexation

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Chitosan is a biocompatible and biodegradable linear polysaccharide of great potential for biomedical applications[1]. We have found that chitosan can form complexes with heparin, an anionic polysaccharide commonly used as an anticoagulant drug.[2] Therefore it may be potentially useful as an agent for neutralization an anticoagulant action of heparin.

However, our model laboratory studies have shown that the efficiency of complexation of unfractionated heparin (UFH) by pristine chitosan is low at the pH values characteristic for blood (pH = 7.4). Therefore we have modified chitosan to obtain its cationic derivative (ChGl). That polymer is very well soluble in water at pH 7.4. The interactions between cationically modified chitosan and heparin (both unfractionated and low-molecular-weight one (LMWH)) have been studied. It was shown that heparin complexation capability by ChGl is comparable to that of protamine sulfate, currently used as a drug of choice for heparin neutralization. Both complexes (ChGl-UFH and ChGl-LMWH) are stable at neutral pH. What is more the thermal sterilization of cationic chitosan (at 1600C for 1 hour) does not influence its capability of heparin binding.

Using dynamic light scattering (DLS) technique we have determined the dimensions of the objects formed as a result of complexation of UFH with PS and ChGl in the aqueous solutions (PBS buffer, pH = 7.4). It has been found that UFH-ChGl complexes have smaller dimensions and lower polydispersity than these formed as a results of heparin complexation with protamine (UFH-PS).

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Induced Fit Homology Model of the 5-HT$_6$ Serotonin Receptor - Application for Virtual Screening

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The 5-HT$_6$ serotonin receptor (5-HT$_6$R) is involved in regulation of variety of physiological processes affecting i.e. cognition, food intake and mood. Therefore 5-HT$_6$R ligands are likely to be effective in therapy of various forms of dementia, schizophrenia, obesity or depression. However, rational design of new 5-HT$_6$R ligands is difficult because of limited knowledge about spatial configuration of 5□HT$_6$R binding site. Homology modeling is a method of choice in such a case. The homology model of 5-HT$_6$R was built using induced fit docking protocol, integrated into Schrödinger Suite 2009 software. This method seems to be very useful in homology modeling of GPCRs since it combines flexible ligand docking with protein structure prediction and side chains refinement [1]. Fair sequence homology between serotonin 5-HT$_6$ and adrenergic β_2 receptors, both of which belong to the same GPCR subfamily, influenced the choice of the latter receptor structure as a template for homology modeling [2]. For this purpose, 14 selective ligands of 5-HT$_6$R with lowest possible conformational flexibility were used [3]. All of these ligands were docked to each obtained model to select the receptors which properly describe interactions in the whole group of ligands. During further validation, a set of ligands with known 5-HT$_6$R affinity (active and non-active) were automatically docked to the selected models in order to verify their usefulness in a virtual screening procedure. Successful verification allowed to carry out the virtual screening trial in which a database of over 17000 compounds has been searched. In order to establish enrichment factor several 5-HT$_6$R high affinity ligands were enclosed within the database [4].

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Evaluation of transcriptional activity of genes encoding IL-6 and its receptor in colon cancer cells treated with phytic acid

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Phytic acid (IP6), a natural dietary component possesses promising cancer preventive and therapeutic activity, of which molecular mechanism remains unclear. Anticancer potential of IP6 has been linked to the modulation of many cellular processes, including its influence on the expression of cytokines. Interleukin-6 (IL-6) is a multifunctional cytokine that has both pro- and anti-inflammatory properties. In normal, adenomatous and cancerous human colon mucosa IL-6 mRNA is expressed at a low level. The aim of this study was to evaluate the effect of phytic acid on the expression of genes encoding IL-6 and its receptor IL-6R in human colorectal cancer cell line Caco-2. The cells were cultured in RPMI 1640 medium supplemented with 10% fetal bovine serum, 100 U/ml penicilline and 100 µg/ml streptomycin. They were grown at 37°C as monolayers in a humidified atmosphere containing 5% CO_2. Cells were treated with 1, 2.5, 5 mM IP6 for 1, 6, 12 and 24 h. Total RNA was extracted from control and IP6 treated cells with the use of TRIZOL® reagent according to the producer's protocol. Quantification of the genes expression was performed by real time QRT-PCR using an Opticon™ DNA Engine Continuous Fluorescence detector (MJ Research, Watertown, MA). The results were recalculated per mg of total RNA. Statistical analysis was performed with the use of Statistica 8.0 software. Phytic acid at concentrations up to 2,5 mM, had no effect on the transcriptional activity of gene encoding IL-6 ($p>0,05$; ANOVA). After 24h, the expression of IL-6 mRNA was up-regulated in Caco-2 cells exposed to 5mM IP6 in comparison with control ($p=0,0026$; Tukey test). At 1h, there were no quantitative changes in the IL-6R gene expression in unstimulated and IP6-stimulated cells ($p>0,05$; ANOVA). The increase in transcriptional activity of IL-6R gene in response to 2,5 mM IP6 after 12h ($p=0,0254$) and 24 h ($p=0,0002$) was observed. Cells treated with 5mM IP6 at 6 - 24h showed a significant ($p<0,05$) increase in the expression of gene encoding IL-6 receptor. In conclusion, IP6 at physiological concentration (1 mM) in the intestinal lumen, failed to induce any alternations in IL-6 and IL-6R genes expression. The increase in IL-6 transcript level was evoked by 5 mM IP6 in the longest-lasting cultures. Along with the increasing IP6 doses and exposure time, Caco-2 cells expressed successively higher IL-6R transcript level.

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Glycosyl O- and S- N-allyl thiocarbamates in the synthesis oligosaccharides.

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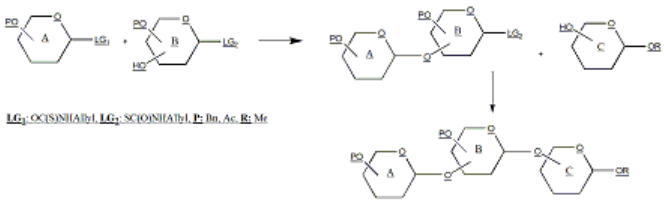
We now report that thiosugars can be obtained by rearrangement of thiocarbamates derivatives of monosaccharides in reaction with commercially available reagents in good yields. Thiosugars obtained by the rearrangement can be easily hydrolyzed, thus thiosaccharides unsubstituted in anomeric position can be obtained by this method as well. This thiocarbamates can be use in the orthogonal glycosylation.

In orthogonal glycosidation a range of glycosyl donors that bear different leaving groups (LG1/ LG2) 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-allyl thiocarbamates (A) readily obtained from anomerically-unprotected sugars are very reactive glycosyl donors[2]. They can be readily activated with bromine. On the other hand the S-glycosyl-N-allyl thiocarbamates (B) can be used as glycosyl acceptors. They can be activated with thiophilic reagents. The application this method to the synthesis of trisaccharides will be presented.



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HPLC determination of cefuroxime in human plasma

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Cefuroxime, (6R,7R)-3-{[(aminocarbonyl)oxy]methyl}-7-{[(2E)-2-(2-furyl)- 2(methoxy- imino)acetyl]amino}-8-oxo-5-thia- 1-aza- bicyclo[4.2.0]oct-2-ene-2-carboxylic acid is a second-generation cephalosporin antibiotic. Although as a second-generation it is less susceptible to ß-lactamase and and so may have greater activity against Haemophilus influenzae, Neisseria gonorrhoeae and Lyme disease. Unlike other second generation cephalosporins cefuroxime can cross the blood-brain-barrier.

A HPLC method with UV detection for the determination of cefuroxime in human plasma has been developed. The method used cefalexin as the internal standard. The drug and the internal standard were isolated from plasma using a protein precipitation. The analysis was carried out on Supelcosil LC-18-DB 250 x 4.6 mm, 5 µm (Supelco) column using a 0.07 M sodium dihydrophosphate solution containing 10 % acetonitrile as the mobile phase. The flow rate was 1.7 mL/min. The linearity was established over the concentration range of 0.2 – 12.0 µg/mL. The method was validated according to FDA and EMEA requirements and all of the investigated parameters met the acceptance criteria.

The developed method can be applied to the pharmacokinetic studies in humans, e.g. bioequivalence studies.

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Synthesis and proapoptotic properties of new casein kinase II inhibitors

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Casein kinase II (CK2) is the most pleiotropic of all protein kinases with more than 300 substrates implicated in a wide variety of cellular functions as signal transduction, proliferation and cell survival. Increased levels of CK2 has been demonstrated in a number of cancers where regulates the activity of various oncoproteins and tumor suppressor proteins. Therefore, CK2 inhibitors could be considered as potential anticancer drugs in monotherapy or in combination with known cytostatics. In this study we used a new strong CK2 inhibitor -4,5,6,7-tetraiodobenzimidazole (TIBI) (IC50 = 38 nM) as well as its 2-substituted derivatives. TIBI was obtained by iodination of benzimidazole with iodine in sulfuric acid-periodic acid mixture. The substitution of 2-bromo-4,5,6,7-tetraiodobenzimidazole with piperidine, piperazine (TIBIPIP) and N-methylpiperazine provided the respective 2-modified 4,5,6,7-tetraiodobenzimidazoles (TBIPIP). The CK2 inhibitor (TIBI) and its two derivatives (TIBIPIP, TBIPIP) were tested for determination of the proapoptotic and cytostatic effects on the promyelocytic leukemia cell line (HL-60). The flow-cytometry results of the tested compounds showed that apoptotic effect was concentration and time dependent. The changes of the mitochondrial membrane potential and cell cycle progression were also observed.

The study was supported by the Foundation for Development Diagnostics and Therapy, Warsaw, Poland (AO i ZK).

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Determination of impurities in medical products containing metformin hydrochloride

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Metformin (dimethylbiguanide) is a biguanide derivative in which two hydrogen atoms at the N1 nitrogen have been substituted with methyl groups. It is the only biguanide derivative currently used in the medicine.

The medical products containing metformin hydrochloride as the active substance have been used in patients with type II diabetes (insulin□independent), in particular in obese patients who cannot achieve normal blood glucose concentrations despite using a diet. In these patients, the pancreas does not produce a sufficient amount of insulin, or the body does not respond normally to insulin, leading to glucose accumulation in the blood. Metformin hydrochloride increases the body's susceptibility to insulin and helps to restore its normal use in the body.

There are many medical products containing metformin as the active substance in the pharmaceutical market. An important element of ensuring their safety of use is to monitor the impurities.

The possible impurities in metformin hydrochloride are the following related substances: cyanoguanidine, melamine, (4,6-diamine -1,3,5-triazine-2-yl)guanidine, N,N-dimethyl-1,3,5- triazine-2,4,6-triamine, 1-methylol biguanidine and N-methyl methan amine.

According to the USP monograph for tablets containing metformin hydrochloride as the active substance, only the level of unidentified impurities is specified. Their amount cannot exceed 0.1%, and the sum of all impurities cannot exceed the value of 0.6% at metformin hydrochloride concentration of 5 mg/ml. According to the Ph.Eur. and USP monographs for metformin hydrochloride (substance), only the cyanoguanidine assay and tests for individual unidentified impurities are performed.

The Polish Pharmacopoeia contains no monograph for metformin hydrochloride.

The object of our study was to identify and to quantify the first four of the above□mentioned impurities (due to the availability of standard substances) in medical products containing metformin hydrochloride, and to determine their limits of detection and quantitation.

The separation of the impurities was performed using a PARTI-

SPHER SCX column and using a spectrophotometric detector (λ = 218 nm). The mobile phase was 1.7% (m/v) ammonium dihydrogen phosphate water solution, with pH adjusted to 3.1 using 85% orthophosphoric acid.

The proposed method is rapid, sensitive and selective, and it can be used to evaluate those medical products for which the impurity tests are not currently performed, as well as those for which only cyanoguanidine or cyanoguanidine and melamine assays are performed.

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Synthesis and biological evaluation of new inhibitors of thioredoxin - thioredoxin reductase.

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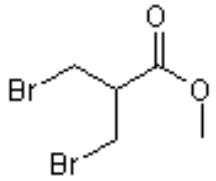
Thioredoxin system (TR) is responsible for regulating redox state in cell. The system is based on two enzymes: thioredoxin and thioredoxin reductase. Thioredoxin helps tumor to manage oxidative stress, the side effect of very quick proliferation.[1] Inhibition of the thioredoxin system will damage this defense system what may sensitize cells to other chemotherapeutics. Some TR inhibitor, such as PX-12, are currently clinically tested in solid tumor therapies.[2]



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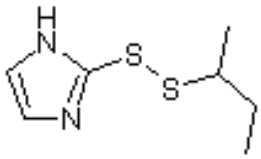

PX-12

In active site of both enzymes there are two cysteine residues which are oxidized or reduced in the disulfide bond formation manner.[3] Those cysteines can be covalently alkylated by alkylating agents i.ex. compound **1**.Alkylated residues are no longer able to create disulfide bond and enzyme activity is blocked. Although **1** inhibits TR in very low concentration, it cannot act as a drug, because of high reactivity and low selectivity.

In our work, to increase the selectivity we have incorporated an electrophilic fragment of compound 1 into peptidemimetic scaffold, using Ugi reaction as a key step. The result of this studies and their biological evaluation of those new inhibitors will be presented.

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17:20 Poster 53

Molecular dynamics of ligand binding and concurrent activation steps in opioid receptors

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Opioid receptors belong to large and diverse family of transmembrane receptor proteins called GPCRs (G Protein Coupled Receptors) which are responsible for transduction of a signal across the plasma membrane. Activated receptor goes through series of conformational changes that enable binding of a G protein in a cytosol. Drugs that interact with opioid receptors cause multiple effects including analgesia, sedation, euphoria and physical dependence. Therefore, the three opioid receptor subtypes: mu (MOR), delta (DOR) and kappa (KOR) are very important pharmacological targets. Discovery of new more potent and selective ligands for each of these three receptor subtypes should suppress the unwanted side effects. Drug design is mostly limited by the scarcity of structural information on receptor proteins. Up to date, structures of only four members of GPCR family have been reported: rhodopsin, β_1-, β_2-adrenergic receptor and adenosine receptor.

Agonist binding is the first step in ligand-induced receptor activation. During activation receptor undergoes a series of conformational rearrangements controlled by molecular switches leading to partially or fully active state of the receptor [1]. To investigate the relationship between the final movements of a ligand in a binding site and the first steps of the activation process in opioid receptors we chose a set of rigid ligands with the structural motif of tyramine (analogs of morphine). The structures of three opioid receptors were built using homology/comparative modeling techniques based on crystallographic structure of inactive rhodopsin. Additionally, N-termini and extracellular loops of KOR were built using the *ab initio* CABS method. Series of agonists and antagonists were docked to the receptor models using simulated annealing procedure and the complexes were simulated in water and lipid environment using GROMACS program. Based on conducted molecular dynamics simulations and on available mutagenesis data we proposed different binding modes for agonists and antagonists. They all initially bind to Y3.33 but only agonists are able to move deeper to H6.52. The movement from Y3.33 to H6.52 induces breaking of the connection between TM3 and TM7 (3-7 lock). Breaking of TM3-TM7 connection was suggested to be the first activation step in rhodopsin [2]. We also observed an action of the extended rotamer toggle switch which can suggest interdependence between those two switches [3].

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17:20	Poster	55

New tachykinin-opioid chimeric analogues as potential new analgesics

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Multidrug therapies become a standard of modern successful therapies in pain treatment in the clinic. However, the different pharmacodynamic profile of the drugs creates limits of using a mixture. Chimerization of tachykinin and opioid pharmacophores in one molecule is a new avenue for analgesic development. In our studies we have demonstrated the relative balance of activities between tachykinin and opioid pharmacophores which will generally determine the net effect of the chimeric molecule as pro-nociceptive, antinociceptive, or neutral. The first combination of multiple active pharmacophores in one molecule which was performed on the SP scaffold showed high antinociceptive effect and was the starting point to design next chimeric peptide called AWL3106. The compound was a hybridized fragment of substance P(7-11) and dermorphin linked by spacer. This chimeric compound displayed analgesic effect without development of tolerance when applied iv and it in Wistar rats. The communication will present the way from an idea through synthesis to pharmacological studies and prospective application of the new chimeric compound called AWL3106 in pain therapy.

Acknowledgement: This work has been supported by 6FP STREP project “NORMOLIFE”

17:20	Poster	57

Functional activity of new derivatives of dextromethorphan as allosteric inhibitors of α3β4 nicotinic acetylocholine receptor.

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Dextromethorphan (DM) is well known the active ingredient in most over-the-counter cough medicines. DM is structurally closely related to levorphanol, codeine, and morphine, but unlike these opiates it has low affinity for opiate receptors and is not considered to be addictive [fig.1]. Its complex pharmacology led scientists to suugest several other potential uses(e.g., for a treatment for Huntington's and Parkinson's disease, stroke and ischemia, seizure disorders, and cocaine dependence). In our studies dextromethorphan and its metabolites were determined as noncopetitive antagionist of neuronal nicotinic acetylocholine receptors, in particular α3β4 subtype which is involved in regulation of dopaminergic pathways in brain sections responsible for addiction development. Extensive molecular modeling studies were performed in our laboratory which identified a binding site within a ion channel and allowed *in silico*

design of N-substituted derivatives of dextromethorphan. Some of these new desigs were synthesized and characterized structurally [Fig.1].

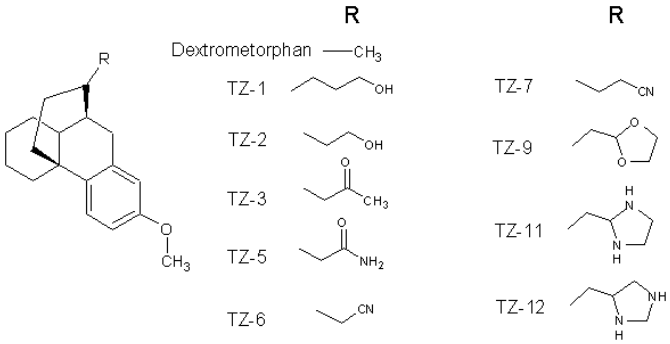

Fig. 1 The structure of dextromethorphan and selected synthesized derivatives.

In current research these compounds are tested for functional activity against α3β4 nAChR in electropfysiological assay. The aim of the study is to perform the *in vitro* tests for these substances using patch-clamp technique. The experiments are carried out on HEK-293 cells stably transfected with rat α3 and β4 neuronal nicotinic receptor genes. Ionic currnets in whole-cellconfiguration is measured using a fast drug delivery syetem DynaFlow. The cells are stimulated by 100μM nicotine in combination with increasin concentration of an inhibitor. The evoked current measurements allows determining sigmoidal drug-response curve and based on such logarithmic graph we can compute such parameters as EC_{50} for an agonist, IC_{50} for DM ant its derivatives, Hill coefficient, dissociation constant ect.

The DM as a potent antagonist of α3β4 neuronal nicotinic receptors could play a pivotal role in developing new more efficient therapy to aim on habenular-interpeduncular (Hb-IPN) system where the α3β4 subtype of nAChR is densely expressed. The most recent results strongly suggest that HB-IPN pathway is associated with withdrawal effect which is mainly responsible for failure in nicotine cessation therapies.

17:20	Poster	59

An asymmetric synthesis of 4-aryloxyazetidin-2-ones and 3,4-benzo-2-hydroxycephams

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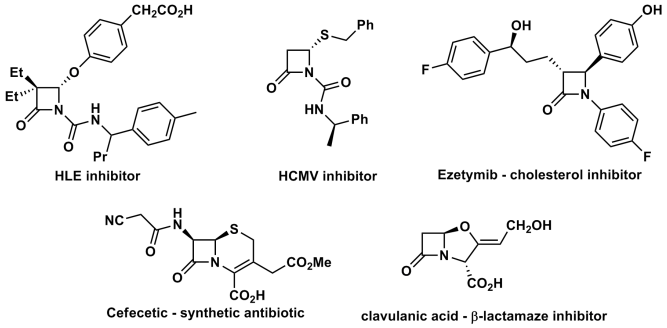
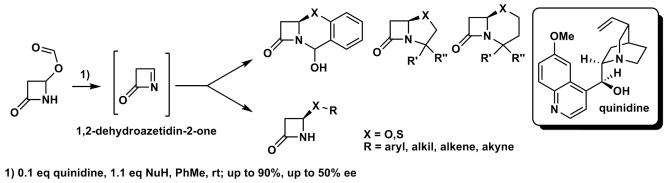
Title compounds represent interesting group of *β*-lactam antibiotics,

β-lactamase and elastase inhibitors as well as activity against HCMV viruses.[1] Owing to their attractive biological activity, the synthesis of novel systems containing the *b*-lactam ring have been extensively investigated.[1]



The most common strategy for the synthesis of such compounds involves nucleophilic substitution at C-4 of the 4-acetoxyazetidin-2-one ring.[2] We have shown previously that cinchona alkaloids are efficient catalyst in the synthesis of enantiomerically enriched 4-aryloxyazetidin-2-ones as well as 3,4-benzo-2-hydoxy-5-oxacephams.[3] We report herein enantioselective, quinidine mediated reaction affording the corresponding mono- and bicyclic *b*-lactams (Scheme 1).



Scheme 1

The scope and limitations of such transformations will be briefly discussed.

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17:20 Poster 61

Pharmacokinetic parameters of amoxicillin in pigs and poultry

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The objective of the paper was: modification of amoxicillin extraction method in serum of pigs and poultry, identification and comparison of amoxicillin pharmacokinetic parameters in pigs and poultry that will facilitate better use of antibiotic with variation in dosage.

The study was carried out on 9 piglets of Polish breed "Polska Biała Zwisłoucha" and on 35 chickens of "Hubart Evolution" breed. The piglets were given drug tested in one dose per os in doses 28 mg/kg body weight as calculated for amoxicillin. The drug was dissolved in water. Blood was sampled from the external jugular vein during after 1, 2, 4, 6, 8, 10 hours of drug administration. The birds were given the drug orally in equal dose of 20 mg/kg of body weight as calculated for the active substance. Blood samples were sampled in the course of exsanguinations when theanimals were pharmacologically deprived of consciousness, after 0,5, 1, 2, 3, 4, 6, 8 hours from the administration of the preparation. The assays performed in the blood serum of the piglets with the HPLC technique proved that minimal concentrations of amoxicillin were present even after 10 hours from the last dose of the drug. The curve depicting the course of amoxicillin concentrations in the drug tested preparation, had an ascending and descending course with the highest level after 2 hours from the veterinary product administration. The highest concentrations (mean values) of amoxicillin after a single administration of the preparation drug tested in chickens were observed after 2 hours. The following pharmacokinetic indices were calculated after application of the studied preparation drug tested in the piglets and slaughter chickens.: T1/2, Cmax, Tmax, AUC, MRT. The performed adaptation of the procedure of amoxicillin concentrations assay in blood serum of piglets and slaughter chickens confirmed the usefulness of chromatographic methods HPLC for determining the concentration levels in blood. Comparative analysis of the tests results performed with HPLC technique confirmed high effectiveness of the methods used in the research by getting comparable results. The calculated pharmacokinetic parameters for the amoxicillin concentrations in blood serum of piglets and slaughter chickens confirm the differentiation of the rate of elimination depending on the species.The obtained levels of amoxicillin concentrations in piglets after the dosage (28 mg/kg of body weight) and in slaughter chickens after the dosage (20 mg/kg of body weight) let us conclude that pharmacokinetics of amoxicillin give a warranty of effective treatment with the applied doses in spite of differences in species.

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Determination of reaction progress in synthesis of EE-3 by NMR technique

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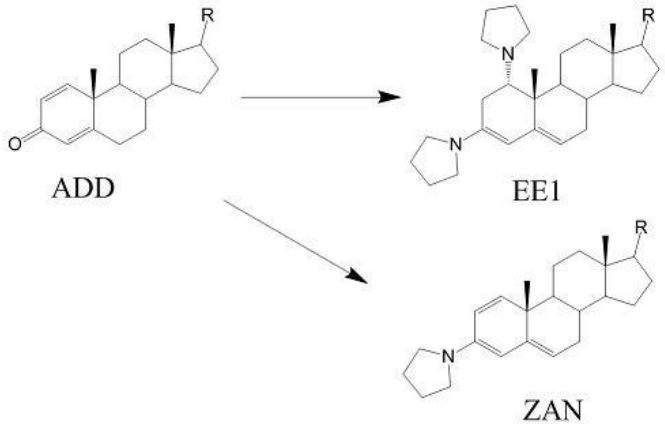
Steroids, natural or synthetic organic compounds, characterized by the molecular core structure of 17 carbon atoms arranged in four fused rings, play a crucial role in biology, chemistry and medicine. Among the synthetic steroids of therapeutic value, there is a large number of anti-inflammatory agents, vitamins, growth-stimulating agents, oral contraceptives and others. Steroids classified as progestogens or aromatase inhibitors are approved for use in breast cancer therapy.

Modifications in the molecular structures of steroid compounds can produce significant differences in their biological actions. The key task in the described synthesis of EE-3 anticancer drug is the activation of an unreactive *6* position in steroid skeleton. The activation

can be achieved by the transformation of substrate ADD into dipyrrolidinyl intermediate EE-1.



Due to the optimization procedures, the reaction time should be controlled. Although different analytical methods might be effective to monitor the reaction progress, the NMR technique seems to be the most relevant in our case. The well separated proton signals, matching with the substrate ADD, product EE1 and impurity ZAN, were observed in ^{1}H NMR spectrum. It allows to apply a single spectrum data for the identification and quantity determination of the above mentioned substances in the reaction mixture.

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Examination of 5-HT$_6$ receptor affinity in the group of arylsulfonamide derivatives

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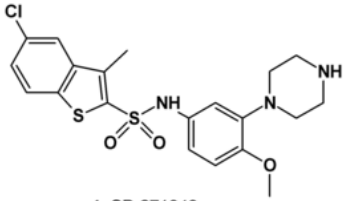
According to the recent research studies 5-HT$_6$ receptors have gained special interest as a drug targets for diseases such as Alzheimer's disease, anxiety/depression and schizophrenia.
This stimulated an intensive research and several new compounds have been developed as a 5-HT$_6$ agents. Due to the arylsulfonyl moiety was a common feature of nearly all published structures, it was proposed that it constitute an important pharmacophoric element, which can influence 5-HT$_6$ affinity. Therefore a series of arylsulfonamide derivatives (2) recently developed as 5-HT$_7$ ligands, were examined additionally at 5-HT$_6$ receptors. The results of receptor binding experiments revealed that the tested compounds displayed a broad range of affinity for 5-HT$_6$ receptor (K_i = 43 – >10 000 nM) and diverse selectivity with respect to other monoamine receptors.



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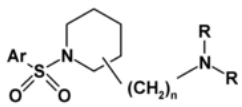

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Application of fermentation in protein hydrolysates utilisation

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Recent studies evidenced that short peptides are able to penetrate gastrointestinal-blood barriers in intact form and modulate number of life processes. Therefore, short peptides might be regarded as very important active component of food. In normal digestion process short peptides are formed from food protein. However, in various pathological conditions diet should be supplemented with protein hydrolyzates as nutraceutics. Previously, we developed pig spinal cord protein hydrolysate as nutraceutic supporting treatments of autoimmunological diseases of the nervous system, like sclerosis multiplex. The active preparations have been obtained in pepsin digestion process. Unfortunately, the digestion resulted in formation of a mixture containing bitter free amino acids that disqualified product as a food component. To resolve this problem we applied yeast fermentation as a second stage of biotransformation. In particular conditions, yeast utilize most of bitter components but leave “good” peptides unchanged. The separated product is well accepted as food component.

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Comparison of accelerated and real-time cyclosporine A release testing from poly(L-lactide-co-trimethylene carbonate) matrices

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Cyclosporine A (CyA) is a cyclic undecapeptide, used in prophylaxis and therapy of graft rejection in all types of solid organ and bone narrow transplantation, as well as in treatment of a number of autoimmune diseases. However, prolonged repeated treatment with CyA may cause many side effects like nephrotoxicity, gingival hyperplasia and neurological disorders. Copolymers of TMC with L-lactide may be interesting in developing alternative delivery systems cyclosporine A. Accelerated CyA release testing is very desirable since long-time degradation of polymeric carrier. It can be achieved by the increase in polymer degradation via acid or alkali catalyzed hydrolysis, addition of surfactants to enhance drug diffusion or increase in temperature [1]. The aim of this study was to evaluate the usefulness of accelerated CyA release testing obtained by elevated

temperature.

Poly(L-lactide-co-TMC) synthesized in Centre of Polymeric and Carbon Materials PASci in Zabrze, with using of $Zr(Acac)_4$ as non toxic initiator of copolymerization reaction was used to prepare matrices with 2, 5 and 10 weight-% of cyclosporine A by solution casting method. Correlation between drug release testing conducted at 70°C the real-time release at 37°C was analyzed. The characteristic of copolymers microstructure during degradation process was conducted by means of high resolution NMR spectroscopy (AVANCE II Ultra Shield Plus, Bruker 600 MHz) [2]. $CDCl_3$ was used as a solvent. The molecular weight (Mn) and molecular weight dispersion (D) were determined by GPC (Physics SP 8800 chromatograph), with chloroform as eluent. The thermal properties were examined by differential scanning calorimetry (DSC) with a TA DSC 2010 apparatus (TA Instruments, New Castle, DE).

The PLATMC characterized random structure (R=0,72). Thermal analysis showed that the used copolymer was amorphous with a glass transition temperature (T_g) at 42°C. Both, the analysis conducted at 37°C and 70°C showed that the highest amount of drug was released from matrices containing 2% of cyclosporine A and the lowest from matrices with the highest initial drug loading (10%). The accelerated cyclosporine A release testing can be a useful method of rapid polymer screening since the obtained results correlated with the real-time release.

Acknowledgement

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17:20 Poster 71

Application of carbohydrate based chiral stationary phase for resolution of fenoterol, clinically used chiral drug

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Fenoterol is a sympathomimetic directly stimulating bronchial β_2-adrenoreceptors [1] used in bronchial obturations. It also is widely used as a tocolytic drug in cases of imminent preterm labour for disruption of uterine contractions [2]. Recently it has been demonstrated that the β_2-AR agonist activity resides primarily with (*R,R*)-fenoterol while (*S,S*)-fenoterol is essentially inactive at this receptor [3]. This compound was already resolved into pairs of enantiomers on carbohydrate stationary phases.

Here we report new validated method for direct chromatographic resolution of (R,R) and (S,S) fenoterol diastereoisomers on amylose tris(3,5-dimethylphenylcarbamate), offering baseline separation of this compound (resolution factor R_S of 3.89). LOD and LOQ for the analysis were determined as 0.2 and 0.5 microgram per mililiter respectively.

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The possible effect of sulforaphane on the bioavailability of drugs

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The nutrients or diet supplements can affected on orally administrated drugs absorption in intestine. Thus, it is extremely important to elucidate which of these food ingredients can impact drugs metabolism

Isothiocyanates (ITC) are found in vegetables from the family Cruciferous and they mainly occur in common consumed vegetables such as broccoli, cabbage, brussels sprouts, cauliflower etc. They are a group of compounds affecting the cells and modulating their metabolism in order to protect them against xenobiotics and other exogenous substances. They act by induction of phase 2 detoxification enzymes like glutathione S-transferases, NADPH: quinine oxidoreductase 1 (NQO1), etc. The gens encoding detoxifying and antioxidant proteins are regulated by nuclear factor-erythroid 2 p45-related factor 2 (Nrf2). Under homeostatic conditions, Nrf2 is mainly sequestered in the cytoplasm by protein – Keap1. Various exogenous substances cause that Nrf2 is released from Keap1 protection and translocates to the nucleus, what results in transcriptional induction of phase 2 genes battery. But, it has already been shown that overexpression of Nrf2 enhances resistance of cancer cells to chemotherapeutic agents such as cispaltin, doxorubicin and etoposide.

In this study we evaluated the influence of naturally occurring ITC sulforaphane (SFN) on the activity of phase 2 enzymes, such as NQO1. The study was carried out in human intestinal Caco-2 cells,

which are widely used as a model in drug development study including its permeability, transport and metabolism. In order to evaluate nontoxic doses of SFN we studied dose and time–dependent changes in viability of Caco-2 sells. We performed a MTT - cytotoxicity test, which is a quantitative colorimetric method for mammalian cell survival and cell proliferation. The NQO1 activity was determined by measuring the NADPH-dependent menadiol-mediated reduction of MTT. In order to study mechanism of NQO1 induction we also examined the time-dependent changes in the subcellular localization of Nrf-2 by immunostaining with anti-Nrf2 antibody and detection the results witch help of confocal microscope.

Our results have shown dose–dependent changes in cell viability and time–dependent changes in NQO1, which indicates that SFN influence the 2 phase enzymes in Caco-2 cells. This in turn indicates that ITC present in nutrients or diet supplements can impact on bioavailability of drugs.

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Opimisation of preparation of TZ-6

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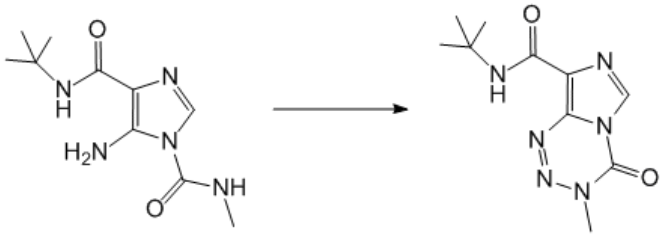
The key step in the synthesis of pharmaceutical substance TZ-S is the reaction involving the diazotization of TZ-5 followed by intramolecular cyclisation leading to the creation of tetrazine ring to obtain compound TZ-6:



TZ-5 TZ-6

After a thorough analysis of the literature data regarding the conditions for the reaction of aromatic amines diazotization, the parameters the change may cause increased productivity and/or purity of the resulting product were set (temperature, used acid, equivalent of reagents, solvents etc.).

Using statistical methods area of the reaction response was designated and then series of experiments were performed. Fixed reaction parameters were changed in specific way.

Purity of the resulting product and overall yield of the process was determined by HPLC methods.

The final results determined the optimal conditions for obtaining compound TZ-6.

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Electrochemical studies of Pt (II) complexes with potential bioreductive properties

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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. Presented poster summarizes electrochemical reduction potentials of the synthesized Pt(II) complexes in comparison with starting nitrodiazole ligands which will be a reasonable indicator of their bioreductive ability[2]. Electrochemical data in connection with cytostatic activities and distribution coefficients (logP) of the compounds tested will be used in further design and synthesis of the new potentially bioreductive Cisplatin analogs.

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An entry to carbapenams via asymmetric Kinugasa reaction involving cyclic nitrones and terminal acetylenes

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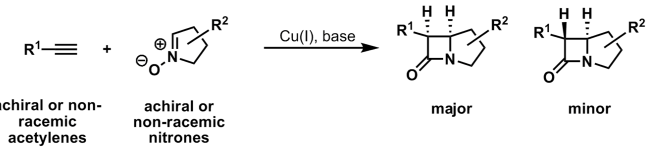
The copper(I) mediated reaction of nitrones and terminal acetylenes, which is known as Kinugasa reaction, represents an attractive method of direct formation of the β-lactam ring.[1,2] This reaction can be performed in many ways including diastereo- and enantioselective versions. In most cases, as 1,3-dipoles simple acyclic nitrones have been used.[3] Number of reactions involving cyclic ones is limited.



Herein, we present our recent studies on Kinugasa reaction involving cyclic nitrones readily available from hydroxy acids or amino acids and terminal acetylenes either achiral or bearing a stereogenic center.[4] All investigated reactions proceeded in good yield and with high diastereoselectivity providing an attractive entry to carbapenams of a potential biological activity.[5] The stereochemical pathway of the reaction and influence of geometry and substitutions in one or both reactants on direction and magnitude of asymmetric induction will be discussed.

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The influence of (24R)-1,24- dihydroxyvitamin D_3 on the anticancer activity of 5-fluorouracil in human colon cancer model

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The results of recent studies allow the suggestion that the new analogs of calcitriol could be applied in an antitumor therapy, especially in combination with cytostatics. In this paper, the effect of (24*R*)-1,24-dihydroxyvitamin D_3 (PRI-2191) on antitumor activity *invivo* of 5-fluorouracil (5-FU) has been evaluated. The experiments were performed in a human HT-29 colon cancer model. Mice bearing subcutaneous colon cancer tumors or tumors implanted orthotopically were treated with PRI-2191 and 5-FU in various schedules. 5-FU was administered to mice repeatedly at dose 75 mg/kg/day i.v. whereas vitamin D analog PRI-2191 was administered s.c. at dose 0,2 or 1 μg/kg/day five or three times per week, respectively. In all experiments it was shown that the analog PRI-2191 improved therapeutic effect of 5-FU but the best effect was observed after administration of PRI-2191 three times per week. Statistically significant inhibition of tumor growth in the combined therapy was observed. What is more, the interaction between both compounds could be showed as synergy.

The analysis of cell cycle and apoptosis in the cells derived from HT-29 subcutaneously implanted tumors showed significant increase in cells number at G1/G0 stage and decrease at cells number at S and G2M stage. Additionally, the tendency to decrease apoptosis in tumor cells from mice treated with PRI-2191 and 5-FU simultaneously was indicated. The general conclusion of this work is that analog PRI-2191 increased antitumor effect of 5-FU in the human colon cancer therapy and it seems that it could be useful for clinical applications.

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In vitro and in vivo evaluation of novel Pim kinase inhibitors with potent anticancer activity

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The Pim serine-threonine kinase family is composed of three members that play an important role in intracellular signaling and contribute to pathways involved in cell survival, proliferation, stress response and cellular motility. Out the three family members, Pim-1 has been studied most extensively and was shown to be a crucial downstream effector of several oncogenes such as Jak2 and FLT3 kinases. Pim-1 overexpression has been also reported in a variety of cancers such as diffuse B cell lymphoma, chronic lymphocytic leukemia, Flt3-mediated acute myelogenous leukemia and solid tumors including prostate and pancreatic cancers. For this reason, Pim-1 kinase emerged as a novel and interesting target of significant potential for therapeutic intervention.

In the current study, the optimization of the initial lead compound - which presented moderate potency in Pim kinase inhibition, low solubility and bioavailability - led to identification of a novel group of orally bioavailable Pim kinase inhibitors. Among the newly synthesized compounds several derivatives exerted increased potency in Pim kinase inhibition with IC50 values below 5 nM. Profiling of best inhibitors on a KINOMEscanTM Max kinase panel revealed superior selectivity towards Pim kinases. Anticancer activity of these new derivatives was investigated in various neoplastic cell lines of hematological and solid tumor origin where the compounds were shown to induce apoptosis both as a single agent, but also synergistically with standard therapies, such as docetaxel, sunitinib and rapamycin. FACS analyses revealed accumulation of subG0 phase, in-

dicating apoptosis in time and concentration dependant manner. In the living cells, two phenotypes could be observed, namely a cell cycle arrest of the cells either in the G0/G1 or G2/M phases, depending on the cell line.

In order to confirm that the observed cytotoxic and cell cycle effects were due to inhibition of the Pim kinases, we performed analyses of direct downstream substrates of these kinases. Pim kinases were shown to phosphorylate p27KIP1, 4E-BP1 and Bad proteins, and treatment of cells in vitro with our new compounds caused a dramatic reduction in the phosphorylation levels of these targets. Best performing compounds were chosen for an efficacy screen in a mouse xenograft model, proving their anticancer activity. Results of Selvita's Pim kinase inhibitor optimization efforts are shown and discussed, supporting further development of this class of inhibitors in oncology indications.

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Synthesis and anticonvulsant activity of new N-Mannich bases derived from 5-cyclopropyl-5-(4-chlorophenyl)-imidazolidine-2,4-diones

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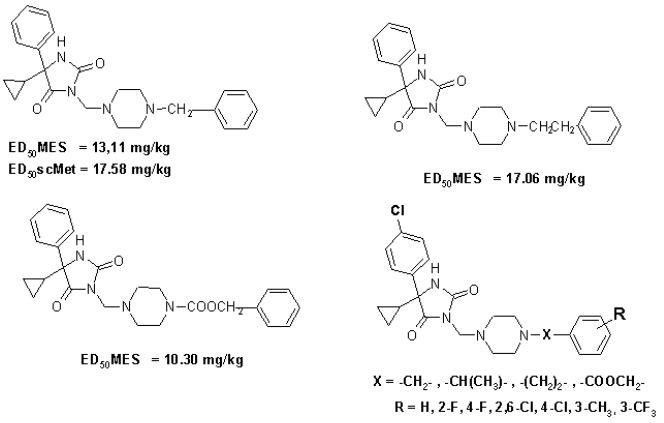
In search for new compounds with predictable anticonvulsant properties our attention was drown to a group of 5-cyclopropyl-5-phenyl-imidazolidine-2,4-diones with 4-substituted piperazine fragment connected to the N3 nitrogen atom through the methylene linker (N-Mannich bases). In this series, many compounds exhibited potent anticonvulsant activity with an ED_{50} values ranging from 5.29 mg/kg to 17.06 mg/kg in the MES test and some of them were also active in the sc.PTZ screen with an ED_{50} ranging from 17.58 mg/kg to 30.78 mg/kg [1].

As a continuation of our research work on structure-activity relationships in the five-member heterocyclic anticonvulsants, we have synthesized series of N-Mannich bases analogues containing 5-cyclopropyl-5-phenyl-imidazolidine-2,4-dione moiety. The main modification was the replacement of a hydrogen atom with an electron-withdrawing chlorine atom at the position-4 of the 5-phenyl ring.

Fig 1.



The target derivatives were prepared by the Mannich-type reaction from the 5-cyclopropyl-5-(4-chlorophenyl)-imidazolidine-2,4-dione, formaldehyde and the appropriately 4-substituted piperazine.

The compounds were evaluated for their anticonvulsant properties within the Antiepileptic Drug Development (ADD) Program, by testing procedures, which have been described earlier [2].

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

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17:20	Poster	87

Growth of human fibroblasts in the presence of 6-hydroxyhexanoic acid

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Biodegradable polymers containing caproyl units belong to the most popular materials used widely in medicine, pharmacy (as drug delivery systems) and tissue engineering. Recently co- and terpolymers obtained from poly-ε-caprolactone have been found to possess a shape memory effect. Shape memory devices are suitable for a wide range of medical applications such as biodegradable stents or surgical clamps. The degradation product of poly-ε-caprolactone hydrolysis is 6-hydroxyhexanoic acid. It is thought to be biocompatible but there are not any experimental data that demonstrate its impact on human cells in vitro.

The aim of our study was todetermine the impact of different concentrations of 6-hydroxyhexanoic acid on human fibroblasts (HGF-1 cell line). Changes in cell morphology and growth rate were analyzed as well as the concentration of tested compound in cell culture medium was assessed.

6-hydroxyhexanoic acid was used at a concentrations range of 0,1–20 mM. Cell proliferation was measured using sulforhodamine B, a dye binding to cellular proteins. Concentration of the studied compound in culture medium was assessed using HPLC technique.

Fibroblasts growing in the presence of6-hydroxyhexanoic acid at all concentrations displayed normal morphology, remained spread on the substratum and any cell detachment was not observed. Some inhibition of cell growth was seen exclusively at the highest concentration of the tested substance.

Generally, results of our study evidence the biocompatibility of the degradation product of poly-ε-caprolactone.

Acknowledgement: This study has been financially supported by MEMSTENT (Grant No: UDA-POIG.01.03.01-00-123/08-01).

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(5-Nitro-2-pyridyl) 1-thioglycosides: application in synthesis of analogues of glycosyltransferases natural substrates

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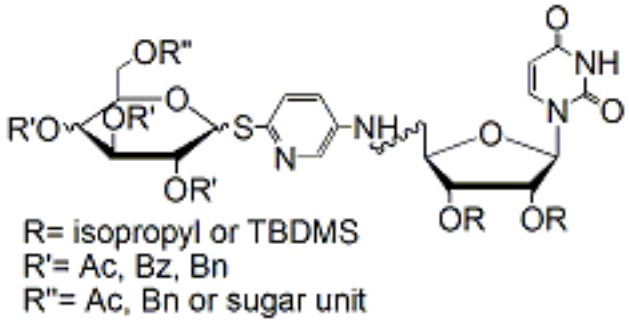
Glycoconjugates play a key role in cell-cell recognition and interaction process. They are also responsible for such events as inflammation, tumor metastasis, bacterial or viral infection or immune system activation. In synthesis of glycoconjugates in biological system enzymes such as glycosidases and glycosyltransfrases are involved. Glycosyltransferases of the Leloir pathway are key enzymes responsible for synthesis of most cell-surface glcoconjugates in mammalian systems. They catalyze the transfer of a sugar moiety from an activated nucleotide sugar to the hydroxyl group of an acceptor which may be a growing oligosaccharide, a lipid or a protein [1]. Inhibition of these enzyme leads to the modulation of oligosaccharide biosynthesis and enables us to study their biological functions. Therefore some of such inhibitors might be of therapeutic interest.



Recently we presented synthesis of some kind of analogues of sugar nucleotides, which were designed to act as glycosyltransferases inhibitors particularly donor substrate analogues [2]. In these glycoconjugates aryl- or heteroaryl 1-thioglycosides derivatives of D-glucose or D-galactose were connected to selectively protected uridine by amide bond with or without a spacer. Some of them exhibited antiviral activity against classical swine fever virus (CSFV) [3]. Now we present next part of our research on changes in structure of already synthesized glycoconjugates and their influence on biological activity of obtained compounds. We add one more sugar unit (derivative of glucose or 2-iodo-2-deoxy mannose) to earlier synthesized glycoconjugates. We also try to construct analogues of glycosyltransferases natural substrates by changing configuration at the anomeric centre of (5-nitro-2-pyridyl) 1-thioglycosides used to connection to uridine derivatives.

Acknowledgement

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Study on lipophilicity of selected uridine derivatives

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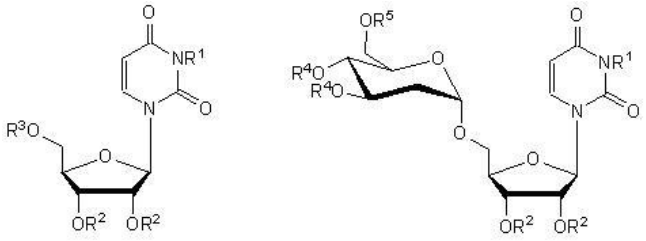
In rational design of new lead compounds correlation between structural or property descriptors of compounds with their activities is of unquestionable meaning. Molecular lipophilicity is one of the most important factors in pharmaceutical research and can be considered as a key determinant of pharmacokinetic properties of a drug and its interaction with macromolecural targets. Determination of partition coefficient is of interest in medicinal chemistry since this quantitative descriptor is frequently used in quantitative structure-activity relationship (QSAR) analysis. LogP can be determined experimentally by a variety of methods or calculated by the use of appropriate software [1]. Among experimental methods RPLC techniques are widely used and replacing traditional shake-flask procedure due to their well-known advantages.

Our goal is to determine octanol-water partition coefficient of selected uridine derivatives experimentally and by means of different computational applications. This work also describes correlation between experimentally determined lipophilicity parameters and calculated logP values. Examined uridine derivatives form a broad range of compounds significantly differentiated in lipophilicity. Some highly lipophilic as well as some highly hydrophilic compounds were present in the examined set. Not for all of the substances partition coefficient can be determined by means of experimental methods because of technical reasons. The main goal is to find the most adequate computational method, that would be predictive for the studied set of uridine derivatives.

Investigated compounds are presented in Figure below.



R^1, R^2, R^3, R^4, R^5 – protecting groups

Correlations were made between two experimentally obtained parameters: $logP_{exp}$ (shake-flask method) and R_{Mw} (RP TLC method) and logP values calculated by nine different algorithms. There was a strong linear relationship between $logP_{exp}$ and R_{Mw} (r=0.96-0.99). For examined set of uridine derivatives the best correlations between R_{Mw} and calculated logP values were found for XLOGP3, ALOGPs and miLogP algorithms. All three methods represent different approach so it can be concluded that various types of computational methods can assure high predictive power for studied set of compounds.

Acknowledgement

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A novel synthesis of 19-nor analogs of vitamin D

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In our continous search for new vitamin D analogs of therapeutic potential in hyperproliferative diseases we have synthesized a new series of analogs of 25-hydroxy vitamin D with truncated methylene unit at carbon atom C-10. The resulting new 19-nor vitamins D are analogous to the existing therapeutic agent from this group. Known syntheses of 19-nor analogs started from 25-hydroxy vitamin D_2 or from quinic acid. However, these syntheses were designed to prepare a selected single analog and did not allowed for the preparation of a series of structurally related compounds to be used for structure-activity analysis. In addition, 25-hydroxy vitamin D_2 is practically unavailable from commercial sources. Our retro-synthetic analysis showed that the target molecule might be conveniently constructed from 19-nor-A-ring synthon, CD-ring system and a side-chain fragment. In our approach the A-ring synthon is first coupled with the indane CD-ring system and then connected with variety of side-chain fragments. This way a series of side-chain modified analogs of 19-nor vitamins D might be obtained in a convergent manner. The yields of key synthetic steps allowed for the synthesis of selected analogs in preparative quantities. Our initial approach of combining first the CD-ring system with the respective side-chain fragment did not gave the intermediates in a yield high enough as for synthesis of potential pharmaceutical substances. The structure of new key intermediates: the CD-ring synthon and the ACD synthon was confirmed by spectroscopic and diffraction methods. The phosphine oxide of A-ring synthon was synthesized from the respective alcohol. This was prepared from an achiral precursor, 1,3,5-trihydroxybenzene. CD-Ring synthon was synthesized by a photolytic degradation of the commercial vitamin D_2. The key diol SCD-2 was converted into the new ketosulfone - synthon CD. Wittig-Horner condensation of this synthon with the A-ring synthon gave the new 19-nor synthon ACD, as the advanced key intermediate. Julia olefination of this synthon with the side-chain aldehydes, followed by dehydroxy-desulfonylation and desilylatioin gave a series of target 19-nor analogs with various alkyls at C-26, C-27 and C-28. New analogs are screened for their cell differentiation and calcemic activity.

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Docking Study of fenoterol derivatives to the β_2 adrenergic receptor. Molecular dynamic simulations for selected agonist-β_2-AR complexes.

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The β_2 adrenergic receptor (β_2-AR) belongs to the large and diverse family A of G-protein-coupled receptors. Both, the bovine rhodopsin (1F88) and the human β_2-AR (2RH1) were crystallized in their inactive states. The human β_2-AR was crystallized with bound inverse agonist – carazolol. The molecular model of the β_2-AR has already been proposed by Furse and Lybrand. This model has been initially constructed by means of comparative modeling techniques based on the rhodopsin template and was further optimized in order to properly reflect experimental results for β adrenergic receptors.

Our study concentrates on comparison of the results of docking studies to the three models of β_2-AR:

1) the complete model of β_2-AR (conformation of N-, C-termini and loop connecting TM5 and TM6 were predicted using de novo methods)

2) the model presenting the active state of β_2-AR (initial receptor structure of complete model of β_2-AR was optimized to preserve receptor-ligand interactions for set of typical agonists applying restrained simulated annealing procedure).

3) the hybrid model of β_2-AR (model based mainly on the Furse and Lybrant model but some of its features were common to the crystal structure of β_2-AR).

In particular, docking studies were applied to the set of fenoterol derivatives (n = 32), in case of which the enantioselectivity plays a crucial role. The ligands were docked into one binding site using several docking procedures. The Molegro Virtual Docker (MVD) software was employed for this purpose. Agonist molecules occupy the

same binding region, located between TM3, TM4, TM5, TM 6 and TM7. The following residues identified by us during docking procedure were experimentally indicated in functional and biophysical studies as being very important for formation of the hydrogen bonds: Ser204, Ser207, Asp113, Asn293, Tyr308 (Figure 1). Docking results were analyzed in terms of scoring functions (MolDock Score, Rerank Score, Similarity Score, Docking Score). Correlations between the function score values and the compound binding affinities (the latter expressed as K_i values) were examined. According to our study, docking in Molegro Virtual Docker offers a good prediction of the binding energies (expressed as the scoring function values). The correlation between the MolDock Score and the experimentally determined pK_i is depicted by the determination coefficients R^2=0.4767 and R^2=0.427 for the hybrid and the crystal model, respectively. In addition, molecular dynamics simulations of obtained complexes were carried out. Systems under consideration consisted of receptors with bound ligands embedded into POPC membrane model in water environment. Trajectories obtained from the MD simulations gave us better insight into receptor-ligand interactions and system evolution. All calculations were performed applying GROMACS 3.3 software package.

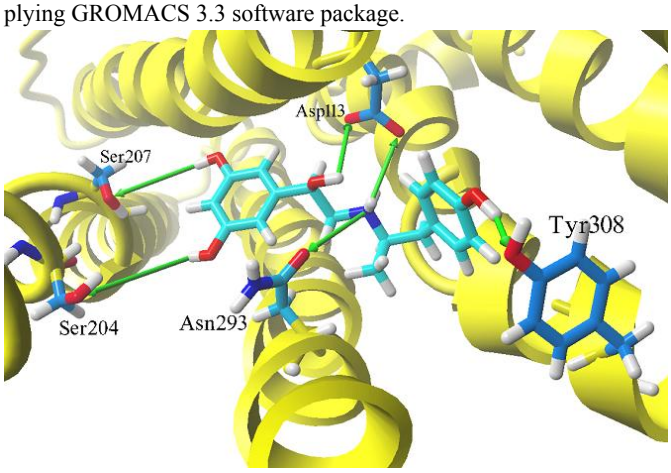

Figure 1. The binding site of β_2-AR and the 3D model of (R,R)-fenoterol-β_2-AR complex.

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Could the calorically restricted ketogenic diet be an effective alternative or supportive therapy for breast cancer?

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Cancer is a complex disease characterized by the loss of the cellular control over the proliferation, caused by the accumulation of genetic and epigenetic defects. Recent findings shows that one of the major alternations found in almost all cancer cells, is a shift in the cellular glucose metabolism. This phenomenon - high rate glycolysis under aerobic conditions is known as "The Warburg Effect" and it is closely related to high metastatic potential of tumors.

Ketogenic diet is a low carbohydrate diet aim to induce ketosis. At this state, liver is using fatty acids to form acetylo-CoA, which is converted into ketone bodies. Ketone bodies are transported to other tissues where they are reconverted to acetylo-CoA, which can by use directly in Krebs cycle. Many cancer cells lack the activity of succinyl-CoA-acetoacetate-CoA transferase and other enzymes needed in ketone bodies metabolism, thus this source of energy is unavailable for them. KetoCal®, ketogenic diet produced by Nutricia is a nutritionally balanced soybean oil diet that was originally developed for managing refractory epilepsy in children. Recent studies showed that KetoCal®, given in restricted amounts reduces plasma glucose levels and elevates ketone bodies levels leading to significant decrease in the brain tumors growth. Additionally, it showed anti-inflammatory, anti-angiogenic and pro-apoptotic activity.

Our goal was to test the possibility of using ketogenic diet in other kinds of cancers. Moreover, we wanted to check the possible synergistic effects between diet and commonly used cytostatic – cyclophosphamide. In our experiment we used mouse breast cancer model 4T1 that closely resembles breast cancer in humans. Ketogenic diet was administered to Balb/c female mice in restricted amounts (1 gram per day per mouse). KetoCal ® (KD) alone and administered with cyclophosphamide (KD+CY) decreased growth of tumors by about 35% and 80%, respectively, compared to the control group (C) which received standard fodder in unrestricted amounts. Ketogenic diet showed additive effect in inhibiting 4T1 growth when combine with cyclophosphamide. Spleens from group KD+CY were significantly smaller in comparison with the control group. Metastasis in lungs were also significantly lower in KD and KD+CY groups. This results indicate that KetoCal® has anti-tumor and anti-metastatic effect in experimental mouse breast tumors when administered in restricted amounts. We can assume that ketogenic diet can be used in the treatment of the other cancers than brain tumors. Moreover, we showed that it could be use not only as an alternative therapeutic option, but also as an supportive therapy during standard chemotherapy.

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Submerged cultivation of Streptomyces sp. 8812 in media containing different sources of nitrogen and carbon

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Since many years we've investigated bioactive metabolites produced by Streptomycetes from the collection of NIZP-PZH. The novel compound with antimicrobial activity, DD-peptidases inhibitor was isolated from culture broth of *Streptomyces* sp. 8812. It is a wild strain of streptomycetes, which was acquired from brazilian soil. It is known that, secondary metabolites with microbial origin are produced in very small amounts. Therefore researches had begun to modify cultivated media for the purpose of increasing the efficiency of bacterial cultures.

By changing the composition media, it was possible to improve in

simple way the efficiency of biosynthesis of bioactive metabolites and optimize bacterial biomass production at the same time.There were investigations on the influence of nitrogen and carbon from various sources (by changing the composition of different media) on *Streptomyces* sp. 8812 biomass growth and on the activity of natural compounds. Different combinations of ingredients were used as a source of nitrogen, e.g.: soybean extract or soytone (papain hydrolizate of soy flour), bacto peptone (papain hydrolizate of meat), bacto tryptone (papain hydrolizate of casein), yeast extract, and corn step liquor. Each product contains different accumulation of amino acids and other amount of total nitrogen (from 3.5 % to 15.5%).

Glycerol, lactose, lactic acid in corn steep liquor and different peptones were used as a source of carbon.

The best strain growth was recorded for media containing soybean extract, bacto peptone and bacto tryptone as a nitrogen source and glycerol as a carbon source. The highest productivity of DD-peptidase inhibitors was detected during cultivation of *Streptomyces* sp. 8812 by the use of special media.

Optimized bacterial biomass growth and the good quality of mycelium were achieved by the use of medium containing soybean extract or soytone, bacto peptone, bacto tryptone, yeast extract, and with (or without) corn step liquor. Whereas glycerol was detected as the best source of carbon.

The highest activity of bioactive metabolites production was received by using the media consisting of bacto peptone, bacto tryptone, yeast extract, and corn step liquor and lactose.

Acknowledgement: This work is co-financed from European Regional Development Fund within Operational Programme „Innovative Economy", the project number UDA-POIG.01.03.01-14-136/09-00

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New amino acid and peptide derivatives of 5H-indolo[2,3-b]quinoline and their biological activity.

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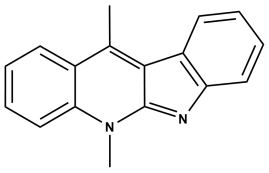
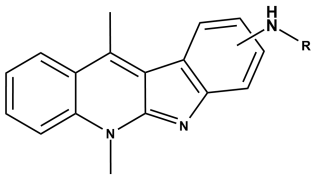
In our earlier studies on chemical and biological properties of indolo[2,3-b]quinoline derivatives we have found that amino acid conjugates of 2-amino- and 9-amino-11-methyl-6*H*-indolo[2,3-b]quinoline display cytotoxic activity [1] and solubility in water contrary to inactive and non soluble in water unsubstituted 11-methyl-6*H*-indolo[2,3-b]quinoline.

Continuing our search of more active cytotoxic compounds we decided to synthesize selected amino acid and peptide conjugates with 2-amino and 9-amino-5,11-dimethyl-5*H*-indolo[2,3-b]quinoline. We designed and obtained a series of novel Gly-, *L*-Pro-, *D*-Pro-, *L*-His-, *D*-His- as well as Gly-Gly-, *L*-Pro-Gly-, *L*-His-Gly-, Gly-*L*-Pro-, *L*-Pro-*L*-Pro- derivatives. Amino acid derivatives were obtained in coupling reactions using TBTU method and peptide derivatives were synthesized through a "step by step" method.



DiMIQ



R= Gly-, *L*-Pro-, *D*-Pro-,*L*-His-, *D*-His-, Gly-*L*-Pro-, *L*-Pro-*L*-Pro-, *L*-Pro-Gly-, Gly-Gly-, *L*-His-Gly-.

New compounds were evaluated for their cototoxic activity againts KB cell line and antimicrobial activity in vitro, according to a routine procedures. The biological tests showed that all amino acid derivatives of 5,11-dimethyl-5*H*-indolo[2,3-b]quinoline display significant biological activity. The peptide derivatives of 5,11-dimethyl-5*H*-indolo[2,3-b]quinoline exhibit similar cytotoxic activity againts KB cells compared to 5,11-dimethyl-5*H*-indolo[2,3-b]quinoline (DiMIQ).

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17:20 Poster 103

Synthesis and in vitro antibacterial evaluation of 1-substituted-4-ethoxycarbonylmethylthiosemicarbazide s and products of their dehydrocyclization

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The emergence of pathogens that are resistant to available drug therapies triggered a clear need for the discovery of new antimicrobials rather than analogs of the existing ones. Traditionally, small molecules have been a reliable source for discovering novel biologically active compounds. Among the family of heterocyclic compounds, thiosemicarbazides and their dehydrocyclization products have received attention as possible antimicrobials. Prompted by these findings and in continuation of our efforts in synthesizing new thiosemicarbazide-like compounds with antibacterial activity, novel 1-substituted-4-ethoxycarbonylmethylthiosemicarbazides and their cyclic analogs were synthesized and tested in vitro for their antibacterial potency.

From 18 compounds tested only 3 showed antibacterial activity; 4-ethoxycarbonylmethyl-1-(naphthyl-1ylcarbonyl)-thiosemicarbazide, N-(2-thiohydantoin-3-yl)amide of indole-2-carboxylic acid, and N-(2-thiohydantoin-3-yl)amide of naphthalene-1-carboxylic acid have weak (MICs of 100-400 μg/mL or higher) potency against gram-positive bacteria. All examined compounds were inactive against gram-negative rods.

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New acridine-tuftsin conjugates with cytotoxic properties and increased specificity toward tumor cells

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Biodegradable polymers or oligopeptides, which are recognized by cell-surface receptors, have been shown to increase specificity and lower systemic toxicity of cytotoxic drugs. The goal of the present study was to evaluate biological properties of several bioconjugates based on tuftsin and its branched analogs combined with 1-nitro-acridines, through a series of different linkers, using the solid phase chemistry. Tuftsin is a natural TKPR tetrapeptide, that has a wide spectrum of biological activities, including stimulation of immune response, phagocytosis, and growth inhibitory activity against bacteria, fungi and tumor cells.

We determined cytotoxic activity and biological effects induced by studied conjugates in two human in vitro tumor models, lung adenocarcinoma A549 and myeloblastic leukemia HL-60. We observed very divergent effects induced by studied tuftsin-acridine conjugates in tumor cells. Two studied conjugates showed comparable cytotoxicity toward both tumor cell types (compounds M8 and M9) or did not produce any cytotoxic effect (compounds M2 and M5). However, conjugate M39 was about 6-fold more cytotoxic toward A549 cells than its corresponding precursor 1-nitroacridine, compound P6. Interestingly, close structural analog of M39, conjugate M29, that differs in only one methylene group in the linker, was about 4-fold less cytotoxic than its acridine precursor, compound P5. We also followed changes in the progression through the cell cycle induced by studied compounds. Exposure of tumor cells to both conjugates and their acridine procursors led to growth arrest in early S and G2/M phases. At longer incubation times, increased fractions of cells undergoing mitotic catastrophe and apoptosis were observed. None of the studied tuftsin-acridine conjugates and their parent acridine precursors showed any effect on DNA topoisomerase I and II activity in vitro.
Together, our results suggest that tuftsin and its analogs may serve as targeting molecules to improve specificity of small molecular weight cytotoxic drugs toward tumor cells.

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The chelation ability of chosen metal ions by protective ointments containing Na_2H_2EDTA and aminoacid

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Protective ointments containing chelating agents are often used by sensitized subjects to prevent the contact allergy dermatitis by chemical binding of allergenic metal ions. The aim of the study was to evaluate the influence of the aminoacid (Gly, Asp, His) ingredient of the protective ointments with Na_2H_2EDTA based on the two formulas (eucerin or hascobase) on the chelation ability of Ni^{2+} and Co^{2+} ions. The *in vitro* test with the diffusion chamber consisting of donor and receptor compartment separated by artificial membrane of chemically modified cellulose was applied. The volume of receptor compartment was filled with the tested ointment and the surface of the membrane was washed with the solution containing Co^{2+} or Ni^{2+} dissolved in water. The concentration of Ni^{2+} and Co^{2+} in the solution was determined by atomic absorption spectrophotometry (AAS vario) after every hour of the extraction. Statistical significance of the observed differences in chelation ability of the analyzed protective ointments was confirmed by analysis of variance. The experiments were run for 5 h. The study showed that the protective ointment containing 10% Na_2H_2EDTA and histidine had the most effective Ni^{2+} and Co^{2+} chelation properties. A histidine of 10% concentration in the ointment was optimal for metal ions binding. Pharmaceutical formula (eucerin or hascobase) of the ointment had no influence on the chelation ability of chosen metal ions.

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Straightforward Methodology for the Stereoselective Synthesis of Benzo[a]- and Indolo[2,3-a]quinolizidines

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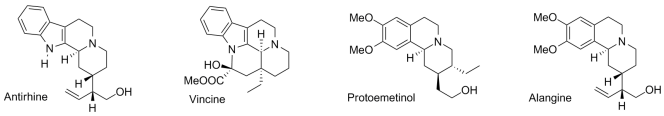
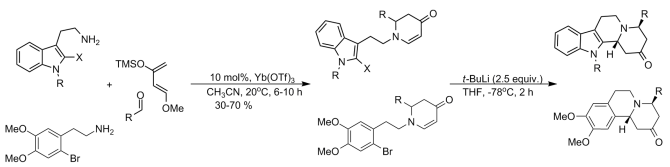
Benzoquinolizidine and indoloquinolizidine skeletons can be found in numerous monoterpenoid-derived alkaloids, many of which possess considerable pharmacological and therapeutic interest.[1]



In connection with our interest in the synthesis of azabicyclic ring systems[2] herein, we report a general and highly stereoselective approach to the construction of Benzo[*a*]- and Indolo[2,3-*a*] quinolizidines based on the lithium-halogen exchange initiated intramolecu-

lar conjugate addition of aryllithiums to dihydropyridones.



A scope, limitation and the stereochemical outcome observed in these cyclizations will be reported.

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17:20 Poster 111

Dendrimeric peptides with affinity to opioid receptors - complexation properties

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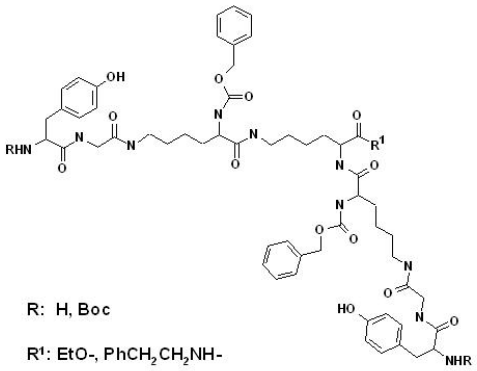
One of the most promising types of molecules allowing practical accomplishment of functional polyvalency are dendrimers - the synthetic macromolecules of nanoscopic dimensions built from several layers of branches located around a central core. This affords location of a high number of functional groups at the surface. Unlike other macromolecular compounds, their unambiguous composition, reliability and versatility of their synthesis, make this type of carriers well-suited to various medical and biochemical applications.

Pharmacological characterization of opioid receptor gave evidence of its complex and multiple structure. Therefore, dendrimeric compounds seem to be well suited for design of ligands that interact efficiently with the nervous system receptors. Here we present convergent synthesis, characterization and biological activity of small library of dendrimers built around $Lys(Lys)_2$ dendron functionalized with N-terminal fragment of enkephalins.



These dendrimers have been used as complexing agents of other peptides known as opioid ligands - biphalin and neurotensin. Application of ESI MS and HPLC methods revealed that in water solution 1:1 complex was formed between dendrimers and biphalin but not with neurotensin. Binding studies to opioid receptors were performed for dendrimers alone as well as for their 1:1 complexes with biphalin. It appears, that the designed dendrimers might be used as new delivery agents for small peptides, ligands for opioid receptors.

Authors acknowledge financial support from the Ministry of Science and Higher Education, grant N204 239436 and EC grant Normolife.

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Analogs of genistein and its possible anti-metastatic properties

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Background: Genistein is a naturally occurring isoflavonoid, which displays antitumor, proapoptotic, antimetastatic and antiangiogenic properties, described in various experimental *in vitro* and *in vivo* models. It is a specific inhibitor of protein tyrosine kinase and topoisomerase II. Genistein can arrest cell growth and proliferation, cell cycle at G2/M phase, invasion and angiogenesis. Integrins comprise a large family of ab heterodimeric cell-surface receptors that present in many species. They are expressed on the wide variety of cells and mediate cell-cell and cell-extracellular matrix interaction.

Objectives: We have examined the effect of genistein, its two new analogs IFG-027 (7-O-alkenyl) and IFG-043 (7-O-arylmethyl) on expression of alfavbeta3 integrins and on adhesion to extracellular matrix protein fibrynogen and fibronectin of human kidney carcinoma A498 cell line.

Methods: Genistein and its analogs were certified synthetic materials obtained from the Pharmaceutical Research Institute, Warsaw, Poland. The human kidney carcinoma cell line A498 was obtained from American Type Culture Collection (ATCC).

The cells were placed in 24-well flat - bottom plates at a density of $1x10^5$ cells per well 24 hours before addition of the tested compounds. The cells were exposed to the test compounds at concentra-

tions of 10 microg/ml for 72 h. After 72 h of incubation, the cells were collected, washed in phosphate-buffered saline (PBS) and counted in a hemacytometer.

The cells were then labeled by alfavbeta3-specific antibodies conjugated with FITC and expression of integrins was analyzed by flow cytometry (Becton Dickinson, San Jose, CA, U.S.A.).

To determine the influence on cell adhesion, the 96-well flat - bottom plates were coated by fibronectin or fibrynogen. Cells (control and treated by genistein or analogs) were placed in plate for 1 hour in 37ºC and then the plate were washed twice by PBS. The bonded cells were then coloured by crystal violet and we measured absorbance on Multiskan RC photometer at 570 nm wavelength.

Results: We have found that genistein and its new analogs influenced the expression of alfavbeta3 integrins and adhesion to fibrinogen and fibronectin. Genistein decreased the expression of integrins by 20%, whereas IFG-027 analog decreased it by 38% and IFG-043 analog revealed only low influence on the expression of the integrins (decrease by 10%). Only analog IFG-043 inhibited the adhesion of A498 cells to fibronectin (47% of inhibition). IFG-027 and IFG-043 decreased the adhesion to fibrynogen (68% and 92% of inhibition respectively), genistein didn't have any influence on cell adhesion. Analogs IFG-027 and IFG-043 could be the possible anti-metastatic agents.

17:20 Poster 115

Natural compositions with antioxidative properties

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The group of flavonilignans so called silymarin is known as a medicine for liver treatment but it also has anticancer and antioxidant properties.

Silymarin is usually obtained from the milk thistle seed husk (Silibum marianum). The post process milk thistle endosperm contains less flavonolignans than husk and a lot of oil (20-30%) which complicates procedure of silymarin isolation.

As a result of ethyl alcohol extraction from the milk thistle endosperm, two fractions were obtained: raw oil fraction and solid fraction that contained silymarin (30-35%), phospholipids (1,2-1,6%) and oil (10-20%) with 80% unsaturated fatty acids. Antioxidant properties of the solid fraction, pure silymarin and derivative of vit. C were tested on Rancimat 679.

Rape seed oil were tested with addition of antioxidants:

-6-O-Palmitoyl-L-ascorbic acid,

-solid fraction of milk thistle endosperm,

-pure silymarin,

-mixtures of listed above compounds.

Comparing results it was observed that:

-6-O-Palmitoyl-L-ascorbic acid i better antioxidant than solid fraction of endosperm of milk thistle,

-solid fraction of milk thistle endosperm should be used in amount bigger than 0,2%

-applying mixture of two above antioxidants gives better results

-solid fraction of milk thistle endosperm is better antioxidant than pure silymarin

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LC/MS/MS determination of exemestane in human plasma

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Exemestane, (6-methylenandrostan-1,4-diene-3,17-dione) is an antiestrogen drug used in an adjuvant treatment of advanced estrogen receptors (ER)-positive breast cancer in postmenopausal women. It is a highly specific and irreversible steroidal aromatase inhibitor, structurally related to the natural substrate androstenedione and metabolized to an intermediate that binds to the active site of the enzyme and inactivates it. It decreases estradiol concentrations, but has no effect on the synthesis of glucocorticosteroids or aldosterone.

A sensitive liquid chromatography – mass spectrometry (LC/MS/MS) method for the determination of exemestane in human plasma is designed for the application to the pharmacokinetic studies in humans, i.e. bioequivalence studies. The method will be validated according to FDA and EMEA requirements.

The development of LC/MS/MS method for the determination of exemestane in human plasma is part of the research project of Pharmaceutical Research Institute entitled „Innovative technologies of oncological medicines of special therapeutic and social importance". The project is supported by the European Regional Development Fund (ERDF).

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Structural characterization of PX-S

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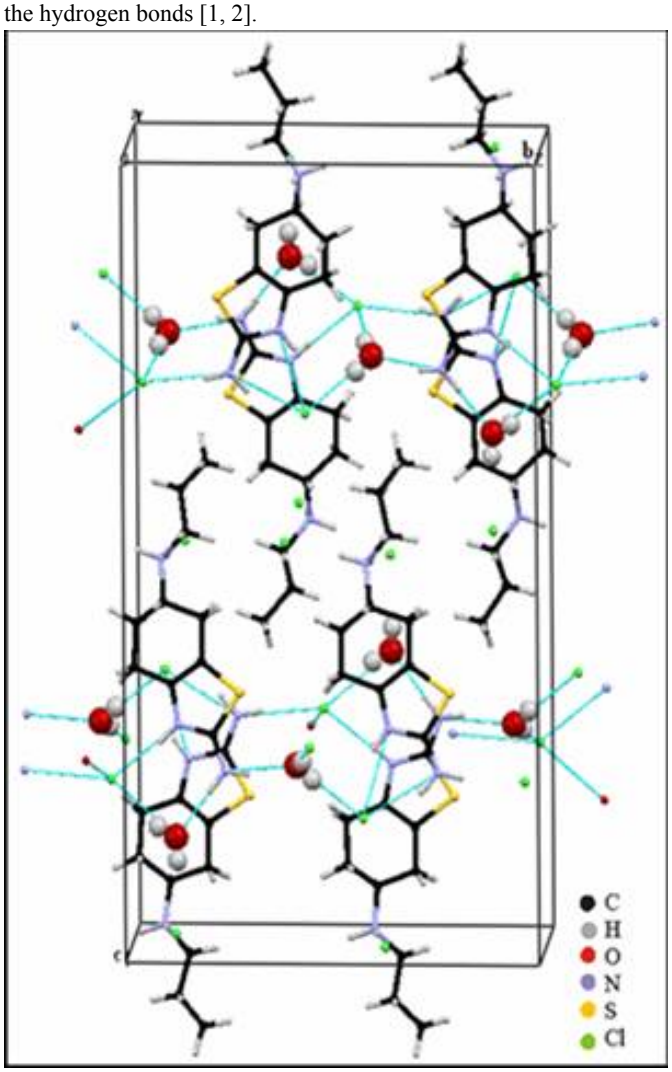
Apart from polymorphs, hydrates of active pharmaceutical ingredient are highly desirable in the pharmaceutical industry. In hydrates the small water molecule can fill structural voids, as well as by acting as a hydrogen donor and/or acceptor it may structuralize a crystal majority into stable crystal structures [1]. Due to high solubility and biocompatibility, amine hydrochlorides are often chosen as drug products. They can easily form hydrates because charged ammoni-

um and chloride ions are in proximity to water molecules through the hydrogen bonds [1, 2].



PX-S is a synthetic aminobenzothiazole derivative. The addition of the N-propylamino group makes PX-S a potent dopamine receptor antagonist [3].

PX-S monohydrate was characterized by nuclear magnetic resonance and X-ray single crystal diffraction methods. It crystallizes in the space group P212121 with unit cell parameters: a=7.0939(2) Å, b=15.1763(3) Å, c=27.2761(6) Å, α=β=γ=90 ^{o}.

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CHO cell line with stable expression of the HTRA1 gene as a tool for studying functional activity of 5-HT$_{1A}$ receptor ligands

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In order to obtain the appropriate CHO (Chinese Hamster Ovary) cell line overexpressing 5-hydroxytryptamine 1A receptor gene (HTR1A gene, human cDNA clone ref. ID NM_000524) pcDNA 3.1 (+) and pCMV6-XL4/HTR1A plasmids were processed with *Not I* restrictive enzyme and ligated. The obtained pcDNA 3.1(+) vector containing *HTR1A* gene was propagated in competent *E. coli* cells on LB-agar plates containing ampicilin. Individual clones containing the pcDNA/HTR1A plasmid were picked up and cultured in the LB Broth medium.

After isolation the pcDNA/HTR1A plasmid has been transferred into CHO-K1 (clone-1)cells with the aid of FuGene reagent. Then the cells were cultured in the presence of geniticine. Selected single cells were cloned and the expression of pcDNA/HTR1A plasmid was evaluated with the aid of Western blot analysis.

The resulting clone CHO-K1 cell lines with stable overexpression of the gene *HTRA1* were used as a model for testing the functional activity of 5HT$_{1A}$ receptor ligands.

Acknowledgements

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Effects of nonenzymatic glycosylation and fatty acids presence on meloxicam binding to human serum albumin

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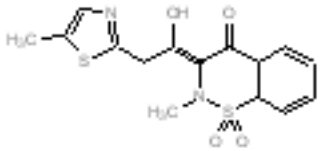
One of the most important factors affecting the distribution and the active concentration of many administered drugs is binding affinity for human serum albumin (HSA). In the bloodstream and tissue fluids, HSA serves as a vehicle for the transport of several endogenous compounds including unesterified fatty acids (FA), hemin, bilirubin and thyroxine all of which bind with high affinity.



The structure of meloxicam

Long-chain fatty acids, a major source of cellular energy, are solubilized and transported in the blood by binding to serum albumin. Crystallographic studies of HSA have mapped at least seven FA binding sites and delineated the overlap with binding sites of some drugs and other endogenous compounds. The interactions of fatty acids with albumin modulates the ligand binding properties of protein by inducing conformational changes in the binding sites I and II, respectively. Thereby, understanding albumin fatty acid interactions is of major clinical and pharmaceutical importance.

In the circulation, HSA becomes nonenzymatically glycosylated by reducing sugars, and the reference range in normal humans is 6–10% glycoalbumin. However, this proportion typically increases to between 20% and 30% in hyperglycemic patients. The principal site of glycosylation of HSA is Lys-525, but the lysine residues in positions 199, 281 and 439 are also susceptible to modification. The effect of nonenzymatic glycosylation can influence on the affinity binding of drugs at site I (subdomain IIA) of albumin molecule.

Meloxicam is pharmacologically important new generation, non-steroidal anti-inflammatory drug (NSAID) of enolic acid class compounds with a minimum adverse gastrointestinal and renal side effects associated with traditional NSAID [1].

The primary function of meloxicam is anti-inflammatory effect but they can also be used as agents in cancer treatment, because in various types of cancer, cyclooxygenase-2 is over expressed. The nature of interaction of meloxicam with HSA involve strong drug–protein interactions with only high affinity site located in subdomain IIA [2,3].

The conformational changes associated with nonenzymatic glycosylation and FA binding may alter the physiological functions of albumin and binding of drugs.

In the present paper the influence of myristic acid and the effect of nonenzymatic glycosylation on meloxicam binding to the albumin using fluorescence quenching method and circular dichroism spectroscopic technique were investigated. The studies have shown that the FA and nonenzymatic glycosylation induce conformational changes in the albumin molecule. These changes affect on the binding ability of meloxicam towards albumin. The association constants in both cases were lower as compared to native protein.

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Synthesis and antitumor activity of novel 6-phenyl-2,7-naphthyridine derivatives.

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Among six structural isomers of naphthyridines, the synthesis and activity of the 2,7 isomer derivatives have not yet been thoroughly investigated. The various pharmacological properties of 2,7-naphthyridine derivatives encourage the search for new methods of their preparation. In the first step, we have determined a way of synthesizing ethyl 8-R-4-hydroxy-1-oxo-6-phenyl-1,2-dihydro-2,7-naphthyridine-3-carboxylates by the Claisen-Dieckman alkoxide-induced rearrangement, according the method reported in our previous paper [1]. Heating obtained before the new pyrrolo[3,4-*c*]pyridine derivatives in a fourfold excess of sodium ethoxide resulted in the rearrangement to a naphthyridine ring and also the transestrification and the substitution of the ethoxycarbonylmethoxy to the ethoxy group. Similarly, from the 4-methyl-2-(2-oxo-2-phenyl-ethyl)-6-phenyl-pyrrolo[3,4-*c*]pyridine-1,3-dione the 3-benzoyl-4-hydroxy-8-methyl-6-phenyl-2H-2,7-naphthyridin-1-one was obtained. Subsequently, new 2,7-naphthyridine derivatives were synthesized by the hydrolysis, ammonolysis, and reaction with hydrazine of the 2,7-naphthyridine esters. A series of Schiff's bases were also produced by treating the obtained hydrazides with the aldehydes. Most of newly synthesized compounds were qualified by the NCI (Bethesda, USA) for *in vitro* antitumor screening. The compounds were evaluated against the 60 different human tumor cell lines, representing melanoma, leukemia, and cancers of the breast, CNS, colon, ovary, kidney, prostate and non-small cell lung. The tested compounds showed variable antitumor activity. The most active were the 2,7-naphthyridine hydrazide derivatives.

The structures of the new compounds were confirmed by elemental analysis and NMR and IR spectra.

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The antiviral and virucidal activity of novel 2-[(4-methyl-4H-1,2,4-triazol-3-yl) sulfanyl]acetamide derivatives

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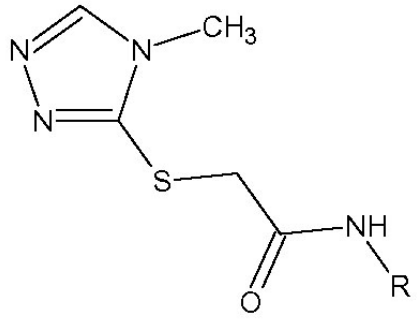
The significant step in antiviral therapy development was the introduction of ribavirin ({1-(β-D-ribofuranosyl)-1H-1,2,4 - triazole-3-carboxamide}) into medical practice. This small and simple, from structural point of view, molecule appeared to have extremely wide antiviral activity spectrum, both in relation to DNA-viruses [1, 2] and RNA-viruses [3-8]. It is known, that ribavirin influences the activeness of many enzymes, like inosine monophosphate dehydrogenases and viral RNA-polymerases [9, 10]. The inhibition of these enzymes causes blocking viral replication. It was obvious that 1,2,4-triazole scaffold, contained in the molecule, influences pharmacological properties of ribavirin. The result of the above observation was numerous research concerning the use of triazole structure in other antiviral drugs [11-13].



General structure of synthesized compounds

Taking into consideration the pharmacological usefulness of 1,2,4-triazole moiety for antiviral activity, we decided to investigate the antiviral properties of our newly-synthesized compounds in relation to DNA-virus (Adenovirus-5) and RNA-virus (ECHO-9-virus).Antiviral and virucidal activity of the title compounds was examined only at concentrations that were non-toxic for HEK-293 and GMK cells. Evaluation of virucidal activity showed that only compound without substituents at amide nitrogen was totally inactive against human adenovirus type 5. Other compounds caused the decrease in the titres of viruses by 0.44-1.56 log (24%-48%). All the tested triazole derivatives affirmed virucidal activity towards ECHO-9 virus.

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Quinolinesulfonamides as potential 5-HT$_7$ and 5-HT$_{1A}$ receptor ligands

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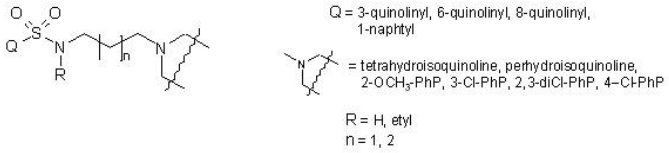
In the recent years a number of studies have been taken to evaluate the role of 5-HT$_7$ receptors. Of particular interests are the facts that several antidepressant and antipsychotic drugs show high affinity for the 5-HT$_7$ receptors.[1] Moreover, the blockade of these receptors potentiates the effect of antidepressants.[2] These observations placed 5-HT$_7$ receptors as potential target for the development of antidepressant agents.

Up to date a number of compounds have been found to bind to 5-HT7 receptors. One of the class of ligands are arylsulfonamides connected by the three or four methylene groups spacer with 4-substituted tetrahydropirydine, 1-arylpiperazine or tetrahydroisoquinoline fragments. Because these structural features are common with other G-coupled receptors ligands, mainly 5-HT$_{1A}$ ones, searching for the new 5-HT$_7$ ligands raises the problem of selectivity.

As a part of our ongoing project to identify new compounds with potential antidepressant activity, we designed series of azinesulfonamides containing different tertiary amines.



Herein, we disclose their synthesis and preliminary biological evaluation as 5-HT$_{1A}$ and 5-HT$_7$ receptor ligands. Starting azinesulfonylchlorides were synthesized according to the previously reported method.[3]

This study was partly supported by the Polish Ministry of Science and Higher Education (MNiSW), Grant No. N N405 378437

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Antiproliferative activity of novel synthetic genistein glycoside derivatives

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Genistein, a naturally occurring soy isoflavonoid, displays antitumor, antioxidant and antiinflammatory properties. It inhibits the activity of topoisomerases and several protein-tyrosine kinases. Genistein is capable of binding to the estrogen receptor. These activities, along with low toxicity, make genistein an important candidate for experimental anticancer therapy, as well as new lead-compound for anticancer drug design [1].

The principal aim of this study was the synthesis of glycoconjugates, which are the drug candidates in antitumor therapy research program. The sugar part is connected to isoflavonoid ring system through a carbonic chain. Our thesis is that the structure modification of glycoconjugates should enhance the bioavailability of these compounds. Therefore, we decided to carry out reactions of glycals with glycosyl acceptors - derivatives of genistein, and we obtained glycoconjugates with high α –stereoselectivity [2].

This new class of substances was shown to possess anticancer activity. Cytotoxic activity of genistein glycoconjugates was evaluated against the model cell lines. Active derivatives were processed for subsequent in vitro toxicity test. For each active derivative IC50 values were obtained. It was found, that all new compounds inhibit proliferation of various cancer cells.

Acknowledgementα

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[2] Polish Patent Application P384578

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Validation of a HPLC method for LI-S analysis

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A new liquid chromatography method has been developed and validated for the analysis of LI-S ((5R)-N-{[3-(3-fluoro-4 -morpholinylphenyl) -2-oxo-5-oxazolidynyl] methyl}acetamide) (Fig.1) and its impurities L7 ((5R)-[3-(3-fluoro-4 -morpholinylphenyl) -2-oxo-5-oxazolidynyl] methanol) and L9 ((5R)-[3-(3-fluoro-4-morpholinylphenyl) -2-oxo-5-oxazolidynyl] metyl azide).

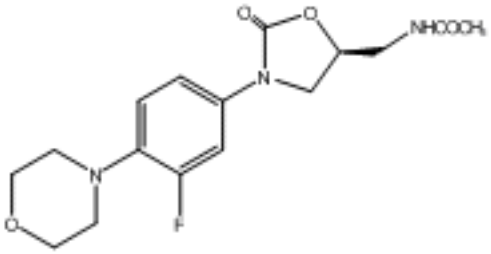

Fig. 1 Molecular structure of LI-S

The separation was achieved on a C18 column with a mobile phase consisted potassium phosphate dibasic buffer, and acetonitrile. In the course of research its was found that the separation between LI-S and L7 is a critical parameter, and its value should be higher than 1.5.

Validation of the method included: selectivity, specificity, method precision, intermediate precision, accuracy (recovery), linearity, limits of detection and quantitation (LOD and LOQ), robustness. The selectivity of the method for chemical impurities determination was demonstrated for 3 analytes: LI-S, L7 (RRT 0.9) and L9 (RRT 2.9). The method for chemical impurities determination was found to be linear (with an R^2 value of 0.999) and precise (with and RSD value of 1.4 %). The LOQ and LOD for LI-S and its chemical impurities were calculated and were found to be 0.01 % and 0.007 % respectively.

The developed and validated method meets the EP criteria of acceptance.

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Iontophoretic delivery of selected antiparkinsonian agents in vitro

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An efficient pharmacological treatment of Parkinson's disease remains a still unsolved problem. The specificity of the disease enforces frequent dose adjustment and employment of complex dose regimes. Oral antiparkinsonian drugs suffer from a marked first pass effect, and a variable absorption in the gastrointestinal tract. In this work we show that the transdermal route can be an advantageous alternative to the standard oral treatment.

Transdermal iontophoretic delivery of six drugs: pramipexole (PMP), piribedil (PIR), selegiline (SEL), trihexyphenidyl (THP), entacapone (ETC) and pergolide (PER) was performed, using side-by-side diffusion cells. Dermatomed pig skin, and human full-thickness skin were employed as membrane models. Samples of receptor solution were analyzed for the drug and electro-osmotic marker content, via HPLC and LCMS. The influence of formulation parameters on the transdermal drug flux was studied. Namely, the effects of: donor solution pH; donor drug concentration in both, single-ion and co-ion situation; current intensity, drug ionization;

and electroosmosis were investigated. Also, the water mobility of ionized drugs and their octanol-water distribution coefficient was measured.
Iontophoresis significantly enhanced the transport of all the drugs, with respect to passive diffusion. Iontophoretic fluxes were proportional to the intensity of the current applied for all the substances examined. This confirms that iontophoresis could allow easy dose individualization. Single-ion fluxes were independent of the drug molar concentration for all the drugs. Drug fluxes dropped markedly with the pH; this was probably due to the increased competition offered by the very mobile hydrogen ions (H+), and reduced skin cation permselectivity. Iontophoretic fluxes usually decreased as competing co-ions were introduced in the donor. However, two distinct behaviours were observed: for one group of drugs fluxes and transport numbers were linearly proportional to mole fraction; while for the other group, only the initial raise in mole fraction resulted in increasing flux. Iontophoretic fluxes took longer to stabilize across full thickness human skin, and were lower than across dermatome pig skin.
Briefly, the best candidates for iontophoretic delivery were pramipexole, selegiline, and piribedil. Trihexyphenidyl, entacapone and pergolide are poor candidates and probably would require patches of impractical size.

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New phenylpropanoic acid derivative with antidiabetic potential acting as a partial PPAR gamma agonist – preclinical studies.

Rafał Derlacz, Urszula Bulkowska, Wojciech J. Gutman, Krzysztof Kurowski, Zbigniew Majka, Katarzyna Matusiewicz, Joanna A. Pawlak, Monika Stupak

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Due to the explosive increase in the number of people diagnosed with diabetes world-wide in the past two decades, we can now speak of diabetes epidemic even if this word seems inappropriate in conjunction with a chronic disease. Diabetes is now considered as one of main threats to human health in the 21-st century. It is a metabolic disorder primarily characterized by insulin resistance and elevated blood glucose levels. Insulin resistance and glucose intolerance are key elements of the metabolic syndrome, which is currently associated with impaired function of PPAR gamma nuclear receptor and excessive production of fat tissue hormones. PPAR gamma is critical transcription factor in regulating lipid and glucose metabolism as well as insulin sensitivity, thus PPAR gamma receptor is considered as a very promising molecular target for developing new antidiabetic compounds.

New investigated compound is a phenylpropanoic acid derivative (non-thiazolidinedione), selective, partial agonist of PPAR gamma nuclear receptor. As a partial PPAR gamma agonist, new compound has less than 30% of the activity associated with currently marketed full PPAR gamma agonist, rosiglitazone. Theoretically, our partial agonist should minimize side effects associates with rosiglitazone treatment by limiting the spectrum of activation of PPAR gamma. Consistent with this prediction, animal studies have revealed that the compound is effective in lowering blood glucose levels and demonstrates a relatively benign adverse event profile at putative therapeutic dose ranges. Doses used in animal models of diabetes such as the db/db mouse and the ZDF rat showed significant efficacy in the control of blood glucose level with minimized body weight gain, when compared to rosiglitazone. Moreover safety pharmacology and toxicology studies with mice, rats, dogs and monkeys suggest a broad safety window in which to study new compound's benefits in humans.

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Computer aided development of dual p53-Mdm2/Mdm4 inhibitors

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The p53 protein respond to a variety of stresses and trigger cell cycle arrest, apoptosis or senescence, thereby protecting against malignant transformation. Almost all of human tumors are believed to harbor a disabled p53, either through mutation of the p53 gene or through aberrant expression of proteins acting as its negative regulators such as Mdm2 or more recently discovered Mdm4. Thus designing molecules to block the MDM2-p53 interaction and reactivate the p53 function has been perceived as a promising therapeutic strategy for the treatment of cancers retaining wild-type p53.

Mdm2 and Mdm4 are closely related, and despite of p53 binding site within Mdm2 and Mdm4 are almost the same, known small-molecule Mdm2 inhibitors like Nutlin-3 are not effective against Mdm4. As a result, their activity is compromised in tumor cells over-expressing Mdm4, preventing these compounds from rescuing the p53 activity.
Herein, we will report our *in silico* approach to progress on development of novel lead compounds active against both Mdm2 and Mdm4 proteins. The approach is based on multiple computational design and screening methods. We will compare their performance and comment several observations that we learned working on this highly demanding targets.

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Experimental and theoretical charge density studies on a model analogue of vitamin D

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SYN1 (Fig. 1) is used in the synthesis of vitamin D analogues. For example calcipotriol, calcitriol and alfacalcidol are good examples

of those analogues that have undergone clinical trials with positive outcome [1,2]. The charge density analysis, and multipole-model based on Hansen-Coppens formalism [3] refinement of electron density, obtained from theoretical and experimental structure factors, shows interesting results.

SYN1 crystallizes in a general position in the orthorhombic $P2_12_12_1$ space group. Molecules are packed in layers, in 3D. The layers are joined by C-H---O hydrogen bond type contacts. There are two types of these bonds: the first one is situated between the oxygen atom from the sulfo group, and hydrogen from the aromatic ring. The second one is situated between the carbonyl oxygen and methylene hydrogen. High resolution experiment allowed obtaining a multipole model of experimental electron density. Subsequently topological analysis was performed. This approach revealed some differences in the interaction energy for the examined interactions and allowed characterizing quantitatively the details of charge density distribution both, in the molecule, and in the crystal lattice.

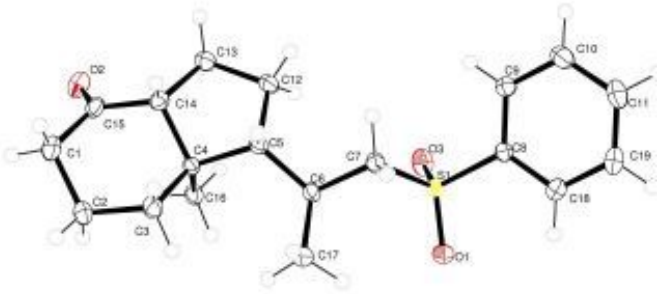

Fig 1. The molecular structure of SYN1 and atomic labeling. Thermal displacement ellipsoids are shown at the 50% probability level.

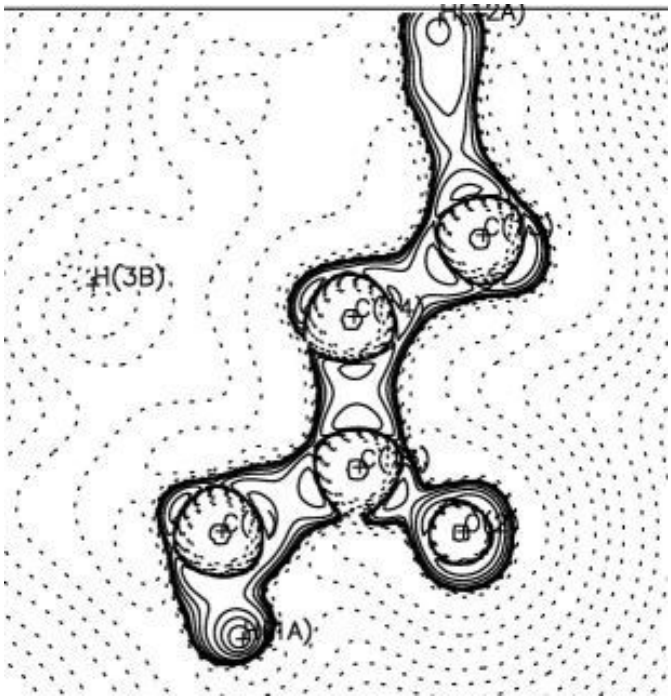

Fig 2. The contour plot of Laplacian of charge density. Plane is set through three atoms: O(2), C(15) and C(4).

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Free time
Monday evening, 10 May, 19:00

Banquet. Announcement of the Stanisław Biniecki Contest Award
Monday evening, 10 May, 20:00
Hyrny Hotel

Tuesday, 11 May

Breakfast
Tuesday morning, 11 May, 7:30

Session IV
Tuesday morning, 11 May, 9:00
Conference room
Chair: Teresa M. Brodniewicz, Karol Gerla

9:00 Invited oral

New solutions and challenges in chromatography and related techniques. Quo vadis separation sciences?

Bogusław A. Buszewski

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The progress observed in modern analytical chemistry is the consequence of civilisation development. Higher and higher expectations as for decreasing the determination level of particular analytes in complex matrices make analysts search for new methodological and instrumental solutions. Combining particular techniques into multi-dimensional and hybrid systems is the future of analytical chemistry. This is possible due to outstanding achievements in chromatography and related techniques, especially: • packing and columns technology, • miniaturisation, • advancement in sample preparation (nanoscale), • application of new selective detection systems, • qualitative and quantitative determinations, • automation and robotization, This contribution is devoted to discussing the above problems.

9:30 Invited oral

New anti-angiogenic drugs

Jozef Dulak

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Traditional anti-tumor therapies have been recently supplemented with substances inhibiting the growth of blood vessels. The rationale for such an approach was the Judah Folkman's hypothesis from the beginnings of 70ies of the last century. He suggested that the growth of tumour is dependent on the process of angiogenesis and proposed that treatment of tumours can be achieved by inhibition of the capillaries growth. Therapy of breast, lung, intestine and kidney cancer was recently improved by introduction of Avastin, the antibody specifically binding the vascular endothelial growth factor. The studies on small molecular weight inhibitors of growth factors receptors are underway, but they reveal the new previously unknown problems. Development of efficient means of inhibition of complex angiogenic activity of tumour cells and strategies allowing. tumour cells to change the mechanisms of angiogenesis during the therapy will be crucial for increasing the effectiveness of current and future anti-angiogenic drugs. During the lecture the own results of studies on mechanisms of angiogenesis regulated by HIF and Nrf2 transcription factors will be discussed.

10:00 Oral

Endothelial P2Y receptors as novel targets for a pharmacological intervention

Katarzyna A. Koziak, Elżbieta Kaczmarek

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Purinergic P2Y receptors (P2R) belong to a large family of receptors activated with extracellular purine and pyrimidine nucleotides. As G protein-coupled receptors they induce Ca^{2+} release from intracellular stores that is followed by activation of several signaling pathways and various cellular responses, such as proliferation, apoptosis or cytokine release. The biological significance of endothelial P2YR has been revealed only recently by showing their role in vascular inflammation, wound healing, angiogenesis and atherosclerosis. We were the first to demonstrate that activation of endothelial P2YR increased intracellular free calcium ion concentrations, induced phosphorylation of focal adhesion kinase (FAK), p130(cas) and paxillin, caused upregulation of α(v) integrin expression and triggered cytoskeletal rearrangements followed by cell migration. Furthermore, we have evidence indicating that endothelial P2YR are linked to AMP-activated protein kinase (AMPK) signaling pathway. AMPK is the key molecule regulating cellular metabolism and is of critical importance in clinical management of type 2 diabetes. Another newly described by us role of P2YR is activation of endothelial nitric oxide synthase (eNOS) that catalyzes the production of nitric oxide (NO) and as such is particularly important for vascular function. It is well established that human endothelium plays a crucial role in the vascular homeostasis and both immune and inflammatory responses, and that likely endothelial P2YR are involved in these processes. Therefore, we propose that P2YR should be recognized as good targets for a pharmacological intervention. Especially that regardless of ubiquitous distribution of various P2 receptors in cells, pharmacological antagonists/agonists of P2R have already been successfully applied clinically, including antiplatelet drug, clopidogrel.

10:20 Oral

Cationic chitosan derivative for heparin reversal - in vitro and in vivo studies in rats

Kamil K. Kamiński[1], Barbara Lorkowska[2], Justyna P. Ciejka[1], Karolina Zazakowny[1], Krzysztof Szczubiałka[1], Maria Nowakowska[1], Ryszard Korbut[2]

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Heparin is a widely used anticoagulant agent, which unfortunately may show some side effects, including hemorrhages, osteoporosis and thrombocytopenia. Moreover, the therapeutic dose of heparin varies significantly depending on the patient and therefore cases of overdose are frequent. At present, if necessary, heparin is neutralized using protamine sulfate. That drug, an exogenous protein, can cause an allergic reaction after administration. So, there is a need for an alternative system which would efficiently neutralize heparin, while not inducing allergic reaction.

Recently, we have developed cationic chitosan derivatives in reaction with glycidyltrimethylammonium chloride [1] which have been shown to bind heparin efficiently in buffer solution and in human blood plasma.

In a current presentation we would like to show the preliminary results of *in vitro* and *in vivo* studies of chitosan derivative (ChGl2) as a heparin antidote using Wistar rat model. Effect of GhGl2 on heparin activity in animal blood was determined using aPTT (activated Partial Thromboplastin Time) tests. Our study revealed that in rat blood *in vitro* ChGl2 can neutralize anticoagulant activity of heparin as revealed by significant aPTT reduction. The dose of ChGl2 required for heparin neutralization was determined. Also, comparative *in vitro* studies on protamine interaction with heparin in animal blood were carried out.

During *in vivo* experiments, defined dose of heparin was injected into femoral vein of the animal. Than, a fixed dose (predetermined in *in vitro* tests) of ChGl2 was intravenously administrated to the animal. Blood samples were collected from the carotid artery at regular intervals and aPTT values in obtained samples were determined. The decrease aPTT levels followed the pattern observed in control experiments in which the protamine sulfate was used as an heparin reversal agent. The studies were completed by the ChGl2 toxicity tests.

Based on the experiments described above it was concluded that chitosan derivative (ChGl2) functions as an efficient heparin antidote in rats. The dose of ChGl2 required to bring down the heparin level in rat model is comparable to that of protamine. Further studies

on larger population of animals are necessary to confirm the above conclusions and to bring the chitosan derivatives to the clinical studies.

References.

1. K. Kamiński, K. Zazakowny, K. Szczubialka, M. Nowakowska, pH-Sensitive Genipin-Cross-Linked Chitosan Microspheres For Heparin Removal *Biomacromolecules***2008***, 9, 3127–3132*

Acknowledgements. Project operated within the Foundation for Polish Science Team Programme (PolyMed, TEAM/2008-2/6) and Ventures Programme co-financed by the EU European Regional Development Fund. Project partially financed by a grant from Polish Ministry of Science and Higher education No. N N204 151336.

Coffee braek

Tuesday morning, 11 May, 10:40
Hyrny Hotel- Patio and Garden

Session V

Tuesday morning, 11 May, 11:10
Conference room
Chair: Barbara Malawska, Józef Dulak

11:10 Invited oral

The use of genistein in treatment of genetic diseases

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Genistein (4', 5, 7-trihydroxyisoflavone or 5, 7-dihydroxy-3-(4-hydroxyphenyl)-4*H*-1-benzopyran-4-one) is a natural isoflavone of various biological activities. Recent studies indicated that this compound can be considered as a potential drug for treatment of some genetic diseases, exemplified by cystic fibrosis (CF) and mucopolysaccharidosis (MPS). Interestingly, molecular mechanisms by which genistein may be beneficial for patients suffering from these diseases are different. In CF, dysfunction of the *CFTR* gene causes a defect in the function of the transmembrane chloride pump, leading to a lack of movement of chloride in nose, sinuses, lungs, intestines, pancreas and sweat glands, and thus, to clinical manifestations of the disease. The most common mutations found in CF patients is DF508, and it appears that the function of the mutated gene product may be partially restored by genistein, which can interact with this protein, especially in combination with phenylbutyrate. MPS is a group of inherited metabolic disorders, caused by mutations leading to dysfunction of one of enzymes involved in degradation of glycosaminoglycans (GAGs) in lysosomes. Due to their impaired degradation, GAGs accumulate in cells of patients, which results in dysfunction of tissues and organs, including the heart, respiratory system, bones, joints and central nervous system. Genistein has been shown to act as an inhibitor of GAG synthesis due to its function of the inhibitor of kinase activity of epidermal growth factor receptor. Therefore, genistein, can be considered as a therapeutic for as complicated diseases as genetic disorders.

11:40 Oral

Microcalorimetric measurements in design of anti-tumor drugs

Jan Mazerski

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DNA is a molecular target of a majority of known anti-tumor drugs. Inhibition of cell proliferation or cell death might be a result of various mode of action on biochemical as well as cellular level. However, in each case formation of physicochemical ligand-DNA complex is a first step of the drug action. Knowledge about the complex stability as well as about nature of interactions resposible for its formation is crucial for rational design of new drugs with better pharmaceutical properties. Thus, a lot of scientific groups, including Department of Pharmaceutical Technology and Biochemistry, Gdańsk University of Technology, develop brought research projects concerning this process. These projects including determination of:

- 3D structure of complexes
- ligand sequential selectivity
- nature of interactions responsible for the complex formation and its stability
- influence of medium composition on the complex formation

Different spectral techniques are mainly used to determine a ligand ability to formation of the complex. However, interpretation of observed spectral changes needs several assumptions about nature of the complex and obtained thermodynamics data have indirect and partially speculative character.

Direct thermodynamics data about the complex formation could be obtained with calorimetric measurements, only. Modern microcalorimeters are able to determine thermal effects on a level of μJ and need relatively small amount of reagents. In addition, direct measure of thermal effects of the complex formation allows to create entirely thermodynamics description including information about nature of interactions responsible for the complex formation. Thus, an application of this techniques rapidly increases.

In this presentation results of microcalorimetric measurements obtained in our Department for selected derivatives and analogs of acridine have been presented together with conclusions come from entirely thermodynamics description. The usefulness of such descriptions for design of new generation of acridine anti-tumor drugs will be also discussed.

12:00 Oral

Design of novel peptide dendrimers: antimicrobial and anticancer activity

Zofia Lipkowska[1], Piotr Polcyn[1], Paulina Zielińska[1], Aleksandra M. Rajnisz[2], Jolanta Solecka[2]

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Dendrimers are macromolecular compounds of branched structure, most widely investigated as prospective drug carriers. Here we present design and biological activity of a novel type of peptide dendrimers based on highly branched tetra-arm core (**A**) with intrinsic antimicrobial properties. They are branched analogs of natural antimicrobial peptides and therefore, are characterized by amphiphilic structure and positive charge.

The tetra-branched dendrimeric peptides described here demonstrate higher activity against Gram(+) and Gram(-) strains, including MRSA and ESBL reference strains, than similarly substituted respective dendrimers built around $(Lys)_2Lys$ scaffolds [1]. More hydrophobic molecules are active against human melanoma cancer cells. Both antimicrobial potency and hemolytic properties strongly depend on molecular structure and character of the R^2 groups. Electron microscopy and DSC studies on interactions with model multilamelar vesicles confirm their activity on phospholipid bilayers [2].

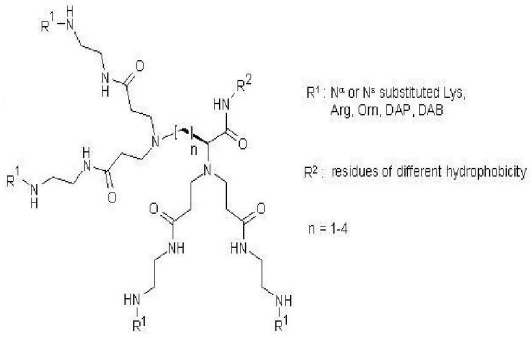

Fig. 1. General structure of dendrimers

These compounds are fully characterized by NMR spectroscopy. The secondary structure of dendrimers was estimated by CD spectroscopy in water, TFE and anisotropic environment of SDS micelles. The designed compounds overcome several disadvantages of the natural antimicrobial peptides including high cost of preparation and low plasma half-life.

Authors acknowledge financial support from the Ministry of Science and High Education, grant N204 239436 and EC project Normolife.

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12:20 Oral

Selvita: Life science solutions

Mateusz Nowak

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Selvita is a product and solution provider for the pharmaceutical and biotechnology industry from Krakow, Poland. We deliver comprehensive solutions to customers from the pharmaceutical and biotechnology industry targeted at lowering the cost of introducing innovative compounds to the market. In cooperation with different laboratories we have initiated several projects from the area of oncology and central nervous system. All projects developed by our company are in the discovery and early preclinical phase. Our most advanced project, SEL24, involves a group of highly potent and selective compounds which are specific inhibitors of Pim-1 kinase, with IC50 at low nM concentration. These are potentially first in class specific Pim-1 inhibitors. SEL24 is currently in the lead optimization phase. First administration to humans is planned to take place in 2011. Selvita also actively explores the area of psychiatric disorders, with a current focus on the schizophrenia and cognitive diseases. Currently, we have an active pre-clinical development project, on a selective antagonist of 5-HT6 receptor, SEL73, which is being developed for indications such as Alzheimer's disease or schizophrenia

12:40 Oral

A Local SCF approach to all-atom semiempirical quantum-mechanical protein modeling and ligand docking

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Molecular modeling and simulation of large systems remains a very difficult task because of complexity and diversity of biological macromolecules. Despite of ever increasing computational capability, investigations at the atomic level pose many unresolved issues. Currently, classical force fields represent most popular approach to studying of biomolecular systems. However, molecular mechanics (MM) force fields are unable to describe the changes in the electronic structure of a system undergoing a chemical reaction. The changes in molecular topology or intermolecular charge transfer require quantum mechanics (QM) for the proper treatment. However, due to high computational cost, the application of ab initio QM is still limited to relatively small systems. Correspondingly, combined QM/MM methods have attracted a significant attention in the past.

However, there are apparent limitations on this path as well. Difficulties in formulation of the QM-MM interaction scheme, bond breaking while defining the QM zone, size of the QM zone, and consistent treatment of solvation bring certain difficulties to the field. Therefore, significant efforts have being made to develop new schemes that would allow treating the entire system at the approximate quantum-mechanical level.

We present a linear scaling semiempirical method LocalSCF [1],[2] and the derivation of the underlying variational finite localized molecular orbital (VFL) approximation [3]. Due to economical use of computational resources LocalSCF method made possible QM calculation of 100,000 atoms systems on a desktop computer. Based on the VFL approximation, and particularly on its ability to treat each linear coefficient of molecular orbital independently, we developed the novel type of the semiempirical QM/QM method in which a part of the system, including ligand and protein active site, are treated at the full self-consistent regimen while the protein bulk is considered as carrying a frozen electronic density matrix. The developed QM/QM method is implemented in the LocalSCF suit of programs [4] and applied toward the protein-ligand binding energy prediction searching through the library of 20,000 ligands represented in total by 200,000 conformations [3]. This remarkable computational performance makes feasible routine application of the QM theory to the computational studies of large biological systems and structure based drug design.

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Dinner

Tuesday afternoon, 11 May, 13:00
Hyrny Hotel - Canteen

Session VI

Tuesday afternoon, 11 May, 15:00
Conference room
Chair: Marek Chmielewski, Wojciech T. Markiewicz

15:00 Invited oral

Molecular properties of active ingredients Impact on bioavailability The biopharmaceutics classification system

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The main target of Biopharmaceutic Classification System (BCS) is to identify molecular determinants that have an impact on bioavailability of studied agents. Solubility and permeability are two properties that have an effect on compounds bioavailability which is the most important for biological activity of drugs in humans. Experimental determination of those parameters is cumbersome and frequently impossible. Therefore we calculated for selected active ingredients the parameters which describe both solubility and the tendency to cross biological membranes. The free enthalpy of solvation (ΔGsolv) 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. Moreover we determined and compared experimental and theoretical log P values which, as first approximation, describe lipophilicity and permeability of pharmacological substances.
The results also usefully serve as description of properties relevant to 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, as an example, to propose BCS categorization for antifungal drugs, as a step to improve the BCS system at the international level.

15:30 Oral

Chemoenzymatic approach to the synthesis of bioactive tripeptide mimetics for treatment of cancer

Ryszard Ostaszewski, Szymon Kłossowski, Michał Mroczkiewicz

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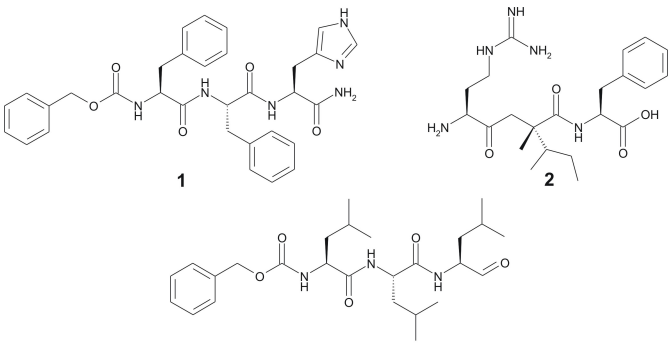
Biocatalysis is a methodology of growing importance among organic chemists aiming at the synthesis of bioactive compounds, resulting in the establishment of many successful synthetic protocols[1]. Main reason for this is an enormous selectivity of biocatalysts.



3 (MG-132)

Tripeptides and tripeptide mimetics are widely investigated due to their biological activity. Among them, an anti-inflammatory agent **1**, an antibiotic **2**, and human rhinovirus 3C protease inhibitors can be found.

Tripeptides with *C*-terminal aldehyde group are of special interest. Compound **3** (Mg- 132) is a potent and selective inhibitor of 20S proteasome, which is often used as a reference in biomedical studies.

The results of our studies on the successful combination of mutlicomponent reactions with enzymatic transformations to the synthesis of bioactive tripeptide mimetics, will be presented [2,3,4].

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15:50 Oral

Comparable antinociceptive effect of biphalin in mice selected for high and low swim stress-induced analgesia after blood-brain barrier disruption caused by hyperosmolar mannitol administration

Anna Klinowiecka[1], Piotr Kosson[1], Anna A. Leśniak[1], Mariusz Sacharczuk[2], Bogdan Sadowski[2], Andrzej W. Lipkowski[1]

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Bidirectional selection of Swiss-Webster mice for high (HA) and low (LA) swim stress-induced analgesia caused substantial differences in endogenous pain inhibition mechanisms triggered by stress. In the HA line endogenous opioid peptides secreted during stress as well as exogenously administered opioid peptides cause considerable analgesia as opposed to the LA line. The mechanism of increased analgesia in HA mice resulting from exposure to stress is still unclear and demanded investigation. A hypothesis has been put forward that these lines differ in blood-brain barrier permeability, hence differences in analgesic potency of exogenous alkaloid and opioid peptides. Moreover, ultramicroscopic studies displayed pathological changes in morphology of BBB in HA and LA mice. In our study we observed a differential response of HA and LA mice to a systemic administration of a dimeric enkephalin analog-biphalin. This study aimed to evaluate whether the analgesic potency of biphalin would alter after blood-brain barrier disruption caused by hyperosmolar mannitol. Intravenous administration of mannitol (20%) before biphalin (10 mg/kg) produced comparable antinociceptive effects in tail flick and hot plate tests in HA and LA mice. In LA mice pretreated with mannitol hot plate and tail-flick latencies were noticeably higher than in saline pretreated mice, whereas in HA mice the effect of mannitol pretreatment was very small. Our study suggests that changes in blond-brain barrier permeability are one of the major factors resulting from selection for high and low stress-induced analgesia.

16:10 Oral

Confocal microscopy - a novel tool in drug analysis

Katarzyna Wiktorska, Małgorzata Milczarek, Elżbieta Anuszewska, Jadwiga Dudkiewicz Wilczynska

National Medicines Institut (NIL), Chełmska 30/34, Warsaw 00-725, Poland

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Confocal microscopy is an advanced modification of optical microscope. It has been widely used in cell biology however, recently it has been applied also in pharmaceutical research. Confocal microscope allows obtaining pictures of greater resolution and contrast. Moreover it allows for a quantitative measurement of fluorescence. The also very useful feature is its ability to optical sectioning allowing for 3D shape reconstruction what enables also an in-depth scanning. All this features make a confocal microscope a promising tool in pharmaceutical science.

Still scanty literature describes a successful application of confocal microscopy in imaging of various pharmaceutical systems including microspheres, colloidal systems, tablets and film coatings. The study includes: the assessment of internal structure of microspheres, quality of coating and also 3D imaging of tablets surface, moreover the active substance distribution and pH change within the microspheres, tablets or bioadhesives. The microscope allows also for time-dependent measurements, e.g live imaging of process of dissolution or interactions of dosage forms with biological barriers. With help of the confocal microscope intracellular or intratissue distribution of drugs can be successfully studied.

The results of our own attempts in confocal microscope application in pharmaceutical research will be presented. The study was performed with help of Olympus IX70 FV500 confocal microscope equipped with Ar laser (488nm) as a light source. The confocal microscope was used to visualize drug crystals and particles suspended in solution. The measurement and assessment of shape, size and quality of insulin crystal surfaces was performed. Also the application of confocal microscope in drug intracellular distribution will be presented. It was possible to study a dose-depend penetration in time of fluorescent cytostatic drugs, adriamycin and its derivative, into the human melanoma ME-18 cells.

Coffee break

Tuesday afternoon, 11 May, 16:30

Poster session II (Presentation of posters with even numbers)

Tuesday afternoon, 11 May, 17:00
Hyrny Hotel

17:00 Poster 2

Determination of total hypericins in St. John's wort and herbal medicinal products

Małgorzata Anyżewska, Anna Kowalczuk, Anna Łozak, Renata Jabłczyńska

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Within the past few years, phytotherapy and herbal drugs have attracted much attention. St. John's wort (Hypericum perforatum L.) is one of the most popular medicinal plants used in traditional medicine all over Europe. According to traditional indications aqueous infusions from St. John's wort containing mainly hydrophilic components: flavonoid glycosides, tanning agents and phenolacids are used in gastrointestinal diseases. On the other hand, ethanolic extracts containing hypericin and hyperforin affect the CNS and are indicated for the treatment of mild depression-like mood disorders.

Different manufacturers use HPLC method or spectrophotometry method to test the quality of medicinal products containing St. John's wort herb. The result of hypericin determination in both methods is expressed as the hypericin content; however, in HPLC method it is the sum of hypericin and pseudohypericin, whereas in spectrophotometry - it is the sum of all derivatives of hypericin. Differences in analytical methods used to determine active substances and in reference materials are the reason why results are presented in a diversified manner, which makes it impossible to compare strength of products.

The work aimed to determine the levels of hypericins expressed as hypericin in the herbal substance of St. John's wort and medicinal products containing extract or powdered herb, by HPLC method. In addition, the amount of hypericins in the infusion prepared from St. John's wort was determined by HPLC and spectrophotometry methods. As evidenced by the study results, the daily dose of hypericins taken by a patients as infusions from St. John's wort herb is 0.30 mg-0.35 mg and it can be compared to the daily dose of hypericins in tablets and capsules indicated for mild depression-like disorders that is 0.29 mg - 0.64 mg.

As a result of lack of a consistent approache to the presentation of the strength of herbal medicinal products confirmed by consistent analytical methods, there is a risk that some indications are omitted in patient information leaflet. As a result of the introduction of a consistent uniformity method to determine hypericins in raw material, preparations and products containing St. John's wort herb using certified reference standards, it would be possible to determine the contents of these substances in products and their strengths in a reliable and unambiguous way.

17:00 Poster 4

Synthesis and in vitro anti-hepatitis B and C virus activity of the new nucleobase analogues containing thiazolo[4,5-d]pyrimidine ring system

Lilianna Becan

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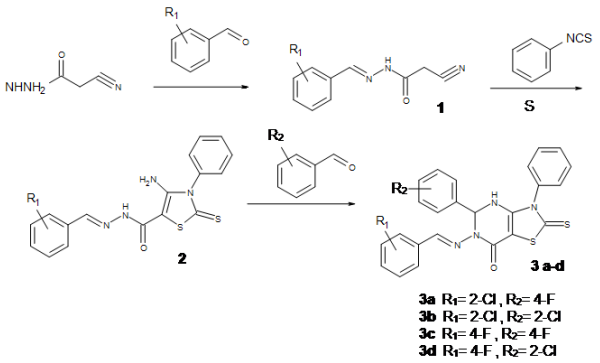
Inflamation of the liver can be caused by viral infections. Hepatitis B virus (HBV) and hepatitis C virus (HCV) are two of the dreadful viruses and have infected billion people worldwide. Therefore huge reaserch effort is being made to discover new, more efficacious, antiviral compounds.

The [4,5-*d*] isomer of thiazolopyrimidines can be considered as 7-thio analogues of guanine and adenine. Thiazolo-[4,5-d]pyrimidines were reported to possess a broad range of biological activity. A number of the new derivatives of thiazolo[4,5-*d*]pyrimidin-2-tione-7one have been synthesized and biologically screened *in vitro* against both hepatitis viruses HBV and HCV. The synthesis of the active compounds is depicted in Scheme 1. The necessary N-(haloaryl-methyleneamino)-2-cyano-acetamides **1** were prepared by reacting 2-cyanoacetohydrazide with appropriate substituted aromatic aldehyde. Thiazoloderivatives **2** were synthesized by the condensation of **1** with sulfur and phenyl isothiocyanate under the reaction conditions described by Gewald. The title derivatives **3a-d** were prepared by heating **1** with aldehyde in the presence of a basic catalyst.

Anti-HCV activity was assessed by the primary HCV RNA replicon assay using Huh 7 cell line. Four compounds **3a-d** showed antiviral activity , they inhibited HCV replication in 48-68%. In further testing **3a** and **3b** exhibited 50% effective contrentration EC_{50}=0,41 μM.

Compound **3b** , specifically tested for inhibition of replication hepatitis B virus in cultured hepatoblastoma 2.2.15 cells exhibited a good toxity/activity profile against HBV by inhibition of the synthesis of extracellular virion release EC_{50}=1,4 μM, EC_{90}=3,6 μM, CC_{50}=148 μM, SI=41 and intracellular HBV replication intermediates EC_{50}=3,5 μM, EC_{90}=15 μM, CC_{50}=148 μM, SI=10, SI= selectivity index was calculated as CC_{50} (toxity) and EC_{90} (activity) ratio. The study of the hepatitis caused by B and C viruses has been hindered by the lack of a small animal model. In 1995, an HBV transgenic mouse model was created to evaluate the antiviral activity of new compounds. As a result of *in vitro* screens compound **3b** is actually tested *in vivo* in the chimeric mouse model suffering from a transgene-induced liver disease. In conclusion, the new thiazolo[4,5-d]pyrimidines exhibit *in vitro* activity against both hepatitis viruses despite the fact that the two viruses have different genomes.

Scheme 1



17:00 Poster 6

The effect of a novel dinuclear platinum complex with berenil and 2-picoline ligands on growth and metabolism of human breast cancer cells

Krzysztof Bielawski[1], Anna Bielawska[2], Bożena Popławska[2], Arkadiusz Surażyński[1], Robert Czarnomysy[1]

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The widespread clinical use of platinum compounds in cancer chemotherapy has prompted a search for new platinum agents. While there has been some success in lowering the toxicity of platinum drugs (carboplatin) and limited success in overcoming acquired cisplatin resistance (oxaliplatin) there has been little success in developing drugs that show activity in cancer cell lines that have a natural resistance to cisplatin and carboplatin. Recent work on the targeting of antitumor agents to DNA by the use of DNA minor groove-binding ligands has shown that this strategy can greatly enhance both the in vitro cytotoxicity and the in vivo antitumor activity of the alkylating moiety, when compared with untargeted compounds of similar reactivity. Berenil (1,3-bis(4'-amidinophenyl)triazene) can exhibit intercalative, as well as minor groove binding, properties when it binds to both DNA and RNA duplexes, while also exhibiting a preference for DNA duplexes with unobstructed minor grooves. Berenil preferentially recognizes and binds to AT-rich DNA sequences and it is strong catalytic inhibitor of mammalian DNA topoisomerase II. Based on this strategy and due to the high affinity of berenil for the minor groove, we are expecting that Pt_2(2-picoline)$_4$(berenil)$_2$ (KB1) would localize in the vicinity of the DNA, and the combined effect resulting from platination and minor groove binding might confer cytotoxic activity of Pt_2(2-picoline)$_4$(berenil)$_2$. Evaluation of the cytotoxicity of a novel dinuclear platinum(II) complex of formula Pt_2(2-picoline)$_4$(berenil)$_2$ employing a MTT assay and inhibition of [^{3}H]thymidine incorporation into DNA in both MDA-MB-231 and MCF-7 breast cancer cells demonstrated that KB1 was more active than cisplatin. The DNA-binding ability of Pt_2(2-picoline)$_4$(berenil)$_2$ was evaluated by an ethidium displacement assay indicated that the complex shows strong specificity for AT base pairs in the minor groove of DNA. We have shown in the present report that Pt_2(2-picoline)$_4$(berenil)$_2$ in opposite to cisplatin is potent catalytic inhibitors of topoisomerase II. KB1 was also compared to cisplatin in respect to collagen biosynthesis, b$_1$-integrin receptor, IGF-I receptor, phosphorylated MAP-kinases (ERK_1/ERK_2 and p38), phosphorylated Akt kinase expression and appearance of apoptosis in MCF-7 and MDA-MB-231 human breast cancer cells. It was found that KB1 was more active inhibitor of collagen biosynthesis than cisplatin. The expression of IGF-I and β_1 integrin receptor, as well as phosphorylated MAPK, (ERK_1 and ERK_2 and p38) was significantly increased in cells incubated for 24 h with 20 mM KB1 compared to the control, not treated cells. The phenomenon was related to the increase expresion of NF-kB by KB1 as shown by Western immunoblot analysis. Flow cytometric analysis and a fluorescent microscopy assay showed that cell death appeared to result from apoptosis, with the possibility of secondary necrosis. These results indicate Pt_2(2-picoline)$_4$(berenil)$_2$ represents multifunctional inhibitor of breast cancer cells growth and metabolism.

17:00 Poster 8

Problems of determination of selected Class 1 residual solvents (according to ICH*)) as process impurities of some Class 3 and 2 solvents in API by GC-HS method

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Class 1 (according to ICH*)) solvents should be determined in API: a) when they are used as starting materials, b) are formed as a by-product from a chemical reaction, or c) arise from another solvent (ICH*). Class 2 or 3) used in the synthesis of pharmaceutical substances (API). It's common knowledge that benzene is a known process impurity of acetone or toluene and some of chlorinated compounds may be process impurities of dichloromethane or chloroform.

The main problem of determination of residues of Class 1 solvents was to find the analysis method because of their very low content in API. On the one hand a task was to find solvent, which guarantee dissolution of tested API, and on the other, a task was to make possible quantity determination of Class 1 solvents in API on very low level.

According to the European Medicines Agency (EMEA) it is considered that amount of said solvents in pharmaceutical product must not exceed the following values: acetone (Class 3) - 5000 ppm, toluene (Class 2) - 890 ppm, dichloromethane (Class 2) - 600 ppm and chloroform (Class 2) 60 ppm. The Class 1 solvents have very low specification limit in API, for example, carbon tetrachloride - 4 ppm and benzene - 2 ppm.

The determination of residues of some Class 1 solvents in API by gas chromatography (GC) was elaborated. Following equipment were selected: headspace injection GC with flame-ionization detector (FID) and DB-624 (60 m long, 0,32 mm ID, 1,8 µm film thickness) column. Variety of conditions of gas chromatography and headspace injection parameters, also conditions of preparation of the sample of tested pharmaceutical substance were tested. The chosen method was validated. Validation included selectivity, specificity, system precision, method precision, intermediate precision, accuracy

(recovery), linearity, limits of detection and quantitation and robustness.

As an example, the development and validation results of method determination of carbon tetrachloride as process impurity of dichloromethane and chloroform will be presented.

*) ICH - The International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use

17:00 Poster 10

New acetylenic derivatives of betulin with anticancer activity

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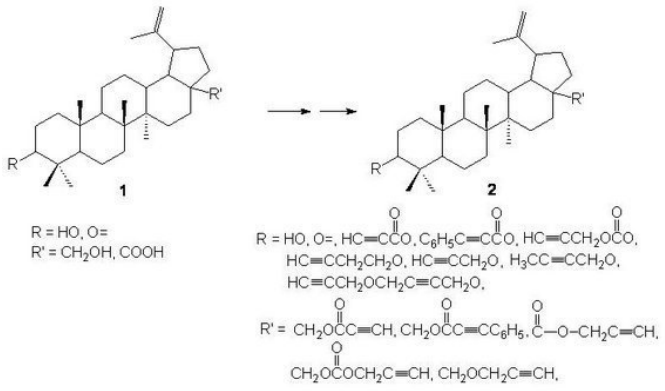
Betulin [lup-20(29)-en-3b,28-diol] is an abundant naturally occurring pentacyclic triterpene type lupane. Large quantities of it are in the external part of birch bark (content 25 – 30 %), from where it was separated in 1788 by Lovitz by sublimation, as one of the first natural products isolated from plants. Betulin and its derivatives have a wide spectrum of biological activities such as anticancer, antibacterial, antiviral, antiinflammatory, hepatoprotective and others [1]. The structure of betulin **1** (R=OH, R'=CH_2OH) has two hydroxyl groups: secondary at C-3, primary at C-28 and isopropylidene moiety at position C-19, where chemical modifications can be easily performed to yield new compounds, which possess various interesting pharmacological properties. So for only little attention has been paid to betulin derivatives containing alkyne groups [2, 3].

As an extension of our work on the development of anticancer agents, we synthesized the series of new derivatives of betulin **2** possessing one or two acetylenic functions. Betulin was isolated from the birch bark and then was oxidized to betulonic acid (R= O=, R'=COOH) and betulinic acid (R=OH. R'=COOH). Compounds **1** in the reactions with acetylenecarboxylic acids or acetylenehalides were converted into the corresponding mono- and diesters or ether derivatives **2**.



The obtained compounds were tested for their anticancer activity *in vitro* against the cells of human and murine cancer cell lines.

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17:00 Poster 12

Synthesis of long-chain arylpiperazine theophylline derivatives as potential 5-HT_7 and 5-HT_6 receptors ligands

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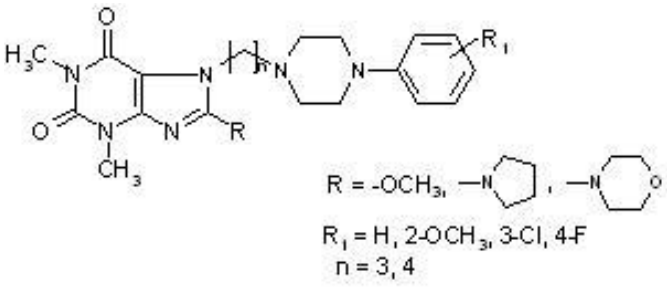
The role of 5-HT_{1A} and 5-HT_{2A} receptors in the pathogenesis of neuropsychiatric disorders is well known. However the most recently identified serotonin receptor subtypes: 5-HT_6 and 5-HT_7 are also reported to have importance in the control of many CNS functions (thermoregulation, circadian rhythms) and dysfunction like migraine, epilepsy and depression [1]. It has been postulated that 5-HT6 ligands may afford useful therapies for the treatment of obesity, as well as cognitive enhancement in schizophrenia and Alzheimer's disease [2].

In the field of serotonergics we have concentrated on the development of long-chain arylpiperazines (LCPs) theophylline derivatives which were active at 5-HT_{1A}, 5-HT_{2A} and 5-HT_7 receptors [3, 4]. Among them the 8-alkoxy derivatives of 1,3-dimethyl-7- (4-arylpiperazinylalkyl)-3,7-dihydropurine-2,6-dione proved to be potent ligands for these receptors and showed anxiolytic and antidepressant activities *in vivo* models [4]. To continue our research and extend the study on 5-HT_6 receptors we designed and synthesized series of the new analogues by modification of the substituent in position 8 of the purine-2,6-dione core. Additionally the *p*-fluoro substituent was introduced into the phenyl ring.



The new analogues are under evaluation for their affinity to the

5-HT_7 and 5-HT_6 receptors. The structure-activity relationship (SAR) will be discussed.

This study is partly supported by a grant PNRF-103-AI-1/07 from Norway through the Norwegian Financial Mechanism.

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17:00 Poster 14

HPLC Methods for Stress Testing of ZL-S Drug Substances

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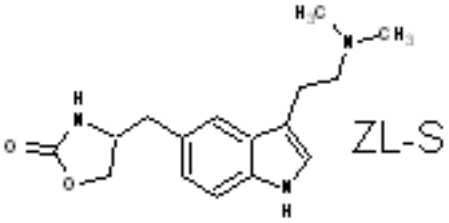
ZL-S, namely the 4-({3-[2-(dimethylamino)ethyl]-1*H*-indol-5-yl}-methyl)-1,3-oxazolidin-2-one is very potent serotonine receptor inhibitor. The primary objectivev ot this research was to study the degradation behaviour of ZL-S under different stress conditions by HPLC with UV detection and to establish stability of this drug substance. The secondary was to establish the stability indicating HPLC methods for assessment of purity and content of ZL-S.



ZL-S was exposed to the recommended by Internetional Conference of Charmonisation stress conditions. The alkaline and acidic hydrolysis, oxidation, photolysis and thermal decomposition stress tests were applied. Almost total degradation occurred in alkaline medium. Partial degradation was observed under oxidative stress conditions, in the acidic medium and in the neutral conditions. The investigated substance was stable under thermal and photo stress conditions.

Satisfying separation of API from degradants formed during stress tests was achieved on a C-18 column using phosphate buffer and acetonitrile as the mobile phase in a gradient mode. Paralelly, the assay of ZL-S was determined in the same chromatographic conditions.

In order to establish the enantiomeric purity of both, ZL-S before and after the stress tests, the separation of drug from its isomer (D) and degradation products was carried out on a Chiralpak AD-H column using a mobile phase consisting of n-hexane, isopropanol, metanol, diethylamine.

Analytical methods used in the stress testing experiments were validated with respect to specifity, linearity, precision, accuracy and robustness. The efficiency of the procedure was verified by its application to standards of potential impurities which emerged in course of the synthesis. The products formed during the stress studies were similar to standards produced by Chemistry Department of Pharmaceutical Research Institute.

17:00 Poster 16

The role of estradiol in reducing the ethanol toxicity

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Estrogens play multifunctional role in the body. The last decades research were focused on its free radicals processes participation. The antioxidative, but also the prooxidative properities of estrogens were the subject of interest. The aim of our research is to investigate whether the estrogens participate in detoxification processes in exposure to xenobiotics, particulary free radicals pathway. The previous study showed that 17-β-Estradiol (E_2) inhibits free radicals processes caused by fluoride, but the mechanism is connected mainly with thiol groups protection than hydroxyl scavenging. The presented study examines the influence of ethanol and estradiol on natural antioxidative barrier like enzymes superoxide dismutase (SOD) and glutathione peroxidase (GPx). In order to evaluate the interaction and the role of estradiol in detoxification the joint effect is examined.

The research was performed in vitro on human blood erythrocytes isolated from blood taken on EDTA (SOD) or heparine (GPx). The SOD activity was measured with Ransod test (Randox), GPx with Ransel (Randox). The concentration of ethanol was 0,4-2,4mg/ml; 17-β-Estradiol 0,75nM, 1,5,10μM. The results were evaluated with statistical analysis (program Statistica 8.0)with Anova or T-Student's test.

It was observed, that ethanol in conc. 0,5 and 2,0mg/ml significantly ($p<0,05$) increased the activity of SOD and GPx in the comparison to the control. The effect of ethanol acute intoxication is quite different then one observed in long-lasting exposure, specially the addiction (decrease). 17-β-Estradiol in physiological level (0,75nM) didn't influence on SOD activity, but in higher conc. (1,0 and 5,0μM) significantly ($p<0,001$) decreased enzyme activity. Similarly estradiol in every examined concentration decreased the GPx activity in erythrocytes. The joint effect didn't show the interaction. The action of estradiol with every concentration of ethanol was comparable with the control. Because ethanol itself increased the enzymes activity, the inhibiting effect of estradiol on oxidative stress caused by ethanol is noted. It is known that oxidative stress stimulate the activity of natural defence system SOD and GPx. The lack of stimulation could be the result of oxidtaive stress inhibition, however the direct effect of xenobiotics on enzyme can't be excluded.

In summary it seems that estradiol partly reduces oxidative stress

caused by ethanol in the influence on natural enzymatic barrier activity.

17:00 Poster 18

Carbocyclic minor groove binders – endonuclease inhibition

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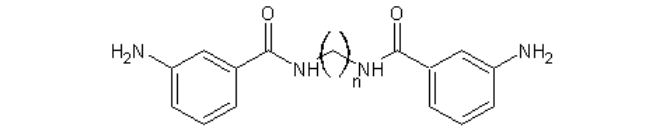
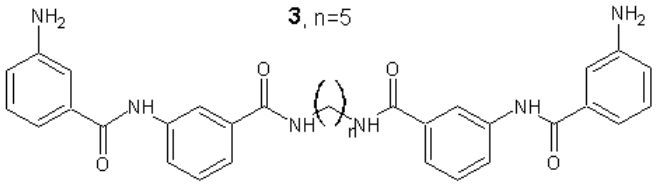
Model of binding of netropsin and distamycin with B-DNA became the inspiration to searches of new compounds with similar interaction to DNA. The class of synthetic heteroaromatic oligopeptides, projected after the models the netropsin and other minor groove binders, information-reading molecules, received the name lexitropsins. Although we observe a huge progress in designing distamycin and netropsin analogues, it does not get compounds so far, which could to apply in therapy.



1, n=3

2, n=4

3, n=5



4, n=3

5, n=4

6, n=5

The present work is in conjunction with our ongoing programme on the syntheses and biological studies of carbocyclic potential minor groove binders. Such lexitropsins, which are readily available, can be modified easily, and are stable under most experimental conditions.

The synthesis carbocyclic potential minor groove binders **1-6** with free aromatic amine groups and their activity in the standard cell line of mammalian tumour MCF-7 were described earlier. The mechanism of action of compounds **1-6** was studied employing the topoisomerase I/II inhibition assay and ethidium displacement assay using pBR322. Determination of association constants were done with using calf thymus DNA, T4 coliphage DNA, poly(dA-dT)$_2$ and poly(dG-dC)$_2$. The effect of compounds **1-6** on the amidolytic activity of plasmine, trypsine, thrombin and urokinase was also examined. Here we present the effect of compounds **1-6** on the activity of different restriction endonucleases.

Financial support from the grant N N405 355537 donated by Polish M.S.H.E. is gratefully acknowledged.

17:00 Poster 20

The influence of novel analogs of vitamin D_3 on the antiproliferative activity of cisplatin or doxorubicin on human promyelocytic leukaemia cell line HL-60

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The analogs of vitamin D_3 named PRI-2201, PRI-2202, PRI-2205 were previously tested for their antiproliferative activity against different cancer cell lines. In general, all compounds have revealed similar or higher activity in cancer cell growth inhibition compared to calcitriol or PRI-2191.

In this paper, the effect in vitro of pretreatment with calcitriol or its above mentioned analogs on antiproliferative activity of cisplatin or doxorubicin on HL-60 cells has been evaluated.

The cells were exposed to various concentrations of calcitriol, PRI-2191, PRI-2201, PRI-2202 or PRI-2205 and cisplatin or doxorubicin. The cytostatic effect was measured by the MTT assay and then the results were calculated as an IC50 (inhibitory concentration 50%). The studies of combined treatment with vitamin D_3 analogs and cisplatin on HL-60 cell line in vitro showed an increase in cell proliferation inhibition when compared to cisplatin alone. This effect was obtained by using lower doses of analogs (10, 1 or 0,1nM) and cisplatin in the dose 1μg/ml.

Calcitriol, PRI-2191, PRI-2201, PRI-2202, PRI-2205 used in 1nM concentration showed synergy or an additive effect in proliferation inhibition when combined with cisplatin (up to 40% of proliferation inhibition in combination than with cisplatin alone) on HL-60 leukemia cells. Analogs PRI-2191 and PRI-2201 used at the dose 1nM in combination with cisplatin showed an synergistic effect, analogs PRI-2202 and PRI-2205 used at concentrations showed a significant proliferation inhibition of HL-60 cells when combined with cisplatin. Comparing the IC50 results for these combined treatment to results for all of these compounds used alone, we can conclude that calcitriol analogs allow to decrease the dose of cisplatin from 1,7 to 6,4 times. In the case of combined treatment with vitamin D_3 analogs and doxorubicin synergistic or a subadditive effect in proliferation inhibition was observed. Especially, lower doses of doxorubicin (0,1 and 0,01μg/ml) combined with lower doses of analogs (10 or 1nM) causes synergy in proliferation inhibition. Even 0,001 μg/ml of doxorubicin combined with PRI-2191, PRI-2201 or PRI-2205 increases the inhibition of cell growth significantly. Using vitamin D_3 analogs we can decrease the dose of doxorubicin for inhibition of cancer cell growth. PRI-2191 allows to decrease the doxorubicin dose up to 12 times, PRI-2201 up to 4 times, PRI-2202 and PRI-2205 up to 3,5 times. We have previously demonstrated synergistic antiproliferative activity of PRI-2191 with some known antitumor drugs in the HL-60 cells. The antitumor effect of PRI-2191, PRI-2202 and PRI-2205 combined with cytostatics in mice mammary and lung cancer models was also evaluated. The results presented suggest that the improved therapeutic effect may be achieved in vivo by the combined

application of the analogues (without calcemic activity) of calcitriol with antitumour agents also on human promyelocytic leukemia cell line.

17:00 Poster 22

Synthesis and antifungal activity of novel S-esters and S,S'-diesters of N'-(2-hydroxybenzoyl) hydrazinecarbodithioic acids

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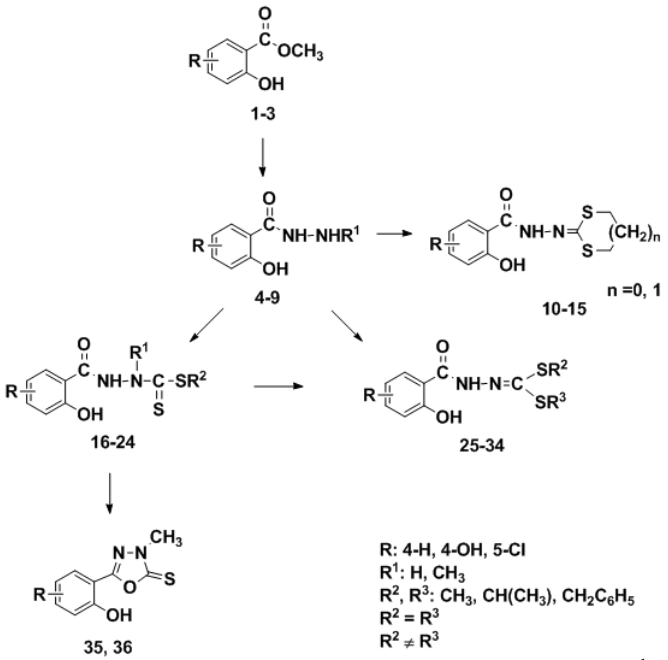
It has been well proved that various derivatives of 2-hydroxybenzoic acid (salicilic acid) exhibit strong antimicrobial activity [1]. Among others same halogenated salicilanilides show high antifungal action [2]. Undertaking studies in that research field we have synthesized a series of novel *S*-esters and *S,S'*-diesters of *N'*-(2-hydroxybenzoyl), *N'*-(2,4-dihydroxybenzoyl), and *N'*-(5-chloro-2-hydroxybenzoyl) hydrazinecarbodithioic acid. Methyl esters **1-3** of appropriate carboxylic acids led to hydrazides **4-6** and *N'*-methylhydrazides **7-9** while treated with hydrazine hydrate or methylhydrazine. Hydrazides **4-6** applied to the reaction with carbon disulfide and appropriate halides in the presence of triethylamine gave cyclic 1,3-dithiolane and 1,3-dithiane derivatives **10-15**, *S*-esters **16, 17** and *S,S'*-diesters **25-34** while *N'*-methylhydrazides **7-9** reacted to *S*-esters **18-24**. In two cases 1,3,4-oxadiazole-5-thiones **35, 36** were formed in side reactions.



The structures of novel compounds were confirmed by IR and ^{1}H NMR spectra. Antifungal activity was examined towards 9 species of *Candida* genus isolated from oral cavity and respiratory tract of patients with infections.

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17:00 Poster 24

Method development and validation of an analytical procedure - control of residual 2-iodopropane in Latanoprost

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Potential genotoxic impurities used in the synthesis of pharmaceutical substances should be identified based on the existing genotoxicity data or the presence of structural alerts and then determined by suitable analytical techniques. 2-iodopropane, halogenated aliphatic compound, has been used during the synthesis of Latanoprost and could be recognized as a potential genotoxic impurity.

The analytical procedure for the determination of residual 2-iodopropane in Latanoprost is described and validated. 2-iodopropane was determined by gas chromatography with the use of flame-ionization detector and DB-624 (60 m long, 0.32 mm ID) column. The program of column temperature was the following: 100ºC, ramp 2ºC/min to 130ºC, ramp 40ºC/min to 240ºC, 10 minutes at final temperature. The developed method allows for the determination of 2-iodopropane in the presence of other solvents arising from the synthesis route of Latanoprost.

The different validation criteria such as selectivity, specificity, system precision, method precision, intermediate precision, accuracy (recovery), linearity, limits of detection and quantitation (in substance), robustness were considered.

17:00 Poster 26

Polyunsaturated fatty acids alter expression of genes encoding antioxidant enzymes in A549 cells exposed to doxorubicin

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Docosahexaenoic acid (DHA, 22:6, n-3) and eicosapentaenoic acid (EPA, 20:5, n-3) exert selective cytotoxicity against various types of cancer cells and inhibit or reverse anticancer drug resistance [1]. Their cytotoxic effect results from lipid peroxidation and forma-

tion of free radicals [2]. Anthracycline drugs, such as doxorubicin (DX), work by generating free radicals and therefore may interfere with intracellular antioxidant enzyme system, which is involved in the development of tumor cells resistance [1, 3]. The loss of antioxidant response to oxidative stress in transformed cells may account for the ability of peroxidizable targets such as EPA, DHA to enhance tumor sensitivity to reactive oxygen species generating anticancer drugs.

The present study was aimed at evaluating of genes expression of superoxide dismutase 1 (SOD1), superoxide dismutase 2 (SOD2), catalase (CAT), phospholipid hydroperoxide glutathione peroxidase (GPx-4), and glutathione S-transferase pi (GST-pi) in human lung adenocarcinoma cells (A549) treated with doxorubicin and supplemented with EPA or DHA.

Viability of A549 cells treated with DX was measured using the XTT tetrazolium salt based assay. Expression of genes encoding the antioxidant enzymes was determined by quantitative reverse-transcription polymerase chain reaction (QRT-PCR) analysis after RNA isolation from A549 cells.

EPA and DHA added to the cultivation medium, increased the antitumor activity of doxorubicin in these cells in a concentration dependent manner. Both, EPA and DHA downregulated SOD1, SOD2, GPx, and GST-pi genes expression in DX treated A549 cells. The observed changes in mRNA levels of CAT were not statistically significant.

The results showed that A549 cells are highly susceptible to EPA and DHA. The altered antioxidant enzymes expression correlated with the sensitization of these cells to the cytotoxic effect of doxorubicin. EPA and DHA incorporated to the tumor cells may serve as possible anticancer therapeutic agents or potential adjuvants to chemotherapy.

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17:00 Poster 28

Molecular properties impact on bioavailability of imidazole antifungal agents.

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The fungal infections became one of the major reason of diseases in the entire world. The azole (imidazole) antifungal agents are the largest class of synthetic antimycotics. These drugs are used in the treatment of fungal infections. The azole antifungal agents selectively inhibit lanosterol demethylase (CYP51) in yeast and fungi. This enzyme converts lanosterol to ergosterol which is bioregulator of fungal cell integrity and fluidity. The activity of imidazole antifungals drugs depends on presence of heterocyclic aromatic five-membered ring. The heterocyclic, basic nitrogen from imidazole ring, forms a bond with the heme iron of active site of CYP51. The lack of ergosterol results in the block of fungal growth.

The pharmacological activity of a particular drug depends on its bioavailability. Two factors: solubility and permeability are very important factors for active substance bioavailability. These two parameters are used as a basic criteria for the create the Biopharmaceutics Classification System (BCS).

In our Laboratory we implemented the use of theoretically derived determinants as a tool for fast chemical substance classification within the BCS. The water solubility determination can be efficiently made based on free enthalpy of solvation (ΔG), while permeability can be described by hydrophobic properties in a first approximation. The hydrophobic properties can be established based on solvation energy in solvents with different polarity. Result of our investigations can also serve as description of other properties relevant to the BCS. The calculated parameters describe both solubility and tendency to cross biological membranes. The free enthalpy of solvation in water and organic solvents and the electrostatic potential surface around molecule in water are a promising tools for fast chemical substances classification within the Biopharmaceutics Classification System which is categorizing active compounds into four classes based on their aqueous solubility and intestinal permeability.

17:00 Poster 30

Basic low molecular dendrimeric peptides designed for better membrane recognition

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Molecular design and development of anti-infective compounds constitute an important area in modern medicinal research. One of the new sources of the prospective alternatives to traditional antibiotics are natural basic antibacterial peptides and their synthetic analogs. Structure-activity studies of this group of compounds showed that they work *via* membrane-lysis mechanism. Necessary condition for effective interactions of these peptides with slightly negative charged bacterias' membranes is conformational change allowing separation of positively charged and lypophilic groups (amphiphatic structure). Previously, we attempted to design such structure using appropriate amino acid building blocks and dendrimeric structure. Such *de novo* approach led to a group of unsymmetrical low molecular weight basic dendrimeric peptides. They showed modest activity against Gram(+) and Gram (-) bacteria [1] and variable cytotoxicity

strongly correlated with degree of branching [2]. This communication presents synthesis and molecular modeling of three diastereoisomeric groups of dendrimeric peptides with C-end modified by aliphatic chains of various length. Microbiological data for this group of derivatives in comparison with the unmodified compounds will be presented. 3D structure of these molecules, respective distances between positively charged groups (amino groups in form of hydrochloride) and lipophylic elements, obtained from molecular dynamics calculations will be discussed.

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17:00 Poster 32

The influence of paclitaxel on hydrolytic degradation process in matrices obtained from aliphatic polyesters and polyestercarbonates

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Biodegradable polymers have become common materials used in pharmacy and medicine due to their properties such as mechanical strength, biocompatibility and non-toxic degradation products. Synthetic homopolymers, copolymers and terpolymers obtained from glycolide, lactide, ε-caprolactone and trimethylene carbonate (TMC) may serve as implants, scaffolds in tissue engineering and drug delivery systems. Different compositions of copolymers and their chain microstructure may have an effect on matrices degradation and thus, the drug release profile. The subject of our study was the influence of paclitaxel content on hydrolytic degradation of terpolymeric matrices. The determined factors may be helpful in designing biodegradable systems of controlled paclitaxel release. Paclitaxel belongs to antiproliferative drugs used in cancer treatment because of binding to the microtubuli and inhibiting cells proliferation. Its effectiveness in preventing of in-stent restenosis has also been reported. With this regard bioabsorbable matrices with suitable paclitaxel release profile may serve as drug-eluting stent or stent coating.

The terpolymers were synthesized in the Centre of Polymeric and Carbon Materials, Polish Academy of Sciences in Zabrze. $Zr(Acac)_4$ was used as the initiator. Matrices containing 5% and 10% (w/w) of paclitaxel were prepared form three kinds of terpolymers: 2 poly(L-lactide-co-glycolide-co-TMC) with different composition (51:26:23 and 62:27:11) and poly(L-lactide-co-glycolide- co-ε-caprolactone) (44:32:24). Hydrolytic degradation of three kinds of matrices prepared from each kind of terpolymer (matrice with 10% of paclitaxel, matrices with 5% of paclitaxel and drug free matrices) was performed in vitro at 37 °C in PBS.

The ^{1}H and ^{13}C NMR spectra of terpolymers were recorded in order to characterize chain microstructure and paclitaxel content. Thermal properties were monitored by differential scanning calorymetry (DSC). Molecular weight dispersity (D) and molecular weight (Mn) were determined using gel permeation chromatography (GPC). The surface morphology of the matrices before and after degradation were studied by means of the scanning electron microscopy (SEM).

The impact of paclitaxel content (5% and 10% w/w) on hydrolytic degradation of matrices was investigated with reference to drug free matrices. Differences in degradation process of each kind of terpolymer were determined. The most significant degradation was observed in case of poly(L-lactide-co-glycolide-co-ε-caprolactone) 44:32:24, which become disintegrated after 2 weeks of incubation. Differences in degradation of matrices with paclitaxel and drug free matrices were noticed. This fact was also confirmed by analysis of surface morphology by means of SEM.

Acknowledgement: This study has been financially supported by MEMSTENT (Grant No: UDA-POIG.01.03.01-00-123/08-00).

17:00 Poster 34

Optimization of BR-8 synthesis

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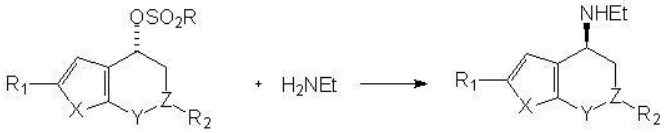
The key step of the **BR-8** API synthesis is the S_N2 type reaction of **BR-7**, in witch the sulfonate group is substituted with ethylamine, to give an aminoderivative with inverse configuration:



The reaction was carried out at laboratory scale under various conditionsin order to achieve high yield and purity of the product. The reaction parameters were sampled into full 3^2 factorial experiment (type of the leaving substituent; the solvent used as a medium) and then following a series of four consecutive screenings of excess of amine and temperature of the reaction following one-dimensional search (keeping one variable constant and changing the other). Molar content of the crude reaction product was found with the use of the mass balance and the corresponding HPLC parameters. Based on the predicted molar content of reaction mixtures a series of reaction response surfaces was calculated. From analysis of original experimental data and the response surfaces it appears that there exists an optimal set of reaction parameters for **BR-8** synthesis securing a high conversion ratio of about 80% yield and 97% purity of the crude product.

17:00	Poster	36

Application of preparative HPLC for protected peptide purification

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Solid phase methods are the most popular for synthesis of bioactive peptides. In such methods, the peptide is released from solid polymeric resin in its final form. In this case, purification of the product is performed by general HPLC methodology applied for peptides, e.g. acetonitrile/TFA/water gradient. However, particular peptides and/or large preparative scale of syntheses of pepitdes may require purification of the protected peptides. It is known, that most of the protective groups are not stable in standard HPLC conditions.

Recently, we developed variants of classical solution method for economical large scale synthesis of peptides. The intermediate compounds are Boc-protected peptides. Unfortunately, classical physicochemical methods of purification (extraction, crystalisations, etc) were not very effective in this case. To purify such peptides in large scale we developed HPLC purification conditions that are very economical and safe for the protected-peptides. This rationalizes application of HPLC in technological scale of peptide production even at intermediate stages.

17:00	Poster	38

Synthesis and anticonvulsant activity of N-substituted 8-azaspiro[4.5]decane-7,9-diones, 3-azaspiro[5.5]unde-cane-2,4-diones and 4-ethyl-4-methylpiperidine- 2,6-diones

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Continued efforts are being in the development of antiepileptic drugs employing a range of strategies, including modification of the structures of existing drugs, targeting novel molecular substrate and non-mechanism-based drug screening of compounds in animal models. To make the discovery of new anticonvulsants more rational many investigators identified structural fragments essential for anticonvulsant properties. One of the important core fragments of anticonvulsants is defined by a nitrogen heteroatomic system usually a cyclic imide, at least of one or two carbonyl groups and phenyl or alkyl substituents attached to the heterocyclic system.[1,2] The previous studies from our laboratory have demonstrated potent anticonvulsant activity in groups of *N*-benzyl-, *N*-phenyl-, *N*-phenylamino-, *N*-pyrid-2-yl- spirossuccinimides and 3,3-dialkyl-pyrro-lidine-2,5- diones.[3-6] These molecules were effective in electrically induced seizures (MES) or/and in the subcutaneous pentylenetetrazole screen (*sc*PTZ) which are recognized as the "gold standards" in the early stages of testing. As a continuation of our systematic SAR analysis in the present studies we have obtained a small library of *N*-substituted spiroglutarimides and 4-ethyl-4-methyl- piperidine-2,6-diones which were designed as analogues of the most active succinimides (**Figure 1**). Such modification enable the examination of influence of the heterocyclic ring size on the anticonvulsant activity.

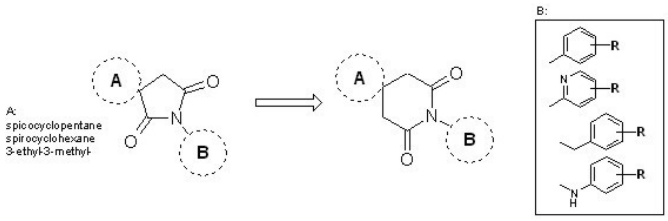

Figure 1.

The desired compounds were prepared by cyclocondensation reaction of 8-oxaspiro[4.5]decane-7,9-dione, 3-oxaspiro- [5.5]undecane-2,4-dione or 3-ethyl-3-methyl-glutaric acid with the respective amines. 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).[7]

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17:00	Poster	40

An approach towards efficient peptide synthesis in aqueous solution

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An approach to efficient procedure of peptide synthesis in aqueous media is of grate importance since the safe disposal of organic solvents is an vital environmental issue, and even more essential, the indispensable ability of proteins, peptides, sugars and other potential drugs to preserve the correct conformation only in environment close to physiological conditions. Unfortunately, there are only few reports presenting resourceful results of peptide synthesis in aqueous solvents. To perform successful peptide synthesis or protein modification the coupling reagent must be water-soluble and maintain its reactivity in water. As one of the best suited to this particular goal were reported 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methyl- morpholinium chloride (DMT-MM) [1], In order to verify this literature reports the studies on versatility of triazine based coupling reagents [2] in aqueous media was been undertaken. We found that opposite to literature reports, synthesis of Z-Aaa-Bbb-OMe in methanol, gave set of amino acid methyl esters instead of expected dipeptides, but confirmed utility of ethanol and isopropanol as co-solvents. Further-

more, the yield of coupling strongly depended on the character of the base used in coupling procedure.

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17:00 Poster 42

The influence of inositol hexaphosphate on the expression of genes encoding matrix metalloproteinases 2 and 9 and their tissue inhibitors in human colon cancer cells

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Tumor metastasis is one of the main cause of the poor prognosis in patients with colon cancer. Degradation of collagen type IV, a major component of the basement membrane, is an essential step in the metastasis to lymph nodes and distant organs. Matrix metalloproteinases 2 (MMP-2) and 9 (MMP-9) belong to zinc and calcium dependent family of enzymes that digeste collagen IV and other components of extracellular matrix. Moreover, MMPs participate in the regulation of cytokines, growth factors and adhesive molecules activity as well as in angiogenesis and apoptosis. The main cellular inhibitors of the matrix metalloproteinases are their tissue inhibitors (TIMPs), supplying a closely regulated mechanism for control, activation and function of MMPs. Inositol hexaphosphate (phytic acid, IP6), a ubiquitous plant component, plays a role in the control of tumor growth, progression, and metastasis. One postulated mechanism by which IP6 prevents the activation of MMPs may be due to its ability to chelate minerals such as iron, copper, zinc, cobalt, and manganese. The aim of the presented study was to evaluate the expression profile of MMP-2, MMP-9 and their tissue inhibitors at the mRNA level in human colorectal cancer cell line Caco-2 treated with phytic acid. A kinetic study of MMP-2, MMP-9 and TIMP-1, TIMP-2 mRNAs was performed on Caco-2 cells after treatment with 1; 2.5; 5 mM IP6 for 1, 6, 12 and 24 h. Quantification of genes expression was carried out using real time QRT-PCR technique. The gene encoding MMP-9 was neither constitutively expressed nor induced by IP6 in Caco-2 cells. IP6 at the concentration of 1mM evoked increase in MMP-2 transcript level, however, its higher doses (2,5; 5 mM) caused a decrease in this gene expression at 1h incubation. In 24 h lasting culture along with increasing IP6 concentrations the cells expressed higher and higher MMP-2 mRNA level. In response to 1 mM at 6h, the cells demonstrated an increased transcriptional activity of the TIMP-2 gene which was accompanied by a decrease in TIMP-1 gene transcription. Treatment of cells with 2,5 mM IP6 at 12h resulted in a strong increase in both TIMP-1 and TIMP-2 expression. The results of this study show that IP6 modulates MMP-2, TIMP-1 and TIMP-2 genes expression in colon cancer cells at the transcriptional level in a way dependent on its concentration and time of interaction.

17:00 Poster 44

Regioselective protection of functional groups in the natural compounds.

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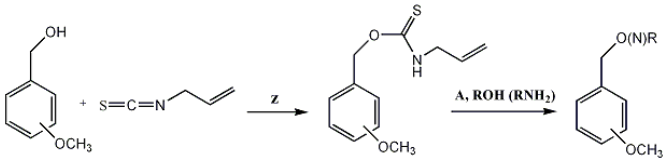
The selection of a protective group and protection strategy are important components of synthetic methodology always where the chemical reaction must be carried out selectively at one reaction side in a multifunctional compound, than other side requires to be temporarily blocked. In the case of natural compound like carbohydrates, nucleosides or steroids, functional groups are hydroxyls and amines, which necessities regioselective protection strategies. Etherification is one of the most fundamental and most frequently used important reaction in synthetic carbohydrates chemistry[1]. Protection of a hydroxyl functionality as the methoxybenzyl ethers is preferred as a temporary protective group when neutral condition of deprotection are required. Additionally this type of protective group in contrast to ester, acetal and silyl protective groups, do not undergo unwanted migration between neighboring functional groups. The metoxybenzyl protection of carbohydrates was already reported in literature with application of different strategies and conditions. Despite that, the search for new efficient etherification method is still a matter of general interest[2,3].

In this communication we report the novel method protection of hydroxyl and amine functionality with p-methoxybenzyl, 2,4-dimethoxybenzyl and 3,4-dimethoxybenzyl groups using methoxybenzyl N-allyl thiocarbamates as donors protective groups. These compounds are readily obtained from methoxybenzyl alcohols by reaction with commercially available N-allyl isothiocyanate[4].



Z: Base, **P**: Bn, **A**: Promoter (Br^+), **R:** alcohols, amines, carbohydrates

Acknowledgement

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17:00 Poster 46

Synthesis, antiprotozoal and antibacterial properties of new polyhalogenobenzimidazoles

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With the increase in antibiotic *resistance,the need for new antibacterial* agents also increases. Benzimidazole derivatives seem to be good candidates for new antimicrobial drugs. In this study we describe antimicrobial activity of new benzimidazole derivatives. Two novel groups of polyhalogenated benzimidazoles were synthesized for such investigations. The first group, composed by 4,5,6,7-tetrachloro- 2□polyfluoroalkylbenzimidazoles, was obtained by chlorination in „*aqua regia*" respective 2-trifluorometylo, -pentafluoroethyl, □heptafluoropropylo i -nonafluorobutylobenzimidazoles. N-Substituted derivatives of 4,5,6,7- tetrachlorobenzimidazole formed the second group. The alkylation of tetrachlorobenzimidazole with bromoacetic acid ethyl ester provided N^1-substituted compound, which underwent further modifications. The respective acid, hydrazide and amides substituted with morpholine, piperidine and N-methylpiperazine, were obtained. Antiprotozoal activity was investigated against *Trichomonas vaginalis*, *Entamoeba hystolitica* and *Giardia lamblia*. The most potent against *T. vaginalis* appeared N-methylpiperazinylamide of 4,5,6,7-tetrachlorobenzimidazole-1-acetic acids (IC_{50} = 0.22 μg/ml). 4,5,6,7-Tetrachloro-2-pentafluoroethylbenzimidazole showed the best activity against *E. hystolitica* (IC_{50} = 0.18 μg/ml). Gram-positive and Gram-negative bacteria were also susceptible to new obtained compounds. Of all derivatives 2-trifluoromethyl- and 2-pentafluoroethyl- -4,5,6,7-tetrachlorobenzimidazoles showed significant inhibition zone diameters (45-50 mm) for *Staphylococcus* and *Enterococcus* species.

The study was supported by the Foundation for Development Diagnostics and Therapy, Warsaw (AO and ZK).

17:00 Poster 48

Steroid profile of Asplenium cuneifolium

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One of the most investigated class of natural compounds are the phytoestrogens. Plant contain several different families of natural products among witch are compounds with weak estrogenic or anti-estrogenic activity toward mammals cells. These compounds include certain isoflavonoids, flavonoids lignans and coumarins. The best-studied dietary phytoestrogens are the soy isoflavones (genistein and daidzein) as the mainly relevant for the human and animal health. Soy is a very important food for Asian population. Epidemic data indicate that the Asian people have lower rates of osteoporotic fractures, cardiovascular diseases, postmenopausal symptoms and certain cancer than the western population.

Plants which are used in the traditional medicine for the treatment of menstrual pain, as contraceptives, or as menopausal remedies probably contain phytoestrogen compounds. Ferns play important role in folklore medicine (1). Literature data are available for different Asplenium species. Asplenium bulbiferum is rich in flavonoids with antioxidant proprieties and this species is used for nutritive properties by Maori people. Asplenium nidus was used in the traditional Vanuatu medicine as a contraceptive as well as to reverse sterility.

In the Italian folk medicine Asplenium trichomanes was used as an expectorant, anti-cough remedy and laxative as well as emmenagogue. In North America the infusion of this species was used as abortifacient and for irregular menses (2).

The aim of our study was to profile steroid, phytoestrogens and lipophilic substances Asplenium cuneifolium, serpentynite ferns from unique habitat occurring in Poland only in Lower Silesia. Plants tested were cultured in vitro on sterile MS medium (3).

Some plants contain steroidal estrogens, however as these are essentially based on the same structures that occur naturally in animals, they are not considered phytoestrogens by the strictest definition (4). Gas chromatography (GC) analyses of methanol extracts of gametophytes Asplenium cuneifolium with using animal sex hormones standards like progesterone, androsteron, estron, pregnenol and plant sterols showed the presence of only common plant sterols: sitosterol and stigmasterol (Fig 1,2). We used most sensitive methods like gas chromatography with mass spectrometry GC-MS to detection lipophilic substance in this fern. Octadecanoic acid, hexadecanoic acid, tocopherol, cholesterol, sitosterol and stigmasterol were detected.

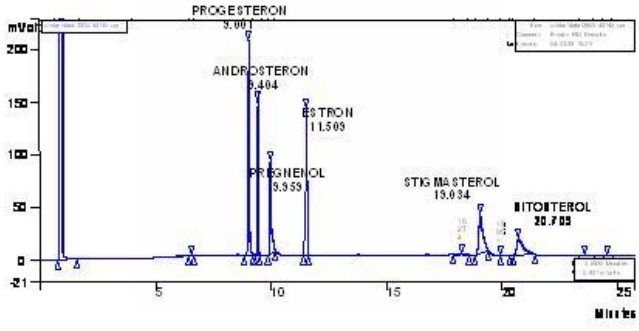

Fig.1.Retention time of standards

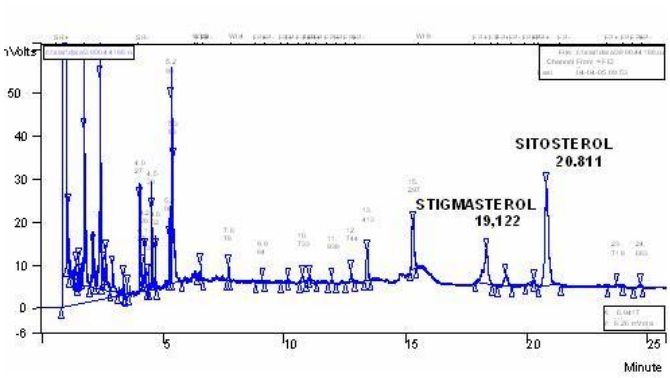

Fig.2.Retention time of Asplenium cuneifolium compounds.

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17:00	Poster	50

H-Dmt-D-Lys-Phe-Phe-OH, a tetrapeptide metabolite of the opioid-neurotensin hybrid peptide PK20, expresses high antinociception

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The clinical treatment of various types of pain relies upon opioid analgesic, however most of them produce, in addition to the analgesic effect, several side effects such as development of dependence and addiction as well as sedation, dysphoria, and constipation. One of the solutions to these problems are chimeric compounds in which opioid pharmacophore is hybridized with other type of synergically active antinociceptor. Neurotensin-induced antinociception is not mediated through the opioid system. Therefore, hybridizing neurotensin with opioid element results in a potent synergic antinociceptor.

Indeed, previously synthesized and examined an opioid-neurotensin hybrid peptide PK20 is a highly potent analgesic. Our *in vivo* studies have shown that PK20 treatment results in long-standing time-dependent antinociception while administered centrally as well as peripherally. This novel opioid-neurotensin hybrid peptide has a significantly intensified analgesic effect, when compared to morphine. The metabolic studies of PK20 indicated formation of quite stable N-terminal tetrapeptide. To evaluate its pharmacological profile the compound (metabolite) has been de novo synthesized and exposed to both *in vitro* and *in vivo* studies, which indicated that this compound expresses a high analgesic activity.

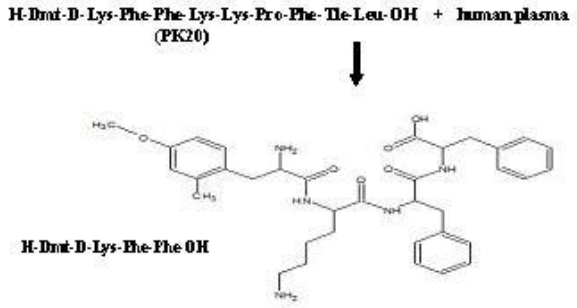

Fig. Degradation of PK20 in human plasma

Acknowledgement: This study has been supported by European 6th SPTRIP Grant

Normolife (LSHC-CT-2006-037733)

17:00	Poster	52

Synthesis and Antimicrobial Activity of P-Triazinylphosphonium Salts

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Antibiotics are one of our most important weapons in fighting bacterial infections and have greatly benefited the health-related quality of human life since their introduction. However, many commonly used antibiotics have become less and less effective against certain illnesses due to emergence of drug-resistant bacteria. To prevent resistance it is essential to continue search for the new agents, expecting minor resistance of pathogens against them.

Long chain quaternary ammonium compounds exert antibacterial activity against both Gram-positive and Gram-negative bacteria, as well as against other pathogenic species of fungi and protozoa [1]. Quaternary ammonium salts belong to group of hard antibacterial agents [2] that are biologically active and non-metabolizable in vivo (soft drugs [3] are defined as drugs, which are characterized by predictable and controllable in vivo destruction to form non-toxic products after they have achieved their therapeutic role). Thus, although the soft analogs have been shown to possess antibacterial activity *in vitro*, it is likely that their *in vivo* activity will be hampered by their chemical instability.

It has been found that quaternary phosphonium salts also showed hard antibacterial activity [4] but scarce motivation to research in this area was caused by difficulties in synthesis of phosphonium salts and limited availability of trialkylphosphines used as starting materials.

In this communication we present results of synthesis and preliminary studies on biological activity of new phosphonium salts obtained in reaction between Bu_3P and PPh_3 and 2-chloro-4,6-disubstituded-1,3,5-triazines.

Acknowledgements: The study was supported by the Ministry of

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Synthesis of fluorinated 2-arylo-3-aroilbenzo[b]furanes

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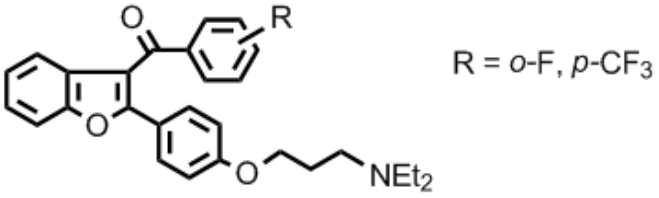
Benzofuran derivatives are actively investigated in the therapy of the Alzheimer disease[1]. We have prepared two new fluorinated benzofuran derivatives[2].



The syntheses started with the preparation and reduction of 2-(4-nitrophenyl)benzofuran to the corresponding amine. The amine was transformed into hydroxy group via diazonium salt. Etherification, followed by the Friedel-Crafts acylation with 4-trifluoromethylbenzoyl chloride and 2-fluorobenzoyl chloride, completed the syntheses. The fluorinated products are potential inhibitors of β-amyloid aggregation, and might be tested in the Alzheimer disease therapy.

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Differences in concentrations of tramadol and its major metabolites after per os sustained release tablet or per rectum suppository application.

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The aim of the present study is to evaluate the pharmacokinetic of T and its major metabolites M1, M2 and M5 after single oral administration of a SR tablet and rectal suppositories in dogs (4-6 $mg \cdot kg^{-1}$ m.c.). The plasma concentration data after SR-tablet and rectal administration were fitted on the basis of mono- and non- compartmental model, respectively. T plasma concentration after SR tablet administration, was quantitatively detected in three dogs, M1 was quantized only in one dog while M2 and M5 were quantized in all the dogs. T showed median values of C_{max}, T_{max} and $T_{1/2}$ of 0.04 (0.17-0.02) $mg \cdot mL^{-1}$, 3 (4-2) and 1.88 (2.21-1.44) hours, respectively. M5 showed median values of C_{max}, T_{max} and $T_{1/2}$ of 0.1 (0.19-0.09) $mg \cdot mL^{-1}$, 2 (3-1) and 4.23 (6.58-1.85) hours, respectively. M2 showed median values of C_{max}, T_{max} and $T_{1/2}$ of 0.22 (0.33-0.08) $mg \cdot mL^{-1}$, 4 (7-3) and 4.49 (6.39-1.57) hours, respectively. Following rectal administration, T was detected from 5 minutes up to 10 h in smaller amount than M5 and M2. T median value of C_{max} was 0.14 ± 0.06 $\mu g \cdot mL^{-1}$ in 0.56 ± 0.41 h (T_{max}). K_{01} $t_{1/2}$ and K_{10} $t_{1/2}$ were 0.27±0.25 h and 2.24±1.82h, respectively. T/M5 and T/M2 ratios were 0.48-0.09 and 0.9-0.13, respectively. M1 was detectable from 5 min up to 2 h showing low values (0.028-0.007 $mg \cdot mL^{-1}$). The present findings suggest oral SR tablet and suppository rectal formulation has similar pharmacokinetic behavior could not has suitable pharmacokinetic characteristics to be administered once-a-day as an effective and safe treatment for pain in the dog.

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In the quest for a new endothelium-protecting drug among escin derivatives

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Herbal remedies, such as horsechestnut (*Aesculus hippocastanum*), have been used as medical treatments since the beginning of civilization. Its active ingredient, escin, despite the broad use, as the remedy for a chronic venous insufficiency, it has not undergone careful scientific assessment. This is probably due to the fact that escin is a mixture of triterpene saponins and the analysis of cellular responses

following its administration may vary depending on the preparation. It is well established that endothelial cell activation caused by exposure to the hypoxic conditions, such as occur during blood stasis in chronic venous insufficiency patients, triggers inflammatory response in the vein. In the search for well-defined and effective venotropic drug with minimum side effects, we will employ several *in vitro* techniques assessing endothelial cell function. These will include analysis of endothelial cell activation, via assessment of various signaling pathways, gene activation and protein expression. We assume that such *in vitro* tests may provide sufficient data to choose best drug candidates to perform *in vivo* assay using murine model of chronic venous insufficiency. Histological techniques will be then used to evaluate the efficacy of newly synthesized escin derivatives as endothelial cell protective agents. The final stage of preclinical studies will focus on pharmacological properties and toxicological characteristics of tested compounds.

17:00 Poster 60

Tiamulin hydrogen fumarate - veterinary uses and HPLC method of determination in premixes and medicated feeds

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In agreement with EU and Polish legislation, both production process of medicated feedstuffs as well as its usage has to be strictly monitored and controlled. The main aims of the process control are determination of active ingredient concentration, homogeneity of the product and accordance with GMP. Main group of actives consist of antimicrobials such as tetracyclines, tylosin, amoxycyline, tiamulin and sulphonamides.

Tiamulin has higher activity than natural pleuromutilin first extracted in 1951 from *Pleurotus mutilus (Fr.) Sacc.* and *Pleurotus Passeckerianus Pil.* It is active against Mycoplasma spp., gram positive bacteria such as streptococci and staphylococci and obligate anaerobes. It is mainly used in treatment of swine (dysentery caused by *Brachyspira hyodeysentreiae,* bacterial pneumonia caused by *Pasteurella multicida*) and poultry.

Tiamulin is a semi-synthetic member of pleuromutilin class of antibiotics. Core of the molecule constitute of three cyclic hydrocarbons with 8 asymmetric carbons. Tiamulin base is lipophilic but in premixes it is mainly used as hydrogen fumarate salt freely soluble in water.

Chemical structure of tiamulin, lack of fluorescence, low UV activity and complexed matrix causes problems in quantitative analysis. Published methods for determination of tiamulin in various materials use thin layer or liquid chromatography as well as gas chromatography, well established is also microbiological assay. The aim of this study was to optimize extraction procedure and HPLC determination method of tiamulin hydrogen fumarate in medicated feedstuffs and premixes available on polish market.

Solid-liquid water extraction of premix samples was used in the procedure. Single or duplicate extraction and different sample size (0.25 – 2.5g) were tested giving recovery over 90%. It was decided to use duplicate extraction of 0.25g sample for further procedures.

1% sodium carbonate solution followed by organic solvent (hexane : ethyl acetate) extraction was employed to free tiamulin from feed matrix. Next step of the procedure was to bring a portion of extract to dryness and dissolve residue in 0.1% tartaric acid solution. Small sample size of 2.0g were tested giving CV% below 6% between 3 series of experiments.

All extracts were analysed on Agilent 1200 series system equipped with Gemini NX, 4.6x150mm column and UV-Vis detection at 208nm. Methanol : acetonitrile : ammonium carbonate (50:25:25) mixture at flow rate of 1.0mL/min was used as mobile phase. Linearity, specificity, precision and recovery level were tested results indicating applicability of the optimized method for tiamulin determination in premixes and medicated feedstuffs.

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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 thioxanthenes, phenothiazines, 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, obtained during a routine state control of pharmaceutical products from the Polish market performed by 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: Nolvadox 20 mg tabl. (tamoxifen), Atorvox 20 mg tabl. (atorvastatine), Rilutec 50 mg tabl. (riluzole), Alpha Vibolex 600 mg caps. (alpha lipoic acid), Deprim forte 425 mg caps. (*Hyperici herbae extr. siccum*), Zokardis 30 mg tabl. (zofenopiril), Fevarin 50 mg tabl. (fluvoxamine), Pernazinum 100 mg tabl. (perazine), Rebetol 200 mg caps. (ribavirine). *S. aureus* was susceptible to over 70% of the drugs listed above. Fluvoxamine and perazine inhibited growth of *S. aureus* in concentration 2 mg/ml and *E. coli* in concentration 2 mg/ml and 4 mg/ml, respectively. *C. albicans* was susceptible to over 60% of the drugs listed above. *C. albicans* showed the strongest susceptibility to antiepileptic substance riluzole (MIC-3 mg/ml). Interestingly, natural product Deprim forte (dry extract from

Hyperici herbae) in concentration 33 mg/ml inhibited growth of *S. aureus*. *P. aeruginosa* was susceptible to fluvoxamine and ribavirine (MIC-10 mg/ml). The antimicrobial activity of all drugs emphasises a necessity of neutralisation of their activity during microbial purity assays of pharmaceutical products.

17:00 Poster 64

Anti-oxidativeproperties of prenylflavonoids isolated from hops (Humulus lupulus L.)

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Hop (*Humuluslupulus L.*) is a genus of flowering plants. The medicinal use of extracts prepared from the parts of the *Cannabaceae* genus dates back to ancient times. The female flowers, commonly called hops, are used as flavouring agents and stabilisers during beer brewing.

At the base of the hop scales there are two hard nuts covered in aromatic, yellow dust called lupulin. Lupulin contains from 5 to 30% bitter substances including acylphloro-glucides, humulones [1], lupulones; essential oil containing mono- and sesquiterpenes, aroma substances, flavonoids, xanthohumol and other chalcones [2,3]. Isolated prenylflavonoids show interesting biological activities witch may be used as cancer chemopreventive agents, anti-oxidative or as antiviral agents [3].

The purpose of this review is to show an overview of the anti-oxidative activities of prenylflavonoids from hops.

Prenylflavonoids were isolated from supercritical carbon dioxide extracted hops. The particular residues were extracted by solvent extraction. The fractions containing xanthohumol and isoxanthohumol were collected, evaporated in vacuo and purified by repeated column chromatography on silica gel. The structures were confirmed by spectroscopic methods: UV-VIS, IR, ^{1}H NMR.

Anti-oxidative property of xanthohumol and isoxanthohumol from hops were compared with other natural antioxidants extracted from the evening primrose (*Oenothera paradoxa* L.) and black chokeberry (*Aronia melanocarpa*).

The induction time and oxidative stability of prenylflavonoids from hops and natural extracts were determined by rancimat test. With the results presented in this study, we concluded that isoxanthohumol and xanthohumol had the highest oxidative stability. Both of those compounds indicate that they are two times stronger in antioxidative activity than the natural extracts from evening primrose and black chokeberry.

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17:00 Poster 66

Identification of Novel 5-HT$_7$R Ligands via Multistep Virtual Screening of Commercially Available Compounds Databases

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In order to find potential new structures of 5-HT$_7$R ligands we used previously developed and tested hierarchical multistage strategy of virtual screening [1]. This workflow was based on two-dimensional (2D) pharmacophore similarity searching, physicochemical scalar descriptors, ADME/Tox filter, three-dimensional (3D) pharmacophore searches and docking protocol. Additionally, in order to increase chemotype's diversity of virtual hits, the chemical topology and pharmacophore topology fingerprints have been applied at the stage of similarity search. The six chemical classes of 5-HT$_7$R antagonists [2] were used as a query structures in double-path virtual screening scheme. The commercially available resources, offered by the ChemBridge [3] and ChemDiv [4] companies, have been adopted and used as a molecular screening space consisting of approximately 1 300 000 compounds. Finally, the best virtual hits were selected and acquired in order to determine their affinity for 5-HT$_7$ receptor. The binding mode of selected virtual hits are shown in comparison to those of known antagonists [2].

This study was partly supported by a grant PNRF-103-AI-1/07 from Norway through the Norwegian Financial Mechanism.

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17:00 Poster 68

Spinal cord peptide epitopes ameliorate immunological reaction in experimental allergic encephalomyelitis /eae/

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Recent studies of gut-blood barrier have showed that short peptides of food proteins are transported to the blood easier than aminoacids. Special system presenting antigens in gut is formed. This process presenting food peptides and suppressing of immunological respons to them is called oral tolerance. Recently, it has been proposed to apply it to treatment of autoimmune diseases as multiple sclerosis, rheumatoid arthritis, uveitis, diabetes type 2.

The aim of our study was to use hydrolizate of spinal cord proteins which is the mixture of neuropeptides obtained after hydrolysis of an undenatured homogenate of proteins digested with pepsin as an antigen for feeding the experimental animals/rats/. After induction of tolerance animals were immunised by injection of guine pig spinal cord homogenate with Freund's adjuvant to evoke experimental allergic encephalomyelitis/EAE/, which is an animal model of sclerosis multiplex. Clinical course have been observed, histopathological study, ultramicroscopic study and metaloproteases determination in blood were done. Study of limphocyte proliferation and level of cytokine Il-4 and Il-10, Inf-γ, and TGF-β were also performed.

Clinical course of EAE post hydrolyzate treatment has been milder than control. MBP and TNF in brains were decreased. Metaloproteases increased in EAE, after hydrolizate treatment were dimished by 30%. Some changes in blood-brain barrier/BBB/ as opened tight junction and other changes in early phase of EAE as karioskeletal damage with vesicular structures in karioplasm, compartmentalisation of the endoplasmic reticulum in perikarium, large cisterns of the Golgi apparatus, increased activity of microglial cells with numbers of phagolisosomes, desorganisation of sheets of myelin, neoangiogenesis of parenchyma of the cerebral cortex has been dimished. Mechanism of this effect is probably through active suppression involving diminishing lymphocytes production of interferon gamma and interleukine-10 as well as increasing production of TGF-α and interleukin-4. Above results indicate on possible clinical implication of oral tolerance in treatment of multiple sclerosis. Results strongly indicate that mixture of neuropeptides in spinal cord hydrolisate given orally diminished immunological response to myelin antigens.

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Assessment of analgesic potency of biphalin in a mouse model of cancer pain

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Malignant, long-lasting pain is an imminent component in advanced cancer. Such pain is still not successfully managed since oftentimes it shows a complex nociceptive-neuropathic mechanism. Morphine is still a major drug of choice in terminal cancer treatment. However, there is much doubt around the use of such therapy because of common undesirable side effects, which may hinder the quality of life to a great extent. Hence, the pressing need for development of novel opioid analgesics demonstrating high analgesic and antitumor potency and simultaneously lack the shortcomings typical for morphine. Particularly, novel neuropeptide analogues offer numerous opportunities for the development of new analgesics and show promise in alleviation of cancer-evoked pain. In a murine skin cancer pain model developed by an intraplantar inoculation of B16T0 melanoma cells signs of thermal hyperalgesia were attenuated by repeated daily injections of biphalin a dimeric enkephalin analog. The antinociceptive effect of biphalin was greater in the tumor bearing paw than in the contralaeral paw. Apart from showing an antinociceptive effect in the periphery, biphalin also exerted a central antinociceptive effect as measured in the tail-flick test. Thus, biphalin, may become a useful drug in cancer pain treatment because it also shows low tolerance liability and relatively high antinociceptive potency.

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17:00 Poster 72

Synthesis and antiproliferative activity in vitro of pyridodiazepine derivatives.

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A novel series of esters and hydrazones was synthesized from 1-phenyl-2-(4-aryl-1,3,4,5-tetrahydropyrido[2,3-b][1,4]diazepin-2-ylidene) ethanones. Subsequent treatment of 1-phenyl-2-(4-phenyl- 1,3,4,5-tetrahydropyrido[2,3-b][1,4]diazepin-2-ylidene) -ethanone, with p-chlorobenzaldehyde furnished azine p-chlorobenzylidenohydrazone 1-phenyl-2-(4-phenyl-1,3,4,5- tetrahydropyrido[2,3-b][1,4]diazepin-2-ylidene)ethanone. Long- standing heating of 4–(p-chlorophenylene)-2-(2-oxo-2-phenylethylidene)-1,2,3,4-tetrahydropyrido[2,3-b][1.4]diazepine-5-carboxylic acid ethyl ester with hydrazine hydrate afforded 3-[1-(p-chlorophenylene)-2-(5-phenyl- 1H-pyrazol-3-yl)-ethyl]-1,3- dihydroimida-zo[4,5-]-pyridin-2-one , the product of thermoisomerisation of hydrazide.

The structures of the compounds were identified by the results of elemental analysis and their IR, 1H NMR and MS spectra. Additionally, the structure of 3-[1-(p-chlorophenylene)-2-(5-phenyl-1H-pyrazol-3-yl)-ethyl]-1,3-dihydroimidazo[4,5-b]pyridin-2-one was confirmed by X-ray diffraction method.

The selected compounds were examined for their antiproliferative activity in in vitro screening assay. The following human cancer lines were used: HL-60 (leukemia), HCV-29T (urinary bladder), SW 707 (rectal), HepG2 (liver) and MES-SA (uterine).

One among tested compounds, 3-[1-(p-chlorophenylene)-2-(5-phenyl-1H-pyrazol-3-yl)-ethyl]-1,3-dihydroimidazo[4,5-b] pyridin-2-one exhibited significant antiproliferative activity against the cells of all cell lines applied. It seems that the activity of compound can be attributed to the presence of pyridine, imidazole and pyrazole rings in the molecule. 4-(p-Chlorophenylene)-2-(oxo-2-phenylethylidene)-1,2,3,4-tetrahydropyrido[2,3-b][1,4]- diazepine-5-carboxylic acid propyl ester and 4-(p-chlorophenylene)-2-(oxo-2-phenylethy-lidene)-1,2,3,4-tetrahydropyrido[2, 3-b][1,4]diazepine-5-carboxylic acid isobutyl ester were active only against two cancer cell lines, namely HL-60 leukemia and HCV-29T urinary bladder cancer. 4–(p-Chlorophenylene)-2-(2-oxo- 2-phenylethylidene)-1,2,3,4-tetrahydro-pyrido[2,3-b][1.4]diazepine-5-carboxylic acid ethyl ester revealed activity exclusively against HL-60 leukemia cells.

17:00 Poster 74

Antiprolfelative and cytotoxic effect of 5-fluorouracil and sulforaphane in Chinese hamster lung fibroblast cell line

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The 5-fluorouracil is the anti-cancer drug widely used for the treatment of colorectal, breast, head and neck, prostate, pancreas cancer. It causes a S phase block in cell cycle by inhibition of thymidylate synthase (TS). As many anticancer agents it's responsible for the production of reactive oxygen species in cancer cells, which induces apoptosis.

Sulforaphane is a naturally derived compound and is considered as a chemopreventive agent. It activates phase II enzymes in many types of cells and is known as indirect antioxidant. At higher doses it exhibits cytostatic and cytotoxic activity by influence on cell cycle and induction of apoptosis. Sulforaphane is widely used as dietary supplement, moreover it is widely present in diet since its main source are Brassica vegetable like broccoli and cabbage. Several studies have shown that sulforaphane can act synergistically with anticancer drugs in cancer cells. The combination treatment is more effective than either agent alone. The combination chemotheraphy can reduce doses of antitumor medicine and its toxicity. Only a few publications showed interactions between similar compounds in the normal cells. In this cases, antagonism is beneficial interaction and leads to a protective effect to normal cells.

The aim of our study was to evaluate if sulforaphane can attenuate toxic effect of cytostatic drug 5-fluorouracil in normal cells. We investigated: antipoliferative, cytotoxic effect of sulforaphane and 5-fluorouracil in normal cell line-Chinese hamster lung fibroblasts using the MTT assay,. The cell viability was studied using staining with FDA/PI and examined with confocal microscope. There were used the two schemes of administration: co-administration of 5-fluorouracil with sulforaphane and pre-treatment with sulforaphane with the subsequent treatment with 5-fluorouracil. The cells were also incubated with substances alone at the concentrations corresponding to the concentrations that were used in a combination.

It was observed that at low range of concentrations 5-fluorouracil is more cytotoxic than mixture or sulforaphane alone. This effect is stronger for the sequence administration than for co-administration. The FDA/PI staining showed that treatment did not caused death of cells since most of them were indicated as living cells.

17:00 Poster 76

Research into transformations of 5-amino-3-methyl-4-isoxazolecarboxylic acid hydrazide under orthoesters influence

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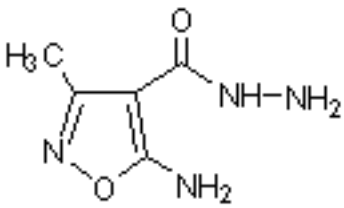
For many years in Chair and Department of Organic Chemistry many derivatives of isoxazole have been synthesized. A number of those compounds have a confirmed immunological effect. The starting material for their synthesis is 5-amino-3-methyl-4-isoxazolecarboxylic acid hydrazide (**1**). Some of its derivatives demonstrated immunosuppressive[1] and immunostimulant[2] activity. To create new 5-amino-3-methyl-4-isoxazolecarboxylic acid hydrazide derivatives, reactions with orthoesters were performed. A purpose of this presentation is to show the results of the researching into chemical transformations of respected hydrazide in reactions with orthoesters.



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17:00 Poster 78

The development of the analytical methods for studying capsules containing Temozolomide active substance

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The aim of this work was to develop analytical methods used for studying Temozolomide, an anti-cancer drug from imidazotetrazine group. Temodal 5 mg, 20 mg capsules manufactured SP Labo N.V. Belgia were used as the reference products.

An RP-HPLC method was developed for the study of assay, dosage uniformity & quantity of the active substance released from the drug form, as well as the purity of the preparation.This method was compared with the spectrophotometric method.

During the purity study, it was observed that the solutions containing Temozolomide were unstable at room temperature. The Temozolomide degradation product was identified as 5(4)-aminoimidazole-4(5)-carboxyamide (AIC).
The described method succesfully separated the observed impurity from the active substance. The metod was used for the routine analysis of Temozolomide capsules.

17:00 Poster 80

Tripeptides with C-terminal arginine as potential inhibitors of urokinase

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The urokinase plasminogen activator system consists of the serine protease urokinase (uPA), its cell surface-associated receptor (uPAR), plasminogen activator inhibitors (PAIs) and the proenzyme plasminogen (Plg). uPA is responsible for the Plg activation to plasmin (Plm) by the Arg561-Val562 bond hydrolysis in Plg.

Plm, the key enzyme of fibrinolysis, is a non-specific trypsin-like protease, which cleaves after numerous Lys or Arg bonds. It attacks fibronectin, fibrin/fibrinogen, clotting factors V/Va and VIII/VIIIa, latent TGF-β, IGF binding proteins and the zymogen forms of several metalloproteases. In contrast, uPA is a highly specific serine protease, which catalyses cleaving single Arg-X or Lys-X bonds in for example the hepatocyte growth factor, fibronectin, diphtheria toxin, uPAR and uPA itself. The two-chain active form of uPA is activated from a single chain precursor (prouPA) by plasmin or possibly via enzymes commonly enriched in cancer cells such as thiol cathepsins. uPA is unique in having its own high affinity cell-surface receptor uPAR. The urokinase receptor is focalized in the cell-cell connection and on the edge of invading cells. Thus, the uPA system plays a pivotal role in degradating and regenerating of the basement membrane which leads directly to tissue remodelling, invasiveness and angiogenesis. The binding of uPA to uPAR also initiates signalling cascades that does not require the uPA catalytic activity but only receptor occupancy. The expression of uPA and uPAR has been demonstrated in essentially every cancer type, such as gastric, colorectal, ovarian, breast, endometrial and prostate cancer.

We present the synthesis and the investigation of effect peptides of general formula H-D-Ser-AA-Arg-OH (AA = leucine, norleucine, izoleucine, valine, norvaline, α-metyloalanine, α-aminobutanoic acid, homoleucine, tert-leucine, neoglycine) on amidolytic activity of urokinase, thrombin, plasmin, trypsin, t-PA and kallikrein. We expected that the use of specific tripeptide sequence to urokinase would cause high urokinase selectivity [1, 2]. The peptides were synthesized on the solid phase manually using standard Fmoc-based strategy.

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17:00 Poster 82

Interaction fingerprints patterns. Binding mode analysis of mGlu2 receptor model based on docking studies

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One of the most troublesome stages of Computer Aided Drug Design (CADD) process is analyzing huge amount of data provided by docking studies. Simple scoring functions alone can provide only shallow information about ligand-receptor interactions, since they do not distinguish neither residues nor single atoms. Very often a visual inspection is the only way to determine binding mode. In this study we would like to introduce an implementation of interaction profiles(1)based on Structural Interaction Fingerprints (SIFt)[(2)]to analyze known ligands docking poses within mGluR2 model. The use of interaction patterns allows precise and rapid binding site description.

The mGluR family consists of eight proteins divided into three groups corresponding to sequence similarities, pharmacology and physiological role. These groups are: I (mGluR1, -5), II (mGluR2, -3) and III (mGluR4, -6, -7, -8). Group II lies in field of our interest due to its potential as therapeutic target for antidepressant and anxiolytic drugs. Research was performed on population of 100

mGluR2 models created on Rhodopsin crystal structure template. Building that many virtual receptors provided us with semi conformational search on residues assembling incriminated receptor. Library of 179 known allosteric modulators of group II mGluR was used for docking studies and thus forging the binding mode.

Acknowledgments

The study was partly supported by a grant PNRF-103-AI-1/07 from Norway through the Norwegian Financial Mechanism, and by the Polish Ministry of Science and Higher Education (MNiSW), Grant No. N N405 184635.

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17:00 Poster 84

Synthesis and anticonvulsant activity of new N-[4-(arylpiperazin 1-yl)]- 1,3-dioxo-1,3-dihydro-2H- isoindole- and 1,3-dioxooctahydro-2H-isoindole-2-carboxamides

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Numerous compounds are synthesized and screened for their anticonvulsant activity each year. To make the discovery of new anticonvulsants more rational many investigators identified structural fragments essential for anticonvulsant properties. One of the structural features that play a significant role in relation to antiepileptic activity is an amide function.[1,2] In the course of developing new anticonvulsant agents as well as taking into consideration the above and vital influence of 4-arylpiperazine moieties on anticonvulsant activity of pyrrolidine-2,5-diones differently substituted at 3-positon of succinimide ring,[3,4] in the present studies we have synthesized a library of 1,3-dioxo-1,3-dihydro-2*H*-isoindole- and 1,3-dioxooctahydro-2*H*-isoindole-2-carboxamides with the 4-arylpiperazine derivatives as an amide function. The structures of compounds obtained are shown on **Figure 1**.

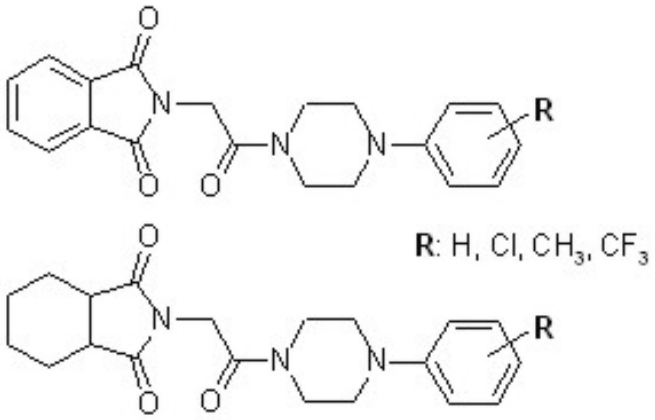

Figure 1.

The desired compounds were prepared by condensation of (1,3-dioxo-1,3-dihydro-2*H*-isoindol-2-yl)- or (1,3-dioxooctahydro-2*H*-isoindol-2-yl)- acetic acids with the appropriately substituted 4-arylpiperazines, in the presence of the *N,N*-carbonyldiimidazole (CDI) reagent. 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).[5]

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17:00 Poster 86

Enzyme-catalyzed synthesis of (R)-2-(1-methyl-2-pyrrolidine)ethanol - clemastine substrate

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Clemastine is an effective antihistamine drug having sedative and anticholinergic effects. The main substrate for manufacturing of clemastine is (*R*)-2-(1-mehyl-2-pyrrolidine)ethanol, which can be also an important chiral building block for the synthesis of biologically active compounds. Starting from the racemic 2-(1-methyl-2-pyrrolidine)ethanol as a substrate, an enzymatic procedure was developed for the efficient synthesis of corresponding highly enantiomerically enriched (*R*) and (*S*)-2-(1-methyl-2-pyrrolidine)ethanol. Various commercially available immobilized and not immobilized lipases were examined and several acyl donors, as a acylating agents in enzymatic kinetic resolution were applied. Some parameters of enzymatic reactions, as temperature, solvent, time and substrates ratio were optimized. Spectroanalysis data, enantiomer excess, specyfic rotation and other physical parameters of obtained compounds were determined.

17:00 Poster 88

Effect of cyclophosphamide or 5-fluorouracil-based therapy supported by cellular vaccines on induction of immunity against transplantable MC38 colon carcinoma

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Despite many improvement efforts, effects of chemotherapy are still unsatisfactory. The combination treatment with the use of cytostatics followed by immunotherapy may augment their antitumor effect. Therapeutic efficiency of two cytostatics cyclophosphamide (CY) and 5-fluorouracil (5-FU) combined with cellular vaccines were compared in mice bearing s.c. growing MC38 colon carcinoma. Vaccines have consisted of bone morrow derived dendritic cells stimulated with tumor lysate (BM-DC/TAg) and IL-2-transduced MC38(MC38/IL-2) cells which produced aproximally 100 LU cytokine/ml/5x10^{6}cells/48h. Cytostatics (150 mg/kg body weight) were administered i.p. on the 14th day after tumor inoculation. Applications of cellular vaccines were given in two consecutive weeks, starting 3 days after drug administration. Administration of CY caused statistically significant higher tumor growth delay compared with 5-FU. Combination of CY with BM-DC/TAg and/or MC38/IL-2 moderately enhanced the effect of the cytostatic activity and percentage of CD8$^+$ cells accompanied by augmented IFN-gamma production by restimulated in vitro splenocytes. No considerable changes in percentage of CD49b$^+$ cells after application of CY and cellular vaccines were observed. On the other hand, the administration of 5FU caused slight increase in percentage of CD4$^+$ among restimulated splenocytes. Besides, 5FU +/- cellular vaccines influenced on augmentation of Treg cell (CD4$^+$CD25$^+$Foxp3$^+$) number in freshly isolated splenocyte population. Our findings suggest that repeated peritumoral application of BM-DC/TAg together with IL-2-producing tumor cells can inhibit tumor growth also by engage of cell influx into tumor tissue. Although cellular vaccine moderately affect tumor growth reduction, they can act as activators able to accelerate effect of anti-tumor response and could be useful as an adjuvant vaccine in combined chemo-immunotherapy of experimental murine tumors.

This work was supported by grant of the Polish Ministry of Science and Higher Education No N N401 235334.

17:00 Poster 90

Glycoconjugates, products of uridine derivatives phosphitylation and oxidation as glycosyltransferases potential inhibitors

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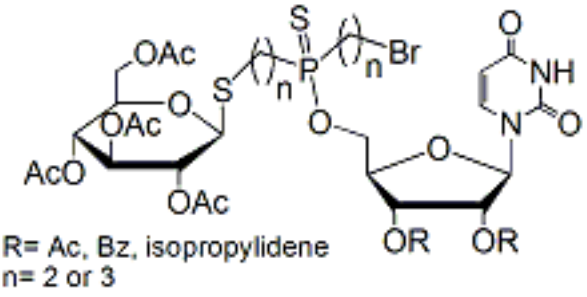
Oligosaccharides play a fundamental role in mammalian cell in many important biological processes for example immune recognition, cell-cell communication and initiation of microbial pathogenesis and are commonly found as glycoconjugates (glycoproteins or glycolipids) [1]. Glycosyltransferases belong to the family of enzymes that create glycosidic bonds in nature. With regard for importance glycoconjugates in function of mammalian cell, control of activity of glycosyltransferases is interesting and evolving course of action.

Previously we proposed protected 5'-uridine derivatives connected with (5-amino-2-pyridyl) 1-thio-β-D-glycosides with a succinic linker as analogues of GTs natural substrate with potential inhibition activity [2]. Some of them were tested for ability to inhibition the propagation of classical swine fever virus (CSFV). Reduction of the number CSFV-infected cells was observed without significant toxicity for mammalian cells. Encouraged these positive results we made new scale of compounds, in which variously protected 5'-uridine derivatives connected with 1-thiosugars with thiophosphoesters fragment.



First stage was phosphitylation – reaction 5'-hydroxyl group of selective protected nucleoside with a phosphitylating agent (N,N-diisopropyl chlorophosphoamidite). We coupled an anomeric phosphoramidites with 2-bromoethanol or 3-bromopropanol and then we oxidized these intermediates with sulphur presence. In next step we used these products to reaction of condensation with 1-thiosugars and received glycoconjugates with potential inhibition activity, which structure was mimic to structure of natural glycosyltrnasferases substrates.

Acknowledgement

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17:00 Poster 92

Development of RP HPLC method for the analysis of calcipotriol in cream and ointment

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Calcipotriol (1S, 3R, 5Z, 7E, 22E, 24S)-24-Cyclopropyl-9, 10 secochola-5,7,10 (19), 22-tetraene-1,3,24-triol is a non-steroidal antipsoriatic agent derived from vitamin D. An accurate and precise high performance liquid chromatography assay has been established for determination of calcipotriol, impurities and degradation products in ointment and cream. Drug products were determined on a C_{18} reversed-phase column with UV detection. The extracts of calcipotriol were prepared by twofold extraction with anhydrous ethanol. The method was statistically validated for linearity, accuracy, precision and selectivity. The linearity for assay of calcipotriol was confirmed in the range 5.1 µg/ml to 15.3 µg/ml. The linearity for the identified impurities was established in the range 0.05 µg/ml to 0.15 µg/ml for impurity A and B, and 0.03 µg/ml to 0.08 µg/ml for impurity D. The mean extraction recovery for the active substance and known impurities was 99.0%. The stability of calcipotriol during processing (autosampler) and in the extract was checked. The method was used for the routine analysis of calcipotriol in cream and ointment.

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17:00 Poster 94

3D-QSAR modeling of binding affinities and functional activities for the set of β_2 adrenergic receptor agonists.

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β_2 adrenergic receptor system is one of the best characterized among G-protein coupled receptors. Our group carries a long term project aimed at development of new selective agonists of the receptor based on the scaffold of fenoterol molecule. Previous results showed that binding affinities strongly depend on the stereochemistry of the molecule. Generally the (R,R)-stereoconfiguration of a derivative is the most effective in binding. In the present study the Comparative Molecular Field Analysis was applied to develop models of binding affinity data generated using two different assays: 1) with [^{3}H]CGP-12177 and 2) with [^{3}H]4-methoxyfenoterol as a marker in radioligand displacement measurements. Two further models were developed for 3) AC_{50} values determined for tested compounds in agonist induced cAMP accumulation data and 4) IC_{50} values determined in agonist induced mitogenesis inhibition of astrocytoma 1321N1 cells.

Although these different sets of data are related to one phenomenon (the interactions of an agonist with β_2 adrenergic receptor) the are not well correlated with each other. Different molecules appear to be the most effective in different assays. This is consistent with the current understanding of activation mechanisms in GPCRs according to which different agonists may induce different receptor activation state and, thus, different downstream signaling pathways in the cell. These diverse modes of interaction within the receptor binding site were characterized using CoMFA approach and significant differences in generated molecular fields were observed depending on the data modeled. The results may be useful in further steps of designing of new derivatives targeted at particular process/therapy.

17:00 Poster 96

Catalytic synthesis and antiproliferative activity in vitro of polisubstituted isoxazolopyridine derivatives.

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The derivatives of isoxazolopyridine have interesting biological properties. These compounds showed bacteriostatic, analgesic, antiinflammatory, cardiotonic and hypotensive, myolytic, antilipidemic and anxiolytic activity. Two of these derivatives were investigated in clinical studies: THIP and THOPO as agonist GABA-receptors are used as anxiolytic agents. Herein, The synthesis of a new series isoxazolo[4,5-b]pyridines is described. 4-Amino-5-benzoyl-3-carbamoylisoxazol was subjected to reactions with selected active methylene compounds in the polar solvent or in the presence of $ZnCl_2$ or $In(OTf)_3$ (under solvent-free conditions), leading to the production of the diverse substituted isoxazolo[4,5-b]pyridines in good yields. The chosen compounds were examined for antiproliferative activity *in vitro* against 8 various human or mouse cancer lines, using SRB technique. Two of them: revealed cytotoxic activity against the cells of all human tumor cell lines applied. Moreover, their ID_{50} values are in the range of the international activity criterion for synthetic agents (4 mg/ml).

17:00	Poster	98

Comparison of antiproliferative activity of the natural isothiocyanates and their mercapturic derivatives.

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A number of natural compounds with the antitumor activity have been found in our diet. Among them there is a large group of compounds known as isothiocyanates (ITC), the majority of which occur in plants, especially in Cruciferous vegetables like broccoli, cauliflower, cabbage and others. Highly reactive isothiocyanate (-NCS) group have an ability to simultaneously modulate multiple cellular targets involved in cancer development and its main targets are cysteine residues. The main metabolic pathway involved in isothiocyanates elimination from cells is the mercapturic acids pathway. The process starts from glutathione, an important redox state guardian, and leads to cysteine and N-acetylocysteine (NAC) conjugates. Interestingly, this metabolic products also exhibits antitumor activity.

We compared the antiproliferative activity of a group of natural isothiocyanates and their metabolites. *In vitro* studies on several lung and breast cancer cells lines were performed. We have showed that isothiocyanates can be strong antiproliferative agents with relatively low cytotoxicity towards normal cells. Moreover, our studies showed that the isothiocyanates metabolites not only posses the antiproliferative activity, but in many cases this activity is greater than activity of ITCs. Because of that, and because of some other reasons (discussed in the paper), we believe that isothiocyanates metabolites could by both anticancer drugs and element of chemopreventive diet.

17:00	Poster	100

The synthesis of ezetimibe with high stereochemical purity

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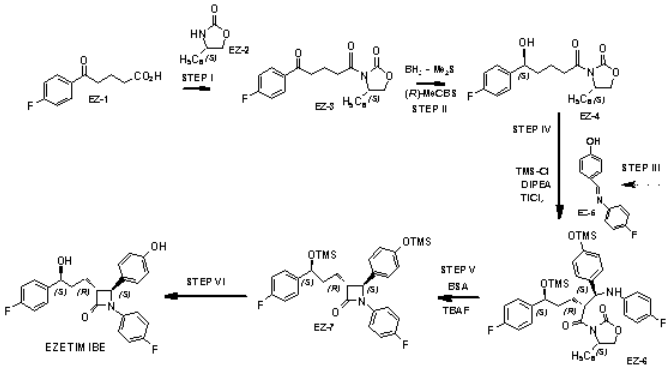
Ezetimibe, (3R,4S)-1-(4-fluorophenyl)-3-((3S)-3-(4-fluorophenyl)-3-hydroxypropyl)-4-(4-hydroxyphenyl)-2-azetidinone, is an antihyperlipidemic drug which is used to lower cholesterol level. It acts by decreasing cholesterol absorption in the intestine.

The three chiral centers in the ezetimibe molecule give rise to eight stereoisomers and the synthesis of stereochemical pure ezetimibe is a significant challenge. The synthesis of ezetymibe is described in many patents and patent applications, however the problem of stereochemical purity of the final product and its intermediates is almost completely omitted.

The synthesis of ezetimibe was realized by a procedure shown below, according to Schering Co. patents No US 6,207,822, EP 1137634:



We have investigated the sterochemical course of all steps of this process and found that for the preparation of optical pure ezetimibe the providing of pure (S,R,S,S) - EZ-6 is cru-cial. This diastereomer (product of anti-condensation of EZ-4 + EZ-5) is usually contaminated with (S,R,R,S) - EZ-6 isomer (syn-condensation), and also with (R,R,S,S) - EZ-6 isomer derived from small amount of (R,S)-alcohol EZ-4 which is usually occurring in required (S,S)-alcohol. The presence of (R,R,S,S) – EZ-6 diastereomer leads to (R,R,S) - "iso-ezetimibe" which is very difficult to remove from ezetimibe.

The synthesis of ezetimibe was optimized, all chemical and sterochemical impurities were isolated and/or synthesized and characterized by NMR, MS and HPLC techniques. The method for the purification of desired key intermediate (S,R,S,S)-6 was elaborated. These al-lowed us to develop the large scale efficient synthesis of pharmaceutical pure Ezetimibe (HPLC > 99,5 %, (R,R,S)-isomer < 0,1 %, single unknown impurity < 0,1 %, total impurities < 0,6 %).

17:00	Poster	102

Comparison of UPLC and HPLC using Flutamide as an example

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Cost and productivity of pharmaceutical quality-control laboratories is of utmost interest for most, if not all, of today's laboratory managers. In a pursuit of speed and excellence many of them moved into modern techniques, such as discussed here UPLC.

Comparison of HPLC and UPLC using Flutamide (API) as an example is presented. Analyses were performed simultaneously by means of HPLC (Waters Alliance 2695) and UPLC (Waters Acquity). Columns used in the study were Phenomenex Luna C18(2) and Waters Acquity BEH C18 respectively. HPLC method was scaled down to UPLC and then the parameters were assessed. No significant loss of method key indicators such as resolution between compounds (R_s) was observed. Moreover it was noticed that sensit-

ivity expressed by signal to noise ratio (s/n) was higher for UPLC. Still the biggest advantage of UPLC over HPLC as shown here is conservation of time and resources which are directly proportional to laboratory efficiency and profitability.

Presented results clearly show the way which quality control laboratories should choose if they are serious about their competitiveness on the market. In contemporary world where more and more laboratories are diving into UPLC/UHPLC. One cannot stay ignorant or will be left behind.

17:00 Poster 104

Synthesis, antimicrobial, and pharmacological properties of thiadiazole derivatives

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The thiadiazole derivatives are known to possess several biological activities.[1] Therefore, thiadiazoles have been synthesized in our laboratory for a long time and their anticonvulsant, antidepressant, analgesic, and antimicrobial potentials have been investigated. Here, we would like to report preliminary evaluation of pharmacological and antimicrobial properties of six thiadiazoles with izoquinoline, indole, or thiadiazole ring at C-5 carbon atom.

Preliminary behavioral study showed that none of the tested compounds was found to show neurotoxic activity because in the dose of 0.1 LD_{50} they did not affect the motor coordination of mice in the "chimney test". All thiadiazole showed depressive activity in mice; they significantly prolonged the thiopental sleeping time in the "thiopental-induced sleep" test. Additionally, some compounds showed strong analgesic activity in the "writhing syndrome" test. Two compounds, which were tested as potential antimicrobials were inactive.

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17:00 Poster 106

Microsatellite markers analysis in the treatment and diagnosis of familial hypertrophic cardiomyopathy

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Familial hypertrophic cardiomyopathy (FHCM) is characterized by an autosomal dominant transmission, left ventricular hypertrophy and myocardial disorganisation. So far, thirteen genetic loci and more than 130 mutations in various genes have been identified. Recent studies suggested the impaired energy production associated with inefficient use of ATP as the main disease cause. In the present study, haplotype analysis with the use of microsatellite markers linked with beta-myosin heavy chain, troponin T, alpha-tropomyosin and cardiac myosin protein C genes in three Polish families (A, B and C) with hypertrophic cardiomyopathy (23 individuals) was performed. This method is based on the analysis of distribution of the disease within the family members and that of the alleles of chosen microsatellite markers. In families A and B, the disease was found to be coupled with beta-myosin heavy chain gene and the presence of the mutation in exon 13 of this gene associated with poor prognosis was excluded by restrictive fragment length polymorphism and multitemperature single strand conformation polymorphism analysis. Genetic carrier of the mutant gene among children in family B was also found. In family C the coupling of the disease with the mutation of alpha-tropomyosin gene was confirmed, no sudden cardiac death was charted and the degree of myocardial hypertrophy was small. The linkage analysis results confirmed by statistical analysis in the examined families was helpful in choosing the appropriate pharmacological therapy to prevent their members from sudden cardiac death.

17:00 Poster 108

Novel bioactive metabolites

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Infectious disease is a therapeutic arena in which there is a constant need for new drugs to combat waves of resistant microorganisms (bacteria, fungi, parasites, viruses) that are selected for by widespread application of any microbial agents. For antibacterial agents the problem has been exacerbated over the past decade both by the spread of clinically significant multiply drug-resistant Gram-negative pathogens and Gram-positive pathogens and the exit of several major pharmaceutical companies from this therapeutic space[1].

Soil microbes represent an important source of biologically active compounds. These molecules present original and unexpected structure and are selective inhibitors of their molecular targets. Many of the products currently used for human or animal therapy, in animal

husbandry and in agriculture are produced by microbial fermentation, or are derived from chemical modification of a microbial products. These products have been obtained after a few decades of intensive screening involving probably millions of microorganisms. Thus, new bioactive metabolites continue to be identified from microbial sources, thanks to the large variety of existing strains[2].

The purpose of this work was to search for novel antimicrobial agents, inhibitors of DD-peptidases (EC 3.4.16.4), microbial secondary metabolites from our collection of terrestrial streptomycetes strains. DD-carboxy-peptidases/trans-peptidases are the enzymes involved in peptide cross-linking during the last stage of bacterial cell wall peptidoglycan biosynthesis. We used DD-peptidase 64-575 II[3]. From the collection of streptomycetes 118 strains (110 of *Streptomyces*, 8 of *Saccharopolyspora*) of 56 species belonging to 45 clusters were tested for production of DD-peptidases inhibitors. 21 % of these strains produced DD-peptidase 64-575 II inhibitors, 27 % of strains produced β-lactamases and 8 % of strains represented both activities.

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Acknowledgement: This work is co-financed from European Regional Development Fund within Operational Programme „Innovative Economy", the project number UDA-POIG.01.03.01-14-136/09-00

17:00 Poster 110

A Novel Method for the Synthesis of Ezetimibe Precursor

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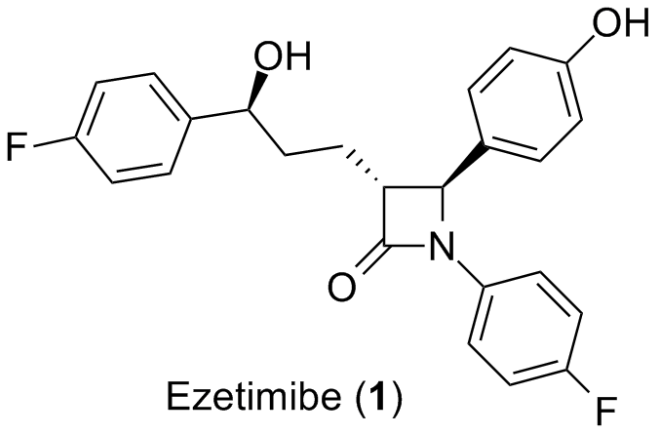
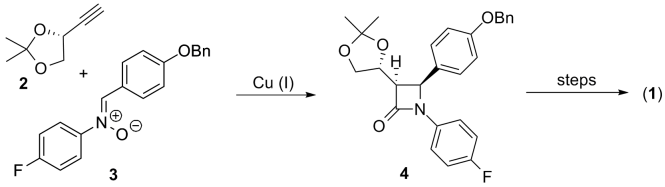
The azetidin-2-one (*β*-lactam) ring is widely recognized as a key structural motif in several families of antibiotics.[1] Ezetimibe (**1**), has recently been commercialized as an effective acyl-CoA cholesterol acyltransferase inhibitor for lowering cholesterol levels. The drug is absorbed into the intestinal epithelial cell and remains associated in great part with the epithelial cell membrane where it is believed to interfere with the putative sterol transporter system.[2] This apparently prevents both free cholesterol and plant sterols (phytosterols) from being transported into the cell from the intestinal lumen.



Ezetimibe (**1**)

The novel structure and potent biological activity of ezetimibe has prompted intense synthetic interest in the synthetic community which led to the development of several syntheses for this molecule. Many of these routes have utilized chiral auxiliary based synthesis and used chiral HPLC for the purification of the intermediates.[3]

In connection with our interest for the synthesis of azetidin-2-one ring[4], we wish to report a facile and efficient method for the preparation of Ezetimibe precursor **4***via* a copper (I)-mediated reaction between diaryl nitrone **3** and chiral terminal acetylene **2**



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17:00 Poster 112

The use of the hyphenated LC-MS/MS technique for the characterisation of impurity profile of Quetiapine during drug development

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As a part of an integrated quality concept for impurities during drug development, the multidimensional evaluation of impurity profiles by LC-MS/MS coupling is presented using Quetiapine active pharmaceutical ingredient (API) as an example. Although the LC-UV is commonly employed for the impurities and degradant compounds determination, the LC-MS/MS technique is proposed in this work as a viable modern alternative for the characterization of these

compounds. The case study outlines the use of LC-MS/MS technique in developing methods for the separation and identification which enable the determination of both process and degradation impurities.

The developed methods of the impurity-profile determination allow monitoring impurity profile changes, resulting from both planned and unplanned changes in the synthesis or the API storage. The developed methods also enable the identity confirmation of the studied API, structure elucidation and identification of the impurities from the synthesis route (isolated, synthetised, and those which were not successfully isolated or synthetised), as well as the impurities resulting from the degradation of the studied pharmaceutical substance which were not obtained as standards. The mass spectra, fragmentation spectra and chromatographic information about the order of elution were all used to identify the impurities. The proposed structures of process impurity were additionally confirmed by analysing the route of synthesis of the studied pharmaceutical substance. On the basis of the proposed structures of API degradation impurities their formation mechanisms were presented.

17:00 Poster 114

New 1-(2-pirydynyl)-6-substituted imidazo[1,2-a][1,3,5]-triazine with potential pharmacological activity.

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The aminocarbonyl derivatives of 1- aryl-2-imidazolidine-2 have significant antinociceptive activity connected with activation of the MOP (mu opioid protein) receptor [1-3].The syntnetic derivatives of triazepine form are various and important group of medicine.In the search for new derivatives with potential pharmacological activity received of new imidazo [1,2-a][1,3,5]triazine. New 1-(2-pirydynyl)-6-fenyl(benzyl)-5,6,8(1H)-dioxoimidazo[1,2-a][1,3, 5]triazine and 1-[2-(5-nitro)pirydynyl]-6-fenyl(benzyl)-5,6,8(1H)-dioxoimidazo[1,2-a][1,3,5]triazine were synthesised in reaction of adequate substituted 1-[(2-pirydynyl)imidazolidine-2-ylideno]-3-fenyl(benzyl)urea with CDI.

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

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17:00 Poster 116

Application of 2-Nitroglycals in the Synthesis of Biologically Active Glycoconjugates

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It can be postulated that low molecular weight ligands, designed as drugs, can also be advantageously modified by chemical glycosylation using natural or synthetic sugars. Suitably selected sugar moieties can render pharmacophoric structures properties desirable in a pro-drug category. In particular, they can serve as: i) an active transport and biodistribution enhancers; ii) protection against phase II metabolism bioconjugation leading to excretion; iii) providers of a lipophilicity element needed for membrane, receptor and binding-pockets docking.[1]

Several classes of plant polyphenols are being studied (flavonols, catechins, isoflavones etc,) as antioxidants, detoxicants, chemoprotectants, immunomodulators, antitumor agents, regulators of lipids metabolism and cardiovascular health promoters.[2] The main reasons for slow progress in pharmaceutical development are their physicochemical properties, low bioavailability and unfavorable metabolism.

Glycals, which are readily available from sugars, can be an attractive starting material for glycoside bond formation. Their nucleophilic character at C-2 permits reactions with oxygen, nitrogen, and sulfur electrophiles that under high substrate stereocontrol generally lead to three-membered rings; ring opening under acid catalysis furnishes the corresponding glycosides [3]. Glycals also can be transformed into derivatives that have at C-2 an electron-withdrawing group and are amenable to Michael-type addition. A good example are 2-nitroglycals. In this case, glycoside bond formation is achieved under base catalysis and leads to 2-deoxy-2-nitroglycosides. These intermediates are readily converted into 2-amino-2-deoxyglycosides [4].

Focusing our attention on soya isoflavonoids, which are drug candidates in antitumor therapy research program, the chemical derivatization of genistein and biological activity study of its new derivatives has been started, aimed at anticancer compounds.

We report herein the regio- and stereoselective synthesis of O-glycosides, and glycoconjugates derivatives of aminosugars and genistein.

The effect of synthesized compounds on proliferation of cancer cells will be presented.

Acknowledgement

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17:00 Poster 118

Molecular modeling of allosteric modulation of nicotinic acetylcholine receptor by different classes of ligands

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Nicotinic acetylcholine receptor (nAChR) is a pentameric protein assembly that belong to the Cys-loop Ligand Gated Ion Channels superfamily. Several neuronal subtypes of nAChR pose a promising target for treatment of such disorders as nicotine addiction, depression, schizophrenia, cognition deficits and treatment of pain. Most of commonly used drug molecules and their metabolites interact with the inner surface of the ion channel by a mechanism of non-competitive inhibition associated with disturbing the flux of ions through the channel. Binding of allosteric ligand molecules to outer and inner surface of the extracellular domain (ECD) of nAChR play important roles in coupling agonist binding to the channel gating. In our project we made two sets of molecular modeling simulations for two distinct part of nAChR and its interactions with ligand molecules. Models of 1) the ion channel domain and 2) the extracellular domain were developed for several subtypes of the receptor and were used in docking simulations of ligands interacting at these locations.

In the project 1) the molecular model of transmembrane domain of the nAChR obtained using cryoelectron microscopy of *Torpedo marmorata* (PDB id: 2BG9) was used. We further modified this model to represent models of the several human neuronal and muscular subtypes. Docking procedures of a flexible ligand into the rigid model of the ion channel were performed and allowed classification of ligands in respect to their binding energies. Obtained result for the interactions with ion channel indicated that ligands stably interact with the surface of the channel formed by an assembly of five transmembrane helices M2. The binding energy estimated in simulations can be related to experimental values.

In the project 2) the molecular models of extracellular domain of nAChR obtained from *Lymnaea stagnalis* (PDB id: 1UV6) and *Gloeobacter violaceus* (PDB id: 3EAM) were used. We constructed the models representing different neuronal subtypes of ECD of nAChR using homology modeling. Obtained models were used for docking simulations of such ligands as galanthamine, physostygmine, codeine, 5-hydroxytryptamine, ketamine and its metabolites. Obtained results suggest that allosteric potential ligands bind to different locations than acetylcholine and affect the function of the receptor.

Studied compounds show different modes of allosteric modulation but all of them play important role in regulation of cholinergic transmission in the brain. Various groups of ligands interact in different modes with distinct regions of the nicotinic acetylcholine receptor.

17:00 Poster 120

CLA derivatives as functional food compounds

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The work presents the results of the study on a synthesis of biologically active isomers of linoleic acid 9c,11t and 10t,12c $C_{18:2}$ (*CLA conjugated linoleic acid*) obtained from poppy seed oil, as well as the results of the study on the influence of preparation obtained on the size of breast cancer tumour nodule.

The aim of the work was to establish the method of biologically active isomers of linoleic acid (9c,11t and 10t,12cC18:2) obtaining from poppy seed oil rich in linoleic acid (9c,12cC18:2) and to determine the influence of that isomers on size changes of breast cancer tumour nodule.

The raw research material was poppy seed oil containing about 72% of linoleic acid (9c,12cC18:2) bounded in triacylglycerols. The isomers of linoleic acid were obtained by the method of positional and geometric isomerisation of linoleic acid. Poppy seed oil isomerisation was conducted in the presence of alkaline catalyst in propylene glycol or glycerin environment. Qualitative identification and quantitative analysis were conducted using the method of gas chromatography with standards of 9c,11tC18:2 and 10t,12cC18:2 isomers of Sigma company. Saponification of triacylglycerols of poppy seed oil to free fatty acids took place concurrently to the reaction of positional and geometrical isomerisation.

The application study of the product obtained was conducted on mice with orthotropically injected cells of breast cancer 16/c. Volume of nodule in mm^3 was studied and compared to the control sample.

As a result of the study conducted, the method of isomerisation of linoleic acid, the main component of poppy seed oil, was established obtaining the product containing mainly 9c,11tC18:2 (about 35%) and 10t,12cC18:2 (about 36%) isomers. Basing on the application study of the product obtained its influence on an inhibition of development of breast cancer tumour nodule of about 30% on average

may be concluded.

The results obtained point the possibility of linoleic acid isomers obtaining from poppy seed oil, and their biological activity towards breast cancer tumour nodules. Since the mixture of CLA isomers was used for the study, it seems to be reasonable to work out a successful method of isomers separation and to obtain the two fractions containing separately both isomers 9c,11t and 10t,12c. Similar study should be conducted for such obtained products in order to determine biological activity of both isomers towards wider group of tumour nodules. As a target, the preparations obtained containing isomers of linoleic acid would be used as functional food components.

17:00 Poster 122

The development of the spectrofotometric and HPLC method for studying enteric coated capsules containing Duloxetine Hydrochloride active substance

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The aim of this work was to develop analytical methods used for studying enteric coated capsules with Duloxetine Hydrochloride – an antidepressant drug.

The reference product was Cymbalta 30 mg, 60 mg capsules manufactured by ELI LILLY Nederland B. V.

The RP-HPLC and spectrophotometric methods were developed for the study of the assay & quantity of the active substance released from the drug form. The above methods were then compared.

The dissolution profiles were determined through the use of spectrophotometric and RP-HPLC methods and compared using the similarity factor *f2* in relation to the reference batch, in the range from the time point 5 minutes to the point when the active substance release was above 85%.

The similarity factors *f2* amounted to above 50 proving that the dissolution profiles were similar. Both methods can be used for the determination of duloxietine content in the tested capsules with Duloxetine.

17:00 Poster 124

Anticancer activity of new coordinative palladium (II) complexes.

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The aim of this study was to establish if newly synthesized palladium (II) complexes possess anticancer properties. The chemical similarity between palladium and platinum leads to the expectation that related palladium complexes might also be active against some cancers. For instance, low but significant activity of palladium chloride complexes with hydroxypyridines against the ovarian cancer cell lines was found [1]. According to many authors, changing the nature of the ligand donor atom may be beneficial. Twelve complexes were studied in various cancer cell lines originating from blood (CCRF – acute human leukemia) and solid tumors (Mcf7 – human breast cancer). Anticancer activity of palladium (II) complexes was determined by MTT test which evaluate viability of cells after treatment with compounds tested. Cells were incubated with increasing concentrations of palladium (II) complexes for 24, 48 and 72 hours. In order to evaluate relative anticancer activity IC50 (inhibitory concentration which decreases viability of cells to 50%) was calculated if possible. Palladium chloride complexes with substituted pyridines were prepared under argon. A known amount of PdCl2 was placed in a flask equipped with magnetic stirrer and Py or substituted XnPy in acetonitrile were added. Reaction was carried out at room temperature for 24 h. The results obtained for these newly synthesized palladium complexes indicate that their anticancer activity differs with respect to their structure. Both cell lines viability, however, was affected and increasing time of incubation enhanced the cytotoxic effect. Palladium (II) complexes can be considered as substances of anticancer properties and further study on their properties and also synthesis of new derivatives is advisable.

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17:00 Poster 126

Analytical control of synthesis and determination of BR-S by HPLC

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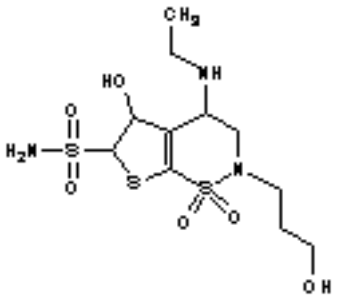
BR-S namely (5R)-5-ethylamino-3-(3-methoxypropyl)-2,2-dioxo-2,6,9-dithia-3-azabicyclo [4.3.0]nona-7,10-diene-8-sulfonamide is a carbonic anhydrase inhibitor used to lower intraocular pressure in patients with open-angle glaucoma or ocular hypertension.



BR-S can be obtained by the seven-step synthesis. Analytical method used for this purpose must ensure fast and efficient determination of the presence of starting materials, product, impurities. The common and widely applied method which meets these requirements is high performance liquid chromatography, especially in reverse phase mode (RP-HPLC). This technique was used for optimization

of synthesis and determination of purity of the API. Finally an HPLC method was elaborated. In course of extensive research it was proven, that the developed method is selective and sensitive for all separated compounds. It fulfills the criteria of European Pharmacopoeia for API analysis and can be used to control the synthesis process.

17:00	Poster	128

HPLC method as an analitycal control of synthesis and determination of TZ-S

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TZ-S (Fig. 1) is an oral alkylating agent which is used for the treatment of Grade IV astrocytoma - an aggressive brain tumor, also known as glioblastoma multiforme.

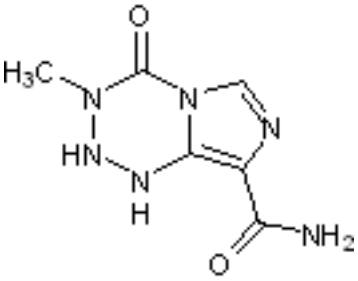

Fig.1 TZ-S, namely 4-methyl-5-oxo-2.3.4.6.8-pentazabicyclo [4.3.0] nona-2,7,9-triene-9-carboxamide.

Sample preparation, particulary with labille compounds, may cause many analytical problems. Therefore, elaboration of suitable analytical mothods for both, routine manufacturing processes, and investigation of novel synthetic routes is very important. RP-HPLC technique was used for the optimization of synthesis and the determination of purity of TZ-S.

Synthesis of the active substance - TZ-S - was conducted in two different ways: the first one, consisted of five synthetic steps, while the other consisted of three steps. The presented methods are useful for both synthetic pathways and, are suitable for determination of product purity, as well as separation and identification of ampurities.

17:00	Poster	130

A novel handle for chemistry on solid support: the Pipecolic linker

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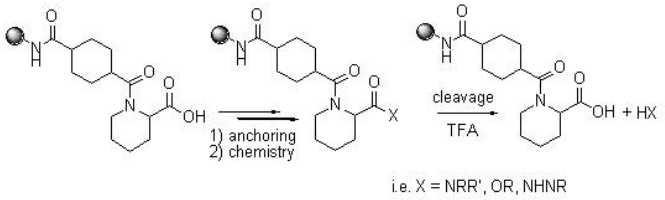
Here we present the design, the synthesis and the use of a new linker for solid-phase chemistry. The pipecolic linker (Pip) was designed on the basis of a side reaction observed on BAL linker functionalized SynPhase Lanterns while synthesizing a focused library of long-chain arylpiperazines.[1] Surprisingly, the TFA cleavage of the Lantern-bound pipecolic acid derivatives did not yield the desired N-acylpipecolyl amides. TFA treatment caused an unprecedented selective hydrolysis of the linker-bound amide bond, which yielded two unexpected products: the free primary amines, and the N-acylated pipecolic acid.

The pipecolic linker is a highly versatile handle as it can immobilize on solid support primary, secondary and aromatic amines as well as alcohols, phenols, and hydrazides.



The advantages of the Pipecolic linker over commercially available ones are very easy and efficient anchoring and high purity of the released products. In addition, solid supports functionalized with pipecolic linker are fully recyclables.[2]

Pipecolic linker can accommodate a wide range of compounds and could be useful in many fields of supported chemistry (i.e. peptide and pseudopeptide synthesis, heterocycle synthesis). The use of this TFA-labile linker is demonstrated for side chain and N-terminus anchoring of peptides and pseudopeptides as well as for the preparation of C-terminal peptide hydrazides.[3]

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17:00	Poster	132

Cyclohexaglycine as a potential ionophore

Paweł Żero[1], Franciszek A. Pluciński[2], Aleksander P. Mazurek[1,2]

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The structure of cyclohexaglycine is an object of different studies from a half of century and is now quite well known. In our work we have searched the possible complexes of cyclohexaglycine with ions

of metals. The studies were performed by *ab initio* method using the Gaussian 03W package. The DFT approach with the 6-31G(d) basis set was used.

We were interested in structure and energy of formation of these complexes. For our research we selected metals that are important from biological point of view, e.g. Na, K, Ca, Fe.

We found that in general there are two possible structures of complex of cyclohexaglycine with ion of metal: endo and exo form can exist. In endo form the ion of metal is situated exactly in the middle of complex and in the exo form the ion is placed on one side of cyclohexaglycine. In most cases the endo form is the preferred one, because of the lower energy of formation.

The complexes of cyclohexaglycine with metallic ions are expected to be stable, due to their negative energy of formation. This quantity has more negative value, when the size of ion or the charge it carries is bigger.

Bibliography:
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[2] Santos M.A., Brennan R.L., Drew M.G.B., *J. Mol. Struct. (Theochem)* 286 (1993) 109

17:00 Poster 134

Stability study of the coated tablets containing clopidogrel bisulfate as the active substance

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Clopidogrel is an oral antiplatelet agent (thienopyridine class) used to inhibit blood clots in coronary artery disease, peripheral vascular disease, and cerebrovascular disease.

The aim of this work was to develop stable formulation containing the crystalline form I of clopidogrel bisulfate as an active substance, with appropriate pharmaceutical bioavailability.

Core tablets were covered by two kinds of coating – Opadry II and AMB. The finished tablets were packed into blisters manufactured from two kinds of packaging materials Alu/Alu and Alu/PVDC. The stability study was conducted in different temperature and humidity conditions during 6 month. An RP-HPLC method was developed for the study of assay, quantity of the active substance released from the drug form, and the purity study of the preparation, whereas stability of Clopidogrel bisulfate I polymorphic form was carried on by FT-IR method. The results show the stability of the active substance in the investigated tablets coated with Opadry II and packed into blisters Alu/Alu after 6 month storage in 40^{o}C temperature and 75% humidity.

17:00 Poster 136

Investigation of unknown impurity of TD-S by HPLC-MS

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(*R*)-2-[3-(diisopropylamino)-1-phenylpropyl]-4-methylphenol (TD-S) is a potent anticholinergic agent used in treatment of urinary incontinence. In this work we show the technological process which leads to obtaining TD-S of pharmaceutical purity.

In Fig.1, the synthesis path of TD-S is presented. Straightforward application of this scheme in the laboratory scale led to obtaining of TD-S with a purity not meeting the requirements of the european regulatory authorities. In particular, the amount of an unidentified impurity (1) characterized by HPLC relative retention time 1,2 was exceeding the acceptable impurity thershold.

Attempts to purify the substance by means of crystallization were found unsuccessful. Thus, it appeared necessary to identify the potential source of impurity in the intermediate TD-5A. Investigations carried out by HPLC-MS-MS technique in both, the final product and the intermediate, allowed elucidating the potential structure of the impurity (1) and linking it to other structures found in the TD-5A intermediate.

Finally it was shown that purification of the intermediate from selected impurities allows obtaining the final product with the pharmaceutical purity.

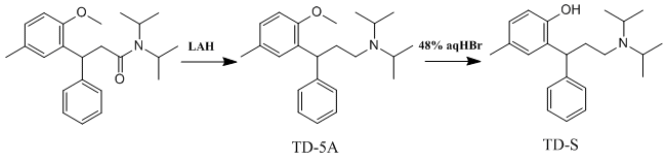

Fig.1 Synthesis path of TD-S

17:00 Poster 138

The new solvates of docetaxel and C_2-C_3 alkyl esters of formic acid and their use for API's preparation.

Witold S. Cieśliński

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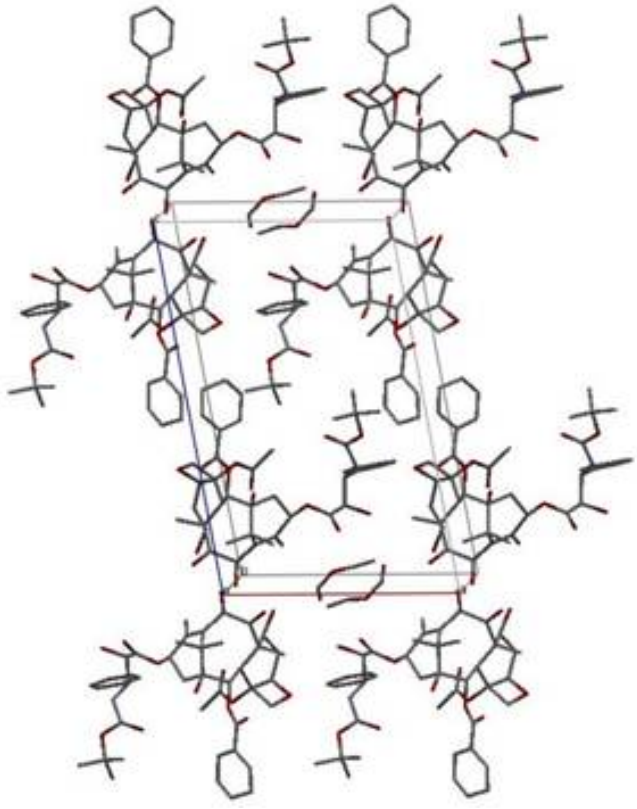
The new solvates of docetaxel with C_2-C_3 alkyl esters of formic acid were identified and fully characterized by methods routinely used in the psudopolymorphs studies, such as: Termogravimetric Analysis (TGA), Differential Scanning Calorimetry (DSC), Fourier Transform Infrared Spectroscopy (FT-IR), X-Ray Powder Diffraction (XRPD). Their crystalline structures were fully confirmed by single crystal X-ray diffraction's studies.

Figure. Crystalline structure of solvate of docetaxel and ethyl formate based on results of x-ray diffraction structural studies of single

crystal.

Thus, obtained crystalline solvates of docetaxel, which chromatographic purity is not less than 99% (determined by HPLC), could be used to prepare pharmaceutical docetaxel as an anhydrous or a hydrate form, ie. trihydrate.

The known methods of purification of docetaxel are time- and labour-consuming. Generally, the purification methods of docetaxel are chromatographic methods and require usage of large amounts of solvents or multiple refining or crystallizations, decreasing the total yields. These problems could be avoided by purification of docetaxel via solvates with C_2-C_3 alkyl esters of formic acid, which are new entities, previously not described in the literature. The desolvation process of solvating solvent - ethyl formate, propyl formate or isopropyl formate, could be accomplished by any known crystallization of docetaxel in accordance to crystallization of the anhydrous or the hydrate, especially the trihydrate.



The new solvates of docetaxel and C_2-C_3 alkyl esters of formic acid, their method of preparation and their use are subject of Polish Patent application no. P-388144. PCT application is also pending.

17:00	Poster	140

Docetaxel trihydrate - polymorphous studies and the new useful method to obtaining one of polymorph.

Witold S. Cieśliński[1], Andrzej Ostrowski[2]

1. *IFOTAM Co. Ltd (IFOTAM), Andrzejewskiej 3, Łódź 92-550, Poland* **2.** *Warsaw University of Technology, Faculty of Chemistry, Noakowskiego 3, Warszawa 00-664, Poland*

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Docetaxel is an antineoplastic agent belonging to the taxoid family and is available on the market in the injection form under the brand name Taxotere®, containing docetaxel trihydrate as an active pharmaceutical ingredient (API). Docetaxel trihydrate crystallizes in two different forms, namely in form A and form B. Both forms have been disclosed by L.Zaske & col. [1], [2]. Their structure dates are available in Cambride Structure Database (code CSD: DARGOT & DARGOT01). The crystalografic data and Cambride Structure Datebase data of both docetaxel trihydrate crystal forms were not fulfilled. They include the space group information and unit cell parameters only.

The aims of this work were:

1. Fully identification of docetaxel trihydrate crystal forms by methods use in the polymorphs studies, such as: Termogravimetric Analysis (TGA), Differential Scanning Calorimetry (DSC), Fourier Transform Infrared Spectroscopy (FT-IR), X-Ray Powder Diffraction (XRPD),
2. The research of new methods of obtaining of docetaxel trihydrate.

The standards of docetaxel trihydrate form A and B have been obtained and identified. TGA date, DSC date, FT-IR characteristic peaks, and XRPD 2theta angels are shown.

The new preparation method of docetaxel trihydrate, form A, was a subject of our further research. The method is based on crystallization of docetaxel from 2-etoxyethanol and water mixture and is especially useful in docetaxel isolation and purification. As result of the direct docetaxel's separation from reaction mixture, the product is obtained without decomposition and in course of a simple procedure. The isolated product has a purity of not less than 98%, depending on the starting purity of the employed substrate, and can be further purified by crystallization only.

The presented preparation-method of the docetaxel trihydrate, form A, is subject of Polish Patent application no. P-388498.

References:

[1] L.Zaske, M.-A.Perrin, F.Laveiller *J.Phys. IV France 11(2001) Pr10-221;*

[2] L.Zaske, M.-A.Perrin, C.Daiguebonne, O.Guillou, *Materials Science Forum vols. 443-444 (2004); pp 411-0*

Przerwa (Break)
Tuesday evening, 11 May, 18:30

Transportation to the Regional Inn
Tuesday evening, 11 May, 19:00

Picnic
Tuesday evening, 11 May, 20:00
Biały Potok Inn - entrance to Dolina Białego Valley, Droga do Białego 7 Str.

Wednesday, 12 May

Breakfast

Wednesday morning, 12 May, 7:30

Session VII

Wednesday morning, 12 May, 9:00
Conference room
Chair: Alekasander P. Mazurek, Lech Kozerski

9:00 Invited oral

Exploration of sugar-based inhibitors of glycolysis to target brain tumors

Waldemar Priebe[1], Marcin Cybulski[1,2], Sławomir Szymański, Izabela Skóra, Stanisław Skóra, Charles A. Conrad, Timothy Madden[3]

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Glycolysis is the major energy producing pathway for fast growing, glycolytically dependent tumors, such as gliomas and ependymomas. Blocking glycolysis is, therefore, an important and clinically unexplored therapeutic strategy when used alone or as a combination therapy to enhance the effects of chemotherapy in energy-starved tumors.

In our studies, we have examined D-glucose antimetabolites 2-deoxy-D-glucose (2-DG), 2-deoxy-2-fluoro-D-glucose (2-FG) and 2-deoxy-2-fluoro-D-mannose (2-FM) and confirmed their ability to block glycolysis and discovered their ability to induce autophagic cell death.
Our more detail evaluation revealed that these compounds do not possess sufficient drug-like properties (reasonable biostability, pharmacokinetic characteristics, or distribution to the target) to warrant further investigations into their clinical effectiveness. In an attempt to improve the pharmacokinetic profile of 2-DG, we have designed, synthesized, and tested a series of prodrugs that enhance biostability, biodistribution, and delivery of 2-DG to its CNS target.
We will present the synthesis and preliminary evaluation of compounds, leading to the selection of WP1122 as our lead compound for further drug development.

9:30 Invited oral

Protein crystallography as a tool in drug discovery

Marcin Nowotny

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Crystallography allows the elucidation of protein's structure and mechanism of action at a molecular level. One example of such studies is our work on structures of RNases H and in particular their complexes with the substrate RNA/DNA. RNase H - a small nuclease that cleaves the RNA strand of RNA/DNA hybrids is an example of an important drug target – it is the only enzymatic activity encoded by the HIV for which no specific inhibitor is available. In the second part of the talk I will discuss how structural information about proteins that are drug targets can be used to improve existing inhibitors or activators or even attempt to design novel ones using computational methods. One such example is the development of antiviral drug Tamiflu. Another important application of crystallography in drug design is a so-called 'fragment-based design'. Computational docking is first used to identify small compounds that could potentially interact with a selected region of the protein surface. Protein crystals are then soaked with these compounds (usually several hundred in total) and the protein structure is solved. Compounds that interact with the protein are observed in the resulting electron density maps and can be identified by their shape. Even weak interactors can be identified and several compounds binding in one region can be combined to produce a tightly binding and specific inhibitor.

10:00 Oral

Novel small molecule STAT3 inhibitors in cancer therapy.

Karolina Dzwonek, Filip Stefaniak, Paweł Gunerka, Monika Lamparska-Przybysz

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Cancer cell proliferation, invasion and metastasis are regulated by an interconnecting signaling pathways involving extracellular ligands, transmembrane receptors, protein kinases and transcription factors. Insights into this complicated signaling network have revealed many novel cancer targets for which chemotherapeutic agents may be developed. On contrary to traditional cancer drugs which interfere with DNA synthesis and repair system, a new class of agents has been developed to aim directly at specific molecular targets involved in oncogenesis (e.g. EGFR). Due to their selective activity they are characterized by significantly different toxicity profile from traditional cancer drugs. However, many cancer therapeutic agents elicit resistance that makes them ineffective and often produce cross-resistance to other drugs. Therefore, we aim to obtain innovative target specific drugs, able to breakdown the cancer cells' resistance and to improve cancer therapy efficiency.
Among recently investigated potential targets for selective cancer treatment are signal transducers and activators of transcription (STATs). STATs are transcription factors activated in response to cytokines and growth factors and are involved in different cellular processes including proliferation, differentiation and inflammation. Of the seven known mammalian STAT proteins STAT3 was shown to be constitutively activated in many human malignancies. Aberrant STAT3 activity promotes tumor progression through transcriptional activation of genes encoding apoptosis inhibitors, cell-cycle regulators and inducers of angiogenesis. Available data indicate that inhibition of STAT3 signaling leads to an attenuation of cancer cell growth and the induction of apoptosis.
The aim of our investigation was to design a novel STAT3 small molecule inhibitor that would selectively inhibit STAT3 activity, re-

ducing expression of its downstream genes. Thus, we performed computational modeling and small molecule docking simulations using X-ray structure of STAT3beta homodimer. Once the model was established and validated we performed a virtual screening of the library of over 3000 compounds. As a result we have chosen 38 promising structures which were then synthesized and their biological activity against STAT3 was tested. We have applied *in vitro* luciferase activity assay exploiting stably transfected cell line with luciferase gene under the control of promoter containing STAT3 binding motif. One of drug candidates - CPL-402-003 showed significant inhibitory effect on STAT3 transcription activity resulting in decreased luciferase expression. Moreover, the same compound revealed low toxicity on non-malignant cells displaying no constitutive STAT3 activation. We are currently investigating CPL-402-003 and its derivatives on different cancer cell lines to establish its anti-proliferative/pro-apoptotic activity. Results of the *in vitro* studies will be subsequently confirmed in mouse xenograft model.

10:20 Oral

Preclinical evaluation of new chemical entity.

Teresa M. Brodniewicz

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Rational drug discovery process starts with two lines of research, which are largely independent and run in parallel, besides, they belong to various domains of life sciences and utilize distinctly different methods – biological (target identification and validation, design of activity tests) and chemical (design of new molecules, hit identification and lead development). While these activities in principle belong to standard exploratory programs performed in innovative big pharma companies under confidentiality clause, they are also a subject of open academic research and even university teaching. Nevertheless, the vast effort of pure and applied sciences combined, bears scanty fruit – number of new launches of innovative drugs remains at unimpressive level of 20 + annually. Although we have better tools than ever in our disposal, including genomics and proteomics on one side and combinatorial chemistry and high throughput screening on the other, the output of pharmaceutical R&D remains well below needs and expectations. It is generally agreed that high attrition rate in the drug discovery and development (DDD) process results mainly from inadequate generating, acquisition and processing of data around the merger point of the two lines of research mentioned above, that is between establishing the lead structure and clinical verification of validated drug candidate. This segment of DDD research, generally described as pre-clinical evaluation, is rather poorly regulated and tendency for regarding particular requirements as „project specific" seems to prevail. Based on case studies and individual experience, we put forward a sequence of task, for an unspecified DDD project, which can lead to safe „first-in-man" application of an investigational new drug, based on regulatory submission. Anotherwords, a set of advices is formulated and discussed, which indicates how to transform a new molecular entity (NME), into properly specified and certified active pharmaceutical ingredient (API), fit for experimental pharmaceutical formulation and manufacturing of clinical batches. At first, "druggability" of a new molecule has to be evaluated, using structural analysis backed up by physicochemical measurements, spectroscopic methods, polymorphism study, SAR analysis, metabolism predictions etc. Then, even more complex tasks follow, with design of biological activity tests at molecular, cellular, tissue and organism levels. Mechanism of biological activity has to be established, biodistribution and metabolism evaluated and pharmacokinetic data determined in an animal model. Last but not least, acute toxicity in two animal species lead to "go/no go" decision point on the road to clinical experiment. Since all these studies have in principle to be carried out under quality assurance system, an interplay between requirements for the tested substance and validation of analytical methods and biological tests, assuring validity of the experimental data, becomes an essential issue in the preclinical segment of drug discovery process.

Coffee break

Wednesday morning, 12 May, 10:40

Sesja VIII

Wednesday morning, 12 May, 11:10
Conference room
Chair: Zofia Mazerska, Jacek Bojarski

11:10 Invited oral

Good Laboratory Practice as the quality system ensuring mutual acceptance of test results in the registration of pharmaceutical products.

Tomasz G. Wasiela

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In 1981 Organisation for Economic Co-operation and Development (OECD) considering the need for and benefits of mutual acceptance in OECD countries of test data used in the assessment of chemicals and other uses relating to protection of man and the environment, have adopted Decision [C(81)30(Final)] on the strength of which data generated in the testing of chemicals in an OECD Member country in accordance with OECD Test Guidelines and OECD Principles of Good Laboratory Practice shall be accepted in other Member countries.

Mutual acceptance of data (MAD) system has been adapted also by European Union Member States. One of the main areas where the requirement of compliance with Good Laboratory Practice exists is studies on pharmaceutical products. Pharmacokinetic and toxicological studies of those products submitted for registration purposes, if not performed in compliance with the GLP Principles, by right cannot be accepted by Regulatory Authorities. The GLP is that necessary quality standard which enable mutual recognition of test results performed by certified testing laboratories in other EU and OECD countries.

11:40 Oral

Homology modeling of G-protein coupled receptors

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

1. *Research Group of Medical Pharmacology and Toxicology, Department of Medical Biology, Faculty of Health Science, University of Tromso, Tromso, Norway (UIT), Tromso N-9037, Norway* **2.** *Narodowy Instytut Lekow (NIL), Chełmska 30/34, Warszawa 00-725, Poland* **3.** *Polish Academy of Sciences. Institute of Pharmacology, Smętna 12, Kraków 31-343, Poland*

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The present presentation will give a short overview of G-protein coupled receptors (GPCRs). In mammalian species, there are three main families of GPCRs. Many drug targets belong to family A of GPCRs, which includes receptors for endogenous compounds including biogenic amines, lipid-like compounds, many neuropeptides, glycoprotein hormones, protease-activated receptors and receptors for exogenous compounds, opsins, odorants and some tastants (Kristiansen, 2004). Homology models of GPCRs were previously constructed by using bacteriorhodopsin x-ray structure (not a G-protein coupled receptor) or a template for family A receptor based on a cryo-microscopy microscopy and sequence analysis of family A receptors (Baldwin, 1997). Today, x-ray structures of several family A receptors have been reported, including bovine rhodopsin (Palczewski et al. 2000), beta1(Warne et al., 2008) and beta2 (Cherezov et al. 2007) adrenergic receptors and A2A adenosine receptor, which has given the opportunity to generate more accurate GPCR models. The x-ray structure of the aminoterminal extracellular domain of family C receptors has also been reported. However, all known x-ray structures of family A receptors represent the inactive state conformation. In the present presentation, a model of the human D2 dopamine receptor constructed by homology with bovine rhodopsin will be discussed. Several antipsychotic drugs were docked into the model by using automatic docking with the modeling software ICM.

Acknowledgements

This study was partly supported by a grant PNRF-103-AI-1/07 from the Norwegian – Polish Research Fund.

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Kristiansen K (2004) Pharmacol Ther. 103:21-80.

Cherezov Vet al. (2007) Science 318:1258-65

Warne T et al. (2008) Nature 454:486-91.

12:00 Oral

Polymer chains as molecular dispensers. Monte Carlo simulations of simple models

Andrzej Sikorski, Łukasz Ołdziejewski

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A simple model of molecular dispenser consisting of protein-like copolymers was designed. A coarse-grained model of polymer chains was used for this purpose. In this model we replaced a real polymer chain with a sequence of statistical segments connected by united atoms while all the atomic details were suppressed. Such a chain was restricted to a lattice of a [310] type, which was frequently used in simulations of polymers and biopolymers. Different macromolecular architectures were studied: linear chains and star-branched chains. Two kinds of polymer segments were defined: hydrophilic and hydrophobic ones (the HP model). The force field used consisted of the long-range contact potential between polymer segments and the attractive potential between a large spherical particle and the polymer. The properties of the model system were determined using the Parallel Tempering (the Replica Exchange) Monte Carlo sampling scheme. The hompolymer chain was adsorbed on the large particle, then polymer segments in loops (non-adsorbed) were changed to hydrophilic ones (process of "colouring") and crosslinks between some hydrophobic segments were introduced. It was shown that a chain prepared in a such way is sensitive to the particle size. The introduction of branching led to the increase of the selectivity.

12:20 Oral

Keratin associated proteins (KAPs) as a new biomaterials for applications in medicine and cosmetology

Andrzej W. Lipkowski[1,3], Katarzyna Kurzepa[3], Anna Grabowska[2,3], Aleksandra M. Szczucińska[3], Barbara Gajkowska[1,3], Marcin Jurga[1], Marta Bochynska[1,3], Katarzyna Michalec[1,3]

1. *Polska Akademia Nauk, Instytut Medycyny Doświadczalnej i Klinicznej (PAN), Warszawa, Poland* **2.** *Instytut Chemii Przemysłowej (IChP), Rydygiera 8, Warszawa 01-793, Poland* **3.** *Polish Scientific Network of Medicinal Chemistry of Peptides, Pawinskiego 5, Warsaw 02106, Poland*

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Micro- and nanobiotechnology are the most explored fields in contemporary life science. This stimulates development of new 3D structural synthetic and semisynthetic materials. Usually, application of proteins as structural biopolymers requires chemical modifications resulting in increased stability of their 3D structures. The biocompatibility of these materials is the most important problem. Our team developed new technology of producing keratin associated protein (KAP) scaffolds from hair, wool and bristle.

Hair keratin associated proteins (hKAP), as well as other biomaterials of this type, are essential for the formation of a rigid and resistant hair shaft. Rigid structures are obtained due to extensive disulfide

bond cross-linking between abundant cysteine residues of hair keratins. These structures in KAPs are extremely resistant to physicochemical and biochemical modifications. In the living organisms such structures are filled with other biological substances, including keratin proteins, that can be removed by enzymatic digestion. Residual microscaffolds could be applied for a number of applications in medicine and cosmetics, including 3D tissue harvesting or as carriers of biological bioactive substances in medicine or cosmetology. The examples of already applied KAPs will be presented.

12:40 Oral

New phenylpropanoic acid derivative with antidiabetic potential acting as a partial PPAR gamma agonist – preclinical studies.

Krzysztof Kurowski, Urszula Bulkowska, Rafał Derlacz, Wojciech J. Gutman, Zbigniew Majka, Katarzyna Matusiewicz, Joanna A. Pawlak, Monika Stupak

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Due to the explosive increase in the number of people diagnosed with diabetes world-wide in the past two decades, we can now speak of diabetes epidemic even if this word seems inappropriate in conjunction with a chronic disease. Diabetes is now considered as one of main threats to human health in the 21-st century. It is a metabolic disorder primarily characterized by insulin resistance and elevated blood glucose levels. Insulin resistance and glucose intolerance are key elements of the metabolic syndrome, which is currently associated with impaired function of PPAR gamma nuclear receptor and excessive production of fat tissue hormones. PPAR gamma is critical transcription factor in regulating lipid and glucose metabolism as well as insulin sensitivity, thus PPAR gamma receptor is considered as a very promising molecular target for developing new antidiabetic compounds.

New investigated compound is a phenylpropanoic acid derivative (non-thiazolidinedione), selective, partial agonist of PPAR gamma nuclear receptor. As a partial PPAR gamma agonist, new compound has less than 30% of the activity associated with currently marketed full PPAR gamma agonist, rosiglitazone. Theoretically, our partial agonist should minimize side effects associates with rosiglitazone treatment by limiting the spectrum of activation of PPAR gamma. Consistent with this prediction, animal studies have revealed that the compound is effective in lowering blood glucose levels and demonstrates a relatively benign adverse event profile at putative therapeutic dose ranges. Doses used in animal models of diabetes such as the db/db mouse and the ZDF rat showed significant efficacy in the control of blood glucose level with minimized body weight gain, when compared to rosiglitazone. Moreover safety pharmacology and toxicology studies with mice, rats, dogs and monkeys suggest a broad safety window in which to study new compound's benefits in humans.

Dinner

Wednesday afternoon, 12 May, 13:00

Session IX

Wednesday afternoon, 12 May, 15:00
Conference room
Chair: Waldemar Priebe, Zdzisław Chilmonczyk

15:00 Invited oral

From unsaturated sugar α,β-lactones to β-lactams. The synthesis. Od α,β-nienasyconych laktonów cukrowych do syntezy β-laktamów.

Marek Chmielewski

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15:30 Oral

Stereoselective synthesis of isoquinoline alkaloids with a quaternary carbon seterocenter

Zbigniew Kałuża

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A number of isoquinoline alkaloids with significant bioactivity have the stereocenter located at the quaternary carbon atom in the a-nitrogen position.[1] The *Erythrina* family e.g. erytramine contain pyrrolo-isoquinoline skeleton, while tetrahydroisoquinoline motif is present in a variety of natural products including cactus alkaloids (Pevoruvic acid), mammalian alkaloids (Salsoline-1-carboxylic acid), *Ecteinascidine* family (ET 743), and spirobenzoquinoline alkaloids (Parfumine, Figure 1).

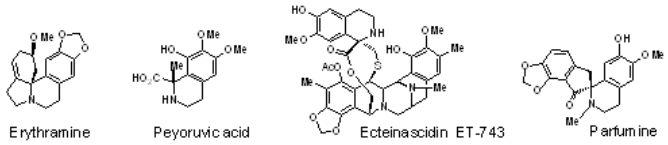
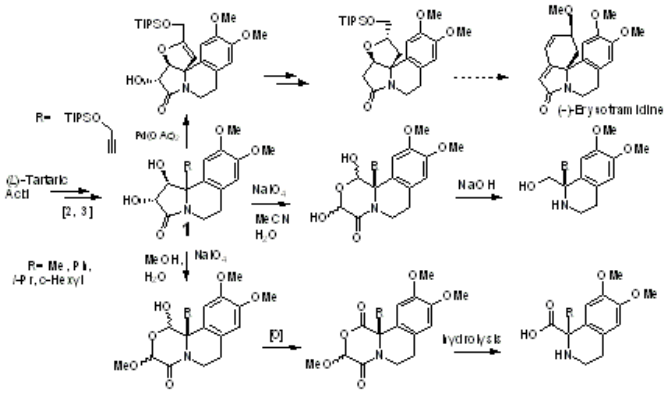


Figure 1.

The stereoselective synthesis of isoquinoline alkaloids with quaternary carbon stereocenter is not trivial, and generally utilize a chiral starting material. We have recently described a simple stereocontrolled synthesis of 10b-substituted hexahydropyrrolo-isoquinolines **1** from *L*-tartaric acid [2, 3]. The easy transformation of **1** into optically salsolinol and salsoline-1-carboxylic acid will be reported. (Scheme 1) The new strategy for the synthesis of *Erythrina* family alkaloids will be discussed.



Scheme 1

References:

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15:50 Oral

Diffusion ordered NMR spectroscopy application to medicines control and research

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DOSY NMR (Diffusion Ordered Spectroscopy) is a powerful method to analyse the mixtures of chemical species in solution.[1] (and references therein) It relies on measurement of translational diffusion coefficients, Dt x 10^{-10} [m^2/s] for species in solution. Each spectral line in 1H NMR spectrum of a given chemical entity is characterised by the same Dt , providing the line is separated from spectral lines of other substance in solution. The larger difference in MW of the two species , the larger the difference in Dt coefficients between them. Separation of lines is better achieved with higher magnetic fields. Large molecules diffuse much slowly than smaller ones.

The lecture will cover the title topics chosen from authors' research and drug control activity.

This, *inter alia*, applies to:

1. Differentiating between oligomers of proteins (insulin) [2]

2. Establishing medicines binding to biomolecules (topotecan) TPT [3]

3. Differentiating between OSCS impurity of ca.18-20 kDa MW range with

LMWH of ca.6 - 8 kDa range or unfractioned heparins.[4]

The results concerning first Ph.Eur. monograph on heparin identity test by 1H NMR will be presented.

1. Nilsson M., Gil A.M. Dellagillo I, Morris G.A. ***Anal. Chem***. **2004**, *76,* 5418-5422

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3. ???

4. I. McEwen, B. Mulloy, E. Hellwig, L. Kozerski, T. Beyer, U. Holzgrabe, A. Rodomonte, R. Wanko, J-M. Spieser **Pharmeuropa Bio 2008-1** 31-39.

16:10 Oral

Polymorphism and the level of P450 gene expression in drug metabolism

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Metabolic pathways of drugs and other xenobiotics are strongly influenced in human organism by the polymorphism of cytochrome P450 and other phase I and phase II enzymes as well as by the level of their gene expression. The highest number of P450 isoenzymes, of various activity, over 40 polymorphic forms, were determined for CYP2D6 isoenzyme. Only two forms of lower activity were found in the case of CYP2C9, whereas *CYP3A4* mutations have not influenced metabolic activity of the enzyme. Three transcription factors PXR, CAR and AhR participate in the expression regulation of P450 genes, *CYP3A4, CYP2B6* and *CYP1A2*, respectively. Receptors PXR, CAR and AhR are transported to the nucleus, and activated to heterodimers. Then, after following coactivation heterodimers bind to PBREM, XREM or DRE regulatory motifs of DNA, respectively.

A variety of drugs, including antitumor agents, were found to modulate the level P450 gene expression by interaction with PXR or CAR receptors, what is a source of drug-drug interactions. Molecular mechanisms of these interactions are still not known in details. However, there were demonstrated that antitumor drug paclitaxel but not docetaxel is an agonist of PXR. Tamoxifen, being the antagonist of the estrogen receptor binds similarly as an estradiol, while cyclophosphamide used together with PXR and CAR agonists rifampicyne and phenobarbital, is metabolized very fast to toxic metabolites.

In our studies we intended to elucidate whether two antitumor agents 1-nitroacridine and triazoloacridinone derivatives affect the level, gene expression and enzymatic activity of CYP3A4 isoenzyme in HepG2 cancer cells. Western blot analysis, RT-PCR procedure and HPLC control of the reaction pathways were applied. The preliminary results indicated that the studied compounds influenced the action of CYP3A4 in the specific manner. The character of this influence seemed to corroborate with the differences in their metabolic pathways studied earlier.

16:30 Oral

Enantioselective reduction of α-dialkylamino ketones

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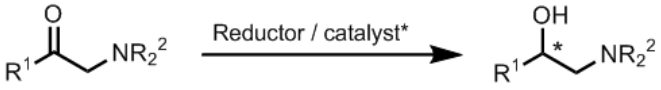
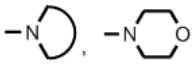
Abstract

Catalytic hydrogenation has been widely applied to achieve asymmetric reductions of aryl-chloromethyl ketones[1,2].
A highly selective and convenient method of asymmetric synthesis of β-N,N-dialkylamino alcohols has been developed by the transfer hydrogenation (ATH)[3]. The importance of this class of physiologically active compounds is increasing. Many of them are β-blockers and exhibite psychotropic activity. The reaction affords the corresponding β-N,N-dialkylamino alcohols with high enantioselectivity, 97-99%.



R^1 = aryl, heteroaryl, alkyl

NR_2^2 = alkyl, 

(-)-Macromerine, a natural alkaloid of the Coryphantha macromeris cactus, has been obtained by the transfer hydrogenation of α-N,N-dialkylamino ketone in the presence of Noyori's ruthenium catalyst with 98% ee.

1) Hamada T., Torii T., Izawa K., Noyori R., Ikariya T., Org. Lett., **2002**, *4*, 4373.
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3) T. Kosmalski, A. Wojtczak, M. Zaidlewicz. „Asymmetric synthesis of α-dialkyloamino alcohols by transfer hydrogenation of α-amino ketones". Tetrahedron: Asymmetry, **2009**, *20*, 1138.

Coffee break
Wednesday afternoon, 12 May, 16:50

Free time
Wednesday afternoon, 12 May, 17:20

Closure of the MKNOL Conference - Closing Banquet
Wednesday evening, 12 May, 19:00

Thursday, 13 May

Breakfast
Thursday morning, 13 May, 7:30

Departure
Thursday morning, 13 May, 9:00

Abstracts
in author alphabetical order

Invited oral

RNAi - breakthrough technology gives hope for breakthrough therapeutics

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The discovery of RNA interference (RNAi) in eukaryotic cells has opened new possibilities for drug development. Target-specific gene silencing via short interfering RNA (siRNA) displays many advantages over traditional pharmaceuticals. The unique siRNA nucleotide sequence benefits in higher drug specificity. Besides designing target-optimized siRNA oligonucleotides, one of the major challenges is to establish the efficient delivery system facilitating selective targeting altered tissues. The proper sequence-delivery combination provides these innovative therapeutics with unrivalled specificity and tolerability.

Another pivotal issue which has to be taken into account designing siRNA drugs is to provide them with an appropriate pharmacokinetic and pharmacodynamic properties. This is often achieved by an introduction of variety of chemical modifications into the siRNA molecules e.g. O-methyl groups added to the 2′ position of the ribosyl ring.

The ongoing extensive studies on RNAi resulted in introduction of several potential siRNA drugs into clinical trials. Celon Pharma takes part in a global research on siRNA therapeutics with its deep pipeline of innovative products including RNAi-based drugs.

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