Book of Abstracts

The Sixth Multidisciplinary Conference on Drug Research

Book of Abstracts: The Sixth Multidisciplinary Conference on Drug Research

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Programme

Sunday, 25 May

Odjazd autokarów z Uczestnikami spod Dworca Centralnego w Warszawie. Przyjazd do Przemyśla około godz. 18:00-19:00
Sunday afternoon, 25 May, 12:00

Rejestracja Uczestników

Rejestracja Uczestników zgodnie z zakwaterowaniem. (Informację o miejscu zakwaterowanie Uczestniczy otrzymają drogą mailową)
Sunday afternoon, 25 May, 15:00

Kolacja

Kolacja w miejscu zakwaterowania
Sunday evening, 25 May, 19:00

Monday, 26 May

Otwarcie Konferencji
Monday morning, 26 May, 8:30

First Session
Monday morning, 26 May, 8:45

8:45 Invited oral

The Concept of the Central Strategic Program - Innovative Medicines

Wiesław Szelejewski

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9:15 Invited oral

Genetic contribution to all cancers: implications for pharmaceutical treatment

Jan Lubiński

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The aim of the study is to verify the hypothesis that genetic polymorphisms are associated with the predisposition to all malignancies. Using as a model breast cancers from the homogenous Polish population (West Pomeranian region) after stratification of 977 patients by age at diagnosis (under 51 years and above 50 years) and by tumour pathology (ductal cancers—low and high grade, lobular cancers, ER-positive/negative) we tested this hypothesis. Altogether 20 different groups of breast cancer cases have been analyzed. The results were compared to a group of unaffected controls that were matched by age, sex ethnicity and geographical location and originated from families without cancers of any site among relatives. Molecular alterations selected for analyses included those which have been previously recognized as being associated with breast cancer predisposition. Statistically significant differences between the breast cancer cases and controls were observed in 19 of the 20 analyzed groups. Genetic changes were present in more than 90% of the breast cancer patients in 18 of 20 groups. The highest proportion of cases with constitutional changes—99.3% (139/140) was observed for lobular cancers. The number and type of genetic marker and/or the level of their association with the specific cancer predisposition was different between groups. Markers associated with majority of groups included: BRCA1, CHEK2, p53, TNRnTT, FGFRnAA, XPD CC/AA and XPD GG. Some markers appeared to be group specific and included polymorphisms in CDKN2A, CYP1B1, M3K nAA, and RS67.

9:45 Invited oral

Unusual application of X-Ray diffraction in drug control – searching for counterfeit and substandard pharmaceuticals

Zbigniew E. Fijałek[1,2], Jan K. Maurin[2]

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Counterfeit and substandard drugs, usually illegally marketed and most probably illegally manufactured have become a noticeable problem in most countries recently. As it might be expected, the spectrum of fake pharmaceuticals ranged from antimicrobial, antihistaminic, hormone through anti-sexual dysfunction remedies. The scope of illegally marketed drugs is different in poor, developing countries and these in North America and Europe. Still in many countries the health care authorities are not fully aware of the problem and health risk for the population, however several initiatives to change this situation have been taken lately. The lack of specific regulations concerning internet marketing and the lack of systematic control of the pharmaceutical market, both legal and illegal, increases the risk. In such case the need of fast, easy, reliable and not expensive methods of drugs screening is essential. No wander that the NIR (near infrared) spectroscopy became popular in this respect.

Our results obtained for counterfeit samples of Viagra® and Traditional Chinese Herbal Medicines show that the X-ray powder diffraction, especially when using new, fast diffraction techniques, including multilayer mirrors and position-sensitive counters, is a method suitable for pharmaceutical market screening control for counterfeit and substandard drugs. This method can easily discriminate fake and original samples, even by visual examination of diffraction patterns, what can be done also by not highly experienced employees. All statistical methods for principal components analysis etc., usually utilized for spectroscopic data evaluation e.g. NIRS, can be also

employed, however are not necessary. Well-resolved picks and their characteristic 2θ values can be used for examining powder diffraction databases in qualitative composition analysis. Diffraction patterns can serve as fingerprints of manufacturers – both legal and illegal – since even small changes in composition are visible. It is worth noting, however, that X-ray powder diffraction, same as NIR spectroscopy, is not a method of trace analysis, and that for those purposes other methods are more sensitive and hence more reliable. The power of XRPD in medicines control demonstrates its unusual application for identification of rubber closures fragments.

10:15 Oral

Focus on drug discovery – new opportunities in life science sector in Poland.

Krzysztof Brzózka

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One of the major problems encountered by Polish scientists working in the drug discovery field in Poland, is a shortage of business partners capable of conducting preclinical development phase. Since only few companies in Poland are working on innovative therapies, the interaction between the academia and industry should be identified as a bottleneck of Polish ingenious drug development and marketing. Therefore companies that bridge the progression in the pipeline using a value-added approach are urgently needed in the Polish medical biotechnology area.

The first company in Poland specialized in preclinical drug development is Selvita. Our mission is to create a multidisciplinary platform which provides wide range of possibilities necessary to translate innovative discoveries into preclinical candidates with high chances of becoming marketed in the future. Expert-based evaluation of the project, decrease in the attrition rates through smart *in silico* target and compound selections, enriched by a wide range of in vitro and in vivo assays performed by our company are the key features allowing us to focus on the rapid and successful advancement of a drug candidate in the pipeline. This presentation provides comprehensive information about Selvita's novel approach in the field of Polish medical biotechnology and our integrated procedure of modern therapeutics development.

10:35 Oral

Registration dossier of chemical active substance

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During the registration process quality of medicinal products is assessed on the basis of chemical documentation of active substance and chemical, pharmaceutical and biological documentation of finished product.

In the European Union a common format of documentation – CTD (Common Technical Document) is applicable for every the type of regulatory procedure (centralized procedure CP, decentralized procedure DCP mutual recognition procedure MRP and national procedure NP).

Data concerning quality of medicinal product are included in Module 3.2.S and must contain the following information:

3.2.S.1 General Information

3.2.S.2 Manufacture

3.2.S.3 Characterisation

3.2.S.4 Control of Drug Substance

3.2.S.5 Reference Standards or Materials

3.2.S.6 Container Closure System

3.2.S.7 Stability

There are three possible ways for submission of documentation of quality of the active substance:

· Active Substance Master File (ASMF)

· Certificate of Suitability of the Monographs of the European Pharmacopoeia

· Module 3.2.S

European legislation requires manufacturing of every active substance according to GMP.

Coffee break

Monday morning, 26 May, 10:55

First Session

Monday morning, 26 May, 11:25

11:25 Oral

The ligand binding to the serotonin transporter

Małgorzata Jarończyk[1], Zdzislaw Chilmonczyk[1], Aleksander P. Mazurek[1], Gabriel Nowak[2], Andrzej J. Bojarski[2], Mateusz Nowak2, Karol Wołosewicz[3], Ingebrigt Sylte[4], Kurt Kristiansen[4], Aina W. Ravna[4]

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Abnormalities in serotonin levels can lead to depression and anxiety, as well as other mental disorders such as obsessive compulsive disorder. The serotonin transporter (SERT) plays a key role in the regulation of synaptic serotonin (5-hydroxtryptamine, 5-HT) levels and therefore is the major target for antidepressants including both the tricyclic antidepressants and selective serotonin reuptake inhibitors. The antidepressants affect the concentration of the serotonin by inhibiting the reuptake of the 5-HT into nerve cells. To examine the molecular mechanism of their different binding affinities the interactions between ligands and serotonin transporter were studied.

In the present study molecular modelling techniques were used to study the interaction between ligands and SERT. The SERT model was based on the crystal structure of the bacterial homologue Na^+/Cl- dependent neurotransmitter transporters from *Aquifex aeolicus* ($LeuT_{Aa}$) [1-3]. For the docking studies two sets of ligands were considered: ligands with quite high affinity, and ligands with a nitro group at the quinoline moiety with much lower affinity for SERT. The ligands were docked to the SERT model using the ICM (Internal Coordinate Mechanics) molecular modelling software.

The docking studies indicate that the binding site of the SERT model constituted amino acids in transmembrane helices (TMHs): 1, 3, 6, 8 for all the studied ligands. The ligands without a nitro group at the quinoline moiety interacted also with amino acids in TMH 10. In complexes of SERT and the ligands with the nitro group at the quinoline moiety a steric interactions between ligand and transporter protein were observed.

Additionally, a putative substrate binding site corresponding to low affinity binding was identified in the pore formed between TMHs 1, 6, 10 and 11. The docking of ligands to this binding site pinpointed an additional region that might be considered for development of new inhibitors.

Acknowledgement
This study was partly supported by the Research Network coordinated by Insititute of Organic Chemistry Polish Academy of Sciences

References

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[3] Jarończyk M., Chilmonczyk Z., Mazurek A.P., Nowak G., Ravna A.W., Kristiansen K., Sylte I., The molecular interactions of buspirone analogous with the serotonin transporter, Biochemical Pharmacology, submitted.

11:45 Oral

Hydrogel coatings and a local drug delivery

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Hydrogel coating as a method for solid substrate surface modification, beside advantages like improving material biocompatibility, hydrophilization and lubrication, brings the additional possibility of the active agent incorporation. Combination of the hydrophilic matrix and the hydrophobic drug seems to be especially promissing.

We have developed a method for polymer coating by water insoluble hydrogel, based on polyurethane (PUR) and polyvinylpyrrolidone (PVP), designed for medical polymeric devices. This hydrogel layer was characterized by the means of the Fourier Transform Infra-Red Attenuated Total Reflection (FTIR-ATR) spectroscopy, static and kinematic friction factor relative to the uncoated backbone material and against porcine tissue counter-face, water wetting angle and microscopic observations.

Our tests confirmed changes in surface composition, superhydrophilicity and enormous lubricity in hydrated state (even 10-fold friction factor reduction). In case of urethral poly(vinyl chloride) catheters with hydrogel coated inner surface the capillary action phenomenon was observed, proving high affinity between coating and water molecules. Experiments with Escherichia coli biofilm growth on unmodified backbone material and hydrogel coated one revealed a significant decrease in a number of adhered bacterial colonies.

Hydrogel modified surface have another advantage over unmodified one, while it can serve as a drug reservoir for a local drug delivery. There are cases when drug dosage time should last at least few hours, but no longer than 3 days. It can be desirable in case of implantation of devices like tracheotomy tubes, when anti-inflammatory active substance should be released at the very beginning to prevent later side effects like tracheal stenosis, but later can not interrupt normal cell divisions in subsequent levels of healing process and epithelium formation. In case of hydrophilic matrix with hydrophobic drug these profile can be easy obtained due to its behavior as the swelling controlled drug release system.

In further investigations we used antibacterial triclosan and anti-inflammatory dexamethasone as model hydrophobic drugs, which were incorporated in two modes: as a component of the solution in any step of the coating formation or through the additional impregnation bath. The recent mode allows also for modification of devices like silicone catheters or polyglycolic acid resorbable sutures without polymeric coating step.

The effects of active agent incorporation were then verified through drug dissolution tests to the phosphate buffered saline (PBS) with 20% methanol or ethanol, extraction, and, in case of germicidal drug, by the inhibited growth zones method. The dependency of the rate of drug dissolution and the load capacity, as well as coating stability on the process parameters was investigated.

We also observed an interesting “spraying effect” during hydrogel swelling, when solid microparticles of the drug were precipitaded out of the coating layer to the surrounding solution. This phenomenon can be utilized in design of drug release systems, reacting on water content increase as the start signal.

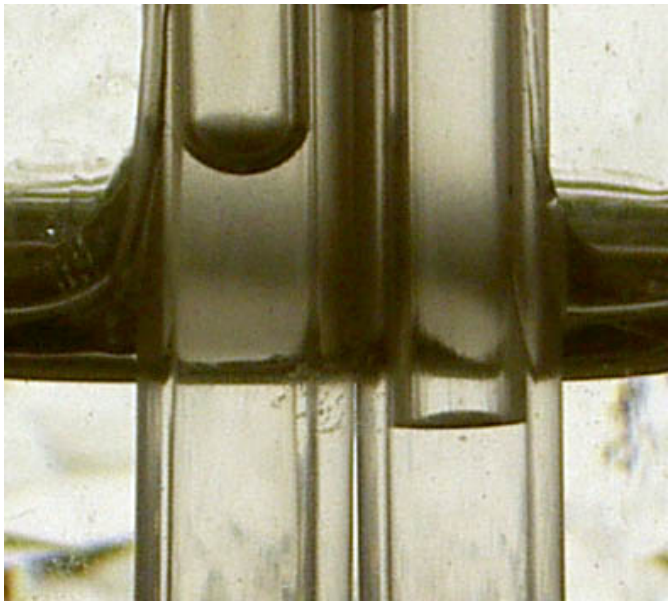

Fig. 1. The capilary action: left - hydrogel coated inner surface, right - unmodified (PVC catheters, outer diameter 5 mm; distilled H_2O).

12:05 Oral

Novel medium for monomer proteins structure elucidation. Human insulin structure in water/ acetonitrile solvent. Search for nonamyloidogenic conditions

Lech Kozerski[1,2], Wojciech Bocian[1,2], Elżbieta Bednarek[2], Robert Kawęcki[1], Jerzy Sitkowski[1,2], Anna Tarnowska[1]

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Here we present evidence that in water /acetonitrile solvent detailed structural and dynamic information can be obtained for important proteins that are naturally present as oligomers under native conditions. An NMR-derived human insulin monomer structure in H_2O/ CD_3CN, 65/35 vol %, pH 3.6 is presented and compared with the available X-ray structure of a monomer that forms part of a hexamer (Acta Crystallogr. **2003** Sec. D59, 474) and with NMR structures in water and organic cosolvent. In particular, the detailed comparison of a structure in 20% acetic acid is presented and discussed. The analysis using PFGSE NMR, temperature-dependent NMR, dilution experiments and CSI proves that the structure is monomeric in the concentration and temperature ranges 0.1 – 3 mM and 10 – 30 ^oC, respectively. The presence of long-range interstrand NOEs, as found in the crystal structure of the monomer, provides the evidence for conservation of the tertiary structure. Starting from structures calculated by the program CYANA, two different molecular dynamics simulated annealing refinement protocols were applied, either using the program AMBER in vacuum (AMBER_VC), or including a generalized Born solvent model (AMBER_GB).
The nonamyloidogenic property of a protein depends essentially on intrinsic propensity of AA sequence to retain hydrophobic core on one hand and on its side chains character determining the solvation shell which should prevent extensive aggregation. The water/ acetonitrile solvent allows to retain tertiary structure exactly matching the native structure of a monomer embeded in a crystal of hexamer and prevents the aggregation. It therefore potentially allows to study the effect of various external factors on misfolded conformation of a monomer in a critical stage leading to amyloid formation.

Acknowledgements: This work was supported by the network "Synthesis, structure and therapeutic properties of compounds and organic substances".

12:25 Oral

Protein modeling in silico: providing new targets for a drug design

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Major bottleneck to develop innovative treatments for human diseases is the lack of new "drugable" protein targets. Most new drugs interact with the same protein targets as the old equivalents, so the progress in diseases treatment is slower than expected. Thus, structures of new drug targets are needed to guide drug discovery and therefore to develop new, even breakthrough, methods of therapy.

Thanks to international effort of genome sequencing projects, last years brought massive amount of new amino acid sequences. Although experimental methods for determining protein structure are providing new structures, they cannot keep the pace at which sequences are resolved. For a large fraction of proteins whose structures will not be determined experimentally, computational methods can provide valuable information.

Selvita's goal is to provide computational methods that accurately predict protein structure and protein complexes. Our modeling technology is based on the CABS model, state-of-the-art approach to protein structure prediction. Our innovative, comparative modeling strategies enable high-accuracy structure prediction using existing, even remote, protein homologs as scaffolds. Combining results of structure prediction and modeling with lead optimization techniques provides a unique opportunity for a new level of rational drug design.

12:45 Oral

Bortezomib in multiple myeloma: treatment and retreatment. A single center experience

Maria Kraj, Krzysztof Warzocha, Ryszard Pogłód, Beata Kwaśniak

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Background. The proteasome inhibitor bortezomib induces apoptosis, reverses drug resistance of multiple myeloma (MM) cells, and affects their microenvironment by blocking cytokine circuits, cell adhesion and angiogenesis. The aim of the study was to evaluate the efficacy and safety of bortezomib in the treatment and retreatment of relapsed MM and its aggressive variant - plasma cell leukemia

(PCL).

Material and methods. We enrolled 4 patients with PCL and 17 patients with relapsed MM who have failed at least two prior lines of treatment, including 4 patients treated with high dose therapy and autologous stem cell transplantation. The time from MM diagnosis to onset of bortezomib therapy varied between 3 and 149 months. Patients received bortezomib 1.3 mg/m^2 as i.v. bolus twice weekly for 2 weeks, on days 1,4, 8 and 11, followed by 1 week without treatment, for up to six cycles. In 6 patients doxorubicin and dexamethasone were added to the regimen (=PAD regimen) and in 2 patients with disease sensitive to the bortezomib therapy, bortezomib was re-administered in the consecutive relapses.

Results: Partial response was achieved in 2 of 6 patients treated with PAD regimen and in 3 of 12 evaluated patients treated with bortezomib alone. In one case of recurrent MM who was three times treated with bortezomib, with preserving a 18-month break between first and second and 8 months between second and third therapy courses, all three treatments resulted in achieving a near complete remission which lasted each 6 months. The patient 120 months since MM diagnosis, further on bortezomib therapy, feels good. In one patient with primary PCL, a near - complete remission was achieved subsequent to induction PAD treatment. Following cyclophosphamide, peripheral blood stem cell were successfully harvested and autologous peripheral blood stem cell trasnsplantation (PBSCT) was performed. Time to neutrophil > 0.5 x 10^6 engraftment was 20 days and time to platelet > 20 x 10^6 engraftment was 17 days. PBSCT led to complete remission which lasted 7 months. Partial remission was achieved subsequently to relapse retreatment with PAD. At present, the patient is further on bortezomib therapy, in partial second remission, 22 months after diagnosis of PCL.

Adverse events: treatment with bortezomib was withheld in 5 patients (after 1, 2, 3, 4 and 5 cycles, respectively) because of skin lesions (erythema multiforme), subileus and aggravation of peripheral neuropathy. Side effects seen in the study included also herpes zoster in 3 patients, pyrexia, infections, nausea, vomiting, abdominal pain, pain in limbs, hypotension, thrombocytopenia.

Conclusions: In relapsed myeloma the rate of response to bortezomib alone is 25 percent, with a duration of response of 6 months. A presented here cases demonstrate the efficacy of repeat bortezomib treatments in the patients with recurrence of myeloma who were previously sensitive to such a treatment. We suggest, bortezomib in combination with other agents may be considered as an initial treatment of primary PCL. PAD regimen is effective and does not prejudice peripheral blood stem cell collection or subsequent engraftment.

Lunch break

Monday afternoon, 26 May, 13:10

Second Session

Monday afternoon, 26 May, 15:00

15:00 Invited oral

Human brain tumor therapy through interference RNA intervention

Jan Barciszewski[1], Stanisław Nowak[2], Ryszard Żukiel[2], Eliza Wyszko[1], Mirosława Z. Barciszewska[1]

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Brain malignancies are a significant source of morbidity and mortality. Glioblastoma multiforme (GBM) is the most common and the most malignant tumor occurring in the central nerwous system and is notorious for its highly infiltrative and invasive behaviour.

Despite surgery, radiotherapy and chemotherapy, about 40% of patients with GBM die within ca 6-8 months after diagnosis. Establishment of a curative treatment for glioblastoma will require better understanding of the molecular mechanisms underlying the proliferation, migration, and invasion of the tumor cells.

Several lines of evidence have been accumulated regarding signal transduction pathways in glioma cells. Amplification of the epidermal growth factor receptor (EGFR) gene occurs in ca 50% of glioblastomas, and the tumor cells usually show overexpression of EGFR. It stimulates the activation of phosphatidylinositol-3-0H kinase (PIK3). Loss of a tumor suppressor gene called phosphatase/tensin homolog on chromosome 10 (PTEN) is also frequently detected in glioblastoma as well as breast, prostate and endometrial carcinomas and melanoma. Recently it has been shown that tenascin-C (TN-C) is strongly expressed in human malignant gliomas and can stimulate glioma cell proliferation and angiogenesis. TN-C is a dominant epitope in GBM.

Disappointing results in the therapy of GBM have fuelled a search for new treatment modalities.

RNA interference (RNAi) is a eukaryotic regulatory mechanism that uses double stranded DNA (dsRNA) molecules as triggers to direct sequence homology-dependent, post-transcriptional gene silencing. RNAi represents a particularly powerful method which includes the RNAi-mediated targeting *in vitro* and *in vivo* for functional studies of various genes whose expression is known to be up regulated as well as the development of novel therapeutic approaches based on gene targeting.

RNAi phenomenon relies on a multistep intracellular pathway which can be roughly divided into two phases. In the first one, endogenous or exogenous dsRNA molecules present within the cell are processed through the cleavage activity of RNase III-type activity (Dicer) into short 20-30 nucleotide fragments called siRNAs. In the second step, siRNAs as well as many proteins including nucleases and helicase form RNA-induced silencing complex (RISC). Through unwinding of double stranded siRNA, the complex becomes activated with single–stranded, noncoding siRNA which guides the RISC complex to its complementary target RNA causing its endonucleolytic cleavage.

We used double stranded interfering RNAs (dsRNAs) to reduce

tenascin-C expression in brain tumor cells. RNAi was injected into postoperative area of 46 patients. The follow up study with MRI and CT clearly show increased survival at better quality of life. The technology we called interference RNA intervention (iRNAi).

Acknowledgements: This work was supported by the network "Synthesis, structure and therapeutic properties of compounds and organic substances".

15:30 Invited oral

Therapeutic monoclonal antibodies in clinical and scientific data*

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Engineered monoclonal antibodies constitute the most rapidly growing class of human therapeutics, with over 20-years history. Since introduction in 1986 of CD3-specific muromonab (OKT3, murine), adverse human anti-mouse antibody (HAMA) immune reaction against the animal protein was one of the major obstacles to overcome. Human monoclonals can be obtained using several molecular biology approaches, including transgenic mice (HuMab-Mouse, Xenomouse), phage display, human hybridomas or immortalized human B-lymphocytes. From strictly of murine source (*-momab*), chimeric constructs (*-ximab*), humanized (*-zumab*) to recently entirely human (*-mumab*), this class of biological response modifiers represents now established and growing market of pharmaceuticals. Antibody drugs are expensive: large expense of drug development and high cost of manufacturing is a limitation despite an impressively high success rate in regulatory approval, about 25%, as compared with approximately 11% success rate for small-molecule drugs. In general antibodies are safe and well tolerated, limitations include infusion reactions, immunosuppressive-like adverse effects. Approximately 500 monoclonal antibodies are pre-clinical, about 160 are in clinical development and over two dozens (including the *in vivo* diagnostic monoclonals) are approved for human use. These include unmodified IgG molecules, Fab fragments, radioimmunoconjugates, antibody-drug conjugates.

*The article refers to the Polish edition of the author's book (copyright Stefan Ball): *Zumaby terapeutyczne przeciwciała monoklonalne* Ed. Wydawnictwo Medyk, Warszawa (2007), 111 pp.

16:00 Oral

Synthesis and Screening of the Library of the Supramolecular Structures Formed by N-Lipidated Oligopeptides for Selection of Host Active as Artificial Esterase

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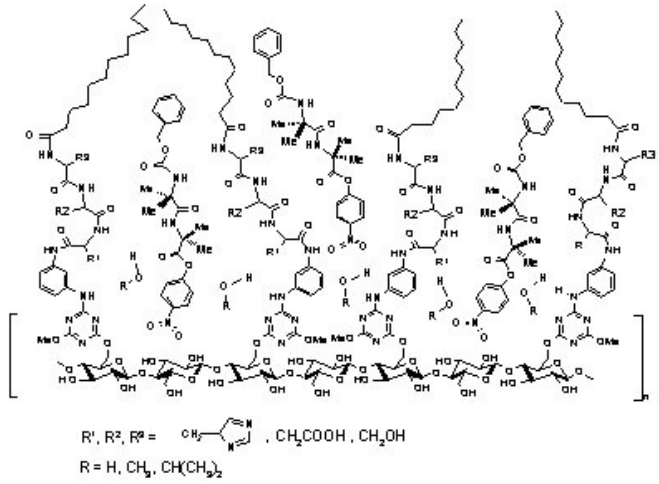
We designed and prepared supramolecular structures formed from N-lipidated oligopeptides immobilized in the regular pattern on the cellulose surface which are capable of specific binding of ligand molecule. Due to the conformational flexibility of the fragments forming the supramolecular structure, the shape and prosperities the binding cavities are adjusted the most effectively to requirements of the guest molecules. The previous studies documented that process of binding guest molecules is highly selective, reversible and competitive. Therefore, we supposed that under favorable circumstances the structures could operate as catalysts if suitable molecular fragment are included inside the binding pocket. In order to verify this hypothesis we prepared library of supramolecular hosts with catalytic triade: His Asp(Glu) Ser, incorporated into the binding pocket [1].



The rate of hydrolysis of *p*-nitophenyl esters of N-protected amino acids was measured by spectrophotometric determination of liberated *p*-nitrophenole in buffered, aqueous methanol and compared with appropriate data obtained in the absence of catalytic structures. It has been found, that several supramolecular structures shown catalytic activity. The most active catalysts were selected from the library and their stability, selectivity and ability for re-use was studied.

Acknowledgement: This work was supported by the Grant **WIDDOK/SC/2007/19**

[1] Piątkowska, N,; Frączyk, J.; Kolesińska, B.; Kamiński, Z.J., XIX Polish Peptide Symposium, Pułtusk 23-27 września 2007, p. 111.

16:20 Oral

Synthesis of selected ^{14}C radiolabelled compounds in recent investigations of new analytical method applied in accelerated drug development (EUMAPP).

Wojciech Łuniewski, Wojciech J. Szczepek

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Accelerator mass spectrometry (AMS) is a method for quantifying rare isotopes and is now being applied in biomedicine. It measures radioisotopes such as radiocarbon, with zepto- or attomole sensitivity and high precision and throughput, allowing safe human pharmacokinetic studies involving microgram doses, compounds

possessing low bioavailability or toxicology studies where administered doses must be kept extremely low. It is used to study absorption, metabolism, distribution, binding, and elimination which can be the measure with high precision after appropriate sample definition. The main application of AMS in drug discovery and development will be in the analysis of 14-carbon. The great sensitivity of AMS analysis allow much lower amounts of ^{14}C to be used than for conventional counting methods. This makes it easier to use ^{14}C for in vitro , preclinical and clinical research programmes. AMS is a technology that should increase human and environmental safety as well as mark up new research directions.

Increasing chemical complexity of new drug candidates results in need to develop innovative synthesis of carbon-14 labelled pharmaceuticals. Limited number of labeled precursors as well as short time-lines force chemists to develop new reagents or to adapt existing methods to labelled syntheses. Selected examples of sumatriptan and compound S 19812–1 syntheses conducted in PRI illustrate some of creative strategies used to overcome these synthetic problems.

Examples of application of common small molecule reagent, such as carbon-14 lebelled potassium cyanide are presented. A few strategies for radiolabelling are also described including the degradation of target molecule for accessing necessary intermediate for synthesis of radiolabelled sumatriptan.

Coffee break
Monday afternoon, 26 May, 16:40

Discussion Panel
Towards new therapies. Introductory remarks, Grzegorz Grynkiewicz: Microdosing - clinical trials phase 0 (FP6 project:EUMAPP)
Monday afternoon, 26 May, 17:10

Free Time
Monday evening, 26 May, 18:40

Gala Banquet
Monday evening, 26 May, 20:30

Tuesday, 27 May

Third Session
Tuesday morning, 27 May, 8:30

8:30 Invited oral

Strategies of the synthesis of oxa- and carba-penams and cephams

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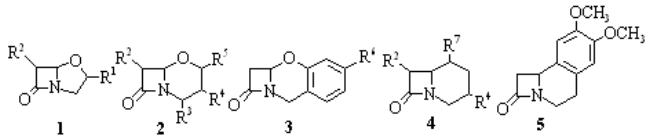
Two synthetic strategies leading to the basic skeletons of the title compounds are discussed. The special attention is focused on the problem of stereocontrol in the formation of a desired configuration of the bridgehead carbon atom. The first one involves cycloaddition reaction between vinyl ethers or alkoxyallenes with chlorosulfonyl isocyanate. The second one is based on the nucleophilic substitution at C-4 of the azetidin-2-ones performed as intramolecular process.



1 2 3 4 5

R^1= Furyl, Phenyl, alkoxycarbonyl; R^2= H, CH_3, =$C(CH_3)_2$; R_3= H, CO_2Et; R^4=H, OH, =CH_2, =O; R^5= CH_3, CH_2OAc; R^6=H, OCH_3; R^7=Allenyl

[2+2]Cycloaddition of chlorosulfonyl isocyanate to chiral vinyl ethers proceeds with excellent diastereoselectivity whereas the same reaction with chiral alkoxyallenes provides moderate diastereoselectivity. In the latter case, however, the exo double bond next to the β-lactam carbonyl atom can be easily transform into variety of substituents.

The second methodology is based on readily available 4-vinyloxy-azetidin-2-one and allows formation only oxa- and carbacephams. Reaction proceeds *via* formation of acyliminium cation generated from the β-lactam fragment, which subsequently is trapped by a nucleophile to produce the corresponding bicyclic skeleton. Attempts to form penam skeletons using that waywere, so far, unsuccessful.

The antibacterial and antifungal properties of all β-lactams obtained were investigated to show, however, moderate activities.

Acknowledgements

This work was supported by a grant PBZ-KBN-126/T09/08/2004 and by a network „Synthesis, structure and therapeutic properties of compounds and organic substances".

9:00 Invited oral

Integrative genomics - an essential tool for development of molecular medicine

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Since understanding the molecular taxonomy of disease requires the introduction of molecular diagnostics into medical practice, molecular medicine would be a natural addition to the post-genomic era. However, current clinical practice employs only elements of molecular diagnostics, usually on the scale of single genes, while testing thousands of molecular markers for association to disease will need to employ quite new, high-throughput technologies used for sequencing, functional genomic and proteomic studies. While the sequence analysis of whole genomes is now possible, the techniques for the measurement of cellular metabolism on a genomic scale are at the early stages of development. Moreover, a vast amount of mo-

lecular information used for routine identification of molecular subclasses of disease will require new computational technologies. But even then, molecular medicine will still deal with methods for analyses of relationship between genetic background and environmental factors, underlying a disease development.

Collection, cataloging and comparison of biological data from genomes and drawing conclusions about their molecular basis are the fundamental roles of bioinformatics and systems biology. Both bioinformatics and systems biology represent the marriage of biology, mathematics, programming and data mining which offer the possibility of evolutionary analysis, description of the function and regulation of a given molecule, and also the *in silico* creation and simulation of molecular interactions within the genomic and proteomic networks. However, on account of the high complexity of the networks responsible for even the simplest phenotypic changes, such global analysis is still being developed. That is why real molecular medicine still needs to create new methodology for the use in medical practice which would allow to identify mechanisms governing the interdependence between genotype and phenotype.

Molecular medicine requires to expand the collection of large numbers of data sets from clinical-molecular, clinical-genetic and pharmacogenomic studies from representative populations of patients in the same stage of a disease. Like no other medical field, it will implement highly efficient methods of molecular imaging on a genomic scale and complex signaling pathway analysis by cooperation between representatives of physicians, experimental biologists and computational biologists. Such collaborative effort should create the fundaments of molecular medicine based on so-called integrative genomics.

Integrative genomics can integrate analytical data, including genetics, transcriptomics, proteomics and metabolomics in a more comprehensive manner by construction complex molecular networks to represent description of molecular phenotypes. In turn, molecular phenotypes may represent intermediate to clinically defined diseased phenotypes.

9:30 Oral

New phosphonate - based strategy for the synthesis of carbocyclic nucleosides: natural Neplanocin A and its unnatural enantiomer.

Maciej Mikina, Marian Mikołajczyk

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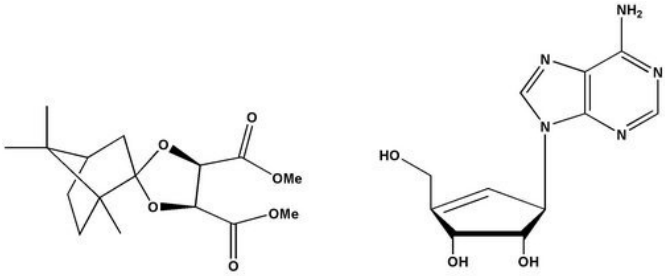
An urgency in the search for agents effective against Human Immunodeficiency Virus (HIV) caused an explosion of synthetic activity in the field of carbocyclic nucleosides and the discovery of several derivatives with potent antiviral activity [1]. Neplanocin A is a member of rich family of carbocyclic nucleosides which exhibit strong antiviral and antitumor activity. It was isolated in 1981 from *Ampullariella regularis.*



1 Neplanocin A

We report herein a new strategy for the synthesis of carbocyclic nucleosides exemplified by the synthesis of enantiomeric (-)- and (+)-Neplanocin A.A key to synthesis of Neplanocin A was the preparation of chiral precursor **1** from meso-tartaric acid . It was obtained from dimethyl meso-tartrate and D-camphor [2]. Then, it was converted into a mixture of diastereomeric 3-(phosphorylmethyl)-cyclopentenones which, after separation, were used in synthesis of both enantiomers of Neplanocin A. In the next step, the Horner reaction with *n*-pentanal afforded dienones, which upon ozonolysis gave the corresponding aldehydes as a result of the cleavage of the exocyclic C=C bond. Aldehyde groups were then selectively reduced to the alcohols using sodium triacetoxyborohydride. Protection of the hydroxyl groups by silylation with t-butyldimethylsilyl chloride and reduction of the ketone moieties gave the corresponding alcohols. Mitsunobu reaction with adenine and full deprotection of the condensation products afforded both enantiomers of Neplanocin A.

[1] Borthwick, A.D.; Biggadike, K. *Tetrahedron,* **1993,** *48,* 571-623

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9:50 Oral

Specific metabolism of antitumor agents with cytochrome P-450 isoenzymes

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Cytochrome P-450 refers to a family of heme proteins present in mammalian cells as well as in plants and prokaryotes. Substrates for mammalian P-450 enzymes (P-450) comprise endogenously synthesized compounds as well as xenobiotic compounds including medicines, food additives and environmental pollutants. The total level of liver microsomal P450s does not vary considerably among humans, however, the interindividual variations in the levels of P450 isoforms (CYPs) are shown. What is more, the variability in expression of CYPs was observed between various tissues and between tumor and normal cells. Such differences indicate the opportunities for the development of prodrugs, which are nontoxic to normal cells and are activated to cytotoxic agents only within the tumor tissue. Gene directed therapy may be also included in this respect. On the other hand, P-450 are capable of deactivating of anticancer drugs, thus, inhibitors of the specific isoenzymes in tumor cells would be developed as modulators of antitumor activity.

In view of all above, investigations on P-450 mediated metabolic transformations of antitumor agents are concentrated on several aspects in many laboratories. Firstly, the studies on the molecular mechanism of metabolic pathways allow to design chemical modification leading to less toxic or more active analogs. Secondly, finding out the contributions of specific human liver CYP enzymes to the activation of antitumor prodrugs will help to modulate the rate of metabolism in patients with various CYP levels and to predict drug selectivity towards normal and tumor cells. Furthermore, the analysis of the individual CYP induction or inhibition allows to predict the drug-drug interactions and helps to design the directed individual therapy in clinical trials.

The following metabolic issues of selected antitumor drugs will be presented: 1. cyclophosphamide was metabolized by CYP2B6, CYP3A4 and tumors with overexpressions of these enzymes were more sensitive to clinical trials, 2. transformation of tamoxifen occurred with CYP3A7 and CYP3A5, whereas this drug was also an inhibitor of recombinant CYP2B6, 3. product of 6a-hydroxylation of taxol was formed with CYP 2C8 and 3A4 and is competitively inhibited by quercetin, however, the pretreatment of taxol with corticoids increased the rate of metabolite formation. 4. the selective mechanism-based inactivation of P-450 was observed for antitumor drug thio-tepa and for other antitumor agents.

Studies on metabolic transformations of acridine antitumor agents are carrying out in our group. We are involved in the identification of metabolic products as well as in the mechanism of P450 level modulation. The transformations are performed with human liver microsomes of individual CYP overexpressions, with selected human *E.coli* recombinant CYPs and with human hepatoma HepG2 cells. The results in the field of CYP mechanism-based inhibition by our compounds and of metabolism in the tumor cells will be presented.

10:10 Oral

N-Phenylamino derivatives of 3-substituted pyrrolidine-2,5-diones as potential anticonvulsant agents

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One of the important core fragments of anticonvulsants is defined by a nitrogen heteroatomic system, usually a cyclic imide, at least of one carbonyl group and phenyl or alkyl groups attached to the heterocyclic system [1]. It was confirmed in our previous studies which have demonstrated the potent anticonvulsant activity among several classes of succinimides. Following these results, as part of our efforts to design new anticonvulsant agents in the present study we have synthesized a library of 60 compounds with *N*-phenylamino pyrrolidine-2,5-dione system as a core fragment and different substituents at the position-3 of imide ring (**Fig**. **1**).

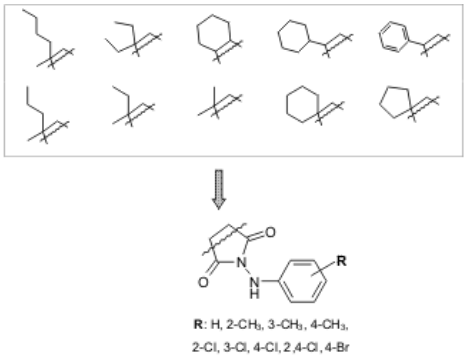

Fig.1.

The initial pharmacological studies were performed within the Antiepileptic Drug Development (ADD) Program in Epilepsy Branch, National Institutes of Health, National Institute of Neurological Disorders and Stroke (NIH/NINDS), Bethesda, MD, USA [2]. The results obtained revealed that majority of compounds investigated showed potent anticonvulsant activity in the animal models of epilepsy. It is noteworthy that several of these molecules revealed protection comparable with the marked AEDs. The results obtained enabled the extensive SAR discussion, which showed that the activity depended mainly on the substitution mode at the position-3 of succinimide as well as the kind of substituents at the phenyl ring.

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10:30 Oral

Cytotoxic and proapoptotic in vitro activity of two novel flavone complexes of ruthenium(II)

Maria M. Kasprzak[1], Elżbieta Zyner[1], Leszek Szmigiero[2], Justyn Ochocki[1]

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Organic complexes of ruthenium have become of great interest as potential antineoplastic drugs. Many of them have confirmed anticancer properties *in vitro* and *in vivo.* They are usually less toxic and more selective than platinum compounds and have different mechanism of action. To the date, two ruthenium complexes: NAMI-A and KP 1019 entered the first phase of clinical trials.

Two novel ruthenium(II) complexes with flavone ligands have been synthetised in our laboratory and named Ru-134 and Ru-138. We also tested their cytotoxic and proapoptotic activity in comparison to cisplatin. The properties were evaluated towards bladder cancer cells EJ and EJcisR (a subline approx. seven times more resistant to cisplatin than EJ cells).

Ru-134 is 12 times less cytotoxic towards EJ cells than cisplatin and 2.5 times less cytotoxic towards EJcisR cells than cisplatin. That

may suggest that it can partly overcome cisplatin resistance. Ru-134 is also 3.5 times less cytotoxic than cisplatin to HeLa cells. Double staining assay shows that Ru-134 can induce apoptosis quicker than cisplatin in their IC_{50} concentrations. Ru-138 is significantly more cytotoxic than Ru-134, it is only three times less cytotoxic than cisplatin towards EJ cells.

Our results and literature data encourage us to continue the research and to investigate the group of ruthenium compounds as potential anticancer agents.

Coffee break

Gromada Hotel
Tuesday morning, 27 May, 10:50

Third Session

Tuesday morning, 27 May, 11:20

11:20 Oral

The role of the Office for Medicinal Products, Medical Devices and Biocidal Products in pharmacovigilance activities in Poland

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Number of medicinal products is steadily increasing, therefore safety of drugs, together with efficacy and quality, is the essential condition of positive assessment of the benefit-risk ratio of the medicinal product.

Clinical trials, which provide the base of evidence in drug approval process, do not describe all the issues of safe drug usage in the post-authorization environment. It is necessary to collect and properly analyze information on adverse drug reactions (ADRs) and perform adequate activities – these are the main goals of pharmacovigilance system. The stakeholders of the system are pharmaceutical companies, healthcare professionals and competent authorities – in Poland it is the Office for Registration, who takes the responsibility. Part of this role is the causality assessment of individual ADR reports from territory of Poland and assessing the information collectively in order to identify if there is any new safety issue arising.

The process of accession to the European Community and required implementation of the European directives provided Polish competent authority tools, which allow performing pharmacovigilance activities and taking adequate decisions when the new safety concern is identified. Polish pharmaceutical law sets certain obligations to marketing authorization holders. Pharmaceutical company, which is planning submission of marketing authorization application is required to provide, as part of the application documentation, the description of pharmacovigilance system within the company and, if required, Risk Management Plan. The aim of the document is to minimize the risk of therapy with the product.

Another important consequence of implementing European directives is the fact, that there will be only one renewal process and after that the marketing authorization will be issued for an unlimited period of time. In this new context, assessment of drug safety data will be the key element, when deciding if the medicinal product needs any special monitoring, what are the conditions of safe therapy with the product or is it necessary to suspend the marketing authorization. This kind of evaluation will also be performed by the Office for Registration of Medicinal Products.

11:40 Oral

Electrically enhanced and controlled drug delivery through buccal mucosa

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The majority of drugs is administrated orally. Patient compliance is very high with this administration route, but bioavailability is often low - from the reason of first pass hepatic effect (e.g. Naltrexone NaCl) and unpredictable - from variable absorption conditions in digestive system. This popular drug form may also lead to negative gastrointestinal side effects (e.g. Galantamine HBr) or to cirrhosis additionally. Because patients usually don't accept injections there is a necessity to work out efficient, painless drug administration technique which will provide a high bioavailability of active substance and reduce the side effects. There is a need to determine new rout of administration and to work out therapeutic system which makes possible to optimize doses and provides an improvement of life comfort for sufferings.

The aim of this work was to determine the possibility of transmucosal iontophoretic delivery of cationic drug and to investigate ex vivo Galantamine HBr and Naltreksone HCl administration via buccal mucosa by applying the iontophoresis and to define of initial donor drug concentration (in the presence and without of competitive cations) and current density influences on drug flux.

The ex vivo iontophoresis through porcine buccal mucosa were conducted at room temperature in a horizontal two-chamber permeation cell with silver/silver chloride electrodes. Chambers contents were stirred to prevent concentration polarization. The donor was a drug solution in artificial saliva buffer or in distilled water. The acceptor was a phosphate buffer saline. The drug concentration was measured by spectrophotometry.

Drug transfer rate was estimated as a molar flux. The increase of current resulted in the increase of drug flux - it was a straight dependence in the range of 0.4 – 1.5 mA/cm2, but over this range it deviated from a straight line. From first and second Faraday's laws the theoretical maximum drug flux was calculated for the system in which the charge is carried by drug cations only. This value was compared to measured drug flux. The result was shown as a drug transport current efficiency which depends on current density. This function reaches plateau above the value 1.5 mA/cm2. The drug flux was independent on drug concentration in the absence of competitive cations in donor solution in whole range of solubility; however the drug flux was an exponential function of drug concentration in

the presence of small inorganic cations, which came from a buffer.

The present work shows that it is possible to deliver a therapeutic dose of drug using the iontophoresis in spite of that the percentage current efficiency reached only the level of 2-10 % under the experimental conditions. Iontophoresis enables control and enhance drug delivery through buccal mucosa. The initial drug concentration and current density provide an easy way to control the rate of drug delivery. These results suggest possibility of design and construction of an intraoral implant for systemic controlled drug delivery.

This work is a part of IntelliDrug Project and was financially supported by European Commission, 6th Framework Programme (www.biomedlab.ichip.pw.edu.pl).

12:00 Oral

Comparison of traditional and new methods of drug discovery

Marek T. Konieczny[1], Anita Bułakowska[2]

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Traditional approach to drug discovery has been based on screening of new chemical entities using relevant, mostly *in vivo*, disease models. The models worked as "black boxes" and consequently, a choice of compounds for screening was independent of our knowledge on etiology and nature of the disease.

Progress in all fields of biology, informatics, medicine and chemistry resulted in a new methodology of drug discovery, where the introductory screening was based on evaluation of interaction between a single receptor, so called target, and the tested molecules. The new approach has two important features, first, the biochemical tests are relatively easy for automation, what makes possible a massive screening of even hundreds of thousands of compounds, and secondly, the known structures of the receptor enable rational design of the tested molecules.

The new method has been in use for almost the past two decades, and comparison of effectiveness of the both approaches seems to be possible. Advantages and disadvantages of the modern and traditional methods are discussed.

12:20 Oral

Synthesis of higher carbon sugars. Unexpected rearrangement of higher sugar allylic alcohols

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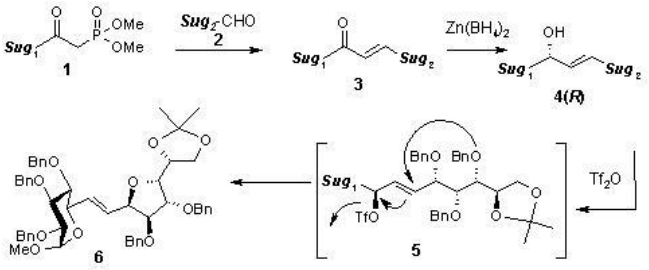
Higher carbon sugars containing more than 10 carbon atoms in the chain, although occuring very rarley in nature , are important components of some antibiotics. Methodology developed in our laboratory allows to obtain rather easily precursors of type **3**, higher sugar enones substituted at both terminal positions with monosaccharide sub-units [1].



Enone **3** was obtained in the reaction of phosphonate **1** with aldehyde **2**. Reduction of the carbonyl function provided two stereoisomeric allylic alcohols with the ***R***-isomer predominating. Attempts to invert the configuration at the carbinol center failed and the rearranged compound **6** was obtained as the sole product in this process.

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12:40 Oral

Newest triazole derivative with antifungal activity: a docking study

Alicja Nowaczyk, Bożena Modzelewska-Banachiewicz

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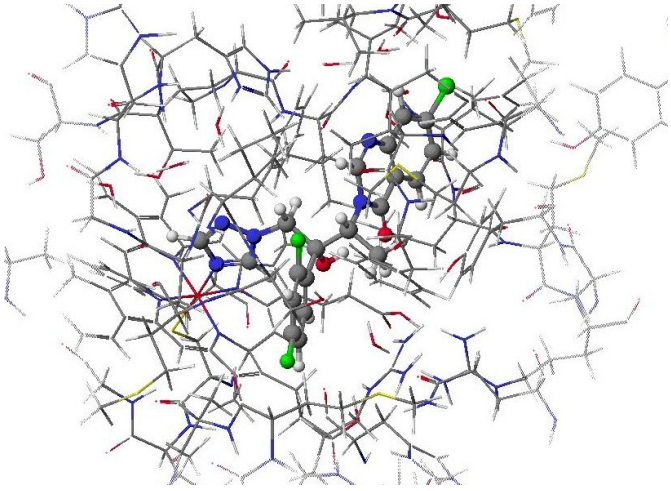
The incidence of fungal infections has increases significantly in the past two decades. The first azoles generation of antifungal inhibitors of CYP51, have revolutionized treatment of some serious fungal infections. Triazoles has been the leading agents for the control of fungal diseases of humans and animals for over 20 years [1]. According to this an azole derivatives are currently the most widely studied class of antifungal agents. The UR-9825 exhibit two important types of activity against certain fungal pathogens *i.e.* activity against yeast and filamentous fungi. Up to date the name of compound UR-9825 is Albaconazole and it is undergoing the Phase II clinical trials [2].

Our recent QSAR study for an azole series shown that the biological activity against pathogenic yeast and some filamentous fungi increases with a decreases of the log P and increase polarizability of the agent [3]. These parameters play an important role not only in the penetration and distribution phenomena but also in the interactions of the compound with receptor.

A knowledge-based strategy was used to perform docking experiments of fluconazole and UR-9825 into the catalytic site of the CYP51. The model was constructed on the basis of the sequence homology relationship with the crystal structure of the CYP51 of *Mycobacteriumtuberculosis* MT-CYP51 (pdb code: *1ea1*) as template [4].



Albaconazole molecule bind to the catalytic site of CA-CYP51 adopting the similar bio-active conformation as observed in fluconazole crystallized complex with the enzyme.

This study was supported by the UMK research grant no. 13/2008.

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Lunch break

Tuesday afternoon, 27 May, 13:00

Fourth Session

Tuesday afternoon, 27 May, 15:00

15:00 Invited oral

Harnessing stem cells and dendritic cells for novel therapies

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Human adult stem cells, which are scarce in various tissues, secure processes of tissue renewal and regeneration. Bone marrow (BM) contains hematopoietic stem cells (HSCs) and mesenchymal stem cells (MSCs), both responsible for the generation of the BM microenvironment. Adult MSC isolated from BM, liver, and heart, can be induced to proliferate extensively *in vitro*, and their progeny retains clonogenicity. In addition, these cells, unlike HSC, can be pushed by cytokines to express features that *in vivo* are associated with derivatives of the 3 germ layers. Yet, such transgermal, multilineage differentiation potential of MSCs proved *in vitro,* requires *in vivo* validation to confirm survival and self-renewal ability after transplantation, without the loss of properties acquired *ex vivo*. The existing differentiated cells in tissue microenvironment strictly limit the expansion of stem cells and affect the direction of differentiation of stem cell progeny. The so-called induced pluripotent cells (iPCs) may be generated from human adult skin cells that had been reprogrammed to an undifferentiated state by inserting just four genes. The iPCs differentiate *in vitro* into the cell types of 3 germ layers, and *in vivo* into teratomas. Success in reprogramming of adult fibroblasts into cells with embryonic stem cell capability to become any cell type in the body, could bypass ethical controversies surrounding the use of human embryos or oocytes. However, before the iPCs can be introduced in the clinic, additional effort is required to avoid employing vectors that integrate into the genome, and oncogenes, for turning back the developmental clock. With the presently used techniques, skin cells reprogrammed into iPCs carry multiple copies of the retroviruses used to insert the genes, what can lead to mutations at the insertion site, and may cause tumors. Human iPCs with retroviral integration are potentially useful for studying disease mechanisms and for drug screening. Once the safety issue is overcome, iPCs will be applicable in regenerative medicine. So far, the most advanced application of stem cells in medicine is hematopoietic stem-cell transplantation (HSCT) in cancer patients and in patients with nonmalignant hematologic disorders. Chemotherapy (CHT) is a double edge-sword that eliminates cancer cells at the price of hematologic toxicity. In addition, many cancers arise from rare self-renewing cells (cancer stem cells) that acquired oncogenic mutations at the stage preceding differentiation. Cancer stem cells being biologically distinct from their more numerous differentiated progeny may be resistant to treatment designed to target differentiated cells. Then, dramatic response of the bulk of cancer for treatment targeting differentiated cells is followed by the re-growth of cancer originating from cancer stem cell reservoir. High-dose CHT potentially improves targeting a reservoir of cancer stem cells, but seriously increases morbidity due to the delay or lack of hematologic recovery. Infusion of autologous or allogeneic HSCs following high-dose CHT, secures hematologic recovery. Allogeneic HSCT may also produce immune-mediated graft-versus-tumor reaction that eradicates cancer. However, allogeneic HSCT is associated with a risk of graft-versus-host disease (GvHD), which is increased for transplants from HLA-disparate relatives. Co-transplantation of *ex vivo* expanded donor MSCs reduces the risk of graft failure in haploidentical HSCT. MSCs inhibit alloreactive T cells, induce dendritic cells (DCs) to tolerogenic function and stimulate proliferation of regulatory T cells. An alternative approach, currently examined in preclinical studies, is based on the administration of tolerogenic DCs or regulatory T cells to prevent or to treat posttransplantational GvHD. DCs generated from HSCs *ex vivo* by stimulation with cytokines have already been used as a natural adjuvant in clinical studies on anti-cancer vaccines.

15:30 Invited oral

Scope and requirements regarding preclinical and human clinical trials of a new medicinal product, including biotechnology and biosimilar products.

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The development of all medicinal products is a complex process involving an evaluation of safety performed in animals (pre-clinical studies) and in human subjects (human clinical trials).

Aim of pre-clinical studies is to assess the potential toxic effect of the tested medicinal products. The studies have to be adequate to characterise toxic effect under the conditions planned to be used during initial starting dose of trials involving human beings. The data obtined during preclinical studies should allow to identify parameters used during clinical trials as the monitoring tools for potential adverse effects.

Human clinical trials are conducted to demonstrate safety and efficacy of **medicinal product** administered in the increased doses and through increasesd duration and/or size of exposed patient population.

During the presentation, principles of plannining of preclinical studies and human clinical trials conduct based on Scientific Guidelines for Human Medicinal Products issued by European Medicines Agency (EMEA) will be presented, with a special emphasis on biotechnology and biosimilar products development.

Coffee break
Tuesday afternoon, 27 May, 16:00

Discussion Panel

New Technologies
Tuesday afternoon, 27 May, 16:30

Free Time
Tuesday evening, 27 May, 18:00

Picnic on the San bank (Gromada Hotel)
Tuesday evening, 27 May, 19:30

Wednesday, 28 May

Fifth Session
Wednesday morning, 28 May, 8:30

8:30	Invited oral

Therapeutic applications of RNAi

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An evolutionary old mechanism of regulation of gene expression called RNA interference (RNAi), was discovered 10 years ago by Craig Mello and Andrew Fire [1]. Both of them were awarded with the Nobel Prize in medicine and physiology in 2006 to admire a deep impact made by their discovery on the molecular biology and potential application in therapy. RNAi is an RNA-dependent posttranscriptional gene silencing process induced in lower organisms by a long double stranded RNA, and in mammals by exogenously delivered short interfering RNAs (siRNAs) or by micro RNAs (miRNAs) coded in genome [2]. The mechanism employing endogenous miRNAs, operates in a cell as a powerful regulatory mechanism for genes switching on and off during various cellular processes, including cell development and carcinogenesis. The siRNA molecules are double-stranded 19-base pairs long RNA fragments with two-nucleotide overhangs at each 3'-end. Such duplexes are able to initiate gene silencing process as components of nucleoprotein RNA-induced silencing complexes (RISC) [3]. One of the siRNA strands, acting as a guide strand, hybridizes to the complementary sequence of mRNA and after RISC-performed cleavage excludes it from the translation process. Since their discovery, siRNA's have been widely used as convenient tools in functional genomics and as potential agents for therapeutic applications [4,5,]). Their silencing activity and specificity are determined by structural features, including thermodynamic stability of the duplex ends and their helical structure ,[6,]). The siRNA silencing activity can be modulated by chemical modifications in the functionally important regions (terminal and central domains of the duplex) [7]. RNAi can be effectively induced by exogenously delivered synthetic siRNAs or by siRNAs generated intracellulary from plasmid- or vector-coded shRNAs. After a long period of very limited success in the field of antisense/ribozyme approach (till now only one drug Vitravene has been approved by FDA), the RNAi technology kindled a new hope for revitalization of therapeutic strategies based on synthetic oligonucleotides. However, the RNAi technology still suffers from many limitations including siRNA intracellular instability, a problem of cell- or tissue-specific delivery and observed side effects. At present, there are a few siRNAs (for example, SiRNA027 and Cand5 designed for therapeutic treatment of the age-related macular degeneration (AMD)) in phase II and III of the clinical trials.

For several years, our research group interests have been focused on development of anti-amyloid strategy for Alzhemier's disease (AD) based on sequence specific silencing of genes of enzymes involved in secretion of beta-amyloid peptides (Abeta). According to the amyloid cascade hypothesis, accumulation of Abeta is the primary factor driving neural degeneration. Abeta is the product of the proteolytic cleavage of the APP (amyloid precursor protein) substrate protein by beta- and gamma-secretases. It was demonstrated that beta-secretase (aspartyl protease Asp2), also called beta-site APP cleaving enzyme (BACE1), is an excellent target for anti-amyloid therapeutic drug design [8]. Till now, several approaches have been evaluated to find an effective inhibitor for human beta-secretase, mostly in the field of peptidomimetic, non-cleavable substrate analogues. Our approach is based on targeting of the BACE mRNA and its down-regulation with small inhibitory nucleic acids (siNAs) [9]. Those include catalytic nucleic acids (ribozymes and deoxyribozymes) as well as siRNAs. Plasmid-coded hammerhead ribozymes and synthetic deoxyribozymes 10-23 were the first investigated as efficient inhibitors of BACE1 gene expression in a sequence-specific manner, measured, both, at the mRNA and protein levels [10,11]. Later on, synthetic siRNA as well as vector-coded shRNAs proved to be useful to down-regulate BACE1 mRNA in human cell lines as well as in adult mice hippocampal neural stem cells

and animal model [11,12].

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9:00 Invited oral

Applications of Mass Spectrometry in the Analysis of Pharmaceuticals

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Since 50-th of the XX century mass spectrometry is one of the most important analytical methods used for qualitative as well as quantitative analyses of organic compounds. Its most intensive development started, however, in the 80-th. The milestones in the development of the mass spectrometric methods were the discoveries of so-called mild ionization techniques like Fast Atom Bombardment (FAB), Electrospray Ionization (ESI) and Matrix-Assisted Laser Desorption Ionization (MALDI). These methods made possible the analyses of compounds which are the main subject of interest in biochemistry and biomedical sciences: peptides and proteins, nucleic acids, sugars, β-lactam antibiotics and many others groups of compounds which previously were out of reach of the mass spectrometry analysis.

Mass spectrometry is also one of the most widely used methods in analysis of pharmaceuticals, cosmetics and raw materials used in their manufacturing. In the present lecture the following topics concerning this subject will be presented:

1. Instrumentation of modern mass spectrometry:
   a) the most widely used ionization and ion analysis methods, their features and applications;
   b) hyphenated techniques: GC-MS and HPLC-MS.
2. Applications of mass spectrometry in the quantitative analyses: mass spectrometer as the selective detector with wide dynamic range.
3. Applications of mass spectrometry for detection of pharmaceuticals and their metabolites in body fluids.
4. Applications of mass spectrometry for detection and identification of impurities in pharmaceuticals, cosmetics and raw materials used in their manufacturing.

The abovementioned topics will be illustrated by the numerous examples from the authors laboratory as well as from the literature.

9:30 Invited oral

Phase Transfer Catalysis in pharmaceutical industry – where are we?

Michał Fedoryński[1], Magdalena Jezierska-Zięba[2], Barbara Kąkol[2]

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Organic synthesis is still the main way to produce pharmaceuticals. Among the reactions used for transformations of substrates into final products, especially important are those in which the abstraction of proton from C, O, N, S, etc. acids under the action of bases results in the formation of the corresponding anions. These anions, being nucleophilic agents enter a variety of reactions with electrophilic partners. It is obvious, that proper selection of the base-solvent system used for reactions induced by bases is of crucial importance.

Phase Transfer Catalysis (PTC) seems to be the most general, efficient and environment-friendly methodology of performing organic reactions in which organic and inorganic anions react with organic substrates. According to this methodology reactions are performed in immiscible two-phase systems. One phase (inorganic) is a source of inorganic anions (if they are available as salts) or base for generation of organic anions. The salts or base are most often used as aqueous solutions, or less frequently in the form of powdered solids. The second (organic) phase contains organic reactants, usually neat or sometimes in appropriate solvents. Upon introduction of a catalyst – tetraalkylammonium salt – continuous transfer of reacting anions, present or produced in the interfacial region, into the organic phase in the form of lipophilic ion pairs with the catalyst cation takes place. All further reactions occur in the organic phase.

Basic concept, special features, numerous and important advantages of PTC, its applications in pharmaceutical industry and perpectives for the future will be presented.

10:00 Oral

Polyhydroxylated decalin derivatives – stereoselective approach from simple sugars via allyltin compounds

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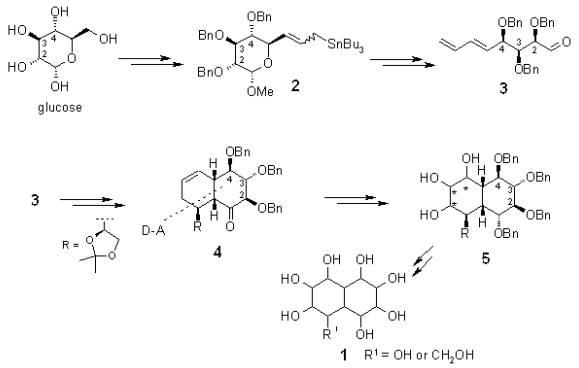
Biological activity of carbocyclic polyhydroxylated derivatives is very well documented. We are interested in the synthesis of optically pure polyhydroxylated decalin derivatives of type **1**, which can be regarded as carbocyclic sugar mimics with rigid structure. The chiron approach leading to derivatives of type **1** from simple sugars is presented in scheme 1 (exemplified by transformations of D-glucose) [1].



[Scheme 1]

In the synthetic route we employed a highly stereoselective transformation of sugar allyltin derivative **2** to dienoaldehyde **3**. The bicyclic structure of **4** was obtained in the intramolecular Diels-Alder cycloaddition. Further stereoselective functionalization of an allylic fragment *via* selenium compounds [2] led to polyhydroxylated decalins **5**. The mechanism of the processes will be discussed.

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10:20 Oral

Tricyclic theophylline derivatives with arylalkylpiperazinyl moiety as CNS agents

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Recently, it was found that annelation of six- or seven-mambered ring at 7,8-position of theophylline change the profile of its CNS activity in comparison to the mother compound (theophylline). Instead of stimulating activity they showed sedative, hypothermic, analgesic and neuroleptic-like properties [1]. In our earlier study a series of arylpiperazinylalkyl derivatives with complex terminal part based on the purine moiety were synthesized. Compounds with pyrimido[2,1-*f*]theophylline fragment showed a significant activity for 5-HT_{1A} receptors and diversified pharmacological profile [2-4].

To study the influence of chemical modyfication of the purine fragment on the CNS receptors affinity, we have synthesized new arylpiperazinylalkyl derivatives of imidazo[2,1-*f*]theophylline. The modyfication of pattern structure were made at 7-position (methyl or phenyl moiety), at alkilene spacer (3-4 methylene units) and kind of the substituent at phenyl ring of the arylpiperazinyl moiety (Scheme). The newly synthesized compounds exhibit multireceptor profile as potent 5-HT_{1A}, α_1 and D_2 receptor ligands. The most potent ligands were tested *in vivo* to evaluate their functional CNS activity and selected compounds were evalueted for their anxiolytic and antidepressant activity. The results indicated that the tricyclic teophylline derivatives linked with arylpiperazinylalkyl moiety are interesting class of compounds for searching a new CNS agents with antidepressant/anxiolytic an neuroleptic like activity.

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10:40 Oral

Two examples of very closely related isoxazoles exhibiting opposite immunological activities

Stanisław Ryng[1], Michał Zimecki[2]

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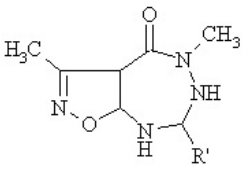
In our studies we showed that a new isoxazolotriazepine derivatives **(I)** [1], in the biological studies have immunosuppressive and antiinflammatory activities, in the mouse model. The compounds inhibited both the humoral and cellular immune response and was effective not only upon *ip* administration but also when given orally, indicating good bioaccessibility and a potential therapeutic application. Other studies revealed the lack of toxicity of the compound even at very high doses.

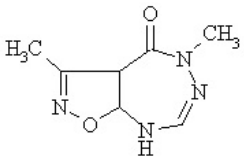
Although immunosuppressory isoxazoles, such as leflunomide, express anti-inflammatory properties and are sometimes used in combination with other immunosuppressors like Cyclosporine A (CsA),

their mechanism of action differs from that of CsA. In particular, the mitogen-induced T cell proliferation is not affected.That was also a case with regard to **I** which stimulated rather than inhibited phytohemagglutinin-induced proliferation of human mononuclear blood cells, in addition, the compound did not affect IL-10 production, in contrast to CsA. Another isoxazole **II**, [2] also stimulated mitogen-induced T cell proliferation, and strongly stimulated both humoral and cellular immune response in mice.

Interesting, the structure **II** is very closely related to that of **I**, differing only in substitution of one group in the isoxazolo[5,4-e]triazepine ring. Therefore, within the family of isoxazoles, both immunosuppressive and immunostimulatory compounds may be found. It is also clear that immunostimulatory activity in some tests i.e. mitogen-induced cell proliferation does not exclude anti-inflammatory activity of a given compounds as in the case of **I.**



I



II

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Coffee break

Wednesday morning, 28 May, 11:00

Fifth Session

Wednesday morning, 28 May, 11:30

11:30 Invited oral

Molecular underpinnings of the targeted therapy for cancer

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Cancer is a real challenge for modern medicine. Biologically, it is of a host origin and therefore its eradication appears not so easy as one could expect to do it. Cancer presents itself with many faces as if it would be Janus the god. The basic knowledge on tumorigenesis at the level of evolutionary science is weak. Additionally, accumulating molecular data are still focused on experimental systems, but more important fact is to determine the molecular pathobiology that could have impact on improvement of control of malignant disease. Poland is among the countries with high cancer morbidity and mortaity. Multidisciplinary approach to detect, control, and treat cancer diseases is the only way to get improved clinical results. Moreover, it is worth pointing out that individual considerations of every patient would offer clinical benefits. Biology of human tumors with the modern armament of molecular and chemical methods would be a help-hand to construct novel drugs. Making a list of crucial pathways worth bloking with their translation into clinical benefits appears to be a great step forward. Chemistry is a real partner to modern medicine due to a technical possibility to have impact on molecules (xenobiotics) that will finally become proved drugs. Combinatorial chemistry offers automated methods for pipeline organic synthsis a large number of chemicals that are further capable of undrtaking investigation at a bed. Many chemicals have been used for more than ten years upon treating various cancer patients. New drugs have various origin , i.e. monoclonal antibodies (Herceptin, Erbitux, Avastin) or small molecules (Glivec, Tarceva, Sutent, Nexavar). We do hope that in the future many new drugs will be available for treatmant of particular disease in relation to genetic characterization of individual patient's tumor. At the same time, we realize the great need for changes in the financial facets of modern individual treatment, and hoping not to hamper the development of new drugs due to the lack of financial solution how to make new and expesive drugs available to many patients.

12:00 Invited oral

Overview of pharmaceutical biotechnology sector in Poland

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12:30 Oral

LC/MS/MS as a tool for profiling metabolites of active substances

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LC/MS/MS is a method of choice for profiling metabolites of active substances during the drug development process. This approach permits to effectively identify and characterize metabolites in complex matrix. First step of the process, e.g. identification of metabolites can be done on ion trap mass spectrometers, and partially on triple quadrupole type instruments.

In contrary to ion traps usually used in a single MS scan mode, triple quadrupole gives more possibilities. Scan modes such as precursor ion scan and neutral loss provide a very selective method for the identification of structurally similar metabolites, even in presence of major background contaminations from complex biological matrices. With these selective scan modes, conventional triple quadrupole can effectively identify metabolite candidates for further characterization analysis. Other triple quadrupole approach for mon-

itoring theoretically possible metabolites is to use Multiple Reaction Monitoring (MRM) experiment, which is an ultra-high sensitivity scan mode.

The next step of the process is the characterization of identified metabolites. This process is mainly performed using ion trap mass spectrometers, due to their great performance in all scan based modes.

In the presentation, above mentioned methods will be compared on real life example and it will be explain why with different approaches it is possible to find different number of metabolites.

12:50 Oral

Anti-glioma activity of selected CK2 protein kinase inhibitors

Maria Bretner[1,2], Malgorzata Makowska[2], Agata Oberek[3], Beata Kaza[3], Bożena Kamińska[3], Zygmunt A. Kazimierczuk

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Human protein kinase CK2 is a ubiquitous serine/threonine kinase involved in many cellular functions, including cell viability, cell proliferation, and malignant transformation. Constitutive activity of CK2, levels of which are frequently elevated in tumours, may contribute to enhanced cell proliferation and resistance to apoptosis. Gliomas are the most common and dangerous brain tumours. Due to lack of effective treatments most patients diagnosed with malignant gliomas survive <1 year.

We compared effects of widely applied CK2 inhibitors: 4,5,6,7-tetrabromobenzotriazole (TBBt), the structurally related 4,5,6,7- tetrabromobenzimidazole (TBBi) and 2-dimethylamino-4,5,6,7-tetrabromobenzimidazole (DMAT) with an efficiency of recently developed derivatives of TBBt, TBBi and pentabromobenzyl-isothioureas on cultured rat glioma cells.

The new derivatives *N*-hydroxypropyl substituted TBBt (*N1*-PrOH-TBBt and *N2*-PrOH-TBBt) and *N1*-PrOH-TBBi were obtained by the alkylation of TBBt or TBBi with 3-bromopropan-1-ol and DBU in acetonitrile. The derivatives of pentabromobenzyl-isothiourea (ZKK-1): *N*-Methyl- (ZKK-2), *N,N*-dimethyl- (ZKK-3), *N*-ethyl- (ZKK-4), *N*-allyl (ZKK-5), *N*-phenyl (ZKK-6), were obtained in the reaction of pentabromobenzylbromide with *N*-substituted thioureas.

New derivatives (ZKK 1-6) and N1-hydroxypropyl derivatives of TBBi and TBBt were more effective than TBBt in inducing growth arrest and cell death in glioma cells when tested at 0.05mM during 48h. TBBi and ZKK-1 strongly induced apoptotic death involving caspase 3 and 7 activation followed by PARP cleavage, while DMAT induced nonapoptotic, programmed cell death. Human gliomas have different genetic alterations, which render them resistant to cell death, thus we evaluated their effects towards cells bearing distinctive alterations of major tumour suppressors: *PT53* and *PTEN*. TBBi or ZKK-1 differentially induced cell death in two human malignant glioma cells. Interestingly, in contrast to ZKK-1, TBBi at doses toxic for glioma cells has no effect on nontransformed astrocytes, suggesting specificity toward tumour cells in cytotoxic action. The more bulky N-phenyl substituent in thiourea fragment of the molecule ZKK-1 reduced cytotoxic activity in pentabromobenzyl-series. Isothioureas carrying Cl or F substitutes on the phenyl ring showed much lower activity.

Collectively taken, the reported data support the idea that suitably modified tetrabromobenzene molecules may be powerful reagents counteracting CK2 tumourigenic potentials.

The study is supported by Ministry of Science and Higher Education (PBZ-MIN 014/P05/2004).

13:10 Oral

Synthesis and antiproliferative activity in vitro of (4-substituted-2-butynylthio)quinolines

Wojciech Mól[1], Małgorzata Matyja[1], Adam Naczyński[1], Katarzyna Szczaurska-Nowak[2], Magdalena Milczarek[2], Joanna Wietrzyk[2], Stanisław Boryczka[1]

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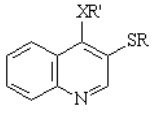
Acetylenic derivatives are an important class of compounds since many of them display interesting biological activities and possess anticancer, antibacterial, antimicrobial, HIV-inhibitory properties. The synthetic methods for their preparation are of interest especially with regard to the synthesis of enediyne antibiotics [1-3]. The natural enediynes are the most potent of anticancer agents discovered, some members of which are three order of magnitude more potent than other anticancer drugs but their clinical use has been limited because of their toxicity and modest selectivity for cancer cells. It has prompted several research groups to design, prepare, and test new simplified, fully synthetic acetylenic analogues, characterized by a similar mode of action [1-4].

As an extension of our work on the development of anticancer drugs, we synthesized the series of new 3,4-disubstituted thioquinolines **1** possessing propargyl, 2-butynyl, 4-bromo(chloro)-2-butynyl and 4-substituted-2-butynyl groups with the aim to obtain more information about the influence of substituents on antiproliferative activity in this class of compounds.



1

X= S, Se

R= CH_3, $CH_2C{\equiv}CH$, $CH_2C{\equiv}CCH_2OH$, $CH_2C{\equiv}CCH_2Cl$

R'= $CH_2C{\equiv}CCH_2OCOC_6H_5$, $CH_2C{\equiv}CCH_2OCOOC_2H_5$, $CH_2C{\equiv}CCH_2OC_6H_4COOH$, $CH_2C{\equiv}CCH_2OCOCH{=}CHC_6H_4$, $CH_2C{\equiv}CCH_2S(Se)CN$, $CH_2C{\equiv}CCH_2NHR''$

The obtained compounds were tested for their antiproliferative activity *in vitro* against the cells of human (colon SW 707, leukemia CCRF/CEM, breast T 47D) and murine (leukemia P388, melanoma

B 16) cancer cell lines. The most active compounds have the ID_{50} values ranging from 0,43 to 4,00 mg/ml comparable to that of referential cis-platin.

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This study was supported by Polish Ministry of Science and Higher Education (Grant No. N405 036 31/2655).

Lunch break

Wednesday afternoon, 28 May, 13:30

Coach transport to Castle Park Complex in Krasiczyn

Wednesday afternoon, 28 May, 15:00

POSTER SESSION

Wednesday afternoon, 28 May, 15:30

15:30 Poster 1

The evaluation of quality of Salicis cortex raw material used in pharmaceutical preparations and its comparison with raw materials collected in natural environment

Małgorzata Anyżewska[1], Elżbieta Wojtasik[2]

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This study aimed to determine the quality of bark willow raw material available in different pharmaceutical preparations. The comparative analysis by Thin Layer Chromatography (TLC) was the base for determination of taxonomical origin of examined raw materials. Salix alba L. was provided from Wroclaw surroundings and Salix purpurea L. was collected in Bieszczady region. A quality evaluation was established by determination of salicin content by HPLC method. The results indicated that salicin content in Salix purpurea from natural environment was 2%. This value increased into 4% after alkaline hydrolysis. This fact is connected with decomposition of salicortin as an influence of alkaline environment of reaction. Moreover, these results were confirmed by comparative TLC analysis of samples prepared in cold extraction, in hot extraction and in hot extraction with alkaline hydrolysis.

The content of salicin in Salix alba from natural environment and in pharmaceutical preparation was 1%. Simultaneously, the presence of derivative with similar retention time to salicin (RRT=1,1) was detected. The different chemical structure was confirmed by UV-VIS analysis with DAD detector. TLC analysis confirmed in all samples, identified as Salix alba, a slight amount of salicin (RT=0,38) and non-identified derivative (RT=0,42), which gives a yellow colour of solution of vanillin.

This derivative was not a result of incorrect samples preparations or thermal decomposition of salicin, which is evidenced by simplified kinetics analysis of hydrolyzate and was not a metabolism product of salicin as well.

Our study demonstrates that it is possible to misinterpret the content of salicin in Salix alba due to the incorrect interpretation of HPLC profiling patterns.

To eliminate possible mistake in calculation, it is suggested to perform a comparative analysis of UV-VIS spectrum or addition a salicin standard into examinated sample.

15:30 Poster 2

Metabolism of antitumor 9-amino-1-nitroacridine derivative C-1748 (Capridine-beta) in human hepatoma HepG2 cells

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Antitumor agent 9-ethylamino-4-methyl-1-nitroacridine, C-1748, belongs to a new set of antitumor derivatives developed in our laboratory and was selected to preclinical studies. Metabolic pathway of a representative 9-amino-1-nitroacridine with rat microsomes was proposed earlier [1]. Compound C-1748, which is less toxic than its analog without 4-methyl group, C-857, was shown to be less reactive with human and rat liver microsomes as well as with human *E. coli* recombinant CYPs [2]. Therefore, we hypothesized that metabolism of C-1748 is a probable reason of its reduced toxicity in animals. In this work we present the studies on metabolic transformations of C-857 and C-1748 in human hepatoma HepG2 cells. The investigations aimed to evaluate whether any new metabolic products are formed in tumor cells in comparison with metabolites identified previous with microsomal and hepatic recombinant enzymes, and whether the differences in HepG2 metabolism between C-857 and C-1748 are similar to those observed earlier for enzymatic transformations. HepG2 cells were incubated with specified concentration of C-857 and C-1748 for 3, 4, 6 and 12 hours. Metabolites were extracted with methanol and RP-HPLC analyses of extracts were performed with UV-VIS and ESI-MS detection. Another part of HepG2 cells incubated with studied drugs were examined under fluorescence microscopy. The obtained results demonstrated that several new metabolites were formed in HepG2 complay to model enzymatic systems. On the other hand, at least one metabolite found in HepG2 cells was identical to that identified previous in enzymatic systems. It was a derivative with additional pyrazole ring closed between nitrogen atoms at positions 1 and 9 of acridine ring. Metabolites of C-857 extracted from HepG2 cells were clearly observed after 3 and 4 hours of incubations, whereas 6 and 12 hours of incubations gave lower concentrations of products. In the contrary, meta-

bolites of C-1748 were observed after prolonged incubation with the drug, until 12 hours. In conclusion, the results showed the presence of reactive metabolites of 9-amino-1-nitroacridines in human hepatoma HepG2 cells and demonstrated also that C-1748 is less reactive than C-857 in this cellular model.

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15:30 Poster 3

Zolmitriptan synthesis and in-process control by HPLC methods

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Zolmitriptan (Zomig®), a single enantiomer 4(*S*)-[dimethylamino)ethyl]-1*H*-indol-5-ylmethyl] oxazolidin-2-one is a novel serotonin 5-hydroxytryptamine receptor agonist that has shown, in an extensive clinical trial program, to be highly effective in the acute oral treatment of migraine with or without aura [1,2]. It works by stimulating serotonin receptors in the brain.

Zolmitriptan is a synthetic indole derivative, which can be obtained by six steps method of synthesis: ZL1 → ZL2 → ZL3 → ZL4 → ZL5 → {[ZL6] + ZL7} → ZL8.

The esterification of L-4-nitrophenylalanine (ZL1) with methanol gives the corresponding ester (ZL2), which is reduced to the (S)-2-amino-3-(4-nitrophenyl)-1-propanal (ZL3). Reduction of the nitro group in ZL3 yielded the corresponding amino derivative ZL4 (step 3), which is cyclized to ZL5 in next step. The diazotation of ZL5 affords the expected hydrazine derivative (ZL6). Finally, hydrazine derivative (ZL6) is reacted with 4-(dimethylamino) butanal diethylacetal (ZL7) to obtain desired product – Zolmitriptan (ZL8).

Different polarity of all products (ZL1-ZL8) determinates application of high selective analytical method for in-process control. Analysis were carried out by reverse phase chromatography (RP-HPLC) with a gradient mobile phase composed of: at first acetonitrile:diethylamine (in a ratio 1:0,1 v/v), and finally water:diethylamine (1:0,1 v/v), on a Phenomenex Gemini C6-Phenyl column. Zolmitriptan (ZL8) and its potential impurities were baseline resolved in the optimized method.

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15:30 Poster 4

Optimization of AR-3 synthesis

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The reaction of quinolinone derivative **1** with piperazine derivative **2** giving **AR-3** was carried out at laboratory scale (10 mmol of **1**) under various conditions in order to achieve high yield and purity of the product. The reaction parameters (**1** : **2** ratio, amount of base, volume of ethanol and the reaction timing) were sampled guided by the D-optimal plans. Molar content of crude reaction product was predicted theoretically with the use of the mass balance and the corresponding HPLC parameters. The theoretical predictions were verified with the potentiometric and thermogravimetric analysis for selected samples. Based on the predicted molar content of reaction mixtures a series of reaction response surfaces was calculated. From analysis of these surfaces it appears that there exists an optimal set of reaction parameters for **AR-3** synthesis for a high conversion ratio of about 99 % and 99.5 % purity of the product.

Keywords: optimization, synthesis, reaction response surface

15:30 Poster 5

Searching for lead molecules as potential new CNS agents in the serotonin 5-HT$_6$ screening assay

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Among 14 different serotonin receptor subtypes 5-HT$_6$ and 5-HT$_7$ ones, simultaneously discovered in 1993, are classified as last members in 5-HT receptor superfamily. They both show high affinity for a wide range of agents used in psychiatry, and many drug discovery programs have been initiated, which were focused on the search of selective 5-HT$_6$ and/or 5-HT$_7$ ligands. In the filed of serotonergics we have successfully concentrated on the development of compounds active at 5-HT$_{1A}$, 5-HT$_{2A}$ and 5-HT$_7$ receptors and recently our studies were extend on 5-HT$_6$ ligands. Strategy that was previously elaborated for investigations of 5-HT$_7$ receptors was applied and both an in-house and external compounds libraries were screened in 5-HT$_6$ receptor binding assay. Several lead molecules

have been identified for further structure optimization on the basis of 5-HT_6 receptor homology model.

This study was partly supported by the Network "Synthesis, structure and therapeutic properties of compounds and organic substances" coordinated by the Institute of Organic Chemistry Polish Academy of Sciences.

15:30 Poster 6

Glycosylation reactions of natural poliphenolic compounds

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Due to their antioxidant potential, many naturally occurring phenolic compounds play an important role in the protection against various diseases. Their biological properties and mode of action may be affected by O-substituents, e.g. the presence of sugar residues. However, the O-glycosylation methods for low-nucleophilic or hindered phenolic compounds are not always satisfactory in term of yield as well as their regio- and stereoselectivity. In the present work, a variety of ribosylation approaches have been studied for two poliphenolic compounds, a biologically significant flavone, quercetin (**1**), and resveratrol (**2**), a compound occurring in the skin of grapes and red wine. Ribosylation of quercetin with 1,2,3,5-tetra-O-acetyl-β-d-ribofuranose in the presence of tin tetrachloride, i.e. under conditions which were successful in synthesis of genistein 4'-O-ribosides [1], failed completely. Similarly, the use of *p*-toluenesulfonic acid as a catalyst did not result in the formation of quercetin ribosides. Some promising results came from ribosylation experiments performed in the presence of boron trifluoride – diethyl etherate. The reactions yielded a mixture of α- and β-ribosides of quercetin, and the study on their structures, ratio and isolation are in progress. In the case of resveratrol, glycosylation with tetraacetylribose and tin tetrachloride gave a complicated mixture of compounds, from which an interesting product, 4-C-α,β-riboside could be isolated in a moderate yield. The application of boron trifluoride – diethyl etherate for ribosylation of resveratrol increases the yield of O-ribosides, as it has been judged from our preliminary experiments.

Acknowledgements: This work was supported by the network "Synthesis, structure and therapeutic properties of compounds and organic substances".

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15:30 Poster 7

2-(1-Arylsulfonylpiperidin-2-yl)ethyl derivatives as 5-HT_7 receptor ligands: synthesis and their affinity for 5-HT_{1A}/5-HT_{2A}/5-HT_7/D_2 receptors

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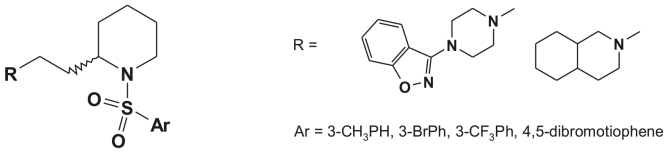
The affinity of several antidepressant and antipsychotic drugs for 5-HT_7 receptor, along with their distribution in CNS, suggested their involvement in the physiopathology of brain disorders. Indeed, some recent studies demonstrated direct involvement of 5-HT_7 receptors in depression, anxiety and mood diseases [1-3].

To better characterize the 5-HT_7 receptor, a new potent and selective compounds are required. Different research centers (among others our Department) are engaged in modeling, design, synthesis and structure-activity relationships studies of new 5-HT_7 ligands.

Here we present a series of 2-(1-arylsulfonylpiperidyn-2-yl)ethyl derivatives with various changes of aromatic substituent in arylsulfonylpiperidine moiety and modifications in terminal amine fragment.

In the competition binding studies of the investigated compounds, both selective 5-HT_7 receptor ligands and that with mixed 5-HT_{2A}/5-HT_7/D_2 pharmacological profile were found. The structure-affinity relationships for all the new derivatives are discussed.



Ar = 3-CH_3PH, 3-BrPh, 3-CF_3Ph, 4,5-dibromotiophene

This study was partly supported by the Ministry of Science and Higher Education (MNiSW), Grant No. N405 026 32/1743

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15:30 Poster 8

SAR studies of novel 5-HT_7R ligands with different spacers between aryl and amine moieties

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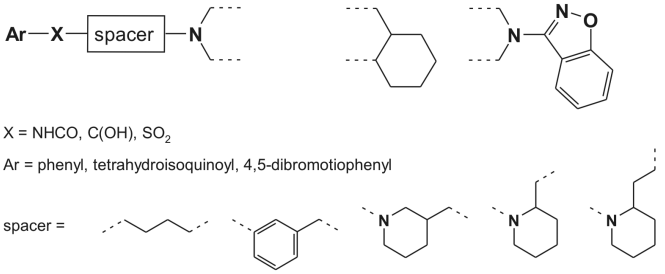
Since the discovery of the 5-HT_7 receptors and their potential therapeutic applications the search for potent and selective ligands has been initiated. More than 10 years of investigations resulted in finding a few selective antagonists like SB-269970-A and SB-656104-A. Several research groups and some pharmaceutical companies are active in this field, and a number of papers describing structure-activity relationship (SAR) [1] as well as some pharmacophoric models [2] have been published.

As a part of our research program directed towards the design and synthesis of potent and selective 5-HT_7 ligands we obtained new series of compounds with flexible and partly constrained spacer between aryl and amine fragment. Radioligand binding study showed that the investigated compounds reveal diverse affinity for 5-HT_7 receptor and selectivity over 5-HT_{1A}/5-HT_{2A}/D_2 sites. The structure-affinity relationships for all the new derivatives are discussed.



This study was partly supported by the Ministry of Science and Higher Education (MNiSW), Grant No. N405 026 32/1743

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15:30 Poster 9

The evaluation of tolterodine tartrate synthesis based on patent EP 0 325 574 B1.

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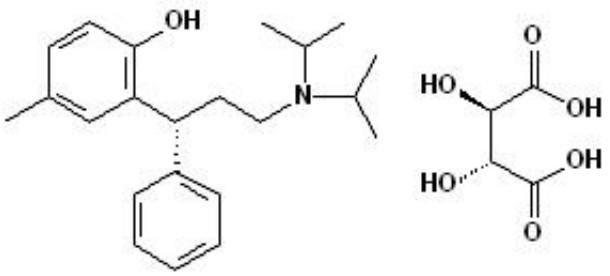
Tolterodine is used to relieve urinary difficulties, including frequent urination and inability to control urination. Tolterodine belongs to a class of medications called antimuscarinics. It acts by preventing bladder contraction. It is sold under the trade names Detrol and Detrusitol. DETROL Tablets are indicated for the treatment of overactive bladder with symptoms of urge urinary incontinence, urgency, and frequency.

The chemical name of tolterodine is (R)-N,N-diizopropyl-3-(2-hydroxy-5-methylphenyl)- -3-phenylpropamine. It is delivered in pharmaceutical form as a tartrate. The empiric formula is $C_{26}H_{37}NO_7$ and the structural formula is represented below:



Synthesis of tolterodine tartrate was firstly revealed in patent EP 0325571 B1 published in 1989. It describes synthesis consisted of seven steps, which starts from p-cresol and trans-cinnamic acid. There is also a modification of first method. This alternative route contains eight synthetic steps. We found that patent description lacks a lot of very important information e.g. purity of intermediates or methods of their purification. It was not mentioned if products were obtained in crystalline, amorphous or oil form.

We decided to investigate both of synthetic routes to gain missing information and to evaluate their usefulness for application in industrial scale.

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Effect of selol on the opioids activity in vincristine induced hyperalgesia.

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The development of vincristine model of chemotherapeutic-induced painful toxic neuropathy provides an opportunity to investigate mechanisms involved in this form of neuropathic pain.

We examined (1) the effect of the opioid receptor agonists and (2) the effect of organoselenium compound (selol), on antinociceptive action of opioid agonists, morphine, fentanyl as well as ago-antagonist with potent analgesic activity, buprenorphine, - in vincristine neuropathic pain model. The changes in pain thresholds were determined using mechanical stimuli - the modification of the classic paw withdrawal test described by Randall-Selitto. Daily administration of VIN (70 μg/kg, iv) resulted in progressive decrease of pain threshold.

In conclusion, the results of present paper suggest, that selol significantly increases analgesic activity of opioids in vincristine model of chemotherapeutic-induced painful toxic neuropathy. This observation can be clinically relevant since selol possess anticancer activity. Therefore, concomitant administration of selenium and opioids may be benefitial in terminal neoplastic states.

15:30 Poster 11

Effect of the magnesium ions on analgesic activity of opioids in vincristine induced hyperalgesia model

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Neuropathic pain is difficult to treat. Classical analgetics, e.g. opioid receptor agonists, possess low activity, therefore other agents, e.g. antidepressants, anticonvulsants or corticosteroids are used.

The changes in pain thresholds were determined using mechanical stimuli - the modification of the classic paw withdrawal test described by Randall-Selitto.

We examined (1) the effect of the opioid receptor agonists on vincristine-induced hyperalgesia and (2) effect of the magnesium ions (Mg^{2+}) on antinociceptive action of opioid agonists in toxic neuropathic pain model.

Daily administration of VIN (70 μg/kg, iv) resulted in progressive decrease of pain threshold.

When administered alone opioid agonist like morphine and fentanyl as well as ago-antagonist with potent analgesic activity - buprenorphine had only a little effect on vincristine hyperalgesia. However pretreatment with Mg^{2+} markedly enhanced the analgesic activity of all three investigated opioids. A practical aspect of this phenomenon is discussed.

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Reactions of diastereomeric sugar derived sulfites with sterically demanding organometallics

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It could be expected that the sulfinylation of the hydroxyl group(s) of a sugar moiety should influence its biological activity. This type of sugar functionalization generate simultaneously interesting diastereomeric *O*-sulfinates, which can be used as substrates for the synthesis of enantiomerically pure sulfinyl derivatives[1]. To verify experimentally both assumptions we decided to check whether the reactions of organometallic reagents with diastereomerically pure (*R*)-1,2-*O*-isopropylidene-3,5-*O*-sulfinyl-a-D-glucofuranose[2] or the sulfite derived from 1,2:5,6-di-*O*-isopropylidene-*α*-D-glucofuranose (DAG) could be stopped at the stage of the corresponding sulfinates.

Su = sugar; M = Li, MgX

In this paper the influence of the reaction conditions on the reaction outcome with sterically demanding organometallics and an X-ray structural analysis of *t*-butanesulfinate derived from (*R*)-1,2-*O*-isopropylidene-3,5-*O*-sulfinyl-a-D-glucofuranose will be discussed and experimental details will be presented

Acknowledgment:

This work was supported by the network "Synthesis, structure and therapeutic properties of compounds and organic substances".

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15:30 Poster 13

High-yielded method of synthesis of voriconazole.

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The description of the efficient synthesis of voriconazole - active substance of antifungal medicine from triazol group with wide-spectrum of activity was presented. High efficiency and the chemical and enantiomeric purity of the product were reached by the modification of the critical steps of the synthesis described by Pfizer company in the basic patent. It was possible due to the use of a structurally advanced intermediate and using Metz Syn[10]

apparatus Radleys company for chemical reaction condition control.

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A high-performance liquid chromatography with electrochemical detection for the determination of olanzapine in human plasma

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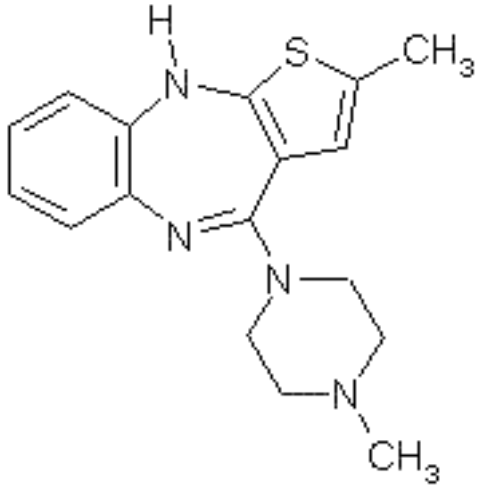
Olanzapine, 2-methyl-4-(4-methyl-1-piperazinyl)-10H-thieno-[2,3-b][1,5]benzodiazepine (**I**)



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is an atypical antipsychotic drug with a high affinity for serotonin ($5HT_{2A/2C}$), dopamine (D_{1-4}), muscarinic (M_{1-5}), histamine H_1, and adrenergic α_1 receptors [1- 2]. Olanzapine is indicated for the treatment of patients with schizophrenia and psychosis of a schizoaffective nature.

We have developed a sensitive and selective HPLC method with electrochemical detection to determine the concentration of olanzapine in human plasma. The analysis was carried out on a reversed-phase column (Symmetry C18, 150 x 4.6 mm I.D., 5 μm) using a mixture of 0.06 M ammonium acetate (pH 5.90), acetonitrile and methanol (40:41:37, v/v/v) as the mobile phase. The flow rate was 0.69 mL/min. The detection voltage was + 0.6 V and the cell and column temperature were 36°C. The drug and internal standard (clozapine) were isolated from plasma using liquid-liquid extraction with ethyl acetate. The mean recovery of olanzapine and internal standard were about 90% and 82%, respectively. Ascorbic acid was added to the samples to inhibit degradation of olanzapine during extraction and storage. The lower limit of quantitation for the assay was established at 0.313 ng/mL. Repeatability, intermediate precision and accuracy were satisfactory.

The method for the assay of olanzapine in human plasma is sufficiently sensitive to apply during the pharmacokinetic and bioequivalence studies of this drug.

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15:30 Poster 15

New serotonin receptors ligands in the group of 8-acylamide derivatives of 1,3-dimethyl-7-[(4-phenylpiperazin-1-yl)-alkyl]-1H-purine-2,6-(3H,7H)-dione

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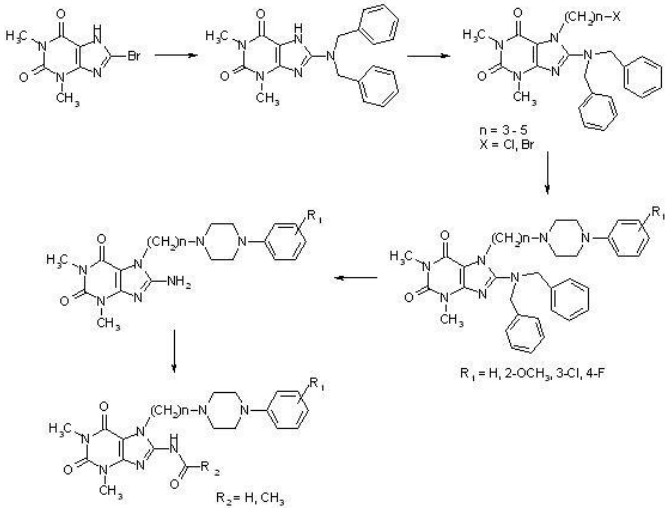
It is known, that many serotonin receptors ligands, especially 5-HT1A and 5-HT_{2A}, posses antidepressant, anxiolitic and antipsychotic activity. As we reported in our previous paper [1] several 8-alkoxy derivatives of 1,3-dimethyl-7-[(4-phenylpiperazin-1-yl)-alkyl]-1*H*-purine-2,6(3*H*,7*H*)-dione have shown moderate to high affinity for 5-HT_{1A} (K_i = 11-19 nM), 5-HT_{2A} (K_i = 15-253 nM) and 5-HT_7 (K_i = 51-83 nM) receptors, depending on the structure of moiety in the 7 position of 1*H*-purine-2,6(3*H*,7*H*)-dione. In our further studies in a group of 8-amino derivatives of 1,3-dimethyl-7-[(4-phenylpiperazin-1-yl) -alkyl]-1*H* -purine -2,6(3*H*,7*H*) -dione we have found out, that replacing the alkoxy substituent in the 8 position with amino substituent strongly decreases affinity for 5-HT_{2A} and 5-HT_7 receptors, but enhances affinity and selectivity for 5-HT1A ones. In order to determine the influence of moiety in 8 position of 1*H*-purine- 2,6(3*H*,7*H*)-dione on the affinity for serotonin receptors we designed and synthesized 1,3-dimethyl-7 -[(4-phenylpiperazin-1-yl) -alkyl]-1*H*-purine -2,6(3*H*,7*H*)-dione derivatives possessing 8-acylamide substituent (Scheme 1).



The new 8-acylamide analogues are under pharmacological trials. The structure-activity relationship will be discussed.

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15:30 Poster 16

Synthesis and serotonin receptors activity of new purine-2,6-dione derivatives with arylpiperazinylalkoxy moieties

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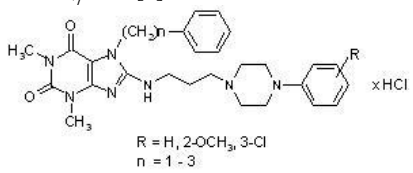
For several years much attention has been focused on the functional importance of serotonin receptors in the pathogenesis of neuropsychiatric and other diseases. Among many classes of serotonin recept-

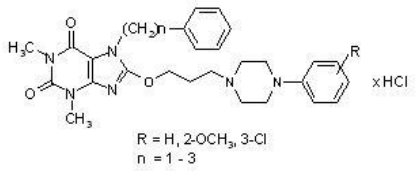
or ligands a long-chain arylpiperazines (LCAPs) containg a different amide/imide terminal fragment were mainly evaluated towards 5-HT1A, 5-HT$_{2A}$ and 5-HT$_7$ receptors.

In our earlier studies it was shown that 7-arylalkyl derivatives of 1,3-dimethyl-3,7-dihydropurine-2,6-dione with 8-[3-(4-arylpiperazin-1-yl)-propylamino] moiety, displayed high to moderate affinity for 5-HT$_{1A}$ receptors and moderate to low affinity for 5-HT$_{2A}$ and 5-HT$_7$ sites [1].



The compounds examined in functional *in vivo* model behaved like postsynaptic 5-HT$_{1A}$ receptor antagonists [1].

To continue our research with this class of purine-2,6-dione analogues we designed and synthesized a novel series of arylpiperazines. Comparing to the previous work structural modifications consisted in replacing the amino group in the 8-position by a ether one.



The new compounds were synthesized in the reaction of previously obtained
1,3-dimethyl-7-arylalkyl-8-bromo-3,7-dihydro-purine-2,6-dione
with the appropriate 3-(4-aryl-piperazin-1-yl)-propan-1-ol. The new analogues are under evaluation for their affinity for 5-HT$_{1A}$, 5-HT$_{2A}$ and 5-HT$_7$ receptors. The most active derivatives will be tested in *in vivo* behavioral models.

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15:30	Poster	17

Synthesis of novel pyrido[1,2-c]pyrimidine derivatives as selective ligands for 5-HT$_{1A}$ receptors

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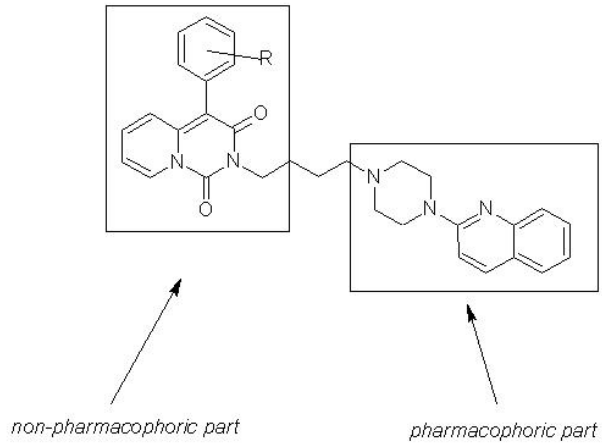
Serotonin (5-HT) is an important neurotransmitter mediator in the peripheral and central nervous system. Serotonin 5-HT$_{1A}$ receptors have been intensively studied because of their implication in several physiological processes and psychiatric disorders such as anxiety and depression. 5-HT$_{1A}$ receptor ligands with serotonin transporter inhibition effect are regarded as potential therapeutics for anxiety, depression. Moreover the chemical structure of synthesized ligands may be responsible for *pre-* and *post*-synapsis agonism or antagonism effect on 5-HT$_{1A}$ receptors.

Although the main investigated structures were buspirone and tandospirone, many modifications have been made in the pharmacophoric and non-pharmacophoric part of potential 5-HT$_{1A}$ receptors ligands.



R= H, 2-Cl, 2-F, 2-Me, 2-MeO, 4-Cl, 4-F, 4-Me, 4-MeO

All novel 4-aryl-2H-pyrido[1,2-c]pyrimidine derivatives containing quinoline substituents were confirmed by ^{1}H and ^{13}C NMR spectra and elementar analysis. Target compounds were assesed for in vitro affinity for serotoninergic 5-HT$_{1A}$ and 5-HT$_{2A}$ receptors and 5-HT-T.

15:30	Poster	18

Homology modeling of the 5-HT$_{1A}$ and 5-HT$_{2A}$ serotonin receptors on the novel ß$_2$-adrenergic receptor template

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Publication of a crystal structure of ß$_2$-adrenergic receptor in November of 2007 [1] seems to be a long awaited breakthrough in discovering three dimensional structure of G-protein coupled receptors, especially in the monoaminergic subgroup. ß$_2$-adrenergic receptor is the second G-protein coupled receptor ever crystalallized and described using rentgenographic methods, after bovine rhodopsin, resolved in 2000 [2]. The newly-published structure revealed fair similarity to the models of monoaminergic receptors obtained on the template of bovine rhodopsin, however some explicit topological differences could be observed. In spite of the fact that localization and shape of the binding site were very similar, divergences in the spatial distribution of the most important interaction points seemed to have potentially significant impact on the ligand recognition pat-

tern.

Considering the fact, that ß$_2$-adrenergic receptor is much more related to the investigated serotonin receptors, especially taking into account the sequence homology of the binding sites, we decided to obtain the homology models of 5-HT$_{1A}$ and 5-HT$_{2A}$ receptors using the novel template. Molecular models were built with Modeller 8v2 software, which allowed the conformational sampling of the binding site, by means of simulated annealing. In order to compare the lignand binding mode and asses the possible benefits of using the novel template, we performed the analysis of ligand-receptor interactions, with the use of docking procedure.

Ligands with known 5-HT$_{1A}$ and 5-HT$_{2A}$ receptor affinity were automatically docked to the multiconformational sets of the obtained receptor models, using both Glide and FlexX software, with and without pharmacophore constraints. Docking was performed with both conformationaly rigidified and flexible compounds, derived from litrerature or synthesized in Department of Pharmeceutical Chemistry. The obtained results were discussed in comparison with data on previously published (5-HT$_{1A}$) [3] or newly obtained (5-HT2A) rhodopsin-based models.

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15:30 Poster 19

Mannich bases, 5-arylimidazolidine-2,4-dione derivatives with arylpiperazine moiety, as 5-HT$_{1A}$ receptor ligands with serotonin transporter affinity

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In recent years there have been a lot of published articles aimed at creating a single hybrid molecule that possesses both 5-HT$_{1A}$ antagonism and serotonin reuptake inhibition [1-4]. Such a molecule could prove to be a better rapidly acting antidepressant than selective serotonin reuptake inhibitors (SSRIs), which are currently used in the therapy of depression [5].

In the course of searching for the new potential antidepressants, which block 5-HT$_{1A}$ receptor and inhibit serotonin reuptake, series of arylpiperazinylalkyl containing 5-aryl-imidazolidine-2,4-dione moiety were synthesized. Then their influence on central serotonergic transmission was examined in a different pharmacological tests. In the radioligand studies the affinity towards central nervous system receptors (5-HT$_{1A}$, 5-HT$_{2A}$ and α_1) and serotonin transporter were evaluated. For selected compounds, which have shown specially promising properties in *in vitro* tests, their anxiolytic and antidepressant activities were investigated in the four-plate test, in the forced swimming test and in the locomotor activity test in mice.

In order to describe the interaction mode of the investigated compounds with 5-HT$_{1A}$ serotonin receptor, *in silico* studies were carried out, comprising automated docking of selected compounds to the molecular model of 5-HT$_{1A}$ receptor. Furthermore the lipophilic character of the compounds (a crucial physicochemical factor for potential CNS activity) was also evaluated by use of RP-TLC and computational methods.

Acknowledgement: The authors thank to Polish Ministry of Science and Higher Education for financial support (grant No 2P05F04329).

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15:30 Poster 20

Quantitation of pseudoephedrine in dosage form

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Ephedrine alkaloids are used for the treatment of asthma and nasal congestion. There are numerous drugs containing pseudoephedrine hydrochloride in combination with paracetamol and other ingredients.

^{1}H NMR spectroscopy in solution application for quantitative determination of the alkaloids from Ephedra species: (-)-ephedrine, (+)-pseudoephedrine, and (+/-)-norephedrine, either singly or in mixtures with each other was reported. The method was specific and accurate.

However, there are a number of situations when it is necessary to determine the concentrations of components in solid-state mixtures without dissolving the sample. Therefore, an attempt was made to quantify pseudoephedrine in a solid formulation with microcrystalline cellulose.

Samples with different fractions (from 5.09 to 95.97 % g/g) were prepared by mixing pure **(3S,2S)-(+)-pseudoephedrine hydrochloride** and pure microcellulose (Avicel-105), one of the most commonly used excipients. To obtain homogeneous physical mixtures, the components were mixed for 5 minutes using an electric

mixer.

Cross-polarization (CP) magic angle spinning (MAS) solid-state ^{13}C NMR spectra were recorded. The CP parameters were used as optimized for experiments with pure (3S,2S)-(+)-pseudoephedrine hydrochloride.

Variable contact time experiments were necessary in order to find "perfect" contact time which ensures quantitation for all carbons of interest (outside the region overlapped by the resonances of cellulose). The peaks chosen for the analysis are these of methyl carbons and quaternary carbon.

^{13}C CPMAS NMR spectra of pseudoephedrine/cellulose mixtures with various compositions were recorded. For statistical reasons, each measurement was repeated three times. A plot was constructed with composition of pseudoephedrine mixtures versus the measured ratio of peak areas. As a next step, the MAS NMR spectra of three drugs containing pseudoephedrine were recorded: Sudafed, Ibuprom-zatoki and Gripex in order to determine, if the content of pseudoephedrine can be determined in the solid commercial formulations.

15:30 Poster 21

Flexible vs partly constrained linkers in NAN-190 and PK-13 analogs investigated as 5-HT$_{1A}$/5-HT$_7$ receptor ligands

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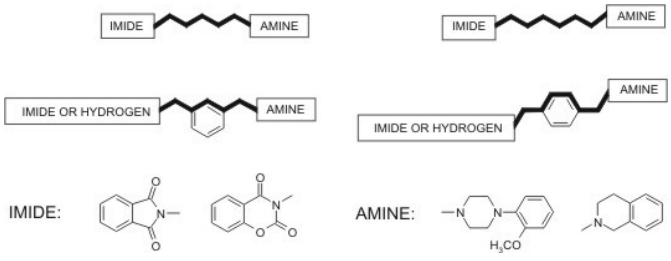
Continuing study of the influence of linker conformation in the structure of long-chain arylpiperazines on the affinity to serotonin 5-HT$_{1A}$ and 5-HT$_7$ receptors [1], new flexible and partly constrained derivatives were synthesized. Well characterized, potent 5-HT$_{1A}$ agents (i.e. NAN-190 and PK 13) were selected as a parent molecules and two series of compounds containing o-metoxyphenylpiperazine or 1,2,3,4-tetrahydroizoquinoline pharmacophores were investigated. Structural modifications involved: elongation of polymethylene chain to five and six carbon atoms and introduction of m-xylene and p-xylene moieties into linker fragment. Results of in vitro binding experiments for 5-HT$_{1A}$ and 5-HT$_7$ receptors are compared to that obtained for previously published analogues with tetrametylene linkers.



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15:30 Poster 22

Chemical characteristics of melanin from the human melanoma malignum

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The *melanoma malignum* is one of the most malignant and often occurring skin tumors, which derives from melanin producing cells – melanocytes. Due to increased melanogenesis in melanoma cells, melanin formation could be used to targeted therapy of the tumor [1]. The increase of the pigment synthesis by *melanoma* cells results in arise of pheo- and eumelanin precursors (5-S-cysteinyldopa and indole derivatives) in blood and urine. These substances may be useful for therapy monitoring as tumor markers. The recognition of related to melanogenesis phenotype of *melanoma* cells could provide a possibility of appropriate selection of therapeutic agents.

The aim of our study was to characterize chemical structure of melanin isolated from the human *melanoma malignum* by two different enzymatic methods of Wilczek et al. [2] and Double et al. [3], with some modifications. Isolated pigment was thermally degraded in the presence of tetramethylammonium hydroxide (TMAH) and thermochemolysis products were analyzed by GC/MS.

Lipid products, especially fatty acids methyl esters and aliphatic and cyclic hydrocarbons were predominant among pyrolysis products of melanin isolated from *melanoma malignum* by the Wilczek method. In contrast, during thermochemolysis of melanin isolated from the tumor cells by the Double method, mainly eumelanin markers (pyrrole and its methyl derivatives, toluene, styrene and benzonitrile) and glycine and alanine methyl derivatives were obtained. The characteristic thermochemolysis products of pheomelanin (sulphur-containing heterocycles) were not observed. The presence of volatile, low-molecular weight compounds such as CO_2, CO, NH_3, H_2S, CH_3SH and SO_2 was confirmed by the analysis of selected mass ions.

Thermochemolysis technique may be useful not only for elucidation of the pigment structure, but it could be also applied to establish the relationship between melanin type and malignancy of *melanoma malignum*.

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15:30 Poster 23

Sulfonates of N-Triazinylammonium Salts as Highly Efficient and Environmentally Friendly Triazine-Based Coupling Reagents

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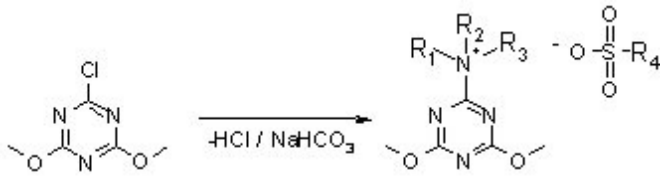
Peptide synthesis depends on the proper combination of protecting groups and the right choice of the coupling method. An optimal coupling reagent should be valid for a wide variety of peptide sequences. It should be soluble and stable in the solvents used for peptide synthesis, efficient in terms of yield, minimize the racemization.

General method of preparation of stable N-triazinylammonium salts opened access to family of N-triazinylammonium sulfonates [1]. Sulfonates of N-triazinylammonium salts consist the family of inexpensive and environmentally friendly Triazine-Based Coupling Reagents. A broad variety of N-triazinylammonium sulfonates can be obtained by treatment of 2-chloro-4,6-disubstituted1,3,5-triazines with sulfonates of tertiary amines.



Up to now, we demonstrated that they can be useful for activation of carboxylic components, with activation rate depending on the structure of the tertiary amine component and sulfonic acid counterion. Participation of triazine "superactive ester", as fundamental intermediate in condensation step, has been proved in model experiments [2]. Herein, we present the studies on screening of family of N-trizainylammonium sulfonates in peptide bond formation in solution and solid phase synthesis. The efficacy of the family of new N-triazinylammonium sulfonates as a coupling reagent in solution was examined using two models systems: Z-Aib-OH + H-Aib-OMe and Z-Leu-Phe-OH + H-Ile-OBu^t. The effectiveness of the family of N-triazinylammonium sulfonates in solid-phase mode was demonstrated in the synthesis of ACP(65-74) (ACP: H-Val-Gln-Ala-Ala-Ile-Asp-Tyr-Ile-Asn-Gly-OH), which is a good example of difficult peptide sequence, because of the development of internal secondary structures.

Acknowledgement: This work was supported by the Grant donated by the Technical University of Łódź.

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15:30 Poster 24

Rate of Binding of Host Molecules to Artificial Receptors Formed by Self-Organisation of Lipidated Oligopeptides

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The arrays of artificial receptors formed by N-lipidated oligopeptides immobilized, in the highly ordered fashion, on the solid support were synthesized and used in the studies on molecular recognition. Thus, even in the case, when the single receptor in a differential array does not necessarily have selectivity for a particular analyte [1], the combined fingerprint response can be extracted as a diagnostic pattern visually, or using any chemometric tools.

The previous studies confirmed that alternation of the binding pattern could detect even tiny structural changes of guest molecules and therefore offer a new tool for SAR studies. An assay of physiological fluids and tissue homogenates has been found useful for diagnosis of thyroid tumors [2].

Further successful application of arrays of artificial receptors for profiling of metabolome depends on efficiency of competitive binding of guest molecules and colored indicator dye. Therefore we attempted to measure the rate of binding of coloured ligands in the search for the most efficient reported dye.

The rate measurements were performed by spectrophotometric method using 96 wells ELISA plate reader.

Acknowledgements: The study was supported by the Polish State Committee for Scientific Research under the Project 2 P05F 03130.

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15:30 Poster 25

Synthesis and tuberculostatic activity of new N'-(6-methoxypyrazine-2-carbonyl)dithiocarbazic acid esters and their amidrazone analogs

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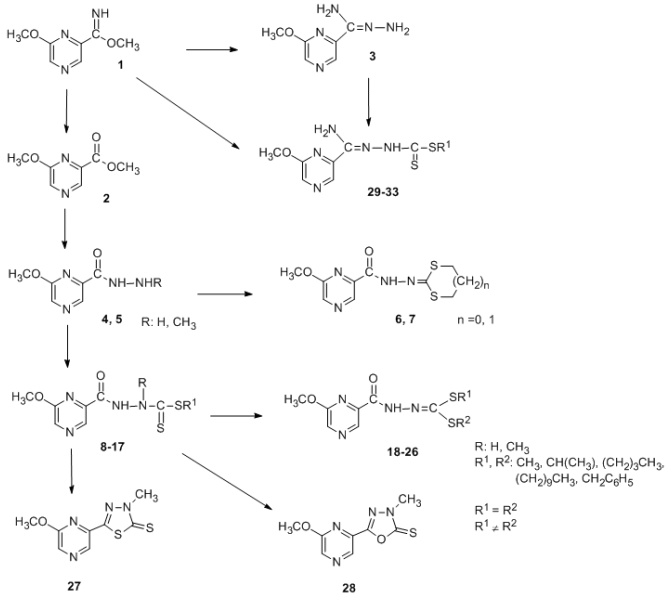
Our previous studies on antituberculosis agents resulted in synthesis of 2-pyrazinecarbamoildithiocarbazate acid esters and amides and also acethylpyrazine thiosemicarbazone derivatives with various tuberculostatic activity [1,2]. Continuing those studies we have synthesized of *N'*-(6-methoxypyrazin-2-carbonyl)dithiocarbazic acid es-

ters and their amidrazone analogs. Acid hydrolysis of methyl 6-methoxypyrazine-2-carbimidate **1** led to methyl ester **2** while treatment with hydrazine hydrate afforded amidrazone **3**. Ester **2** applied to the reaction with hydrazine hydrate or methylhydrazine gave corresponding hydrazides **4**,**5** which when treated with carbon disulfide and appropriate halides in the presence of triethylamine reacted giving *N'*-(6-methoxypyrazine-2-carbonyl)dithiocarbazic acid esters **6-26**. Similar reactions performed for amidrazone **3** resulted with corresponding structural analogs **29-33**.



The structures of novel compounds were confirmed by IR, ^{1}H NMR and MS spectra. The tuberculostatic activity was tested using *M. tuberculosis* strain H_{37}Rv and wild strains isolated from tuberculotic patients and resistant to common applied antituberculosis drugs.

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15:30	Poster	26

Validation of an analytical procedure – control of residual 8 solvents in a pharmaceutical substance

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Residues of methanol, n-pentane, ethanol, tert-butylamine, 2-propanol, acetonitrile, dichloromethane and toluene were determined by headspace gas chromatography with the use of flame-ionization detector and DB-624 (60 m long, 0.32 mm ID) column. Oven temperature in headspace was 100° C for 30 min.

According to the European Agency for the Evaluation of Medicinal Products it is considered that amount of said solvents in pharmaceutical product must not exceed: methanol 3000 ppm, n-pentane 5000 ppm, ethanol 5000 ppm, 2-propanol 5000 ppm, acetonitrile 410 ppm, dichloromethane 600 ppm, and toluene 890 ppm.

Specification limit has been established for tert-butylamine at the level of 1000 ppm.

Validation of the method included: selectivity, specificity, system precision, method precision, intermediate precision, accuracy (recovery), linearity, limits of detection and quantitation (in substance), robustness.

15:30	Poster	27

Molecular Properties Relevant to Bioavailability of Tioconazole and its Derivatives

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The molecular properties of the active substance have an impact on their pharmacokinetics and bioavailability. To simplify prove of bioequivalence of generics with reference to medicinal products various exemption procedures and rules were implemented. One of them is substitution of in vivo bioequivalence study by in vitro dissolution similarity determination. This makes a base for so called Biophamaceutical Classification System (BCS). The main two properties are considered: solubility and permeability. Experimental determination of those parameters is cumbersome and frequently impossible. Therefore theoretically derived determinants are promising tool for fast chemical substance classification within BCS. The water solubility determination can be efficiently made based on free enthalpy of solvation (ΔG), while permeability can be described by the hydrophobic properties in a first approximation. The hydrophobic properties can be established based on solvation energy in solvent with different polarity. In this study we focused on the important from therapeutic view-point antimycotic medicinal products containing the imidazole ring. Here we calculated ΔG of solvation by water and chloroform molecules at the Hartree-Fock 6-31G* level using SCI-PCM model. For tioconazole and –OH, -CH_2OH and ring derivatives and hydrochloride the values of ΔG of solvation by water molecules appeared discriminative. The values are as follows: tioconazole ΔG = -0.51 kcal/mol, -OH in 4 position of imidazole ring ΔG = -3.25 kcal/mol, -CH_2OH in 4 position of imidazole ring ΔG = -1.58 kcal/mol, hydrochloride ΔG = -44.17 kcal/mol. In chloroform, solvent in dielectric constants similar to an octanol the respective values of ΔG are: -5.25 kcal/mol, -2.72 kcal/mol, -3.32 kcal/mol and -27.17 kcal/mol. Analyzed structural changes give hints to synthesis of compounds with better pharmacokinetic properties of antimycotic agents, the OH substituent appeared to be promising way of parent structure modification.

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Novel 4-aryl-pyrido[1,2-c]pyrimidines with dual SSRI and 5-HT$_{1A}$ activity. Part 2.

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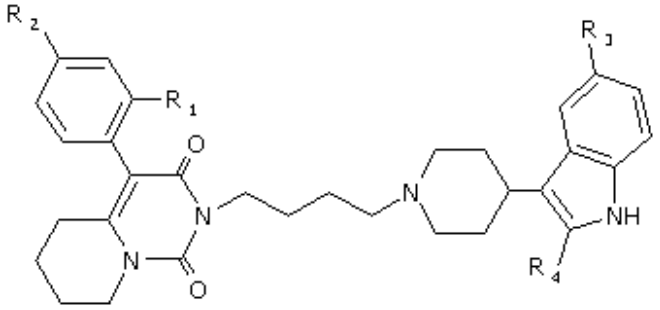
In our previous studies on the pirydo[1,2-*c*]pyrimidines we obtained a series of new compounds with dual SSRI and 5-HT$_{1A}$ agonist activity. As a continuation of our research programme to develop faster acting antidepressants, we synthesized new derivatives of 4-aryl-5,6,7,8-tetrahydro-pyrido[1,2-*c*]pyrimidines with 3-(piperidin-4-yl)-1*H*-indole moiety.

The structures of new compounds were confirmed by ^{1}H and ^{13}C NMR spectral data as well as by C, H, N analyses.

Target compounds were tested for their affinity for 5-HT$_{1A}$ receptor and 5-HT reuptake inhibition using radioligand binding assay. Compounds **8a-k** revealed affinity for 5-HT$_{1A}$ receptor which varied from high to moderate ($K_{i5\text{-HT1A}}$: 10,4 – 53,8 nM), while compounds **8l-t** were almost inactive. Compounds **8a-t** displayed moderate to low affinity for 5-HT-T receptor ($K_{i5\text{-HT-T}}$: 71,6 – 943,7nM).

Compounds **8a**(20mg/kg), **8d** (20mg/kg), **8f** (10mg/kg) and **8g** (10mg/kg) were found to be presynaptic 5-HT$_{1A}$ receptor agonists in induced hypothermia test in mice, while compound **8b** (20mg/kg) displayed presynaptic 5-HT$_{1A}$ receptor antagonistic activity.

Compounds **8d**(20mg/kg), **8f** (10mg/kg) and **8g** (10mg/kg) revealed their postsynaptic 5-HT$_{1A}$ agonist activity in forced swimming test in mice.



R_1 R_2 R_3 R_4: **8a**: H, CH_3, OCH_3, H; **8b**: H, OCH_3, H, H; **8c**: H, OCH_3, OCH_3, H; **8d**: F, H, OCH_3, H; **8e**: H, Cl, OCH_3, H; **8f**: H, CH_3, H, H; **8g**: F, H, H, H; **8h**: CH_3, H, H, H; **8i**: CH_3, H, OCH_3, H; **8j**: H, F, OCH_3, H; **8k**: Cl, H, H, H; **8l**: F, H, H, CH_3; **8m**: H, H, H, CH_3; **8n**: OCH_3, H, H, CH_3; **8o**: CH_3, H, H, CH_3; **8p**: H, CH_3, H, CH_3; **8q**: H, OCH_3, H, CH_3; **8r**: H, Cl, H, CH_3; **8s**: Cl, H, H, CH_3; **8t**: H, F, H, CH_3;

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Novel 4-aryl-pyrido[1,2-c]pyrimidines with dual SSRI and 5-HT$_{1A}$ activity. Part 1.

Franciszek Herold[1], Andrzej Chodkowski[1], Łukasz Izbicki[1], Jerzy Kleps[1], Marek Król[1], Gabriel Nowak[2,3], Katarzyna Stachowicz[2], Małgorzata Dybała[3], Agata Siwek[3]

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One approach toward new antidepressants with better clinical parameters is the synthesis of compounds with dual SERT inhibitor/ 5-HT$_{1A}$ activity [1]. Among many concepts in this field, the idea of incorporating a 5-HT$_{1A}$ antagonist component into the structure of an SSRI is thought to be the most advantageous. However, there is a concern that the use of 5-HT$_{1A}$ receptor antagonist without discriminating between its pre- and postsynaptic activity could cancel the benefits of enhancing presinaptically the 5-HT function. Therefore an alternative method is the design of compounds with dual 5-HT reuptake blockade/5-HT$_{1A}$ receptor agonist action, which could desensitize presynaptic 5-HT$_{1A}$ receptors and postinaptically enhance serotonin transmission [2-4].

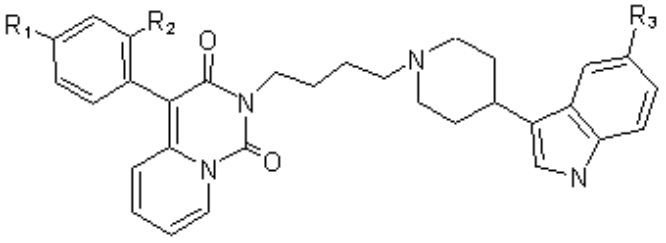
The aim of this work was the design, synthesis and biological evaluation of new compounds with dual 5-HT$_{1A}$ and 5-HT-T affinity.

The structures of the new compounds were confirmed by ^{1}H and ^{13}C NMR spectral data as well as by C, H, N analysis.

Target compounds were tested for their affinity for 5-HT$_{1A}$ receptor and 5-HT reuptake inhibition using radioligand binding assay. Compounds **7j** and **7k** revealed very high affinity to 5-HT$_{1A}$ and 5-HT-T receptors (**7j** $K_{i5\text{-HT1A}}$ = 4,8nM / $K_{i5\text{-HT-T}}$ = 0,7nM, **7k** $K_{i5\text{-HT1A}}$ = 5,8nM / $K_{i5\text{-HT-T}}$ = 0,3nM). Other ligands showed high to moderate affinities.

Compounds **7d** (20mg/kg), **7g** (10mg/kg), **7i** (20mg/kg), **7j** (20mg/kg) and **7k** (20mg/kg) were found to be presynaptic 5-HT$_{1A}$ receptor agonists in induced hypothermia test in mice.

Compounds **7g** (20mg/kg), **7i** (20mg/kg) and **7j** (10mg/kg) revealed their postsynaptic 5-HT$_{1A}$ agonist activity in forced swimming test in mice.



R_1 R_2 R_3: **7a**: H, H, OCH_3; **7b**: H, OCH_3, H; **7c**: H, OCH_3, OCH_3; **7d**: H, Cl, H; **7e**: H, Cl, OCH_3; **7f**: F, H, OCH_3; **7g**: H, F, H; **7h**: H, F, OCH_3; **7i**: H, H, H; **7j**: F, H, H; **7k**: H, CH_3, H; **7l**: H, CH_3, OCH_3;

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Amphiphilic dendrimeric peptides as efficient broad spectrum antimicrobial agents

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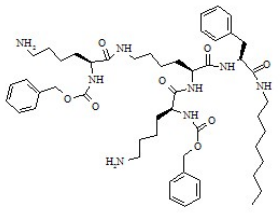
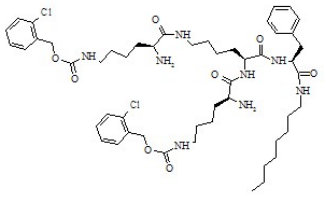
Limited treatment options with the use of conventional antibiotics to combat the antibiotic resistance, created a growing need to invent, new potent broad-spectrum antimicrobial drugs. One of the new sources of the prospective alternatives to traditional antibiotics are natural basic antibacterial peptides 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 microbial 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 [1,2] and variable cytotoxicity strongly correlated with degree of branching. This communication presents synthesis and results of microbiological tests of the two diastereoisomeric groups of dendrimeric peptides (A and B) with C-end modified by aliphatic chains of various length.



A



B

The above compounds express high antimicrobial activity, particularly against Gram(+) bacteria including MRSA strains and fungi from *C. albicans* family. Studies of circular dichroism (CD) showed that these molecules undergo conformational change in presence of micelles that mimic bacterial membranes.

Financial support from Ministry of Education and Informatics of Poland, Grant 3T09B 115 28 is acknowledged.

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Separation of enantiomers of Z and E 3-phenylglycidic acid t-butyl ester

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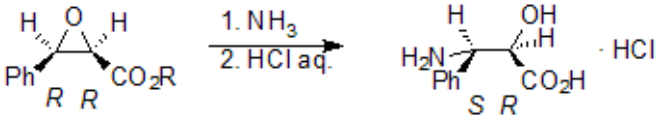
Looking for a new method of synthesis of (*2R,3S*)-3-phenylisoserine hydrochloride – an important intermediate on the route to *Paclitaxel* – we turned our attention to the reaction of (*2R,3R*)-3-phenylglycidic acid esters with ammonia, which should lead to (*2R,3S*)-3-phenylisoserine.



Glycidic esters are most conveniently prepared in Darzens reaction of α-halogenoacids esters with carbonyl compounds, carried out in the presence of a base.

Working on a practical synthesis of (*2R,3R*)-3-phenylglycidic acid *t*-butyl ester we needed a good analytical method of determination of the content of enantiomers of both *Z* and *E* isomers of 3-phenylglycidate in reaction mixtures.

The aim of the presented work was to elaborate the conditions for chiral separation of the enantiomers mentioned using high performance liquid chromatography (HPLC).

We tested three analytical columns and investigated the chiral separation depending on the composition of the mobile phase. The following mixtures were used as mobile phases in different proportions: *n*-hexane / 2-propanol, *n*-hexane / methanol, *n*-hexane / *t*-butyl methyl ether.

The best chiral separation of the enantiomers of *Z* and *E* 3-phenylglycidic acid *t*-butyl ester was achieved using the following conditions:
Chiralcel OD-H column, packing composition: Cellulose tris (3,5-dimethylphenylcarbamate) coated on 5µm silica-gel, 250×4.6mm.
Mobile phase: *n*-hexane / 2-propanol solvent mixture (98.2 : 0.8 v/v).
Detection: UV/VIS, 235nm.
Flow rate: 0.5ml/min
Temp.: 25°C.

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Determination of the enantiomeric purity of epinephrine in pharmaceutical preparations

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Epinephrine [(R)-(-)-1-(3,4-dihydroxyphenyl)-2-methylamino-ethanol] is known to exhibit high affinity to β-adrenergic receptors. It is released from the adrenal medulla and can be found in central as well as in peripheral nervous system. Epinephrine is a "fight-or-flight" hormone, and plays a central role in the short-term stress reaction when danger threatens or in an emergency. When secreted into the bloodstream, it rapidly prepares the body for action in emergency situations. It increases heart rate and stroke volume, dilates the pupils, and constricts arterioles in the skin and gut while dilating arterioles in skeletal muscles. It elevates the blood sugar level by increasing catalysis of glycogen to glucose in the liver, and at the same time begins the breakdown of lipids in fat cells [1]. Epinephrine is used as a drug to treat anaphylactic shock (e.g. after insect stings or adverse reaction to medication) , cardiac arrest and other cardiac arrhythmias resulting in diminished or absent cardiac output.

As a method of *S* enatiomer determination European Pharmacopeia 5.0 recommends specific rotation measurement. Since specific rotation is a parameter depending on several factors which often are difficult to control (e.g. optically active impurities) new methods such as capillary electrophoresis [2,3] and high performance liquid chromatography [4] are being developed.

In the present communication we report the application of chiral stationary phase high performance liqud chromatography to the enantiomeric purity determination of epinephrine in pharmaceutical substances and preparations.

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[3] H. Li, W. Luo, X. Hu. *Se Pu.Chin. J. Chromatogr.* Determination of enantiomeric purity for epinephrine by high performance liquid chromatography. **17**, 403-405 (1999).
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Isolation and purification of (2R,3S)-3-phenylisoserine hydrochloride

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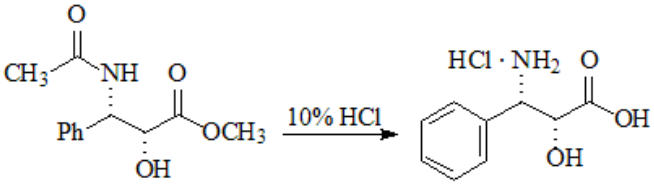
The aim of this work was to develop a method of separation and purification of (*2R,3S*)-3-phenylisoserine hydrochloride (FIS), obtained from methyl *N*-acetyl-(*2R,3S*)-3-amino-2-hydroxy-3-phenyl propionate by acidic hydrolysis.



We found that isolation and purification of FIS can be effectively realised by extraction of impurities, present in the aqueous phase after acidic hydrolysis, by organic solvents. The best results were achieved using diethyl ether or *t*-butyl methyl ether as solvents. The aqueous phase after extraction was then evaporated to dryness. The crude solid FIS thus obtained crystallizes with difficulty from aqueous or alcoholic solutions. Good results of purification were obtained using *n*-propanol as a solvent, however the yield of crystallization was only 54%.

Removal of the impurities present in the solid FIS can be achieved much more efficiently by simple stirring of the solid with ethyl acetate or methylene chloride at 50 ºC for 2 hours. Using this procedure we obtained FIS with 90% purity and 95% purification yield.

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The New Analogues of Nitrogen Mustard With One, Two or Three 2-Chloroethylamino Fragments. Reactions with Nucleophiles

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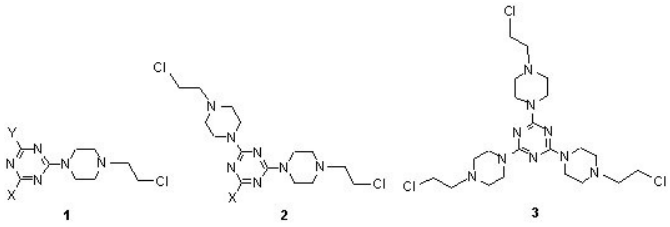
Nitrogen mustards (NM) with 2-chloroethylamino fragment are used as antineoplastic agents in therapy of cancer as nonspecific DNA alkylating agents. The antitumor activity of nitrogen mustards has been connected with their ability to cross-link the twin strands of DNA which if not repaired, can inhibit DNA replication and transcription, eventually leading to cell cycle arrest, apoptosis, and the inhibition of tumor growth. Classical NM drugs don’t operate selectivity, causing high levels of inadvertent DNA damage in normal

cells, toxic and mutagenic side effects, and in some cases, leading to secondary malignancies Therefore, the search for the new analogues is continued expecting improvement of therapeutic index by modification of NM structure. In our previous report [1] we presented results of synthesis of triazines substituted with one, two or three 2-chloroethylamine moiety **1-3** and studies on the determination of apoptotic index and cell viability on the standard cell line of mammalian tumor MCF-7 by the compounds obtained.



The studies shown that triazine derivatives **1-3** inhibits 50% of colony formation at the concentration in the range 13,88 µM to 146,79 µM, while the IC50 of chlorambucil is 24,6 µM. Herein, we present the studies on alkylation of broad range of nucleophiles with triazine NM **1-3.**

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15:30	Poster	35

Fucosyl thiocarbamates, synthesis and application in glycosylation

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Fucose is a deoxyhexose that is present in the L-configuration of many N- and O-linked oligosaccharide structures of membrane as well as soluble glycoproteins and glycolipids produced by mammalian cells. The fucose molecule is present in ABH blood group antigens and in some oligosaccharide structures. Fucose can be α1,2, α1,3, α1,4, and α1,6 linked to the glycans of glycoconjugates. This predisposes fucose to play a crucial role in biological recognition events, such as cell-cell and cell-matrix interactions [1]. For that reasons the chemical synthesis of fucosyl glycosides, especially an efficient method for the formation of 1,2-cis glycosidic bond is a challenging task. In this communication we report the novel and stereoselective fucosylation of using O-fucosyl N-allyl thiocarbamates as glycosyl donors. These compounds are readily obtained from anomerically-unprotected fucose by reaction with commercially available N-allyl isothiocyanate [2]. The application this method for the synthesis of alkyl glycosides, disaccharides, trisaccharides and glycoconjugates of fucose will be presented. This method was used in the synthesis 2'-*O*-α-L-fucopyranosyl-lactose important component of human milk.

Acknowledgments

Financial support from the Polish State Committee for Scientific Research (Grant No. 3 T09B11229) is gratefully acknowledged.

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15:30	Poster	36

Structure and Molecular Dynamics of Benzodiazacoronads in the liquid phase - study of preorganization mechanism leading to planar chirality of crystals.

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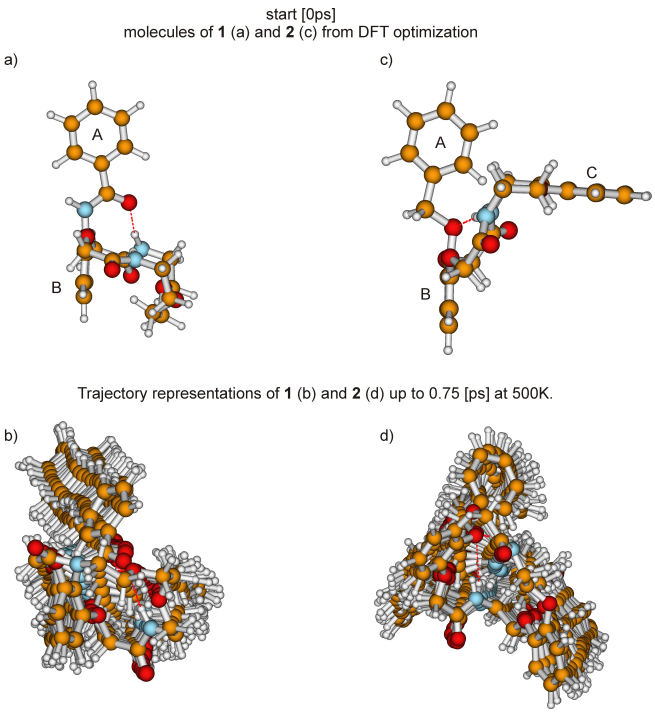

Chiral crystals formed from achiral molecules have recently received a great deal of attention due to their attractive structural properties and prospective applications in chemistry [1]. For instance, such compounds can be used as ligands in enantioselective reactions or models for investigation of molecular recognition [2]. Understanding the details, which govern the process of formation of chiral crystals, chiral induction, and th mechanism of spontaneous resolu-

tion of racemic compounds, is one of the most general questions touching the problem of the origin of life [3].

As a part of our interest in the problem of formation of crystals with planar chirality [4] we presented the structural studies for two diazacoronands **1** and **2** [5] in the solid phase. It was apparent, that crystals of **1** and **2** have to be considered as a two-component system consisting of an organic unit and a water molecule in 1:1 ratio.

Both components play an important role in the crystal structure. The strong (O-H...O, N-H...O) and weak (C-H...O) intermolecular hydrogen bonds are responsible for phase organization and, in consequence, formation of chiral or achiral crystals. The alignment of the water molecule with respect to the macrocycle is different for samples **1** and **2**, so the water molecule can be an important achiral cofactor responsible for chiral crystallization. It is worthy to note, that process of removal of water from the crystal lattice of **1** is reversible.

In this work, we report the results of the investigation of the structure and molecular dynamics of two benzodiazacoronads **1** nas **2** in the liquid phase. The major aim of our project is to understand the origin of distinction between these compounds and answer the question what is the role of water molecules in the process of formation of chiral crystals during the preorganization step? For this purpose, NMR spectroscopy and molecular dynamics calculations appear to be a powerful methods. Both, the results of relaxation parameters (T_1, T_2 and NOE) measurements and MD Calculations, will be presented and discussed.

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[4] a) Kalisiak, J.; Jurczak, J. Synlett 2004, 9, 1616-1618. b) Piatek, P.; Kalisiak, J.; Jurczak, J. Tetrahedron Lett. 2004, 45, 3309-3312. c) Kalisiak, J.; Skowronek, P.; Gawronski, J.; Jurczak, J. J. Eur. Chem. 2006, 12, 4397-4406. d) Kalisiak, J.; Jurczak, J. Cryst. Growth Des. 2006, 6, 22-24.
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First attempts at the enzyme-promoted hydrolysis of N-acyl phosphonoamides

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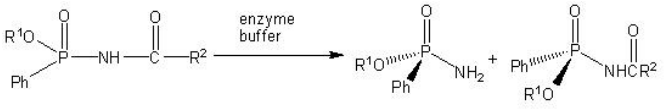
P-Chiral organophosphorus compounds play an important role both in mechanistic studies and as biologically active species. Among a variety of methods of their synthesis those based on enzyme-mediated transformations have recently become a valuable supplement [1]. Continuing our earlier works on the application of hydrolytic enzymes in the synthesis of functionalized chiral organophosphorus derivatives [2], we became interested in using this methodology in the synthesis of P-chiral non-racemic phosphonoamides, the compounds which are difficult to obtain via traditional chemical methods. To achieve this, we screened a series of hydrolytic enzymes for the stereoselective hydrolysis of a variety of N-acyl phosphonoamides under kinetic resolution conditions.



Although a number of enzymes proved unreactive towards particular substrates, some of them were found to catalyze the desired reaction. The influence of the substituents at phosphorus and the kind of enzyme on the reaction outcome will be discussed.

Acknowledgment:

This work was supported by the network "Synthesis, Structure and Therapeutic Properties of Compounds and Organic Substances".

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15:30 Poster 38

Determination of the stereoisomers of racemic α-tocopherol in pharmaceutical products by high-performance liquid chromatography and gas chromatography

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The molecule of α-tocopherol is characterised by three chiral centres (C-2, C-4', C-8') which yield eight optically active stereoisomers. The two compounds of the highest biological activity and the greatest practical significance are the naturally occurring RRR-α-tocopherol and the synthetic all-rac-α-tocopherol, which is a mixture of the following stereoisomers: SSR, SSS, SRS, SRR, RSS, RRS, RRR, RSR.

Most pharmaceutical products whose composition includes vitamin E contain racemic α-tocopherol or its esters (mostly acetate). It is therefore important to determine stereoisomeric purity of the pharmaceutical products containing this compound.

Previous papers describe methods of determination of the stereoisomers of α-tocopherol in biological material.

The aim of our study was to separate all-rac-α-tocopherol into indi-

vidual stereoisomers in pharmaceutical products containing dl-α-tocopherol acetate.

We performed hydrolysis of dl-α-tocopherol acetate to α-tocopherol, followed by precolumn derivatisation of the standard and the pharmaceutical products with dimethyl sulphate. Derivatisation was performed to change the polarity of the analyte. We then separated the resulting methyl derivatives of α-tocopherol by HPLC, yielding 5 peaks corresponding to the 2S (SRS+SSR+SSS+SRR) and RSS, RRS, RRR, RSR stereoisomers. We then collected the 2S stereoisomer fraction with a fraction collector and analysed it by gas chromatography.

The combining of the two chromatography techniques (HPLC with GC) allowed us to separate α-tocopherol derivatives (α-TMe) into 8 stereoisomers. HPLC additionally enabled unequivocal identification of these stereoisomers. Our results confirm the correct selection of raw materials used for the manufacture of the pharmaceutical products we investigated.

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Stereoselective synthesis of highly oxygenated derivatives decalins and cyclohexanes from monosaccharides

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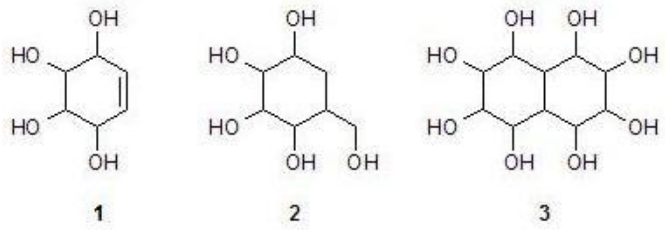
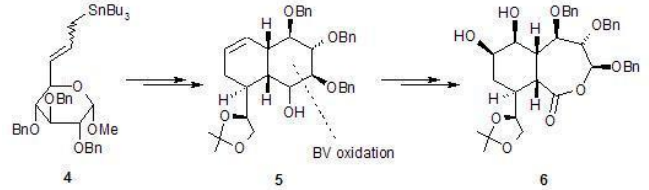
Polyhydroxylated monocarbocyclic derivatives (such as 1 or 2) are known as structures of high biological activity. The bicyclic analogues, houever, although much less known possess also interesting activity. This types of compounds are considered as inhibitors of glycosidase and glycomimics.



Compound 5 has been prepered from the alliltin derivative 4 by a metodology developed in our laboratory [1].

Functionalization of either ring in 5 will be presented a leading example of such functionalization is converrsion of 5 into cyclohexane 6 via Baeyer-Villiger oxidation [2].



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15:30 Poster 40

Synthesis of Building Blocks Containing Glycosyl Moieties by Using Triazinylammonium Sulfonates

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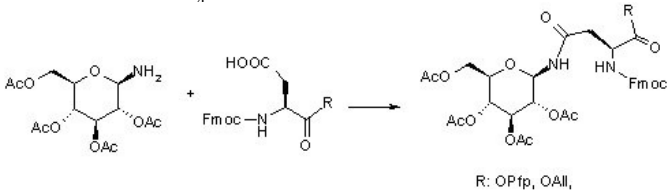
Posttranslational modifications are covalent processing events that change the properties of a protein by the addition of a modifying group to one or more amino acids [1]. Growing evidences indicate that N-glucosylation is a posttranslational modication (PTM) that may play a fundamental role in a large number of biological events [2]. The most well-known modifying groups in eukaryotic proteins are glycans. Protein glycans are classified into two groups: N-linked glycans attached to asparagine (Asn) residues and O-linked glycans attached to Ser or Thr residues. Currently, the synthesis of building blocks containing glycosyl moieties, linked to the carboxyl function of aspartic acid by an amide bond, typically requires glycosylamines and efficient coupling reagents. In our previous studies [3], we examined triazine-based coupling reagent in synthesis of N-glycosylated-Asp derivative. A comparative study of the coupling reaction between glucosamine and aspartic acid was performed using DMT/NMM/BF_4, CDMT, HATU, TBTU, and BOP.



Herein, we present the studies on screening of family of N-triazinylammonium sulfonates in synthesis of building blocks containing glycosyl moieties.

Acknowledgement: This work was supported by the Grant donated by the Technical University of Łódź.

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Transformation of Tertiary Amines into N-Triazinylated Analogues by Treatment with Chloro-1,3,5-Triazines

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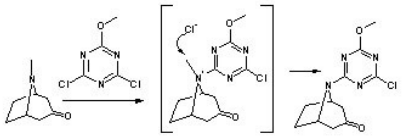
A survey of novel small-molecule therapeutics reveals that the majority of them result from analogue design and that their market value represents two-thirds of all small-molecule sales. Formally, definition of analogue allows the establishment of three categories: analogues possessing chemical and pharmacological similarities (direct analogues); analogues possessing structural similarities only (structural analogues); and chemically different compounds displaying similar pharmacological properties (functional analogues) [1]. Another approach for efficient search of new therapeutics involves hybrids that combine two pharmacophores expecting that the dual mode of action could be demonstrated for both of them. Recently we found conditions for mild, highly efficient N-dealkylation-triazinylation process proceeding with defined stereochemistry and high yield, involving reaction of tertiary amines with chloro-1,3,5-triazine. Considering potential activity of many triazine as topoisomerase inhibitors we attempted to introduce into triazine ring an additional structural motifs. Herein we used this approach for modification of native structure of alkaloids.



In the case of a sterically constrained bicyclic system of tropinone N-demethylated –triazinylated product with monosubstituted triazine was obtained offering a reactive centre in the triazine ring convenient for the further modification.

Acknowledgement: This work was supported by Ministry of Science and Higher Education, Grant PBZ-KBN-126/T09/15.

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15:30 Poster 42

Application of privileged structures in new drug discovery

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A success in development of new drug strongly depends on the early stage of the process, namely on the selection of a good lead compound. A failure of several "structure diversity" oriented drug discovery campaigns turned attention of medicinal chemist to other approaches, including so called privileged structures. According to the original definition the term describes "a single molecular framework able to provide ligands for diverse receptor" (*J. Med. Chem*. **1988**, *31*, 2235) and in a more descriptive form relates to "... certain classes of compounds that are highly represented in the overall bioactive compound population, have demonstrated a wealth of biological activity in addition to sound drug-like properties ..." (*Comb. Chem. High Through. Scr*. **2004**, *7*, 473).

The concept of privileged structures will be presented.

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The Crystal Structure of distrontium salt of 5-[bis(carboxymethyl)amino]-3-carboxymethyl-4-cyano-2-thiophenecarboxylic acid

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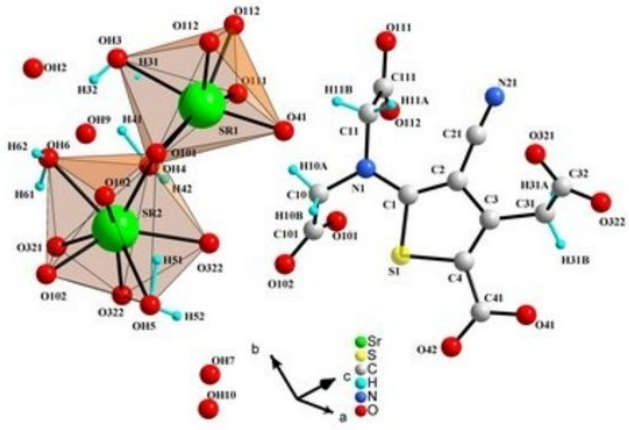
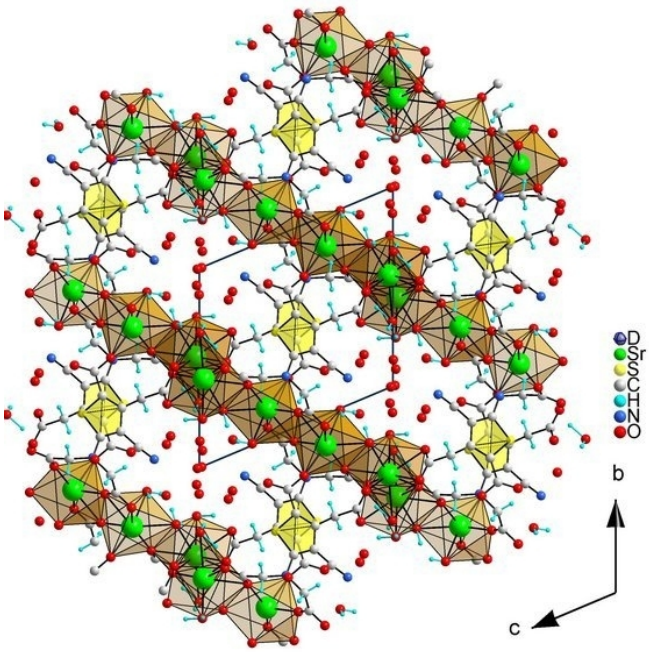
Strontium ranelate, i.e. distrontium salt of 5-[bis(carboxymethyl)amino]-3-carboxymethyl-4-cyano-2-thiophen ecarboxylic acid is licensed for the treatment of postmenopausal osteoporosis to reduce the risk of vertebral and hip fractures. It reduces the risk of vertebral and non-vertebral fractures compared with placebo but has not been directly compared with other osteoporosis treatments [1].

Its crystal structure has been determined from X-ray diffraction data at 150K (using the single crystal diffractometer with CCD area detector). Very small (0.01*0.02*0.28mm) single crystals of strontium ranelate octahydrate have been obtained according to the procedure described in Example 1d (second method) disclosed in EP 415 850 B1 [2]. The crystal structure was solved by direct method [SHELXL 97] and was refined by full matrix least squares. The experimental crystallographic data are following:
Space group: triclinic, P-1(2), with the lattice parameters at 150K:
a = 8.2580(17) Å, *b* = 11.5140(23) Å, *c* = 12.4270(25) Å
α = 111.95(3) º, *β* = 102.62(3) º, γ = 91.27(3) º
The projection of ranelic acid anion with the coordination polyhedron of Sr ions and the packing of the molecule in the crystal lattice along b axis are illustrated in the bottom figures.





The distances and angles in the strontium ranelate molecule are comparable to those found in the tetraethyl ester of ranelic acid [3]. The crystal structure contains 8 molecules of water. The symmetry independent Sr(1) and Sr(2) ions are complexed to oxygen 7 and 9, respectively, coming from carboxylate oxygens and the water molecules. The polyhedrons of Sr ions form the layers in the (01–1) plane. The water molecules occupying the positions between layers are strongly disordered [4].

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15:30 Poster 44

Synthesis, structure and biological activity of 1-[(imidazolidin-2-yl)imino]azoles*

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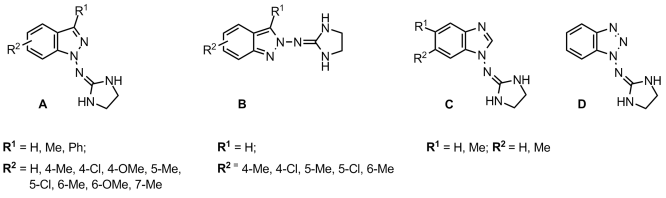
In connection with our previous studies on novel imidazoline compounds [1-3] with alpha$_2$-adrenoceptor and/or imidazoline I$_1$/I$_2$ receptor affinity, we have prepared a series of new indazole **A** - **B**, benzimidazole **C** and benzotriazole **D** derivatives bearing (imidazolidin-2-yl)imino moiety at position 1 or 2.



The *in vitro* assays involving investigation of the affinity and selectivity of the newly synthesized imidazoline ligands for alpha$_2$-adrenoceptors, imidazoline I$_1$ and imidazoline I$_2$ binding sites showed very low or no affinity for imidazoline I$_2$ receptors. 1-[(Imidazolidin-2-yl)imino]indazole (*marsanidine*), the most active agent at alpha$_2$-adrenoceptors (K_i = 14 nM), displayed a very high alpha$_2$/I$_1$ selectivity ratio = 3879.

The *in vivo* cardiovascular properties of the selected indazole derivatives of type **A** were evaluated after intravenous infusion in anaesthetized male Wistar rats. Among the tested compounds the highest hypotensive activity was found for 1-[(imidazolidin-2-yl)imino]-7-methylindazole (Δ MAP = - 43.5 mmHg at dose 10 μg/kg, ED_{50} = 0.6 mg/kg), which also exhibited a good affinity for alpha$_2$-adrenoceptors (K_i = 53.5 nM) and moderate affinity for imidazoline I$_1$ receptors (K_i = 387 nM).

These results suggest that *marsanidine* may find variety of medicinal uses, especially as an alpha$_2$-agonist for organoprotection and anaesthesia, while its 7-methyl analogue is a good candidate for further development as a centrally acting antihypertensive drug.

*This research was supported by the Polish Ministry of Science and Higher Education (Grant N40500532/0458). Sączewski F., Kornicka A., Rybczyńska A., Hudson A.L., Miao S.S., Gdaniec M., Boblewski K., Lehmann A. 1[(Imidazolidin-2-yl)imino]indazole (*Marsanidine*) – highly alpha$_2$/I$_1$ selective agonist: synthesis, X-ray structure and biological activity - accepted for publication in *J. Med.Chem*. **2008**; Sączewski F., Kornicka A., Rybczyńska A., Hudson A.L. Nowe pochodne 1-[(imidazolidyn-2-ylo)imino]indazolu i sposób ich otrzymywania. *Patent Application***P 383955**.

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The determination of chromatografic purity of Pramipexole Dihydrohloride Monohydrate

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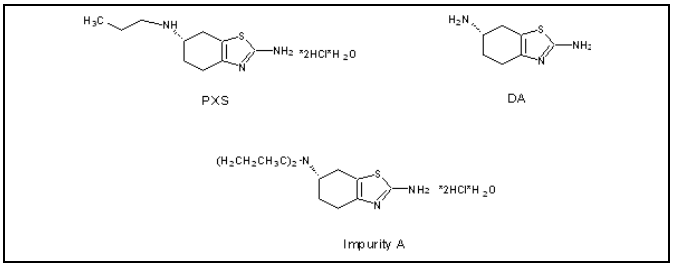
The aim of this study was to develop the chromatographic method (HPLC) for determination of pramipexole and its two impurities: S(-)-4,5,6,7-tetrahydrobenzothiazol-2,6-diamine (DA) and dihydro-

chloride monohydrate (S)(-)-4,5,6,7-tetrahydro-N^6,N^6dipropylbenzothiazole-

2,6-diamine (Imp. A). The chemical structures of tested substances are presented in Figure 1.



Several methods have been tested and finally the method using C18 column and the mobile phase containing 1-octanosufonic acid salt was chosen. The method has been validated and the following parameters were tested: specificity and selectivity, linearity, detection and quantitation limits, precision, intermediate precision, accuracy and robustness. The acceptance criteria were fulfiled.

The method has been applied in testing the stability of pramiexole under stress conditions. The tests included: the effect of oxidation on API and stability in the acidic and alkaline conditions.

It was found that the influence of 1 M HCl on PXS is minimal, the influence of 3 %H_2O_2 is noticeable and the influence of 1M NaOH is significant. The additional peaks of unknown substances appeared in the chromatograms of the tested solutions.

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Efficacy and safety of thalidomide in the treatment of relapsed and refractory multiple myeloma

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Background: Thalidomide can directly inhibit the growth and survival of myeloma cells. It also targets marrow stromal cells, alters IL-6 and TNF-a production and decreases adhesion of myeloma plasma cells .Due to modified cell –to- cell contacts and interactions within bone marrow microenvironment, malignant plasma cells may become resistant to therapy and may develop tendency to disseminate and infiltrate other tissues in the periphery. The aim of study was to assess thalidomide treatment efficacy and pay attention to appearance of severe, especially cardiac, complications and possibility of development of extramedullary myeloma manifestation following thalidomide therapy.

Methods: We enrolled 21 patients (8 females and 13 males, aged 47- 78 years) with refractory/relapsed multiple myeloma who have failed at least one prior line of treatment. The time since myeloma diagnosis to onset of thalidomide therapy ranged from 7 to 120 months. In 10 patients thalidomide alone at doses from 100 to 400 mg/day was applied as long-term therapy, in 3 thalidomide was given together with high-dose dexamethasone pulses, in 7 patients in form of combined treatment with melphalan and prednison (MPT program) and in one- together with cyclophosphamide and dexamethasone (CTD program) .

Results: Of 10 patients treated with thalidomide only, partial remission was achieved in 2 patients, disease stabilization in one, and in 4 patients disease progressed, including one in whom myeloma evolved into plasma cell leukemia. Time to disease progression since the onset of thalidomide therapy ranged from 5 to 10 months. In 3 patients the treatment was interrupted due to adverse events: Morgagni-Adams-Stokes-syndrome, bradyarrhythmia- atrial fibrillation, sudden loss of hearing and enormous debilitation, respectively. In 3 patients, in whom thalidomide was administered together with high-dose dexamethasone, partial remission was achieved after two months of treatment. In one of those patients partial remission lasted 10 months but the treatment had to be stopped due to bradycardia while in two remaining patients partial remission lasted 3 months and ended with plasma cell leukemia in one patient, and with plasma cell infiltration of lymph nodes in the other one. In all assessed patients treated according to MPT or CTD regimens a partial response was achieved. In this group, the treatment was stopped in two patients; in one due to extensive skin lesions, and in the other one due to atrial fibrillation recurrences.

Conclusions: In relapsed/refractory myeloma the rate of response to thalidomide alone is 28%, with median duration of response of 6 months. Our findings suggest that thalidomide is effective in initially reducing more mature plasma cell compartment confined to the marrow and allows a relatively immature myeloma cell compartment to escape marrow microenvironment. In one third of patients treatment with thalidomide is interrupted due to adverse events.

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Examination of antibacterial and antifungal activity of selected non-antibiotic products

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Various compounds which are involved in the management of diseases of non-infectious aetiology have shown some antimicrobial activity *in vitro*, against bacteria and other microorganisms. Such compounds are called “non-antibiotics”. So far, a lot of attention has been focused on phenothiazines, thioxanthenes and other agents with affinities to cellular transport systems or agent showing other inhibition mechanism. Some authors confirmed that some non-antibiotics are helper compounds which enhance the *in vitro* activity of certain antibiotics against specific bacteria (e. nizatidine and omeprazole enhance the effect of metronidazole on *Helicobacter pylori*). The aim of this study was to detect and characterise the antimicrobial activity of non-antibiotic drugs, selected from the pharmaceutical products analysed during state control performed in National Medicines Institute in Poland. 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 ex-

amined strains: Citabax 40 mg tabl. (citalopram), Clopidix 75 mg tabl. (clopigrel), Doculax 50 mg caps. (sodium docusate), Glucobay 100 mg tabl. (acarbose), Nalgesin 550 mg tabl (naproxen), Simvacor 10 mg tabl. (simvastatine), Sylicaps 100 mg tabl. (sylimarine) and Vesicare 5 mg, 10 mg tabl. (solifenacin succinate). *Staphylococcus aureus* was susceptible to over 70% of the drugs listed above. The lowest inhibitory concentration was found for docusate sodium (3 mg/ml). Other chemical compounds showed activity against this microorganism in concentrations between 5 and 70 mg/ml. Acarbose and citalopram in concentration 7 mg/ml showed the strongest activity against *E. coli*. *C. albicans* showed the strongest susceptibility to simvastatine (MIC- 5 mg/ml). Interestingly, natural product – sylimarine extracted from *Silybum marianum* inhibited *S. aureus* in concentration 67 mg/ml. *P. aeruginosa* was resistant to all of the examined active substances. The antimicrobial activity of all drugs emphasises a necessity of the neutralisation of their activity during the microbial purity assays of pharmaceutical products.

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Determination of the enantiomeric purity of norepinephrine in pharmaceutical preparations

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Norepinephrine [(R)-(-)-2-amino-1-(3,4-dihydroxyphenyl)ethanol] is known to activate mostly α-adrenergic receptors. It can be found in central as well as in peripheral nervous system. It is also released by the adrenal medulla causing a contraction of peripheral blood vessels, a dilation of coronary vessels and exhibiting positively inotropic activity. As a stress hormone, norepinephrine affects parts of the brain where attention and responding actions are controlled. Along with epinephrine, norepinephrine also underlies the fight-or-flight response, directly increasing heart rate, triggering the release of glucose from energy stores, and increasing blood flow to skeletal muscle. As a drug it used in acute circulation insufficiency, in myocardial infarction and anaphylactic shock.

As a method of *S* enatiomer determination European Pharmacopeia 5.0 recommends specific rotation measurement. Since specific rotation is a parameter depending on several factors which often are difficult to control (e.g. optically active impurities) new methods such as capillary electrophoresis [1,2] and high performance liquid chromatography [3] are being developed.

In the present communication we report the application of chiral stationary phase high performance liqud chromatography to the enantiomeric purity determination of norepinephrine in pharmaceutical substances and preparations.

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[3] M. H. Hyun, S. C. Han, B. H. Lipshutz, Y.-J. Shin, C. J. Welch. *J. Chromatogr. A* Liquid chromatographic resolution of racemic amines, amino alcohols and related compounds on a chiral crown ether stationary phase. **959**, 75-83 (2002).

15:30 Poster 49

Disubstituted indolo[2,3-b]quinoline derivatives - the cytotoxic activity in vitro against various human tumor cell lines.

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In our study double substituted indolo[2,3-b]quinoline derivatives bearing (dialkylamino)alkyl chains at N-6 and C-2 or C-9 position were tested against various human tumor cell lines and their drug-resistant sublines: human colon cancer (LoVo) and doxorubicin-resistant LoVo/DX (P-gp-dependent, MRP-, LRP-dependent multidrug resistance), uterine sarcoma (MES-SA) and MES-SA/DX5 (P-gp-dependent resistance to doxorubicin), human promyelocytic leukemia cell line (HL-60) and HL-60/MX2 (P-gp-independent and topoisomerase II-dependent resistance).

The results of our investigations showed that all these compounds were able to overcome the barrier of P-gp-dependent drug resistance. The most effective of the all tested diubstituted indolo[2,3-b]quinoline derivatives were derivatives substituted in position C-2. The tested compounds did not overcome the barrier of topoisomerase II-dependent drug resistance. Only compound ISS-101 showed ability to overcome the barrier of drug resistance against human promyelocytic leukemia cell line.

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Mono substituted 5H- and 6H-indolo[2,3-b]quinoline derivatives and their ability to overcome the barrier of drug resistance.

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A series of novel 5-H- and 6H- indolo[2,3-b]quinoline derivatives bearing (dialkylamino)alkyl chains at C-2 or C-9 position attached to indoloquinoline core via amine, amide or ether bond were prepared and tested for ability to break multidrug resistance in cancer cells.

In our research we studied the cytotoxic activity of tested compounds on three various human cancer cell lines and their drug-resistant sublines: human colon cancer (LoVo) and doxorubicin-resistant LoVo/DX (P-gp-dependent, MRP-, LRP-dependent multidrug resistance), uterine sarcoma (MES-SA) and MES-SA/DX5 (P-gp-dependent resistance to doxorubicin), human promyelocytic leukemia cell line (HL-60) and HL-60/MX2 (P-gp-independent and topoisomerase II-dependent resistance).

The results of our investigations showed that all these compounds were able to overcome the barrier of drug resistance. The compounds of this group had the highest activity against leukemia cell line. Only three compounds did not show ability to overcome the barrier of drug resistance against human colon cancer (ISS-22 and ISS-103) and uterine sarcoma cell line (ISS-22 and IQ6/9/2). However, against leukemia cell line the ability to overcome this barrier, was similar to other compounds. The most effective derivatives of the all 5-H indoloquinolines tested were ISS-104 (C-2) and ISS-89 (C-9) those which were connected with indoloquinoline core via amine bond. In series 6H- indoloquinolines the most effective derivatives were ISS-43 (C-2) and ISS-26 (C-9) which were connected with indoloquinoline core also via amine bond.

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Synthesis new 1,2,4-triazoline- and 1,3,4-tiadiazole - isoxazole derivatives with immunomodulatory activities

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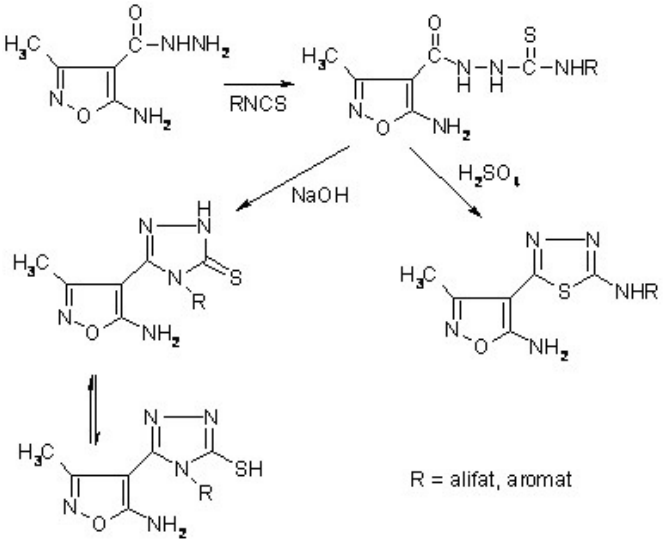
Intensive studies on new, biologically active isoxazole derivatives, have been conducted for the last few decades. We synthesized a group of monocyclic and bicyclic isoxazole derivatives. In the group of monocyclic compounds, the pharmacophore structure was a fragment of 5-amino-3-methyl-4-isoxazolecarboxylic acid. The importance of the pharmacophore structure of 5-amino-3-methylo-4-isoxazolecarboxylic acid, contained in N'-substituted hydrazides, semicarbazides, semithiocarbazides of that acid with immunosupressory activities and isoxazoleoxadiazol, isoxazolopirazol and isoxazolopirol, with high immunomodulatory activities, was confirmed in several patents.

Looking for active immunomodulators we synthesized new 4-substituted
3-(5-amino-3-methyl-4-isoxazole)-1,2,4,-triazoline-5-tione and 5-substituted 2-(5-amino-3-methyl-4-isoxazole)-1,3,4-tiadiazole derivatives. We obtained the compounds in reaction of 5-amino-3-methyl-4-isoxazolecarboxylic acid thiosemicarbazides with conc. sulphuric acid or sodium hydroxide.



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Synthesis of a Polyaminooligonucleotide Combinatorial Library and Studies of Properties of Polyaminooligonucleotides

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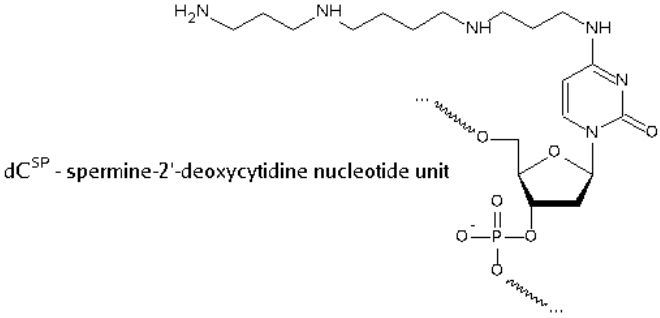
Automatic synthesis of polyaminooligonucleotides (PAOs) utilizing fully protected polyamino-2'-deoxynucleoside 3'-phosphoramidites was carried out. PAO structures were analyzed by FAB-MS and MALDI-TOF. A polyaminooligonucleotide combinatorial library (SPAOCL) containing spermine-2'-deoxycytidine units (dC^{Sp}) attached to polystyrene support was synthesized. Analysis of hybridization properties of PAOs in SPAOCL with fluorescently labeled complementary probe revealed the stabilizing effect of spermine modification of cytosine residue upon duplex formation. Moreover, hybridization experiments using SPAOCL revealed influence of PAOs overall charge upon their hybridization properties. The question of unspecific hybridization was addressed and PAOs were shown to be a new example of cationic oligonucleotides.



In order to study in more detail the stabilizing effect of dC^{Sp} established by analysis of the combinatorial library SPAOCL all 16 members of that library were synthesized. Then, their ability to form complementary duplexes was studied by measuring melting temperatures (Tm) The collected data corroborate high stabilizing effect of

one spermine modification in a chain (increase of Tm by ca 8 °C). More strikingly, two spermine residues in a chain still stabilize the duplex with a complementary d-AAAAGGGG in comparison with unmodified duplex. The latter case is quite interesting not only that the close neighborhood of two modifications still allows for a stabilizing effect but also because this oligonucleotide carries a much smaller overall electric charge (-1 in contrary to -7 of unmodified d-CCCCTTTT).

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15:30 Poster 53

The role of hepatic cytochrome P-450 enzymes in metabolism of imidazoacridinone antitumor agent, C1311 (Symadex^{R}): studies with the hepatic NADPH:cytochrome P450 reductase null mice

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Antitumor agent, 5-diethylaminoethylamino-8-hydroxyimidazoacridinone, C-1311, (Symadex^{R}) developed in our laboratory , is under Phase II clinical trials. We showed earlier that C-1311 metabolism *in vitro* with rat liver microsomes led to four main metabolites [1]. However, the incubation of the studied compound with human *E. coli* recombinant cytochrome P450 isoenzymes (CYPs): 1A2, 2C9, 2C19, 2D6 and 3A4 did not result in significant amount of metabolic product [2]. Therefore, we suspected that cytochromes P-450 were not crucial enzymes involved in rat liver metabolism of Symadex^{R}. In order to elucidate the role of hepatic CYPs in metabolism of C-1311 we compared the elimination of the drug with blood and urine occurred in wild-type (WT) and hepatic NADPH: CYP oxidoreductase null-type (HRN^{TM}) mice. Mice were dosed at 50 mg/kg by ip injection, blood and urine samples were taken at 10, 20, 40, 60 minutes and 2, 4 and 6 hours and were analyzed by HPLC. Moreover, Metabolic transformations of C-1311 and its less active analog, C-1330 with liver microsomal fractions of HRN and WT mice were also compared. WT and HRN^{TM} mice liver microsomes were incubated with C-1311 and C-1330 and the obtained reaction mixtures were analyzed by HPLC with UV-vis or MS/MS detection. The results revealed slightly slower elimination of C–1311 in blood and urine of HRN^{TM} than of WT mice. In the presence of WT mice liver microsomes C-1311 was transformed to at least one product m/z 365 identified as C-1311 derivative with additional oxygen atom in aromatic ring and probably the second one, m/z 323, resulted from deethylation of the side chain. After incubation with HRN mice liver microsomes HPLC peaks of metabolites were lower. Similar relations between metabolite amounts with HRN and WT microsomes were found in the case of C-1330 compound. In addition, C-1330 turned out to be a prodrug of C-1311 as the metabolite m/z 351 equal to M+1 of C-1311 was found. In conclusion, the differences in elimination rates of C-1311 observed between HRN^{TM} and WT mice in blood and urine indicates that liver cytochromes P450 are involved in metabolism of Symadex to a small extent. If so, dealkylation of the side chain and some oxidative transformation of aromatic ring occur. However, extra-hepatic transformations of C-1311 may play a significant role.

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A novel synthesis of antineoplastic drugs docetaxel and paclitaxel

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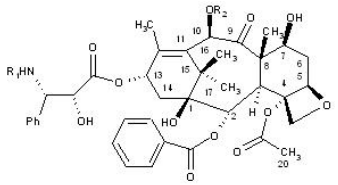
Application of the allyloxycarbonyl protecting group has no precedence in taxane chemistry. We have demonstrated that it's use simplifies the synthetic pathways leading to the title drug substances and can be treated as the technical process innovation.

Novel route for the synthesis of docetaxel and paclitaxel consists of: esterification of 7,10-*O*-diallyloxycarbonyl-10-desacetylbaccatin III or 7-*O*-allyloxycarbonylbaccatin III by (4*S*,5*R*)-*N*-allyloxycarbonyl-2-metho-xyphenyl-4-phenyl-1,3-oxazo-lidine-5-carboxylic acid, subsequent one step removal of all allyloxycarbonyl protecting groups in the esterification products, the cleavage of oxazolidine ring, and finally the conversion of 13-[(2*R*,3S)-3-phe-nylisoserinyl]-10-desacetyllbaccatin III to docetaxel in the presence of di-*tert*-butyl dicarbo-nate or 13-[(2*R*,3S)-3-phenyliso-serinyl]-baccatin III to paclitaxel in the presence of benzoyl chloride.



docetaxel $R_1 = (CH_3)_3COCO$, $R_2 = H$
paclitaxel $R_1 = C_6H_5CO$, $R_2 = COCH_3$

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New calcipotriol analogs, their toxicity and antitumor activity in vivo in comparison to the affinity with VDR and DBP

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Calcitriol, [1α,25-dihydroxyvitamin D_3], the seco-steroid hormone, has been proven to be a potent antiproliferative agent against various normal and neoplastic cells. Moreover, calcitriol and other vitamin D analogs revealed the ability to induce differentiation of many human cancer cells. Biological activity of these compounds is mediated by the nuclear vitamin D receptor (*n*VDR). Calcitriol is carried with plasma Vitamin D Binding Protein (DBP). The affinity with DBP could be responsible for the toxicity and bioavailability. Such biological properties suggest the potential therapeutic application for these agents, including antitumor therapy. The undesired hypercalcemia after calcitriol application which is a serious limitation to its clinical use, explains the motivation to develop new analogs. Two of the promising calcipotriol analogs: PRI-2202, PRI-2205 and the paternal compound have been the objects of our intensive studies. In this work, we present results of the affinity of different analogs of vitamin D with Vitamin D Receptor (VDR) or with Vitamin D binding Protein (DBP) using Molecular Operating Environment (MOE) program. PRI-2205 analog exhibited the highest affinity with VDR and DBP. Furthermore, the toxicity, calcemic activity and antitumor activity of these analogs in the LLC mice tumor model were tested. PRI-2205 analog exhibitad both the low toxicity and calcemic activity and the highest antitumor activity with comparison to other derivatives [1,2].

In conclusion,based on these results, we could formulate the hypothesis about the positive correlation between the antitumor activity of new calcioptriol analog and its affinity with VDR. Moreover, its lower toxicity and calcemic activity seems to be correlated with its higher affinity with DBP.

The authors are grateful to Chemical Computing Group Inc for making the MOE program available for free testing work.

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Homology modeling of metabotropic glutamate receptor 2

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Many studies show involvement of metabotropic glutamate receptors (mGluRs) in synaptic excitation transduction. 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 stroke and pain drugs. Primary goal of our research is to describe a binding mode of positive modulators within a transmembrane part of mGluR2, and to develop a viable virtual model of investigated receptor, so it can be used for further studies.

The lack of crystal structure of transmembrane domain of mGluR2 caused that our approach is based on homology modeling. MGluRs are part of superfamily of G protein coupled receptors (GPCRs) and thus their sequence is similar to Rhodopsin, which crystal structure has been published in PDB database. Rhodopsin structure has been used as a template for homology modeling of mGluR2 receptor. 400 conformers have been generated as a base for molecular docking of selected ligands. As a first step in developing mGluR2 ligand binding mode several high affinity modulators were intensively docked with various pharmacophoric constrains. The best models will be used for virtual screening of our chemical database and for a complete description of interactions of modulators within transmembrane binding site.

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The nature of the interactions of oxprenolol with polypyrrole sorbent

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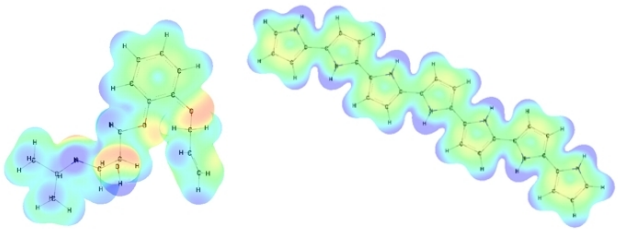
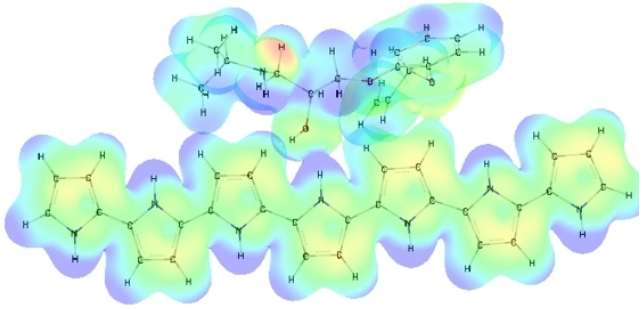
The solid phase microextraction (SPME) developed by Pawliszyn [1] allows one to monitor the drug concentration in blood by the simple and effective way. In this work we have investigated the nature of interaction between oxprenolol (**1**) and polypyrrole (**2**) on the theoretical level. The former is a beta-adrenergic antagonist drug used in treatment of hypertension, angina pectoris, and arrhythmias. The later is a polymer used as an active sorbent of the drug.



1 **2**

As a representative for the polypyrrole we have used the a-a N-*anti* heptamer. Geometries of separated molecules were optimized on the DFT B3LYP/6-31G** level of theory. The Interaction were determined by the minimization of the energy of system consist of two separated molecules (**1**) and (**2**). and analysis of the electrostatic potential distribution in resulting complex (**3**).



3

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This work was financially supported by professor's subsidy awarded (BB) by the Foundation for Polish Science (FNP)

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Molecular modeling explains differences in binding affinity of new potent and selective 5-HT$_{1A}$ ligands: arylpiperazinylalkylthiobenzoxazole derivatives

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Binding modes of series of new compounds containing a benzoxazole moiety bridged to an arylpiperazine by different thioether chains to 5-HT$_{1A}$ receptor were investigated by means of molecular docking. The correlation of the binding affinity with the length of thioether spacer was observed experimentally: three-unit spacer caused at least 100-fold decrease in K_i compared to longer spacers. Automated docking with pharmacophoric constraints revealed the structural cause of this correlation. Possible interactions between Tyr7.43 and thioether fragment of the spacer caused the weakening of interactions from arylpiperazine part of the ligand. Additionally, the benzoxazole moiety of three-unit spacer compounds could hardly form any interactions with the transmembrane part of the receptor. On the contrary, for the compounds with longer spacers, not only the arylpiperazine moiety occupied optimal position in the binding pocket, but also benzoxazole was shown to form favorable interactions (H-bonds, π-π stacking) with residues in the third and seventh transmembrane helices. It is also shown, that the extended conformations for those flexible, long-chain molecules are both observed by MNR measurements and predicted by modeling techniques.

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Pharmacophore model of group II metabotropic glutamate receptor modulators

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Metabotropic glutamate receptors (mgluRs) are members of large G-protein coupled receptor (GPCR) family, activated by L-glutamate, an excitatory neurotransmitter. They are responsible for normal signal transduction in central nervous system as well as patophysiological processes. No crystal structure of complete metabotropic glutamate receptor is known so far, although it is believed, that all mgluRs manifest similar three-dimensional organization. They consist of large extracellular domain with glutamate binding site, a cysteine-rich linker and typical for GPCR's trans-helical domain containing allosteric site [1]. mGluR group II receptors are potential targets for anti-schizophrenic drugs, as well as for generalised anxiety disorder [2]. The orthosteric ligand binding site has been extensively studied and shown limited usability as a drug target, because of marginal selectivity between receptor types. Another, allosteric

binding site located on extracellular part of trans-membrane domain exhibits more diversity, and thus specificity. The search for mgluR modulators (i.e. compounds showing the affinity to allosteric site) yielded vast amount of pharmacological data, facilitating computer-assisted approaches. Until today, pharmacophore models for metabotropic glutamate receptors has been elucidated only for mGluR I [3].

We created a pharmacophore model of positive mGluR II modulator using openly available bio-assay results. For creating a 3-D model we utilized Catalyst software from Accelrys. Some key interactions responsible for binding and specificity were proposed. The mapping of pharmacophore on the 3D model of the binding site is also presented.

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15:30	Poster	60

Synthesis and anticonvulsant activity of new N-[(4-arylpiperazin-1-yl)-methyl]-3-phenyl-pyrrolidine-2,5-dione derivatives

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As has been shown in our previous investigations on the search for new anticonvulsant agents in a group of *N*-[(4-arylpiperazin-1-yl)-alkyl] 3-substituted pyrrolidine-2,5-diones, the introduction of aromatic area at the position-3 of the imide ring caused considerable growth of anti-seizure activity. It was especially noticeable in case of molecules with chloro atom attached at position-2 to the phenyl ring. In this series of compounds the most active were *N*-[4-(3-chloro-phenylpiperazin-1-yl)-methyl]-3-(2-chlorophenyl)-pyrrolidine-2,5-dione which showed ED_{50} value of 14.18 mg/kg in the MES test [1]. Following these finding, in the present work, we have synthesized two series of analogues, containing the chloro atom at the position-3 of the phenyl ring as well as molecules without substituents at the aromatic fragment (**Fig**. **1**).

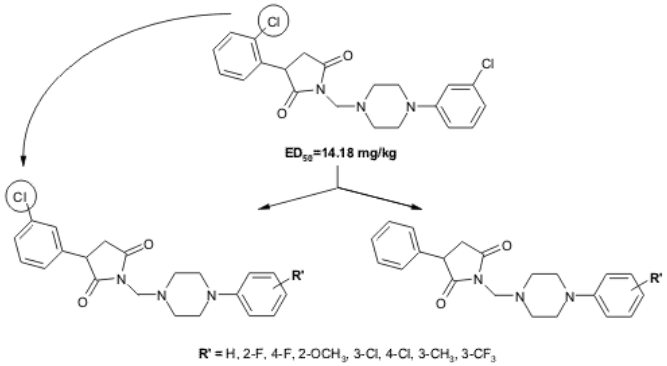

Fig. **1**.

The initial pharmacological screening was performed within the Antiepileptic Drug Development (ADD) Program in Epilepsy Branch, National Institutes of Health, National Institute of Neurological Disorders and Stroke (NIH/NINDS), Bethesda, MD, USA [2]. The results obtained revealed that anticonvulsant activity depended on the presence of chloro atom at position-3 of the phenyl ring as well as the kind of substituents at the 4-arylpiperazine fragment.

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15:30	Poster	61

Synthesis of novel, peptidic kinase inhibitors with cytotoxic activity

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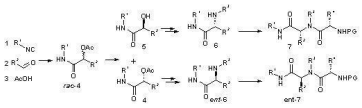
The promising use of small peptides as therapeutics suffers seriously from the problems with farmacokinetics of these compounds. They include: proteolytic instability and high polarity, which prevents small peptides from crossing cell membranes. The synthesis of peptidomimetics aims at the solution of these problems. The changes, which are usually introduced into the structure of the peptide are: *N*-alkylation of peptide bond [1], introduction of non-coded amino acids [2] and introduction of special functional groups at the terminae of the peptide [3].

A novel method for the preparation of peptidomimetics **7** has been recently developed in our laboratory (**Scheme 1**) [4]. It uses Passerini multicomponent reaction for the preparation of racemic scaffold **rac-4** which is then enantioselectivelly hydrolyzed by hydrolytic enzymes to enantiomerically enriched alcohols **5.** These compounds are functionalised towards amines **6**, which are used as substrates in the synthesis of peptidomimetics **7**.



This methodology was applied for the synthesis of novel, peptidic kinase inhibitors with cytotoxic activity towards tumor cells. Studies on the influence of main structural features of studied compounds on biological activity will be presented. The efforts to determine which enantiomer is responsible for the activity will also be presented.

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Searching for the 5-HT$_7$ receptor ligands in the series of new 1,2,3,4-tetrahydroisoquinolines with imide fragment

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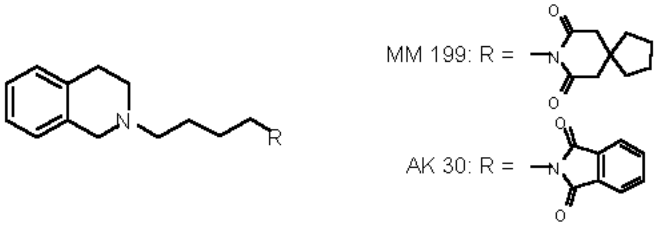
1,2,3,4-tetrahydroisoqunolines are of great importance to many different biological targets [1] and are frequently used as tool compounds to investigate the ligand–serotonergic receptor interactions [2,3]. The screening of our library of tetrahydroisoquinoline derivatives for 5-HT$_7$ receptor affinity led to the identification of MM 199 (tetrahydroisoquinoline analog of buspirone) and AK 30 (tetrahydroisoquinoline analog of NAN-190) having good 5-HT$_7$ receptor affinity.



To continue our studies on development of potent and selective 5-HT$_7$ receptor ligands we designed and synthesized a series of MM 199 and AK 30 analogs with modified tetrahydroisoquinoline fragment. We studied the effect of the introduction of methyl and benzyl substituent into the 2 position of amine moiety or spacer elongation on 5-HT$_7$ receptor affinity. Moreover, the structural variations included also changes of terminal imide fragments. The affinity for serotonergic receptor subtypes 5-HT$_7$ and 5-HT$_{1A}$ were determined and some structure–affinity relationships are discussed.

This study was partly supported by the Ministry of Science and Higher Education (MNiSW), Grant No. 2 P05F 019 30.

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15:30 Poster 63

Synthesis and molecular modeling of 1,2,3,4-tetrahydroisoquinoline-based arylsulfonamides as potential 5-HT$_7$ receptor ligands

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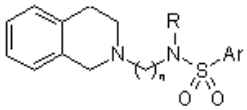
The 5-HT$_7$ receptor is the latest identified serotonin (5-hydroxytryptamine, 5-HT) receptor subtype. The brain distribution of 5-HT$_7$ receptors suggests their significant role in many mental diseases [1]. Thus, the novel and selective 5-HT$_7$ receptor ligands which may have potential therapeutic implications are a putative targets for novel drug discovery [2[.

Here we report the synthesis of a series of 1,2,3,4-tetrahydroisoquinolines with mono- or disubstituted arylsulfonamide moiety. The structure of new compounds was confirmed by ^{1}H NMR spectra as well as by C, H, N analysis. For all compounds the 5-HT$_7$ receptor affinity was determined and some structure-affinity relationships are discussed.



n = 2 - 4
R = H, $(CH_2)_2$-Y, $(CH_2)_3$-Y
Ar = phenyl, 1-naphthyl, 2-naphthyl

Molecular modeling techniques were used to rationalize the structure-activity relationships. The studied compounds were docked to the homology model of 5-HT$_7$ receptor and the binding modes were described.

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15:30 Poster 64

Uridine derivatives of 1-thioglycosides as analogs of glycosyltransferases natural substrates

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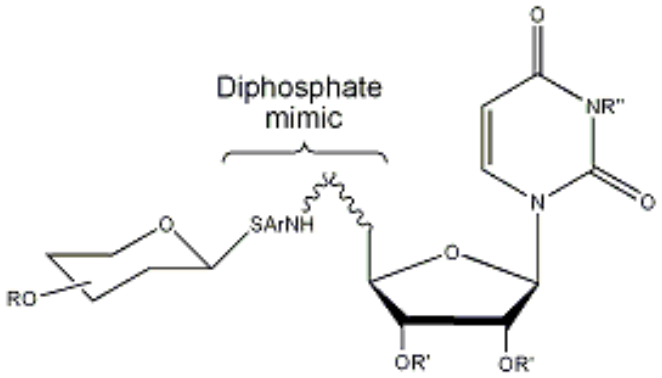
Complex oligosaccharides present in different classes of glycoconjugates are involved in numerous cell-cell recognition and communication processes. They are also responsible for intercellular adhesion in inflammation, bacterial or viral infection and activation of the immunity system. Formation of glycosidic linkage in biosynthesis of oligosaccharides usually occurs under action of glycosyltransferases (GTs). They catalyze sugar-transfer reaction in a regio- and stereospecific manner. Inhibition of GTs leads to the modulation of oligosaccharide biosynthesis and enables us to study their biological functions. Therefore some GTs inhibitors might be of therapeutic interest. GTs inhibitors are generally designed based on analogies

between the three different moieties composing NDP sugar natural substrates: carbohydrate part, the diphosphate linkage and the nucleoside moiety [1].



We present herein a part of our current program on the synthesis of some kind of analogues of sugar nucleotides, which were designed to act as GTs inhibitors particularly donor substrate analogues. Such inhibitors contain a glycosyl unit (usual glucose or galactose) and a nucleoside diphosphate mimic, which is essential for binding to the enzyme. We construct analogues of GTs inhibitors using aryl and heteroaryl 1-thioglycosides as glycosyl units which are connected to uridine with or without a spacer. We used succinic, glutaric and malonic acid as a spacer. We believe that presence of sulfur atom instead of glycosidic oxygen atom increases the stability of sugar-aglycon linkage against enzymatic cleavage [2]. This way we obtained several uridine derivatives containing different types of diphosphate mimic linkers.

Acknowledgement

Financial support from the Polish State Committee for Scientific Research (Grant No. 1 T09A 08630) is gratefully acknowledged.

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15:30	Poster	65

Uridine derivatives of heteroaryl 1-thioglycosides: synthesis and biological activity against CSFV glycoproteins

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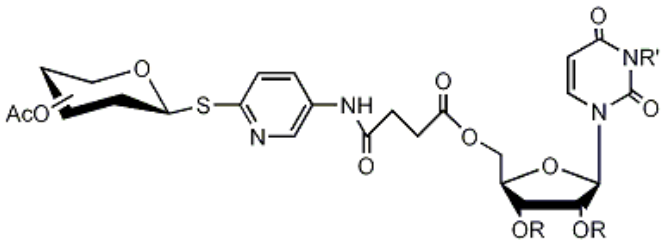
In search for effective inhibitors of sugar processing enzymes the uridine derivatives of (5-amino-2-pyridyl) 1-thioglycosides attract our attention as substrate analogs. Along this line, we have reported different methodology for the synthesis of compounds in which heteroaryl 1-thioglycosides derivatives of D-glucose and D-galactose were connected to selectively protected uridine by amide bond. As a spacer between these two parts we have chosen a succinic acid. In order to obtain uridine with ester bond connected succinic acid in good yield we applied microwave irradiation. The construction of amide bonds was performed using DCC/DMAP [1], ethyl chloroformate [2] or DMTMM as condensing agent [3]. This way we obtained uridine derivatives **GP-U1**, **GP-U2** and **GP-U3** and then we tested their biological activity.



The three inhibitors tested by us exhibited relatively low toxic effect on cells in *in vitro* tests using swine kidney (SK6) cells. High antiviral activity against classical swine fever virus (CSFV) was demonstrated by inhibiting the propagation of the virus. We have investigated the formation of envelope glycoproteins of CSFV after inhibitor treatment by immunoperoxidase monolayer assay and by immunoblotting. We showed that they can influence, not only glycosylation, but also the stability of E2 protein, effectively inhibiting the formation of glycoprotein complexes.

Acknowledgement

Financial support from the Polish State Committee for Scientific Research (Grant No. 1 T09A 08630) is gratefully acknowledged.

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15:30	Poster	66

Crystal structure of β-adrenergic receptor as a new template in homology modeling of GPCR. Application to serotonin 5-HT$_{1A}$ and 5-HT$_7$ receptors

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When dificulties in receiving crystal structures appear, homology modeling aproach is the best way to obtain three dimensional structure of a protein. Procedure is realtively simple, but results depend on degree of similarity between a target and a template sequence. Since 2000 there was one available crystal structure of transmembrane domain of GPCR protein: the bovine rhodopsin. We, and others have previously shown that regardless of relatively low sequence identity, it was possible to obtain useful rhodopsin based models of serotonin receptors, such as 5-HT$_{1A}$ and 5HT$_7$. In October 2007, a high resolution structure of another GPCR, beta-2 adrenergic receptor, was solved. Very close evolutionary relationships between 5-HT

receptors and beta adrenoreceptors give us possibility to improve comparative models of serotonin receptors and to verify our previous results. New homology models will be used in a process of drug design and virtual screening.

Acknowledgement
This study was partly supported by the Network "Synthesis, structure and therapeutic properties of compounds and organic substances" coordinated by the Institute of Organic Chemistry Polish Academy of Sciences.

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New rosiglitazone salts

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The description of the new pharmaceutical salts of rosiglitazone was presented.

Rosiglitazone is an oral drug that reduces the glucose level in the blood. It is used for treating patients with type 2 diabetes and is in a class of anti-diabetic drugs called thiazolidinediones.

The possibilities to obtain of rosiglitazone salts with pharmaceutically acceptable organic acids were tested. The conditions of the synthesis for three possible new salts to obtain and also the technology for one of them were worked out.

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Syntheses of N,S-substituted 4-chloro-2-mercapto-5-methylbenzenesulfonamide derivatives with potential biological activity

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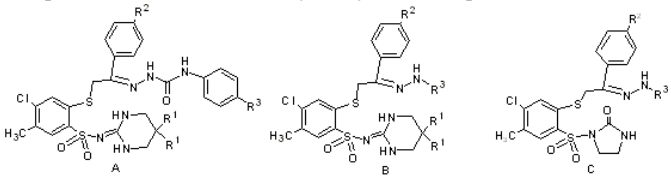
As a part of our research on chemical and biological properties of 2-mercaptobenzenesulfonamides with potential anticancer and/or anti-HIV activities [1-4], the syntheses of *N,S*-substituted benzenesulfonamide derivatives were performed. Reactions of the previously described
2-(2-mercaptobenzenesulfonylimino)tetrahydropyrimidines [5] or 1-(2-mercaptobenzenesulfonyl)-2-imidazolidinone [6] with the appropriate 2-bromoacetophenones afforded 4-chloro-*N*-(tetrahydropyrimidin-2-ylidene) -5-methyl-2-[2-oxo-2-(4-R^2-phenyl)ethylsulfanyl]benzenesulfonamides **1-3** and 1-{4-chloro-5-methyl-2-[2-oxo-2-(4-R^2-phenyl)ethylsulfanyl]benzenesulfonyl}imidazolidin-2- ones **4-5**, respectively. The subsequent reactions of sulfonamides **1-3** with suitable semicarbazones or hydrazines and **4,5** with hydrazines led to the target title compounds of type **A, B** and **C**. The structures of new compounds were determined by analytical and spectral methods.



Ten of the obtained compounds were screened at Institute of Pharmacy University of Greifswald for their in vitro activity against a panel of 12 human cancer cell lines. The prominent benzenesulfonamide showed the selective activity toward cell lines of bladder cancer (IC_{50} values in range 2.9-8.1 μM).

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Development of HPLC and GC methods for analysis of Zolmitriptan of pharmaceutical purity

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Zolmitriptan (4(S)-4-[3-2-dimethyl amino-ethyl)-1H-5-indolyl-methyl]-1,3-oxazolan-2-one) belongs to group of medicines known as Serotonin 5-HT1D receptor antagonist. Zolmitriptan is used to treat severe migraine headaches. This cure is available on market as convetional tablets (Zomig), or nasal spray (Zomig nasal spray).

For the determination of pharmaceutical purity of Zolmitriptan, high performance liquid chromatography with spectrophotometric detection is recommended as an analytical technique. The chromatographic separation was achieved on a Waters XTerra RP Column, (250mm, x 4,6 mm, x 5μm) column using linear gradient solutions. As mobile phase – 20 mM ammonium hydrogen orthophosphate and acetonitrile was chosen. In the developed HPLC method, the resolution between Zolmitriptan and its potential impurities, ZL3, ZL4, ZL5, ZL7, werefound to be greater than 3. Obtained product, as pharmaceutical substance, should contain less than 0.5% of total impurities, and no more than 0.10 % of an individual unidentified impurities (acc. ICH). The detection limit (0.5 mg mL^{-1}) for compoud ZL7 obtained using the developed HPLC method with spectrophotometric detection is unsatisfactory. Because of that, for determination of this compound the different method of analysis need to be used. The developed GC method gave an accepted limit of detection for

analysis of this potential impurity (75 ppm). The proposed methods, owing to its satisfactory precision and accuracy as well as selectivity, and can be applied for the determination of impurities in pharmaceutical substance.

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Synthesis and biological properties 1,3,7-triaryl-5-methyl-1,2,3,4-tetrahydropirimid [4,5-d] pirimidine derivatives.

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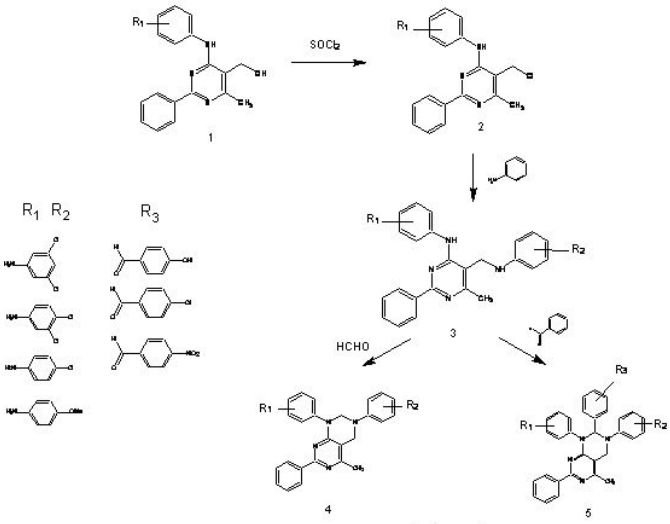
A substrate used in the study was 5-hydroxymethyl-6-methyl-2-phenyl-4- phenylaminepirymidine (**1**), which with $SOCl_2$ was transformed into 5-chloromethyl derivative of pirimidine **2**. The derivative **2** was treated with various aromatic amines, obtaining amino-derivatives **3**, which in turn were entered into Mannich reaction, producing derivatives of 1,2,3,4-tetraliydropirimid [4,5- d] pirimidine **4** and **5.** Those newly obtained compounds were microbiologically tested in selected strains of: *Bacillus subtilis, Escherichia coli, Proteus vulgaris, Pseudomonas aeruginosa, Enterococcus faecalis, Staphylococcus epidermidis, Stapylococcus aureus, Klebsiellapneumoniae.* Anti-fungal tests were also realised on *Candida albicans.* Interesting microbiological results were achieved, both antibacterial and antifungal.



Scheme 1

15:30 Poster 71

Synthesis of novel, thioredoxin - thioredoxin reductase inhibitors

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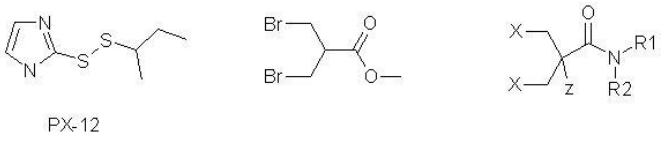
Thioredoxin-1 (Trx-1) is a protein which is over-expressed in many human tumors. The increased level of this protein in cells results in aggressive tumor growth and decreased patient survival.

Because of its role in promoting cell survival, proliferation and tumor angiogenesis, Trx-1 is an attractive molecular target. Therefore many studies have been done in order to obtain a selective inhibitor of Trx-1. One of such compounds is 2-[(1-Methylpropyl)dithio]-1H-imidazole (PX-12) (1). It is a small molecule which causes the oxidation of cysteine residues both in thioredoxin reductase (TR) and thioredoxin. But it irreversibly inhibits only Trx-1. [1] The second inhibitor of TR-Trx system is *b*,*b*-dibromoisobutyric acid methyl ester(2). [2]

Although biological activity of these two compounds is well described in literature, still not much is known about the mechanism of inhibition.



We will present the results of our studies on the synthesis of peptidomimetics possessing
b,b-dihalogeno-*iso*-butyric group (3) and on the influence of main structural features of studied compounds, such as leaving group (X=Cl, Br, I, Ms, Trf) and substituents (R1, R2) on biological activity.

Acknowledgment:
Supported by a grant from the Ministry of Science and Higher Education no. N N405 3202 33

References:

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Fischer, Stephen Chan and Stewart Martin, *Mol Cancer Ther* 2008, *7*, 143

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15:30 Poster 72

Selective monoacylation of alkane-α,ω-diols using crude acetone powders of animal tissues

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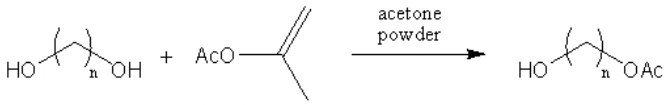
Monofunctionalization of symmetrical substrates bearing two identical substituents continues to be a real challenge for organic chemists, despite a number of attempts that have been described in the literature [1]. Of particular importance is the monoacylation of polymethylene α,ω-diols, since the resulting products are crucial building blocks in the synthesis of various biologically active deriv-

atives, e.g. insect sex pheromones [2].

In this paper a new methodology of the monoacylation of alkane-α,ω-diols will be presented, which rests upon the use of crude animal tissues (liver or kidney) acetone powders as the source of ester-forming enzymes.



n = 4, 8, 10

The reaction was performed in various solvents using isopropenyl acetate as the acetylating agent. It should be stressed, that certain crude acetone powders proved superior to a variety of commercially available enzymes. For example, the use of bovine liver acetone powder (BLAP) in the acetylation of octane-1,8-diol in toluene allowed to reach the 87% conversion with a monoacetylation excess of 95%. The influence of the solvents and reaction conditions on the reaction outcome will be discussed and experimental details will be presented.

Acknowledgment:

This work was supported by the network "Synthesis, Structure and Therapeutic Properties of Compounds and Organic Substances".

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Title: Sex dependent antinociceptive activity and blood pressure changes in SHR, WKY and WAG rats strains after diclophenac treatment.

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The experiments on drugs acting in females become more common. The complication of interpretation these data is resulted by the estrus cycle. This complexity application of analgesic's influence on blood pressure in different rat strains.

Antinociceptive action of diclophenac (non-opioid analgesic) was investigated in male and female normotensive (WKY), genetically established hypertensive (SHR) and WAG rats. The aim of this study was the confirmation of the hypothesis regarding fluctuations of blood pressure level during estrus cycle in rat's female and determination of blood pressure changes after diclophenac treatment.

Diclophenac was administered subcutaneously 10 mg/kg body weight and per os 20 and 40 mg/kg.

Statistically significant differences in pain threshold were reported between male and female rats of investigated strains. Phases of sex cycle in female rats of three strains were determine along with the pain threshold level. Arterial hypertension control in different strains females performed in parallel showed considerable changes in blood pressure after diclophenac administration in the estrous phase. There were several correlated effects in blood pressure and pain threshold after diclophenac administration.

Obtained results may explain the non-uniformity in antinociceptive action of analgetics in both sexes during long-term therapy.

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Application of confidence intervals to bioanalytical method validation

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Pharmacokinetic studies, e.g. innovative drug pharmacokinetic evaluation or generic drug bioequivalence testing, can be requested by the regulatory authorities during the registration process of medicinal products. The drug determination in biological fluids is performed by means of the instrumental analysis, including high-performance liquid chromatography (HPLC). To assure the reliability of results a selected bioanalytical method should be validated according to the specific requirements [1]. A detailed drug stability in biological matrix testing is one of the important steps within the validation. The FDA Guidance describes a standard approach of direct comparison of analytical results for the studied and reference samples, but also suggests that other statistical approaches may be used.

The presented method of statistical evaluation of stability is based on the application of confidence intervals. The idea, based on the assumption of equal variances for the studied and reference samples, was first reported by Timm et al. in 1985 [2]. To allow application of confidence intervals - when this condition is not met - a new method is proposed. Prior to statistical analysis the outliers in measurement results are identified and discarded. After logarithmic transformation of original data, F-Snedecor test is applied to compare variances in both groups of samples. The 90% confidence interval is calculated either using pooled variance (in the case of equal variances) or individual variances of the studied and reference samples [3,4].

As an example of confidence intervals application, freeze and thaw stability of gemcitabine in rat plasma was presented. After the 1st freeze-thaw cycle, variances in the studied and reference samples were equal and the 90% C.I was 102-108%. After the 3rd freeze-thaw cycle, the condition of equal variances was not met and the 90% C.I was 96-108%. Both confidence intervals for stability were within acceptance limits of 85-115%.

The described method of stability testing could be considered superior to the standard approach because confidence intervals include uncertainty of measurement. Application of confidence intervals during bioanalytical method validation can improve the quality of data obtained during pharmacokinetic studies.

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Structure-activity relationships in group of 2,4-dioxo-1,2,3,4-tetrahydropyrido[2,3-d]pyrimidine derivatives

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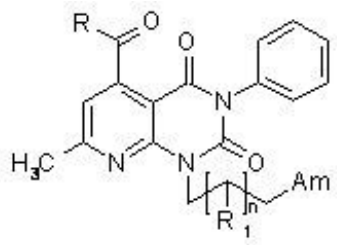
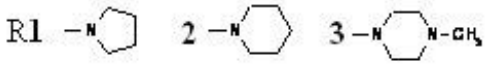
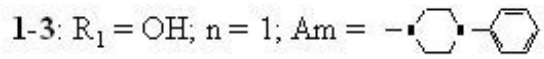
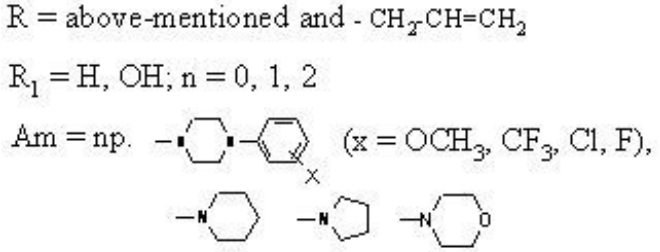
The structures of 2,4-dioxo-1,2,3,4-tetrahydropyrido[2,3-d]pyrimidine derivatives synthesized at the Department of Chemistry of Drugs consist of the three basic fragments: the aliphatic spacer linking two pharmacophore termini: pyrido[2,3-d]pyrimidine nucleus and N-phenylpiperazine or other cyclic amine. From our investigations in this field it follows that all above presented fragments have influence on biological activity. The findings obtained for amides of 1-[2-hydroxy-3-(4-phenyl-1-piperazinyl)propyl]-7-methyl-3-phenyl-2,4-dioxo-1,2,3,4-tetrahydropyrido[2,3-d]pyrimidine-5-carboxylic acid **1-3** [1] indicate that the strength of the analgesic action depends on the structure of the amide group. All compounds **1-3** displayed in pharmacological screening strong but different antinociceptive properties in the "writhing syndrome" test (values of ED_{50} are as follows 1.44 (**1**), 13 (**2**), 6.1 mg/kg (**3**)). Having to regard to it we synthesized then series N,N-dialkyl(dialkenyl)amides [2]. All obtained substances showed also strong analgesic activity in above mentioned test (ED_{50}=1.53-5.31 mg/kg). In the further investigations we introduced pharmacophoric substituents (Cl, F, OCH_3, CF_3) into the phenyl at N-4 of piperazine [3,4]. Most of them exhibited significant analgesic activity and this activity was comparable or superior than that of aspirin. Next modification concerned the structure of alkyl spacer. It was stated [5] that the prolongation of the side-alkyl chain at N-1 to C-4 and elimination of the hydroxy group weakened the analgesic properties and increased toxicity. Continuing investigations in this group of compounds we wanted to know which influence on analgesic activity and toxicity would have: 1) shortening of the alkyl linker to C-2 with simultaneous replacement of N-phenylpiperazinyl substituent by other cyclic amines (piperidine, pyrrolidine...); 2) introduction of pharmacophoric substituents (CH_3, CF_3) into the phenyl at N-4 of piperazine in above mentioned butyl derivatives.







Next compounds:



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15:30 Poster 76

Determination of sibutramine and desmethylsibutramine in Chinese Herbal Medicines by HPLC-ED, HPLC-MS/MS end XPRD methods

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The contemporary societies of the developed countries are prone to use traditional far-east medicines as remedies for all diseases. Some of them, such as obesity, might be classified as civilization diseases. Combating the problem, people try not only several miraculous diets but also herbal infusions (teas) and variety of "herbal" preparations. All these believing that such treatment is healthy and harmless as far as it is "natural". Leaving out of the way the question if herbal medicines can be taken safely without doctors' control the query arises if the common preparations are strictly natural and herbal.

Here we report examples of quality studies of such medicines using HPLC-ED, HPLC-MS/MS and X-ray powder diffraction (XRPD) methods. Especially the XRPD assisted with an optical microscopy

seems to be useful as a fast screening method of general sample composition of such preparations. First of all it can discriminate between capsules containing pure herbal materials and those with some chemical additives. Secondly, different chemical substances, having different powder diffraction patterns, can be easily identified when deposited in powder databases. In the case of mixtures of different chemical additives further studies such as HPLC-ED and HPLC-MS/MS are helpful.

Our experience proved that the most often used additive in different herbal preparations is sibutramine hydrochloride (~28 mg/capsule by HPLC).

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New amino acid derivatives of 6H-indolo[2,3-b]quinolines

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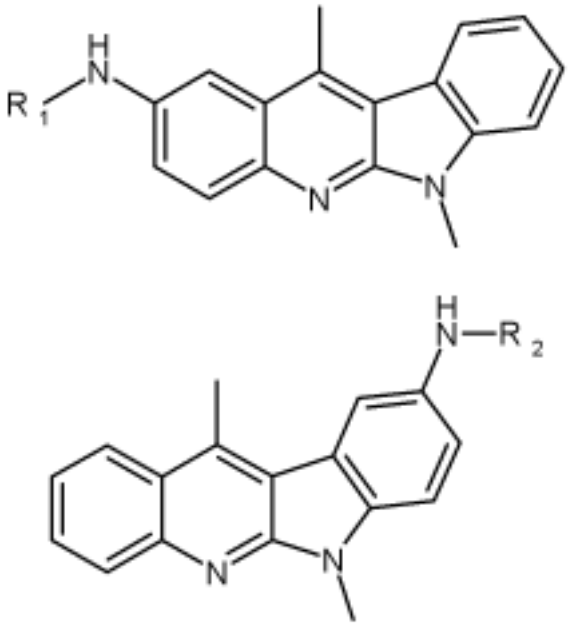
Some of alkyl, dialkylaminoalkyl and saccharide derivatives of 6*H*-indolo[2,3-b]quinoline manifest a cytotoxic activity. A wider application of these compounds is limited because of their low water solubility.

Amino acids and peptides can be used as substituents to optimize drugs. These substituents increase selectivity and bioavailability of many substances. It is well known that anthracyclines, which have a very similar structure to indolo[2,3-b]quinolines, have been successfully conjugated with *L*-leucine. For example, doxorubicin (Dox) has been transformed into the pro-drug N-*L*-leucine-doxorubicin which shows more selective biological antitumoural activity than free doxorubicin. A usage of peptide substituents as vectors (e.g. somatostatin) has been also investigated. For example, peptides were used for transporting doxorubicin through a blood-brain barrier and a peptidic vector can exert an antiproliferative effect. Another strategy to selectively deliver oncostatic drugs was coupling two peptides with doxorubicin. One peptide containing an α-integrin-binding motif, the Arg-Gly-Asp (RGD), and the other one containing an Asn-Gly-Arg (NGR) motif, enhanced the efficacy of doxorubicin against human breast cancer and also reduced the toxicity.

Continuing the search of more active *in vivo* compounds we undertook the investigations of amino acid conjugates with 9-amino- and 2-amino-6*H*-indolo[2,3-b]quinolines. This paper presents the method of synthesis of amino acid derivatives of 9-amino- and 2-amino-6*H*-indolo[2,3-b]quinolines with selected amino acids such as glycine, *L*-alanine, *L*-leucine, *L*-serine, *L*-methionine, *L*-lysine, *L*-proline and *L*-histidine. Three classical methods of coupling: DCC/HOBt, mixed anhydrides, and TBTU, were first evaluated. Finally, TBTU method was selected as it gave best yield and short reaction time, and therefore was used in all coupling reactions.



R_1 = Gly-, *L*-Leu-, *L*-Met-, *L*-Lys-, *L*-Pro-, *L*-His-
R_2 = Gly-, *L*-Ala-, *L*-Leu-, *L*-Ser-, *L*-Met-, *L*-Lys-, *L*-Pro-, *L*-His-

New compounds were evaluated for their cytotoxicity against KB cell lines *in vitro* according to a routine procedure. The biological tests showed that amino acid derivatives of 6,11-dimethyl-6*H*-indolo[2,3-b]quinoline possess significantly enhanced biological activity in comparison with the original 6,11-dimethyl-6*H*-indolo[2,3-b]quinoline.

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Antimicrobial properties of 3-(2-methylfuran-3-yl)-4-substituted-Δ^2-1,2,4-triazoline-5-thiones

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The biological activities of 1,2,4-triazoles have been extensively studied. In particular, their antimicrobial and CNS activities have been extensively documented. Although limited, there are also examples of the antibacterial, antinociceptive, anti-inflammatory properties of furan derivatives We have hypothesized that 3-(2-methylfuran-3-yl)-4-substituted- Δ^2-1,2,4-triazoline-5-thiones might show antimicrobial and antifungal activity. To test this hypothesis we have synthesized four new analogous with phenyl, 2/4-tolyl, 4-methoxyphenyl and cyclohexyl substituents in the position 4 of 1,2,4-triazoline-5-thione.

The assessment of the antimicrobial action of the synthesized com-

pounds was performed using the disc-diffusion method and the minimal inhibitory concentration (MIC). Minimum inhibitory concentrations (MICs) were defined as the lowest concentration of the compounds that inhibited visible growth of microorganisms after 18 h incubation at 35°C. Microorganisms used in this study were as fallows: Gram-positive bacteria: four strains of *Staphylococcus aureus* (NCTC 4163, ATCC 25923, ATCC 6538, ATCC 2921), *Staphylococcus epidermidis* ATCC 12228, *Bacillus subtilis* ATCC 6633, *Bacillus cereus* ATCC 11778, *Enterococcus hirae* ATCC 10541, *Micrococcus luteus* ATCC 9341, *Micrococcus luteus* ATCC 10240 and Gram-negative rods: three strains of *Escherichia coli* (ATCC 10538, ATCC 25922, NCTC 8196), *Proteus vulgaris* NCTC 4635, three strains of *Pseudomonas aeruginosa* (ATCC 15442, NCTC 6749, ATCC 27863), *Bordetella bronchiseptica* ATCC 4617. For testing antifungal activities of the compounds, fallowing reference strains were tested: *Candida albicans* ATCC 10231, *Candida albicans* ATCC 90028 and *Candida parapsilosis* ATCC 22019. The results revealed that all tested compounds were inactive against all tested yeasts. 4-(2-Tolyl)-3-(2-methyl-furan-3-yl)-Δ^2-1,2,4-triazoline-5-thiones exhibited activity against Gram-positive bacteria, especially against *M. luteus* ATCC 9341 (MIC 50 µg/ml).

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Transcriptional activity of interferon γ and two subunits of its receptor as molecular markers of myocarditis

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Inflammatory cytokines have an important role in the development of myocarditis, but still little is known about their prognostic importance. The aim of this study was to evaluate the prognostic value of transcriptional activity of IFN γ and two subunits of its receptor measured in the biopsy of hearts of patients with inflammatory myocariditis.

Sixty three patients with clinically confirmed myocarditis were divided into three groups according to therapy: twenty eight patients received immunosuppressive therapy (prednison and azathioprine), twenty patients were treated with atorvastatin, fifteen received only conventional therapy (without steroids and statins). Total RNA was extracted from the biopsy of specimens and the transcriptional activity of IFN γ and two subunits of its receptor (IFNRα and IFNRβ) were assessed by QRT-PCR reaction with the use of ABIPRISM 7700 and SYBRGreen chemistry. The specificity of reaction was confirmed by the melting curve profile, sequence and length of QRT-PCR products analysis.

Initial values (before therapy) of transcriptional activity of IFN γ and the ratio of two subunits of its receptors (IFNRβ/IFNRα) were statistically different between three analysed groups of patients ($p = 0.0001$ and $p = 0.002$, respectively). The prognostic value of these parameters was assessed by the logistic regression analysis. We studied the relationship between the transcriptional activity of IFN γ or the ratio IFNRβ/IFNRα in the heart (independent variable) and the probability of progresion myocarditis which require steroids (value 1) or statin (value 0) therapy. The odds ratios for IFN γ and IFNRβ/IFNRα were 57 and 3.8 respectively.

The results of the present study suggest a positive association between the elevated value of transcriptional activity of IFN γ and the ratio IFNRβ/IFNRα in the heart biopsy and the progression and severity of myocarditis. These parameters are useful in the choice of the appropriate pharmacological therapy for given patients based on its inflammatory status.

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JS-1, a novel isoquinoline alkaloid with antimicrobial activity isolated from Streptomyces sp. 8812

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The increasing bacterial resistance to antibiotics is at present a great therapeutic problem. Multi-resistant pathogenic bacteria occur more and more frequently. Hence, there is a necessity for the discovery of new classes of antibiotics for the treatment of drug resistant bacterial infections. One of the solution of this problem, apart from combinatorial chemistry, rational drug design and computer assisted design technology involves screening for microorganisms producing novel antimicrobial drugs. Streptomycetes are well known producers of antibiotics and other bioactive metabolites. Enzymes and structures taking part in cell wall biosynthesis have proved to be excellent targets for antibacterial agents because the cell wall pathway is conserved among bacterial pathogens and is absent from mammalian cells. β-lactam antibiotics are the most important class of DD-peptidases/penicillin-binding proteins inhibitors and antimicrobial agents. Moreover, are known only γ-lactams and pyrazolidinones led to moderate antimicrobial activity through the inhibition of the DD-peptidases. In our screening program for novel inhibitors of DD-peptidases from microbial secondary metabolits from our collection of streptomycetes strains, we used DD-peptidase 64-575 II [1]. The novel compound with antibacterial activity JS-1, DD-peptidases inhibitor, member of isoquinoline alkaloids was isolated from the culture broth of *Streptomyces* sp. 8812. It was purified by acetone protein precipitation from the culture supernatant, used of anion exchanging resin column and RP HPLC with C18 modified column. The molecular formula $C_{10}H_9NO_4$ was deduced from HRESI-MS and NMR data. JS-1 is inhibitor of exocellular DD-peptidases 64-575 II from *Saccharopolysporaerythraea* 64-575 II, R61 from *Streptomyces* R61 and R39 from *Actinomadura* R39. JS-1 exhibited

significant activity against the Gram-negative bacteria [2].

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Acknowledgement: This work was supported by network "Synthesis, structure and therapeutic properties of compounds and organic substances", Poland. We are grateful to Prof. Jean-Maria Ghuysen (Centre for Protein Engineering and Laboratoire d'Enzymologie, Université de Liège, Belgium) for kindly providing us with DD-peptidases R61 and R39.

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Proline analogue of nitrosourea as a new cytotoxic prodrug

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Carmustine (bis-chloroethyl-nitrosourea) is anticancer compound in the nitrosourea class commonly used in the treatment of malignant gliomas, multiple myeloma and breast cancer. Carmustine undergoes hydrolysis in vivo to form reactive metabolites which cause alkylation of biologic molecules and cross-linking of DNA. High toxicity and low selectivity of carmustine reduces its safe use in the cancer therapy. Therefore, there is need for new compounds characterized by similar therapeutic activities but less harmful sides effects. As a result, proline analogues of nitrosourea have been synthesized. It has substituted one chloroethyl group by proline and reveals higher selectivity than carmustine. Our aim was to compare the influence of carmustine and new proline analogue of nitrosourea (N-[N'-(2-chloroethylo)-N'-nitrosocarbamoyl]-proline) (AB) on the antioxidative system of fibroblasts and cancer cells MOLT4, to examine redox potential mechanisms of this proline analogue. Administration of carmustine and AB compound caused decrease in activities of antioxidative enzymes in fibrobasts as well as in MOLT4 and these changes were accompanied by increase in hydrogen peroxide level as well as lipid peroxidation marker - MDA. Moreover, administration of carmustine caused increase in fibroblasts glutathione concentration. Increase in dityrosine-marker of protein oxidative modification in fibroblasts and MOLT4 was also observed after administration of both compounds. Finally, it may be concluded that, new proline analogue of nitrosourea causes number of changes in antioxidative system of cancer cells probably by increase in generation of hydrogen peroxide. It was confirmed by enhancing the process of oxidative modification of proteins and lipids. Lack of significant differences in fibroblasts and MOLT4 antioxidative system reaction of examined compounds result from the similarly activity of prolidase – hydrolase which cut off proline residue.

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Polyhydroxylated indolizidine and pyrrolizidine alkaloids. Synthesis and bioactivity.

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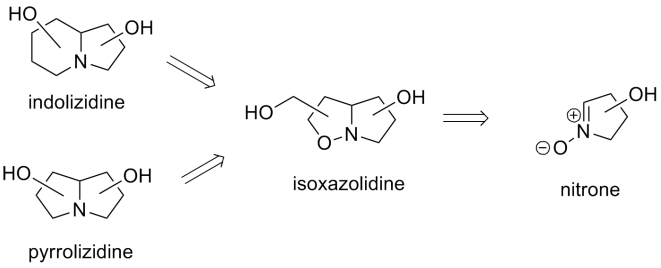
Number natural polyhydroxyleted alkaloids belonging to the pyrrolidine and piperidine classes (iminosugars) and their nitrogen bridgehead bicyclic analogues (pyrrolizidines, indolizidines), as well as a considerable number of non-natural structural analogues, are important glycomimetics [1]. They behave as an enzyme inhibitors, being able to interact with glycosidases and glycosyltransferases – enzymes deputed to the cleavage or formation of glycosidic linkages in the biosynthesis of glycoproteins. Several glycosidase inhibitors have pharmacological relevance, being currently tested or approved in the treatment of diabetes, Gaucher's disease, osteoarthritis, HIV infection, viral infection, or cancer [1].



We have pointed out that the 1,3-dipolar cycloaddition reactions of cyclic nitrones to unsaturated 5- and 6-membered lactones [2] represent an attractive entry to variety of different type of polyhydroxylated alkaloids [3]. The used strategy consists of a cleavage of the N-O bond of isoxazolidines obtained *via* [3+2] cycloaddition followed by the intramolecular *N*-alkylation. A replacement of 5-membered nitrone by 6-membered one allows to obtained indolizidine as well as quinolizidine alkaloids [3].

Several polyhydroxylated alkaloids (iminosugars) were obtained *via* above methodology. Bioactivity of synthesized compounds was tested toward commercially available glycosidases (*α*- and *β*-D-glucosidase, *α*-D-mannosidase, *β*-D-galactosidase, *β*-D-glucuronidase and *α*-L-fucosidase) [3].

Acknowledgements: This work was supported by a grant PBZ-KBN-126/T09/08/2004 and by a network „Synthesis, structure and therapeutic properties of compounds and organic substances".
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Isolation, structural elucidation and characterization of impurities in latanoprost

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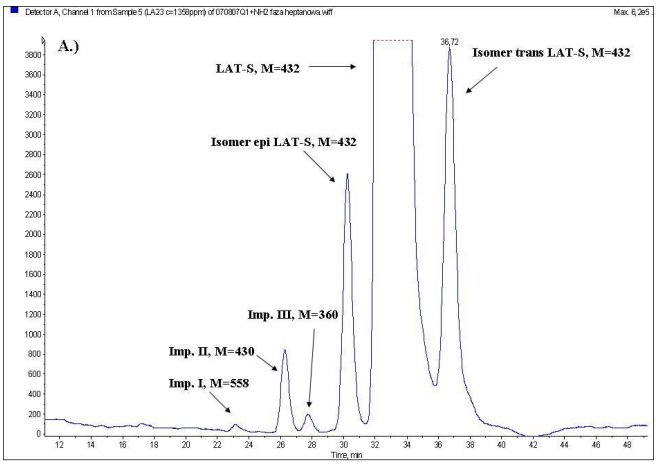
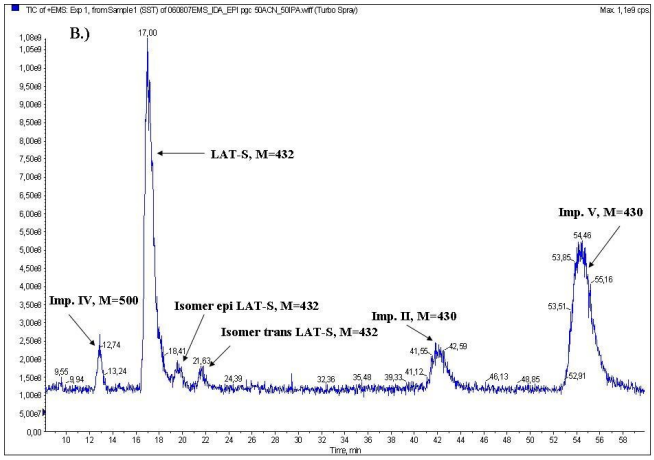
Conventional LC-MS/MS technique has become an indispensable tool in process of identification of isomers, drug metabolites, impurities, degradation products, reaction by-products, etc. Usually, the complete structural identification of undesirable by-product is a difficult and time consuming task. Often, separation of the impurity and its further analysis by complementary techniques, such as NMR, to fully assign a structure are strongly required. One way of overcoming these difficulties is the implementation of high resolution MS and ion trap MS in order to obtain the elemental composition and fragmentation of ions, which provides significant help in the structural identification process.

In this work, we test the identification of impurities of latanoprost by spectroscopic methods (MS and NMR). Unknown impurities in latanoprost samples have been detected by normal phase and reverse phase positive ion ESI and APCI LC-UV-MS/MS with the quadrupole / linear ion trap hybried system as shown on the chromatograms below. One of these impurities was isolated from sample of latanoprost using preparative HPLC and analyzed by NMR. Structural elucidation of compounds, proposed MS fragmentation pattern and possible ways of formation of latanoprost impurities have been discussed.



A.) Normal phase – UV chromatogram



B.) Reverse phase – TIC ion chromatogram

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The cytotoxic activity of glycosides of indolo[2,3-b]quinoline derivatives.

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The cytotoxic activity *in vitro* of glycosides of indolo[2,3-b]guinoline (5*H* and 6*H* series) were tested against cervix carcinoma cell line (KB).

The cells were placed in 96-well flat-bottom plates at a density of $1x10^4$ cells per well in 100 ml of cultured medium 24 hours before addition of the tested compounds. The cells were exposed to the test compounds at concentrations 0.1, 1, 10, and 100 mg/ml for 72 h. The *in vitro* cytotoxic effect of all agents was examined using the SRB assay.

We found that all tested indoloquinolines showed cytotoxic activity against KB cells and the most potent were two of them: IBR-15 (ID50: 0,29±0,12 mg/ml) and IBR-17 (ID50: 0,33±0,08 mg/ml). The 5*H* serie was more active against cervix carcinoma cell line than 6*H* one.

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The new analogs of genistein decrease mitochondrial membrane potential and activate caspase-3

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Background: Genistein is a naturally occurring isoflavonoid, which displays antitumor, antimetastatic and antiangiogenic properties, described in various experimental *in vitro* and *in vivo* models [1]. 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 [2].

Objectives: We have examined the effect of genistein, its new analogs IFG-027 and IFG-043, and complexes with polymers Xyloglucan x Genistein (XYL) and Schisophylan x Genistein (SCH) on mitochondrial membrane potential and activity of caspase-3 on human promyelocytic leukemia HL-60 cell line.

Methods: Genistein and its analogs and complexes were certified synthetic materials obtained from the Pharmaceutical Research Institute, Warsaw, Poland. The human promyelocytic leukemia cell line HL-60 was obtained from European Type Culture Collection by courtesy of Professor Spik and Dr Mazurier (Laboratory of Biological Chemistry USTL, Lille, France).

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 concentrations of 1, 5 and 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 ($2x10^5$) were washed twice with PBS. To determined the caspase-3 activity the cells were incubated with PE-conjugated monoclonal rabbit anti-active caspase-3 antibody and to determined the mitochondrial membrane potential the cells were incubated with JC-1. Data analysis was performed by flow cytometry.

Results: All tested compounds decreased mitochondrial membrane potential and the most potent were the analogs, they decreased the potential already in concentration 5 microg/ml (only analog IFG-027 decreased the potential in concentration 1 microg/ml – statistical significant in comparison to control and genistein $p \leq 0.05$). Only IFG-027 and IFG-043 (in concentration 10 microg/ml) activated the caspase-3, the more potent was analog IFG-043 - it activate caspase-3 already in concentration 5 microg/ml (statistically significant $p \leq 0.05$ as compared to control and genistein).

Conclusions: The analogs of genistein IFG-027 and IFG-043 have very strong proapoptotic activity and may be of potential use in anticancer therapy.

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15:30 Poster 86

New imidazo[1,2-a][1,3,5]triazine with potential pharmacological activity

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In the search for new derivatives with potential pharmacological activity received of 1-aryl-6-aminocarbonylmethyl-5,7(1H0-2,3-dihydroimidazo[1,2-a][1,3,5]-triazine.

New compounds were synthesised in reaction of 1-aryl-2-aminoimidazolidine wtith ethyl isocyjanato acetate and received isomeric 1-(1-arylimidazolidine-2-ylidene)-3-ethylo- carbonylurea and 1-aryl-2-imine-3-etoxycarbonylmethylaminocarbonylimidazolidines. In the reaction of 1-(1-arylimidazolidine-2-ylidene)-3-ethylocarbonylureas with ammonia we have obtained carbonyloaminomethyl urea. 1-Aryl-6-aminocarbonylmethyl-5,7-(1H0-2,3-dihydroimidazo[1,2-a][1,3,5]-triazine were obtained with condensation amides with carbonylodiimidazol (CDI).

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

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Synthesis and biological properties of a new family of agmatine analogues.

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Imidazolines belong to a numerous and long known family of compounds with wide therapeutic applications. They are the active ingredients of many antiallergic, antihypertensive, antidiabetic, sympatholytic, analgetic and anxiety-relieving drugs. The therapeutic potential of these agents is mediated by two types of receptors: α_2-adrenoceptors and I-receptors. According to the most recent hypothesis three types of imidazoline receptors (I_1, I_2, I_3) in Rostral

Ventrolateral Medulla (a part of medulla oblongata) and in peripheral tissues mediate the desired actions of imidazoline like drugs, and the α_2-adrenoceptors are mainly responsible for the side effects [1].

In early 90thies agmatine, a decarboxylated metabolite of arginine, that was long known to be synthesized in plants, bacteria, many invertebrates and fish [2], was discovered in mammalian tissues [3,4]. This natural polyamine was proposed as an endogenous ligand of the I-receptors and a novel neurotransmitter in mammalian brain [3,5].

Agmatine affects also several other receptors (NMDA, nicotinic, 5-HT) and enzymes (NOS, ODC). This diversity of molecular targets is responsible for various biological properties of agmatine: antihypertensive, antidiabetic, anticonvulsant, antidepressant, antinociceptive, antiapoptotic [6].

We have developed a hypothesis, that compilation of the imidazoline ring and the structure of agmatine (the natural ligand of imidazoline receptors) into one formula, may improve biological properties of the resultant compounds. The final compounds are 4(5)-derivatives of previously unpublished imidazolines.

In this work the synthetic approach and the biological evaluation of this group of compounds will be presented.

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15:30 Poster 88

Synthesis and cytostatic activity of new 1-substituted 6H-pyrido[4,3-b]carbazole derivatives

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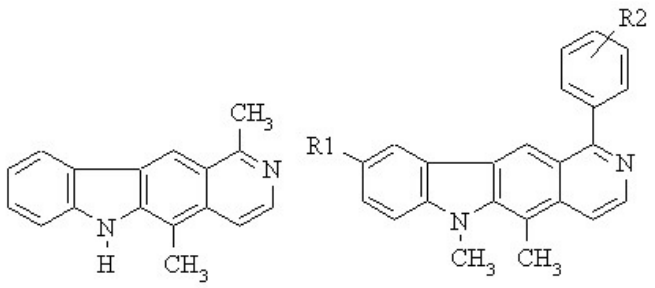
Olivacine **1** is a natural alkaloid isolated from the bark and stem of *Aspidosperma olivaceum* Müll. Arg. [1], which exhibits anticancer activity. 1,5-Dimethyl-6*H*-pyrido[4,3-b]carbazole **1** is an intercalating compound and has high DNA binding affinity, which is responsible for its pharmacological properties.

Based on the literature data and on our personal results of research [2-4] we have designed the syntheses of some new 1-substituted pyrido[4,3-b]carbazole derivatives. Presented paper described the synthesis and antitumor activity of some new 1-phenyl 9-hydroxy(or methoxy) 6H-pyrido[4,3-b]carbazole derivatives **2**.



1 **2**

Cytostatic activity of these compounds was performed in the Institute of Immunology and Experimental Therapy Wrocław Poland.

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Biological action of methyl 2-[5-oxo-3,4-di-(-2-pirydyl)-1,4,5,6-tetrahydro-1,2,4-triazine-6-ylidene)acetate

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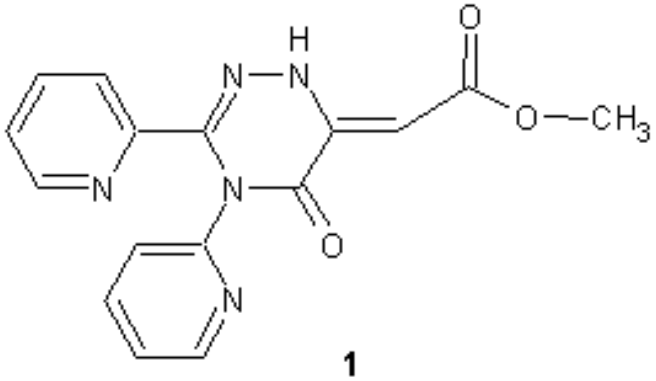
The reaction of the N^3-substituted amidrazones with dimethyl acethylenedicarboxylate in absolute ethanol at the temperature of -10°C led to the formation of derivatives of dimethyl 2-[(1-aryl-amino-1-arylmethylidene)hydrazono]succinate. Only amidrazone containing two 2-pirydyl substituents gave methyl 2-[5-oxo-3,4-di-(-2-pirydyl)-1,4,5,6-tetrahydro-1,2,4-triazine-6-ylidene)acetate **(1)** under above-mentioned conditions. Cyclization of other linear products was carried out in methanol solution in the presence of triethylamine [1].



1

Depending on the type of substituent 5-oxo-1,2,4-triazine derivatives show different fungi- and bacteriostatic activity. The highest activity exhibited compound **1**, which inhibited the growth of *Staphylococcus aureus, Escherichia coli, Brucella abortus, Mycobacterium smegmatis* and *Candida albicans* in concentration of 125-250 μg/ml. The acute toxcity of this compound in mice was low (>2000 mg/kg i.p.) [1].

Considering to these findings we continuated studies on the antifungal activity of compound **1** using 22 strains of *Candida albicans* (6 strains resistant to fluconazole and itraconazole) and 7 strains *Candida* non-*albicans* (2 strains resistant to fluconazole and itraconazole) isolated from various clinical materials at the University Hospital in Bydgoszcz. Antifungal activity was measured by the disc-diffusion method under standard conditions using Mueeller-Hinton agar medium. The results were read after 24 hours incubation at 35°C. Antifungal activity expressed as minimal inhibitory concentration (MIC) values in μg/ml. It was observed that the growth of only one strain of *Candidaalbicans* one strain of *Candida* non – *albicans* were inhibited by compound **1** in a relatively low concentration 128 and 256 μg/ml respectively.

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Synthesis and biological activity of the product obtained in the reaction of the N^3-substituted amidrazones with cis-1,2-cyclohexanedicarboxylic anhydride

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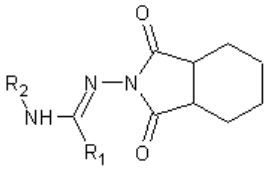
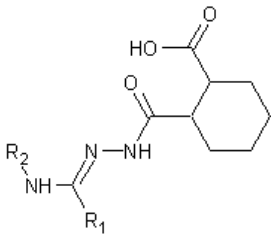
Amidrazones - hydrazones of acid amides have been shown to be important precursors or intermediates to obtain various compounds with industrial aplications. Moreover NCNN group is an essential part of molecules bearing high biological activities and it is template in drug discovery. Open-chain or cyclic derivatives of the amidrazones and also their metal complexes are known to exhibit following activities: antithrombotic, antiinflamatory, antibacterial, antimalarial, antifungal, anticancer and insulin-mimetic.

The subject of our present study was biological activity of hexahydro-1*H*-isoindole-1,3(2*H*)-diones **(1-3)** and 2-cyclohexanecarboxylic acid derivatives **(4, 5)**. Products were obtained in the reaction of some N^3-substituted amidrazones with cis-1,2-cyclohexanedicarboxylic anhydride [1,2].





1 R_1=2-C_5H_4N, R_2=C_6H_5; **4** R_1=2-C_5H_4N, R_2=4-NO_2-C_6H_4;
2 R_1=C_6H_5, R_2=4-NO_2-C_6H_4 **5** R_1=2-C_5H_4N, R_2=2-C_5H_4N;
3 R_1=C_6H_5, R_2=C_6H_5;

The chemical structure of compounds was confirmed by IR, ^{1}H NMR, EI-MS, elemental analysis and X-ray crystallography. Their purity was confirmed by chromatographic methods.

The products (**1-5**) were tested *in vitro* against bacterial and fungal species. MIC_{50} values were determined as the lowest concentration of compound which inhibited 50% of growth of the microorganism tested. The sensitivities to the derivatives **1**, **4**, and **5** were similar (MIC_{50} 100-250 mg/ml) for the following strains: *Nocardia spp.*, *Enterococcus faecalis* ATCC 29212, *Sarcina lutea*, and *Yersinia enterocolitica* O3. Product **1**, MIC_{50} 250 mg/ml, was also active against *Staphylococcus aureus* ATCC 25923. Compound **3** was shown to be effective against the Gram-positive bacteria *Staphylococcus aureus* ATCC 25923, *Sarcina lutea* and *Enterococcus faecalis* ATCC 29212 as well as against the fungi *Aspergillus niger* and *Candida albicans* at a concentration of 250 mg/ml [2]. Derivative **2** exhibited a relatively high antibacterial potency against *Sarcina lutea* (MIC_{50} 75 mg/ml), *Pseudomonas aeruginosa* ATCC 27853 and *Yersinia enterocolitica* O3 (MIC_{50} 200 mg/ml). The examined compounds did not inhibit the growth of the fast growing *Mycobacterium smegmatis* or strains used as drug resistant markers, *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 (except derivative **2**).

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15:30	Poster	91

Interactions between glycosyltransferases and 2-deoxy glycosyl derivatives of uridine simulated by molecular docking

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Glycosyltransferases (GTs) are enzymes involved in the biosynthesis of oligosaccharides, polysaccharides and glycoconjugates. Modulation of GTs activities by efficient inhibitors is promising for the control of various molecular recognition processes including bacterial and viral infections. Therefore selective inhibitors of GTs are of interest because they may lead to the development of novel therapeutic agents [1]. Identification of potent inhibitors has been developing very rapidly during the last two decades since the 3D structures of several GTs were found [2] and catalytic mechanism proposed [3].

The use of computer-aided structure-based approach has been very useful for the optimization and *de novo* discovering inhibitors of enzymes. As a rational design of lead compounds this report focused on the investigation of an interaction between 2-deoxy sugar derivatives of uridine and active site of target proteins, GnT I and GalT I. The ligand-protein interactions were simulated with a docking GOLD 3.2 software [4]. A small but diverse library containing 28 ligands was assembled. Structures in the set possessed common uridine motif and two moieties of 2-deoxy sugar connected by α-(1→3)-, α-(1→4)- or α-(1→6)-linked glycosidic linkages and can be synthesized in a totally stereoselective manner [5]. Uridine fragment is supposed to ensure reasonable interactions with enzyme, similar to that of natural substrate. All ligands were docked into the active sites of target proteins and the bound conformations inside the active sites were visually examined. Visual analysis of the ligand-protein complexes as well as the scores from docking simulations suggest that some structures bind the enzymes active sites by a similar mode as their natural substrates. Selected compounds will be synthesized and subjected to bioactivity assays in traditional enzymatic tests.

Acknowledgement

Financial support from The Polish State Committee for Scientific Research (Grant No. 1 T09 A 08630) and The Board of Faculty of Chemistry (Grant BW-RCh-2/2008) are gratefully acknowledged.

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15:30 Poster 92

The effect of inositol hexaphosphate on NFkB expression in human colon cancer cells

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Phytic acid, a hexaphosphorylated inositol (IP6) is a major fiber-associated component of wheat bran and legumes. A number of recent studies revealed strong anti-cancer activity of IP6, and hence, the molecular mechanisms of its action are still under investigation. IP6 is hypothesized to target cancer through multiple pathways, i.e., modulation of cell signal transduction, inhibition of cell proliferation, and cell cycle progression, and activation of apoptosis, and induction of cell differentiation. IP6 may also be involved in many nuclear processes, including DNA repair, transcriptional regulation and mRNA transport.

Nuclear factor kB (NF-kB), a member of transcriptional factors, plays an important role in regulation of the expression of genes employed in these processes. It has been shown that NF-kB is constitutively activated in several types of tumors including colorectal cancer.

The aim of this study was to evaluate the influence of IP6 on the expression of genes encoding p65 and p50 subunits of NF-kB and of its inhibitor IkBα in human colorectal cancer cell line Caco-2.

The cells 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 with a SYBR Green I chemistry (SYBR Green Quantitect RT-PCR Kit, Qiagen) using an Opticon™ DNA Engine Sequence Detector (MJ Research, USA). The results were recalculated per mg of total RNA. Statistical analysis was performed with the use of Statistica 6.0 software.

Experimental data revealed time dependent changes in transcriptional activities of *IκBα* and *p65* occurring in both control cells and in cells incubated with IP6. The expression of *IκBα* inhibitor did not show any significant changes in response to 1.0, 2.5, and 5 mM IP6 at 1 h incubation, compared to the expression of this gene in the control cells ($p=0,7353$; ANOVA). Treatment of cells with 5 mM of IP6 resulted in strong increase in *IκBa* expression observed at 6h ($p=0,0009$; Tukey test), 12h ($p=0,0002$) and 24h ($p=0,0011$). The level of *p65* transcript after 1 h was lower in the cells exposed to 1, 2.5, and 5 mM IP6 than in the control cells ($p<0,05$; Tukey test). There was no statistically significant difference between *p65* mRNA quantities in the cells exposed to increasing concentrations of IP6 for 1 h. However, the increase in transcriptional activity of *p65* gene in response to 5 mM IP6 after 6h ($p=0,0009$) and 12 h ($p=0,048$) was observed. Cells treated for 24h with 2.5 mM ($p=0,0026$) and 5 mM IP6 ($p=0,0112$) showed a significant decrease in expression of *p65* gene. There were no quantitative changes in the *p50* gene expression in the cells treated with IP6 compared to the control cells ($p>0,05$; ANOVA). High correlation between the expression of *IκBα* and *p65* ($R=0,72$, $p=0,000$; Spearman's rank correlation test) was observed. There were any correlations between *IκBα* and *p50* ($R=0,37$, $p=0,1465$) as well as between *p50* and *p65* transcript levels ($R=0,39$, $p=0,1220$).

In summary, the findings of this study show that IP6 alters *p65* and *IkBα* genes expression in colon cancer cells. Changes in transcriptional activities of *IkBα* and *p65* depend on IP6 concentration and time of interaction. These results suggest that the ability of 5 mM phytic acid to inhibit colon cancer cells proliferation may be mediated through the stimulation of *IκBα* expression at the mRNA level.

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New derivatives of thiosemicarbazide and 1,2,4-triazole-5-thione with potential antimicrobial activity

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1,2,4-Triazoles have been reported to associate with antimicrobial, fungicidal, anti-inflammatory, antiparasitic, insecticidal, herbicidal, antiviral, antitumor, anticonvulsant, antidepressant, hypotensive effects and plant growth regulatory activities. On the other hand, nitroimidazole like metronidazole, ornidazole, secnidazole and tinidazole are widely used in the treatment of diseases caused by protozoa and anaerobic bacteria. Furthermore, some 5-nitroimidazoles have been shown activity against *Helicobacter pylori.* In the design of new compounds, the development of hybride molecules through the combination of different pharmacophores in one frame may lead to compounds with increase antimicrobial activity. Herein, we have synthesized novel compounds containing (4-nitroimidazol-1yl)methyl fragment at the position 3 of the 4-substituted-1,2,4-triazole-5-thiones.

Six compounds were screened for their antimicrobial activity against reference strains of bacteria (9 species) and fungi (5 species). Two compounds inhibited the growth of Gram-positive *Micrococcus luteus* ATCC 10240 with MIC = 250 mg L^{-1} and MIC = 500 mg L^{-1}, respectively. The most effective against Gram-negative bacteria was 4-phenyl-1-[(4-nitroimidazol-1-yl)acetyl]thiosemicarbazide with MIC = 500 mg L^{-1} for *E. coli* ATCC 25922 and about 36 to 65% reduction of the growth of *Klebsiella pneumoniae* ATCC 13883 and *Proteus mirabilis* ATCC 12453 at lower concentrations (7.81 - 250 mg L^{-1}). None of the compounds had influence on the growth of reference strains belonging to *Staphylococcus, Bacillus* or *Pseudomonas* species. The tested compounds had no activity against fungi, besides 4-(4-tolyl)-1-[(4-nitroimidazol-1-yl)acetyl]thiosemicarbazide showing moderate inhibitory effect against *Trichophyton menthagrophytes* ATCC 9533 with MIC = 250 mg L^{-1} and about 30-70% reduction of the growth of this dermatophyte at lower concentrations (7.81 - 125 mg L^{-1}). Moreover, this compound exerted about 0-70% inhibition of the growth of reference strains of *Candida* spp. and *Aspergillus niger* ATCC 16404 at concentrations of 7.81 - 500 mg L^{-1}.

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HPLC as a method for analytical control of synthesis and determination of tolterodine (TD-S)

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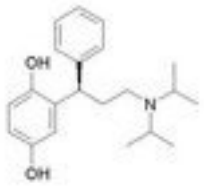
Physical preparation or complex structure of API may cause many analytical problems. Therfore elaboration of suitable analytical methods for both routine manufacturing processes and investigation of novel synthetic routes is very important.

Analytical method used for this purpose must ensure fast and efficient determination of the presence of starting materials, product, impurities and side products. Nowadays, 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 tolterodine tartrate – an active substance administered in urinary incontinence therapy.



Tolterodine

Synthesis of the active substance – tolterodine - was conducted in two different ways: first one consisted of seven synthetic steps, and second one of eight steps. The method described in this poster is useful for both synthetic pathways and suitable for determination of product purity, as well as separation and identification of impurities and side products.

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Solid-phase synthesis and preliminary biological investigation of arylpiperazine library as 5-HT_{1A} and 5-HT_{2A} receptor ligands

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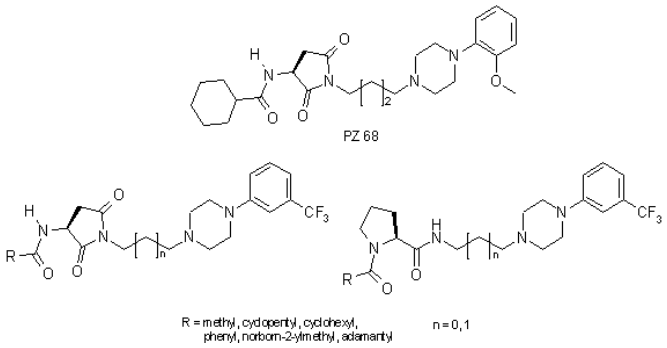
We have previously reported on successful application of combinatorial chemistry techniques for generation of rationally designed libraries of serotonin receptor ligands, namely arylpiperazine derivatives containing N-acylated amino acid fragments [1,2]. It was found, that the kind of substituent at aromatic ring influenced receptor affinity and compounds in vivo 5-HT_{1A} intrinsic activity. Finally, the project allowed selecting a lead compound (PZ 68), pre- and post-synaptic 5-HT_{1A} agonist, which demonstrated distinct anxiolytic-like and antidepressant-like effects in the respective animal models.

Taking advantage of the solid-phase chemistry for quick generation of compound libraries, we have designed and synthesized analogs of previously reported long-chain arylpiperazines containing N-acylated amino acid fragments (aspartic acid, proline).



A 24 member library of trifluromethyl derivatives was synthesized on solid-support. The previously reported synthetic methodology was now adopted for a BAL-type PL-MBHA resin. Library generation was performed manually by using Bill-Board set [3]. This equipment keeps the solid-phase reactions organized in a grid and simplifies repeated cycles of reactions, washings, cleavage, and finally solvent evaporation step. Selected library representatives were evaluated for their 5-HT_{1A} and 5-HT_{2A} receptor affinities. The results obtained followed by the discussion on the influence of the modifications applied on receptor affinity will be presented.

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Oxidation status of ALDH3A1 and antioxidant capacity correlation for human saliva.

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Aldehyde dehydrogenase isozyme (ALDH3A1) is an enzyme oxidizing mainly long- and medium-chain aliphatic as well as aromatic aldehydes. The salivary aldehyde dehydrogenase was postulated to play an important role in deactivation of higher aldehydes of plant origin, and may be involved in the prevention of chemical carcinogenesis [1,2].

It is also well documented that ALDH3A1 can be induced several-hundred-fold in some neoplastic states of different cancers e.g. liver, breast, colon and oral [3].

Activity of ALDH is unstable in the absence of thiols, but can be stabilized by 1 mM glutathione. Inactivated enzyme can be re-activated within 10 minutes by treatment of 0.5 mM DTT [4].

Saliva samples were collected to buffer stock solution containing various thiols, and assayed in the presence of fluorogenic substrate 6-methoxy-2-naphthaldehyde and NAD^+.

The oxidative stress has been associated with increased risk of many diseases, including different kinds of cancer. It can be measured by many methods, like ORAC (Oxygen Radical Absorbing Capacity) method [5], which gives information about total antioxidant capacity of body fluids. ORAC-FL test uses fluorescein as a fluorophore. The decay of the fluorescence emission over time due to exposition to the peroxyl radical source (AAPH) is measured. In the presence of diluted saliva sample the fluorescence decay is delayed due to the presence of antioxidants present in the saliva.

In present studies the ORAC value of human saliva of oral cavity surgical patients and healthy subjects was correlated with the ALDH3A1 activity.

We observed no distinct direct correlation between antioxidant capacity and ALDH3A1 oxidation status of human saliva in all healthy patients. However, in smoking patients and in patients with definite diet the correlation was observed.

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15:30 Poster 97

"Preliminary study of silicone gel sheets containing onion (Allium cepae) extract for treatment of scars"

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Hypertrophic scars and keloids are formed during abnormal scarring process and result from excessive collagen deposition. Creams containing onion extract are very common in therapy of the scars and lately occlusive silicone dressings become a popular treatment option. However, the mode of pharmacological action of such preparations still remains unclear.

Although silicone preparations are used since 1983, there has been no reports about adhesive silicone-based sheet containing any active compounds. Our aim was to develop silicone adhesive gel sheet containing dry onion extract. Another innovative aspect of this project is to use dry onion extract instead of a liquid ethanolic extract, which may allow for introducing higher concentrations of active compounds in the prepared formulation.

Silicone sheets were formed, using silicone components from Dow Corning (Wiesbaden, Germany) and from Silikony Polskie (Nowa Sarzyna, Poland). Dry onion extract was obtained by spray drying of a liquid ethanolic extract of fresh onion bulbs (*Allii cepae bulbus*, var. Armstrong).

The content of quercetin and quercetin glucosides in dry and in liquid ethanolic extracts was compared using high-performance liquid chromatography (HPLC) in order to optimize the method of obtaining onion extract.

Occlusive properties of prepared silicone gel sheets were examined by measuring the amount of water (50 ºC, 48 h), evaporated from a vial sealed with the studied preparations. Occlusion factors were found to be 60-70 %. Using texture analyser TA XT Plus (Stable Micro Systems, Godalming, Great Britain) it was demonstrated that adhesive properties do not change after washing silicone sheets using warm water with soap, as it is recommended while used by a patient.

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Stereoselective acetylation of racemic 1-chloro-3-(2-chloro-5-methylphenoxy)propan-2-ol

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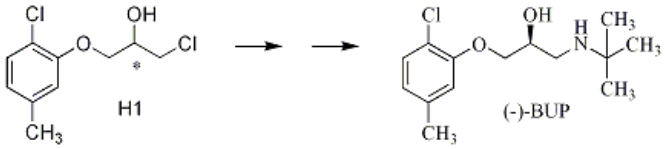
Reactions catalyzed by various types of hydrolases are predominant among biotransformations. Lack of sensitive cofactors, which have to be recycled, makes them particularly attractive for organic synthesis. Among hydrolytic enzymes lipases and esterases are frequently used because they accept a broad range of substrates and often exhibit high enantioselectivity. Lipase-catalyzed reactions in organic solvents are becoming increasingly important in enantioselective synthetic chemistry, as the reactions which are sensitive to water can be effectively carried out in organic media.

Biocatalytic methods of obtaining homochiral *β*-blockers that are focused on production of versatile precursors are widely described in literature. Halohydrins are the established intermediates in the preparation of optically active *β*-blockers. Their resolution by esterhydrolases has been described as a standard alternative in preparation of the homochiral propranolol, atenolol, practolol [1].

In this study we report lipase-catalyzed kinetic resolution of a chiral intermediate H1 of bupranolol (BUP):1-chloro-3-(2-chloro-5-methylphenoxy)propan-2-ol.
Bupranolol demonstrates antagonistic activity against all known subtypes of *β*-adrenoceptors (*β*-AR), including the low affinity state of *β1*-AR (also known as atypical *β*-adrenoceptors) [2]. It contains a chiral center and studies *in vitro* proved that among 12 *β*-blocking agents - levorotatory enantiomer of BUP is the most potent [3]. The structure of BUP is similar to that of most other *β*-blockers; a tertiary butyl group replaces the more common isopropyl group and it contains substituents in *ortho*- and *meta*- positions of phenyl ring.



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Acknowledgements

This work was supported by the Polish Ministry of Science and Higher Education grant No N405 018 32/1604.

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Preparation of enantiopure (R)-hydroxy metabolite of denbufylline catalysed by immobilized Lactobacillus kefiri

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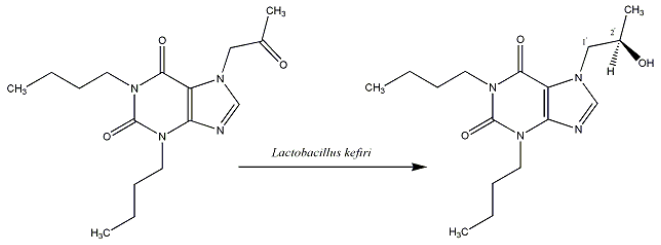
The xanthine derivative denbufylline (DBF: 1,3-dibutyl-7-(2-oxo-

propyl)- 1*H*-purine-2,6(3*H*,7*H*)-dione) as a cAMP-phosphodiesterase (PDE4) inhibitor has been shown to possess relaxating effects on bronchial smooth muscles as well as anti-inflammatory activity. Therefore it has attracted attention as a remedy for asthmatic patients. DBF has also been shown to ameliorate learning or memory impairment, to increase cerebral blood circulation, and to facilitate cerebral glucose metabolism and improve intracerebral oxygen tension (pO_2). These findings suggest that DBF may be effective in the treatment of cerebral disorders including hypoxic conditions such as cerebral ischemia as well as neuropsychiatric symptoms associated with these diseases. DBF is also effective against osteoporosis in animal models. Pharmacokinetic studies in human have shown that DBF is converted to several metabolites. The most important, pharmacologically active metabolite is (*R*,*S*)-1,3-dibutyl-7-(2-hydroxypropyl)-1*H*-purine-2,6(3*H*,7*H*)-dione (BRL-31532), which is a chiral compound.



DBF (*R*)-hydroxy metabolite of **DBF**

Biological methods for the synthesis of chiral compounds offer some advantages in comparison to chemical methods, e. g. remarkable chemo-, regio-, and enantioselectivity. As biocatalysts alcohol dehydrogenases (ADHs) have gained increasing interest. They can be applied as isolated enzymes or incorporated in whole cells. In comparison to isolated enzymes, whole cell biocatalysts are usually more stable due to the protective cell matrix envelope for the enzyme. For many industrial applications they can be immobilized, *via* very simple and cost-effective protocols, in order to be re-used over very long periods of time. Whole cells can be immobilized in a matrix by covalent bonds, by physical adsorption or by gel entrapment. This last technique is widely used. Many natural synthetic polymers have been described, such as calcium or barium alginate, κ-carrageenan or polyurethane foams etc.

In this study we report the use of immobilized *Lactobacillus kefiri* DSM 20587 for the enantioselective reduction of the methyl ketone group of denbufylline for the preparation of the enantiopure (*R*)-hydroxy metabolite: (*R*)-1,3-dibutyl-7-(2-hydroxypropyl)- 1*H*-purine-2,6(3*H*,7*H*)-dione. As DBF is insoluble in water (logP 2.64) different co-solvents were used for biotransformation. The influence of co-solvents on the yields and enantioselectivity of the bioreduction process was examined.

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Comparison of properties of cyclic and linear glycine derived structures and their phosphorus analogues - theoretical studies

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The cyclic structures are characteristic for many classes of compounds, which exhibit pharmacological activity and are used as medicines (cyclosporin A, polyene antibiotics, etc.). A creation of cyclic structure may modified susceptibility of peptide compounds to bioavailability and enzymes' action.

The study include quantum-chemical computations of stability of cyclic structures built from n glycine residues connected with peptide bonds or n glycine analogue moieties, in which nitrogen atom was replaced with phosphorus atom. The calculations were conducted with application of Hartree-Fock method and STO-3G and 6-31G* basis functions. All calculations were preformed with the GAUSSIAN 03W program package.

The cyclic structure of hexaglycine containing six identical intramolecular hydrogen bonds (Figure 1.) was found to be the global energetic minimum. By the analogy to this structure a series of cyclic peptides was built. The small cyclopeptides up to hexaglycine are circle like, whereas larger compounds lose this symmetry. Also their linear equivalents and phosphorus analogues were designed.

Computed energies per one created peptide or quasipeptide bond decrease with the increase of molecule's size. For cyclic structures stand out two local energetic minima by compounds built of six or twelve units. For peptides they are even more energetically favourable than linear compounds. These findings suggest that such cyclic peptides should occur in nature and should be fairly stable.

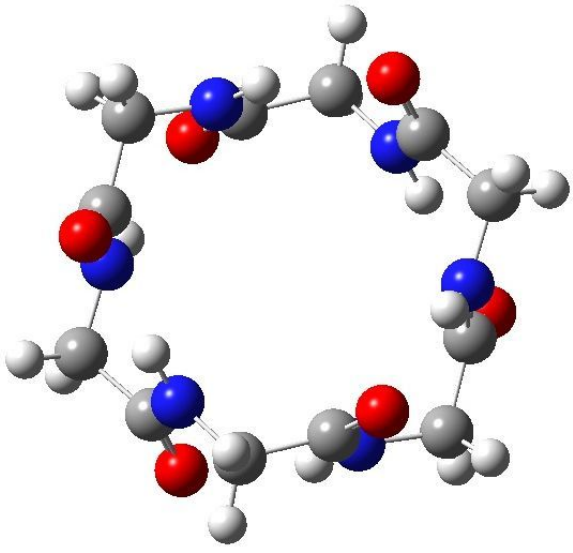

Figure1. Cyclohexaglycine

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New azaphenothiazine derivatives - synthesis and antibacterial properties

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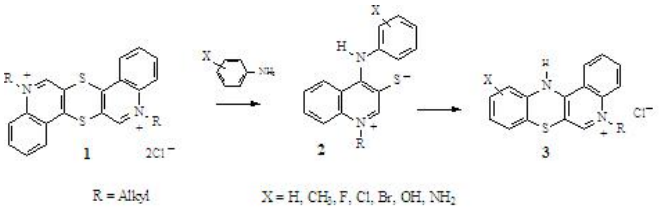
Many phenothiazine derivatives have been used as drugs. Modifications of the main structural fragment of phenothiazine drugs reveal interesting chemical properties and potent biological activity.[1] Extensive research has been conducted on new methods of synthesizing potentially useful phenothiazine derivatives with pharmacological activity. New applications of phenothiazine derivatives have also been found.[2]



The reaction of bis-salts (**1**) with primary aliphatic amines and anilines led to 1-alkyl-4-aminoquinolinium-3-thiolates (**2**). 1-Alkyl-4-arylaminoquinonolinium-3-thiolates in the presence of oxygen and hydrogen chloride undergo cyclization to 5-alkyl-12*H*-quino[2,3-b][1,4]benzothiazinium chloride (**3**).[3,4] This reaction is a new method of synthesizing phenothiazine derivatives.Antibacterial studies of the compounds (**3**) were conducted on *Staphylococcus aureus, Escherichia coli, Enterococcus faecalis* and *Pseudomonas aeruginosa*. Correlations of antibacterial activity tested compounds with chemical structure and lipophilicity were observed.

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Coffee break
Wednesday afternoon, 28 May, 16:00

POSTER SESSION
Wednesday afternoon, 28 May, 16:30

Violin Concert
Wednesday afternoon, 28 May, 17:15

POSTER SESSION
Wednesday afternoon, 28 May, 17:45

Free Time
Wednesday evening, 28 May, 18:30

CEREMONIAL DINNER
Wednesday evening, 28 May, 20:00

Thursday, 29 May

Śniadanie dla Uczestników wycieczki do Lwowa
Thursday morning, 29 May, 5:00

Odjazd autokarów z Uczestnikami wycieczki do Lwowa
Thursday morning, 29 May, 5:30

Śniadanie dla pozostałych Uczestników
Thursday morning, 29 May, 7:00

Odjazd autokaru z Uczestnikami do Warszawy
Thursday morning, 29 May, 9:00

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