Book of Abstracts

The Fifth Multidisciplinary Conference on Drug Research

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Published May 2006, ISBN 83-89585-10-3

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Revision: 14.5.27, 2006-05-04 00:15 GMT

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07:00 Śniadanie i Rejestracja

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Sesja I

Przewodniczą: Osman Achmatowicz, Aleksander P. Mazurek

09:45 00:45 Invited Oral Jan Lubiński THE LATEST ADVANCES IN CLINICAL GENETICS OF BREAST CANCER.

10:30 Przerwa na kawę

Sesja I cd.

Przewodniczą: Osman Achmatowicz, Aleksander P. Mazurek

10:50 00:20 Oral Andrzej J. Bojarski NEW DUAL 5-HT / 5-HT LIGANDS WITH ANTIDEPRESSANT AND ANXIOLYTIC-LIKE ACTIVITY

11:10 00:20 Oral Ryszard Andruszkiewicz NOVEL ANITIEPILEPTIC DRUG - DISCOVERY STORY AND PERSONAL REMARKS

11:30 00:20 Oral Lech Kozerski NMR INVESTIGATIONS OF HUMAN INSULIN AGGREGATION AND STRUCTURE

11:50 00:20 Oral Desmond O' Grady AUTOMATED DESIGN OF ROBUST BATCH COOLING CRYSTALLIZATION USING REAL TIME ANALYTICS LASENTEC FBRM AND ReactIR

12:10 00:20 Oral Katarzyna Kieć-Kononowicz DESIGN AND SYNTHESIS OF HAPTEN TO GENERATE CATALYTIC ANTIBODIES THAT REDUCE PENTOXIFYLLINE

12:30 00:20 Oral Agnieszka Bartoszek DIETARY INTERVENTION WITH RED BEET JUICE DURING CANCER CHEMOTHERAPY WITH DOXORUBICIN AS MEANS OF COMBATING TOXIC SIDE EFFECTS RESULTING FROM OXIDATIVE STRESS

13:00 Przerwa obiadowa

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Przewodniczą: Jan Lubiński, Ignacy Siemion

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15:45 00:20 Oral Michał Fedoryński LARGE SCALE LABORATORY SYNTHESIS OF 1-(4-CHLOROPHENYL)-1-CYANOCYCLOBUTANE - A SIBUTRAMINE PRECURSOR

16:05 00:20 Oral Zbigniew J. Kamiński AN APPLICATION OF THE ARRAY OF LIPIDATED OLIGOPEPTIDES RESEMBLING ARTIFICIAL RECEPTORS FOR MOLECULAR RECOGNITION. THE STUDIES ON THE LIPOPHILICITY OF SOME PIPERAZINES

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Przewodniczą: Jan Lubiński, Ignacy Siemion

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10:40 Przerwa na kawę

Sesja III cd.

Przewodniczą: Andrzej Kutner, Wojciech Kostowski

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15:55 Przerwa na kawę

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May 17th, Wednesday

07:00 Śniadanie

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10:40 Przerwa na kawę

Sesja V cd.

Przewodniczą: Grzegorz Grynkiewicz, Waldemar Priebe

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13:00 Przerwa obiadowa

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17:15 Przerwa na kawę

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09:00 Wyjazd

Programme

Sunday, 14 May

Rejestracja
Sunday afternoon, 14 May, 15:00

Kolacja i Rejestracja
Sunday evening, 14 May, 19:00

Monday, 15 May

Śniadanie i Rejestracja
Monday morning, 15 May, 7:00

Otwarcie Konferencji
G. Grynkiewicz - Wystąpienie okolicznościowe; O. Achmatowicz - Rozstrzygnięcie ,,Konkursu o Nagrodę i Medal im. Stanisława Binieckiego"
Monday morning, 15 May, 8:30

9:00 Opening lecture

CAN A QUEST FOR A NEW DRUG OF POLISH ORIGIN BE SUCCESSFUL?

Wiesław Szelejewski

Instytut Farmaceutyczny (PRI), Rydygiera 8, Warszawa 01-793, Poland

E-mail: kontakt@ifarm.waw.pl

Sesja I
Monday morning, 15 May, 9:45
Chair: Aleksander P. Mazurek, Osman Achmatowicz

9:45 Invited Oral

THE LATEST ADVANCES IN CLINICAL GENETICS OF BREAST CANCER.

Jan Lubiński

Pomorska Akademia Medyczna, Zakład Genetyki i Patomorfologii, P.Wielkopolskich 72, Szczecin, Poland

E-mail: lubinski@sci.pam.szczecin.pl

Przerwa na kawę
Monday morning, 15 May, 10:30

Sesja I cd.
Monday morning, 15 May, 10:50
Chair: Aleksander P. Mazurek, Osman Achmatowicz

10:50 Oral

NEW DUAL 5-HT$_7$/5-HT$_{1A}$ LIGANDS WITH ANTIDEPRESSANT AND ANXIOLYTIC-LIKE ACTIVITY

Andrzej J. Bojarski[1], Jan Boksa[1], Beata Duszyńska[1], Piotr Brański[2], Agnieszka Pałucha[2], Joanna Wierońska[2]

1. *Institute of Pharmacology Polish Academy of Sciences, Department of Medicinal Chemistry, Smętna 12, Kraków 31-343, Poland* **2.** *Institute of Pharmacology Polish Academy of Sciences, Department of Neurobiology, Smętna 12, Kraków 31-343, Poland*

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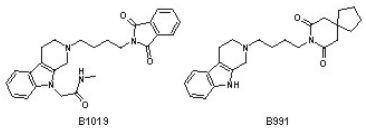
In the course of searching for the new, potent 5-HT$_7$ antagonists, a group of 14 new tetrahydro-β-carboline derivatives with tetramethylene linker and different (cyclic imide/amide, benzotriazole) terminals were synthesized and evaluated for 5-HT$_7$ and 5-HT$_{1A}$ receptor affinity. Compounds with ftalimide, indolinone and 8-azaspiro[4.5]decane-7,9-dione fragments were among the best 5-HT$_7$ ligands and they also showed a significant activity for 5-HT$_{1A}$ receptors. For these selected compounds functional profile for 5-HT$_7$ receptors was determined in adenylate cyclase assay, and their therapeutic potential was further examined in forced swimming, tail suspension and plus-maze tests. Of the analyzed ligands B1019 displayed antidepressant properties whereas for B991 anxiolytic-like activity was detected.



This study was partially supported by the research grant no. 012/2002 from the Polish Pharmacy and Medicine Development Foundation, given by the POLPHARMA Pharmaceutical Works.

11:10 Oral

NOVEL ANITIEPILEPTIC DRUG - DISCOVERY STORY AND PERSONAL REMARKS

Ryszard Andruszkiewicz

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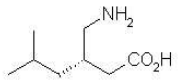
Novel drug, known under the brand name Lyrica and produced by Pfizer, has recently been approved for the management of neuropathic pain associated with diabetic peripheral neuropathy, postherpetic neuralgia and adjunctive treatment of partial seizures in adults with epilepsy. It is currently available in about 25 countries and 25 more countries have approved it and should be selling it by summer this year. Lyrica

shows also high efficacy in the treatment of generalized anxiety disorder, fibromyalgia, as well as in sleep quality disorder. The story of a novel medication started in the late 1980s, when the author of this presentation came to Northwestern University in Evanston, near Chicago, to begin his post doctoral studies in R. Silverman laboratory in the Department of Chemistry. The project started with the synthesis of a series of substituted γ-amino butyric acid (GABA) derivatives and L-glutamic acid analogues which, then, were tested as GABA g-aminotransferase inhibitors. Moreover, these compounds, showed, unexpectedly, *in vitro* activation of brain L-glutamic acid decarboxylase (GAD) and anticonvulsant activity in mice. One of the selected compounds, i.e. (*S*)-3-isobutyl-4-aminobutyric acid exerted exceptionally high anticonvulsant activity and has been selected for clinical trials in Parke-Davis, then, in Pfizer and became known as pregabalin.



Pregabalin

After intensive clinical trials, Pfizer in 2004 received approval from the European Medicines Agency (EMEA) to market Lyrica in all European Union member states, then in 2005 received the similar approval from the US Food and Drug Administration (FDA) to sell Lyrica in the US. This drug has a newly definied mechanism of action. In this short presentation some aspects of the discovery of the active compound will be presented.

11:30 Oral

NMR INVESTIGATIONS OF HUMAN INSULIN AGGREGATION AND STRUCTURE

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

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A number of physicochemical techniques have been utilized over the last decade to characterize the state of insulin aggregation, namely; cryo-electron microscopy, nano-spray ESI MS, small angle X-ray scattering, and NMR.

Various attempts were undertaken to find conditions for the monomeric structure in solution. The water/acetic acid (80/20 vol %), trifluoroethanol and water/actonitrile (65/35 vol %) were found appropriate media to observe good quality 1D-NMR spectra suitable for undertaking the structural studies. However, none of these solvents was characterized in detail with respect to aggregation state and existing equilibria. The solution structure of native human insulin was so far only solved in the water/acetic acid (80/20 vol %).

In our laboratory the NMR investigations were undertaken into two directions; *i,* find a direct spectroscopic evidence for the aggregation equilibrium in solution and, *ii*, find a solvent which would allow the structure elucidation while the tertiary structure should remain conserved. The COSY and TOCSY spectra of $Thr^{A8, B27, B31}$ region were found suitable to observe separately individual aggregates and characterise semiquantitatively their population in solution.

The PFGSE (Pulsed Field Gradient Spin Echo) technique was used to monitor the aggregation in solution by means of establishing the diffusion coefficient, D_i, for each species in solution.

The water/actonitrile mixture, as a solvent, was found to simultaneously satisfy requirements of high resolution insulin spectrum and monomeric conditions over the wide concentration range of insulin. These topics wil be presented in a lecture.

11:50 Oral

AUTOMATED DESIGN OF ROBUST BATCH COOLING CRYSTALLIZATION USING REAL TIME ANALYTICS LASENTEC FBRM AND ReactIR

Brian O Sullivan, Paul Barrett, Jochen Schoell, Desmond O' Grady

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Running crystallizations in a robust manner is key for most end product and intermediate processes. However, in practice crystallization process design often omits a sound design of the process due to short term time limits. In this paper, we present an automated approach to design and run relative supersaturation control for the example of a cooling crystallization. A two step procedure is applied to run a supersaturation controlled crystallization, using an automated crystallizer with real time analysis of both solid and liquid phase. The aim of this paper is to underline that understanding and designing a robust crystallization process can be achieved rather rapidly when using an automated approach based on real time analytics.

12:10 Oral

DESIGN AND SYNTHESIS OF HAPTEN TO GENERATE CATALYTIC ANTIBODIES THAT REDUCE PENTOXIFYLLINE

Tomasz Wójcik, Elżbieta Pękala, Katarzyna Kieć-Kononowicz

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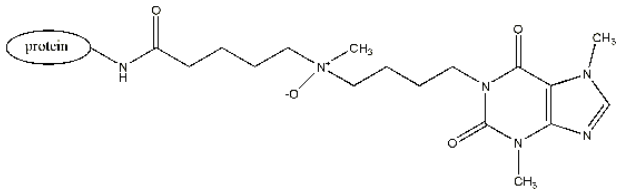
Antibodies are structural and functional relatives of enzymes. Catalytic antibodies have been shown to catalyze a great number of chemical processes, with specifity, stereoselectivity and the ability to direct reaction through a disfavored chemical pathways. Of primary importance in successful catalytic antibody production is the rational design of the hapten that will be used for immunization. One of the most applied strategies employs antigens designed as stable analogs of transition-state of the target reactions [1]. In general, three different chemical groups are used to mimic tetrahedral reactions intermediates: amine or amide group, phosphonate group and sulfonyl group. Several classes of chemical reactions are, so far, catalyzed by abzymes formed against stable Transition State Analogs, like reduction reactions, hydrolysis reactions, cationic cyclizations, Diels-Alder reactions.

As a continuation of our previous studies [2,3], concerning the enantioselective reduction of pentoxifylline to 1-(5-*R*-hydroxyhexyl)-3,7-dimethylxanthine (lisofylline), we proposed method based on catalytic antibodies. For that purpose the two haptens were designed to generate monoclonal antibodies with following important features of binding pockets: a hydrophobic binding pocket for the xanthine ring and an acidic residue complementary to the oxyanionic transition state. Also, the haptens possesses a hydrocarbon linkers with carboxylic residues for a carrier protein coupling to provide sufficient immunogenicity of the antigens to elicit an immune response.



The N-oxide hapten has been synthesized from theobromine in four-step method. This hapten will be used for immunisation of mice as a keyhole limpet hemocyanin (KLH) conjugate for monoclonal antibodies production and for enzyme linked immunosorbent assay (ELISA) screening as a bovine serum albumin (BSA) conjugate.

Acknowledgements:

Financial support from the Jagiellonian University Medical College CR-122/2005 grant is gratefully acknowledged.

[1] Xu Y., Yamamoto N., Janda K.D. *Bioorg. Med. Chem.***2004**, *12*, 5247-5368

[2] Kieć-Kononowicz K., Pękala E. (CMUJ). 2004, patent application P-369904

[3] Pękala E., Wójcik T.*Sci. Pharm.***2005**, 73(2), Supp.1, S67

12:30 Oral

DIETARY INTERVENTION WITH RED BEET JUICE DURING CANCER CHEMOTHERAPY WITH DOXORUBICIN AS MEANS OF COMBATING TOXIC SIDE EFFECTS RESULTING FROM OXIDATIVE STRESS

Jolanta Łukowicz[1,2], Grażyna Peszyńska-Sularz[1,2], Anna Cieślak[1], Anita Piasek[1], Stefan Popadiuk[2], Włodzimierz Grajek[3], Agnieszka Bartoszek[1]

1. *Gdansk University of Technology, Department of Pharmaceutical Technology and Biochemistry, ul. Narutowicza 11/12, Gdańsk 80-952, Poland* **2.** *Medical University of Gdansk, ul. Debinki 1, Gdańsk 80-523, Poland* **3.** *The August Cieszkowski University of Poznań, Department of Biotechnology and Food Mocrobiology, Wojska Polskiego, Poznań 60-657, Poland*

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Research carried out over past two decades revealed a number of substances present in plantborne foods with cancer preventive properties, antioxidative activity in particular. It has been also long recognized that toxic side effects of cancer chemotherapy are associated with the ability of anticancer drugs to stimulate oxidative stress in the human organism. Therefore, a number of attempts have been undertaken to combine cancer treatment with the application of natural antioxidants to improve patients' quality of life. The substances tested include antioxidant vitamins, some isolated polyphenols, as well as plant extracts e.g. from spinach or grape seeds. This approach, though based on edible food components, still is rather pharmacological than dietary. In contrast, our research represents a purely nutritional approach and is aimed at finding out whether special design of patients' diet, i.e. enriched in a food item with high antioxidative potential, can influence the final outcome of cancer therapy. The experiments were carried using tumor bearing mice fed with red beet juice ad libitum instead of water and treated with doxorubicin, an antitumor drug whose toxicity, especially cardiotoxicity, is known to result from massive induction of reactive oxygen species. Prior to experiments in vivo, we demonstarated in cultured cells that red beet juice does not interfere with doxorubicin activity. Several experimental schemes were tested with juice given to mice before or/and after treatment of two

types of tumors: melanoma B16 resistant and leukaemia L1210 sensitive to doxorubicin. In the first case, we observed slight improvement of therapeutic affect of doxorubicin in mice given red beet juice 7 days before and 7 days after therapy, however it was not statistically significant. Interestingly, this improvement occurred despite appparent stimulation of tumor growth in mice drinking juice. In general, in all mice groups tested, those drinking red beet juice were more lively, displayed better appetite as if they tolerated better both the disease and chemotherapy. In leukaemia bearing mice, at least in some experiments, the significant increase (around 30% over the mice given doxorubicin alone) of lifespan was observed for animals fed with red beet juice 7 days before and 7 days after therapy and also their body mass decreased less. All our observations, including those escaping quantification, suggest that appropriate dietary intervention may considerably augment cancer chemotherapy and improve patients' quality of life. In separate series of experiments, we collected serum, leukocytes and hearts from animals treated with doxorubicin in or without conjunction with red beet juice application. These samples are currently processed to determine such markers of oxidative stress as lipid peroxidation, oxidative damage to proteins, genotoxicity in leukocytes and heart tissue.

Przerwa obiadowa

Monday afternoon, 15 May, 13:00

Sesja II

Monday afternoon, 15 May, 15:00

Chair: Ignacy Siemion, Jan Lubiński

15:00 Invited Oral

CANCER CHEMOPREVENTIVE AGENTS - DRUGS FOR THE 21st CENTURY ?

Wanda Baer-Dubowska

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After a quarter of a century of rapid advances in cancer research, the focus of oncological drug development has shifted from cytotoxic chemotherapy to rationally designed agents that target specific molecules associated with malignant cells or their environment. Carcinogenic process is driven by mutation, but there are many epigenetic variables which could be the targets of early intervention before invasion and metastasis occur. Chemoprevention is the inhibition, retardation or reversal of carcinogenic processes by pharmacological or natural agents targeting these pathways in high-risk individuals. This approach was developed more than 30 years ago and its credibility was enhanced by the positive results of clinical trials involving subjects with risk of developing breast cancer and head and neck second primary tumors. So far however, not many clinical trials provided satisfying results, not only because of the lack of efficacy or side toxic effects of chemopreventive agents (promising celocoxib and rofecoxib has to be withdraw from the word market), but also the lack of precise biomarkers monitoring their effects. In spite of all these obstacles, the field of cancer chemoprevention is very active, not only because of its accelerating scientific base, but also because is vitally needed. New information from molecular studies has identified specific molecular targets for chemopreventive agents. These include regulatory molecules such as Nrf2, epidermal growth factor receptor kinases, phosphatidylinositol 3-kinase, components of the Janus kinase-signal transducers and activators of transcription (JAK-STAT) pathway, nuclear factor-kB, and cyclin D. The development of new drugs for the control of these targets that are both safe and effective will be important for the future of cancer chemoprevention.

The presentation gives an overview of conceptual basis of chemoprevention and perspectives of its application.

15:45 Oral

LARGE SCALE LABORATORY SYNTHESIS OF 1-(4-CHLOROPHENYL)-1-CYANOCYCLOBUTANE - A SIBUTRAMINE PRECURSOR

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

1. *Warsaw University of Technology, Faculty of Chemistry, Koszykowa 75, Warszawa 00-662, Poland* **2.** *Industrial Chemistry Research Institute (ICRI), Rydygiera 8, Warszawa 01-793, Poland*

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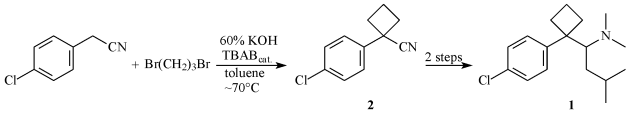
Sibutramine (**1**) is an antidepressant used also for the treatment of obesity and Parkinson disease. Known methods for preparation of crucial intermediate in the synthesis of **1** - 1-(4-chlorophenyl)-1-cyanocyclobutane (**2**) - consist of cycloalkylation of 4-chlorophenylacetonitrile with 1,3-dibromopropane in the presence of such base/solvent systems as: NaH/DMSO/ether, solid KOH/DMSO/ether, solid KOH/toluene/quaternary ammonium salt as a catalyst (solid - liquid phase transfer catalysis, PTC). All of them are inconvenient for large scale applications. On the other hand, it is now commonly accepted, that liquid-liquid PTC (conc. aq. solution of NaOH as a base) is the best practical procedure for alkylation of carbanions of arylacetonitriles and their derivatives. However, with 1,3-dibromopropane under typical PTC conditions (50% NaOH aq. as a base) yields of 1-aryl-1-cyanocyclobutanes are low, b-elimination of HBr from the alkylating agent is often observed, leading to mono- and diallylated arylacetonitriles as by product, which are difficult to separate.

We found, that a simple change of 50% NaOH for 60% KOH overcomes all the difficulties mentioned above. Cycloalkylation of 4-chlorophenylacetonitrile with 1,3-dibromopropane carried out in the presence of 60% KOH and tetrabutylammonium bromide (TBAB) as a catalyst in toluene as a solvent proceeds in high yield, b-elimination of HBr from the alkylating agent practically does not proceed and isolation of **2** is very easy. This process was performed on a 10 mole scale, with the yields exceeding 70%.



After separation of the phases and addition of an appropriate amount of KOH to aqueous phase it is possible to use it again in the next synthesis.

16:05 Oral

AN APPLICATION OF THE ARRAY OF LIPIDATED OLIGOPEPTIDES RESEMBLING ARTIFICIAL RECEPTORS FOR MOLECULAR RECOGNITION. THE STUDIES ON THE LIPOPHLILICITY OF SOME PIPERAZINES

Justyna Kolesińska[1], Anna Musiał[2], Barbara Malawska[2], Zbigniew J. Kamiński[1]

1. *Technical University of Łódź, Institute of Organic Chemistry (PŁ), Żeromskiego 116, Łódź 90-924, Poland* **2.** *Jagiellonian University, Collegium Medicum, Department of Pharmaceutical Chemistry, Medyczna 9, Kraków 30-688, Poland*

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We found that supramolecular structures formed from N-acylated peptides attached on the a regular basis to the CH_2OH groups on the surface of cellulose support via aminophenylamino-1,3,5-triazine very efficiently recognize small guests molecules, resembling the artificial receptors. In the contrary to the rigid structure of the most of artificial receptor described in the literature [1] (especially those, prepared by imprinting in the polymer matrix), the supramolecular structure is highly flexible. Thus, it is expected, that the host structures adjust their shape to fit the guests molecules most efficiently.

The array of the receptors wave been synthesized and used in the studies. Thus, even in the case, when the single receptor in a differential array does not necessarily have selectivity for a particular analyte, the combined fingerprint response can be extracted as a diagnostic pattern visually, or using chemometric tools.

In order to study the mechanism of the molecular recognition, we compared the selectivity of binding of triphenylmethyl dyes, Paclitaxel®, arylpiperazine derivatives with gradually increased lipophilicity and their fluorinated analogues respectively. Arylpiperazine derivatives have been chosen as model compounds with amide group, ester moiety, amine group, and lipophilic fragment in a molecule. These different fragment of a molecule, as well as lipophilicity might be responsible for interacting in a target site.

The interactions of colorless guest with the array were visualized by the subsequent processes of competitive adsorption-desorption of appropriate dye.

We found, that the binding process depends on the structure of amino-acids as well as lipidic fragment of the receptor and analyte structure. The complex nature of the host-guest interactions interactions will be discussed.

Acknowledgements: The study was supported by the Polish State Committee for Scientific Research under the Project 4-T09A 189 25.

[1] a) Zimmerman, S.C., et al. 2002. Synthetic hosts by monomolecular imprinting inside dendrimers. Nature 418, 399-403; b) Wright A.T.; Anslyn, E.V. , Differential receptor arrays and assays for solution-based molecular recognition, Chem. Soc. Rev., 2006, 35(1), 14-28.

Przerwa na kawę

Monday afternoon, 15 May, 16:25

Sesja II cd.

Monday afternoon, 15 May, 16:45

Chair: Ignacy Siemion, Jan Lubiński

16:45 Invited Oral

THE DISCOVERY AND DEVELOPMENT OF BLOOD-BRAIN BARRIER (BBB) PENETRATING ANTHRACYCLINES FOR THE TREATMENT OF BRAIN TUMORS

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Malignant gliomas are devastating cancers, which due to infiltration and location are difficult to treat and also represent significant mortality in young populations. Development of effective chemotherapeutic strategies has been limited in part by the inaccessibility of the CNS to pharmacological intervention. As a possible target, topoisomerase II (topo II) overexpression has been documented in human gliomas and correlated to poor survival, but no effective topo II poison capable of reaching the target tissue after systemic administration has yet been developed. Immunohistochemical studies of primary and secondary glioblastomas and their astrocytic precursor tumors have demonstrated that like topo II, ATP-binding cassette (ABC) transporters like MRP1, LRP, and P-gp are overexpressed in glioblastomas. We hypothesized that

the presence of ATP-binding cassette (ABC) transporters in the blood-brain barrier (BBB) might, in part, be responsible for limiting the CNS penetration of most anticancer drugs, while their presence in tumor tissue also confers resistance to wide range of drugs at a cellular level. To identify new agents effective in vivo against glioblastomas, we have developed an innovative approach combining our modular design of DNA-binding agents allowing for the creation of unique libraries of DNA binders and potential topo II poisons. By systematically screening such libraries, we have identified highly apoptotic compounds that can circumvent Pgp and MRP1-mediated resistance mechanisms, suggesting that such compounds will be potent cytotoxins against gliomas, while possessing the ability to cross the BBB. We prepared and screened a selected library of over 400 DNA binding agents against a panel of cells overexpressing P-gp and MRP1, identifying < 10 compounds possessing the necessary characteristics from which the compounds WP744 and WP769 were selected for more detailed evaluation. Both compounds are structurally related to the well-known anticancer drug doxorubicin (DOX), but they possess in vitro and in vivo properties that are very different from those of DOX. WP744 and WP769 are significantly more apoptotic than DOX against both wild-type tumor cells and multidrug-resistant tumor cell lines with the MDR1 and MRP1 phenotypes. WP769 is also a significantly more potent topo II poison than either DOX or WP744. Both WP744 and WP769 cross the BBB, reaching CNS and tumor concentrations that exceed that of plasma. In vitro, both are effective at nanomolar concentrations in inhibiting growth of the glioma cell lines U87MG, D54MG, and U251MG. Because of their unique biological characteristics (potent topo II poisons, ability to cross BB barrier, activity against multidrug resistant tumors) these agents are uniquely placed to become effective therapeutic agents for the treatment of GBM. We will discuss the design, synthesis, screening, selection, and unique properties of compounds WP744 and WP769. Currently, WP744 is entering Phase I clinical studies in humans.

17:30 Oral

THE IMPORTANCE OF THE TYPE OF ISONIAZID ACETYLATION IN MONITORING OF TUBERCULOSIS TREATMENT.

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Isoniazid (INH) is a main agent in the treatment of tuberculosis in combination with other drugs or alone as a prophylactic agent. The major pathway for INH metabolism is acetylation by a hepatic enzyme N-acetylotransferase, discovered over 40 years ago following differences observed in tuberculosis patients to isoniazid toxicity and bioavailability. The biochemical basis for this observation relates to substrate specificity and molecular genetics of two distinct N-acetyltranferase isoenzymes NAT1 an NAT2. The genetically polymorphic NAT2 is responsible for INH metabolism and individuals can be classified as rapid and slow acetylators. In our last study in 237 Tb patients treated by standard course of chemotherapy with dose of INH 300 mg/day we observed that 1. majority of the patients (70 %) shown fast type of INH acetylation and 2. after the same doses the three parameters of INH bioavalability in a serum of fast and slow acetylators had significant differences: INH concentrations, pharmacokinetic and biovailability factors. The objective of the present study was to apply the genotyping of the fast and slow acetylators for personalized therapeutic dose. Materials and methods: blood samples were taken from the patients and volunteers before (time 0) and 1,3,6,24 hrs after drug administration. Plasma concentrations of INH were determined with biological method in the authors modification.This method warrants high accuracy and secured repeatable results. The lowest measurable concentration was 0,25 mcg/ml. For marking INH concentrations in human serum was applied as a pattern strain *Mycobacterium aurum* REB . Two indicators of acetylation rate I_3 and C_6 have been used to determine an acetylation type. Genomic DNA was isolated from the blood samples. DNA extracted by Gustinicich method and amplified by PCR by Spurr with two primers: P1: 5'-GCTGGGTCTGGAAGCTCCTC-3' and P2: 5'-TTGGGTGATACATACACAAGGG-3'. After initial amplification, the PCR product was cut separately with 3 different restriction enzymes: Kpn1, Tag1, and BamH1. A loos of a Kpn1 restriction site denotes NAT2*5 allele, a Tag1 restriction site denotes NAT2*6 allele, and a BamH1 restriction site denotes NAT2*7 allele. We separated the products on non-denaturing poliacrylamide gels and then stained them with ethidium bromide and visualized them on an UV transilluminator. The presence of any 2 mutant alleles defines the slow-acetylator genotype, whereas rapid acetylators have 1 or 2 wild-type NAT2*4 alleles. In a third part of patients with fast acetylation phenotype INH concentration in serum did not reach a value of 1 mcg/ml after 3h from therapeutic dose administration. In a third part of patients with slow acetylation phenotype INH concentration in serum exceeded a value of 2 mcg/ml after 6h from therapeutic dose administration. Four different NAT 2 alleles were detected in the study population. For all treated patients concentrations of isoniazid observed in rapid acetylators were considerably lower the those found in slow acetylators. On the basis of our results we suggest the using of NAT 2 genotyping for discrimination of the fast and slow acetylators in monitoring of tuberculosis therapy.

Praca finansowana z grantu KBN nr. 2 PO5B 136 27

17:50 Oral

SEARCHING FOR OPTIMAL METHOD FOR SOLUBILITY PREDICTION AMONG BIOLOGICALLY ACTIVE PHENYLPIPERAZINE DERIVATIVES OF PHENYTOIN

Jadwiga Handzlik, Katarzyna Kieć-Kononowicz

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The aqueous solubility of compounds is one of the most important factors in determining their biological activity. The solubility is particularly relevant to pharmacokinetic properties (ADMET). Thus, a prediction of compounds solubility is desirable to assess the concentrations that the drug will achieve in the target area, to establish the therapeutic level and to prevent toxicity. During the search for new antiarrhythmic agents among phenylpiperazine derivatives of phenytoin, it has been observed a significant influence of the compounds solubility on their pharmacological properties [1]. Furthermore, the aqueous solubility has determined a way of compounds administration during tests in vivo. Low soluble compounds can be tested only after i.p. administration, that needs much more amount of compounds and longer time of observation than that of i.v. administration. The results of previous investigations [1] prompted us to search for accurate methods to determine aqueous solubility among the phenylpiperazine phenytoin derivatives. On this way, some selected compounds were examined on their aqueous solubility using different methods. The aqueous solubility was predicted in silico using computer programs: MMProPlus, Chem3D, ACD/LogD Solubility Suite, Ched/Slippper as well as basing on semi-empirical methods (Yalkowsky's equations [2, 3]). To solve the semi-empirical equations, lipophilicity values of the compounds were needed. Thus, octanol-water partition coefficients of the compounds were evaluated using experimental shake-flask method. Furthermore, an experimental method of aqueous solubility estimation among considered compounds was elaborated. Based on Yalkowsky's equations and experimental results, a new semi-empirical equation for the solubility prediction of the compounds studied was found. Results of theoretical, semi-empirical and experimental methods were compared to find an optimal method for solubility prediction among considered compounds. [1]. Dyląg, T.; Zygmunt, M.; Maciąg, D.; Handzlik, J.; Bednarski, M.; Filipek, B.; Kieć-Kononowicz, K. Eur. J. Med. Chem. 2004, 39, 1013; [2]. Pinal, R.; Yalkowsky, S.H. J. Pharm. Sci., 1988, 77, 518; [3]. Jorgensen, W.L.; Duffy, E.M. Adv. Drug Deliv. Rev., 2002, 54, 355.

Bankiet
Monday evening, 15 May, 20:00

Tuesday, 16 May

Śniadanie
Tuesday morning, 16 May, 7:00

Sesja III
Tuesday morning, 16 May, 8:30
Chair: Andrzej Kutner, Wojciech Kostowski

8:30 Invited Oral

DOES OXIDATIVE DAMAGE TO DNA AND ANTIOXIDANT STATUS HAVE CLINICAL SIGNIFICANCE?

Ryszard S. Oliński, Rafal Rozalski, Daniel Gackowski, Marek Foksinski, Jolanta Guz, Agnieszka Siomek

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Oxidative damage to DNA is the seemingly inevitable consequence of cellular metabolism. Furthermore, despite protective mechanisms, cellular levels of damage may increase under conditions of oxidative stress, arising from exposure to variety of physical or chemical insults. Elevated levels of oxidatively damaged DNA have been measured in numerous diseases, and as a result, it has been hypothesised that such damage plays an integral role in the aetiology of that disease.

Many epidemiological studies have reported inverse association between vegetable and fruit consumption and occurrence of cancer and other degenerative diseases. One of the possible mechanisms of this protective effect is by exerting antioxidative activities of such plant food constituents as vitamins A, C and E. These antioxidant vitamins are effective radical scavengers therefore they should protect biomolecules such as DNA from oxidative damage.

In our recently published works have been found that the levels of oxidative DNA damage in leukocytes were significantly higher while the concentrations of the antioxidant vitamins were significantly lower in colon and lung cancer patients than in control group. Moreover, the same direction of the changes has been found in patients with adenoma. This, in turn, suggests that the changes in aforementioned biomarkers of oxidative stress are characteristic for cancer development. Results of investigating relationship between oxidative DNA damage biomarkers and antioxidants in aging process will also be presented.

Although at present it is impossible to answer directly the question concerning involvement of oxidative DNA damage in etiology of different diseases it is likely that oxidative DNA base modifications may serve as a source of mutations

that initiate carcinogenesis and other pathological conditions (i.e. they may be causal factors responsible for the process).

9:15 Invited Oral

NEW PERSPECTIVES ON THE TREATMENT OF NEURODEGENERATIVE DISEASES

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Neurodegenerative diseases are a major clinical and social problem in our times, hence an intensive search for neuroprotective drugs seems fully justified. Recent decades have witnessed fast development of the biochemical theories of neuronal death and survival and in consequence new approaches to the treatment of neurodegenerative diseases based on a cell and a gene therapy have been proposed. The classic excitotoxic cascade theory still remains valid, but has been enriched with new findings on the mechanism of apoptosis. Besides, a stronger emphasis has been put on the role of mitochondria in neuronal damage. Apart from agents that interfere with particular phases of excitotoxic cascades such as, e.g., pathological activation of ionotropic and metabotropic glutamatergic receptors, activation of voltage-dependent calcium and sodium channels, free radicals formation and activation of neuronal nitric oxide synthase, some new potential neuroprotective drugs have been recently suggested, e.g. neuropeptides, endocanabinoids, protein kinase inhibitors, erythropoietin, prostanoid receptor agonists, targeted neurotrophins and some immunosuppressants. However, despite indisputable progress in molecular and genetic sciences, no neuroprotective drug has been designed so far. There are at least two main reasons for this failure. First, our knowledge of the mechanism of neuronal death and survival is still incomplete. Genomic and proteomic analyses provide us with a plethora of new data, but there are still serious problems with their correct and straightforward interpretation. Moreover, better animal transgenic models of neurodegenerative diseases are required, and *in vitro* models should be based on human rather than animal neuronal cell lines, since the composition of protein targets for new drugs may show remarkable species differences. Second, there are no clear indications how these drugs should be used in the clinic. A number of putative neuroprotective drugs possess a narrow time-window, have dual activity (pro- or antiapoptotic, depending on the concentration, the type of a neuronal tissue and the stage of its development), or unfavourable pharmacokinetic parameters. Therefore it seems that further improvement of the outcome of neurodegenerative disease treatments may depend not only on a better understanding of the molecular mechanism of neuronal pathology and survival, but also on the beneficial biopharmaceutical properties of neuroprotective agents and on well-designed clinical trials.

10:00 Oral

METABOLIC TRANSFORMATIONS 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-450s) comprise endogenously synthesized compounds as well as xenobiotic compounds including medicines, food additives and environmental pollutants. There are many various isoforms of P450s, which have overlapping but distinct substrate specificities.

Although the total level of liver microsomal P450s does not vary considerably among humans, genetic polymorphism and inducibility of a given P-450 isoenzymes gives the interindividual variations in the levels of P450 isoforms (CYPs). 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-450s are capable of deactivating of anticancer drugs, thus, inhibitors of the specific isoenzymes in tumor cells would be developed as modulators of antitumor activity.

Considering all above, studies on metabolic transformations of antitumor agents are concentrated on their susceptibility to transformation with individual forms of cytochrome P-450. There was reported earlier that cyclophosphamide was metabolized by CYP2B6, whereas transformation of tamoxifen occurred with CYP3A7 and CYP3A5 giving rise different products in each case.

In our group we have studied metabolic transformations of acridine antitumor agents, especially compounds C□1311 and C□1748, which are under II and I phase of clinical trials, respectively. We are involved in the search of individual P450 isoenzymes responsible for metabolic transformations of C□1311 and C□1748 as well as in the identification of metabolic products formed after incubation with various CYPs. Transformations were carried out with human and liver microsomes of individual CYP overexpressions and with selected P450s recombinated in *E.coli*. CYP inhibition by acridine

drugs were also studied. We showed that CYP2 family of P□450 took part in metabolism of both compounds, however, each one underwent of various pathways of metabolic transformations.

10:20 Oral

STERICAL PREREQUISITES FOR PARTIAL ANTAGONISTS OF GLUTAMINERGIC GLUR5/6 RECEPTORS

Dariusz Matosiuk[1], Zbigniew Karczmarzyk[2], Agnieszka Kaczor[1], Christiane Kronbach[3], Klaus Unverferth[3]

1. *Department of Synthesis and Chemical Technology of Pharmaceutical Substances, Medical University, Staszica 6, Lublin 20-081, Poland* **2.** *Department of Chemistry, University of Podlasie, ul. 3 Maja 54, Siedlce 08-110, Poland* **3.** *Elbion AG, Meissner Strasse 191, Radebeul 01445, Germany*

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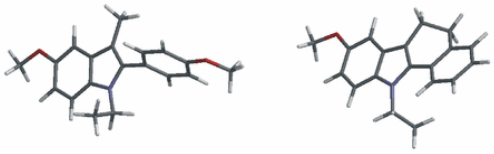
Glutamate is one of the most important excitatory neurotransmitters in the central nervous systems and plays significant role in the pathophysiology of numerous neurological and psychiatric disorders [1,2]. Indole derivatives with aromatic substituent at C2 make new class of the non-competitive, partial GluR5/GluR6 antagonists. Between them 1-ethyl-2-(methoxyphenyl)-3-methyl-5-methoxy-indole was found to be the most potent (EC_{50} = 0.2 mm) in contrary to the derivative having rigidly bonded C2 pharmacophoric aromatic substituent, which show no activity.



Basic structural and conformational information obtained from X-ray investigations were used to explanation the dramatic changes in the glutamate receptor GluR5/6 affinity with the changes of the spatial location of the C2 substituent. The molecular modeling studies using molecular mechanic method and MNDO-AM1 approximation were undertaken to investigate the conformational preferences of searched derivatives.

[1] S. Bleich, K. Romer, J. Wiltfang, J. Kornhuber, *International Journal of Geriatric Psychiatry*, **2003**, 18 (Suppl. 1), 33.

[2] M. Nedergaard, T. Takano, A. J. Hansen, *Nature Reviews Neuroscience*, **2002**, 9, 748.

Przerwa na kawę

Tuesday morning, 16 May, 10:40

Sesja III cd.

Tuesday morning, 16 May, 11:00

Chair: Andrzej Kutner, Wojciech Kostowski

11:00 Oral

GENE EXPRESSION-TARGETED ISOFLAVONE THERAPY FOR MUCOPOLYSACCHARIDOSES

Grzegorz Węgrzyn[1], Ewa Piotrowska[1], Joanna Jakóbkiewicz-Banecka[2], Sylwia Barańska[1], Anna Tylki-Szymańska[3], Barbara Czartoryska[4], Alicja Węgrzyn[2]

1. *University of Gdansk, Dept. of Molecular Biology (KBM-UG), Kladki 24, Gdańsk 80-822, Poland* **2.** *Institute of Biochemistry and Biophysics, Polish Academy of Sciences, Lab. affiliated to UG, Kladki 24, Gdańsk 80-822, Poland* **3.** *The Childrens Memorial Health Institute, Aleja Dzieci Polskich 20, Warszawa 04-730, Poland* **4.** *Institute of Psychiatry and Neurology, Department of Genetics, Aleja Sobieskiego 9, Warszawa 02-957, Poland*

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Mucopolysaccharidoses (MPS) are inherited, severe, progressive, metabolic disorders caused by deficiencies in different enzymes involved in degradation of glycosaminoglycans (GAGs). Although enzyme replacement therapy (ERT) has recently been available for MPS type I, and clinical trials have been performed in ERT for MPS II and MPS VI, it is little chance that this kind of treatment may be effective for neurodegenerative forms of MPS (due to inefficient delivery of enzymes to central nervous system through the blood-brain barrier), hence currently there is no effective therapy available for them. Sanfilippo disease (MPS III) seems to be especially problematic as this condition is associated with severe learning difficulty and behavioral disturbance and only relatively mild somatic involvement. In most affected patients the progressive nature of the disease leads to death in the second (or rarely third) decade of life. As the disorder primarily affects the brain and nervous system, attempts to cure the disorder have not been possible and the best that can be currently offered is palliative or symptomatic care. Therefore, we aim to develop an alternative therapy for mucopolysaccharidoses. Apart from provision of the deficient enzyme, another possible strategy for treatment of lysosomal storage disorders (including MPS) is reduction of the substrate whose degradation is impaired. For such a substrate reduction therapy (SRT) an analogue of a monomer that is incorporated into a complex macromolecule is usually considered. It may be a competitor of the natural substrate for an enzyme synthesizing the macromolecule. However, in the process leading to synthesis of GAGs, the monomers are carbohydrates or their derivatives which are also involved in many other metabolic pathways. Therefore, a putative competitor that could block activity of

one of enzymes taking part in GAG synthesis, would most probably interfere with many other metabolic pathways, by blocking other biochemical reactions, thus giving potentially serious side effects. Therefore, to avoid this problem, we aimed to develop SRT based on regulation of expression of genes coding for specific GAG synthetases. We found that genistein (4',5,7-trihydroxyisoflavone or 5,7-dihydroxy-3-(4-hydroxyphenyl)-4 *H*-1-benzopyran-4-one), an isoflavone occurring naturally in relatively large amounts in soy, inhibits synthesis of GAGs considerably in cultures of fibroblasts of MPS patients (types I, II, IIIA and IIIB were tested). Prolonged cultivation of these cells in the presence of genistein resulted in reduction of GAG accumulation and normalization of cells as estimated by biochemical tests and electron microscopic analysis, respectively. Importantly, genistein is able to cross the blood-brain barrier to some extend. Since genistein inhibits kinase activity of epidermal growth factor (EGF) receptor, which is required for full expression of genes coding for enzymes involved in GAG production, we propose to consider a substrate deprivation therapy for mucopolysaccharidoses, which is referred to as "gene expression-targeted isoflavone therapy" (GET IT).

11:20 Oral

THE BIOLOGICAL ACTIVITY OF NEW TUFTSIN DERIVATIVES - INDUCTION OF PHAGOCYTOSIS.

Anna Wardowska[1], Krystyna Dzierzbicka[2], Andrzej Myśliwski[1]

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Phagocytosis is one of the essential arms of host defense against bacterial or fungal infections. The phagocytic process consists of several major stages: 1. chemotaxis - migration of phagocytes to inflammatory sites, 2. attachment of particles to the surface of phagocytes, 3. ingestion - phagocytosis and intracellular killing of by oxygen-dependent and oxygen-independent mechanisms. This process can be induced by many phagocytosis stimulating factors. One of them is an endogenous tetrapeptide - tuftsin, that occurs in the blood of humans including human beings. Being an integral part of a heavy chain of IgG, tuftsin is liberated by the action of two specific enzymes: splenic tuftsin endocarboxypeptidase and leukokininase. Tuftsin is capable of potentiating granulocyte and macrophage functions such as: phagocytosis, motility, chemotaxis as well as bactericidal and tumoricidal activity. Due to high plasma instability of tuftsin, many derivatives of this peptide has been already synthesized and examined. Some of them are equally active as tuftsin or even display better biological properties.

The other particle able to induce phagocytosis is muramyl dipeptide (MDP) the smallest synthetic glycopeptide of bacterial origin that possesses an immunogenic activity. MDP is known to affect most functions of macrophages. The activation of those cells results mainly in increased reduction of oxygen to the superoxide anion (O_2^-) and then to hydrogen peroxide, which is involved in phagocytosis.

The aim of this study was to evaluate the impact of new tuftsin and MDP derivatives (one tuftsin analogue and four conjugates of tuftsin and muramyl dipeptide or nor-muramyl dipeptide) on the induction of the phagocytosis process through the influence on the activity of phagocytic cells.

We tested phagocytosis stimulating properties of a new group of derivatives on the peripheral blood mononuclear cells (PBMC) isolated from the venous blood of healthy, young donors. We performed Phagotest®, which enables the qualification of phagocytic activity of monocytes and granulocytes in heparinized whole blood.

The results of the study show that newly synthesized derivatives of tuftsin and MDP may influence the first step of immune response. Increased phagocytic activity of neutrophils and monocytes indicate that the examined compounds can be useful in curing bacterial infections.

11:40 Oral

NEW CORES FOR THE SYNTHESIS OF NEW GENERATION OF DENDRIMERIC PEPTIDES

Piotr Polcyn, Zofia Urbańczyk-Lipkowska

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Dendrimers provide an excellent platform to tailor molecular diversity by appending numerous biologically active elements at terminal positions. Recently, we proposed a novel approach, where not only end of branches but also the whole dendrimer tree was used to locate active groups responsible for interactions with microbial membranes (aromatic and positively charged amino acid residues). Recently, dendrimeric peptides based on lysine as a trivalent core and branching element have been developped [1]. They expressed wide spectrum of antimicrobial activity against Gram-positive (S. *aureus*), Gram-negative (*E. coli*) and *C. albicans* strains. This confirmed our postulate that bioactive conformation could be achieved by selection one out of many topologically relevant structures.

Cytotoxicity studies showed that better properties have compounds with higher degree of branching ("dendrimeric ef-

fect") [2]. Therefore, several new *N*-propylamino derivatives of basic amino acids were designed and used as cores in the synthesis of a new generation of dendrimeric peptides.

The present communication will present synthetic approach towards preparation of analogs containing higher number of active elements within the dendrimer tree and therefore, more rigid structure.

This project was supported by grant No. 3T09B 115 28 from the Ministry of Education and Science.

1. Janiszewska J, Swieton J, Lipkowski AW, Urbanczyk-Lipkowska Z., *Bioorg.Med. Chem. Lett.,***2003**,*13*, 3711-3713.

2., Klajnert B., Janiszewska J, Urbanczyk-Lipkowska Z, Bryszewska M., Shcharbin D, Labieniec M, *Intern.J. Pharmaceutics***2006***,309*, 208-217.

12:00 Oral

NEW SYNTHESIS OF REPAGLINIDE

Barbara Szechner, Osman Achmatowicz, Michał Odrowąż-Sypniewski, Krzysztof Wiśniewski

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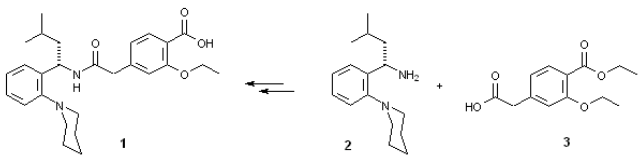
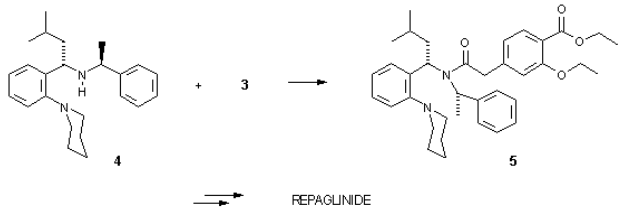
Repaglinide (**1**) belongs to a new class of orally administered drugs for treatment of type 2 diabetes. It was launched in 1997 under brand names Prandin (USA) and NovoNorm (elsewhere). Repaglinide (**1**) was obtained by several related ways. In each case it involved condensation of (*S*)-amine **2** with substituted phenylacetic acid **3** and hydrolysis of an ester group in the resulting amide [1].



In this communication we report on the new method of repaglinide (**1**) synthesis [2] rendering preparation of amine **2** unnecessary.

Our approach is based on the improved method of (*S*,*S*)-amine **4** preparation**,** its condensation with an acid **3** yielding amide **5** and removal of an ester group and chiral auxiliary, *N*-(1-phenyl)ethyl substituent from the latter.



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Przerwa obiadowa

Przygotowanie do II sesji posterowej
Tuesday afternoon, 16 May, 13:00

Sesja IV

Tuesday afternoon, 16 May, 14:30
Chair: Łukasz S. Kaczmarek, Wanda Baer-Dubowska

14:30 Invited Oral

STRENGTHS AND LIMITATIONS OF INTEGRATIVE GENOMICS EXEMPLIFIED BY STUDIES OF BARRETT'S ESOPHAGUS

Jerzy Ostrowski

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The availability of complete human genome sequence and powerful methods for systemic profiling of gene expression, in connection with computational strategies for integrating analyses of large-scale data sets, provide a promising opportunity for elucidating the molecular mechanisms of disease. However, we still do not know what is the best way to study genomic networks. Microarray and mass spectrometry (MS), two different technologies used for the analysis of global gene expression, demonstrate both strengths and limitations. Patterns of transcript levels seem to be consistent among tissue samples of the same origin, and therefore, besides many other potential applications, microarrays are useful for molecular fingerprinting and for classification of various stages of a disease. However, its multivariable data are often noisy, which can result in difficulties of data interpretation, and both array quality and choice of analytical processing methods have a major impact on differential expression analysis of microarray data. Although MS is a powerful tool for rapid protein identification and for analysis of protein modifications, also this method reveals significant limitations in profiling cellular proteomes on a genome-wide scale.

It has been proposed recently that gastroesophageal reflux disease patients may be categorized into three distinct groups exhibiting nonerosive reflux disease, erosive reflux disease, and Barrett's esophagus (BE). Genetic predisposition to the development of BE may result from contributing of low penetrated genetic alterations. Understanding of genetic changes that cause BE would have significant diagnostic, prognostic and therapeutic benefits. To better define the differences

between esophageal squamous epithelium and metaplastic columnar-lined esophageal epithelium in BE patients, transcript levels were measured using Affymetrix U133A 2.0 microarray and validated by quantitative RT-real-time PCR, while protein expression was examined by gel-free shotgun liquid chromatography tandem mass spectrometry (LC-MS-MS) runs. The methodological aspects, results of gene expression profiling, and prospects and challenges for using integrative genomics to systematically discover entire gene networks that underlie and modify reflux disease will be discussed.

15:15 Oral

CLICK CHEMISTRY? WE LIKE IT

Marek T. Konieczny, Wojciech Konieczny

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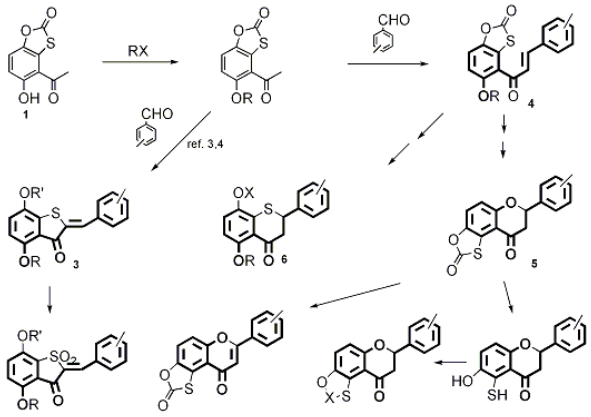
Modern methodologies of drug development create a need for synthesis of large number of compounds. Ideally, the compounds should be easily accessible, structurally related and, at the same time, diversified - a dream summarized as *Click Chemistry* [1,2].

Our program is aimed at search for new analogs of flawonoids bearing biological activity. Concerning chemistry, we explore syntheses starting with 4-acetyl-5-hydroxy-2-oxo-benz[1,3]oxathiole (**1**) and leading to compounds comprising o-hydroxy substituted flavonoid unit **2**.

The four-substituted benzene derivative **1** provides access to a remarkable number of diversified chemical entities comprising the desired structural fragment **2** (Scheme).

Scheme



We have already described compound **1** as a convenient substrate for synthesis of large group of thioaurones **3** [3,4], some of the compounds posses significant cytotoxic activity. We have also prepared chalcones **4**, flavanones **5** and thioflavanones **6**. The prepared compounds could be elaborated further, some possibilities are shown in the Scheme. Ultimately, starting from benzoxathiole **1**, we are able to prepare compounds comprising flavonoid structural fragments **2**, and characterized by different rigidity, lipophilicity, acidity and other pharmacologically important parameters.

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15:35 Oral

THE INFLUENCE OF SIMVASTATIN IN HIGH DOSE AND DILTIAZEM ON MYOCARDIUM IN RABBITS

Magdalena Jasińska, Jacek Owczarek, Marzena Ratyńska, Anna Zielińska, Krystyna Marek, Michał Karasek, Daria Orszulak-Michalak

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3-hydroxy-3-methyl-glytaryl coenzyme A (HMG-CoA) reductase inhibitors have been proved to be extremely useful in the management of hypercholesterolemia, as well as in prevention of primary and secondary coronary heart disease. However, they may produce rare but severe muscle-related symptoms such as myopathy and rhabdomyolysis. Recent findings *in vitro* have shown that statins could reduce cardiomyocyte viability. The correlation between statin cardiotoxicity, assessed *in vitro*, and cardiac efficiency, investigated *in vivo* has not been estimated so far. Diltiazem as CYP3A4 inhibitor, is well recognized risk factor of skeletal muscles myopathy, if co-administered with simvastatin. It is not known whether such interaction affects myocardial efficiency. A

combination of pathological and biochemical studies were performed in myopathy model induced by simvastatin as well. causing haemodynamic changes. The aim of the experiment was to establish the impact of simvastatin, at the dose provoking myopathy of skeletal muscles, and co-administered with diltiazem, on myocardium efficiency, defining as cardiac output (COI), after continuous infusion of dopamine as well as the influence on the histopathological and biochemical changes. The experiments were performed on New Zealand white rabbits. The animals were divided into four groups receiving: 0.2% MC (control group); diltiazem (5 mg/kg); simvastatin (50 mg/kg); simvastatin + diltiazem, for 14 days (*po)*. Dopamine did not cause a statistically significant increase in COI in rabbits receiving simvastatin alone. Diltiazem significantly increased the cardiac output index, maximally by 23%, if given simultaneously with simvastatin. The combined administration of simvastatin with diltiazem caused a marked increase in cardiac output index, as compared to simvastatin alone, too. Simultaneous administration of simvastatin and diltiazem caused 23-fold increase ($P< 0.01$), in rabbit serum CK levels and 20-fold increase ($P=0.056$) in TnI levels, as compared to the initial values. Also in these rabbits significant increase in CK (12411,60 *vs* 839,87 IU/L) and TnI (0,26 vs 0,014 ng/mL) as compared to control group were observed. Significant increase in CK (12411,60 *vs* 1100,92 IU/L) and TnI (0,26 vs 0,012 ng/mL) as compared to diltiazem alone were noted, too. Several lesions such as necrosis with macrophagic infiltration and single muscle degradation in myocardium were found. However, those changes seem to had no influence on cardiac efficiency which was even improved. It may suggest another mechanism, of drug-drug interaction than the one based on CYP3A4 inhibition considering the statin impact on skeletal and cardiac muscle.

Przerwa na kawę

Tuesday afternoon, 16 May, 15:55

Sesja IV cd.

Tuesday afternoon, 16 May, 16:15

Chair: Łukasz S. Kaczmarek, Wanda Baer-Dubowska

16:15 Invited Oral

NANOPARTICLES FROM POLYESTER-CONTAINING BLOCK COPOLYMERS AS CARRIERS OF BIOACTIVE COMPOUNDS

Stanislaw Słomkowski[1], Izabela Radomska-Galant[2], Mariusz Gadzinowski[1], Stanislaw Sosnowski[1]

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Many compounds used as active substances are decomposed or deactivated before they reach their targets. Delivery of oligopeptides and proteins is particularly difficult. Oral delivery is often ineffective due to protein or oligopeptide disintegration in digestive tract. Delivery by intravenous or subcutaneous injection is inconvenient whereas delivery by inhalation often leads to allergic response. Transdermal delivery is quite ineffective due to inefficiency of protein transport through skin.

There are hopes that some of the mentioned above problems could be solved by packing proteins into small particles with degradable cores and shells protecting nanoparticle content from premature contact with an organism. Proper chemical structure of the shell should eliminate or at least reduce induction of immunoresponse of the organism.

Recently, we developed methods suitable for synthesis of block copolymers composed of biodegradable polylactide and polyether (poly(ethylene oxide) and polyglycidol) biocompatible blocks [1]. In this work we will report on formation of nanoparticles from these copolymers and on their loading with drug models.

Nanoparticles were made by two methods. The first one did consist on self-assembly of copolymer molecules above critical aggregation concentration (CAC). For investigated copolymers CAC ranged from 8 mg/l to 0.15 g/L. According to the second method the particles were formed by dialysis. Namely, polymer solution in organic water miscible solvent (e.g. acetonitrile) was dialyzed against water. Slow exchange of organic solvent for water resulted in gradually decreased solvent quality and led to formation of nanoparticles. Diameters of obtained particles ranged from 20 to 300 nm, depending on copolymer structure and method of particle formation.

Self-assembly of block copolymers carried on in presence of hydrophobic compounds (*e.g.* pyrene used as a model) leads to formation of loaded particles. The effective loading with proteins was achieved by dialysis of protein and copolymer solutions. In this way particles with 30 wt% protein content were obtained.

It was found that not loaded and protein loaded particles are stable in pH range from 2 to 10. Only in a 1 M NaOH (*i.e.* at conditions not encountered in the organism) the polylactide-b- polyether nanoparticles are quickly decomposed.

Hydroxyl groups in colpolymers containing polyglycidol segments could be used for further functioonalization (*e.g.* in reaction with cyclic anhydride). CAC for nanoparticles formed from copolymers containing carboxyl groups was dependent on presence of Ca^{+2} cations that did act as particle stabilizers. Modification of copolymers by introduction of azobenzene moieties allowed obtaining light sensitive nanoparticles. Proper tailoring of chemical structure of chromophores may

allow formation of nanoparticles releasing bioactive compounds under irradiation with near-visible infrared light that is able to penetrate few centimeters through the soft tissue.

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Acknowledgement: This work was supported by the State Committee of Scientific Research, grant No. BZ-KBN 070/T09/2001/3.

17:00 Oral

ANTIFUNGAL DRUGS AND MULTIDRUG RESISTANCE - AN OPEN PROBLEM

Sławomir Milewski, Roland Wakieć

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The most important challenges for the modern antifungal chemotherapy are: a rapidly growing number of cases of fungal disseminated infections in immunocompromised patients, a very limited repertoire of available effective antifungal chemotherapeutics to cure such diseases and an emerging problem of fungal resistance, especially of the multidrug character. Our research activity has been for recent years aimed at search for novel targets for antifungal chemotherapy and new antifungal drug candidates, demonstrating high activity against multidrug-resistant (MDR) fungal cells. In the present studies we compared the *in vitro* growth-inhibitory activity of several antifungal drugs, including ketoconazole, fluconazole, clotrimazole, voriconazole, Amphotericin B and 5-fluorocytosine, against sensitive and multidrug-resistant *Candida albicans* clinical isolates, as well as against model *Saccharomyces cerevisiae* strains harbouring or lacking *C. albicans* genes, encoding particular drug-effluxing membrane proteins. Relative affinity of antifungal drugs to the main *C. albicans* drug transporters, CaCdr1p and CaCdr2p, was determined using a competitive fluorescent assay. In parallel studies, the same methodology was applied for several potential antifungal drugs having amino acid or peptide structure.

Our results show that presence and activity of multidrug transporters: CaCdr1p, CaCdr2p or CaMdr1p, makes fungal cells cross-resistant to all tested azole and triazole antifungal agents, including the newest, second-generation triazole derivative - voriconazole. Voriconazole and other antifungal azole and triazole derivatives were shown to compete effectively with the fluorescent probe, Rhodamine 6G, for the binding sites in CaCdr1p and CaCdr2p. On the other hand, the MDR cells did not demonstrate resistance to Amphotericin B and 5-fluorocytosine. Finally, we found that presence of CaCdr1p or CaCdr2p made the fungal cells more susceptible to the action of peptidic antifungal agents: nikkomycin, FMDP-peptides and oxalysyl peptides and to some antifungal amino acids. The results of our comparative studies allowed to formulate some general conclusions concerning novel strategies overcoming fungal MDR.

17:20 Oral

INCIDENCE OF JAW OSTEONECROSIS IN MYELOMA PATIENTS TREATED WITH BISPHOSPHONATES

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Background: Jaw osteonecrosis has recently been identified as complication in bisphosphonate-treated patients. In a Web-based survey conducted to assess the risk factors for osteonecrosis of the jaw of 1203 respondents, 904 had multiple myeloma (MM) and 299 breast cancer and among them 152 patients had osteonecrosis of the jaw or suspicious findings, including bone erosions and spurs plus exposed bone. It was found that osteonecrosis of the jaws was most strongly associated with use of Aredia and/or Zometa. With data censored at 36 months, the estimated incidence among patients receiving zoledronic acid was 10 percent and that among those receiving pamidronate was 4 percent (Durie et al. N Engl J Med 2005; 353: (1) 99.

The aim of the study was assessment of occurrence of jaw osteonecrosis in 113 MM patients treated with different bisphosphonates which have been evaluated in prospective study performed at our institution.

Methods: Sixty one MM patients received clodronate (Bonefos; Schering AG) per os 2,4g/24 hrs. Median treatment duration amounted to 17 months and in 14 patients treatment duration exceeded 24 months. Forty six MM patients received pamidronate (Aredia; Novartis) intravenously 60 mg monthly. All 46 patients included in pamidronate study were followed up until death or at least for 6 years. Six MM patients received zoledronic acid (Zometa; Novartis) intravenously either 4 or 8 mg every 3 to 4 weeks for 13 months.

Results: In none case treated with clodronate or pamidronate there was observed occurrence of jaw osteonecrosis. Osteonecrosis of the mandible developed in 2 of 6 patients treated with zoledronic acid. The duration of treatment with zoledronic acid in each case was 13 months with a cumulative zoledronic acid dose in one case of 72 mg and in the other of 144 mg. The lesions were refractory to conservative debridement, surgey and antibiotic therapy.

Conclusions: Clinicians should be aware of the potential serious complication of bone necrosis in MM patients receiving

long-term treatment with potent bisphosphonates. It is recommended a dental examination to identify and correct predisposing conditions before bisphosphonate treatment is started.

Sesja Posterowa I

Tuesday afternoon, 16 May, 17:40

17:40 Poster I-1

THE ROLE OF C-RING OF FLAVONOIDS FOR BIOLOGICAL ACTIVITY TOWARDS HCK AND COX-2

Rafał Augustyniak[1], Dariusz Maciej Pisklak[1], Iwona Wawer[1], Grzegorz Grynkiewicz[2]

1. *Medical University of Warsaw, Faculty of Pharmacy, Department of Physical Chemistry, Banacha 1, Warszawa 02-097, Poland* **2.** *Pharmaceutical Research Institute (IF), Rydygiera 8, Warszawa 01-793, Poland*

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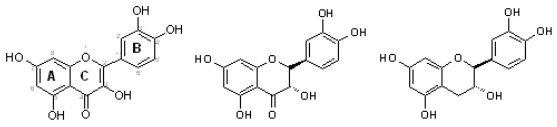
Flavonoids are polyphenolic compounds widely distributed in plants. Their benefits to human health are attributed to a wide range of biological activities which include antimutagenic, anticarcinogenic, immune-stimulating, antiinflammatory and artheriosclerosis inhibiting effects. The polyphenols are able to act as antioxidants, inhibiting lipid peroxidation and scavenge superoxide or hydroxyl radicals [1].

Another mechanism for the endogenous defense against free radicals (oxidative stress) has been proposed via the modification of signal transduction pathways.

It was proved that quercetin, binding to the active site of the enzyme, inhibits protein kinase Hck which plays significant role in signal transduction in the immune system [2]. Additionally, flavonoids have antiinflammatory properties and could interact with cyclooxygenase (COX-2) as selective inhibitors [3].

Taxifolin, a natural dihydroflavonol (found in grapefruit, orange, the wood of the Siberian and Dahurian larches), quercetin (present in onions, apples and red wine) and epicatechin (common in black and green tea) are structurally similar with the same 5,7,3',4' hydroxylation pattern.

We have investigated selected flavonoids: quercetin, taxifolin and epicatechin in order to find structural features that govern the specific binding to the Hck and COX-2. These compounds differ in the structure of C-ring; the double bond between C2 and C3 in the ring C may play a dominant role on their biological activities. Molecular modeling confirmed that the geometry of C-ring is an important factor for intermolecular interactions with these enzymes.



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17:40 Poster I-2

THE SEARCH FOR ENANTIOSELECTIVE REDUCTION OF PROPENTOFYLLINE BY WINE'S YEAST BIOCATALYSIS IN WATER AND IN ORGANIC SOLVENTS

Elżbieta Pękala, Tomasz Wójcik, Gniewomir Latacz

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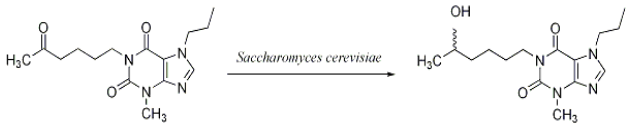
Propentofylline (PPT, 3-methyl-1-(5-oxohexyl)-7-propyl-xanthine) has been reported to be a beneficial for treatment of both vascular dementia and dementia of the Alzheimer type. PPT was synthesized to increse its solubility in lipids by substituting methyl group with a propyl one in the position 7 of the purine backbone of pentoxifylline. The pharmacological effects of PPT may be exerted *via* stimulation of the nerve growth factor, increased cerebral blood flow, and inhibition of adenosine uptake. PPT also enhances extracelullar adenosine concentrations and decreases extracellular levels of glutamate *in vivo* during ischemia.

In contrast to the known pharmacological effects, up to now few clinical pharmacokinetic and metabolism studies of PPT were reported in the literature. The short half-life time of PPT at the terminal elimination phase and poor bioavailability after oral administrations to rabbits suggest that this drug takes the extensive first-pass metabolism in the liver. *In vivo* it was metabolized to the racemic compound - (±)-1-(5-hydroxyhexyl)-3-methyl-7-propyl-xanthine (HOPPT) [1].



In this work we are presenting the results of stereoselective reduction of PPT to HOPPT catalysed by whole cells of baker's and a few strains of wine's yeasts in water and in organic solvents. The yeasts for microbiological reduction were carried out under non-fermenting conditions. The stereoselectivity of this biotransformation process was determined using a HPLC with a chiral column. It was stated, that the different yeast strains preferred biotransformation of PPT to the different enantiomers. The wine's yeast in the presence of water metabolised PPT to a (*R*)-3-methyl-1-(5-hydroxyhexyl)-7-propyl xanthine, with the exception of Burgund 38 strain (it formed *S*-isomer). In this reaction selected baker's yeast strains in water and in organic solvents, gave mainly an excess of enantiomer (S) - 3-methyl-1-(5-hydroxyhexyl)-7-propyl xanthine. Here the exception was Ihirondelle strain in *n*-hexane, which preferred PPT conversion to (*R*) -isomer.

Acknowledgements:

The research was supported by a scholarship from the Rector's Found of the Jagiellonian University.

Authors are grateful to *Intervet* Company for a free sample of PPT and P. Zamojski for wines yeasts supply.

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17:40 Poster I-3

SYNTHESIS, PHYSICOCHEMICAL AND ANTICONVULSANT PROPERTIES OF NEW N-AMINOPHENYL AZASPIRANE AND PYRROLIDINE-2,5-DIONE DERIVATIVES

Krzysztof Kamiński[1], Jolanta Obniska[1], Agnieszka Dzierżawska-Majewska[2], Janina Karolak-Wojciechowska[2]

1. *Jagiellonian University, Medical College, Department of Pharmaceutical Chemistry, Medyczna 9, Kraków 30-688, Poland* **2.** *Technical University Institute of General and Ecological Chemistry, Żwirki 36, Łódź 90-924, Poland*

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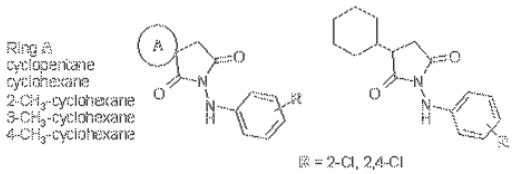
Previously the anticonvulsant properties many of 2-azaspiro[4.4]nonane- and [4.5]decane-1,3-diones differently substituted at the imide nitrogen atom were described. Among those derivatives the most active was N-aminophenyl-2-azaspiro[4.4]nonane-1,3-dione, with ED_{50} =76,27 mg/kg in the maximal electroshock test (MES) [1-3]. Based on these findings, in effort to obtain compounds with enhanced anticonvulsant activity new series of 2-azaspiro[4.4]nonane- and [4.5]decane-1,3-diones with aminophenyl moiety at the nitrogen atom have been synthesized. On the other hand, to investigate the influence of the cycloalkyl system, attached to the imide through the C3 spiro carbon atom, on the anticonvulsant activity, two analogues with cyclohexyl moiety as a flexible fragment at position-3 of pyrrolidine-2,5-dione ring were obtained.



The compounds were evaluated for their anticonvulsant activity within the Antiepileptic Drug Development (ADD) Program (Epilepsy Branch, Neurological Disorders Program, National Institute of the Neurological and Communicative Disorders and Stroke (NINCDS), Bethesda) [4]. The lipophilic character of the molecules, which is one of the crucial physicochemical parameters of bioactive compounds, was estimated by use of RP-TLC, RP-HPLC methods and Pallas 3.1 computer program. The data obtained enable to evaluate the correlation between different measurements of lipophilc properties. The structural characterization of the selected compounds has been done by crystal X-ray structure analysis to determine the parameters important for anticonvulsant activity.

References:

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17:40 Poster I-4

SYNTHESIS AND ANTICONVULSANT ACTIVITY OF NOVEL N-PYRIDINE-2-YL AND N-AMINOPHENYL DERIVATIVES OF 3,3-DIALKYL-PYRROLIDINE-2,5-DIONES

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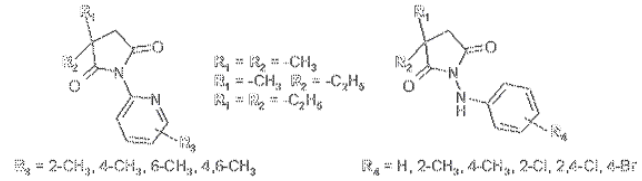
In the recent years we have synthesized a great number of spirosuccinimides with anticonvulsant activity by changing substituents at the imide nitrogen atom [1, 2]. In the current study a new series of N-pyridine-2-yl and N-aminophenyl de-

rivatives of 3,3-dialkyl-pyrrolidine-2,5-diones were synthesized. These molecules were designed as analogues of 3-ethyl-3-methyl-pyrrolidine-2,5-dione (ethosuximide), to evaluate the influence of such modifications on anticonvulsant activity.



The compounds were evaluated for their anticonvulsant and neurotoxic properties within the Antiepileptic Drug Development (ADD) Program by testing procedures, which have been described earlier [3]. One of the current approaches in rational drug design is to estimate the lipophilic character of the molecules. Therefore for all compounds the lipophilicity was determined by use of the Pallas 3.1 computer program. The results obtained provided a good basis for the evaluation of structure-lipophilicity relationships as well as the correlation between clog*P* values and anticonvulsant activity.

References:

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2. Kamiński K., Obniska J., Zagórska A., Maciąg D.; Arch. Pharm.
in press (2006).
3. Kupferberg H.J.; Epilepsia 30 (suppl.) (1989) 51-56.

17:40 Poster I-5

SYNTHESIS AND ANTIPROLIFERATIVE ACTIVITY OF DIACETYLENIC THIOQUINOLINES

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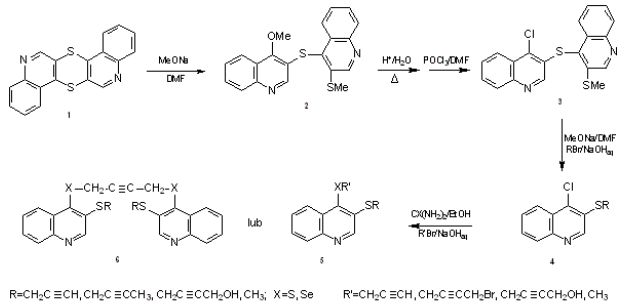
Acetylenic derivatives are an important class of compounds that has attracted increasing attention as a source of new anticancer agents. The synthetic methods for their preparation are of interest especially with regard to the synthesis of enediyne antibiotics and their analogues.[1] Recently we have carried out a new synthesis of propargyl thioquinolines which exhibited significant *in vitro* cytotoxicity.[2-3]

In the present study a series of new 3,4-disubstituted thioquinolines **5-6** which possess one or two the same or different O, S, Se-propargyl, 4-bromo-2-butynyl, 4-hydroxy-2-butynyl groups were synthesized. The obtained compounds were tested for their antiproliferative activity *in vitro* against the cells of human (colon cancer SW 707, leukemia CCRF/CEM) and murine (leukemia P388, melanoma B16) cancer cell lines. The most active compounds **5** have the ID_{50} values ranging from 0.2 to 4.5 μg/ml comparable to that of referential cisplatin.



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17:40 Poster I-6

SYNTHESIS AND CYTOTOXIC ACTIVITY OF 10-DEACETYL-10-PROPARGILPACLITAXEL

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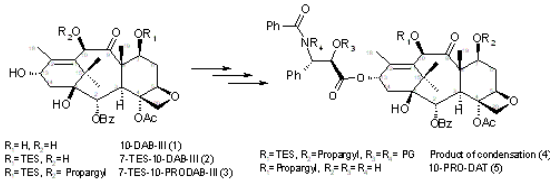
Paclitaxel is one of the most important chemotherapeutic agent for clinical treatment of ovarian and breast cancer. Due to its high cytotoxic activity and unique mechanism of action, and also because of its drawbacks (poor water solubility and iduction of multi-drug resistance), paclitaxel has become the subject of extensive structure-activity relationship studies, in order to obtain better insight into its mechanism of action at the molecular level and to prepare new, more active analogues with better pharmacological properties. Many derivatives of paclitaxel have been reported to data, some of which have been found to be more, and some less potent than paclitaxel.

Here we wish to report the synthesis of paclitaxel derivative with propargyl group at C-10 as an example of alkynyl pharmacophore which may significantly modify the chemical, physical and biological properties of such substances. Compouns **5** was prepared in 4 steps starting from 10-deacetylbaccatin III (10-DAB-III) (**1**) isolated from natural resources (needles of *Taxus baccata*). Silylation and next propargylation of **1** gave 7-TES-10-PRODAB-III (**3**). Compound **3** was condensated with protected taxol side chain, using DCC as a condensing agent. Hydrolysis of the product of condensation **4** provided 10-propargyldeacetylpaclitaxel (**5**).



The obtained compounds were tested for their antiproliferative activity *in vitro* against the cells of human lung carcinoma A549, human breast carcinoma MCF-7 and mice melanoma B16-F0. The compound **5** was active only against MCF-7 and **3** was active against all tested cell lines. However, all compounds were about 100-1000 fold less active than paclitaxel.

17:40 Poster I-7

SYNTHESIS AND ANTI-HIV-1 ACTIVITY OF NOVEL SERIES 1,4,2-BENZODITHIAZINE DERIVATIVES

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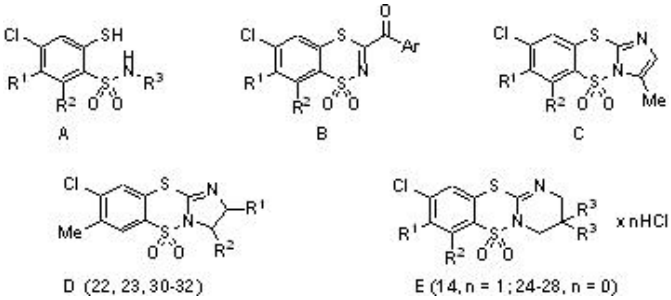
Variously substituted 2-mercaptobenzenesulfonamides (**A**) and 3-aroyl-1,1-dioxo-1,4,2-
benzodithiaznes (**B**) were synthesized in our laboratories and described as a novel class of potent HIV-1 integrase inhibitors [1,2]. Recently, we have found that the compound of type **C** (R^1 = Me, R^2 = H) showed also remarkable anti-HIV activity with 50% effective concentration EC_{50} value of 0.94 μM and no significant cytotoxicity at 200.0 μM [3].

The above findings prompted us to develop new methodologies for the syntheses of the compounds depicted as **D** and **E**. The syntheses of the target compounds were achieved by convenient three or four step procedures starting from the corresponding 4-chloro-3-methylthio-1,4,2-benzodithiazine 1,1-dioxides.



The *in vitro* anti-HIV activity of compounds **14, 22-25, 27, 30** and **31** has been tested at the NCI (Bethesda, USA). The selected compound with remarkable anti-HIV activity (EC_{50} = 0.09μM) and very high therapeutic index (TI=1177.7) was 9-chloro - 2,3,4 - trihydro - 7 - methyl - 6,6 - dioxopyrimido[1,2-*b*][1,4,2]benzodithiazine (**25**).

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This study was supported by Polish State Committee for Scientific Research (grant no 2 P05 035 27).

17:40 Poster I-8

SYNTHESIS OF 25- HYDROXYVITAMIN D_3 - CALCIFEDIOL FROM VITAMIN D_2

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Vitamin D is essential for the absorption and utilization of calcium and phosphate and aids in the mobilization of bone calcium and maintenance of serum calcium concentrations. 25-Hydroxyvitamin D_3 is the major circulating metabolite of vitamin D_3.[1]

It is produced in the liver and is the best indicator of the body's vitamin D stores. It is effective in the treatment of Rickets and Osteomalacia, both in azotemic and non-azotemic patients. Calcifediol also has mineralizing properties and it is used in clinical situations.

We described herein synthesis of calcifediol from vitamin D_2 based on the Barton-Hesse and their collaborators[2,3] strategy[2] with some modifications of this pathway. Our synthesis of calcifediol can be suitable for the large scale preparation of this important vitamin D analogue.

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17:40 Poster I-9

ENANTIOSELECTIVITIES OF SOME 1,4-DISUBSTITUTED PIPERAZINES ON AMYLOSE CARBAMATE

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Chromatographic behaviour of several chiral 1,4-disubstituted piperazine derivatives with hypnotic-sedative activity has been examined on amylose tris-(3,5-dimethylphenyl)carbamate (Chiralpak AD) with hexane-isopropanol and hexane-ethanol mobile phases and the results were compared with those obtained on cellulose tris-(4-methylbenzoate) (Chiralcel OJ). On Chiralpak AD column the chiral resolution has been obtained for 10 out of 11 tested compounds whereas on Chiralcel OJ 3 compounds remained unresolved. Mobile phase influence on enantioselectivity was more pronounced on Chiralpak than on Chiralcel stationary phase. The difference between the stationary phases lies in that amylose has helical while cellulose linear structure and amylose carbamate (contrary to cellulose benzoate) may serve as hydrogen bond donor. As a result for both stationary phase types one can observe different mobile phase influence on stereoelectronic interactions between analite and stationary phase.

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8-ALKOXY-PURINE-2,6-DIONES WITH 7-PHENYLPIPERAZINYLALKYL AND 7-TETRAHYDRO-ISOQUINOLINYLALKYL MOIETIES AS 5-HT$_{1A}$, 5-HT$_{2A}$, 5-HT$_7$ RECEPTOR LIGANDS

Grażyna Chłoń-Rzepa[1], Paweł Żmudzki[1], Maciej Pawłowski[1], Andrzej J. Bojarski[2], Sijka Charakchieva-Minol[2], Beata Duszyńska[2], Agnieszka Nikiforuk[2]

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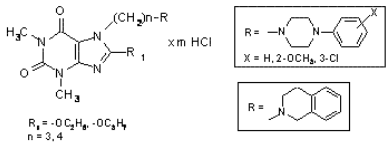
Based on our previous systematic studies on the structure-activity relationships in arylalkylpiperazine group of serotonin receptor ligands [1-3] we designed and synthesized a set of new 8-alkoxy-1,3-dimethyl-purine-2,6-dione derivatives with 7-(4-phenyl-1-pipera-zinyl)-alkyl and 7-(4-tetrahydroisoquinolinyl)-alkyl moieties. Previously obtained 1,3-dimethyl-7-(3-chloroalkyl)-8-alkoxy-purine-2,6-diones, in the reaction with appropriate piperazine derivatives and 1,2,3,4-tetrahydroisoquinoline yielded final products, which were then converted into water soluble hydrochloride salts. The purity of the compounds were controlled by TLC, the structures were confirmed by spectral (^{1}H-NMR, MS, UV), and C, H, N analyses.



The new compounds were tested in competition binding experiments for 5-HT$_{1A}$, 5-HT$_{2A}$ and 5-HT$_7$ receptors. It was found that some of 7-(4-phenylpiperazinyl)-butyl derivatives were potent 5-HT$_{1A}$, and/or 5-HT$_{2A}$ receptor ligands (K_i = 11-28 nM), and also highly active 5-HT$_7$ ligands (K_i = 75-90 nM). The compound with 7-(4-tetra-hydroisoquinolinyl)-butyl moiety was selective 5-HT$_7$ receptor ligand (K_i = 106 nM) with moderate 5-HT$_{1A}$, and low 5-HT$_{2A}$ receptor affinity. Some behavioral models demonstrated that selected compounds may be classified as partial agonists of 5-HT$_{1A}$ receptor.

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17:40 Poster I-11

DESIGN AND SYNTHESIS OF NEW 7-PHENYLPIPERAZINYLALKYL AND 7-TETRAHYDROISOQUINOLINYLALKYL DERIVATIVES OF PURINE-2,6,8-TRIONE AS POTENTIAL SEROTONIN RECEPTOR AGENTS

Grażyna Chłoń-Rzepa[1], Paweł Żmudzki[1], Maciej Pawłowski[1], Andrzej J. Bojarski[2], Sijka Charakchieva-Minol[2], Beata Duszyńska[2]

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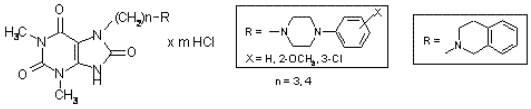
It is known that pharmacophoric arylpiperazine fragment is well recognized by 5-HT$_{1A}$, 5-HT$_{2A}$, as well as 5-HT$_7$ receptors. Although the terminal amide fragment significantly affects binding of 1-arylpiperazine derivatives with serotonin receptors, its role is not clear yet. In our earlier attempt to find new 5-HT$_{1A}$ / 5-HT$_{2A}$ receptor ligands several series of arylpiperazinylalkyl theophylline derivatives have been synthesized and their affinities for 5-HT$_{1A}$ and 5-HT$_{2A}$ were determined [1-3]. The selected compounds were potent 5-HT$_{1A}$ receptor ligands [2]. In order to explain the influence of theophylline (purine-2,6-dione) moiety on serotonin receptors affinity, purine-2,6,8-trione analogues were obtained. Additionally the arylpiperazine fragment was modified by introduction of 1,2,3,4-tetrahydroisoquinoline.



Previously obtained 1,3-dimethyl-7-(3-chloroalkyl)-8-methoxy-purine-2,6-dione in the reaction with appropriate piperazine derivatives and 1,2,3,4-tetrahydroisoquinoline yielded final products, which were isolated as a hydrochloride salts. The purity of the compounds were controlled by TLC, the structures were confirmed by spectral (^{1}H-NMR, MS, UV), and C, H, N analyses.

The 7-{4-[4-(3-chlorophenyl)-piperazin-1-yl]-butyl}-1,3-dimethyl-7,9-dihydro-3H-purine-2,6,8-trione was preliminary evaluated for its affinity to 5-HT$_{1A}$, 5-HT$_{2A}$ and 5-HT$_7$ receptors. It was found that this compound was 5-HT$_{2A}$ receptor ligand (K_i = 63 nM) with moderate 5-HT$_{1A}$ (K_i = 263 nM), and low 5-HT$_7$ (K_i = 1820 nM) receptor affinity.

References:

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3. P. Zajdel, A.J. Bojarski, H. Byrtus, G. Chłoń-Rzepa, et al.: Biomed. Chromatogr. 17, 312, 2003.

17:40 Poster I-12

ROPINIROL - GENERIC DRUG FOR PARKINSON'S DISEASE

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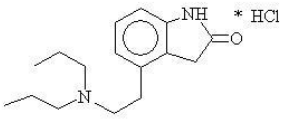
Multistep synthesis of 4-[2-(dipropylamino)-etyhl]-1,3-dihydro-2*H*-indol-2-one hydrochloride (ropinirole hydrochloride) was elaborated in Pharmaceutical Research Institute. From ten steps of synthesis - reduction of starting 2-methyl-3-nitrobenzoic acid to the corresponding alcohol, reaction of chain extension and reductive cyclization to indolone ring play significant role in the process of the formation find product.



Development of synthetic steps one to four was based on the literature data [1] and steps five to ten were carried out according to the patent procedure [2].

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17:40 Poster I-13

SYNTHESIS OF NEW 4,5-DIHYDRO-3aH-IMIDAZO[1,5-a]QUINOLINE DERIVATIVES AS 5-HT$_{1A}$ SEROTONIN RECEPTOR LIGANDS.

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Long-chain arylpiperazines (LCAPs) with an amide or imide moiety represent one of the most important classes of 5-HT$_{1A}$ receptor ligands (e.g. buspirone, tandospirone, WAY 100135, WAY 100635, NAN-190, flesinoxan). Buspirone, an

arylpiperazine derivative with high 5-HT$_{1A}$ receptor affinity, was the first agent in this class to be approved for clinical use. Most of the ligands with high affinity for the 5-HT$_{1A}$ receptor exhibit a high level of undesired affinity for the α_1-adrenergic receptor [1].

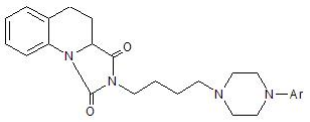
The aim of the present study was to synthesize new ligands with higher affinity and selectivity to 5-HT$_{1A}$ receptor. In this study buspirone was the key structure to which certain modifications were made, namely by introducing the imidazo[1,5-*a*]quinoline-1,3-dione residue. Other modifications were made by introducing different substituents at the piperazine ring nitrogen [2-7].

Multi-stage preparations were used to obtain 4,5-dihydro-3aH-imidazo[1,5-*a*]quinoline-1,3-dione, being the starting compound for further modification. N-alkilation of the imide group in 4,5-dihydro-3aH-imidazo[1,5-*a*]quinoline-1,3-dione followed, using 1,4-dibromobutane to yield monobromobuthyl derivative.

New targets were obtained by condensation of appropriate arylpiperazine with the above described monobutyl derivative.

The structure of the new compounds has been determined on the basis of spectra
(^{1}H and ^{13}C NMR, IR) and elemental analysis.

The new compounds will be submitted to screening test to elucidate their affinity for
5-HT$_{1A}$ receptor.

	**Ar = phenyl, 2-pyridyl, 2-pyrimidinyl, 2-fluorophenyl, 2-tolyl, 2-chlorophenyl, 2-methoxyphenyl, 3-fluormethylphenyl**

[1] Trumpp-Kallmeyer S. et al.; J. Med. Chem. 35, 3448 (1992)
[2] Kuipers W. et al.; J. Med. Chem. 40, 300 (1997)
[3] Lopez-Rodriguez M. L.; J. Med. Chem. 42, 36 (1999)
[4] Abou-Gharbia M. A.; J. Med. Chem. 42, 5077 (1999)
[5] Herold F. et al.; Farmaco 57, 959 (2002)
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17:40 Poster I-14

NEW PERHYDRO-PYRROLO[1,2-a]PYRAZINE DERIVATIVES WITH AN ARYLPIPERAZINE MOIETY AS LIGANDS FOR 5-HT$_{1A}$ RECEPTOR.

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Long-chain arylpiperazines (LCAPs) represent one of the most important classes of
5-HT$_{1A}$ receptor ligands [e.g. buspirone, tandospirone, NAN-190, flesinoxan, WAY 100135]. Most of the ligands with high affinity for the 5-HT$_{1A}$ receptor exhibit a high level of undesired affinity for α_1-adrenergic receptor [1].

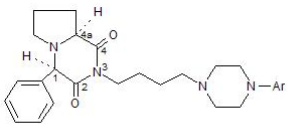
Structural modification within LCAPs occurs mainly at the two opposite ends of the molecule and can significantly influence both their affinity and functional profile at 5-HT$_{1A}$ receptors [2-4].

The aim of the work, which is a continuation of our previous study, was to synthesize new analogues of buspirone (key structure), with higher affinity and selectivity to 5-HT$_{1A}$ receptors [5-7]. A series of new 4-aryl-perhydro-pyrrolo[1,2-*a*]pyrazine derivatives with an arylpiperazine moiety have been obtained. Multi-stage preparations were used to obtain pure diasteromer (*1R,4aS*) 4-phenyl-perhydro-pyrrolo[1,2-*a*]pyrazine-2,4-dione, being the starting compound for further modification. N-alkilation of the imide group in (*1R,4aS*) 4-phenyl-perhydro-pyrrolo[1,2-*a*]pyrazine-2,4-dione, with 1,4-dibromobutane was used to yield monobromobutyl derivative.

The final new ligands were obtained by the condensation of appropriate arylpiperazine with the above described monobutyl derivative.

The structure of new compounds was confirmed by ^{1}H and ^{13}C NMR spectral data and X-ray diffraction data as well as by C, H, N analysis.

The final compounds will be submitted to screening test to elucidate the affinity for 5-HT$_{1A}$ receptors.

	**Ar = phenyl, 2-pyridyl, 2-pyrimidinyl, 2-fluorophenyl, 2-tolyl, 2-chlorophenyl, 2-methoxyphenyl, 3-trifluormethylphenyl**

[1] Trumpp-Kallmeyer S. et al.; J. Med. Chem. 35, 3448 (1992)
[2] Perrone R. et al.; Bioorg. Med. Chem. 8, 873 (2000)
[3] Caliendo G. et al.; Bioorg. Med. Chem. 8, 533 (2000)
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[5] Herold F. et al.; Farmaco 57, 959 (2002)
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[7] Herold F. et al.; Eur. J. Med. Chem. in press 2006

17:40 Poster I-15

SYNTHESIS OF NEW DERIVATIVES OF THIAZOLO[4,5-d]PYRIMIDINE AS POTENTIAL ANTITUMOR AGENTS

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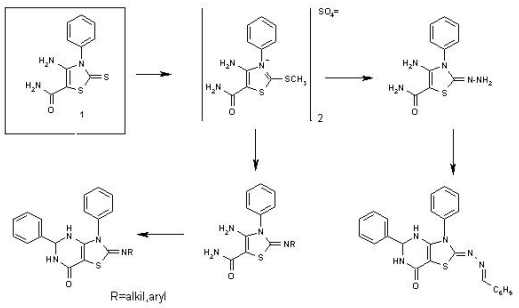
Fused heterocyclic derivatives with thiazole moiety are very prospective objects in modern drug discovery. Isomer [4,5-d] of thiazolopyrimidines can be considered as a 7-thia-analogue of the naturally occurring purine bases, adenine and guanine and have been reported to possess broad spectrum of activities . Futhermore, based on the fact that many Schiff's bases and hydrazones exert potential anticancer acitivity we we planned to synthesize a series of novel thiazolo[4,5-d]-pyrimidine derivatives as an interesting structure, especially as potential antitumor agents. The presence of fluorine often increases the lipid solubility and therapeutic efficiacy of a drug. We introduced fluorophenyl moiety into 2 and 7 positions of the prepared compunds. We described methods developed to synthesize and modify the structure of the thiazolo[4,5-d]pyrimidine ring system with hope of discovering biologically active, selective and less toxic compounds. The synthetic strategies adopted to obtain the target compounds are depicted in Schemes 1 and 2.

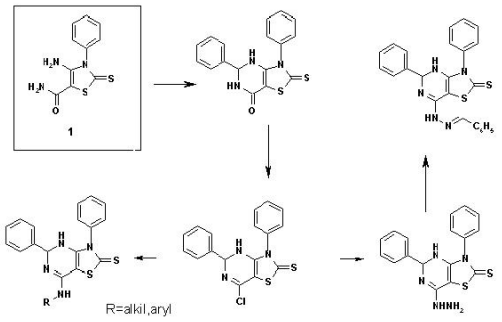
The necessary aminothiazole compound **1** was prepared by reacting phenyl isothiocyanate with powdered sulphur and methyl cyanoacetate in the presence of triethylamine. Treatment of aminothiazole with dimethylsulphate for the replacment of the 2-tioxo group with an active methylene moiety followed by reaction of the produced 2-methylthiothiazolium salt with hydrazine hydrate gave the 2-hydrazonoderivative. This compound was cyclized to the thiazolo[4,5-d]pyrimidine using a benzaldehyde. The intermediate 2-methylthiothiazolium salt with the various amines, especially 4-fluoroaniline, gave 2-iminoderivatives which were cyclized to bicyclic system under the same conditions as described above.

Scheme 1



The second parent thiazolo[4,5-d]pyrimidine substituted at position 7 was prepared by cyclization of aminothiazole with benzaldehyde, but the reaction required the presence of a basic catalyst. Chlorination of products with phosphorus oxychloride gave 7-chlorothiazolo[4,5-d]pyrimidine which upon treatment with amines gave the 7-aminoderivatives. The replactment of 7-chloro with 7-hydrazino group by using hydrazine hydrate gave 7-hydrazinothiazolo[4,5-d]pyrimidine derivative. Condensation of the latter compound with benzaldehyde yielded 7-benzylidenehydrazino derivative.

Scheme 2



The structures of prepared compounds were confirmed by elemental analysis, IR, ^{1}HNMR. They will be evaluated for their anticancer activity in the NCI screening program.

17:40 Poster I-16

ENHANCED STABILITY OF HIGHLY PURE PHAGES WITH BLOCK COPOLYMER OF ETHYLENE OXIDE AND PROPYLENE OXIDE

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Poloxamers and meroxapols were introduced to the market in the 1950s by the BASF Corp. under the names Pluronic S and Pluronic R. Poloxamers have found wide application in technology, medicine and cosmetics industry.

Bacteriophages used in therapy must be free of impurities that might cause adverse reactions *e.g.* endotoxins. The appropriate phage strains can be obtained and purified, for example, by the methods described in the Polish patent applications of the Institute of Immunology and Experimental Therapy PAN in Wrocław: P.348740; P 354822; P.355355; PCT/ PL02/000053.

The pyrogen free bacteriophages (against Gram negative bacteria) were stored at 4 ^{o}C over periods of five and ten months, during which they maintained 100% of their initial lytic activity. Bacteriophages stored in culture broth at +4 ^{o}C lost their lytic activity insignificantly. Bacteriophages stored in a physiological saline solution lost their activity significantly after five months.

The highly purified bacteriophage preparation suffer from lack of stability. Stabilization is therefore an important problem, which we solve by addition polymers (which are accepted for use on humans).

17:40 Poster I-17

SYNTHESIS AND BIOLOGICAL ACTIVITY OF SOME 1, 5, 5-TRIMETHYL-3-CYCLOHEPTENE DERIVATIVES

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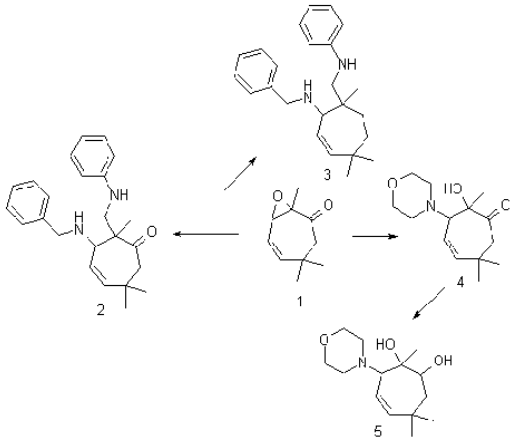
During aminolysis reaction of 2, 3-epoxy-2,6,6,-trimethyl-4-cyclohepten-1-one some new hydroksyalkylo and dialkyloamino derivatives of 1, 5, 5-trimethyl-3-cycloheptene were obtained. The screening investigations of these compounds showed their various pharmacological activities. Particularly interesting are antiulcer properties of comound **3** and soporific activity of compound **5**.



17:40 Poster I-18

SYNTHESIS AND BIOLOGICAL ACTIVITY OF PYRIMIDINE 5-TERPENO DERIVATIVES

Jerzy Cieplik[1], Katarzyna Paulus[1], Zbigniew Rykowski[1], Olaf Gubrynowicz[2], Janusz Pluta[2]

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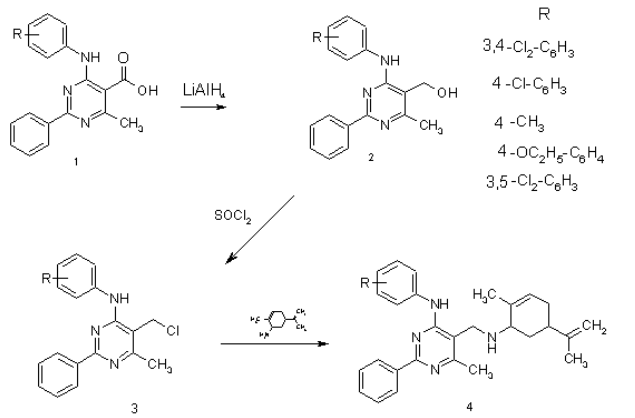
The parent substance in our studies was 2-phenyl-4-phenylamino-5-hydroxymethyl-6-methylpyrimidi ne (1) which, by treating with $SOCl_2$,was converted into pyrimidine 5-chloroderivative (2). The chloroderivative obtained was then condensed with terpene amine. The pyrimidine terpenoderivatives were tested on seven selected bacterial strains*: Bacillus subtilis, Escherichia coli, Proteus vulgaris, Pseudomonas aeruginosa, Enterococcus faecalis, Staphylococcus epidermidis, Staphylococcus aureus, Klebsiella pneumoniae.* Antifungal tests were also carried out on *Candida albicans*. Some interesting microbiological results were obtained, both antibacterial and antifungal.



17:40 Poster I-19

A NEW, EFFICIENT AND ECONOMIC METHOD FOR PREPARATION OF PRAMIPEXOLE.

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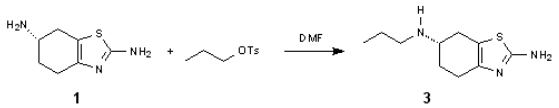
Pramipexole is a novel nonergot dopamine agonist which has high selectivity for interacting with dopamine D_2 receptors. It is effective in early Parkinson,s disease as monotherapy and as adjunctive therapy with L-dopa in advanced stages of the disease.

Known, two-steps method for preparation of pramipexole (3) is based on acylation reaction of diamine 1 with propionic anhydride. The obtained amide 2 is subsequently reduced using borane to give final product 3 with 65% yield.

Now, we present novel, more economic and safe procedure for obtaining pramipexole. Our one-step method requires only alkylation of 1 using n-propyl tosylate. Dangerous reduction with borane is eliminated and the final compound is obtained with similar yield as in a previous method.



17:40 Poster I-20

^{1}H AND ^{13}C NMR DATA FOR INDOLO[2,3-b]QUINOLINES - AMINOGLYCOSIDE HYBRIDS, A NOVEL POTENT ANTICANCER DRUG FAMILY .

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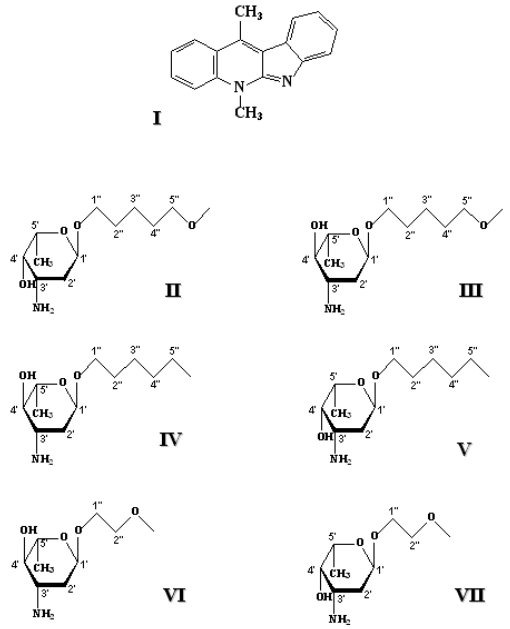
The family of indolo[2,3-*b*]quinolines exhibit significant cytotoxic activity. These tetracyclic, nitrogen heterocycles are synthetically obtained analogs of naturally occuring neocryptolepine, that is used for treatment of infectious diseases. 5,11-Dimethyl-5*H*-indolo[2,3-*b*]quinoline (DIMIQ) (**I**), displaying a structure remarkably close to that of antineoplastic alkaloid ellipticine, was shown to have significant antineoplastic effects on three of experimental tumors. Moreover the derivatives belonging to the *5H*- and *6H*-indoloquinolinium salts demonstrated significant activity against procaryotic and eucariotic organisms and were cytotoxic *in vitro*. The active compounds increase DNA denaturation temperature and stimulate the formation of calf thymus topoisomerase II mediated DNA cleavage.

We focused our work on construction an effective anticancer drug by modification of the parent 5H- and 6H-indoloquinoline system. The modification should lead to derivatives combining DNA intercalating and topoisomerase II inhibiting activity with more pronounced affinity to specific sites in polynucleotide chain (DNA sequence reading drug). Such a goal might be achieved in hybrid molecules composed of two parts, which have a defined functions: sequence specificity associated with the groove binding moiety and nuclear targeting promoted by the intercalating indoloquinoline system.

We synthesized aminoglycoside derivatives of indoloquinoline as novel hybrid compounds with potentially pronounced antitumor activity. New molecules consist of intercalating indoloquinoline skeleton and aminocarbohydrate with alkyloxy spacer (**II-VII)**.

The complete assignments of title compounds was carried out by extensive use of 1D and 2D NMR techniques (^{1}H, ^{13}C, GCOSY, GHSQC, GHMBC).



17:40 Poster I-21

IDENTIFICATION AND DETERMINATION OF ANGIOTENSIN II RECEPTOR ANTAGONISTS WITH DENSITOMETRIC METHOD

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Hypertension is one of the most widespread diseases of cardiovascular system. The angiotensin II receptor antagonists (ARA) are effective in all stages of hypertension.

The aim of this study was elaboration of a simple and sensitive chromatographic method allowing to identify and determine the compounds from the ARA group in pharmaceutical preparations.

DRUGS AND METHODS

The identification of ARA: potassium losartan, irbesartan, candesartan cilexetil, telmisartan, valsartan and eprosartan methanesulphonate with thin-layer chromatography have been elaborated.

Two optimal systems have been chosen for experiments:

System 1: HPTLC F_{254} chromatographic plates and the mobile phase: methylene

chloride - ethyl acetate - ethanol - glacial acetic acid - water

(45:40:5:1:0.5)

System 2: RP-Diphenyl chromatographic plates (Wattman) and the mobile phase:

acetonitrile - methanol - 0.1 mol/l ammonium acetate - 25% ammonia

(30:20:50:0.5)

System 1 were used to elaborate the densitometric method for the determination of the studied compounds in pharmaceutical preparations.

The quantitation limit was 0.5mg for potassium losartan, irbesartan, candesartan cilexetil and valsartan, and 1mg for telmisartan and eprosartan methanesulphonate. The detection limit was 0.01µg for candesartan cilexitil, 0.02µg for irbesartan, telmisartan and valsartan, 0.05µg for eprosartan methanosulphonate and potassium losartan.

The recovery was satisfactory; the mean per cent of recovery were close to 100 and the results were within 98-102%.

SUMMARY

Determination of the content of the studied angiotensin receptor antagonists in the pharmaceutical preparations: Lorista tablets, Aprovel tablets, Blopress tablets, Micardis tablets, Diovan tablets and Teveten tablets was performed with densitometric method. Statistical data obtained for the elaborated method show satisfactory precision and accuracy. The elaborated densitometric methods are simple, less expensive compared to the HPLC method and can be applied in laboratory practice.

17:40 Poster I-22

SYNTHESIS AND ANTICONVULSANT ACTIVITY OF 4-ARYLPIPERAZINYL METHYL DERIVATIVES OF 5-CYCLOPROPYL-5-PHENYL HYDANTOINS

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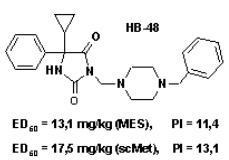
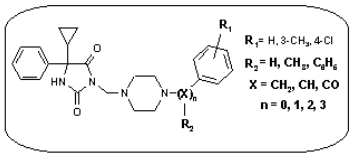
The classical anticonvulsant **Phenytoin** (DPH, 5,5-diphenylhydantoin) is a effective drug in the treatment of *grand mal* epilepsy. During our studies on new anticonvulsant agents we found that some derivatives of 5-cyclopropyl-5-phenylhydantoin (CPPH) of Mannich base exhibited interesting activity. In this series of compounds the most potent was 3-[(4-benzylpiperazin-1-yl)-methyl]-5-cyclopropyl-5-phenylhydantoin (HB-48). It was protective in the maximal electroshok seizure (MES) test in rats with an oral ED_{50} of 13,1 mg/kg, and in the subcutaneous pentylenetetrazole test (scMet) ED_{50} of 17,5 mg/kg. Protective Index, (PI; TD_{50}/ED_{50}) 11,4 MES and 8,5 scMet respectively.





The principal objective of the present investigation was the preparation of analogs of HB-48. Starting from CPPH, formaldehyde and corresponding piperazine derivatives, a series of new compounds - Mannich base was synthesized. Lipophilicity of the all synthesized derivatives CPPH, calculated by a computer method [1] was moderate and suggested their good biological barrier penetration.

Anticonvulsant activity of the obtained compounds was tested in vivo in mice and rats in Anticonvulsant Screening Project (ASP) [2]. On the basis of the obtained results all the compounds were classified to 1st and 2nd class of ASP.

Introduction of 3-CH_3 group on the aromatic ring of benzylpiperazine led to an increase of activity in MES-test and decrease neurotoxicity. Substitution of 4-Cl decrease activity in MES test and increase neurotoxicity. Replacement of the benzylpiperazine by benzhydrylpiperazine led to decrease activity in MES-test and neurotoxicity. The study has shown that the terminal benzylpiperazine group has determined anticonvulsant activity.

1. PALLAS (version for Windows 1.2) distributed by Compu Drug Chemistry Ltd 1995

2. Kupferberg, H.J. Epilepsia, 30, (suppl.), 51-56, 1989

This work was partially supported by CMUJ Research Programme (Wł 309/P/F)

Acknowlegements: We thank Dr J. Stables from National Institute of Neurological Disorders and Stroke, Bethesda, Maryland USA for anticonvulsant testing in the frame of ASP.

17:40 Poster I-23

SYNTHESIS AND PHARMACOLOGICAL ACTIVITY OF NEW 5,5-DISUBSTITUTED HYDANTOINS

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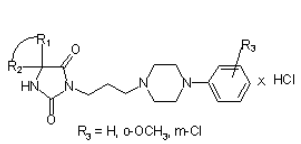
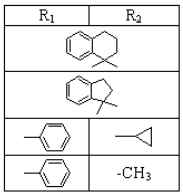
Arylpiperazine is a core fragment of many bioactive compounds exhibiting a variety of pharmacological effects. One of the extensively studied group of arylpiperazine derivatives, called long-chain arylpiperazines (LCAPs), has been found as serotonin 5-HT_{1A} and 5-HT_{2A} receptor ligands [1].

In our earlier studies, it was shown that majority of obtained compounds containing 5,5-dialkyl-, or 5-spirohydantoin connected to phenylpiperazinealkyl moiety exhibited high 5-HT_{1A} and 5-HT_{2A} receptor affinity (K_i<50 nM) and represented various profile of pharmacological activity [2, 3, 4]. Based on this data, we have synthesized new α-tetralonohydantoin, α-indanohydantoin, 5-cyclopropyl-5-phenyl-hydantoin and 5-methyl-5-phenyl-hydantoin derivatives.





The newly synthesized compounds, as soluble in water hydrochlorides, were tested *in vitro* for their 5-HT_{1A} and 5-HT_{2A} receptor affinities. Majority of them are potent 5-HT_{1A} or 5-HT_{2A} receptor ligands (K_i<50 nM). Additionally, for selected compounds affinity to dopaminergic D2 receptors were checked. Pharmacological *in vivo* studies directing to 5-HT_{1A} or 5-HT_{2A} receptor activity profiles were also assayed.

This study is supported by Polish Ministry of Scientific Research and Information Technology, grant No 2P 05F04226.

Anna Czopek is a scholar of the project which is co-financed from the European Social Fund and national budget in the frame of The Integrated Regional Operational Programme.

1.Lopez-Rodriguez M. L., Ayala D., Benhamu B., Morcillo M. J., Viso A.: Curr. Med. Chem., 9, 443 - 469, 2002

2.H.Byrtus, M. Pawłowski, S. Charakchieva-Minol, B. Duszyńska, M. J. Mokrosz, J. L. Mokrosz, A. Zejc: Arch. Pharm. Pharm. Med. Chem., 329, 283, 1996

3.H.Byrtus, M. Pawłowski, B. Duszyńska, A. Wesołowska, E. Chojnacka-Wójcik, A. J. Bojarski: Pol. J. Pharmacol., 53, 395-401, 2001

4.H.Byrtus, M. Pawłowski, A. Czopek, A. J. Bojarski, B. Duszyńska, G. Nowak, A. Kłodzińska, E. Tatarczyńska, A. Wesołowska, E. Chojnacka-Wójcik: Eur. J. Med. Chem., Eur. J. Med. Chem., 40, 820 - 829, 2005

17:40 Poster I-24

STEROIDAL SAPONINS FROM CONVALLARIA MAJALIS

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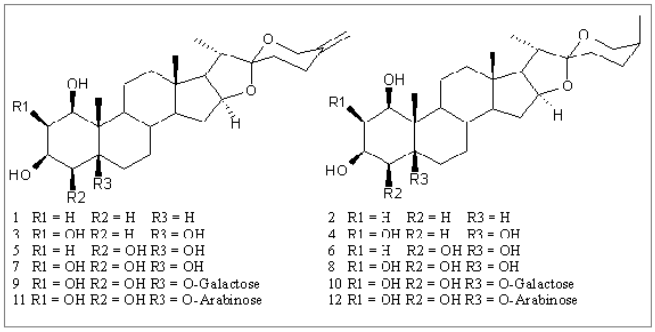
Saponins are glycosides, widely distributed in the plant kingdom, consisting of a sugar moiety linked to a triterpene or steroid aglycone (sapogenin). They are constituents of many plant drugs and folk medicines, especially from the Orient.

New spirostanol sapogenins and saponins with several OH groups were isolated from *C. majalis* from the family *Liliaceae*. In Poland, *Herba C. majalis* is used as a medicinal material due to cardiac glycosides content. Recently, several new tetra- and penta-hydroxylated sapogenins and saponins have been isolated (from the roots and rhizomes), and identified using 1D and 2D NMR techniques. These new saponins were tested for antiangiogenic activity and the convallamaroside was proved to be effective [1]. Biological properties of polyhydroxy compounds are related to their ability to form hydrogen bonds with water molecules.

The octanol-water partition coefficient (log P) was calculated for model compounds with different number and localization of OH groups in A-ring using semi-empirical method implemented in HyperChem 7.0 package.



Intramolecular and intermolecular hydrogen bonds formed by three, four or five OH groups as well as their influence on low-energy conformation have been analyzed.

1. Nartowska J., Sommer E., Pastewka K., Sommer S., Skopińska-Różewska E. Anti- angiogenic activity of convallamaroside, the steroidal saponin isolated from the rhizomes and roots of *C.majalis* L. Acta Polon.Pharm. Drug Res**.** 61 (2004)279-282

17:40 Poster I-25

COMPARISON OF THE STABILITY OF 5-AMINOLEVULINIC ACID AND HIS ESTERS

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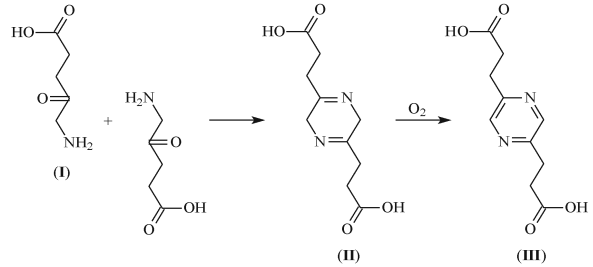
5-Aminolevulinic acid (ALA) **I** is commonly used in photodynamic therapy (PDT) and photodiagnosis (PD). Applied exogenously it is selectively metabolised in neoplastic tissues to yield actual photosensitizer: protoporphyrin IX (PpIX). The compound under light irradiation in reaction with molecular oxygen dissolved in tissue environment gives singlet oxygen responsible for necrosis of tumour tissues.

5-Aminolevulinic acid is used for therapy of skin diseases: Bowen's disease, basal cell carcinoma (BCC), acne; is also used for therapy and diagnosis of bladder, lungs and stomach tumours, in gyneacology and laryngology.

ALA has limited stability. The compoud in aqueous solution at physiological pH condenses to yield dihydropyrazine (**II**) and pyrazine (**III**) derivatives. The rate of the condensation is dependent on concentration of compound, temperature and pH value. Scheme of the reacion is shown below.



The aim of the work was comparison of the stability of ALA and chosen esters: methyl, ethyl, butyl and hexyl at different pH values (5,0; 5,5; 6,3 and 7,2). Concentration of 5-aminolevulinic acid and esters at time was determined by HPLC method.

17:40 Poster I-26

THE RESEARCH ON BIOLOGICAL ACTIVITY OF ANTOXID. PART I. THE INFLUENCE ON FERRIC REDUCING ANTIOXIDANT POWER (FRAP)

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1. *Wroclaw Medical University, Department of Toxicology, ul. Traugutta 57/59, Wrocław 50-417, Poland* **2.** *Medical University, Department of Pharmaceutical Botany, J. Kochanowskiego 10, Wrocław 51-601, Poland*

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INTRODUCTION

Antoxid is a new herbal free radical scavenger obtained from Radix Scutellariae baicalensis Georgi. The water-alkoholic extract of Radix obtained according to described procedure is very rich in flavonoids with baicaline as a main one (72% approximately). Antoxid is the main component of BAICADENT gel used in stomatology. There are several parameters reflecting the antioxidative properties of agents in blood. One of them is FRAP (Ferric Reducing Ability of Plasma). However the extract from Scutellariae baicalensis, standarised on baicaline, was a subject of various investigations, particularly evaluating the antioxidative properties, there were no reports on its influence on FRAP.

The aim of study was to determine the influence of Antoxid on ferric reducing ability of plasma (FRAP) and the comparison of the results with vitamin C influence on FRAP.

EXPERIMENTAL

The material was blood plasma from patients of Surgery Dept. in Medical University Clinic. The blood was taken on anticoagulant. The patients did not receive any drugs. FRAP was evaluated by the measurements of Fe^{+2}/TPTZ-complex by colorimetric method with spectrophotometer. The Antoxid was dissolved in methanol/water and used in concentration as follow: 5,0; 10,0; 20,0; 30,0 and 50,0 μg/ml.The results were evaluated with t-Student test.

RESULTS AND DISCUSSION

The comparison of Antoxid ferric reducing capacity with plasma ferric reducing capacity showed in general lower activity of Antoxid than plasma. However the date of FRAP for plasma and Antoxid in concentration 20-50 μg/ml were close to each other. The Antoxid in lower concentration (5-10 μg/ml) had much lower ferric reducing ability (FRAP) than plasma.

The examination of Antoxid influence on plasma ferric reducing capacity showed strong antioxidative ability of extract. Antoxid in concentration 10-50 μg/ml statistically significant ($p=0.000016$) increased FRAP. The strongest effect was obtained with 30 μg/ml concentration of Antoxid.

The antioxidative properties of various flavonoids are well known. They are good chelators of metals ions, particularly iron or cuprum metals, Fenton reaction catalyzer. The antioxidative properties of baicaline are connected with xanthine oxidase inhibition.

Flavonoids influence also free radical generation by chelating transition metal ions, which catalyze the reaction. It is reported that high flavonoid intake shows significant effect on the liver, but not on the brain. It is probably because the liver is the main metabolic organ of flavonoids. Many flavonoids have hepato-protective effect when the liver is under pathological condition.

Recently Firuzi examined *in vitro* the influence of various flavonoids on FRAP, using the artificial model of blood (TPTZ/$FeCl_3$). The most active was baicaleine. It looks that it plays more important role in FRAP influence than flavonoid baicaline. So it seems that the Antoxid's ferric reducing ability is determined mainly by baicaleine with the participation of baicaline.

CONCLUSIONS

1. Antoxid in conc.> 5 μg/ml increases ferric reducing ability of human plasma.

2. Antoxid in conc. 30 μg/ml is more active than vitamin C in influence on FRAP.

17:40 Poster I-27

THE RESEARCH ON BIOLOGICAL ACTIVITY OF ANTOXID. PART III. THE INFLUENCE ON CHEMICALLY INDUCED LIPID PEROXIDATION.

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1. *Wroclaw Medical University, Department of Toxicology, ul. Traugutta 57/59, Wrocław 50-417, Poland* **2.** *Medical University, Department of Pharmaceutical Botany, J. Kochanowskiego 10, Wrocław 51-601, Poland*

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INTRODUCTION

There are many reports on antioxidative properties of flavonoids presented in Radix Scutellariae baicalensis Georgi. The most active are baicaline and baicaleine. There are also some investigation on antioxidative properties of Antoxid (AX), the water-alcoholic extract obtained from Radix Scutellaria baicalensis in crystalline form, standardized on baicaline. There are no reports on Antoxid utility in oxidative stress caused by chemicals e.g. environmental aromatic hydrocar-

bons.

The aim of study was the investigation of AX influence on lipid peroxidation stimulated by the chemicals: t-butyl peroxide or xylene. The results were compared with vitamin C activity.

EXPERIMENTAL

The study was performed on *in vitro* model, human placental mitochondria. The mitochondria were isolated by Radi method from mature placenta obtained after physiological delivery from Medical University Clinic. The proteins in mitochondria were measured by Lowry method. The AXwas dissolved in mitochondrial buffer (TRIS-HCl - pH-7,4) and used in following concentrations: 1,5; 3,0; 6,0; 12,0 and 30 µg/ml. The lipid peroxidation was evaluated by malondialdehyde level measured spectrophotometrically with thiobarbituric acid method (TBARS).

RESULTS AND DISCUSSION

At first the antioxidative properties of AX were examined at mitochondria stimulated with 1% t-BOOH. It was observed that AX inhibits lipid peroxidation in three from five concentration - in dose 6,0; 12,0 and 30 µg/ml but not in dose 1,5 and 3,0 µg/ml. It means that AX in doses higher than 6 µg/ml is able to reduce such free radical process as lipid peroxidation leading to MDA level decrease. The decrease of MDA level was dose dependent ($p<0,001$).

The aim of study was to examine the utility of AX in oxidative stress stimulated by environmental chemicals like aromatic hydrocarbons, for example xylene. The next experiment was performed in order to explain whether simultaneous exposition to xylene and AX doesn't give harmful interaction in free radicals process. The results show that simultaneous mitochondria treatment with xylene in conc. 17,64 µg/ml and AX in conc. 6,0 or 12,0 µg/ml leads to statistically significant ($p<0,001$) decrease in MDA level in comparison to the control without AX. The obtained results points at high effectiveness of AX in reduction of lipid peroxidation stimulated by aromatic hydrocarbon-xylene.

It was also interesting to explain whether the effectiveness of AX depends on time of toxic action. It means whether AX is more useful as preventing agent (giving before the exposition to hydrocarbon) or repairing agent (giving after the exposition to hydrocarbon). In order to examine the repairing effect, AX in conc. 6,0 or 12,0 µg/ml was added to mitochondria incubated with xylene in dose 17,64 µg/ml 30 min. after the exposition. The results show that only higher dose (12,0 µg/ml) was able to reduce MDA level significantly ($p< 0,001$) (ryc. 3). Different results were obtained when AX was given 30 min before exposition to xylene. Preincubation of mitochondria with AX in conc. 6,0 or 12,0 µg/ml successfully prevents MDA level increase.

CONCLUSIONS

1. Antoxid in conc. >6 µg/ml significantly inhibited lipid peroxidation caused by t-BOOH or xylene.

2. The effectiveness of Antoxid depends on time of exposition.

3. Antoxid given before or simultaneously with xylene is more active than given after xylene.

4. Antoxid has preventing and repairing activity, but acts stronger as preventive agent.

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THE COMPARATIVE STUDIES ON ADENOSINE RECEPTORS AFFINITY OF PYRIMIDO AND PYRAZINO PURINEDIONES

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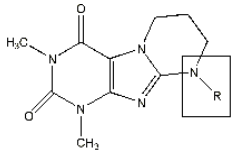
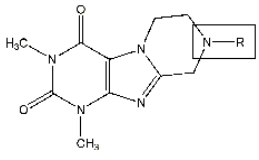
To date, four adenosine receptor AR subtypes: A_1, A_{2A}, A_{2B} and A_3 have been cloned and pharmacologically characterized.

Searching for new selective ligands we have synthesized tricyclic purinediones with oxygen or nitrogen containing annelated ring (1)(2).

As a continuation of this research two groups of compounds with N-alkyl (cycloalkyl) pyrimido and pyrazino purinediones have been synthesized and their spatial and physicochemical properties examined by X-ray structure determination and theoretical calculation.





To compare biological activity their affinity toward A_1 and A_{2A} AR was evaluated.

The location of N in fused ring determinated AR affinity and selectivity. Pyrimido ring is beneficial for A_{2A} activity.

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Supported in part by Polish State Committee for Scientific Research (Grant № 2 P05F 022 26).

17:40 Poster I-29

SYNTHESIS, CRYSTAL STRUCTURE AND BIOLOGICAL ACTIVITY OF COPPER (II) COMPLEXES WITH CHELATING BIDENTATE 2-SUBSTITUTED BENZIMIDAZOLE LIGANDS.

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Complexes based on copper have demonstrated various biological activities and are among the most potent antivirial, antitumor and antiinflammatory agents. Anticancer copper (II) complexes synthesized recently appeared to act as either the inducers of apoptosis on human tumor cell lines or agents that alter the cell cycle and decrease the telomerase activity. Moreover, chemical species of this type possess Cu,Zn-superoxide dysmutase (SOD) mimicking properties.

The aim of this study was to synthesize a new series of copper complexes of ligands incorporating bidentate a-diimino moiety, such as 2-(imidazolin-2-yl)- (**8**, **10**), 2-(tetrahydro-pirymidin-2-yl)- (**9**), 2-(oxazolin-2-yl)- (**11**), 2-(oxazin-2-yl)- (**12**, **13**) and 2-(pirazin-2-yl)- (**14**) benzimidazoles.

Structures of the complexes obtained were confirmed by spectral (IR) data and C,H,N analysis. Important structural features were determined by X-ray crystallographic studies of compounds **12**, **13** and **14** (Figure).

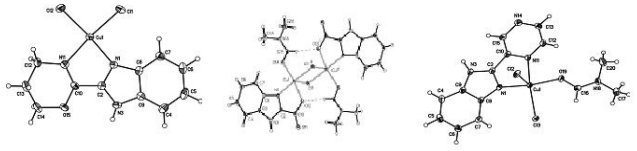

Figure. ORTEP drawings of complexes **12**, **13** and **14**.

2-(4,5-Dihydro-1*H*-imidazol-2-yl)-1*H*-benzimidazole $CuCl_2$ (complex **8**) showed a very potent Cu,Zn-SOD activity in vitro with IC_{50} of 0.09 μM, comparable to those described in the literature for best low molecular weight CuZnSOD mimics.

The in vitro biological tests against seven human cancer cell lines showed that the most active 2-(4,5-Dihydro-1*H*-imidazol-2-yl)-5-nitro-1*H*-benzimidazole $CuCl_2$ (complex **10**) possessed antitumor properties with IC_{50} of 4.76 - 10.84 μM, depending on the cell line.

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INFLUENCE OF SODIUM BUTYRATE ON ANTIOXIDATIVE ENZYMES ACTIVITY IN CACO-2 CELL LINES.

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Butyric acid, the product of bacterial fermentation of complex carbohydrates in the large intestine, is a preferred energy source for normal colonocytes and stimulator of their proliferation in vivo. In contrast, butyrate has been shown to inhibit proliferation and induce apoptosis in a number of colorectal tumor cell lines. Among the possible mechanisms by which butyrate may exert anti-carcinogenic effects, its antioxidant activity has recently been suggested. Reactive oxygen species (ROS) are involved in many physiological and pathological processes, such as cell proliferation control, apoptosis and gene expression induction. An overproduction of ROS or their limited inactivation may cause cellular damage resulting in genomic changes and carcinogenesis process induction.

The aim of this study was to evaluate the impact of sodium butyrate (NaB) on antioxidative enzymes glutathione peroxidase (GPx) and superoxide dismutase (SOD) activity in colon cancer cell line Caco-2. Cells ($1x10^6$/dish) were cultured in Minimum Essential Medium supplemented with 10% fetal bovine serum, 100 units/ml penicillin, 100 μg/ml streptomycin, and 10 mM HEPES at 37 ^oC in a humidified atmosphere with 5% CO_2. The cells were incubated for 3 days to adhere to the plates, and then they were exposed for 4 days to 1mM and 10 mM NaB. Afterwards, the adherent cells were washed with phosphate-buffered saline, harvested and sonicated. Cell supernatants were used for GPx and SOD activity assay with the use of Ransod and Ransel kits (RANDOX). Morphological analysis of cell nuclei was evaluated based on their characteristic changes visualized after staining with 5 μg/ml acridine orange and observed under fluorescence microscope (Olympus BX-60). Cellular differentiation of NaB-treated cells was evaluated by measuring alkaline phosphatase (AP)

activity.

The results of this study showed that butyrate changed GPx activity in Caco-2 cells. The highest activity of GPx was observed in colonocytes incubated with 1 mM butyrate.

The highest SOD and AP activities were observed in Caco-2 cells treated with 10 mM butyrate.

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THE STUDY OF ROTAMERS IN PRILS AND PRILATES BY HPLC AND NMR METHODS

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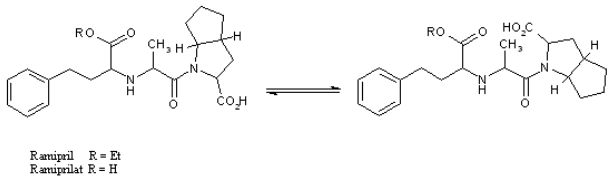
A very important class of antihypertensive drugs are angiotensin converting enzyme (ACE) inhibitors. They are generally known as the prils. Most of ACE inhibitors are administrated into the body as prodrugs; chemically they are monoesters of diacids which metabolize to real enzyme inhibitors - diacids, which are called prilates.

It is well known from NMR studies that both, priles and prilates, as a substituted ami-des, can exist in solution as *cis* and *trans* rotamers and may interconvert around the amide bond (see for example, rotamers of ramipril and ramiprilat).



Ramipril R = Et
Ramiprilat R = H

Recently, it has been surprisingly found that during HPLC analysis the *cis-trans* interconversion can also proceed, which influences the peak shape or even causes peak splitting.

In our laboratory the chromatographic behaviour of some ACE inhibitors (prils and prilates) was studied. We worked out mobile phases in which *cis-trans* rotamers may be observable. We also studied how column temperature influences rotamers separation and the rate of isomerization. Simultaneously, preliminary NMR studies were carried out for solving the structure of rotamers and establishing which rotational isomer dominates in solution.

These investigations are crucial for proper determination of studied ACE inhibitors in mixture. The *cis-trans* isomerization process observed under HPLC condition can lead to misinterpretation of the identity of a peak, because peak broadening or even splitting can be easily attributed to an impurity. This should be taken into account when describing ACE inhibitors and their metabolism products in Pharmacopoeia.

17:40 Poster I-32

SYNTHESIS AND ANTITUBERCULOUS ACTIVITY OF NEW 3,5-DISUBSTITUTED-1,2,4-OXADIAZOLES

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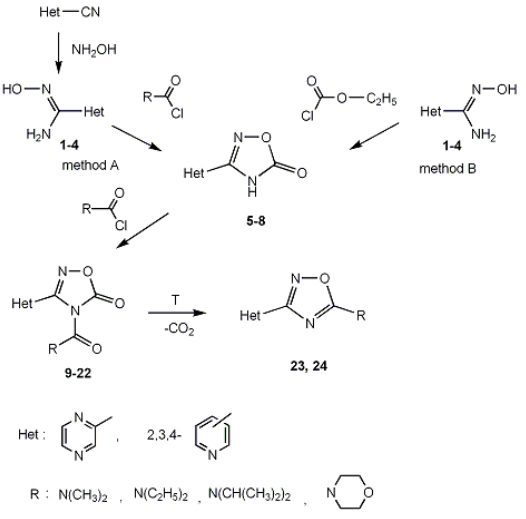
The 1,2,4-oxadiazole is a heterocycle which has seen utility in producing bioactive compounds extensively used in many research programs. Among others some 1,2,4-oxadiazole derivatives are known as antibacterial agents active against *Mycobacterium tuberculosis*.[1]

Our previous studies on antituberculosis agents resulted in synthesis of 3-pyrazine-1,2,4-oxadiazoles with high tuberculostatic activity even against resistant strains. [2] That fact prompted us to synthesize a series of new disubstituted 1,2,4-oxadiazoles bearing pyrazine or pyridine ring in 3-position and amine or amide functional group in 5-position. The amides were obtained from appropriate amidoximes in reaction with carbamoyl chlorides. Some of the amides have undergone thermal decarboxylation to tertiary amines. All reactions were performed according to the following scheme:



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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17:40 Poster I-33

BIOTRANSFORMATION OF PRAZIQUANTEL BY HUMAN CYTOCHROME P450 3A4 (CYP 3A4)

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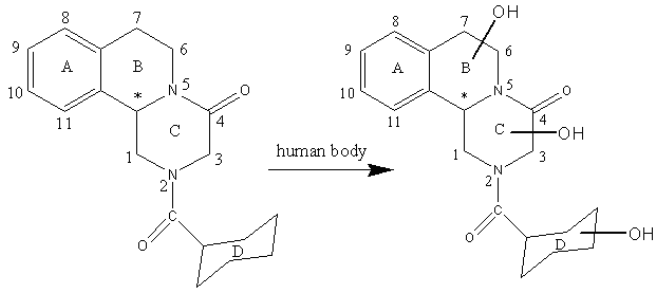
Praziquantel (PZQ) is the drug of choice for the treatment of human schistosomiasis. It is estimated that about 200 million people in the world are currently affected by this tropical disease. Now it is also used in malaria treatment. The usefulness of PZQ as antimalarial drug is important because of rapid development of resistance to usually applied drugs. PZQ undergoes extensive metabolism in human body, mainly in liver by two cytochrome P-450 isoenzymes 2B1 and 3A [1]. As the result of these biotransformations numerous mono- and dihydroxylated derivatives in B, C and D ring are formed. One metabolite has been fully identified and described, it is *cis*- and *trans*- 4-hydroxypraziquantel [2,3]. Up to now there were created many different in vitro and in vivo models of PZQ biotransformations[4]. In our research we have created in vitro model of PZQ biotransformation by using of human cytochrome P-450 3A4 expressed in *Escherichia coli*[5] and *Saccharomyces cerevisiae*[6].



In the first experiment was used human cytochrome P-450 3A4 from *Escherichia coli* (isolated on Ni-NTA-column). In the second experiment we used microsomes isolated from Saccharomyces cerevisiae containing coexpressed human CYP 3A4, human CYP-reductase and human cytochrome b5. The reactions were monitored by HPLC and MS.

The research suppotred by Polish State Committee for Scientific Research, grant no. 2 P05F 005 28

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17:40 Poster I-34

WHOLE CELLS OF LACTOBACILLUS KEFIRI AS BIOCATALYST FOR THE SYNTHESIS OF ENANTIOPURE 1-(5R-HYDROXYHEXYL)-3,7-DIMETYL-XANTHINE

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Recent developments in biology, biochemistry and medicine have demonstrated that in most chiral compounds only one enantiomer is active. Subsequently, pharmaceutical and cosmetic industries, as well as food and agrochemical industries, have developed great interest in the stereoselective production of asymmetric compounds, as well as new systems that are able to perform it. Lisofylline (1-(5*R*-hydroxyhexyl)-3,7-dimethyl xanthine (LSF)), represents a new methylxanthine which is a stereospecific metabolite of pentoxifylline (1-(5-oxohexyl)-3,7-dimethyl xanthine (PTX)). LSF is an agent with anti-inflammatory properties, that was originally developed to reduce cellular damage due ischemic reperfusion, hypoxia or autoimmune diseases. Only the (*R*) stereoisomer is biologically active. As LSF is not commercially available in our country we had to obtain it by new methods [1,2]. In this study we reported biotransformation of PTX to LSF in the presence of whole cells of *Lactobacillus kefiri* (DMS 20587). These microorganisms possess NAD(P)H-dependent alcohol dehydrogenase (LKADH) (E.C.1.1.1.1.) which displays a broad substrate range and high stereoselectivity, therefore could convert prochiral ketone (PTX) by the asymmetric reduction to the chiral alcohol (LSF).

Lactobacillus kefiri DMS 20587 cells were grown in a MRS culture medium (pH 6, 30 ^{o}C, 48 h, under an atmosphere of nitrogen). Bioreductions were performed with resting cells of *L. kefiri* in 0.2 M potassium phosphate buffer (pH 6.5, 30 ^{o}C, 50 g_{DCW}/L). For the comparison co-substrates in 0.2 M potassium phosphate buffer with 5mM $MgCl_2$, with different concentracions of glucose (225, 450, 675 mM) and with different v/v concentacions of 2-propanol (2.5, 5, 10, 10%), respectively, were used. Yields of transformations and amounts of each enantiomer formation were examined by means of chiral HPLC.

The research was supported by Polish State Committee for Scientific Research (grant No 2 P05F 002 27)

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17:40 Poster I-35

THE NEW WATER SOLUBLE SALTS, PRONE TO SPONTANEOUS DECOMPOSITION WITH FORMATION OF 2-CHLOROETHYLAMINO MOIETY (NITROGEN MUSTARD DERIVATIVES)

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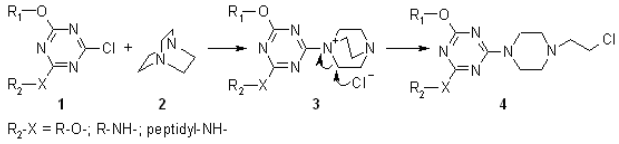
The variety of compounds with 2-chloroethylamino fragment (nitrogen mustard analogues) with strong alkylating activity terminate the proliferation of the cells, however, due to the strong toxic and mutagenic side-effects, their therapeutic value is substantially limited. Therefore, the search is provided for the non-alkylating precursors prone to conversion under physiological conditions with formation of the mustard moiety, as potential pro-drugs for the cancer therapy.

Recently, we found that some N-triazinylammonium chlorides **3** easy accessible from broad range of triazines **1** and 1,4-diazabicyclo[2.2.2]octane (DABCO) (**2**) rearranged with a formation of **4** bearing 2-chloroethylamino fragment**.**



The most of N-triazinylammonium salts **3** were obtained in almost quantitative yield. Rearrangement of **3** to **4** proceeds spontaneously at room temperature slowly and is accelerated in non-aqueous media and at elevated temperatures. Both **3** and **4** were isolated and identified. Compounds **4** were investigated in the standard cell line of mammalian tumor MCF-7.

17:40 Poster I-36

KERATINE PREPARATIONS FOR WOUND HEALING

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Wound associated with diabetes, bed rest injury or post burn wound create real challenge in development of new healing stimulating substances and procedures. Our new approach is to combine new bandage with combination of complementally medicines. The new bandage is a specially prepared keratin powder impregnated with antimicrobial and healing stimulating substances that could be applied directly on wound surface. The keratin preparation forms scab keratin that eliminates body fluids loss and isolates wound from invading microbes. In addition the keratin forms skeleton for reconstructing wound tissues. The active substances that are released from the keratin speed up healing process.

Acknowledge: Project partially supported by Polish Ministry of Education and Science grant no. 1352/TO9/2005/29

17:40 Poster I-37

A STRUCTURAL AND SAR STUDY OF 2-[4-(SUBSTITUTED-PHENYL)PIPERAZIN-1-YLMETHYL]ISOTHIAZOLO[5,4-b]PYRIDINES WITH ANALGESIC ACTIVITY.

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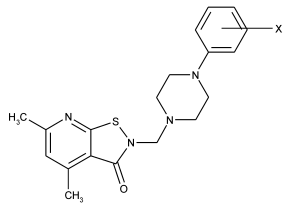
Arylpiperazine derivatives of isotiazolopyridines of Mannich base type **1a-h** exhibited differentiated analgesic activity in pharmacological screening [1].



1a - h

a - X = H, **b** - X = 2-OCH_3, **c** - X = 2-CH_3, **d** - X = 4-NO_2, **e** - X = 2-Cl, **f** - X = 2-OC_2H_5, **g** - X = 2-CH_3-5-Cl, **h** - X = 3-CF_3

To explain the observed discrepancy of biological effects within series **1a-h**, we studied structure/analgesic activity relationship with a view of determining which geometry and portion of the side chain in molecules are important in an analgesia. In our SAR study we found that the conformation of arylpiperazine substituent and steric as well as substituent effects in its arylpiperazine part may contribute to the pharmacological activity of compounds **1a-h**. Basic structural and conformational information were obtained from X-ray investigations and molecular modeling studies [2].

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17:40 Poster I-38

THE ANTIMETASTATIC EFFECT OF BACTERIOPHAGE T4* ADMINISTRATED IN COMBINED TREATMENT WITH SELECTED CYTOSTATICS IN MICE WITH B16 MELANOMA

Katarzyna Szczaurska[1], Krystyna Dąbrowska[1], Marta Celka1, Aneta Kurzępa[1], Dmitry Nevozhay[1], Joanna Wietrzyk[1], Kinga Świtała-Jeleń[1], Danuta Syper[1], Adam Opolski[1,2], Andrzej Górski[1,3]

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The bacteriophage T4 preparation (BP T4)* was obtained and purified at the Ludwik Hirszfeld Institute of Immunology and Experimental Therapy in Wrocław. BP T4 purification enabled concentration of the phage suspension and partial removal of most of its contaminants. However, endotoxins (LPS), despite their relatively low molecular weight, were not completely removed and their concentration in the final bacteriophage preparation was less than 10 EU/mL [1].

BP T4 is a biologically active agent exhibiting antitumor effect. As previously shown, BP T4 binds to cancer cells and shows antitumour and antimetastatic effect in murine experimental tumor models [2, 3]. This binding may explain the mechanism of bacteriophage-eukaryotic cell interactions and the observed biological effect. According to our hypothesis, this binding is mediated by the gp24 phage head protein and b3 integrins expressed on mouse tumor cells. Bacteriophage gp24 contains KGD amino-acid motifs, i.e. RGD homologues able to bind b3 integrins' receptors [4].

The aim of this study was to verify the possible effect on experimental lung metastases in mice injected *i.v.* with B16 melanoma. The effect of BP T4 administration on the effectiveness of known anticancer agents (cyclophosphamide, cisplatine, or 5-fluorouracile) was studied. C57Bl/6 mice were daily injected intraperitoneally with BP T4 in a dose of 10^9 pfu/mouse for 21 days. We used two control groups, one injected with physiological saline and one with a solution of LPS in a concentration as in BP T4. The bacteriophage T4 preparation was applied together with a single dose of cyclophosphamide, cisplatine, or 5-fluorouracile (in doses of 50, 5, or 100 mg/kg, respectively). The mice were maintained under standard laboratory conditions. They were sacrificed by cervical dislocation 21 days after tumor inoculation and the experimental metastatic lung colonies were counted.

A decrease in the number of metastasis formations was observed in the lungs of the mice treated with cyclophosphamide combined with BP T4 (72% compared with the control, $p<0.01$). The decrease in lung metastasis loci in the mice treated with BP T4 alone was in the range of 35% to 50% in two independent experiments ($p>0.05$). These results corresponds with our previous data [2, 3]. The biological effect of LPS used alone was observed whereby the number of metastases was reduced by 18% or 42% ($p>0.05$). We did not observe any enhanced antimetastatic effect of BP T4 applied together with cisplatine or with 5-fluorouracile, although the antimetastatic effect of these cytostatics was observed (63% and 20%, respectively).These data show that our bacteriophage preparation can influence the antimetastatic activity of cyclophosphamide. We suppose that the modifying effect of the bacteriophage preparation could be related to the drug's mechanism of action. This phenomenon requires further study.

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2. Dąbrowska K. et al., *Acta Virologica*, **2004.**

3. Dąbrowska K. et al., *Anticancer Research,***2004**.

4. Górski A. et al., *Medical Immunology,***2003**.

17:40 Poster I-39

CLUSTERING AS A SUPPORTING TOOL FOR STRUCTURAL DRUG DESIGN

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In this work we show the applicability of a structural clustering in a molecular docking calculations. Our hierarchical clustering protocol is able to distinguish between correct and incorrect geometry of a ligand interacting with a receptor. For a test case (PDB code 1CKA) we generated multiple models of protein-ligand complexes. High resolution reduced model of protein conformational space was used for fully flexible ligand docking. In these models we observed different mutual orientations between the molecules. However, the most populated cluster exhibited the lowest distance from the crystal structure. This opens new possibilities in structure-based rational drug design and studies of macromolecular assemblies.

17:40 Poster I-40

SUBMERGED CULTIVATION OF STREPTOMYCES TSUKUBAENSIS IN MEDIA COMPOSED OF WASTE PRODUCTS OF FOOD INDUSTRY.

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The *Streptomyces tsukubaensis* was found in the early 1980s near Mt. Tsukuba, Ibraki, Japan. In 1985 *S. tsukubaensis* was found to produce a 23-membered macrolide antibiotic - Tacrolimus (also FK-506, Fujimycin, Prograf®), a potent immunosuppressive agent. It was introduced into the clinic in the late 1980s for use as both primary and rescue therapy in patients receiving solid organ transplants. Its main function is to reduce the activity of the patient's immune system after allogenic organ transplant and therefore the risk of organ rejection. Tacrolimus hydrate is isolated from the whole fermentation broth of a bacterium *Streptomyces tsukubaensis* cultivated in submerged culture.

Our research project has been aimed at developing an original, novel method of biosynthesis of the macrolide by means of submerged cultivation using liquid media containing degradation products of food industry.

In our research the strain of *Streptomyces tsukubaensis* (FERM BP-927) has been used. Several media have been tested to find the relationship between the composition of the cultivation medium and the mycelial growth and tacrolimus productivity by the microorganism.

The method of determination of FK 506 by High-Performance Liquid Chromatography has also been studied in this research. Main problems in HPLC determination of Tacrolimus are caused by two kinds of conformational heterogenity of this compound. First kind is a cis-trans conformational isomerization involving restricted rotation of the amide bond in a pipecolic moiety, second is reported for aqueous solution epimerization of FK-506 to an intermediate tautomer I (cis) which is converted into tautomer II (cis-trans). For quantitative chromatographic method it creates a problem when there is a difference in water content between a sample and a standard solution. To avoid problems caused by the epimerization and isomerization during inprocess monitoring of bulk drug substance during the Tacrolimus biosynthesis, combined methods of Akashi [1] and Nishikawa [2] were used in our research.

The best strain growth was recorded for media containing starch as a carbon source and soybean extract as a nitrogen source. For cheap media, containing beat molasses and grain worth as carbon and nitrogen source, growth of cultivated strain was two times lower, however productivity of tacrolimus biosynthesis observed for these cultivations was comparably good.

The highest productivity recorded during our preliminary tests was equal 2,8 mcg/ml of medium. Obtained productivity is two times lower than that declared in Okuhara et al. US patent [3]. We hope that optimization of cultivation conditions and culture media will positively affect the macrolide productivity.

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[3] Okuhara et al., US Patent 4,894,366 (1990)

17:40 Poster I-41

TRIAZINO AND TRIAZEPINO[4,3-f]PURINEDIONES AS A_1 AND A_{2A} ADENOSINE RECEPTOR LIGANDS

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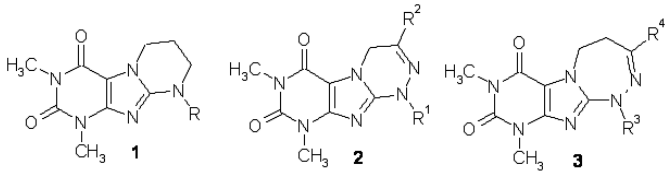
In the last years numerous studies have confirmed the potential ability of A_1 and A_{2A} adenosine receptor antagonists to prevent acute renal failure, neurodegenerative diseases such as Parkinson's and Alzheimer's disease, ischemic brain damage and epilepsy [1]. Methylxanthines such as theophylline and caffeine have been known to enhance locomotor activity; however, these compounds are nonselective antagonists and have weak affinity for A_{2A}AR. Potent A_{2A}AR antagonists were discovered among 8-styrylxanthines: **KW-6002** similarly potent to **MSX-2**, now is in the phase II clinical trials [2-4]. Compounds envisaged as constrained bioisosteric analogues of 8-styrylxanthines, pyrimido[1,2-*a*]purinediones (**1**), appeared to be active ligands [5].

To investigate the influence of the additional nitrogen atom and substituents in the annelated cycle series of [1,2,4]triazino- (**2**) and [1,2,4]triazepino[4,3-*f*]purinediones (**3**) were designed, synthesised and investigated in radioligand binding assays: A_1AR affinity - rat cortical membranes using ^{3}[H]CCPA, A_{2A}AR affinity - rat brain striatal membranes using ^{3}[H]MSX-2. Molecular modelling and SAR studies were performed to investigate structure-activity relationships using programs CAChe 6.1, HyperChem 7.5 and Alchemy2000.



[1,2,4]Triazino[4,3-*a*]purinediones (**2**) have shown affinity toward A_1AR. Compounds with C3-phenyl substituent were most active, introduction of N-acyl group has improved selectivity. In turn, N-phenyl derivatives were inactive toward both AR subtypes. The N-unsubstituted analogues in the group of 1,2,4-triazepino[4,3-*f*]purinediones (**3**) are A_{2A}AR ligands, while the N-substituted compounds are A_1AR ligands. The character of the linkage of N-substituent is important for the selectivity while the length is important for affinity. Generally, compounds possessing ethyl moiety at C3-atom of triazepine ring were more active.

The research was partly supported by Polish State Committee for Scientific Research Grant No. 2 PO5F 01428

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17:40 Poster I-42

MICROWAVE-ASSISTED SYNTHESES OF SUBSTITUTED COUMARINS

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The use of microwave energy in organic synthesis is becoming very popular and many reactions are becoming accessible. An advantage of microwave-catalyzed reactions, is that they can take place under solvent-free conditions (eco-friendly process) compared to conventional heating; in addition, lower reaction times and higher yields are generally obtained.

The procedures are easy to perform and the reactions can be conducted in solvent free conditions and lead to almost quantitative yields of products.

We report the microwave-assisted synthesis of an useful byproduct: 8-acetyl-7-hydroxy-4-methylcoumarin (see Fig. 1).

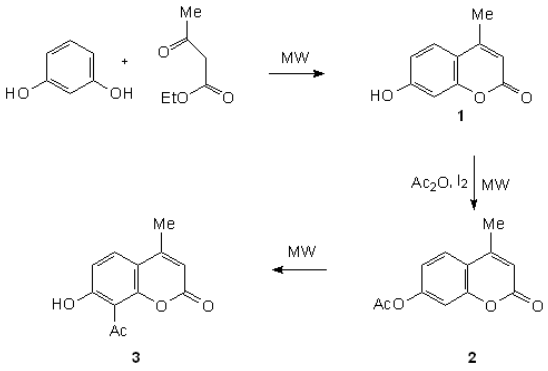

Fig. 1. Scheme of synthesis.

^{13}C CP/MAS NMR spectra of compounds **1-3** were analyzed to elucidate the structural details.

17:40 Poster I-43

NOVEL 4-ARYL-2H-PYRIDO[1,2-c]PYRIMIDINES WITH 5-HT-TRANSPORTER AND 5-HT$_{1A}$ AFFINITY

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Depression is a mood disorder, which affects an estimated more then 100 million people worldwide. The treatment of depression has been revolutionized by the introduction of SSRIs that possess fewer side effects than tricyclic antidepressants. The major drawback with the current line of SSRIs is that they have a delayed onset before the beneficial therapeutic effect is observed.

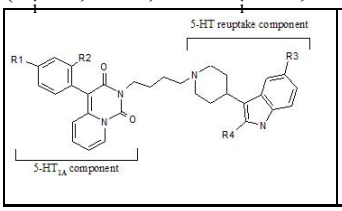
One approach to create a fast-acting SSRI is to combine, in a single molecule, an agent with SSRI activity and 5-HT$_{1A}$ antagonist activity [1-4].

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 structure of new compounds was 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.

The tested compounds showed relatively high affinity for the 5-HT$_{1A}$ receptor and serotonin reuptake inhibition at nanomolar concentration for dual activities (K_i = 4,8 - 10,9 and K_i = 15,3 - 35,2 respectively).



R1 = H, CH$_3$, OCH$_3$, F
R2 = H, CH$_3$, OCH$_3$, F
R3 = H, OCH$_3$ R4 = H, CH$_3$

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17:40 Poster I-44

SYNTHESIS OF 8-FURFURYLTROPANE BENZAMIDE DERIVATIVES WITH POTENTIAL ANTIPSYCHOTIC ACTIVITY

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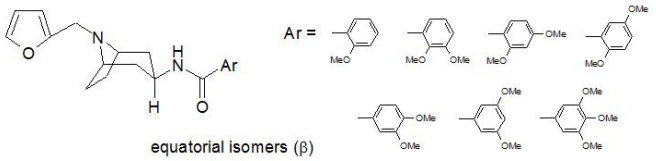
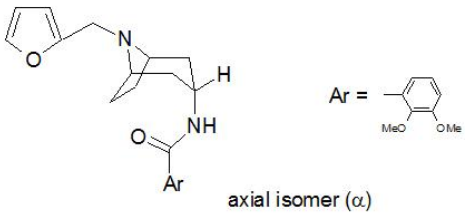
Amisulpride and Sulpiride are benzamide derivatives, both comprising a group of atypical antipsychotic drugs owing to their low level of side effects. Another interesting compound among benzamide derivatives is the Tropapride (MD 790501), an antagonist of D_2 postsynaptic receptors. It is less likely to cause sedation, catalepsis and extrapyramidal symptoms (EPS) than the classical neuroleptic agents. In this paper we selected Tropapride as the leading structure for the performed syntheses. Modifications consisted in substituting the benzyl substituent with the 2-furanomethyl system, and also included certain changes in pharmacophore benzamide substituent where the methoxyl groups were located in different positions. The purpose of the synthesis was mainly to obtain equatorial isomers (β) because of their higher pharmocological activity. To confirm stereoselectivity of the performed syntheses, one axial isomer (α) was also obtained.





The structure of the compounds obtained was confirmed by elemental analysis and also by IR spectra, ^{1}H and ^{13}C NMR analyses.

Further investigations are in progress aimed at examining the above compounds as concerns their affinity to D2 and 5-HT receptors.

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17:40 Poster I-45

RELATIONS "STRUCTURE-ACTIVITY" IN PLATINUM-BASED CYTOSTATICS

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The computer-aided QSAR calculations are commonly applied to rational designing of many group of drugs except of Pt-based agents. We present the results of seeking for the QSAR correlation between antiproliferative activity and molecular descriptors of Pt-drug analogs.

The main goal of this study was to complete Hansch equation [1] showing the relations between hydrophobicity (independent variable) and biological activity (dependent variable) in the series of neutral platinum(II) complexes. The object of these studies were two groups of dicarboxylate platinum complexes, one with primary N-donors (ethylenediamine), and the second with tertiary N-donors (1-alkylimidazole).

The hydrophobicity (log P) of the series of compounds was determined using a shake-flask method; the platinum content of the organic and aqueous phases was measured by ICP. The biological activity was expressed as 1/C, where C was the concentration of drug required to kill 50% of the treated cells. The cytotoxicity concentration (IC50) of platinum complexes was determined by SRB and MTT assays [2] on the human breast cancer (MCF-7) and leukemia (HL-60) cell lines.

As we found earlier [3] the studied complexes differed significantly in their crosslinking ability when they interacted with plasmid DNA. To find the reason of such behavior we evaluated the representative complexes on ovarian carcinoma OvBH-1 cells by immunohistochemical staining using monoclonal antibodies.

The results of SRB or MTT assays demonstrate that new platinum(II) complexes exhibit wide range of cytotoxicity (IC50), between 4.8 and 70.3 microg/ml. In general, they reveal higher antiproliferative activities than referential carboplatin against both cell lines, and the leukemia cells HL-60 are more sensitive target than MCF-7. The regression analyses based on Hansch equation indicate the correlation between the measured value of logP and cytotoxicity, expressed by log 1/C (for IC50). The results of immunohistochemical evaluation, which present the influence of platinum complexes on expression of multi-drug resistant proteins (P-gp, MRP, LRP), apoptosis related proteins (Bax, p53, Bcl-2) and mismatch repair gene product (hMLH1), showed the big differences in the action of platinum species. This is in agreement with differences at crosslinking DNA observed earlier by us on another way [3].

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17:40 Poster I-46

SYNTHESIS AND CYTOTOXIC ACTIVITY OF NEW 1-SUBSTITUTED PYRIDO[4,3-b]CARBAZOLE DERIVATIVES

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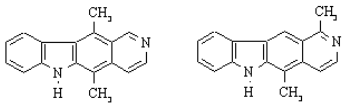
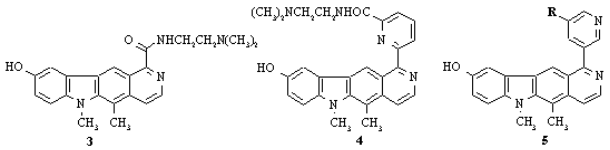
Since many years, alkaloids such as ellipticine **1** and olivacine **2,** are known of theirs antitumor activity.



1 **2**

They have inspired many groups of researchers to modify their chemical structure in the aim of improving the therapeutic index. Many of obtained compounds exhibited stronger antiproliferative activity then alkaloids mentioned above. Our investigations was concerned with modification the olivacine structure. One of derivatives we obtained **3** has entered II phase of clinical trials. Its analogues **4** also displayed strong activity against L1210. This prompted us to undertake a study on the synthesis of the title compound **5**.



3 **4** **5**

Cytotoxic activity of some compounds was performed in the Institute Curie (FRANCE) (for L-1210) and Institute of Immunology and Experimental Therapy (POLAND)(for A-549

lung cancer).

17:40 Poster I-47

THE INFLUENCE OF TEMPERATURE AND SOLVENT ON DARZENS CONDENSATION STEREOSELECTIVITY

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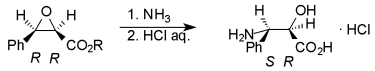
Glycidic esters are versatile intermediates in organic synthesis. These compounds are most conveniently prepared in Darzens reaction of α-halogenoacids esters with carbonyl compounds[1], carried out in the presence of a base.

Looking for new method of synthesis of (2*R*, 3*S*)-3-phenylisoserine hydrochloride - an important intermediate on the route to *Paclitaxel* - we turned our attention to the reaction of (2*R*, 3*R*)-3-phenylglycidic acid with ammonia, which should lead to (2*R*, 3*S*)-3-phenylisoserine **1**.



2 **1**

In the presented work we studied the influence of the solvent and temperature on the stereoselectivity of Darzens reaction between t-butyl chloroacetate and benzaldehyde carried out under phase transfer catalysis (PTC) conditions (conc. aq. solution of NaOH as a base in the presence of a catalytic amount of quaternary ammonium salt).

Previously we tested many chiral quats derived from cinchona alkaloids. We selected *N*-2,4-dichlorobenzylcynchonine bromide and investigated the influence of parameters mentioned on the reaction course. We examined toluene, diisopropyl ether, methylene chloride and THF at temperatures -15, 1 and 8˚C.

Until now, the higher conversion of benzaldehyde (95%), Z/E ratio of isomers of **2** (2/1) and enantiomeric excess of (2*R*,3*R)* isomer (20%) were obtained in toluene at 1˚C after 8 h. Further investigations of this reaction are in progress.

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17:40 Poster I-48

MOBILE PHASE INFLUENCE ON CHROMATOGRAPHIC BEHAVIOUR OF SOME 1,4-DISUBSTITUTED PIPERAZINES

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Chromatographic behaviour of several 1,4-disubstituted piperazine derivatives on cellulose and amylose chiral stationary phases as well as mobile phase influence on solute-stationary phase complexes have been examined. The influence of hydrophobic aliphatic substituents appeared to be much stronger on cellulose than on amylose phase. Also mobile phase influence exhibited pronounced differences. For isopropanol-hexane mobile phases higher solute-stationary phase complex stabilities on cellulose than on amylase stationary phase were observed. On the other hand, on cellulose stationary phase in isopropanol-hexane mixtures the complex stabilities were bigger than in ethanol-hexane mixtures, while on amylose different relationship was observed. The significance of the obtained results will be discussed.

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NEW SYNTHESIS OF 11-(1-PIPERAZINYL)-DIBENZO[b,f][1,4]THIAZEPINE, A CRUCIAL INTERMEDIATE IN QUETIAPINE PRODUCTION.

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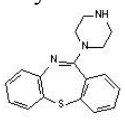
Quetiapine is a novel antipsychotic drug, showing higher activity and fewer side effects than the classical antypsychotic agents. 11-(1-Piperazinyl)dibenzo[b,f][1,4]thiazepine is the crucial intermediate in the synthesis of quetiapine:



Our invention[1] relates to the process of the preparation of this intermediate. In comparison with the classical industrial method[2] ours offers several advantages, i.e.:

- reduction of operations number and time; - higher total yield (75% vs. 60%); - lower consumption of solvents; - reduction

of waste material quantity;

All these advantages result in considerable reduction of manufacturing costs.

[1] Ł. Kaczmarek, K. Badowska-Rosłonek, E. Stolarczyk, W. Szelejewski, " Process for preparation of 11-(1-piperazinyl)dibenzo[b,f][1,4]thiazepine, an intermediate in the synthesis of the antipsychotic drug Quetiapine.", PCT/EP2004/051520 (2004).

[2] EP 0240228

17:40 Poster I-50

APPLICATION OF HPLC FOR ANALYTICAL CONTROL OF LARGE SCALE SYNTHESIS OF (2R, 3S)-3-PHENYLISOSERINE HYDROCHLORIDE

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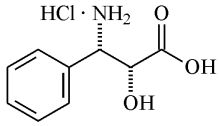
(2*R*, 3*S*)-3-phenylisoserine hydrochloride **1** is an important intermediate in the synthesis of *Paclitaxel* - an anticancer drug.



1

In NS-3 Department ICRI we elaborated technology of compound **1** in ½ technical scale (20 - 25 kg per year). The preparation of this compound was performed according to the six-step procedure.

The aim of this study was elaboration of method and conditions for analysis of (2*R*, 3*S*)-3-phenylisoserine hydrochloride by high-performance liquid chromatography (HPLC).

We tested two analytical columns: Zorbax Eclipse XDB-Ph and Zorbax Eclipse XDB - CN (250 mm x 4,6 mm, f-5m). The isocratic elution of separated compounds was carried out by mobile phase - methanol & sodium phosphate buffer 90:10 v/v, using constant flow rate 1,0 cm^3/min. Chromatographic analysis was performed on a Merck LaChrom D-7000 Liquid Chromatograph (detector UV, l=210nm).

The influence of solvent, pH and ionic strength of mobile phase on separation and peak symmetry was studied.

17:40 Poster I-51

OPTIMIZATION OF CHIRAL SEPARATION ON VARIOUS POLISACCHARIDE STATIONARY PHASES

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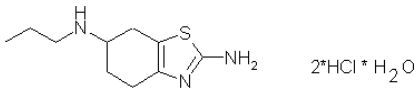
Only
)-2-Amine-4,5,6,7-tetrahydro-6-(n-propylamine)benzothiaz (*S*ole dihydrocloride monohydrat (**1**) is a new dopaminergic agonist for the treatment of Parkinson disease. It has been effective in early Parkinson's disease as monotherapy and as adjunctive therapy with L-dopa in advanced stages of the disease [1] (chemical formula is presented in Figure 1).



1 is a chiral compound and its (+) enantiomer is the low-affinity dopamine agonist.

An analytical method for the determination of the enantiomeric purity of synthetic product is required.

In the development of a chiral HPLC method, it is usually desirable to use a chiral stationary phase (CSP) for direct separation of enantiomers because of to the simplicity of operation. There are various types of CSPs available. Among them cellulose and amylose based CSPs have been proved to be quite versatile [2,3]

In this work we have compared separations of **1** obtained on various amylose and cellulose columns. The effect of the alcohol used as mobile phase modifier and the addition of small amount of various amines to the mobile phase has been investigated. To optimise the analytical procedure the effect of temperature on retention and selectivity on various columns using a variety of mobile phases composition was also studied.

[1] Medline.com

[2] Y. Okamoto, Y. Kaida, J. Chromatogr. A, 666 (1994) 403

[3] Y. Okamoto, E. Yashima, Angew. Chem., Int. Ed. Eng. 73 (1998) 1020

17:40 Poster I-52

OPTIMIZATION OF THE CHROMATOGRAPHIC SEPARATION BY HPLC METHOD AND CONFIRMATION OF THE IDENTITY OF CHOSEN ESCITALOPRAM OXALATE INTERMEDIATE PRODUCTS

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Escitalopram (fig. 1) is an oral drug that is used for treating depression and generalized anxiety disorder. It works by affecting neurotransmitters in the brain, the chemical messengers that nerves use to communicate with one another. Neurotransmitters are made and released by nerves and then travel to other nearby nerves where they attach to receptors on the nerves. Some neurotransmitters that are released do not bind to receptors and are taken up by the nerves that produced them [1].

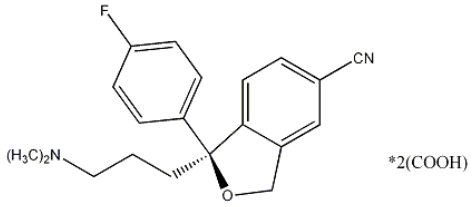

fig. 1

An optimization of the method for the separation of the previously selected intermediate products by High Performance Liquid Chromatography (HPLC) is described. The determination of the chemical identity of the isolated compounds was performed. Different spectroscopy methods were used for this propose: Infrared Spectroscopy (FT-IR) and Nuclear Magnetic Resonance (NMR).

[1] E. Saltiel, Pharm D. MedicineNet.Com

17:40 Poster I-53

THE VALIDATION OF ANALYTICAL METHODS OF A PHARMACEUTICAL ACTIVE SUBSTANCE PRODUCED IN SMALL SCALE PLANT (SSP). THE EXAMPLE OF PIOGLITAZONE HYDROCHLORIDE.

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Pioglitazone hydrochloride is an oral antidiabetic agent that improves sensitivity to insulin. It is used in the management of type 2 diabetes mellitus.

Requirements for drug active substances are specified in documents from International Conference of Harmonization. According to these guidelines quality of the pharmaceutical compounds must be confirmed by validated analytical methods [1,2].

The quality of pioglitazone hydrochloride batches manufactured in Pharmaceutical Research Institute was examined by the following methods:

- Gas Chromatography (GC) was used for the quantitative determination of the following solvents: 2-propanol, acetone, acetic acid.
- High Performance Liquid Chromatography (HPLC) was used for determination of the purity and quantity of the active substance,
- Potentiometric titration was used for quantitative determination of chloride anion.

Adapted methods were validated to prove that all the procedures used to generate analytical data are suitable for their purpose.

[1] Dyrektywa 75/318/EEC z aneksem 91/507/EEC.

[2] J. Pawlaczyk, M. Zając, "Walidacja metod analizy chemicznej - przykłady walidacji metod", AM Poznań 2005, wyd. 3

17:40 Poster I-54

HPLC SEPARATION AND DETERMINATION OF ZIPRASIDONE

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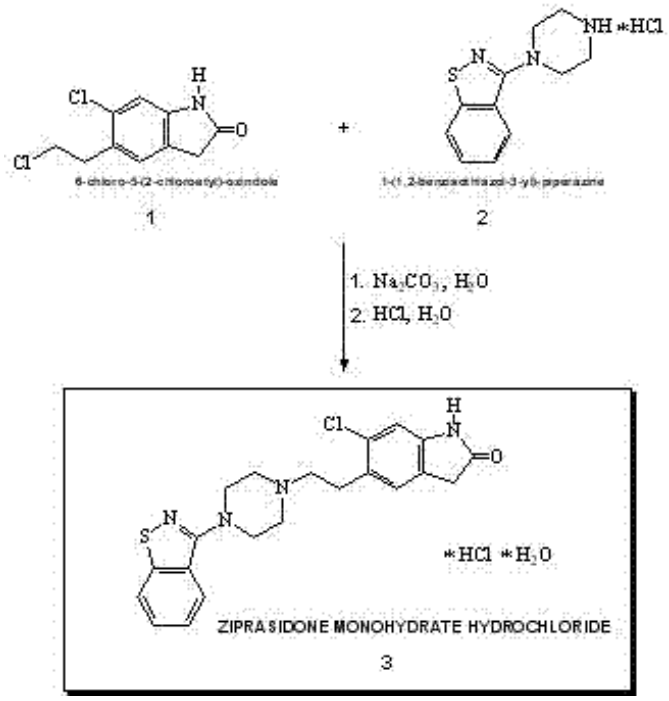
Ziprasidone (5-(2-(4-(1,2-benzisothiazol-3-yl)piperazinyl)ethyl)-6-chloro-1,3-dihydro-2(1*H*)-indol-2-one hydrochloride monohydrate (**3**)) with the chemical formula $C_{21}H_{21}ClN_4OS^* HCl^*H_2O$) is a new antipsychotic with combined dopamine and serotonin receptor antagonist activity. Clinical trials suggest that the drug is an effective antipsychotic in schizophrenia and schizoaffective disorder with a beneficial effect on negative symptoms and symptoms of depression.



The monohydrate hydrochloride (**3**) was obtained from the appropriate base prepared in condensation reaction of starting materials **1** and **2**. For the final product purity determination a simple analytical method was developed.

High-Performance Liquid Chromatography (HPLC) is a simple analytical technique for the separation and determination of organic analytes in pharmaceutical samples.

The optimalization of the reversed phase high-performance liquid chromatographic separation of ziprasidone and starting materials is described. This method was successfully applied to the analysis of compound purity. The method is sufficiently simple and rapid for the quality assurance of this type of compounds. The peaks in the chromatograms of a mixture were identified based on the retention times of isolated components injected separately.

The X-Ray powder diffraction method (XRPD) and infrared spectroscopy (IR) were used to identify and characterize the samples of substance and tablets of Ziprasidone toward the polymorphic form.

17:40 Poster I-55

QUINONES AND HYDROQUINONES SEPARATION AND DETERMINATION BY HPLC CHROMATOGRAPHY

Ewa Chojecka-Koryn, Hanna M. Beczkowicz, Wojciech J. Szczepek, Wojciech Łuniewski

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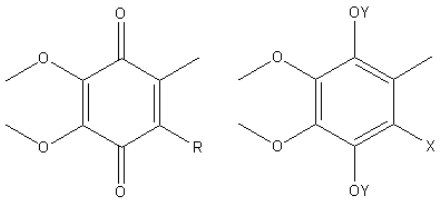
Quinones and hydroquinones are widely used in the syntheses of organic compounds including pharmaceutical substances preparations. There is currently considerable interest in the role of this group of substances as antioxidants and biologically active components.

For detection of their presence in the reaction products and to evaluate the purity of the prepared samples, a simple analytical method was developed.

High-Performance Liquid Chromatography (HPLC) is an analytical technique appropriate for the separation and determination of organic analytes in various samples, especially biological, pharmaceutical, environmental, and industrial.

Figure 1



Synthesized compounds representing structures I and II (Figure 1) were well determined and separated by reversed phase high-performance liquid chromatography in isocratic and gradient conditions. The optimization of the simultaneous determination of six compounds, shown on the Figure 1, was performed. The peaks in the chromatograms of a mixture were identified based on the retention times of isolated components injected separately. Additionally chemical structure of examined compounds was established using spectrometric methods.

17:40 Poster I-56

VALIDATION OF AN ANALYTICAL PROCEDURE - CONTROL OF RESIDUAL ETHANOL, 2-PROPANOL AND ETHYL ACETATE IN PHARMACEUTICAL SUBSTANCE

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Residues of ethanol, 2-propanol and ethyl acetate 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 90° 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:

ethanol 5000 ppm

2-propanol 5000 ppm

ethyl acetate 5000 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.

17:40 Poster I-57

APPLICATION OF GC/MS FOR IDENTYFICATION OF THE SIDEPRODUCTS IN A PROCESS OF PREPARATION OF PRAMIPEXOLE.

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Contemporary drugs are usually prepared in a multistep organic syntheses. Already, at the early development stage, there is a high demand for quick separation, identification and confirmation of the structure of reaction products. The GC/MS is a perfect analytical tool to meet these requirements.

Alkylation of 2,6-diamino-4,5,6,7- tetrahydrobenzothiazole with n-propyl tosylate was investigated. Besides of peaks corresponding to the starting material and the desired product (6-n-propylamino derivative), other mono-, di- and tri-n-propyl substituted 2,6-diamino-4,5,6,7- tetrahydrobenzothiazole derivatives were also observed.

In a separate experiment, acylation products of 2,6-diamino-4,5,6,7- tetrahydrobenzothiazole were investigated.

Conclusions from these experiments were usefull for the development of the process in a laboratory scale.

17:40 Poster I-58

VALIDATION OF AN ANALYTICAL PROCEDURE - CONTROL OF RESIDUAL 9 SOLVENTS IN PHARMACEUTICAL SUBSTANCE

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Residues of methanol, ethanol, acetone, dichloromethane, hexane, ethyl acetate, tetrahydrofuran, pyridine 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, ethanol 5000 ppm, acetone 5000 ppm, dichloromethane 600 ppm, hexane 290 ppm, ethyl acetate 5000 ppm, tetrahydrofuran 720 ppm, pyridine 200 ppm and toluene 890 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.

17:40 Poster I-59

SYNTHESIS OF SOME 5-SUBSTITUTED ETHYL 3-METHYLISOTHIAZOLE-4- CARBOXYLATE DERIVATIVES

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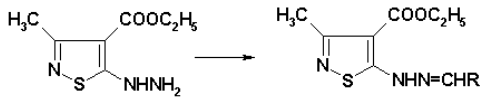
For many years isothiazoles are of interesting as a class of heteroaromatic compounds with pharmacological activity. One of them is Vratizolin, which has found, its use in medicine. Continuing our research on derivatives of isothiasole ring, diamide derivatives which showed antiviral, anticancer, antiinflamatory and immunosuppressive inspired our interest in the syntheses of new isothiazole compounds.

Starting material for this synthesis is 5-hydrazinoester. In the present research new series of 5-hydrazino substituted derivatives were obtained.



The project is financially supported by the Community of Wrocław Medical University GR-1311.

17:40 Poster I-60

SYNTHESIS AND ANTICONVULSANT EVALUATION OF SOME N-ALKENYL / ALKINYL PHTHALIMIDE DERIVATIVES

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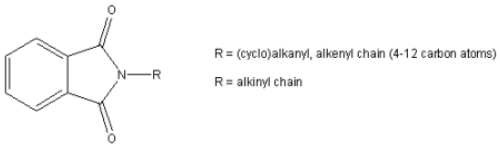
Epilepsy is one of the most prevalent neurological syndromes in the word. The classical anti-epileptic drugs comprise

phenobarbital, phenytoin, carbamazepine and valproic acid. All currently approved antiepileptic drugs have dose-related toxicity and idiosyncratic side effects. The search for new antiepileptic drugs with lower toxicities and fewer side effects continues. Among compounds studied for anticonvulsant activity, there are phthalimide derivatives. The phthalimide pharmacophore was developed by Vamecq et al. [1]. As a continuation of our research on phthalimide derivatives [2] now we report on the synthesis and evaluation of anticonvulsant and toxicological profiles of the new phthalimide derivatives.



A series of alkanyl, alkenyl and alkinyl derivatives of phthalimide was prepared starting from phthalimide. Compounds containing 1-3 double bonds or triple bond were screened for anticonvulsant activity in the maximal elektroshock screen (MES) and the subcutaneous pentylenetetrazole (ScPTZ) test according to Anticonvulsant Drug Discovery Program National Institutes of Health, Bethesda. Among the new derivatives evaluated, alkinyl derivative emerged as the most active compound as indicated by the protection in the MES and ScPTZ sreens. Configuration and length of the alkenyl chain influenced the activity. Cycloprpyl ring was beneficial for the MES seizure screen.

This work was partly supported by Project No BBN 501/P/185/F by Polish State Commitee for Scientific Research.

[1] Vamecq J.; Bac P.; Herrenknecht Ch.; Maurois P.; Delcourt p.; Stables J.P.: J. Med. Chem. 2000, 43, 1311-1319.

[2] Marona H.; Kieć-Kononowicz K.: Pharmazie 1998, 53, 9, 603-606.

17:40 Poster I-61

THEORETICAL STUDIES ON THE STERICAL PREREQUISITES FOR ACTIVE DOMAIN OF THE SEROTONERGIC 5-HT$_{1A}$ RECEPTOR.

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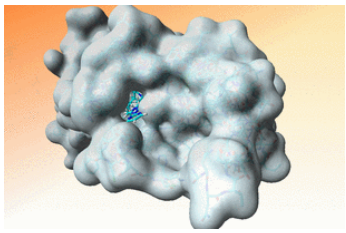
The main goal of the research was to identify and describe the bonding domain of the serotonergic 5-HT$_{1A}$ receptor and to explain the possible mechanism of interaction between derivatives of N-substituted imide of 3,7-dimethyl-5-oxobicyclo[2.2.2]octane-1,2-dicarboxylic acid - the buspirone analogs, and receptor.

At the beginning, using the homology method based on the best-scored sequence alignement between the receptor protein and the rodopsine, 3D structure of 5-HT$_{1A}$ receptor was constructed with help of MODELLER software.



In next step group of ten ligands of know affinity with some structural analogy to the compounds investigated was selected. In this groupe buspirone, tandospirone and NAN 190 was included. The energy minimum optimazed 3D structures of ten standards and 12 derivatives of the imide of 3,7-dimethyl-5-oxobicyclo[2.2.2]octane-1,2-dicarboxylic acid was docked to the receptor with use of the PathDock software.

The results allow to point out the bonding domain for each compound showing also differences in their interactions with receptor.

17:40 Poster I-62

INVESTIGATION OF THE RELATIONSHIP BETWEEN LIPOPHILICITY AND THE STRUCTURE OF PERHYDROPYRIMIDINE DERIVATIVES BY THIN-LAYER REVERSED-PHASE CHROMATOGRAPHY.

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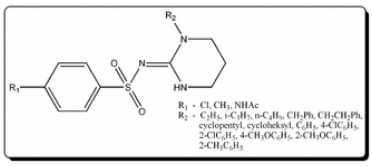
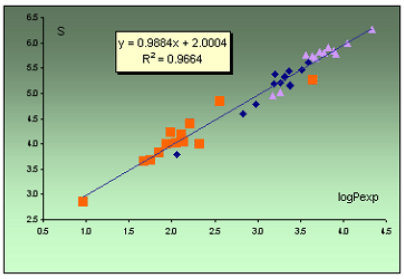
The main goal of the research was determinatin of the lipophilicity (lpg*P*) of forty alkyl, cycloalkyl, arylalkyl and aryl derivatives of perhydropyrimidine of the following structure.



Experimental lipophilicities were determined by RP-TLC method with three different polar modifiers and were correlated with lipopholicity coefficients calculated by different algorithms. Additionaly molecular lipophilicity potential

(MLP), molecular hydrophobicity index (IML), molecular electrostatic potential (MEP), polar surface (PSA) and solvent accessible surface (SAS) were calculated.



Correlation of the experimental lipophilicity logP_{EXP} with the S (slope) values in acetonitryl-water solvent system shows that the R_1 substituent is the one determining the lipophilicity of the whole molecule.

17:40 Poster I-63

DETERMINATION OF IMPURITIES IN PHARMACEUTICAL PREPARATIONS CONTAINING FOLIC ACID

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It is recognized that some years ago at the time of submission marketing authorization dossiers for medicinal products containing folic acid, there were no part of test procedure for its impurities determination.

In most cases, in the single-component preparations, the spectrophotometric or spectrofluorometric methods are used for folic acid determination. In multivitamin preparations the microbiological method was the method of choice for folic acid determination. However it was impossible, using these methods, to determine folic acid impurities at the same time.

Development of modern instrumental methods such as HPLC, GC, EC makes possible folic acid impurities determination. But it isn't possible to determine, in the same chromatographic conditions, both: folic acid and its impurities simultaneously.

The aim of this work was to determine the conditions for HPLC procedure for identification and quantitative determination of folic acid impurities: p-aminobenzoic acid and N-4-aminobenzoyl-L-glutaminic acid.

Chromatographic separation was achieved on Waters Spherisorb S5ODS1 column (250 x 4.6 mm). Detection was performed using UV/VIS detector at wavelength of 269 nm. A solution of: potassium dihydrogenphosphate water solution: tetrabutyloammoniumhydroxide methanol solution : methanol as a mobil phase was used (pH = 5.0). The injection volume was 25 ml.

The concentration of reference substances mixture in mobile phase was: 0.5 mg/ml of p-aminobenzoic acid and 2mg/ml of N-4- aminobenzoyl-L-glutaminic acid.

Analysed samples were alkalized with 12% ammonium hydroxide prior to extraction with mobile phase solution.

The proposed HPLC method, owing to its satisfactory precision (low relative standard deviation) and accuracy as well as short retention times (4.1 min for p-aminobenzoic acid and 5.3 min for N-4- aminobenzoyl-L-glutaminic acid), can be applied to determine the impurities of folic acid in pharmaceutical preparations.

17:40 Poster I-64

A SIMPLE PREPARATIVE HPLC ISOLATION OF CONJUGATED LINOLEIC ACIDS (CLAs).

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Conjugated linoleic acid (CLA), a natural polyunsaturated fatty acid, is one of the major components of nutraceutics, mainly taking into account the terapeutical applications; many studies have shown that CLA may play an important role in helping fight obesity, cancer, diabetes, as well as hardening of the arteries.

In natural occurring products a very low concentration (≤ 7%) of this fatty acid is noticed, therefore it is necessary to isolate it while nutraceuticals production. However, every method of the isolation of conjugatd linoleic acid is rather arduous and expensive (e.g by super-critical extraction connected with crystallization using urea we are able to achieve only 11% of CLA, which is the maximum value). Thus it seems to be crucial to find the proper method which will be efficient, especially during the large-scale isolation process.

In general, preparative HPLC, due to its costs, it is not considered as an economical method which might be used in nutraceutics isolation. However, we have developed a simple HPLC method with the implementation of methanol, in which we are able to obtain as well as separate "blocks" of various fatty acids, including conjugated unsaturated fatty acids.

This method may be used for the preparative isolation of conjugated unsaturated FA (diens or triens) derived from either natural sources (milk, oils) or chemical izomerization products.

17:40 Poster I-65

PURITY AND ASSAY VALIDATION OF PHARMACEUTICAL SUBSTANCE TACALCITOL BY HPLC METHOD

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Quality control of pharmaceutical substance is very important and complicated problem because of consequences of propable mistakes during particular analytical determination. Methods used for purity and assay control should be highly specific and precise because of low active substance concentration. Method validation is the process of proving that an analytical method is acceptable for its purpose. During each validation minimum requirements for the method should be set. Checked parameters are, for example: presicion, LOQ, LOD, linearity, selectivity. This work presents tacalcitol purity and assay validation by HPLC - method that is used in routine analysis of this substance. Tacalcitol prevents the excessive growth rate of keratinocytes (a type of skin cells) that leads to scaling of the skin characteristic of psoriasis. Purity and assay validation of this pharmaceutical substance by HPLC, was performed to prove that applied method is highly specific, selective and allows to obtain reliable results.

17:40 Poster I-66

OPIOID PEPTIDE ANALOGUE AA2016 IN MOUSE MODEL OF INDIVIDUAL PAIN SENSITIVITY

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It is widely appreciated among clinicians and researches that sensitivity to pain and responses to analgesics are highly variable. In the process of discovery of new analgesic this phenomena should be taken into account. However, tendency to eliminate of any individual variability is visible even in preliminary stages of selection of drug candidates using animal tests. Over twenty years ago Sadowski et al. developed genetic mouse lines selectively bred for high and for low swim stress-induced analgesia (SSIA). These animal lines may well simulate human high and low sensitivity to both, pain and stress response.This communication will present different response to opioid peptide analogue AA2016 by mouse SSIA without any stressor. The possible reasons of observed differences in analgesic effects will be discussed.

17:40 Poster I-67

EFFECT OF GENISTEIN SYNTHETIC DERIVATIVES ON REGULATION OF GLYCOSAMINOGLYCAN SYNTHESIS IN SANFILIPPO DISEASE (MUCOPOLYSACCHARIDOSIS TYPE III)

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Mucopolysaccharidoses (MPS) are rare genetic, metabolic disorders caused by accumulation of mucopolysaccharides (glycosaminoglycans, GAGs) in lysosomes. This accumulation is caused by a deficiency in one of several specific enzymes involved in GAGs degradation. The disease causes severe problems in virtually all tissues and organs and usually leads to death in childhood. Currently, an effective treatment is available for MPS I (Aldurazyme®) and clinical trials for MPS II and MPS VI have been completed. The treatment is based on administration of the lacking enzyme (enzyme replacement therapy, ERT). The delivery of enzyme molecules to the central nervous system is highly inefficient because of the blood-brain barrier. However, in many MPS types (MPS IH, MPS II, MPS IIIA, MPS IIIB, MPS IIIC, MPS IIID, MPS VII), central nervous system is also affected and ERT seems to be ineffective in treatment of neurological symptoms. The aim of this project is to develop an alternative, gene expression targeted isoflavone therapy (GET-IT), which could be used either alone or in combination with ERT. It seems that expression of at least some of genes coding for enzymes involved in GAGs degradation might be controlled through pathways, which are dependent on estrogen receptor protein and its tyrosine kinase activity (PTK). It has been reported that genistein (a natural soy isoflavone) inhibits activity of the tyrosine kinase (PTK). Basing on several human fibroblast culture experiments, we found that genistein and some of its synthetic derivatives inhibit GAG production up to ten times in fibroblasts of patients suffering from various types of MPS. We observed a significant decrease in levels of GAGs accumulated in fibroblasts of MPS III patients treated with gen-

istein for several days. Moreover, genistein was reported to cross the blood-brain barrier in rats with efficiency of several percent after intravenous administration. It is of our interest to find out if any other natural isoflavones (e.g. daidzein, kaempferol, apigenin, naringenin) or genistein synthetic derivatives reveal inhibitory effects on GAGs synthesis, combined with a relatively high potential in blood-brain barrier penetration, especially in mucopolysaccharidosis IIIA and IIIB fibroblasts, as severe neurological injury is observed in these MPS types. We tested 20 synthetic genistein derivatives with MPS III human fibroblast cultures and 5 of them, presenting the highest inhibitory effect on GAG synthesis (similar to genistein or even higher) were chosen for further experiments. In conclusion, it appears that gene expression targeted isoflavone therapy (GET IT), based on administration of specific natural isoflavones or genistein synthetic derivatives, may potentially be an effective treatment of MPS III patients.

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THE BIOLOGICAL TARGET DERIVED PHARMACOPHORIC MODEL FOR 5-HT$_7$ SEROTONIN RECEPTOR ANTAGONISM

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The biological target derived pharmacophoric model is presented for 5-HT$_7$ serotonin receptor antagonism. It was generated based on results of automated docking of examples of all known antagonists classes to the conformational ensemble of rhodopsin based receptor models. The methodology reflects conformational flexibility of both ligand and receptor. Current pharmacophoric model is divided into two sub-models: (1) "affinity" model - including features common for all (nonselective and selective) antagonists; (2) "selectivity" model - explaining which pharmacophoric features are responsible for selectivity toward 5-HT$_7$ receptor. Nonselective antagonists, described by the model (1), are situated along TM3, occupying the cavity formed by TMHs 4-6 and interacting specifically with Asp3.32, Phe6.61, Phe6.62, Ser5.42 and optionally Phe3.28, Tyr7.43. Selective antagonists form the network of interactions with the residues from TMHs 3 and 7: Asp3.32, Phe3.28, Tyr7.43 and Arg7.36 and optionally Phe6.61, Phe6.62. It is postulated that if the latter interaction pattern dominates over the former one, selectivity toward 5-HT$_7$ receptor is enhanced.

Acknowledgement

This study was partially supported by the research Grant no. 012/2002 from the Polish Pharmacy and Medicine Development Foundation, given by the POLPHARMA Pharmaceutical Works.

17:40 Poster I-69

SYNTHESIS AND SEROLOGICAL INTERACTIONS OF H.PYLORI UREASE FRAGMENT 321-339 IMMOBILIZED ON THE CELLULOSE SUPPORT

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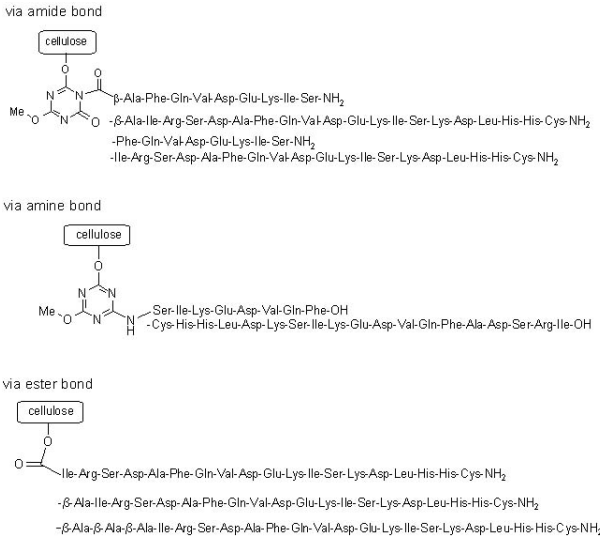
H. pylori is a major etiological agent of gastroduodenal ulcer diseases. One of the significant pathogenic factor of *H. pylori* is urease production and anti-urease antibodies might be responsible for inflamatory reaction proceeding atherosclerosis [1,2].

In order to study the recognitions of *H.pylori* UreB epitopes by sera of atherosclerosis patients we prepared 321-339 urease fragments attached to the cellulose plate with N-terminus as well as with C-terminus.

The F8 epitope: SIKEDVQF and UB-33 epitope: CHHLDKSIKEDVQFADSRI [3] were synthesized directly on the cellulose plate by using triazine based condensing reagent [4].



The peptides were treated with sera of patients with medically confirmed arteriosclerosis and then with anti-human antibodies labelled by horse-radish peroxidase HRP, followed by ad-

dition 4-chloronaphtol and hydrogen peroxide.

Peptides anchored with N-terminus to cellulose gave positive reactions with patients sera more often. Results also indicated that recognition of peptides by anti-urease antibodies strongly depended on the method of bonding of epitope with cellulose plate and the structure and the length of linker between cellulose matrix and peptides.

Acknowledgement: The study was supported by the Polish State Committee for Scientific Research under the Project 4-T09A 189 25.

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17:40 Poster I-70

AN APPLICATION OF CHIRAL COUPLING REAGENTS FOR ENANTIOSELECTIVE ACTIVATION OF RACEMIC N-BENZOYL-2-HYDROXYMETHYL-2-AMINO ACIDS

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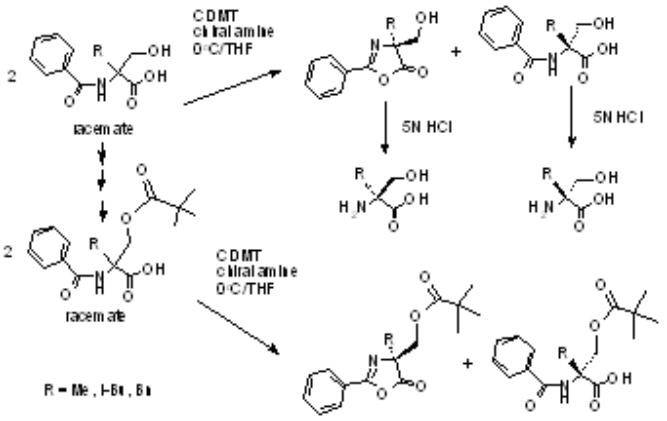
Numerous enantiomerically pure α-substituted serines were isolated from pharmaceutically interesting natural products. Due to the presence of two side chains attached to the α-carbon atom every one enantiomer of chiral α-substituted serines, (chimaeras of serine and other amino acids) belongs simultaneously to L and D family of amino acids. This cause severe problems with an application of enzymes for the resolution of readily accessible racemic α-substituted serines.

Herein, the results of enantioselective activation of racemic α-substituted serines with chiral reagents are presented. In accord to the previous studies chiral coupling reagents (accessible *in situ* by treatment of triazines with chiral tertiary amines) were found very efficient in the enantioselective synthesis of optically active products directly from racemic proteinogenic amino acids [1].



Brucine, strychnine, quinine, quinidine, sparteine, and nicotine were used as chiral auxiliary for preparation of enantioselective coupling reagents. The optical purity of products was determined by HPLC on chiral stationary phase.

In the case of α-methylserine (R=Me) and its O-trimethylacetyl derivative, poor enantioselectivity has been observed for all amines used in this studies.

The study was supported by the Polish State Committee for Scientific Research under the Project 3 T09A 189 25.

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17:40 Poster I-71

SYNTHESIS AND APPLICATION OF 2-IMINOIMIDAZOLIDINES TO THE SYNTHESIS OF NOVEL FUSED HETEROCYCLIC RING SYSTEMS*

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As a part of our research on chemical and biological properties of imidazoline derivatives [1-4], we report the synthesis of 2-(imidazolidin-2-ylideneamino)anilines **3a-d** and [2-(imidazolidin-2-ylideneamino)phenyl]methanol (**7**). Compounds **3a-d** are further subjected to the reaction with carbon disulfide in the presence of triethylamine to give]q
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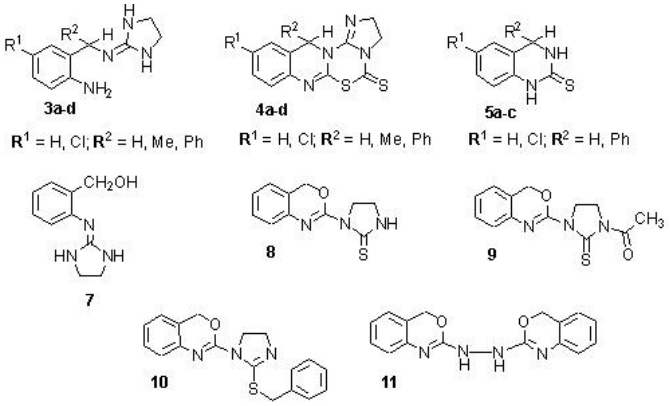
2,3-dihydro-12*H*-imidazo[2',1':4,5][1,3,5]thiadiazino[2,3-*b*n-5-thiones **4a-d** and 3,4-dihydro-1*H*-quinazolin-2-thiones **5a-c**. A similar reaction of **7** with carbon disulfide leads to the

formation of 1-(4*H*-3,1-benzoxazin-2-yl)imidazolidin-2-thione (**8**). Compounds **9** and **10** are obtained by reacting **8** with acetyl chloride and benzyl bromide, respectively. Treatment of **8** with hydrazine hydrate or methyl hydrazine affords hydrazine derivative **11**.



Structures of the compounds **3a-d**, **4a-d**, **7-11** were established by IR, NMR and MS spectroscopic data as well as X-ray analysis of **3a**, **4b** and **10**.

The compounds **4b-d**, **8-9** and **11** were submitted to the US National Cancer Institute (Bethesda) for screening against human tumour cell lines.

*This work was supported by the Polish State Committee for Scientific Research (Grant 6 PO5F 03821).

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17:40 Poster I-72

SYNTHESIS OF A SERIES OF 3-PHENYL-PIPERIDINE-2,6-DIONE DERIVATIVES AS POTENTIAL ANXIOLYTICS

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The class of arylpiperazines have been largely investigated as a group of compounds according to their ability to modify serotonergic neurotransmission via $5HT_{1A}$ receptor interactions.

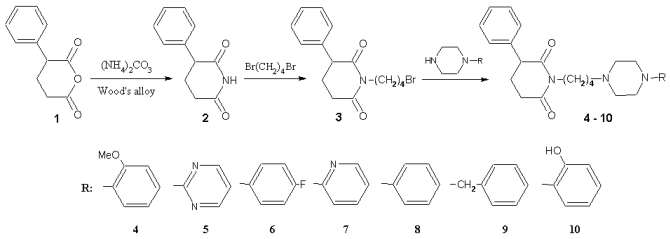
Searching for new chemical substances expected to have an anxiety-relieving character, we have designed a new series of 4-[4-aryl/heteroarylpiperazin-1-ylbutyl) derivatives of 3-phenylpiperidine-2,6-dione **2**, analoges of gepirone [1,2].

By alkylation of compound **2** with 1,4-dibromobutane, 4-(4-bromobutyl) substituted derivative **3** was obtained. Next the latter was condensed with appropriate amines to yield compounds **6-10**.

The structure of all derivatives of compound **2** have been established on the basis of elemental analysis and ^1H NMR spectra.

Compounds **4** and **6** were given to the Department of Crystalography, UMCS in Lublin, for X-ray single-crystal analysis, performed by prof. dr hab. A.E. Kozioł.

Several compounds were given to the Department of Toxicology, The Medical University of Lublin, for pharmacological screenings, performed by prof. dr hab. E. Jagiełło-Wójtowicz.



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17:40 Poster I-73

SYNTHESIS OF SOME N-SUBSTITUTED DERIVATIVES OF 1-(1H-PYRROLE-1-YL-METHYL) - 10-OXA-4-AZATRICYCLO-[5.2.1.0$_{2,6}$]DEC -8-ENE-3,5-DIONE WITH AN EXPECTED ANXIOLYTIC ACTIVITY

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The best anxiolytic drug without side effects is still being investigated. The new generation of anxiolytics are derivatives of buspirone.

Buspirone displays high affinity for the $5\text{-}HT_{1A}$ and D_2 re-

ceptor types and is therefore widely used in the treatment of psychotic and neurotic disordes.

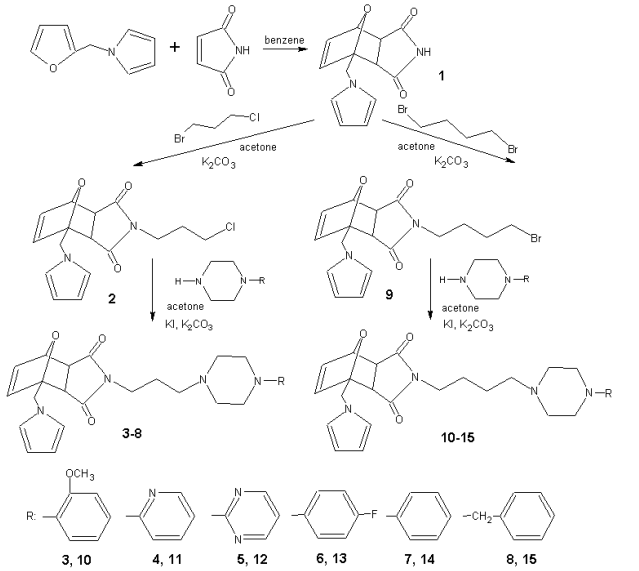
We have synthesized a range of new compounds **3-8** and **10-15**, that are supposed to demonstrate an anxiolytic activity.

Our starting material was isoindole **1** obtained in Diels-Alder reaction of 1-(2-furylmethyl)-1H-pyrrole with maleimide. By alkylation of the isoindole **1** with 1,4-dibromobutane or 1-bromo-3-chloropropane, respectively N-(4-bromobutyl) and N-(3-chloropropyl) substituted derivatives **2** and **9** were obtained. Next the compounds **2** and **9** were condensed with appropriate amines.

The structures of new derivatives of imide **1** have been established on the basis of ^{1}H NMR and elemental analysis.

Compounds **1** was given to the Department of Crystalography, UMCS in Lublin for X-ray single-crystal analysis, performed by prof. dr hab. A. E. Kozioł.

Several compounds will be given to the Department of Toxycology, The Medical University of Lublin for pharmacological screenings, performed by prof. dr hab. E. Jagiełło-Wójtowicz.



17:40 Poster I-74

DEVELOPMENT OF NEW TACHYKININ-OPIOID CHIMERIC ANALOGUES AS POTENTIAL NEW ANALGESIC

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Chimerization of tachykinin and opioid pharmacophores offers a new avenue for analgesic development. The complexities of such design are illustrated by the analgesic efficacy (via different mechanisms) of chimeras that combine pharmacophores with opioid activity and substance P activity, as well as those that combine opioid agonist and substance P antagonist moieties. Although the interaction between substance P and opioid neural systems is more complex than a simple one-way inhibition, the relative balance of activities between tachykinin and opioid pharmacophores will generally determine the net effect of the chimeric molecule as pro-nociceptive, antinociceptive, or neutral. Intriguingly, endomorphins - mu opioid receptor agonists with high intrinsic activity - may owe some of this high activity to weak but significant antagonist properties at tachykinin receptors, implying that these native peptides are endogenous chimeric opioid agonist + tachykinin antagonist compounds. The modification of basic endomorphin I sequence resulted in analogues with increased affinities to tachykinin, especially NK1 receptors.

17:40 Poster I-75

STUDIES ON THE SYNTHESIS AND BIOLOGICAL ACTIVITY OF COLCHICINE ANALOGUES

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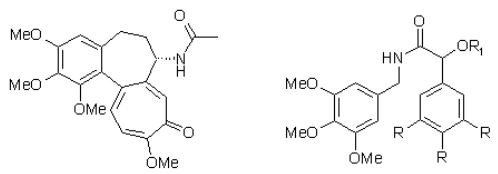
Colchicine (1) is an alkaloid isolated from the dried corns and seeds of *Colchicum autumnale*. It is important to note that colchicine binds to tubulin founded in leucocytes and inhibits the migration of the white blood cells into the inflamed area, causing reduction in pain and inflammation. In the course of our studies a new colchicine analogues, compounds 2 were synthesized. These compounds bears a structural resemblance to colchicine 2 and are potential inhibitors of tubulin polymerization.



General method for the synthesis of compounds 2 will be presented together with biological activity profiles of its selected derivatives.

Acknowledgements

This work was supported by Polish State Committee for Scientific Research, project PBZ-MIN-007/P04/2003.

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DETERMINATION OF OTC IN BIOLOGICAL MATRICES

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Oxytetracycline (OTC) has been used for years in medical field. Chemically, it is 4-(dimethylamino)-1,4,4a,5,5a,6,11,12a-octahydro3,5,6,10,12 ,12a-hexahydroxy-6-methyl-
1,11-dioxo-2-naphthacenacarboxamide. OTC is bacteriostatic agent with broad-spectrum of antimicrobial activity. OTC is used in the treatment of respiratory, urinary and alimentary tract infections, because of its activity, low toxicity and good penetration into tissues [2,3].

This paper presents a simple, highly sensitive, rapid and economical HPLC method for determination of OTC in plasma.

The chromatographic system used was a Varian liquid chromatograph (Varian, Walnut Creek, CA, USA). It consisted of a solvent delivery pump (STAR 9002), a 10 µL volume manual injector, a variable wavelength UV-VIS detector (all Varian Analytical Instruments, USA). Chromatographic separations were performed using a Varian ChromSep HPLC OmniSpher 5 C18 (250 × 4.6 mm) column. A centrifuge (MPW 210), an analytical balance (Sartorius BP 61S), cartridges (Shimadzu C18, 500 mg), a vacuum pump (AGA Labor, Warsaw, Poland), Vortex (WL-1, Bio-mix, Warsaw, Poland), and extraction chamber SPE (Varian, USA, 16 × 75 mm) were also used.

Sample preparation:1 mL of plasma was mixed with 1 mL of methanol. After centrifugation (15 min. at 5500 rpm) the upper supernatant layer was discharged. The solution was diluted to 30 ml using the buffor 0.01 M EDTA-McIlvaine. Next following reagents were applied to a cartridge (in reported sequence): methanol, deionized water, buffor, an analyte and buffor. After this analyte was eluted using ACN-buffor 0.01M EDTA-McIlvaine solution. Sample was evaporated and redissolved in mobile phase, and the analysis was preformed by HPLC. A mobile phase consisted of ACN-MeOH-$(HCOO)_2$ (17.5/17.5/65, v/v/v) (pH=2) was pumped at a flow rate of 1.4 mL/min. The variable wavelength UV detector was set at 280 nm [1,4]. The method was validated by the determination of the following parameters: linearity (r=0.999) precision and accuracy (n=4), limit of detection (LOD=3.58 ng/mL) and limit of quantification (LOQ=11.93 ng/mL). The equation for the curve was
y = 55.035 x + 28.875. The calculated standard deviation (SD) was 1.40 ng/mL and relative standard deviation (RSD) was 1.39 %. The calculated of OTC recovery was 92.50%.

Described HPLC method for the measurement of OTC in plasma was fully validated and showed good sensitivity, reproducibility, linearity and selectivity. This makes it valuable and adequate in many applications, particularly in veterinary medicine studies. Other authors determined residues of tetracyclines (including oxytetracycline) in animal tissues, milk and cheese using HPLC method. According to our best knowledge the recovery has never reached the level of 90%. As was already mentioned the recovery was 92.50%.

It can be concluded that the developed method in the present study can be successfully applied for analysis of OTC in plasma.

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17:40 Poster I-77

PROBIOTICS - AN ALTERNATIVE FOR ANTIBIOTICS?

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Probiotics are defined as the viable microorganisms that exhibit a beneficial effect on the health of the host by improving its intestinal microbial balance. Probiotic strains, which are commonly use include *Lactobacillus sp. (L. johnsonii, L. acidophilus, L. casei*), *Bifidobacterium* (*B. bifidum, B. infantis B. animalis)* and the only fungal strain *Sacharomycesboulardii*. Following FAO recommendations probiotic strains must be able to be manufactured under industrial conditions and have to survive and retain their functionality during storage of the products: genera of the human or animal origin; stability against bile, acid and enzyme; non-pathogenicity and antibiotic resistance characteristics; ability to adhere to intestinal mucosa; colonization potential in gastrointestinal tract; production of antimicrobal substances and demonstrable efficacy and safety [1].

During the past ten years, a large number of extensive sci-

entific researches have been carried out to demonstrate the beneficial effects of probiotic lactic acid bacteria both in humans and specially domestic animals. The main reported beneficial effects including: antimicrobial activity, enhanced immune system, balancing of colonic microbiota, reduction of fecal enzymes involved in cancer initiation, treatment of diarrhoea associated with antibiotic therapy, control of colitis and prevention of ulcers related to *Helicobacter pylori*.

There are different mechanisms: the competition for substrates, the prevention of pathogen adherence by specifically competing for the same receptor sites on the epithelium or mucus, or alternatively by providing an aspecific steric hindrance as a barrier to mucosal colonization, an enhancement of the host immune response against pathogens, the production of antimicrobial substances (inhibitory metabolites like organic acids (*e.g.* formic acid), ethanol, carbon dioxide, diacetyl, hydrogen peroxide; bacteriocins, bacteriocin-like, or non-bacteriocin substances) to remove pathogens from the intestine [2].

In addition, bacteriocins produced by lactic acid bacteria are in general active towards Gram-positive bacteria, but when the structure of their cell surface is disturbed, also Gram-negative bacteria can become sensitive. On the other hand, several probiotic strains produce antibacterial, acidic, low-molecular-mass, heat-stable, non-proteinaceous compounds with an inhibitory spectrum including both Gram-positive and Gram-negative bacteria [3].

However, the challenge for the food and pharmaceutical industries will be to conduct well-designed, multidisciplinary and multicenter clinical investigations to ascertain the health benefits or therapeutic efficacy of selected lactobacilli and bifidobacteria strains to be used as functional additives or biotherapeutic agents [4].

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17:40 Poster I-78

BIOTECHNOLOGICAL APPROACH TO THE SYNTHESIS OF IMPORTANT DRUG INTERMEDIATES

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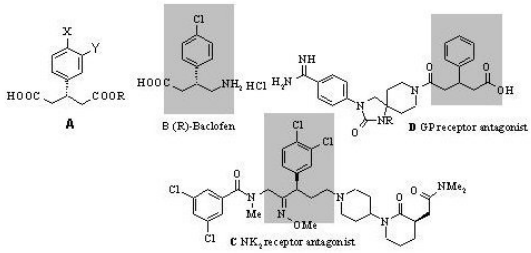
Recently we have focused our attention to the synthesis of optically active 3-arylglutaric acid derivatives **A**, which are important building blocks in synthesis of a number of biologically active compounds and drugs. The most prominent examples are (*R*)-Baclofen (**B**) - a selective $GABA_B$ receptor agonist and G-protein coupled NK-receptors antagonist **C**. Intensive search for a new glycoprotein antagonist led to compound **D** bearing 3-phenylglutaric unit and possessing improved pharmacokinetic properties.



Biologically active compounds possessing 3-arylglutaric unit.

The results of our studies on applications of native and functionalized enzymes for the key biotechnological biotransformation leading to 3-arylglutaric acid derivatives A will be presented. The possibilities to extend this methodology on other drug intermediates will be discussed and some preliminary results will be presented.

Acknowledgements

This work was supported by Polish State Committee for Scientific Research, project PBZ-MIN-007/P04/2003.

17:40 Poster I-79

EFFICACY AND SAFETY OF BORTEZOMIB (VELCADE) IN THE TREATMENT OF RELAPSED MULTIPLE MYELOMA

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Background: The proteasome is a multi-enzyme complex that provides the main pathway for degradation of intracellular proteins in eukaryotic cells. Proteasome - mediated proteolysis has been shown to play a key role in the regulation of cell-cycle progression, genomic transcription, apoptosis, chemotaxis, cell adhesion and angiogenesis. Bortezomib (Velcade), a boronic acid dipeptide, is a novel, potent, selective, and reversible inhibitor of the proteasome that has been shown to have antimyeloma activity.

Methods: We enrolled 5 patients (2 female and 3 male, in age 35, 50, 64, 65, 70 years) with relapsed multiple myeloma

who have failed at least two prior lines of treatment, including 2 patients treated with high dose therapy and autologous stem cell transplantation. The time since myeloma diagnosis to onset of bortezomib therapy in particular patients was 27, 27, 28, 84, 125 months. Patients received bortezomib 1.3 mg/m^2 as an 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 (18 weeks). In 2 patients with progressive disease dexamethason was added to the regimen.

Results: In one patient a near - complete (immunofixation-positive) response was achieved. The time to response was 63 days and the duration of response was 180 days. Three patients had stable disease and 1 patient had progressive disease while receiving bortezomib. Six months after completing bortezomib therapy all 5 patients are alive, 4 with progressive disease and 1 with stable disease. The median time to progression of disease was 7 months. Adverse events: treatment with bortezomib was withheld from 2 patients (after 2 and 4 cycles, respectively) because of skin lesions (erythema multiforme) and aggravation of peripheral neuropathy. Side effects seen in the study included also pyrexia, infections, nausea, vomiting, abdominal pain, pain in limb, hypotensio, thrombocytopenia.

Conclusions: In relapsed myeloma the rate of response to bortezomib alone is 20 percent, with a duration of response of 6 months.

17:40 Poster I-80

SYNTHESIS AND ANTIBACTERIAL ACTIVITY OF DL-THREO-1-(1-METHYL-4-NITRO-PYRROLE-2-YL)-2-DIFLUOROACETAMIDO-PROPANE-1,3-DIOL

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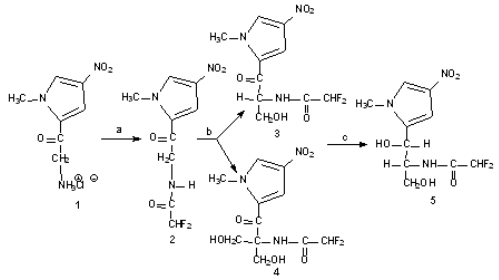
Among over 2000 synthesized compounds of the structure similar to chloramphenicol [1], only three found use in therapy: thiamphenicol, azidamphenicol and florfenicol. The heteroaromatic analogs of chloramphenicol are insignificantly active or inactive. The only exception is the thiophene analogue with the activity of 50% in relation to the racemic chloramphenicol.

Pyrrole analogs of chloramphenicol are known to exhibit the selective significant activity only against strains from the *Enterobacteriaceae* family and high activity against Gram-positive and Gram-negative bacteria [2,3].

The synthesis of comound **5** was performed according to the procedure showen in the scheme below.



Reagents and conditions**: (a)** CHF_2COOH, $SOCl_2$, Et_3N, temp. 0 ^oC, THF; **(b)** HCHO, $NaHCO_3$, EtOH; **(c)** $(i\text{-PrOH})_3Al$, i-PrOH

The structure of compounds **2**-**5** were confirmed by means of ^1H NMR and ^{13}C NMR spectrometry. Compound **5** (DL-*threo*) presents insensibility antibacterial activity.

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17:40 Poster I-81

EVALUATION OF ANTIMICROBIAL ACTIVITY OF SELECTED NON-ANTIBIOTIC DRUGS

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A variety of pharmaceutical preparations, which are applied in the management of the non-infectious diseases, have shown in vitro some antimicrobial activity. These drugs are called "non-antibiotics". So far, a lot of attention has been focused on phenothiazines, thioxanthenes and other agents with affinities to cellular transport systems. Some authors confirmed that non-antibiotic compounds enhance the in vitro activity of certain antibiotics against specific bacteria, for instance nizatidine and omeprazole enhance the effect of metronidazole on Helicobacter pylori. The search of "non-antibiotics" among drugs, distributed at Polish pharmaceutical market have been performed in Drug Institute for several years. The aim of this study was to detect and characterise the antimicrobial activity of non-antibiotic drugs, recently analysed in National Institute of Public Health. Over 180 of pharmaceutical preparations were randomly chosen from different groups of drugs. The surveillance study was performed on standard ATCC microbial strains used for drug control: Staphylococcus aureus, Escherichia coli, Pseudomonas aeru-

ginosa and Candida albicans. It was shown that the drugs listed below inhibited growth of at least one of the examined strains: Abutol 200 mg tabl. (acebutolol), Acecor 400 mg tabl. (acebutolol), Amlopres 5 mg, 10 mg tabl. and Normodipine 10 mg tabl. (amlodipine), Cipramil 20 mg tabl. (citalopram), Clodron saure 400 F tabl. (clodronate), Coordinax 1 mg/ml oral susp. (cisapride), Cuprenil 250 mg tabl. (penicillamine), Dilzem ratard 120 mg tabl. (diltiazem), Fenactil 4% solution (chlorpromazine), Fitoprost 150 mg caps. (Serenoae repens fructus extr.), Gastrografin 760 mg /ml (amidotrizoic acid), Ginsepan tabl. (15 mg ginsenosides - Papax ginseng), Metocard 100 mg tabl (metoprolol), PectoDrill 750 mg tabl. (carbocysteine), Perfenil 8 mg tabl. (perphenazine), Phloderm 0,3% ointment (hamamelitanine from Hamamelis sp cortex extr.), Polfenon 300 mg tabl. (propaphenone), Prostamol uno 320 caps. (Sabalis serrulate extractum), Sedalin 35 mg tabl. (acepromazine), Tetryvil 0.05 %, 0.1% nose drops (tetrahydrozoline), Tisercin 100 mg tabl. (levomepromazine), Ulfamid 20 mg tabl. (famotidine) and Velafax 75 mg tabl. (venlafaxine). Staphylococcus aureus was susceptible to over 70% of the drugs listed above. Tetrahydrozoline and amlodipine inhibited growth of S. aureus in concentrations 0.05 and 3 mg/ml, respectively. Other chemical compounds showed activity against this microorganism in concentrations between 5 and 100 mg/ml. Famotidine in concentration 2 mg/ml showed the strongest activity against E. coli. Pseudomonas aeruginosa was resistant to the most of examined chemical substances, except: cisapride, penicillamine and amidotrizoic acid (MICs: 0.05, 62 and 76 mg/ml respectively). C. albicans showed the strongest sensibility to chlorpromazine and diltiazem (MICs: 20 and 26 mg/ml respectively). Amidotrizoic acid showed activity against all examined strains (MIC: 38-76 mg/ml). Interesting activity was showed for natural product extracts. Extracts from Serenoae repens inhibited S. aureus (MIC: 6 mg/ml), extract from Sabalis serrulate inhibited C. albicans (MIC: 13 mg/ml) but extract from Hamamelis sp in concentration 1 mg/ml inhibited growth of three strains: S. aureus, E. coli and P. aeruginosa. The antimicrobial activity of non-antibiotic drugs emphasises a necessity of the neutralisation of their activity during the microbial purity assays of pharmaceutical products.

17:40 Poster I-82

DETERMIANTION OF LIPOPHILICITY OF THE IMIDAZO-, PYRIMIDO-, AND DIAZEPINOPURINEDIONS - ADENOSINE RECEPTORS LIGANDS

Anna Drabczyńska[1], Kamil J. Kuder[1], Miriam Fast[2], Christa Müller[2], Elżbieta Pękala[1], Britta Schumacher[2], Silvia Scolari[2], Katarzyna Kieć-Kononowicz[1]

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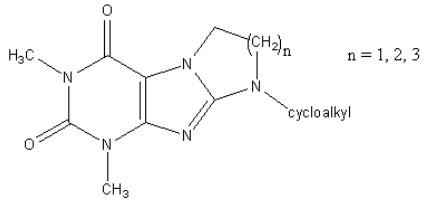
Adenosine receptors ligands are currently being developed as promising agents for CNS disorders (Parkinson's, Alzheimer's, morbus, ischaemia) [1].

The lipophilicity is a parameter regarded as an important factor significantly influencing CNS bioavailability.

Searching for new selective ARs ligands, we have synthesized tricyclic derivatives with imidazo-, pyrimido- and diazepino annelated ring and *N*-cycloalkyl moiety.



The obtained compounds were bioassayed for the affinity towards adenosine A_1 and A_{2A} rat receptors in *in vitro* binding tests, showing A_{2A} selectivity.

In aim to evaluate the structure-bioactivity relationships, we determined their lipophilicity expressed by R_{M0} values using planar RP-TLC method. The theoretical partition coefficient parameters (logP) were also calculated using computer programs: HyperChem, PALLAS, CAChe Project Leader, SciLogP for Alchemy [2]. The correlation between parameters of lipophilicity (R_{M0}, logP) and activity K_i was examined.

Supported in part by Polish State Committee for Scientific Research (Grant No 2P05F02226)

References:

[1]. S. Hess; Recent advances in adenosine receptor antagonist research. *Expert. Opin. Ther. Patents***2001**, 11, 1-29

[2] HyperChem 7.5 by Hypercube Inc., PALLAS 3.2.2.1 (demo version) by CompuDrug, CAChe Project Leader 6.1 by Fujitsu Ltd., SciLogP 3.0 for Alchemy (demo version) by SciVision

17:40 Poster I-83

HISTAMINE H_3 RECEPTOR ACTIVITY OF 4-N-SUBSTITUTED PIPERAZINE-1-N-ALKYL ETHERS

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Histamine H_3 receptor is constitutively active G_i - protein coupled receptor mostly expressed in central nervous system (CNS), and described as a presynaptically located auto- and heteroreceptor. Blockade of this receptor leads to increased levels of histamine and other neurotransmitters such as: Ach, NA, 5-HT in CNS. Therefore histamine H_3 receptor antagonists may provide novel therapies for treating such CNS diseases as: Alzheimer disease, ADHD, dementia, epilepsy, obesity [1].

First known histamine H_3 receptor antagonists contained imidazole group, which could cause a number of side effects mostly because of cytochrome P_{450} interactions. On the other hand imidazole derivatives may tend to have low bioavailability. Among other proposed, piperazine moiety has been shown to be a suitable bioisosteric replacement of an imidazole moiety. Since prior researches described mainly 4-*N*-acylated piperazine derivatives of Ciproxifan [2], compounds (Fig. 1) were designed.

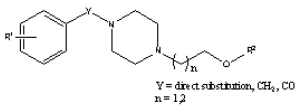

Fig.1

The aim of this work was to synthesize (un)substituted 4-*N*-benzoyl- or aryl(alkyl)-piperazine-1-*N*-alkyl derivatives (Fig.1) to study the influence of 4-*N*-piperazine substituents, alkyl chain length and (cyclo)alkyl or arylalkyl lipohilic residue on histamine H_3 receptor activity. Compounds were obtained with microwave oven method, using proper (un)substituted *N*-piperazinepropan-, or ethan-1-ol derivatives as starting material and *via* modified Williamson synthesis in KOH/DMSO system.

The novel compounds were evaluated for the histamine H_3 receptor activity *in vitro* in a binding assay for the human histamine H_3 receptor stably expressed in CHO-K1 cells. Two of the tested compounds showed good antagonist activity at the histamine H_3 receptor.

For the series of compounds physicochemical properties such as lipophilicity by means of logP values and distribution coefficient (logD) were predicted using computer programs [3]. As there is a strong need to describe the ADME parameters for the compounds in the early stages of research, for the representative compounds metabolites were predicted using the program METEOR 6.0.0. [4].

[1] Stark, H. *et al*. Mini Rev. Med. Chem. 2004, 4, 965-977; Cowart, M. *et al*. Mini Rev. Med. Chem. 2004, 4, 979-992

[2] Esbenshade, T. A. *et al*. J. Pharm. Exp. Ther. 2003, 305, 887-896

[3] PALLAS ver. 3.1 demo, CompuDrug; CAChe ProjectLeader 6.1, Fujitsu Ltd.;

[4] METEOR ver. 6.0.0, Lhasa Ltd

17:40 Poster I-84

DICARBOXYLATOPLATINUM(II) COMPLEX - SIX TIMES LESS TOXIC THAN CARBOPLATIN

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In continuation of our search for higher generation platinum drugs, we examined a series of complexes, well soluble in water, weakly reactive with glutathione and resistant against hydrolysis. Such properties are expecteded to result in lower toxicity of these compounds.

There was obtained a group of dicarboxylatoplatinum(II) complexes of general formulae:[Pt(A)2(X2)] or [Pt(A2)(X2)], where A = monodentate (or A2 - bidentate) amine and X2= dicarboxylate dianion.

As amine neutral ligands there were used: ethylenediamine, 1-ethylimidazole, 1-propylimidazole. In this way we synthesized two kinds of complexes, "classical" having NH2 donor group, and "non-classical" having tertiary donor nitrogen atom. Considering our earlier studies [1], we expected that new "non-classical" cisplatin analogs may be the source of drugs overcoming the resistance of cancer cells.

As anionic ligands there were introduced dianions originating from acids which differ significantly in their hydrophobic properties: (L-,D-, or DL-) malato-, 2-oxo-glutarato- and 2-etoxyglutarato- ligands should cause the modification of pharmacokinetic properties of complexes and thus deliver the broader and more varied samples for testing.

The antiproliferative effects were determined by SRB and MTT assays [2] against human neoplastic cell lines such as: A549 (nono-small cell lung carcinoma), SW707 (rectal adenocarcinoma), HCV29T (uroepithelial cancer), T47D (breast cancer). On the base of results obtained from in vitro studies there were pre-selected five malatoplatinum complexes for in vivo toxicity evaluation. Toxicity (subacute toxicity, three-week observation) was measured as lethal dose [LD50] and as mouse mortality [%] in dependence on dose of platinum compound. It was shown that four compounds revealed lethal toxicity similar to carboplatin (referential drug), whereas the one, bis(1-ethylimidazole)L-malatoplatinum(II), was six times less toxic than carboplatin.

Concluding, the new synthesized dicarboxylatoplatinum(II) complexes seem to be very promising group of potentially antitumor active carboplatin analogs, in particular with regard on its unusually low toxicity and "non-classical" chemical character among platinum drugs.

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Acknowledgement: Authors thank for financial support by KBN-Pr UE/DIE 621/2004

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17:40 Poster I-85

EVALUATION of p53, Bax, Bcl-2 and hMLH1 EXPRESSION in OvBH-1 CELL LINE TREATED WITH A NEW PLATINUM-BASED REGIMEN

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Platinum-based antitumour agents play critical role in the treatment of ovarian carcinomas. The initial response rate is high but one-third originally responsive patients develop chemoresistance during the course of their treatment. This gives an incentive to seek other Pt complexes with lesser side effects and strong disease-oriented action. Such requirements seem to be fulfilled by the hydroxydicarboxylatoplatinum complexes which are the object of our study: ethylenediamine(L-malato)platinum(II), (Pt-en-L-mal), and bis(1-ethylimidazole)(L-malato)platinum(II), (Pt-etim-L-mal).

The present study was undertaken to examine the influence of cisplatin and new cytotoxic platinum complexes on expression of multi-drug resistant proteins (P-gp, MRP, LRP), apoptosis related proteins (Bax, p53, Bcl-2) and mismatch repair gene product (*hMLH1*) in OvBH-1 cell line.

The expressions of all proteins were assessed and compared on OvBH-1 cells, before and after treatment with drugs, by immunohistochemical staining using monoclonal antibodies. The results of immunohistochemical study show the big differences in the action of platinum species. In particular, only exposure to cisplatin increased expression of P-gp, MRP and LRP whereas the expression of apoptosis related protein, Bcl-2, was strongly stimulated only by Pt-etim-L-mal. However, the nuclear accumulation of p53 was found in a similar percentage of all treated and untreated cells. Finally, the above facts together with observation of big and different influences of platinum species on *hMLH1* expression may indicate that, in OvBH-1 cells, some of examined drugs activate the DNA mismatch repair mechanism on the way independent on p53 expression. The presented results will be confronted with the settlements from the study of biological properties of malatoplatinum complexes on cellular and DNA levels.

Using AFM technique we studied and imagined the influence of platinum drugs on the surface of OvBH-1 cells. It was found that the surface relief of ovarian cell membrane in nanometer scale differs from the membrane of platinum-treated cells. We also could notice the differences between action of cisplatin and carboplatin on the cell surface which might dependent on P53 status in OvBH-1 cell line. The differences seem to be a result of various changes caused by these drugs in membrane protein structure.

Acknowledgement. The financial support, as a grant for V.A. F., by KASA im. J. Mianowskiego - in Warsaw (Poland) is greatly appreciated.

17:40 Poster I-86

PRELIMINARY EVALUATION OF CNS EFFECTS OF ARYL- AND HETEROARYLPIPERAZINYL DERIVATIVES OF SELECTED ARRANGEMENTS AZATRICYCLOUNDECANE

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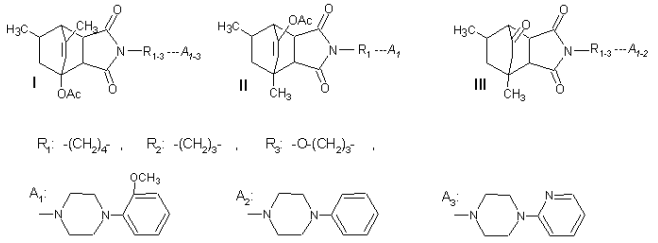
The anxiolytics of new generation, like tandospirone and bus-

pirone, exhibit their therapeutic properties through activation of 5-HT_{1A} subtype of serotonin receptors.

Many analogs have been synthesized and those that contain the 4-aryl/heteroaryl-piperazinylalkyl group attached to a cyclic imide, have demonstrated an anxiolytic and antidepressive activity.

Recently we obtained new derivatives of the above agents with 4-aryl/heteroaryl-piperazinylalkyl group attached to a complex imide systems which synthesis has already been reported [1].



All the amine derivatives were converted to hydrochlorides and evaluated in vitro for their affinity for 5-HT_{1A} and 5-HT_{2A} receptors. The tested compounds showed high affinity for 5-HT_{1A} (K_i ranged from 10 to 115 nM) and lower affinity for 5-HT_{2A} (K_i > 181 nM).

Since it is well established that 5-HT_{1A} ligands possess anxiolytic and antidepressive properties, ten selected compounds were further examined in some behavioural tests in mice.

The investigated new derivatives of selected arrangements azatricycloundecane were administered intraperitoneally (ip) as suspensions in 1% Tween 80 in a constant volume of 0.1 ml/kg. Control groups were given appriopriate amounts of the solvent. Of all the investigated compounds only 1-(2-methoxyphenyl)piperazinylbuthyl derivative of imide I produced antidepressive and antiserotoninergic effects. In the remaining pharmacological tests none of the investigated derivatives did not produce statistically significant effects.

1. J. Kossakowski, B. Kuran, Annals of the Polish Chem. Society, (2003) 284-8

17:40 Poster I-87

SYNTHESIS OF CONFORMATIONALLY CONSTRAINED ARYL- OR HETEROARYLPIPERAZINYL DERIVATIVES OF SELECTION IMIDES AS 5 HT RECEPTOR LIGANDS.

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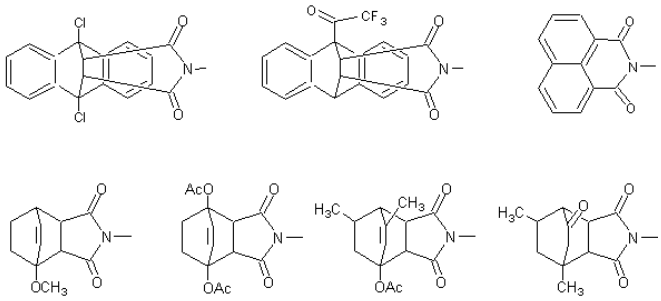
The present work describes synthesis of aryl- or heteroarylpiperazinyl derivatives of selected imides with constrain alkyl chain.

Last years studies shows that chain constraintion has significant influence on the serotonin receptors affinities [1, 2].

This work is continuation of our studies in searching of compounds with an anxiolytics and antidepressive activity.

New compounds were obtained by using cis- and trans-1,4-dichloro-2-butene and 1,2-bis(chloromethyl)benzene and amines: 1-(2-methoxyphenyl)piperazine, 1-(2-pyrimidyl)piperazine and selected imides:



The structures of all compounds were established by elementary and spectroscopic analysis.

1. A. J. Bojarski et al. J. Bioorg. Med. Chem. Lett. 14 (2004) 5863-6

2. A. J. Bojarski et al. Bioorg. & Med. Chem. Vol.14 Is.5 (2006) 1391-1402

17:40 Poster I-88

THE COMPARISION OF ACTION OF NOVEL POTENTIAL ANTIDIABETIC DRUGS (AD10 369, AD10 371, AD10 797, AD10 798 AND AD10 1025) ON PLASMA LIPIDS AND GLUCOSE LEVELS IN db/db MICE.

Krzysztof Kurowski[1], Urszula Bulkowska[1], Katarzyna Matusiewicz[1], Rafał A. Derlacz[2], Sylwia Rybkowska[1], Monika Stupak[1]

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Thiazolidinediones, synthetic ligands for the peroxisome proliferator-activated receptor-g (PPAR-g) are antidiabetic drugs which improve insulin resistance and ameliorate consequences of metabolic syndrome. Therefore in the present study we investigated the metabolic response to novel PPARg agonists. These compounds were chosen from the library of 8000 molecules designed by Organic Synthesis Laboratory, Adamed Ltd. according to competition binding test with the recombinant PPARg Ligand Binding Domain and adipogenic potential performed on mouse embryonic fibroblast 3T3 L1

line.

We measured the effects of orally administrated novel PPARg agonists (10 mg/kg body weight for two weeks): AD10 369, AD10 371, AD10 797, AD10 798 and AD10 1025 on glucose, triglyceride, total cholesterol, LDL and HDL plasma levels in *db/db* mice - an animal type 2 diabetes model. Additionally we estimated the effect of novel PPARg agonists on *de novo* glucose and lactate synthesis in primary cultured renal tubule cells isolated from rabbits and growing in hormonally defined medium. The metabolic action of novel PPARg agonists were compared with the effect of rosiglitazone - a compound licensed for use in selected diabetes patients.

17:40 Poster I-89

SEARCH FOR NEW ANTIMICROBIAL PEPTIDES FROM HYDROLIZATES OF SEED PROTEINS.

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Plants are constantly exposed to a large array of pathogenic organisms and the survival in these conditions demands quick defense responses which include the synthesis of defense peptides and proteins with antimicrobial properties. Among these compounds several low molecular weight proteins or peptides with antibacterial or antifungal activity have been isolated in recent years from various plants and are believed to be involved in a defense mechanism by inhibiting microorganisms growth through diverse molecular modes.

The present study has been focused to isolate small proteins/ peptides for antimicrobial activity from the plants of *Aesculus hippocastanum* and *Sylibum marianum*. The hydrolysates of seed proteins were prepared by using neutral proteases and antimicrobial active fractions were isolated. Low molecular weight peptides generated by cleavage of seed proteins from studied plants showed broad-spectrum antimicrobial activity, inhibiting the growth of bacteria such as Staphylococcus aureus, Pseudomonas aeuroginosa, Escherichia coli and fungi such as Candida albicans.

These antimicrobial proteins in the present study may have commercial value as a natural preservative agent for use in foods and cosmetics and may be used for agricultural or pharmaceutical applications.

17:40 Poster I-90

HYDROLISATE OF PIG SPINAL CORD AS A NUTRACEUTIC IN MULTIPLE SCLEROSIS.

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Recent studies of gut-blood barrier have showed that short peptides of food proteins are transported to the blood easier than aminoacids. Special system presenting antigens in gut is formed. This process presenting food peptides and suppressing of immunological respons to them is called oral tolerance. Recently, it has been proposed to apply it to the treatment of autoimmune diseases as multiple sclerosis, rheumatoid athritis, uveitis and diabetes type 2. The aim of our study was to use hydrolisate of pig spinal cord proteins which is the mixture of neuropeptides obtained as a result of hydrolisis of an undenatured homogenate of proteins as an antigen for feeding experimental animals/rats/.After induction of oral tolerance, animals were immunised by injection of guine pig spinal cord homogenate with complete Freund s adjuvant to evoke experimental allergic encephalomyelitis /EAE/, which is an animal model of multiple sclerosis. Clinical course was observed, histopathological study, ultramicroscopic study and metaloproteases determination in the brain was done.Results. Clinical course of EAE post hydrolisate treatment has been milder than control one. MBP and TNF alfa in the brain were decreased. Metaloproteases increased in EAE, after hydrolisate treatment were diminished by 30 %. Some changes in blood brain barrier/BBB/ as opened tight junction and other changes in early phase of EAE as karioscelетal damage, compartmentalisation of the endoplasmic reticulum, large cisterns of the Golgi apparatus, increased activity of microglial cells with numbers of phagolisosomes, desorganisation of sheets of myelin, neoangiogenesis of parenchyma of the cerebral cortex , have been dimished. Above results indicate on possible clinical use of oral tolerance induced by pig spinal cord hydrolisate as a supportive treatment of multiple sclerosis.

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CHARACTERIZATION OF ARIPIPRAZOLE SOLVATE WITH ETHANOL AND POLYMORPHIC FORMS PRODUCED DURING ITS HEATING

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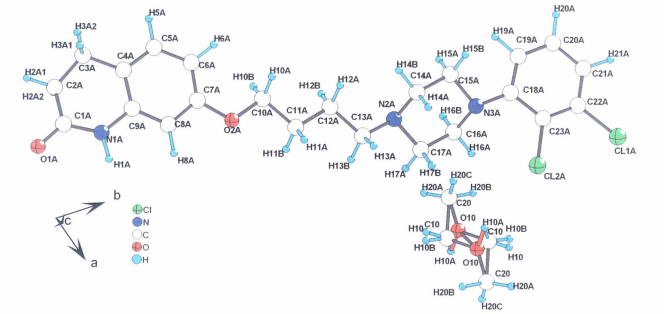
Aripiprazole (7-[4-[4- (2,3-dichlorophenyl) piperazin-1-yl] butoxy]-3,4- dihydro- 1H quinolin-2-one) is an anti - psychotic drug.

In this work a solvate with ethanol of Aripiprazole ($2(C_{23}H_{27}Cl_2N_3O_2)\cdot C_2H_5OH$) was fully characterized by means of the single crystal and the powder X-ray diffraction [1]. The studies revealed that the solvate crystallizes in space group P-1, Z=2 with unit cell parameters: *a*=7.796(2), *b*=10.694(4), *c*=15.608(7) Å and a=84.61(3), b=80.35(3), g=73.43(3)^{o}. The ethanol molecule is incorporated in dimmers of Aripiprazole between chloro-derivative aromatic rings.

Thermal analysis and IR techniques additionally confirmed a presence of the ethanol molecule in Aripiprazole. XRPD, IR techniques revealed that the polymorph I can be obtained by a heating the solvate at the temperature of 100 ^{o}C but DSC analysis showed a presence of another form dash - the polymorph II [2]. Probably a quantity of polymorph II was too small for XRPD and IR detection. Further heating, up to 145 ^{o}C (temperature between two endo- peaks on a DSC curve), brought about a growth of polymorph II seeds what was observed by XRPD, IR and DSC techniques.



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17:40 Poster I-92

NONNATURAL D-AMINO ACIDS AS A BUILDING BLOCKS OF NEW PEPTIDOMIMETICS

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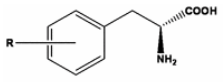
Peptidomimetics are the molecules bearing identifiable resemblance to peptides, that as ligands of biological receptors can imitate or inhibit the effect of natural peptides. Many of the D-amino acids can be incorporated in place of the natural L-amino acids, either at a specific position, or throughout the whole peptide to increase peptides stability to ward proteases. Nonnatural amino acids may also increase *in vivo* half life time and potency of peptides[1,2]. Because of the non-polar nature and steric bulkiness of its side chain phenylalanine is one of the preferred amino acids in peptidomimetics. Such bioactive peptides as: third-generation modified GnRH antagonist (Cetrorelix), antibiotics (Bacitracine A, Gramicidine S), synthetic somatostatin analogue (Octreotide) are containing D-phenylalanine and its derivatives [3,4]. Predicting that pharmaceutical demand in this field may expand we have obtained following D-phenylalanine derivatives using enzymatic hydantoinase method:



R= 2-Br, 3-Br, 4-Br,
2-F, 3-F, 4-F,
2-CH_3, 3-CH_3, 4-CH_3, 2,4-CH_3

Hydantoinase method consist of three steps: **1.** chemical and/or enzymatic racemisation of D,L-5-substituted hydantoins, **2.** opening of the hydantoin ring to the N-carbamoyl-D-amino acid by D-hydantoinase (E.C.3.5.2.2), **3.** converting of the N-carbamoyl-D-amino acid to the D-amino acid by diazotization or by the next enzymatic reaction catalysed by N-carbamylase (E.C.3.5.1.) [5]. D,L-5-substituted hydantoins were obtained as the result of the appropriate aldehydes and hydantoin Knoevenagel condensation, followed by arylidene bond reduction.

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LIPOPHILICITY OF ANTITUBERCULOTIC 5-ARYLIDENE DERIVATIVES OF (THIO)HYDANTOIN EVALUATED IN SILICO AND BY MEANS OF RP-TLC

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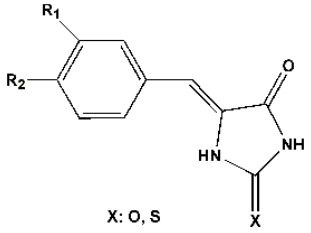
Tuberculosis (TB), since the introduction to the therapy streptomycin and other several anti-TB agents, is curable disease. However, nowadays it is estimated that one third of the world's population is infected with TB which could progress to active disease within their lifetime. It is also observed the development of "drug resistance" forms of the disease (especially "multi-drug resistant" TB). That is why the new selective anti-TB drugs are necessary [1].

Lipophilicity (log *P*), one of the factors determinating the amount of drug reaching the place of its action, can be evaluated by the traditional "shake-flask" method or by chromatographic techniques (RP-HPLC or RP-TLC). Reversed phase thin layer chromatography (RP-TLC) is very convenient and speed method for use.

The tested 5-arylidene-(thio)hydantoin derivatives showed different *in vitro* activity against *Mycobacterium tuberculosis* from 0% to 97% inhibition as reported earlier [2, 3].



Lipophilicity of these compounds and TB-drugs (*Isoniazid* and *Thioacetazone*) was evaluated on silica gel RP-18 F_{254s} plates. A mixture of acetone and water with acetone content in the range 60-80% were used as mobile phase. The values of the experimental lipophilicity R_{M0} *for hydantoin derivatives were in the ranges 2.475 to 4.263 and much lower for* Isoniazid (1.273) and *Thioacetazone* (0.667).

The partition coefficient log *P* was also calculated by means of computer programs (exp. KnowWin, ClogP, Interactive analysis, ChemDraw and Ched). The calculated values of lipophilicity (log P_{cal}*) were mostly lower than the experimental* R_{M0} values.

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This work was partly supported by grant no. BBN 501/P/185/F.

17:40 Poster I-94

ANALYTICAL DETERMINATION OF HOMOCYSTEINE AND RELATED AMINOTHIOLS IN HUMAN PLASMA BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY WITH UV DETECTION

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Elevated levels of plasma homocysteine (Hcy) are associated with increased risk of cardiovascular disease though it is uncertain whether increases in Hcy represent a cause or a consequence of the disease process. Plasma Hcy exists in reduced, free oxidized, and protein-bound forms, that together comprise total Hcy (tHcy). Free reduced Hcy is thought to be the atherogenic, though minor, sub-fraction of tHcy.

We present a highly selective and sensitive method for the determination of total and free reduced homocysteine and related aminothiols that play important roles in health and disease. The key step in the analysis is derivatization with 2-chloro-1-propylpyridinium iodide followed by separation from other thiols derivatives by high-performance liquid chromatography (HPLC) with detection at 312 nm.

Homocysteine is clearly separated from other thiols, the retention time being 6,23 min, total analysis time is 10 min. To determine total homocysteine it is necessary to cleave disulphide bonds by the use of tris(2-carboxyethyl)phosphine hydrochloride in order to form free sulfhydryl group.

The described method has several advantages: simple sample preparation procedure, simultaneous determination of low-molecular-mass aminothiols during the procedure, fast chro-

matography procedure, simple and prevalent mobile phases .

Our method was used in routine clinical analyses of homocysteine in plasma in patients with acute myocardial infarction.

Keywords: L-homocysteine; L-cysteine; gluthatione; HPLC; UV; CMPI;

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SYNTHESIS AND HYPOLIPIDEMIC ACTIVITY OF ASARONE ANALOGS IN MALE AND FEMALE RATS

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A series of alpha-asarone analogs were synthesized and tested for hypolipidemic activity (against Clofibrate as a reference drug) in male and female rats. Compounds 5-((1E)-but-1-enyl)-1,2,3-trimethoxybenzene (7), 5-((1E)-okt-1-enyl)-1,2,3-trimethoxybenzene (11), 2,6-dimethoxy-4-[(1E)-prop-1-enyl]phenyl nicotinate (21), 2,6-dimethoxy-4-[(1E)-pent-1-enyl]phenyl nicotinate (22) and {2,6-dimethoxy-4-[(1E)-pent-1-enyl]phenoxy}-4-oxobutanoic acid (24) reduced the total cholesterol, LDL cholesterol and increased the HDL cholesterol in male rats. Furthermore, compounds (11), (22) and (24) decreased triglyceride levels. Interestingly, compound (22) reduced the total cholesterol, LDL cholesterol and increased the HDL cholesterol also in female population. Thus, compound (22) 2,6-dimethoxy-4-[(1E)-pent-1-enyl]phenyl nicotinate may be considered as a good candidate for effective hypolipidemic drug. QSAR studies were conducted to examine the influence of structural properties on the compounds hypolipidemic activity.

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NEW IMPROVED METHOD OF POLYMORPHIC α FORM OF IMATINIB MESYLATE (GLEEVEC®) SYNTHESIS.

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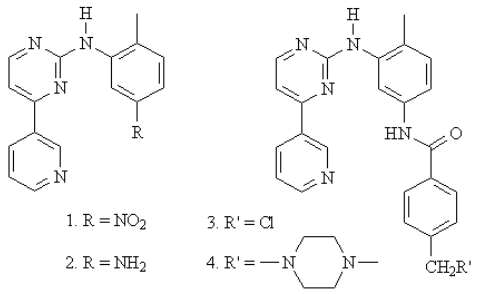

Derivatives of N-phenyl-2-pirimidineamines 2 are valuable intermediates in manufacturing of biologically active compounds. For example, they are used in synthesis of tyrosine kinase inhibitors such as Imatinib, which is approved to treat a rare cancer called Chronic Myeloid Leukemia (CML). On XLVIII Meeting of Polish Chemical Society we revealed improved methods of compound 1 obtaining. Now we would like to show further steps, which lead to polymorphic α form of Gleevec®.

We developed such process conditions, which guaranteed high yields of all synthetics steps as well as simple methods of products' isolation and purification. In our procedure compound 1 is catalytically reduced to amino-derivative using aqueous hydrazine solution. [1] Then compound 2 undergoes reaction with 4-chloromethylbenzoyl chloride in the presence of base, followed by condensation of obtained product 3 with N-methylpiperazine. Resulting reaction mixture is diluted with water or organic solvent chosen from lower aliphatic alcohols or ketones and optionally neutralized with aqueous solution of inorganic base. Precypitated product 4 is then filtered. [2,3] Finally compound 4 free base is converted into methanesulphonic acid salt and crystallized from proper solvent to give desired product in polymorphic form α.[4]

Literatura:

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[2] Sposób wytwarzania pochodnych N-fenylo-2-pirymidynoamin P-376691

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[4] Process for preparation of imatinib monomesylate crystalline Form α PCT/PL2005/000024

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DISUBSTITUTED INDOLO[2,3-b]QUINOLINE DERIVATIVES. THE CYTOTOXIC ACTIVITY IN VITRO AGAINST VARIOUS HUMAN TUMOR CELL LINE.

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In our study disubstituted 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: human colon (LoVo), uterine (MES-SA), promyelocytic leukemia (HL-60), lung (A-549) and human melanoma (Hs294T), as well as normal mouse fibroblast cell line (BALB/3T3).

We found that all indoloquinolines showed cytotoxic activity against the cells of all cancer lines used. ID_{50} values of tested compounds against the normal mouse fibroblast cell line (BALB/3T3) were comparable to the results obtained for the tumor cell lines. It suggests that the compounds show similar cytotoxic activity against normal and tumor cell lines.

The studied indoloquinolines were highly active against the cell of human promyelocytic leukemia (HL-60) and human uterine sarcoma cell line (MES-SA). The compound ISS-101 showed the highest of all the derivatives tested cytotoxic activity against the tumor and normal cell lines.

Table 1. Cytotoxic activity in vitro of indolo[2,3-b]quinolines against the cells of LoVo, HL-60 , MES-SA, A-549, MCF-7, Hs294T and BALB/3T3 cell lines.

compound	Cell line/ ID_{50}*±SD [µg/ml]						
	LoVo	HL-60	MES-SA	A-549	MCF-7	Hs294T	BALB/3T3
ISS-101	0,05±0,01	nd	0,019±0,305	0,068±0,022	0,19±0,00	0.14±0,02	0,14±0,01
ISS-91	0,11±0,02	0,052±0,004	0,041±0,304	0,12±0,02	0,43±0,02	0.29±0,04	0,24±0,01
ISS-73	0,14±0,04	0,023±0,001	0,042±0,306	0,10±0,03	0,15±0,02	0.26±0,03	0,13±0,02
ISS-71	0,25±0,08	nd	0,037±0,304	0,24±0,04	0,33±0,04	0.27±0,03	0,28±0,02
ISS-56	0,26±0,03	0,017±0,006	0,033±0,305	0,08±0,03	0,27±0,02	0.31±0,06	0,23±0,02
ISS-74	0,26±0,04	0,051±0,007	0,052±0,303	0,13±0,01	0,33±0,03	0.37±0,05	0,30±0,02

ID_{50}*-the dose of compound that inhibits proliferation rate of the tumor cells by 50%

Table2. Structures of disubstituted indolo[2,3-b]quinoline derivatives.

compound	Position of substitution	substituent
ISS-101 ISS-91	C-2 C-9	$-NH-(CH_2)_2-N-(CH_3)_2$
ISS-73 ISS-71	C-2 C-9	$-NH-CO-CH_2-N-(CH_3)_2$
ISS-56 ISS-74	C-2 C-9	$-O-(CH_2)_2-N-(CH_3)_2$

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IMMUNOLOGICAL ACTIVITY OF DISUBSTITUTED SEMICARBAZIDE AND THIOSEMICARBAZIDE ISOXAZOLE DERIVATIVES

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Title compounds were tested for their ability to affect the proliferative response of mouse splenocytes to concanavalin A (Con A) and the secondary, humoral immune response of splenocytes to sheep red blood cells (SRBC), measured as the number of antibody-forming cells (AFC). Cyclosporin A (Cs A) served as a reference compound. The compounds demonstrated differential activities in the proliferation test. Some of them were universal stimulators (M5, M6, M9) in all the studied models. Other compounds were regulatory (M2, M3, M4, M7, M8), since they stimulated to various degree the proliferative response of cells at 1-10 mg/ml, whereas the proliferation at high (100mg/ml) concentration was inhibited. Among the stimulators, compounds M1 and M6 deserve special attention, since they strongly stimulated the proliferative response at 100ug/ml (by 4-and 3-fold, respectively). On the other hand, in the humoral immune response *in vitro* all the compounds exhibited more or less uniform dose-dependent suppressive properties, M2, M5 and M8 being the most potent. Structure-activity relationship of the investigated compounds is discussed. The results of compound M1 on the spontaneous proliferative response of splenocytes and mitogen-induced splenocyte proliferation were described [1].

These studies were supported by the Wrocław Medical University, grant 1312.

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17:40 Poster I-99

SYNTHESIS OF NEW 3-PHENYLPROPIONIC ACID DERIVATIVES HAVING ANTIDIABETIC ACTIVITY

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Diseases such as hyperlipidemia, atherosclerosis, obesity, and type 2 diabetes become the serious concern not only for developed industrial societies. It is estimated that more than 150 million people worldwide suffer from type 2 diabetes, and this number is expected to double by 2025. In Poland, currently about 2 million people suffer from this disease, and the same number is at risk of developing it. Costs of medical care in diabetic patients reach 6 to 8 percent of total medical care budgets.

More than 20 years ago, the thiazolidinedione group of compounds was discovered, showing the activity in rodent models of type 2 diabetes and insulin resistance. Although their mechanism of action was not known, the compounds have been successfully used in therapy of type 2 diabetes. Publications demonstrating that they exerted their effect via the nuclear PPAR gamma receptor were published only in the middle of 90's. Now, it is well known that intracellular receptor proteins of the PPAR family control the expression of genes involved in the regulation of lipid-carbohydrate metabolism.

We synthetised three groups of compounds with antidiabetic activity. Experiments showed the high affinity of obtained compounds to PPARg receptor.

17:40 Poster I-100

PURIFICATION AND ISOLATION OF PPAR LIGANDS BY HIGH PERFORMANCE LIQUID CHROMATOGRAPHY

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In a process of receiving new chemical compounds applied to biological tests purification is essential. There is variety of efficient purification methods in organic chemistry such as: precipitation, evaporation, extraction or chromatographic methods like Flash, TLC, and HPLC. In the case of library of small samples -quantity of milligrams - classic methods can not be applied easily.

Usefulness of reversed-phase gradient HPLC method to isolation of PPAR libraries is shown in this publication. Fast and efficient separation of several compounds with very good yield and purity has been achieved. Duration of analysis is about 20 minutes (2 injection per sample). Identities of isolated substances have been verified by HPLC/MS analysis. The molecular ions of mean analytes and theoretical mass calculated from chemical equation have been compared. Purity of isolated compounds was 80 - 100 % depending on the initial contamination of the sample. Quantity of isolated substance was from 1 mg to 100 mg .

Direct scaling from analytical to semi-preparative HPLC has been done. This allows predicting retention times in the preparative system just by analyzing sample in the analytical system. Limitation of preparative columns has been noticed - larger particles size causes worse resolution.

Piknik
Tuesday evening, 16 May, 19:30

Wednesday, 17 May

Śniadanie
Wednesday morning, 17 May, 7:00

Sesja V
Wednesday morning, 17 May, 8:30
Chair: Grzegorz Grynkiewicz, Waldemar Priebe

8:30 Invited Oral

RECENT TRENDS IN ORAL DRUG IMPROVEMENT

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Oral route still remains the favourite route of drug administration in many diseases and up today it is the first way investigated in the development of new dosage forms.
The major problem in oral drug formulations is low and erratic bioavailability, which mainly results from poor aqueous solubility. This may lead to high inter- and intra subject variability, lack of dose proportionality and therapeutic failure. It is estimated that 40% of active substances are poorly soluble in water. The improvement of bioavailability of drugs with such properties presents one of the greatest challenges in drug formulations.
Various technological strategies are reported in the literature including micronization, solid dispersions or cyclodextrines complex formation and different technologies of drug delivery systems. Oral drug delivery systems are also primary topics in pharmaceutical industry.

There are two categories of oral drug delivery systems: controlled-release preparations and targeting preparations. Recently, many novel oral drug delivery systems have been invented which are prepared in traditional dosage forms such as tablets or capsules. The controlled release may be achieved by changing the physical and chemical properties of drugs and excipients as well as those of dosage forms. Numerous new concepts of the technologies are proposed.

Various technological solutions are connected with modified push-pull osmotic systems and capsules containing different multicompartmental systems. To deliver slightly water-soluble drugs, two-layered push-pull oral osmotic pumps were developed and marketed. They can deliver drug in a controlled manner over a long period of time. A several new options are available in this field, including modified system for simultaneous delivery of two drugs to reduce the problems with multidrug therapy (1).

To achieve desired results after oral administration, micro- and nanotechnologies are also developed. Insoluble drugs, which may require large doses to promote absorption can be administered as a micro- or nanoparticles with a lower frequency and smaller quantity (2) Biodegradable and biocompatible polymer nanoparticles can be used as drug carriers. Their advantage lies in the fact that they can be absorbed in an intact form in the gastrointestinal tract after administration. As a drug delivery systems they can be widely applied in different diseases where continous and controlled drug administration is important (3).

Another approach is the incorporation of the drug into inert lipid vehicles. Recently much attention has been focused on self-emulsifying drug delivery systems, mixtures of oil and surfactant and cosurfactant that have the ability to form fine o/w emulsions when exposed to aqueous media upon mild agitation. The incorporation of self-emulsifying systems in pellets is a promising proposal for delivery of a nonaqueos system in novel solid dosage forms.

In situ-gelling pectin formulations are the next technological solutions of oral sustained delivery. Gels are formed after oral administration of aqueous solution of drugs. It is essential for drugs that they should be absorbed primarily in the stomach.

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9:15 Invited Oral

ASYMMETRIC SYNTHESES AND TRANSFORMATIONS - TOOLS FOR CHIRALITY MULTIPLICATION IN DRUG SYNTHESIS

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With increasing demand for the use of enantiomerically pure substances as drugs we witness rapid development of synthetic protocols which are capable to provide such products efficiently, at large scale and with concern for environmental issues.

Nature is the originalnal supplier of chiral non-racemic compounds. Direct transformation of natural products, such as carbohydrates, amino and hydroxy acids and terpenes, is frequently used for drug synthesis. Less frequent appears the use of natural products as chiral auxiliaries in asymmetric syntheses whereas enantiomer separation by diastereomeric salts or complexes is still of great practical importance. However, the structural variety of natural products is quite limited so their rational use in the synthesis of structurally diverse drugs is questionable.

Chirality multiplication with the use of nature-derived pool of chiral compounds (including catalysts and biocatalysts) is a much more flexible strategy for the synthesis of enantiomerically pure products, with no limit to their structural varieties. Chirality multiplication can be achieved either by asymmetric catalysis or by deracemisation processes.

Asymmetric catalysis with chiral ligand-metal complexes has been developed in the past 25 years to such an extent that the formation of a C-C, C-O or C-N bond at the chiral center presents now no problem. These processes encompass a wide range of reactions, including asymmetric hydrogenations (AH), asymmetric epoxidations (AE), asymmetric dihydroxylations (AD), asymmetric aminohydroxylations (AAH), asymmetric aldol reactions (AA), asymmetric Diels-Alder reactions (ADA), asymmetric reductions, asymmetric alkylations, and many other. With regard to chiral catalysts, some of them have been useful for a range of applications (so called *privilegedchiral catalysts*), and interestingly a number of them employs C_2-chiral ligands derived from either tartaric acid, *trans*-1,2-diaminocyclohexane or 1,1'-bi-2-naphthol.

Chiral organocatalysts are emerging now as highly competitive to chiral metal complexes. They present less environmental hazard and work under less stringent regimes. Nature derived cinchona alkaloids and amino acid proline are best known representatives of this class of chiral catalysts.

Enzyme catalysed chemical transformations are now widely recognized as practical alternatives to traditional organic synthetic methods. In many cases otherwise intractable synthetic problems can be solved by biocatalysis and its use for industrial synthesis has now become routine. Lipases, esterases, dehydrogenases, and amidases are frequently applied for carbon-heteroatom bond formation while aldolases are applied for carbon-carbon bond formation.

Both enzymes and simple chiral organic compounds can be used for kinetic resolution of racemates - a highly effective al-

ternative compared to classical resolutions of diastereomers. Ultimately, chemically or enzymatically catalysed racemisation combined with kinetic resolution can transform a racemate into a single enantiomer - a procedure well suiteded for industrial applications.

There is no single method of chirality multiplication which can be universally applied to solve any synthetic problem. Rather, as numerous examples show, one needs to tailor chirality multiplication strategies to specific needs and requirements.

10:20 Oral

EUROPEAN CRITERIA FOR QUALITY ASSESSMENT OF CHEMICAL AND BIOLOGICAL MEDICINAL PRODUCTS DURING THE REGISTRATION PROCESS

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Before being placed on a market each medicinal product is evaluated during the process of registration. Four registration procedures are applicable in the European Union, namely: centralized procedure (CP) under responsibility of the EMEA (European Medicines Agency) located in London, decentralized procedure (DCP), mutual recognition procedure (MRP) and national procedure under responsibility of national regulatory authorities, in Poland of the Office for Registration of Medicinal Products, Medical Devices and Biocides. The Quality of medicinal product is assessed on the basis of chemical, pharmaceutical and biological documentation provided by an applicant and for an active substance by its manufacturer (closed part of DMF) in most cases directly to the regulatory authority. The above mentioned documentation forms Module 3 of Common Technical Document (CTD) and its summary is included in Module 2 as Quality Overall Summary. CTD consists of five modules containing also documentation on safety (Module 4) and efficacy (Module 5) of medicinal products. The Assessment of chemical, pharmaceutical and biological documentation is performed with respect to its conformity to the requirements of EU Directives, Regulations (in Poland Pharmaceutical Law and Regulations of the Minister of Health), European Pharmacopoeia (or, failing this to pharmacopoeia of a Member State) and guidelines of CPMP, ICH and WHO. These legal acts and guidelines define in details the way the tests should be performed as well as the interpretation of the results. According to Annex to Module 3 (part A of the Annex) 10 general and 46 detailed guidelines are in force. Detailed requirements for biotechnological substance as well as for medicinal products containing these substances are much wider in comparison to synthetic substances. Manufacturing of active substances on the basis of biotechnological methods using cell banks of microorganisms, plants or animals origin is so specific that the additional guidelines (14 general and 27 detailed) included into part B of the above mentioned Annex were predicted. The Assessment of quality of medicinal products on the basis of chemical, pharmaceutical and biological documentation provided for procedures CP, MRP and DCP is completed by issuing Quality Assessment Report (English language, strictly formalized structure of the document). For national procedure the assessment of quality documentation may be written in national language (in Poland only in Polish version). The information on structure and content of registration dossier is available on the following websites: www.emea.eu.int and pharmacos.eudra.org

Przerwa na kawę
Wednesday morning, 17 May, 10:40

Sesja V cd.
Wednesday morning, 17 May, 11:00
Chair: Grzegorz Grynkiewicz, Waldemar Priebe

11:00 Invited Oral

FACTS AND MYTHS CONCERNING CLINICAL TRIALS OF ONCOLOGICAL DRUGS

Piotr T. Siedlecki

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First I will deal with the genesis of the title of my presentation. Then I will describe the criteria of the response used in subsequent phases of clinical trials and their usefulness for creation of clinical standards and daily practice. The role of EBM (evidence base medicine) and its variety - eloquence and eminence based medicine - will be described.

Personal opinion about sources of credible data (Cochran Library, NICE, PDQ) will be presented. Critical evaluation of the levels of evidence of the data and usefulness of a meta analysis and historical control (Will Rogers phenomenon) will be discussed. Then, using epoetines as an example, I will present the relationship between the pharmaceutical industry, medical journals and official institutions such as FDA and EMEA .

Panel dyskusyjny
Z udziałem: W. Szelejewskiego, G. Grynkiewicza, A. J. Bojarskiego i P. T. Siedleckiego na temat: ,,CAN A QUEST FOR A NEW DRUG OF POLISH ORIGIN BE SUCCESSFUL?" (,,Czy mamy szansę uzyskać nowy polski lek oryginalny?")
Wednesday morning, 17 May, 11:45

Przerwa obiadowa
Wednesday afternoon, 17 May, 13:00

Sesja VI
Wednesday afternoon, 17 May, 14:30
Chair: Jerzy Ostrowski, Katarzyna Kieć-Kononowicz

14:30 Oral

PROGRESS IN CARDIOVASCULAR DISEASES TREATMENT

Grzegorz Opolski[1], Marcin Grabowski[2]

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Up-to-date pharmacological standard for primary and secondary prevention of cardiovascular diseases is called with acronym 2ABS and includes treatment with (A) antiplatelet agents (acetylsalicylic acid, thienopyridines), (A) angiotensin-converting enzyme inhibitors, (B) beta-blockers and (S) statins. However new pharmacological options for cardiovascular patients are being currently tested. Also modern invasive treatment is associated with aggressive pharmacological treatment with several drugs. New aspects and trends in management of hart failure, coronary artery disease and acute coronary syndromes will be discussed in presentation.

15:15 Oral

ANTICONVULSANT PROPERTIES AND 5-HT$_{1A}$, 5-HT$_{2A}$ RECEPTOR AFFINITY OF NEW N-[(4-ARYLPIPERAZIN-1-YL)-ALKYL] - 2 - AZASPIRO[4.4]NONANE- AND [4.5]DECANE-1,3-DIONES

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There is growing evidence that serotoninergic neurotransmission modulates a wide variety of experimentally induced seizures and is involved in the enhanced seizure susceptibility observed in some genetically-epilepsy-prone rats GEPR$_S$ [1]. The anti-seizure activity many of 5-HT receptor ligands would indicate that an anticonvulsant effect can be obtained not only by the GABA and glutamate systems but also by potentiating serotoninergic neurotransmission [2]. Moreover, serotonin may play a role in the mechanism of action some antiepileptic drugs such as carbamazepine which increase extracellular 5-HT at anticonvulsant doses in GEPR$_S$ [3]. On the other hand, it has also been shown that fluoxetine, a selective serotonin reuptake inhibitor (SSRI$_S$) exhibited anticonvulsant effect both in the experimental animals and in man [4].

Based on these data, in the course of our research on development new potentially anticonvulsant agents as well as potent 5-HT$_{1A}$ and 5-HT$_{2A}$ receptor ligands, we designed and synthesized a series of long chain arylpiperazines (LCAPs) containing as a cyclic amide fragments 2-azaspiro[4.4]nonane- and [4.5]decane-1,3-dione moiety [5, 6].

Among these compounds several anticonvulsant active derivatives were found, on the other hand some of them were high 5-HT$_{1A}$ and 5-HT$_{2A}$ receptors ligands (K_i ranged from 3.1 nM to 94 nM and 29 nM to 82 nM respectively). According to the results obtained, we can suppose that anticonvulsant activity of compounds described above, may resulte from activation of 5-HT$_{1A}$ or 5-HT$_{2A}$ receptors but further studies are required to confirm this hypothesis.

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15:35 Oral

LONG-TERM CYCLOSPORINE A (CyA) AND SIROLIMUS RELEASE FROM BIODEGRADABLE MATRICES AS A RESULT OF OPTIMAL ADJUSTMENT OF COPOLYMER CHAIN MICROSTRUCTURE

Janusz Kasperczyk[1], Katarzyna Jelonek[1], Piotr Dobrzynski[2], Bożena Jarząbek[2]

1. *Śląska Akademia Medyczna, ul. Narcyzów 1, Sosnowiec 41-200, Poland* **2.** *Centrum Chemii Polimerów PAN, Curie-Skłodowskej 34, Zabrze 41-800, Poland*

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Biodegradable copolymers of glycolide, L-lactide, ε-caprolactone and trimethylene carbonate (TMC) are valuable approach in the use for local immunosuppressants delivery. The appropriate comonomers combining allows to obtain long-term drug release from polymeric matrice as a result of slow degradation process. However, development of method for adjusting the concentration of released therapeutical agent

is very needed, too. The aim of this study was to assess the in vitro release of Sirolimus and CyA from different kinds of biodegradable copolymer matrices as well as correlation between the copolymers microstructure and drug release profile.

Different kinds of copolymers were used to prepare matrices containing 10% of CyA (92% ε-caprolactone 8% glycolide; 75% L-lactide 25% ε-caprolactone; 70% TMC 30% L-lactide) or Sirolimus (75% L-lactide 25% ε-caprolactone; 70% L-lactide 30% TMC; 70% TMC 30% L-lactide).The concentration of drug release from polymeric matrices in in-vitro environment has been determined by means of UV-Vis spectroscopy for 35 weeks. Analysis of changes in copolymers chain microstructure during the degradation process was conducted based on the parameters determined from ^{1}H NMR and ^{13}C NMR spectra: the average length of the lactidyl, carbonate, glycolidyl and caproyl blocks (L_{LL}, L_{T}, L_{GG} L_{Cap}), intermolecular transestrification ratio (T_{II}) and the degree of chain randomization (R) [1,2].

According to the expectations, the used copolymers provided prolonged release of drugs in all cases. The process proceeded very steadily for Sirolimus, which correlated with small changes in copolymer chain microstructure during degradation. There were some more alterations in CyA release, as a consequence of more significant changes in copolymer chain structure. The differences between two studied drugs release have been noted even for the same kind of polymeric matrice as presented in Table 1

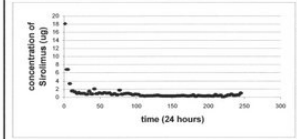
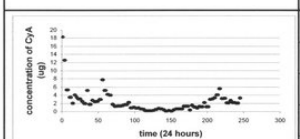
Immunosuppressants release profile	Time (weeks)	Polymer degradation				
		Lactide content (%)	L_{LL}	L_{T}	T_{II}	R
	0	27	1,56	4,15	0,63	0,56
	2	27	1,56	4,15	0,63	0,56
	5	26	1,49	4,24	0,69	0,57
	10	26	1,51	4,30	0,65	0,56
	26	26	1,47	4,18	0,51	0,58
	0	27	1,47	4,18	0,55	0,58
	2	27	1,47	4,18	0,55	0,58
	5	28	1,55	3,98	0,75	0,57
	10	23	1,40	4,69	0,60	0,56
	26	24	1,42	4,50	0,67	0,57

Table 1. The comparison of release profile of Sirolimus and CyA from L-lactide/TMC polymeric matrice and characteristic of copolymer chain microstructure changes during degradation.

This indicates the necessity of individual research for every therapeutical agent having different physico-chemical features, that incorporated in polymeric material may influence degradation process.

The results of the study demonstrate that CyA and Sirolimus release is strongly influenced by copolymeric chain microstructure and proceeds steadily according to small changes during the degradation.

References

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15:55 Oral

MOLECULAR INTERACTIONS BETWEEN NICOTINIC ACETYLCHOLINE RECEPTOR AND ITS LIGANDS - CHROMATOGRAPHIC AND MODELING APPROACHES

Krzysztof Jóźwiak[2], Ruin Moaddel[1], Irving W. Wainer[1]

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Neuronal Nicotinic Acetylcholine Receptor (nAChR) is a ligand gated ion channel playing an important role in the cholinergic system. A broad range of various CNS drugs interact *directly* with the neurotransmitter binding sites or *allosterically* on several other binding domains of the receptor modulating its synaptic activity. Fast and reliable methods of measuring and characterization of this modulation is of great interest in CNS pharmacology and drug discovery.

New experimental and theoretical methods to characterize these interactions has been developed. Affinity chromatography technique, with the nAChR immobilized on the surface of the chromatographic stationary phase, can be used to describe the affinity of small ligands towards the receptor and the kinetics of this process. We used this method in fast screening of the series of constrained nicotine analogs and the method was able to successfully sort out the compounds with activity on nAChR. High agonistic activities were further confirmed in regular functional assays for these compounds.

Bioaffinity chromatography was also employed to determine the affinity of non-competitive (allosteric) inhibitors (NCIs) towards the receptor. This application is particularly important since other methods are hardly applicable to characterize the strength of binding for this class of ligands. The data collected for the series of non-competitive inhibitors was used to

generate QSAR models describing the affinity of ligands towards the internal channel domain of nAChR and to predict functional inhibition in *in vitro* studies.

The molecular mechanisms of non-competitive inhibition were also elucidated with computational modeling techniques. The inner surface of the nAChR channel is regarded as the most common active site for binding and inhibition by allosteric NCIs. The molecular model of the channel domain was generated using homology modeling. Molecular docking procedure was employed and the series of active site - ligand molecular complexes were developed. The estimated values of free energy changes of binding (D*G*) obtained in these simulations were found to be well correlated with inhibitor affinities measured in chromatographic experiments.

The results of the computational simulations and QSAR modeling suggested the alternative mechanism of blocking action for the non-competitive inhibitors of ion channels. The tandem of chromatographic approach and computational modeling can be successfully employed for fast screening of pharmacological modulation of nAChRs by CNS drugs and new drug candidates. Various subtypes of the receptor are of increasing interest for medicinal chemists as potential drug targets and presented methods of ligand characterization can be used in drug discovery and design.

16:15 Oral

MODULATION OF TRANSCRIPTIONAL ACTIVITY IN ADIPOSE TISSUE OF db/db MICE AFTER ORAL ADMINISTRATION OF NOVEL POTENTIAL ANTIDIABETIC DRUGS (AD10369, AD10371, AD10797, AD10798 AND AD101025).

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Peroxisome Proliferator Activated Receptor gamma is a member of the nuclear receptors superfamily of ligand-activated transcription factors that have a central role in the storage and catabolism of fatty acids. PPAR gamma agonists promote adipocyte differentiation and have insulin - sensitizing effects in animal and diabetic patients.

Effects of potential PPAR agonists were tested on mouse type 2 diabetes models. Compounds (library of 8000 molecules designed by Organic Synthesis Laboratory, Adamed Ltd.) were preselected with help of competition binding test with PPAR gamma receptor. Recombinant PPAR gamma Ligand Binding Domain was used in ligand binding assay to determine the ability of subjected chemical compounds (potential PPAR protein ligands) in order to displace radioligand bound to receptor (tritiated reference compound with known strength of binding). Next the ability of these molecules to differentiate of 3T3-L1 cells into mature lipid bearing adipocytes was tested.

Diabetic *db/db* mice -an animal type 2 diabetes model - were orally administered with 5 chosen molecules: AD10369, AD10371, AD10797, AD10798 and AD101025 capable of both LBD PPAR gamma binding and 3T3-L1 fibroblasts differentiation. After 2 weeks of treatment total RNA was prepared from perigonadal adipose tissue and the expression levels of genes involved in the metabolism of glucose and lipids was determined by comparative real-time PCR. The expression of diabetes-related genes was measured in untreated and drug-treated *db/db* mice and compared to rosiglitazone treated *db/db* mice.

16:35 Oral

PHARMACOPOEIA - SET OF QUALITY REQUIREMENTS FOR MEDICINAL PRODUCTS

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Correct quality of medicinal products is connected with full filing all the criteria of quality of: identification, purity, content of active substance and functionality which are included in suitable documents, e.g. in pharmacopoeial monographs. General and individual monographs compose the Pharmacopoeia, however the national and international legislation makes them obligatory standards at a given territory.
Current requirements of European Pharmacopoeia (Ph. Eur.) are contained in its 5th edition with Supplements 5.1, 5.2 and 5.3., and of Polish Pharmacopoeia - in its 6th edition (Ph. Pol. VI). The published in 2005, "Supplement 2005" of the 6th edition of Polish Pharmacopoeia is a link between European and Polish Pharmacopoeias. It contains Polish versions of Ph.Eur. monograph titles as well as Ph.Eur. monographs of water for pharmaceutical use. The Polish version of European Pharmacopoeia is currently prepared in the Office for Registration.

16:55 Oral

NEW 3-ALKENYL DERIVATIVES OF RIFAMYCIN ANTIBIOTICS

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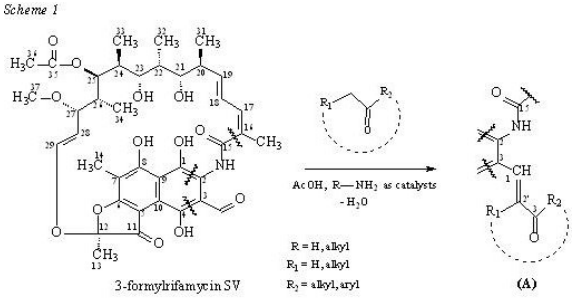
Within the framework of our Polish-German co-operation we have studied the chemistry of new biologically active semi-

synthetic rifamycin antibiotics useful in the therapy of tuberculosis and other diseases caused by pathogenic mycobacteria. Thus, we have broadly elaborated the reaction of 3-formylrifamycin SV with monoalkyloamines and ketones: R_1-CH_2-CO-R_2 [1]. It resulted in the synthesis of some dozen of interesting crystalline or amorphous 3-alkenyl derivatives of rifamycin SV (*Scheme 1* - **A**) with a substituent at C(3) of a,b-unsaturated ketone character [2]. Their structures have been determined on the basis of mass spectrometry results as well as (1D) and (2D) ^{1}H- and ^{13}C-NMR analysis.

Scheme 1



The obtained compounds were tested *in vitro* for activity against different strains of *Mycobacterium tuberculosis* with respect to standard rifampicin and rifabutin samples. They were also tested against different types of MOTT (Mycobacteria other than *Tuberculosis*) sensitive or resistant to rifampicin. These derivatives, showing a marked but clearly lower antimycobacterial activity, than that of the reference drugs, can serve as potential substrates for further modifications. We have already obtained their new analogues with very high activity against mycobacteria [3].

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[3] Patent Application - in preparation.

Przerwa na kawę

Wednesday afternoon, 17 May, 17:15

Sesja Posterowa II

Wednesday afternoon, 17 May, 17:35

17:35 Poster II-101

INVESTIGATIONS OF LIPOPHILICITY OF ANTICANCER-ACTIVE THIOQUINOLINE DERIVATIVES

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The biological activity of molecules is related to their chemical structure and physicochemical properties. It is well known that lipophilicity plays an important role in several aspects of absorption, distribution, metabolism, and excretion (ADME) of compounds [1-3]. The aim of our study was the estimation of the lipophilicity of propargyl thioquinolines, and search for relationships between their lipophilicity and *in vitro* anticancer activity. The synthesis of propargyl thioquinolines and thier antiproliferative activity against the breast line T47D have been described previously [4]. The lipophilicity of the series of the anticancer propargyl thioquinoline derivatives has been investigated using chromatograhic and computational methods. The parameters of relative lipophilicity (R_{MO} and $\log k'$) of the tested compounds was determined experimentally both by reverse-phase thin layer (RP-TLC), and high performance liquid chromatographic methods (RP-HPLC, LiChrospher RP-18 column), with mixtures of acetonitile and water as mobile phases. Their phospholipophilicity ($\log k'_{IAM}$) was determined using immobilized artificial membrane HPLC (IAM.PC DD2 Regis column). Acetonitrile in mobile phases was used in the concentrations: 50-90% (RP-TLC), 55-90% (RP-HPLC) and 35-60% (IAM-HPLC). The R_M, $\log k$ and $\log k_{IAM}$ values of the investigated compounds were linearly dependent on the acetonitrile concentration. The analysis allowed us to calculate the parameters: R_{MO}, $\log k'$ and $\log k'_{IAM}$ values for each of the tested compounds. Their partition coefficients ($\log P$) were also calculated with the Pallas (ver. 1.2 Compu Drug Chemistry Ltd, 1995) and CAChe (6.1.10) programs. The experimental and theoretical data has been compared and the relationship between the structure and the lipophilicity has been found. The quantitative structure-activity relationship studies have indicated that for the tested compounds there are dependencies between their lipophilicity and *in vitro* anticancer activity, expressed as ID_{50} [mg mL^{-1}], against the breast cancer cell line (T47D).

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17:35 Poster II-102

ANALYSIS OF GLUTATHIONE AND GLUTATHIONE DISULFIDE STATUS IN SENSITIVE AND RESISTANT HUMAN MELANOMA CELLS TREATED WITH DOXORUBICIN

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The development of resistance to multiple drugs used on cancer chemotherapy is a serious limitation of this form of treatment. In resistant cells there is correlation between decrease drug accumulation and expression of a 170kD cell surface glycoprotein which is designate P-glycoprotein, encoded by the MDR1 gene and 190KD glycoprotein - multidrug resistance protein - MRP1.

There is evidence that the MRP1 gene encodes a human GS-X pump. MRP1 contains multiple transmembrane domains plus two intracellulary localized ATP - binding cassettes, suggesting that it functions as an efflux pump for drug elimination, including doxorubicin.

Cancer cell resistance to antitumor agents has often been found associated with increases in intracellular glutathione (GSH) level.

In the present study we investigated the effect of doxorubicin on GSH level in human melanoma cells, sensitive and resistant. The GSH content was measured by BIOXYTECH® GSH/GSSG - 412™ Kit.
The preliminary study indicate that GSH content in both kinds of cells is many times greater than GSSG content, also human melanoma cells resistant to doxorubicin contain 3 x more GSH than sensitive ones, after 72 h incubation.

17:35 Poster II-103

THE COMPARISON OF TWO VALIDATED HPLC METHODS FOR THE DETERMINATION OF (+)-CLOPIDOGREL HYDROGENSULFATE PURITY WITH THE USP METHOD.

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The comparison of two validated high performance liquid chromatography (HPLC) methods for the determination of chemical impurities and enantiomeric purity of (+)-clopidogrel hydrogensulfate with the USP method is presented.

The determination using the developed methods were performed on Nova-Pak Phenyl and Chiral AGP columns. The selectivity of the method of chemical impurities determination was demonstrated for 6 analytes: impurity A (RRT 0.5), D (RRT 0.7), B (RRT 1.3), E (RRT 1.7), substrate Cl-5 (RRT 0.4) and clopidogrel. Method for chemical impurities determination and method for chiral analysis are linear with R^2 = 0.999 and R^2 = 0.996 and precise with RSD 1.4 % and 2.3, respectively. The LOQ and LOD were calculated respectively on the 0.02 % and 0.006 % for chemical impurities and LOQ = 0.3 % for (-)-clopidogrel determination. Both methods are not affected by broad range of changes of chromatographic parameters, thus proving their robustness.

The developed method for chiral analysis allows to obtain the results as good as using USP method. However, concerning the chemical impurities determination the developed method presents much better selectivity than USP method.

17:35 Poster II-104

PREPARATION OF PELLETS WITH TRAMADOL HYDROCHLORIDE OF MODIFIED RELEASE

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The results constitute a part of project aiming at working out method of obtaining modern multiparticulate drug in form of compressed pellets with tramadol hydrochloride (TH). That form is to ensure a more effective, slower TH release and contribute to pain treatment pharmacotherapy optimization. It was assumed that the TH contents in pellets cores will

amount to at least 50%. Pellets, directly with the drug were obtained by means of extrusion and spheronization. Microcrystalline cellulose was used as a basic TH carrier. Powdered cellulose, hydroxypropylomethylocellulose, carmellose sodium, povidone and guar gum were also examined as fillers in the process of water granulation. Colloidal silica and glyceryl behenate were added to the contents of the powder mixture in order to decrease agglomeration of the granulated mass. The attempts of using hydrophilic polymer, e.g. PVP K - 30, failed due to too intensive powder mixture binding. It was found that cores having very good sphericality and smooth surface, deprived of the *"shark skin"* effect, may be obtained at 60% TH contents in pellets. After examining a dozen or so formulations optimum core contents (%) was selected: TH (60.0); Avicel PH 101 (35.0); Aerosil R972 (2.0); glyceryl behenate (3.0). Formulations with 70% TH contents in pellets had smooth core surface but significantly worse sphericality compared to 60%. The effect of modified slow TH release rate from cores was obtained by coating them with film Eudragit NE. Using also other copolymers of methacrylic acid, ethylcellulose and shellac is planned to this end. Evaluation of film endurance and diffusion properties according to the fact if polymers are showered on cores in the form of solution or dispersion in a water or organic solvent will also be conducted. Selected formulations of coated pellets will be directly compressed into tablets using laboratory rotation tabletting machine with the power of monitoring compression forces and the force of tablet expulsion from matrix.

17:35 Poster II-105

DESIGN, SYNTHESIS AND ACTIVITY OF NEW ORGANOPHOSPHORUS INHIBITORS OF UREASE

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Urease (E.C. 3.5.1.5) is an enzyme, which catalyses the hydrolysis of urea into ammonia and carbamate. Enhanced activity of urease is claimed to be a pathogenic marker in case of many infections of urinal and digestion tracts. This hydrolase is also crucial for the bacterium *Helicobacter pylori* existence, a pathogen causing stomach ulcers and finally tumors. This fact originates from local increase of pH caused by the catalyzed reaction, forming this way microenvironment suitable for bacterium survival and proliferation. Thus, the development of potent and selective inhibitors of urease provides perspective biomedical applications.

Several classes of compounds are known to show considerable inhibitory activity against urease. Hydroxamic acids have been the most extensively studied, whereas phosphordiamidate and thiophosphordiamidate transition state analogues of the enzymatic reaction are those of the highest activity. However, the main disadvantage of phosphorus acid diamide derivatives is their low stability in water solutions caused by rapid hydrolysis.

Here, we present the rational design, synthesis and enzymatic activity of novel organophosphorus inhibitors of urease. They represent *P*-methylphosphinate and phosphonamidate analogues of phosphordiamidate phenyl esters. These transition state analogues of improved hydrolytic resistance will serve as new lead compounds for the development of more extended urease inactivators.

17:35 Poster II-106

SEARCHING FOR NEW INHIBITORS OF CHOLINESTERASE IN GROUP OF CARBAMATES

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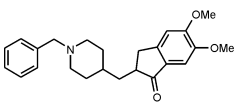
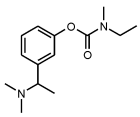
Alzheimer's disease (AD) is the most common form of dementia among older people, which initially affects the parts of the brain that control thought, memory and language. Therefore its first symptoms are: confusion, disturbances in short-term memory, personality changes and language difficulties. They are thought to be related to the degeneration of cholinergic neurons in the cerebral cortex and subcortical structures. Since putting forward this hypothesis, known today as the cholinergic theory, the substances which enhance the cholinergic transmission have been searched. Among possible strategies cholinesterase inhibitors are the only class of compounds to date, that have consistently proven to be efficacious in treating the cognitive and functional symptoms of AD. Four of these belonging to different chemical groups have been approved for the symptomatic treatment of mild to moderate AD: tacrine (aminoacridine), donepezil (benzylpiperidine), galantamine (a tertiary alkaloid) and rivastigmine (carbamate). However, none of these is efficient enough and consequently it is necessary to search for new ones [1, 2].

DONEPEZIL

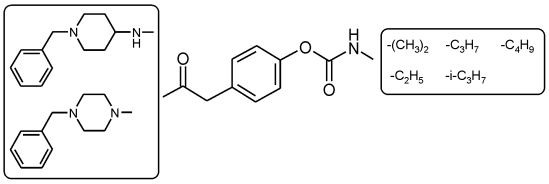


RIVASTIGMINE



Our research group has managed to synthesize potential new inhibitors of cholinesterase. After analyzing the available

data, considering especially the interactions between certain structures and active site of cholinesterase, we designed and obtained two series of compounds. Taking rivastigmine as a structural model, both series contain carbamoyl moiety attached directly to the phenyl ring, and one of them contains also benzylpiperidine fragment which is crucial for the activity of donepezil.



Both series will be tested with Ellman's method to examine their cholinesterase inhibition activity.

1. M. Bolognesi; J. Med. Chem., 2004, 47, 5945-52

2. M. Recanatini; Curr. Pharmac. Des., 2004, 10, 3157-66

17:35 Poster II-108

SYNTHESIS AND ANTIPROLIFERATIVE ACTIVITY IN VITRO OF NEW 2-AMINOBENZIMIDAZOLE DERIVATIVES. PART 3. REACTIONS OF 2-ARYLIDENE-AMINOBENZIMIDAZOLE WITH SELECTED 1,3-DIKETONES.

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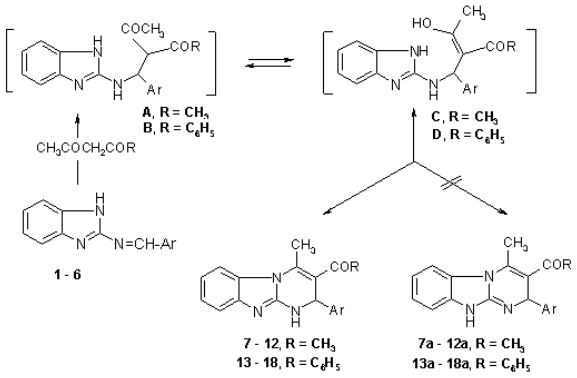
A series of pyrimido[1,2-a]benzimidazole derivatives has been synthesized in the reactions of 2-aminobenzimidazole Schiff bases **1-6** with selected β-diketones; acetylacetone **7-12** or benzoylacetone **13-18**. The structures **4, 7-18** were confirmed by the results of elemental analysis and their IR, ^{1}H NMR and MS spectra. Compounds **4**, **7-18** were examined for their antiproliferative activity *in vitro* against 3 cancer cell lines: P338 (mice leukemia), A549 (non-small cell lung carcinoma), SW707 (rectal adenocarcinoma), using SRB (sulphorhodamine B) or MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) technique. The Schiff base **4** and tricyclic derivatives **9**, **14**, **16**, exhibited the highest cytotoxic activity *in vitro.*

Table 1. Comparison of calculated energies and dipole moments for selected compounds.

Compound	Total energy /Hartree/	Dipole moment /D/
9	-1310.7818324	1.81
9a	-1310.7747886	5.02
Difference E_9-E_{9a}	-0.0070438 = -18.5 kJ/mol	
15	-1502.5141959	1.76
15a	-1310.5066769	4.08
Difference E_{15}-E_{15a}	-0.0075190 = -19.7 kJ/mol	



17:35 Poster II-109

PRIMARY CYTOTOXICITY EVALUATION OF NEW HETEROCYCLIC SYSTEMS WITH PYRIDODIAZEPINE.

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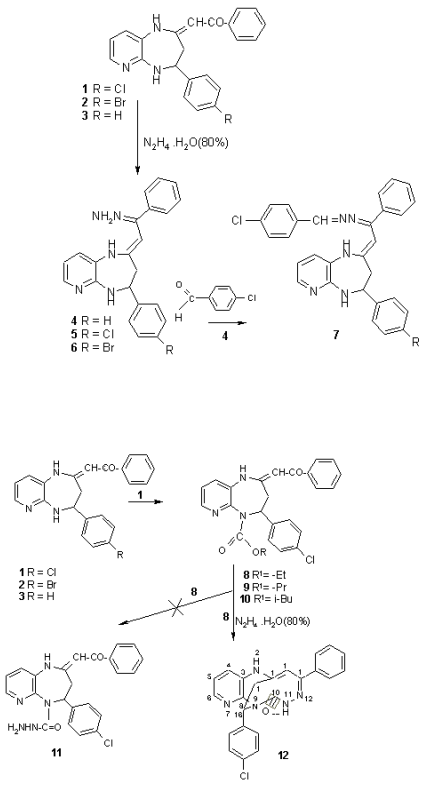
New esters **8-10** and hydrazones **4-6** were synthesized from 1-phenyl-2-(4-aryl-1,3,4,5-tetrahydropyrido[2,3-b][1,4]diazepin-2-ylidene)ethanones (**1-3**). Subsequent treatment of hydrazone **4** with *p*-chlorobenzaldehyde furnished azine **7**. The prolonged heating of ester **8** with hydrazine hydrate afforded a polyheterocyclic compound with fused and bridged rings **12**. The structures of new compounds were determined by analytical and spectral (IR, ^{1}H NMR, MS) methods. Compounds **8 - 10** and **12** were examined for their antiproliferative activity *in vitro* against the cells of 5 human cancer cell lines, using SRB or MTT technique. One out of all tested compounds **12** revealed cytotoxic activity *in vitro* against all cell lines applied with ID_{50} (inhibitory dose 50%) values lower than 4 mg/ml, which is an international activity criterion for synthetic compounds. All compounds inhibits the proliferation of HL-60 human promyelocytic leukemia cell

line . Carbamates **9** and **10** were active only against two cancer cell lines, namely HL-60 leukemia and HCV-29T urinary bladder cancer. Compound **8** revealed activity only against HL-60 leukemia cells.



17:35 Poster II-110

CONJUGATES OF METHOTREXATE AND DEXTRANS IN MOUSE LEUKEMIA MODEL WITH MULTIPLE DOSE SCHEDULE

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Methotrexate (MTX) is one of the widely used agents in chemotherapy of oncological and hematological diseases. Low plasma half-life, toxicity for normal proliferating cells and other limitations of the drug impel scientists to search for improved forms of the MTX. Conjugation of the drug with macromolecular carriers is one of the strategy seems to be perspective in obtaining preparations with amended properties. The aim of this work was to compare two dextrans T10 and T40 as potential carriers for MTX. We hypothesized that the difference between conjugates would be evident in multiple-dose schedule due to distinct molecular weights (MW) of these macromolecules and allow us to select more prospective carrier.

Conjugates were synthesized using dextrans with MW of 10 and 40 kDa (preparations coded T10-MTX and T40-MTX, respectively). The level of substitution was 0.022 mol of MTX per 1 mol of glucose for each preparation. Female (C57Bl/6 x DBA/2)F1 mice, 12-20-week-old, weighing 21-27 g were applied. All animals were randomly divided into four groups and injected with 10^6 leukemia (P388) cells i.p. (day -1). Then animals were administered with appropriate agents on the 0^{th}, 3^{rd} and 6^{th} days of the experiment. The mice in control and MTX treated group were administered with 0.9% saline solution and 20 mg/kg/day of free MTX, respectively. Another two groups were administered with 20 mg/kg/day of T10-MTX or T40-MTX conjugates, respectively. All doses were based on the molarity of the MTX in the conjugates. Mice body weight and survival data were collected daily. Overall toxicity in experimental groups was assessed using a number of treated mice, which died earlier the day of first death registered in control group. Two experiments with identical protocols were conducted and after obtaining comparable results were pooled and analysed together. Survival data were compared using the Cox's F test with Bonferroni correction for multiple comparisons.

There were not early deaths due to toxicity in groups treated by either of conjugates. Data on weight changes show that both tested compounds have approximately the same toxicity as free MTX when applied in multiple-dose schedule. Notably, the antileukemic effects of conjugates were not the same. Mice treated by T10-MTX compound had lower median survival time in comparison with both free MTX and T40-MTX treated groups, and these differences were statistically significant ($p < 0.01$ and $p < 0.0001$ respectively). However, T40-MTX treated mice had median survival time similar to free MTX treated mice and there was not statistically significant difference in survival between these two groups.

We explain this diversity of the properties of T10-MTX and T40-MTX conjugates by the difference in the MW of their carriers. It is known that dextran T10 is excreted unrestrictedly, while dextran T40 is not able to pass unchanged through the pores of the glomerular capillary walls due to its larger molecular size. We suggest that the T10-MTX conjugate is excreted faster than T40-MTX. Therefore, significantly lower amount of T10-MTX have time to degrade inside tumor cells in comparison with T40-MTX. The difference between two conjugates becomes well pronounced in multiple-dose schedule, when advantage of T40-MTX over T10-MTX is cumulated during the administration course. These data confirm our previously published results that MW of the carrier is the critical parameter and should be taken into an account while designing new conjugates. We are currently conducting stud-

ies in solid tumor models, to further investigate the possibility of using the conjugates as prolonged forms of the parental drug. The advantage of T40 over T10 dextran carrier is also should be confirmed in pharmacokinetics studies.

17:35 Poster II-111

THE CYTOTOXIC GLYCOSIDES OF INDOLO[2,3-B]QUINOLINE DERIVATIVES. IN VITRO AND IN VIVO STUDIES

Aleksandra Nowicka[1], Małgorzata Piskozub[1], Joanna Wietrzyk[1], Katarzyna Badowska-Rosłonek[2], Łukasz S. Kaczmarek[2], Wanda Peczyńska-Czoch[3]

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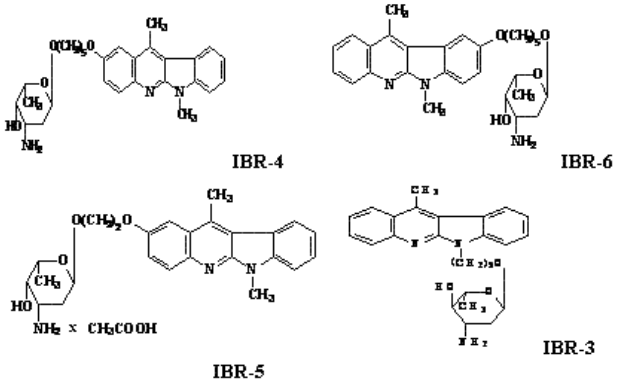
The cytotoxic activity *in vitro* of glycosides of indolo[2,3-b]guinoline having daunosamine or acosamine moiety was evaluated. In our research we used human colon cancer (LoVo), uterine sarcoma (MES-SA), promyelocytic leukemia (HL-60), lung cancer (A-549) and melanoma (Hs294T) cell lines. We found that all the indoloquinolines studied were cytotoxic against the cells of all cancer lines used. They showed the highest cytotoxic activity against human leukemia cell line (HL-60) and the lowest against the human melanoma cell line (Hs294T) as well as against the human colon cell line (LoVo) (Table1.)

Table1. *Cytotoxic activity in vitro of the studied compounds against the cells of LoVo, HL-60, MES-SA A-549, MCF-7, and Hs294T cell lines*

Compound	Cell line/ ID50±SD [μg/ml]					
	LoVo	HL-60	MES-SA	A-549	MCF-7	Hs294T
[IBR-4]	3,12±0,21	0,26±0,04	0,32±0,03	2,74±0,61	2,32±0,57	3,33±0,06
[IBR-6]	3,39±0,07	0,32±0,01	2,19±0,67	2,93±0,45	2,56±0,39	3,29±0,09
[IBR-5]	2,89±0,33	0,31±0,006	2,34±0,49	2,87±0,31	2,37±0,41	3,22±0,10
[IBR-3]	3,19±0,30	2,57±0,36	3,0±0,18	3,32±0,12	2,86±0,22	3,43±0,14

Table2. *Structures of double substituted indolo[2,3-b]quinoline derivatives*



To define antitumor activity the compound IBR-5 in *vivo* we applied mouse leukemia cell line (P-388). The results of our research show toxicity of studied compound in higher range of doses (30, 50 and 100 mg/kg). The average survival time (AST) of mice, which was administered with the compound IBR-5 in higher doses was 7 days and for control mice 12 days. Additionally in lower range of doses (10, 5, 1 mg/kg) the compound did not show antitumor effect. The average survival time of mice which were administered with the compound in lower doses was comparable to AST of control mice.

17:35 Poster II-112

MONO SUBSTITUTED 5H-INDOLO[2,3-B]-QUINOLINE DERIVATIVES AND THEIR ABILITY TO OVERCOME THE BARRIER OF DRUG RESISTANCE.

Aleksandra Nowicka[1], Joanna Wietrzyk[1], Wojciech Łuniewski[2], Agnieszka Ciesielska[2], Łukasz S. Kaczmarek[2], Wanda Peczyńska-Czoch[3]

1. *Institute Immunology and Experimental Therapy Polish Academy of Science (IITD), Rudolfa Weigla 12, Wrocław 53-114, Poland* **2.** *Pharmaceutical Research Institute (IF), Rydygiera 8, Warszawa 01-793, Poland* **3.** *Wroclaw University of Technology Department of Chemistry Faculty of Chemistry, 27 Wybrzeże Wyspiańskiego, Wrocław 50-370, Poland*

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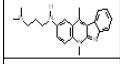
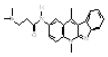
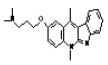
A series of novel 5-H indolo[2,3-b]quinoline derivatives bearing (dialkylamino)alkyl chains at C-2 or C-9 position attached to indoloquinoline core via nitrogen, amide or ether bond were prepared and tested for ability to break multidrug resistance in cancer cells.

In our research we used 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 the compound ISS-22 did not show ability to overcome the barrier of drug resistance against human colon cancer and uterine sarcoma cell line. 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 those which were connected with indoloquinoline core via ether bond.

compound	Cell line/RI*		
	(LoVo/DX)/LoVo	(HL60/MX2)/HL60	(MES-SA/DX)/MES-SA
ISS-103 	2,46	0,79	0,68
ISS-90 	1,45	1,05	1,1
ISS-42 	1,37	0,064	0,83
ISS-22 [LKS-15] 	2,04	0,11	6,8
ISS-68 	1,2	0,1	1,25
ISS-81 	1,14	0,06	1,2

RI* *was calculated according to the formula RI=(ID50 estimated against resistant cell line)/(ID50 estimated against non-resistant cell line); values range: 0<RI<2-indicate that the tested compound is able to overcome drug resistance; 2<RI<10 - defines the moderate ability of the compound to overcome drug resistance; RI>10 - defines no influence on the drug resistance phenomenon.*

17:35 Poster II-113

NEW ARYLPIPERAZINE DERIVATIVES OF 3-PHENYLPYRROLIDINE-2,5-DIONE AS POTENTIAL ANTICONVULSANT AGENTS

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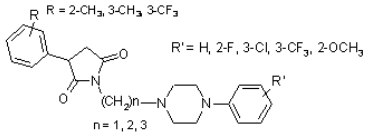
Recently it has been demonstrated that compounds with 4-aryl or 4-methylpiperazine moiety connected to 3-phenylpyrrolidine-2,5-dione fragment by the alkylene spacer exhibited high anticonvulsant activity, with ED_{50} from 14 mg/kg to 49 mg/kg in the MES test [1, 2].

As a continuation of our research, in the present study, we designed and synthesized a new series N-[(4-arylpiperazin-1-yl)-alkyl]-3-phenylpyrrolidine-2,5-diones with different length of alkylene chain between two nitrogen atoms. On the other hand, we introduced two kind of substituents, electron-donating CH_3 and electron-attracting CF_3 at the phenyl ring connected with pyrrolidine-2,5-dione moiety.



comp. **1-16**

The target compounds with ethylene and propylene chain between nitrogen atoms were synthesized by one-pot cyclization reaction of the prepared 3-phenylsuccinic acids and appropriately substituted N-aminoalkyl-4-arylpiperazine. Compounds with methylene bridge were obtained in the Mannich-type reaction from the respective 3-phenylsuccinimides, formaldehyde and the corresponding 4-arylpiperazines. The structure determination was based on ^{1}H-NMR spectral data and C, H, N analysis. The newly synthesized compounds (**1-16**) were evaluated for their anticonvulsant activity within the Antiepileptic Drug Development (ADD) Program by testing procedures described earlier [3]. The derivatives obtained revealed anticonvulsant activity in the MES-test, that depended on the character of substituents at the aromatic ring, as well as the length of linker between two nitrogen atoms.

References:
1. Obniska J., Zagórska A. Il Farmaco 58 (2003) 1227-1234.
2. Obniska J., Jurczyk S., Zejc A., Kamiński K., Tatarczyńska E., Stachowicz K. Pharmacol. Rep. 57 (2005) 170-175.
3. Kupferberg H. J. Epilepsia, 30 suppl. (1989) 51-56.

17:35 Poster II-114

NEW ADAMANTACYL DERIVATIVES OF DINITROIMIDAZOLE

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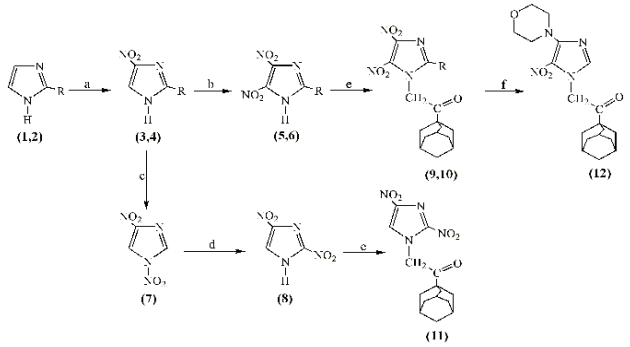
Nitroimidazole derivatives have been prepared and investigated for therapeutic and prophylactic applications. Numerous compounds have been used as antibacterial, antiprotozoic, antimycotic, immunosuppressive and radiosensitizing agents. It has been usually established that 5-nitro derivatives are more active than 4-nitro isomers.

Our earlier investigations have been devoted to synthesis and antifungal as well as antibacterial properties of *N*-phenacyl-4,5-dinitroimidazole derivatives [1,2]. In this communication we report on the synthesis of new compounds with adamantacyl group at the *N*-1 position of heterocyclic ring. The syntheses of new compounds **(9-11)**, are based on analogous reaction. The starting materials 4,5-dinitro-, 2-methyl-4,5-dinitro- and 2,4-dinitroimidazoles have been prepared according to the methods described in the literature [3-5]. Dinitroimidazoles **5, 6** and **8** were alkylated with adamantyl bromomethyl ketone in absolute ethanol in the presence

of sodium etoxide. The treatment of *N*-substituted dinitroimidazole **(9-11)** with cyclic amines (e.g. morpholine), led to the derivative of 4-amino-5-nitroimidazole (e.g. **12**). Similar products were also formed in the reaction of respective *N*-phenacyl derivatives [6]. All newly obtained compounds are expected to show antiviral activity.



where: R = H, CH_3; a = 65% HNO_3/H_2SO_4, boiling; b = 100% HNO_3/H_2SO_4, boiling; c = $CH_3COOH/HNO_3/(CH_3CO)_2O$, r.t., 48 h; d = chlorobenzene, 115-120 °C, 10 h; e = adamantyl bromomethyl ketone, Na/EtOH, boiling; f = morpholine, THF, r.t.

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Acknowledgements: This work was supported by KBN Grant No. 2 PO5F 039 27.

17:35 Poster II-115

THE ANTITUBERCULAR ACTIVITY OF 7-NITROIMIDAZOOXAZOLES

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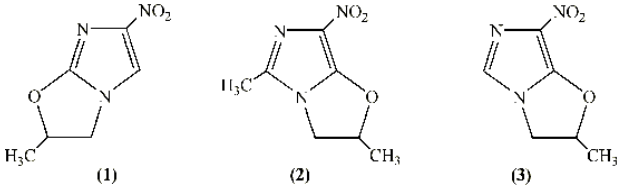
Mycobacterium tuberculosis is the greatest single infectious cause of mortality worldwide, killing roughly two million people annually [1]. Estimates indicate that one-third of the world population is infected with latent *M. tuberculosis* [2]. In most parts of the world people who suffer from TB are restricted to combinations of only five drugs to treat this illness effectively. These are rifampicin, isoniazid, ethambutol, streptomycin and pyrazinamide [3]. A series of bicyclic nitroimidazooxazoles, originally investigated as radiosensitizers for use in cancer chemotherapy, were found to posses activity against cultured replicating *M. tuberculosis* [4].

By the present time only 2,3-dihydro-6-nitroimidazo[2,1-b]oxazoles with different substituents at C-2 position have been tested as to their antitubercular properties. One of these compounds - CGI 17341 has shown particular promise in clinical trials [5]. However, the activity of 2,3-dihydro-7-nitroimidazo[5,1-b]oxazoles hasn't been examined. In our earlier works [6,7] we have described the synthesis of series of 2,3-dihydro-7-nitroimidazo[5,1-b]oxazoles. At present, two of these compounds (2, 3) are being tested against *Mycobacterium tuberculosis.* Their structural analogue (1) from 6-nitroimidazo[2,1-b]oxazole series showed an MIC against Mtb H37Rv of 1.95 µg/ml [4] and this was the lead compound of that studies.



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Acknowledgements: This work was partially supported by KBN Grant No. 2 PO5F 039 27.

17:35 Poster II-117

GROWTH OF HUMAN CHONDROCYTES ON BIODEGRADABLE SYNTHETIC POLYMERS

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Biodegradable polymers such as polylactic acid and polyglycolic acid are commonly utilized to produce surgical fibers

and devices for bone fracture internal fixation. They have also been considered to be useful for tissue engineering and as the carriers for controlled release of drugs [1]. These materials must be biocompatible to be well tolerated by the body as well as must support cell adhesion, growth and differentiation. However, traditional methods of their synthesis employ highly toxic tin compounds as initiators of polymerization. Complete elimination of these compounds from the polymers is practically impossible which results in their slow penetration into patients blood circulation system [2]. Moreover, the implant composed of polymeric material may often induce severe inflammatory reaction [3]. The aim of our study was to examine the growth of human chondrocytes on a set of novel biodegradable materials from copolymers of L-lactide, glycolide, ε-caprolactone and trimethylene carbonate. Their synthesis was carried out with the use of nontoxic zirconium acetylacetonate as an initiator of polymerization. The cells were isolated from costal cartilage from a rib of three years old patient subjected to the plastic operation of the chest. They were plated into 96-well plates coated with the uniform thin polymer films and cultured for 4 days. Cell number was measured with a DNA binding fluorescent dye, CyQuant GR (Molecular Probes). Chondrocyte proliferation on 85:15 poly(L-lactide-co-glycolide) (PLG, M_n 75 600) and 70:30 poly(L-lactide-co-trimethylene carbonate) (PLT, M_n 36 000) did not differ significantly from the control. Microscopic observation of cultures revealed that these substrata considerably supported adhesion and spreading of the cells. However, plating efficiency on 85:15 PLG and 70:30 PLT, measured 3 hours after cell seeding, revealed that the cells were attaching to these substrata more slowly compared to control. Chondrocytes cultured on 30:70 poly(caprolactone-co-trimethylene carbonayte)(M_n 31 500), 70:30 poly(L-lactide-co-caprolactone)(M_n 60 300), 30:70 poly(L-lactide-co-trimethylene carbonayte)(M_n 17 500) and 90:10 poly(caprolactone-co-glycolide)(M_n 63 000) displayed slower proliferation compared to control although the cells efficiently attached and spreaded on these substrata. Generally, our results indicate that all the polymer materials used in this investigation can provide a suitable substrate for chondrocyte growth.

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17:35 Poster II-118

SYNTHESIS AND ANTIBACTERIAL ACTIVITY OF NEW TRIMETHOPRIM ANALOGUE

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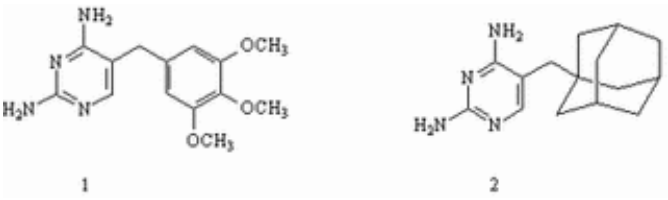
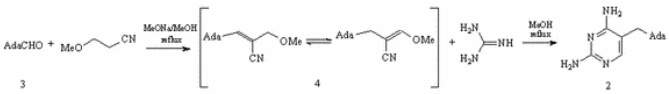
Certain benzylpyrimidines, such as trimethoprim **1** are potent and selective inhibitors of bacterial dihydrofolate reductase, the enzyme responsible for the NADPH-dependent reduction of 7,8-dihydrofolate to 5,6,7,8,-tetrahydrofolate. The latter is essential for various biosynthetic reactions *e.g.* biosynthesis of nucleic acids and certain amino acids. Trimethoprim is used solely or in combination with sulphonamides (synergic effect) to treat a wide range of bacterial infections in humans [1].



The adamantyl moiety present in numerous agents and drugs improves their lipophilicity due to aliphatic cage-like structure. Such compounds could be much better taken up by cells, and have enhanced blood-brain barrier penetration and increased accumulation in lipids [2].



Our previous investigation revealed that the combination of an adamantyl moiety with pyrimidine ring leads to compounds of significant antimicrobial properties [3]. It has inspired us to synthetize a new adamantane analog of trimethoptim **2**.

The known 1-formyladamantane **3** was prepared here from 1-hydroxymethyladamantane by a Swern oxidation (yield > 95%). The conversion [4] of the aldehyde **3** into β-methoxypropionitrile **4** was followed by condensation with guanidine base in methanol [5] affording 5-adamantan-1-ylmethyl-pirimidine-2,4-diamine **2**.

Compound **2** was tested against selected Gram-negative and Gram-positive strains.

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17:35 Poster II-119

SIMVASTATIN INTENSIFY HEART RATE DEPRESSION AFTER METOPROLOL AND ATROPINE ADMINISTRATION IN NORMOCHOLESTEROLAEMIC RATS

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The use of statins has been for many years connected with significant clinical benefits due to cholestrerol synthesis inhibition and increase of LDL - lipoprotein expression. In recent years, growing attention is being paid to the pleiotropic properties of statins. The mechanisms responsible for blocking prenylation of small G proteins are the underlying explanation of the above mentioned properties of statins. The existence of an interaction on the intracellular signalling level between the simulation of beta - adrenergic receptors and statin administration has been demostrated. The aim of the study was heart rate and blood pressure evaluation after metoprolol administration in rats given normal diet and simastatin for two weeks. The study was performed on Wistar outbred rats. After a two days adaptation period, simvastatin in a dose 10 mg/kg was administered into the stomach for a period of two weeks. The last administration drug was on the day prior to the heart rate and blood pressure of the tests. Heart rate and blood pressure measurements were performed with the use of HSE Haemodyn equipment. Before immobilization on the operating table, the rats were anaesthesized with the use of penthobarbital administered intraperitoneally in a dose of 30 mg/kg. Need be, general anaesthesia was sustained by one administration of penthobarbital (10 mg/kg of body mass dose), until complete lack of response to pain was achieved. After parameter stabilization (approximately 15 minutes), metoprolol in a dose of 1.0 mg/kg, and atropine in dose of 0.5 mg/kg were given intraperitoneally. After the administration of metoprolol and atropine, evalution of parametres was performed for another 15 minutes. Heart rate, systolic, diastolic and mean blood pressure inital were similar. After metoprolol and atropine administration, heart rate in rats receiving simvastatine was significantly decrease compared to rats without simvastatin. Blood pressure (systolic, diastolic, mean) values, after metoprolol and atropine administration in both groups, were statistically insignificant. It seems that the basis of the above mentioned interaction is the influence of simvastatin on the autonomic nervous system or modulation of intracellular transmission.

17:35 Poster II-121

4,6-DISUBSTITUTED 2-(4-METHYL-1-PIPERAZINYL)PYRIDINES: SYNTHESIS AND THEIR BINDING TO SEROTONIN 5-HT$_{1A}$, 5-HT$_{2A}$, AND 5-HT$_7$ RECEPTORS

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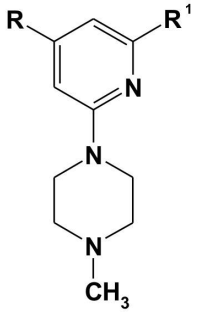
The role of the 5-HT$_7$ receptors in the CNS and the periphery has not been fully clarified since to date there are only limited number of the selective-active ligands. During screening of our compounds library against the 5-HT$_7$ receptor from rat hypothalamic membranes, it was found that a lot of agents, besides 5-HT$_{1A}$ and/or 5-HT$_{2A}$ receptors activity, displayed also a significant affinity towards 5-HT$_7$ sites. The 4-mono, and 4,6-disubstituted 2-(1-piperazinyl)pyridines were particularly interesting, since compounds of such structure have never been reported as 5-HT$_7$ receptor ligands.



R: Ph, 2-thienyl, 3-thienyl, 2-OCH_3-Ph, 3-OCH_3-Ph, 4-OCH_3-Ph
R^1: Ph, 2-thienyl, CH_3

Here we report the 5-HT$_7$ receptor affinity for some previously described 4,6-disubstituted 2-(1-piperazinyl)pyridines as well as a series of newly designed and synthesized analogues. The target compounds were synthesized using the benzotriazole-assisted Katritzky method. The affinity for three serotoninergic receptor subtypes (5-HT$_{1A}$, 5-HT$_{2A}$ and 5-HT$_7$) were determined and some structure-affinity relation-

ships are discussed.

In addition, an automated docking to homology model of 5-HT$_7$ receptor was performed, and it was found that in the best PMF-scored complexes ligands were placed between helices 2, 3 and 7, revealing a strong interaction formed by heteroatom and indole nitrogen of Trp7.40.

17:35 Poster II-122

STRUCTURAL MODIFICATIONS OF SOME ARYLPIPERAZINE LIGANDS TOWARDS IMPROVED SELECTIVITY FOR 5-HT$_7$ RECEPTORS

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The latest discovered subtype of serotonin receptors - 5-HT$_7$ - emerged as a new valuable therapeutic target. Based on its distribution and pharmacological studies, the 5-HT$_7$ receptors has been implicated in many different functions in CNS (like circadian rhythm, learning and memory, mood, endocrine regulation) as well as in the periphery. It was found that many of the previously described serotonin ligands showed a high level of 5-HT$_7$ receptor activity. The latter was observed for 5-HT$_{1A}$ agents, in particular in the group of long-chain arylpiperazine derivatives (LCAPs).

As part of our research program directed toward development of potent and selective 5-HT$_7$ receptor ligands, we synthesized a novel series of LCAPs analogues. The structural modifications included replacement of terminal amide fragment by aryl sulfonamide moiety and/or changes of aromatic substituent in arylpiperazine fragment.

All the new compounds were evaluated for affinity at 5-HT$_7$ and 5-HT$_{1A}$ receptors and preliminary structure-affinity relationships are presented.

17:35 Poster II-123

1-THIOSUGARS: SYNTHESIS AND BIOLOGICAL ACTIVITY AGAINST CSFV GLYCOPROTEINS.

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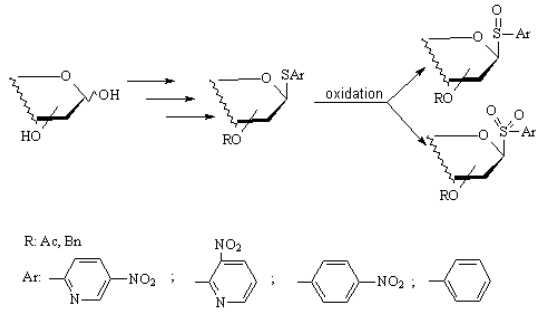
In search for effective inhibitors of sugar processing enzymes the *S*-glycosides and products of their oxidation attract constant attention as viable substrate analogs.

Along this line, we have reported a simple and efficient methodology for the synthesis of thioglycosides, derivatives of nitroaromatic halides, via aromatic nucleophilic substitution of halogen with 1-thiosugar derivatives [1,2]. Phenyl thioglycosides used in our study were prepared as described in literature, in reaction of acylglycosyl halides with thiophenol under phase transfer conditions [3].

The oxidation of glycosyl sulfides respectively to sulfoxides or sulfones was achieved using common oxidising agent [4]: *m*-chloroperbenzoic acid (*m*-CPBA) in CH_2Cl_2 at room temperature. During oxidation to sulfoxides *m*-CPBA was added in equimolar amount in low temperature (-20 ^oC) to avoid sulfone formation.



Desired oxidation products were obtained in a good yield and their structures were confirmed by NMR spectra.

Biological studies with these inhibitors were divided into two parts. First, using the neutral red cytotoxicity assay, we established the optimal doses of inhibitors when the viability of swine kidney cells (SK6) was higher than 50%. In the next experiments, we examined the effect of concentration of inhibitors on penetration and propagation of classical swine fever virus (CSFV). The best results were observed for GP5 inhibitor. Even low doses of this inhibitor (5 μg/ml), when the

viability of SK6 cells is higher than 90%, inhibited the propagation of CSFV virus and the viral yield was decreased by over 70%.

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17:35 Poster II-124

SOLVENT-FREE THIONATION OF FRAGRANT HETEROKETONES UNDER MICROWAVE CONDITIONS

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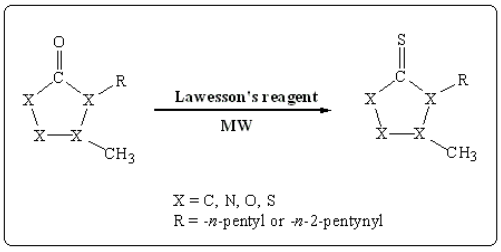
Jasmone is well-known of natural origin ketone from jasmonoids group and is important representative of jasmine fragrant compounds, derived from polyunsaturated fatty acids as a result of biosynthesis in plants [1]. The synthesis of thioanalogues of ketones, flavones, lactones, amides and esters has received considerable attention due to biological and commercial importance of these molecules and their usefulness as precursors for the synthesis of various organic compounds. The common procedures for the chemical oxygen-sulfur exchange in the carbonyl compounds require the use of dry aromatic hydrocarbon solvents, lengthy reaction times and usually excess of thionathion reagent [2].

The starting heterocyclic analogues of jasmone based on oxazolidinone, pyrrolidinone, pyrazolidinone, pyrazolinone and thiazolidinone cycles were successfully prepared under microwave irradiation [3]. In the present investigation we report a rapid, solvent free, high yielding and cleaner conversion of heteroanalogues of jasmone to the corresponding thioanalogues by using Lawesson's reagent.



Odor quality and durability of obtained jasmone hetero- and appropriate thioheteroanalogues were evaluated and compared with fragrance properties of typical floral odor of jasmone.

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Acknowledgements: We are grateful for financial support to the Polish State Committee for Scientific Research (KBN Grant N^{o} 2PO5F 031 29)

17:35 Poster II-125

MICROWAVE-ASSISTED SYNTHESIS OF UNSATURATED HETEROCYCLIC FRAGRANT COMPOUNDS RELATED TO JASMONE

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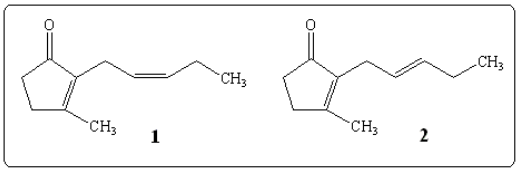
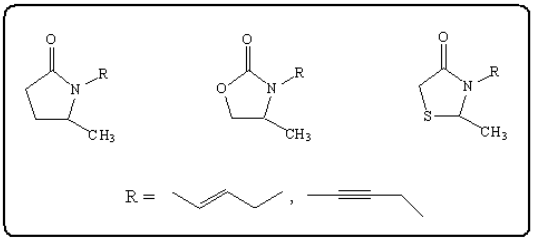
Jasmone is well known component of plant volatiles and is very important substance which determines the odor of jasmine flowers [1]. This compound can be obtained from natural extract or by using various synthetic methods [2]. From the chemical point of view it is a 2,3-disubstituted derivative of cyclopentenone with pentenyl side chain. Natural jasmine extract contains two geometrical isomers: *cis*-jasmone **1** and *trans*-jasmone **2**.



Recently we reported microwave synthesis of structural and fragrant heteroanalogues of jasmone containing saturated alkyl side chain with five-carbon atoms [3]. Saturated derivatives of pyrrolidinone, oxazolidinone and thiazolidinone have exhibited the most interesting odor and durability. The aim of the present work is preparation of successive fragrant heteroanalogues with *trans*-double and triple bond in the side chain.



As a result of the microwave-assisted reactions the series of new jasmone heteroanalogues comprising unsaturated double or triple bond in the side chain were obtained. New fragrant compounds demonstrated an interesting, specific odor which

was compared with floral, typical jasmine odor of jasmone.

References
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Acknowledgement: We are grateful for financial support to the Polish State Committee for Scientific Research (KBN Grant N^{o} 2PO5F 031 29)

17:35 Poster II-126

ANTITUMOR EFFECT OF CISPLATIN OR FLUOROURACIL IN COMBINED TREATMENT WITH (24R)-1,24-DIHYDROXYVITAMIN D_3

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Much efforts have been made to obtain active and less toxic vitamin D analogs for new clinical applications. In the previous studies, we have demonstrated that 1,24-$(OH)_2D_3$ revealed a potent antiproliferative effect against various cancer cell lines *in vitro* and this effect was stronger than obtained with parental 1,25-$(OH)_2D_3$. Moreover, we have shown that this effect could be attributed to induction of cancer cell-differentiation. Our results indicate, that 1,24-$(OH)_2D_3$ exhibits lower calcemic activity as well as toxicity than calcitriol.

In the present study we have evaluated the antitumor effect of combined treatment with cisplatin or fluorouracil and vitamin D analog and analyzed the serum calcium level in tumor (WEHI-3) bearing mice.

Single therapy with cisplatin reduced tumor volume with tumor growth inhibition (TGI) of 74%. For 1,24-$(OH)_2D_3$ we obtained 45% TGI value. However, combined treatment led only to subadditive effect. For fluorouracil we obtained 67% TGI value and for combined treatment 91% - what meant synergistic effect between cytostatic and vitamin D analog. Combined treatment did not cause an increase in life-span in comparison to applying either agent alone. The toxicity of combined treatment was the same as a singular drug treatment.

In conclusion, combined treatment of cisplatin and 1,24-$(OH)_2D_3$ lead to subadditive effect and fluorouracil with this vitamin D derivative lead to synergistic effect in reducing tumor volume.

17:35 Poster II-127

SUBSTRATE DEPRIVATION THERAPY - ALTERNATIVE TREATMENT OF MUCOPOLYSACCHARIDOSES

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Mucopolysaccharidoses (MPS) are inherited disorders of glycosaminoglycan (GAG) metabolism caused by deficiency of certain lysosomal enzymes involved in GAG degradation. This inadequacy leads to storage of GAGs in most tissues, including brain in many types of MPS, and causing serious malfunction of organs. Enzyme replacement therapy (ERT), developed for a few of lysosomal storage disorders (LSDs), including three types of MPS, has been successful in treating somatic pathology and ameliorating clinical conditions of patients. However, since the intravenously injected enzyme is unable to cross the blood-brain barrier (BBB), disorders causing neurological impairment remain refractory. Substrate deprivation aims to reduce the intracellular load of substrate (GAG in MPS) for the deficient enzyme to digest, by decreasing the initial synthesis of the substrate. The soy isoflavone genistein was reported to affect, in an inhibitory way, the synthesis of GAGs via both estrogen receptor (ER) and protein tyrosine kinase pathway (PTK). Since genistein is a relatively small molecule and it was found to penetrate BBB, it should be considered as an alternative mean for MPS treatment. Here, GAG levels were measured in cell extracts of fibroblasts isolated from MPS I, MPS II, MPS IIIA and MPS IIIB patients as well as a control (healthy person) within several days of incubation with pure genistein, soy extract or Aldurazyme (a drug used in ERT for MPS I). Genistein was found to inhibit GAG synthesis in fibroblasts. Moreover, within several days, a considerable decrease in the amount of stored GAGs was observed in all types MPS cells (in MPS I fibroblasts the level of total GAG after genistein treatment was lowered comparably to the reduction gained with Aldurazyme). Therefore, administration of genistein may be

considered as a possible supportive treatment of MPS, especially types in which central nervous system is affected and for which no clinical trials of ERT were yet initiated.

17:35 Poster II-128

CONFORMATIONS OF ARYL PIPERAZINES AS STUDIED BY NMR AND DFT CALCULATIONS

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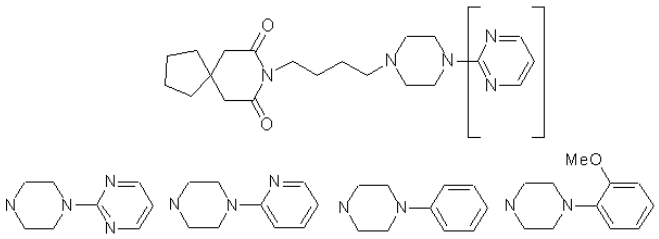
Buspirone and some of the newer buspirone-like antidepressants have become the alternative to the benzodiazepines and the treatment of choice for patients with anxiety disorders. These drugs are composed of two pharmacologically important parts, arylpiperazine and imide linked by aliphatic chain. Structural modifications involve changes: in the arylpiperazine moiety, in the imide part and in the length of aliphatic chain. The development of new biologically active structures is connected with deeper understanding of steric and electronic factors.



Considering the interaction of a ligand with 5-HT1A receptor, it was supposed that the distances between the aromatic substituent, nitrogen atom of piperazine and carbonyl group of imide moiety are important for binding of buspirone-like compounds [1,2]. Therefore, the data on the structure, conformational flexibility and properties of functional groups are of interest. Our interest was focused on the configuration of piperazine nitrogen attached to the aromatic moiety. The combined NMR and theoretical studies enabled an improvement of the model of pharmacophore. The results indicate that biological activity of buspirone-like ligands may dependent on the piramidal or flat configuration of the nitrogen atom.

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17:35 Poster II-129

PPARs REPRESS VEGF AND MMPs EXPRESSION IN HUMAN MELANOMA CELLS: IMPLICATIONS FOR CANCER ANGIOGENESIS AND INVASION.

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Peroxisome proliferator-activated receptors (PPARs) are well characterized nuclear hormone receptors (NHRs) in the superfamily of ligand-activated transcriptional factors. PPAR ligands have recently been demonstrated to affect proliferation, differentiation and apoptosis of different cancer cell types. The growth of any solid tumor depends on angiogenesis. Vascular endothelial growth factor (VEGF) plays a prominent role in vesical tumor angiogenesis regulation. VEGF is regulated by diverse developmental and hormonal signals, including eicosanoid ligands of PPAR. Previous studies have shown that the peroxisome proliferator-activated receptors (PPARgamma, alfa and beta) are expressed in four melanoma cell lines (A375, WM35, WM9, WM239).

The present study was undertaken to investigate whether PPAR ligands could inhibit proliferation of melanoma cell lines and influence expression and activity of VEGF and matrix metalloproteinase's MMP-9 and MMP-2.

Melanoma cells were treated with a PPAR ligand for 48 h. VEGF, MMP-9 and MMP-2 expression was analysed on mRNA level. In addition secreted VEGF protein was quantified by immunoassay and MMPs gelatinolytic activity was determined on zymograms. The proliferation of all the studied cell lines was inhibited by natural and synthetic ligands PPAR - linoleic acid, ciglitazone, fenofibrate, and carbacycline. Their application led also to decreased expressions of VEGF, MMP-9 and MMP-2 in all the cell lines. In addition VEGF protein secretions as well as gelatinolytic activities of MMP-9 and MMP-2 in the four melanoma cell lines were significantly inhibited by natural and syntetic PPARs ligands. We conclude that since PPAR ligands significantly inhibited proliferation of melanoma cells in vitro, and downregulated VEGF, MMP-9 and MMP-2 expression they potentially could as well inhibit invasion of treated cells through ECM. Agonists of this nuclear receptor might be exploited pharmacologically to inhibit pathological vascularization and migration of tumor cancer such as melanoma.

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PREPARATION AND EVALUATION OF PHYSICOCHEMICAL PROPERTIES OF MUCOADHESIVE, FREEZE-DRIED BUCCAL SYSTEMS WITH SELEGILINE

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Selegiline, a selective, irreversible inhibitor of monoamine oxidase type B, is widely used in the treatment of Parkinson's disease. This drug, after oral administration, undergoes extensive metabolism (mainly in the liver) and therefore the absolute bioavailability is only about 10% [1]. This problem can be solved by special drug formulations that are not designed for swallowing but release selegiline in the mouth and buccal absorption prevents first - pass effect [2]. Until now neither of these formulations provides an extended release of selegiline.

The aim of the study was to develop and evaluate a new mucoadhesive, buccal dosage form for selegiline, prepared by lyophilization, which enables the release of the drug during a few hours (optimally for 4 - 6 h) by means of the multiparticulate structure.

The study comprised of: selection of a mucoadhesive polymer for preparation of the drug carrying matrix of the lyophilized system, preparation of prolonged-release particles with selegiline (microspheres or coated pellets) and incorporation of these forms in the matrix. The microspheres of selegiline were prepared by emulsification method, using ethylcellulose as the polymer forming microspheres and the mixture of acetone and ethanol as solvent. The microspheres were formed by emulsifying the solution of drug and polymer in liquid paraffin and evaporation of the volatile solvents. Pellets were made by extrusion - spheronisation method and coated in a fluid - bed coater. Aqueous dispersion of ethylcellulose was chosen as a coating material. The obtained extended release forms of selegiline were incorporated into the carbomer viscous solution, which was dosed into blisters.

Experimental methods comprised of: visual and microscopic inspection, drug loading and release profiles both from the microspheres or pellets and from the freeze - dried system as well as organoleptic and bioadhesion analysis of the placebo systems. In vitro studies of mucoadhesion, using texture analyzer are also planed.

The obtained results show, that it is possible to prepare freeze - dried systems with prolonged release of the drug. Incorporation into the mucoadhesive matrix small polymeric reservoirs: microspheres and pellets containing the selegiline hydrochloride enables to obtain system, which will release the drug for a period of 5 - 6 hours. Carbomer can be used as the adhesive component although time of its mucoadhesion in vivo was only about 2 hours and the composition requires modification. The mucoadhesive properties of the system can be also explained by the texture analysis.

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17:35 Poster II-131

SYNTHESIS AND ANTI-HIV ACTIVITY OF 4-CHLORO-2-MERCAPTO-N-(1,4,5,6-TETRAHYDRO-1,2,4-TRIAZIN-3-YL)BENZENESULFONAMIDES

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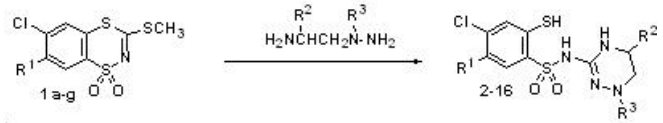
The sulfonamides constitute an important class of therapeutic agents in current medicinal science and various structurally novel sulfonamide derivatives have recently been reported to show substantial anti-HIV activities. Our extensive research program is aimed at the synthesis of 1,1-dioxo-3-methylthio-1,4,2-benzodithiazines and their subsequent transformation into 4-chloro-2-mercaptobenzenesulfonamides with the nitrogen atom of sulfonamide moiety attached to variety of heterocyclic ring systems. These compounds, depending on structure, displayed either anticancer [1,5] or anti-HIV activities [2,5] and have been described by Neamati as a novel class of potent HIV-1 integrase inhibitors [3,4]. These findings have increased our interest on new anti-HIV agents and led us to synthetize variously substituted 4-chloro-2-mercapto-*N*-(1-alkyl-1,4,5,6-tetrahydro-1,2,4-triazin-3-yl)benzenesulfona mides. The reactions of substrates **1 a-g** with the appropriate (2-aminoethyl)alkylhydrazine carried out in methanol afforded title compounds **2-16.**



R^1 = Me, COOMe, CONH-i-Pr, CONH-i-Bu, CONHPh, CONHPh-4-Me, CONHPh-4-Cl,
R^2 = H, Me,
R^3 = Me, Et, Bu, Hex.

The compounds tested for their *in vitro* anti-HIV activity in the US National Cancer Institute were essentially inactive, while 4-chloro-2-mercapto-*N*-(1-metyl-1,4,5,6-tetrahydro -1,2,4-triazin-3-yl)benzenesulfonamide displayed significant activity (IC50 = 158 μM, EC50 = 25.4 μM, TI50 = 6.21).

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THE RESEARCH ON BIOLOGICAL ACTIVITY OF ANTOXID. THE INFLUENCE ON HYDROXYL RADICAL GENERATION.

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The flavonoids present in extract from Radix Scutellariae baicalensis Georgi are very effective antioxidants. It was proved that xylene has an influence on hydroxyl radical generation, increasing its level.

The aim of present study was to explain whether antioxidative properties of Antoxid (AX) are connected with its influence on hydroxyl radical generation and whether the mechanism connected with $^{\cdot}OH$ generation is responsible for biological effect of AX. This radical is very aggressive and could initiate lipid peroxidation. It isn't deactivated with superoxide dismutase or scavenge by other active molecule. So it was interesting to look into AX mechanism of inhibition of lipid peroxidation caused by xylene.

The study was performed on *in vitro* model, human placental mitochondria. The mitochondria were isolated by Radi method from mature placenta obtained after physiological delivery from Medical University Clinic. The proteins in mitochondria were measured by Lowry method. The Antoxid was dissolved in mitochondrial buffer (TRIS-HCl - pH-7.4) and used in following concentrations: 1.5, 3.0, 6.0, 12.0 and 30 μg/mL. The hydroxyl radical was measured by deoxyribose degradation.

At first the effect of AX on $^{\cdot}OH$ generation in mitochondria by *t*-BOOH was measured. The statistically significant ($p<0.001$) decrease in $^{\cdot}OH$ level was noted after AX treatment in doses 1.5-12.0 μg/ml. It looks, that inhibiting effect of AX on lipid peroxidation provoked by *t*-BOOH, expressed as MDA level, could be caused by inhibition of hydroxyl radical generation by AX. Quite different seems the mechanism of AX effect towards lipid peroxidation provoke by xylene. It looks that the pathway connected with $^{\cdot}OH$ generation is not responsible for MDA decrease resulted after AX treatment. When mitochondria exposed to xylene in dose 17.64 μg/mL were treated with AX in doses 1.5, 3.0, 6.0 or 12.0 μg/mL any decrease in $^{\cdot}OH$ generation was observed. Quite apposite the low doses (1.5 and 3.0 μg/mL) even stimulated the $^{\cdot}OH$ formation, whereas higher doses (6.0 or 12.0 μg/mL) don't show any influences.

The mechanism of AX preventing action was also examined by treatment of mitochondria with AX, 30 min. before exposition to xylene. The results demonstrate that also the preventing activity towards lipid peroxidation caused by xylene is not connected with inhibition $^{\cdot}OH$ generation by AX. The results show that $^{\cdot}OH$ generation was even increased by AX, but it didn't correlate with MDA increase.

Conclusions.

1. Antoxid decreased hydroxyl radical generation in exposure to *t*-BOOH but not to xylene.

2. The preventing and repairing effect of Antoxid in oxidative stress caused by xylene is not connected with hydroxyl radical scavenging.

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THE RESEARCH ON BIOLOGICAL ACTIVITY OF ANTOXID. THE SYNERGISTIC EFFECT OF VITAMIN C AND ANTOXID ON FRAP DEPENDS ON THE RATIO OF COMPONENTS.

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INTRODUCTION

Our previous investigation showed the stronger effect of Antoxid than vitamin C on ferric reducing plasma ability (FRAP). Antoxid is the water-alcoholic extract obtained from Radix Scutellariae baicalensis Georgi according to special procedure. The final step in preparation was the crystallization. Antoxid is the main component of BAICADENT gel used in stomatology. It looks that the most important components which determine the FRAP activity of Antoxid are flavonoids baicaleina and baicaline.

The aim of presented study was to examine the joint effect of Antoxid with vitamin C. It is interesting to know whether the

common use of those two agents gives harmful interaction or brings some advantages.

The material was blood plasma from patients of Surgery Dept. in Medical University Clinic. The blood was taken on anticoagulant. The patients didn't receive any drugs. FRAP was evaluated by the measurements of Fe^{+2}/TPTZ-complex by colorimetric method with spectrophotometer. The three combinations of vit. C (C) and Antoxid (A) mixture were used for examination: M1 (5 µg/ml A + 5 µg/ml C); M2 (15 µg/ml A + 5 µg/ml C); M3 (5µg/ml A + 15 µg/ml C).

The results were evaluated with t-Student test.

Every mixture (M1, M2 and M3) statistically significantly increase the value of FRAP (p=0.02 - 0.002). It looks that the mixture of Antoxid and vitamin C in various concentrations posses antioxidative properties. There is no doubt that the most advantageous is the combination of low doses of agents in stechiometric ratio (1:1). The Antoxid in dose 5 µg/ml didn't show the influence on FRAP. The influence of 5 µg/ml vitamin C on FRAP was also very weak. The mixture of Antoxid in concentration 5 µg/ml with 5 µg/ml vitamin C showed strong synergistic effect on FRAP increasing its value significantly (p=0.00002). There are some observation that the presence of flavonoids and vitamin C in diet is very useful specially in the prophylaxis of cardiac diseases.

CONCLUSIONS

1. The mixture of Antoxid and vitamin C shoved antioxidative properties measured by increase of human plasma FRAP value in various concentrations.

2. The synergistic action of components was observed for stechiometric ratio of Antxid with vitamin C.

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SYNTHESIS OF PIOGLITAZONE HYDROCHLORIDE OF PHARMACEUTICAL PURITY AT SMALL PLANT SCALE

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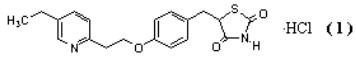
Pioglitazone hydrochloride (**1**) is an antidiabetic agent for the treatment of noninsulin dependent diabetes mellitus (NIDDM). It decreases hyperglycaemia and acts by increasing insulin sensitivity.

Reduction of
(E)-5-{4-[2-(5-ethylpyridin-2-yl)ethoxy]benzylidene}thiazolidene-2,4-dione with sodium borohydride and cobalt(II) complex in dimethyldioxyme and a subsequent conversion of a free base with HCl afforded pioglitazone hydrochloride in high yield (about 95%) and purity (above 99%).



(1)

The scale-up was performed taking into account the results of a laboratory study on the optimization of a key process of the synthesis, i.e. the reduction of the double bond in benzylidenethiazolidenedione [1,2].

A summary of the small plant scale synthetic procedure will be given. More details are available through the polish patent application No. P-376857 [3].

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SYNTHETIC ANALOGUES OF NETROPSIN WITH POTENTIAL ALKYLATING PROPERTIES

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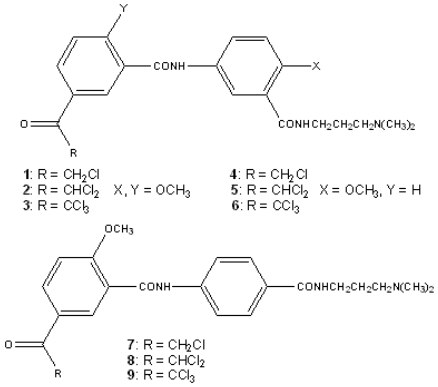
Several antitumour agents act by binding within the minor groove of double stranded DNA and interfering with both replication and transcription. One of most studied minor groove binders are the oligopeptide antibiotics netropsin and distamycin. The attachment of reactive moieties to the *N*-terminus of these compounds increased the affinity of the attached group to the minor groove. From this point of view, netropsin, distamycin and their synthetic analogues, named lexitropsins, are excellent carriers of alkylating elements [1]. Carbocyclic lexitropsins possess greater curvature of molecules and bind to DNA more weakly than parent netropsin and distamycin, but they interfere with catalytic action of topoisomerases and show antiproliferative activity [2].

In the course of our investigations of carbocyclic lexitropsins [3], compounds **1-9** with chloroacetyl, dichloroacetyl and trichloroacetyl as the *N*-terminal groups were obtained. The alkylating activity in the Preussmann's test [4] and cytotoxic effect on the proliferation of MCF7 cell cultures were studied.



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17:35 Poster II-136

3,3'-DIINDOLYLMETHANES - SYNTESIS AND STRUCTURAL ANALYSIS

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3,3'-Diindolylmethane is a promising cancer chemopreventive agent derived from *Brassica* food plants, and its mode of action is widely examined. Therefore we have chosen it as a template structure in our search for new compounds with better pharmacological profile.

In course of our studies we are interested in optimization of synthetic procedure leading to 5,5'- or 6,6'-disubstituted *bis*-indoles (Fig. 1) [1].

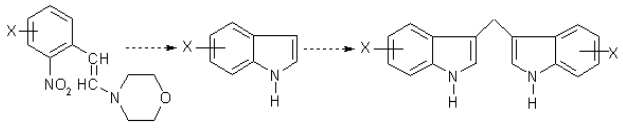

Fig. 1. Intermediates in the synthesis of *bis*-indoles.

The ^{1}H and ^{13}C NMR spectra of reagents in solution show standard resonances and enabled us to confirm the synthetic pathway.

The ^{13}C CP/MAS NMR spectra of products represent various signal patterns. This could have been caused by:

- the presence of two energetically similar conformers in the solid state

- the different packing mode of both indole rings in one conformer

- the presence of only one conformer with magnetically equivalent C atoms in both indole rings

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17:35 Poster II-137

OPTIMALIZATION OF THE METHOD FOR DETERMINATION OF AFLATOXINS IN HERBAL DRUGS

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Aflatoxin (AF) is the strongest know naturally occurring carcinogen. It can be present in various foods, spices or medicinal plants. No legal limits have yet been provided for medicinal herbs and there are not available standards method for its assay, although it is well known that some plants could be frequently contaminated.

The method of Reif and Metzger (1995) was adopted and it was optimized for determination of aflatoxins in herbal drugs from Poland. The modified method has three major steps. The first, the samples were extracted with 70 % (v/v) methanol in water and centrifuged. The supernatant was then filtered through a folded filter paper. The second, the clear filtrate was diluted with 10 % (v/v) Tween20 / PBS and cleaned up with an immunoaffinity column (IAC). After washing the IAC with wather, the aflatoxins were eluted from the column with methanol. The final step was the separation and determination of AF by reversed-phase LC and detected by fluorescence after post column derivatization (PCD) involving bromination. PCD was achieved with an electrochemical (Kobra) cell by addition of bromide to the mobile phase. The extraction procedure was optimized in order to obtain the best recovery and minor changes to HPLC conditions were intoduced to improve the method. The use of twice larger volume of extraction solvent and addition of a surfactant improved aflatoxins recovery.

Statistical analysis of the data was performed to determine recoveries and repeatability of the method. Recoveries of AFs

(B1, B2, G1) added to *cortex salicis* and semen lini were over 70% at two levels of fortification (higher level: 2 ng/g for every type of AFs, lower level (only cortex): 0.5 ng/g, for every type of AFs except for the case of B1 in semen lini (68%). The recoveries of G2 were 34-37%. For cortex salix, relative standard deviations for repeatability (RSD) for all aflatoxins ranged from 1. 4 to 5% for two contamination levels. For semen RSD values for all aflatoxins ranged 0. 4 to 3. 8 %.

This validated assay was applied to determine the degree of contamination of AFs in 16 various herbal medicinal products currently marketed in Poland. The method was successfully carried out with all herbal products diversified as to compositions and dosage forms. The results revealed that ten of herbal samples were contaminated and in two samples detectable amount of the total AFs was higher then the current legislative level for foods. The assay will be continued.

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LC-MS DETERMINATION OF TAMSULOSIN IN HUMAN PLASMA

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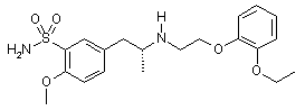
Tamsulosin (-)-(*R*)-5-{2-[[2-(*O*-ethoxyphenoxy)ethyl]amino]propyl}-2-methoxybenzenesulfonamide - is a selective α_{1A} adrenoreceptor antagonists, which is used clinically as an oral medication to ameliorate the dysuria associated with prostatic hypertrophy [1, 2]. The aim of the study was to adapt and validate HPLC-MS method for determination of tamsulosin in human plasma.



Detection was performed on a quadrupole mass spectrometer LCMS-2010 (Shimadzu). The electrospray ionization (ESI) with capillary voltage at 4.9 kV, nitrogen flow rate at 4.5 L/min, CDL (*Curved Desolvation Line*) voltage at 40 V, temperature of CDL and block set at 190 °C and 260 °C, respectively, were used. Target ions were monitored in positive mode at *m/z* 409.05 for tamsulosin $[M+H]^+$ and at *m/z* 573.20 for I.S. $[M+H]^+$.

Sample preparation was based on liquid-liquid extraction with hexane-ethyl acetate (1:1) after addition of saturated sodium bicarbonate solution to 0.5 mL of plasma. Organic layer was evaporated and reconstituted in mobile phase.

The separation from endogenous compounds was performed on a Nucleosil C18, 5 m m (125 mm x 4.0 mm i.d.) column with oven temperature at 40 °C. Mobile phase consisted of methanol and 0.05 M ammonium acetate buffer pH 3.7 (6:4, v/v, flow rate at 0.5 mL/min). Internal standard (IS) was (*R*)-5-{2-[Bis[2-(2-ethoxyphenoxy)ethyl]amino]propyl}-2-methoxybenzenesulfonamide.

The limits of detection and quantification were determined at 0.1 ng/mL and 0.7 ng/mL, respectively. The calibration curves were obtained by weighted (1/x) linear regression analysis and were linear up to 35.0 ng/mL.

Validation parameters calculation was based on assaying three different concentrations of tamsulosin (2.1, 14.0 and 28.0 ng/mL). The recovery of both tamsulosin and I.S. was ca. 70-80%. No matrix effect influencing selectivity, sensitivity and precision of the method was observed. Accuracy during the day and within three days ranged 91-103% and 98-108%, respectively. Precision of the method within one day and within three days was determined at 2.1-3.1% and 2.4-5.9%, respectively.

Stability of stock and working solutions of tamsulosin and I.S. was confirmed. No influence of storing plasma samples neither for 4 months at -20 °C, nor for 4 hours at room temperature, on stability of tamsulosin was observed. Three freeze-thaw cycles did not effect quantitation of tamsulosin. Obtained results confirmed stability of processed samples in autosampler (18 hrs).

The method was validated according to regulatory authority requirements (EMEA, FDA) and was subsequently applied to bioavailability studies of 0.4 mg tamsulosin modified release capsules.

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17:35 Poster II-139

PRELIMINARY STUDIES ON IMMUNOTROPIC ACTIVITY OF THE NEW LEAD STRUCTURES IN THE ISOXAZOLE HETEROCYCLIC SYSTEM

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Title compounds were preliminary tested for their immunotropic activities in several experimental models such as: the secondary humoral immune response of mouse splenocytes to

sheep red blood cells (SRBC), the proliferative response of splenocytes to concanavalin A and lipopolysaccharide (LPS)-induced production of tumor necrosis factor alpha (TNF alpha) and interleukin 6 (IL-6) in cultures of human peripheral blood mononuclear cells (PBMC). Each of the four studied compounds showed different characteristics in affecting the tested immune parameters. Our studies on these activities and their potential application are discussed.

This work was supported by the State Committee for Scientific Research (KBN) Grant No. 3 P05F 012 24

Reference

Ryng S, Zimecki M, Maczynski M, Chodaczek G, Kocieba M.: Immunosuppressive activity of an isoxazolo[5,4-e] triazepine - compound RM33. I. Effects on the humoral and cellular immune response in mice. *Pharm Rep*, **2005**, *57*, 195-202

17:35 Poster II-140

FIRST ROUND STUDIES ON LEWIS LUNG CARCINOMA ACTIVITY OF THE NEW LEAD STRUCTURES IN THE ISOXAZOLE HETEROCYCLIC SYSTEM

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In our previous studies, two types of isoxazole derivatives weres tested on anticancer activities [1, 2, 3]. In this communication the effect *in vivo* of the new lead structures applied intraperitoneally on the growth of experimental Lewis lung carcinoma (LLC), transplanted subcutaneously, is presented. We observed the tendency of growth inhibition of LLC cancer by compound R13 with the tumor growth inhibition (TGI) achieving 30%. In the case of other compounds tested (R11, R12, R14 and R15) we observed no effect or stimulation of LLC tumor growth. The increase in life span (ILS%) of mice, which was administered with the compounds was 7-17% and was not statistically significant. Observation of body weight shows no toxicity of compounds tested. In conclusion, we show that compound R13, may inhibit tumor growth. Other compounds in these conditions, may stimulate Lewis lung cancer growth. Our studies on these activities and their potential application are discussed.

This work was supported by the State Committee for Scientific Research (KBN) Grant No. 3 P05F 012 24

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17:35 Poster II-141

SIMULTANEOUS DETERMINATION OF MIDAZOLAM AND ITS α-HYDROXY METABOLITE IN HUMAN PLASMA BY LC-MS USING AN AUTOMATIC SOLID-PHASE EXTRACTION SYSTEM

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Midazolam (8-chloro-6-(2-fluorophenyl)-1-methyl-4*H*-imidazo[1,5-*α*][1,4] benzodiazepine), a short-acting benzodiazepine with hypnotic properties, is used for conscious sedation to perform short diagnostic and endoscopic procedures and for induction and maintenance of general anaesthesia [1]. Midazolam is metabolized by the cytochrome P-450 enzyme system to several metabolites including an active metabolite, α-hydroxymidazolam [2].

We have developed a simple and reproducible HPLC-MS/APCI method to determine the concentration of midazolam and its α-hydroxy metabolite in human plasma. The method consists of a solid-phase extraction procedure (SPE) as the sample preparation step.

The SPE operations were performed automatically by means of a sample processor equipped with a robotic arm (Gilson ASPEC XL). The ASPEC was programmed to condition each Bakerbond SPE C2 extraction column with 1 mL of methanol followed by 1 mL of water and 1 mL of 1% acetic acid just before use. A 0.5 mL of plasma sample containing the internal standard (flunitrazepam) was then applied on the cartridge. The washing step was performed with 2 x 1 mL of 0.01 M ammonium acetate. Finally, the analytes were eluted with 2 x 1 mL of 0.2 M ammonium acetate (pH 9.0) in methanol (10:90,v/v). The eluent was evaporated to dryness. The sample residue was dissolved in 200 µL of mobile phase,

transferred to autosampler vial and 60 µL aliquot was injected onto a LC-MS system. The chromatography separation was achieved on a 100 x 4.6 mm Chromolit RP-18e column operated with a mobile phase of acetonitrile and 0.01 M ammonium acetate buffer pH 4.3 (52:48,v/v) at a flow-rate of 1 mL/min. The mass spectrometer equipped with atmospheric pressure chemical ionization (APCI) source was run in the positive ion mode and set with a selected ion monitoring method (SIM) of ion m/z = 325.75, 341.75 and 313.75 for midazolam, α-hydroxymidazolam and flunitrazepam, respectively

The recoveries from plasma samples for midazolam and α-hydroxymidazolam ranged from 84.8 to 97.7% and from 85.8 to 88.4%, respectively. The limit of detection was established as 0.1 ng/mL for both compounds. The linearity was assessed in the concentration range 1.5-100 ng/mL for midazolam and 1.5-60 ng/mL for α-hydroxymidazolam. The intra- and inter-run precision and accuracy values were less than 10% for both compounds. No matrix effect was observed.

The sensitive and reproducible procedure described offers possibility to measure plasma level of midazolam and α-hydroxymidazolam after oral administration during the pharmacokinetic studies in humans.

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17:35 Poster II-142

ANTIMICROBIAL ACTIVITIES OF 4-SUBSTITUTED 3-(PIPERIDIN-4-YL)-Δ^2-1,2,4-TRIAZOLINE - 5 - THIONES

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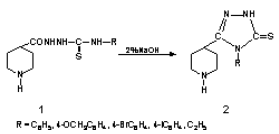
The cyclization reaction of 4-substituted 1-[(piperidin-4-yl)-carbonyl]thiosemicarbazides **1** gave 4-substituted 3-(piperidin-4-yl)-Δ^2-1,2,4-triazoline-5-thiones **2**.



1 2

R = C_6H_5, 4-$OCH_3C_6H_4$, 4-BrC_6H_4, 4-IC_6H_4, C_2H_5

Cyclic compounds **2** with R = C_6H_5, 4-BrC_6H_5, 4-I-C_6H_5 were screened for their antimicrobial activity *in vitro* against 14 species of aerobic bacteria and 12 species of fungi. MIC (Minimal Inhibitory Concentration) values defined as the lowest concentration of compounds required to inhibit the visible growth of the tested microorganism, was estimated by agar-dilution method. Petri dishes containing Mueller-Hinton agar (for bacteria) or RPMI 1640 agar (for fungi) and the compounds at a concentration of 31.25-500 mg/mL were inoculated with a culture size no. 0.5 McFarland Standards and incubated at 37 ^oC for 18 h for bacteria and at 30 ^oC for 5 days for fungi. The growth of microorganisms were compared with a control culture without the tested compounds. The compounds **2** with R = C_6H_5 and R = 4-I-C_6H_5 were not active against bacteria, however, compound **2** with R = 4-BrC_6H_5 partially inhibited the growth of *Staphylococcus* sp. species (by about 80-95%) with values ranging from 125-500 mg/mL. All compounds were active against *Trichophyton* sp. with MIC values of 62.5-500 mg/mL, depending on the species. The tested compounds had not activity against yeast-like fungi and moulds. Only compound **2,** R = 4-BrC_6H_5 affected the growth of *Aspergillus niger* with MIC value of 62.5 mg/mL.

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SYNTHESIS OF SOME NEW 4-SUBSTITUTED - 1,3 - DIPHENYL-1,2,4-TRIAZOLINE - 5 - THIONE WITH POTENTIAL PHARMACOLOGICAL ACTIVITY

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Compounds with 1,2,4-triazole moiety have received considerable attention among medicinal chemists because molecules with this structural feature have been found to display a wide range of potent biological activities, such as antifungal, antibacterial, antiviral, anti-inflammatory and antitumor. One of the method to synthesize these compounds is the reaction of condensation of amidrazone hydrochlorides with isothiocyanates. The above method was applied to synthesis of ethyl ester of 1,3-diphenyl-1,2,4-triazoline- 5-thione-4-acetic acid **1**, which was next used to prepare new compounds by transformation in the ester group. Thus, **1** was transformed first into the corresponding hydrazide **2** and then into the respective thiosemicarbazides **3**. Next, the base or acid catalyzed intramolecular dehydrative cyclization of **3** furnished compounds **4** composed of two 1,2,4-triazoles linked through the methylene group or compounds **5** consisting of 1,2,4-triazole and 1,3,4-thiadiazole linked in the same way.

Compounds with the similar structure show a weak toxicity and have sedative activity. We expected that new analogues also could have sedative activity on the central nervous system.

The structures of the new compounds were established by the

elemental analysis, infrared and nuclear magnetic resonance data.

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SYNTHESIS AND ANTITUMOR ACTIVITY OF NOVEL S,N-DISUBSTITUTED 2-MERCAPTO-BENZENESULFONAMIDES

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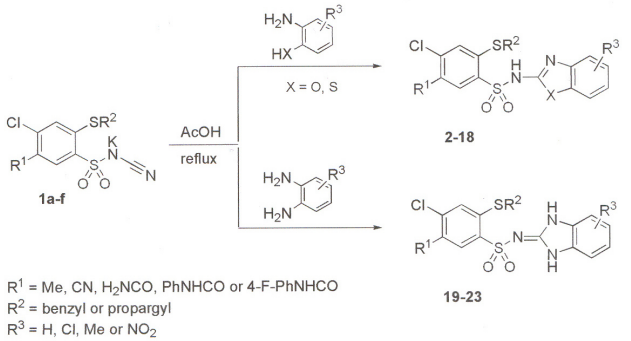
Our recent studies on 2-mercaptobenzenesulfonamides revealed that a number of their *S,N*-disubstituted derivatives possess substantial in vitro antitumor activity [1-4]. This prompted us to develop a method for the synthesis of a new series of sulfonamides bearing heterocyclic rings attached to the sulfonamide nitrogen atom.

The synthesis of the target compounds **2-23** were achieved by reacting the corresponding *N*-(2-alkylthiobenezenesulfonyl)cyanamide potassium salts **1a-f** with suitable *ortho*- substituted anilines, such as *o*-aminophenoles, *o*-aminothiophenols or *o*-phenylenediamines under reflux in glacial acetic acid.



Seventeen of obtained compounds were screened at the NCI (Bethesda, USA) for their *invitro* activities against a panel of 60 human tumor cell lines. Ten of these compounds exhibited a pronounced activity against numerous tumor cell lines. The prominent 2-benzylthio-4-chloro-5-(4-fluorophenylcarbamoyl)-*N*-(benzoxazol-2-yl)benzenesulfonamide **11** showed significant activity toward some cell lines of non-small cell lung cancer, melanoma and ovarian cancer (GI_{50} values in the range 0.1-0.6 μM).

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This study was partially supported by Polish State Committee for Scientific Research (grant no 2 P05 035 27

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NOVEL, BIOLOGICALLY ACTIVE INHIBITOR OF DD-PEPTIDASE 64-575.

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The use of penicillin G, β-lactam-containing antibiotic has began the era of antibacterial therapy. The therapeutic use of penicillin G preserved the millions patients. Thousands of derivatives have been designed around the simple 2-azetidinone ring. Despite discoveries, syntheses and administration of new β-lactam derived and other antibiotics, the war against infectious bacteria continues. Within a short time after introduction of each new antibiotic, nature seems to deliver a counter punch in the form of newly resistant bacteria. Microbes have altered their permeability barriers, drug target binding sites or even induced synthesis of β-lactamases to destroy antibiotics. While continued effort is needed to extend the effectiveness of current antibacterial arsenal and to identify alternate drug targets, innovative approaches to antibacterial treatment are urgently needed.

The previously conducted experiments showed that strain *Streptomyces* sp. from National Institute of Hygiene Collection is producer of inhibitors of DD-peptidase 64-575 and the inhibition is not degraded by β-lactamases of classes A, B, and C and in wide range of pH. The inhibitor of DD-peptidase was obtained on the way of biosynthesis. Culture of *Streptomyces* sp. was elaborated by centrifugation to obtain supernatant. Futher steps of purification: acetone protein precipitation, liquid chromatography (anion-exchange, pH gradient), HPLC (C18 phase). The inhibitor was characterised as follow: physico-chemical properities: the MW of 207.1 was obtained from MS, some structural information (heterocyclic compound, carboxylic group) was obtained with NMR. Inhibitor showed antibacterial activity against *Proteus vulgaris* and *Escherichia coli*). Futher experiments will be done to establish the chemical structure of the novel β-lactams (grant MNiE 3 PO5F 033 24).

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THE ADVERSE DRUG REACTION - THEORY AND PRACTICE

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One of the most important tasks of contemporary pharmacy is the search for new drugs characterised by high activity and being safe to use. Unfortunately, the majority of drugs show some adverse drug reaction (ADR) or toxic effects, usually related to intake of an overdose, taking a drug for a too long time or hypersensitivity to a given drug components. Side effects can be often minimised by administering another pharmaceutical form of drug or an alternative drug from another chemical group. This solution is particularly important in treatment of the elderly whose enzymatic efficiency is reduced. An important element of correct hospitalisation is to report and register all unwanted side effects of a given drug, which would eventually lead to a shortening of the treatment, reduction of its cost and help avoid the undesired effects in other patients. Each report on the undesired side effect of a given drug or on the frequency and conditions of its occurrence contributes to the knowledge on a given drug and permits optimisation of the pharmacotherapy. The character and intensity of the adverse drug reaction differ from very mild to highly serious. The widest range of side effects has been observed to accompany the use of cytostatics, antibiotics and antiinflammatory drugs. The most often developing ADR include dysfunctions of the alimentary track, allergic reactions and skin changes. The undesired side effects can be caused the mechanism of the drug activity (cough after of inhibitors ACE), the chemical structure (skin changes after application of coxibes, stomach dysfunctions after acetylcysteine) or by a combination of these two factors (stomach ulcers after administration of non-steroid antiinflammation drugs).

In the years 2000-2004 at the Department of Dermatology a project was undertaken to monitor patients who developed skin changes as a result of ADR of the drugs administered. In the five years 57 patients, including 30 men and 27 women were observed. The majority of the patients developed macular - papular rash (16), urticaria medicamentosa and Quincke's oedema (16) and erythema multiforme (15). The other cases included erythroderma (5), anaphylactic shock (2), lichenoid dermatosis (1), vesicular eruption (1) and Hoigne's syndrome (1). The most probable factors producing these changes were concluded to be antibiotics (45%), non-steroid antiinflammation drugs (33%) and the other drugs responsible were from different pharmacological and chemical groups (carbamazepine, diltiazem, hydrocortisone, methotrexate and herbs). The frequency of occurrence of skin changes of particular type in age groups were also analysed. The greatest number of skin changes in response to drug therapy was in the age group 41 - 60, while for the age group > 60 ADR revealed the greatest diversity in the clinical picture. The most probable explanation of these findings are the polypragmasy applied in the elderly patients and the interdrug interaction as well as decreased efficiency of the organic clearances.

The conclusion is that although the cases of drug rashes made a small percent of those in hospitalised patients of the Department of Dermatology, the fact of their occurrence as a result of adverse drug reaction should be taken into consideration in planning pharmacological treatment.

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DELAYED TYPE HYPERSENSITIVITY TO PENICILLIN IN A PATIENT WITH MORPHEA - CASE REPORT

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Localized scleroderma (morphea) is a relatively benign skin condition, characterized by well-defined, round, irregular or linear sclerotic plaques. The disease affects all age groups, although the peak incidence is observed between 20-40 years of age. The etiology of morphea has not been yet fully understood, but there is evidence pointing towards autoimmune, hereditary, chemical and traumatic factors acting as causative agents. There is no standard treatment scheme in morphea and in less severe cases local therapy alone may be sufficient. Systemic therapy includes, among different measures repeated courses of intramuscularly (i.m.) administered procaine penicillin, which in many patients results in softening of affected skin.

We describe a case of a 40-year old patient with localized scleroderma, whose first course of i.m. treatment with a single dose (2 400 000 i.u.) of procaine penicillin was disturbed after 11 hours by the appearance of maculopapular ex-

anthem, localized on the trunk and extremities, with co-existance of pruritus and oedema. Intradermal tests with penicilloylpolylysine (PPL) and 2% procaine performed before penicillin administration were negative after 15, 30 and 60 minutes and within 24 hours of observation.

3 months after the remission of skin lesions diagnostic procedures were performed including skin prick tests (negative with 2% procaine, PPL, benzylpenicillin (BP) and amoxicillin), intradermal tests (negative with 2% procaine, PPL, amoxicillin and positive after 3 hours with BP) and patch tests (negative with amoxicillin and ampicillin and positive after 48 hours with BP). Skin biopsy was obtained from the area of positive intradermal test result, showing mixed dermal inflammatory infiltrate with the majority of eosinophils.

Penicillins may be responsible for various types of allergic reactions, classified as immediate and nonimmediate, based on the time interval between drug administration and their onset. Both patch and intradermal tests are regarded by several authors as useful in evaluating delayed type hypersensitivity to penicillins, particularly maculopapular exanthems. In the case of our patient, maculopapular rash could be caused by minor penicillin determinant mixture (PPL - negative, BP - positive). Delayed hypersensitivity to beta-lactams is a long-lasting condition, therefore further observation of the described patient is planned in order to evaluate the duration of skin tests positivity.

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INTERACTION OF THE HISTIDINE WITH PACLITAXEL SEMIEMPIRICAL COMPUTER SIMULATION STUDY

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Taxol® (paclitaxel) is currently considered one of the most important drugs in cancer chemotherapy. Paclitaxel activates tubulin by binding to the microtubules, stabilizing the assembled form, and blocking the microtubule dynamics necessary for cell function. The binding site of paxclitaxel with β-tubulin was recently defined by electron crystallography [1] and such electron-density map of refined complex integrated in modeling strategy by J. P. Snyder from Emory Univeristy Atlanta, Georgia, USA [2]. The fundamental reason for activity of paclitaxel is related to the location of the β-tubulin's His-229 in the taxoid binding pocket [3].

The stability of complexes between the paclitaxel and $N^{\delta 1}$, $N^{\varepsilon 2}$ tautomers of the histidine as a binding center of the β-tubulin protein receptor in vacuo has been analysed. The His-229 has been isolated computationally from Snyder's receptor and converted into *N*-acetyl-*N*-methylamide derivative using the molecular modeling package HyperChem7. In calculation the two different histidine derivative molecules have been analyzed, due to the possibility of the imidazole ring tautomerism. Both structures have been kept in unchanged position referring to the backbone using restraints. Two torsion angles crucial to the spatial position of the imidazole ring have been changed simultaneously from 0 to 360° with step of 30°.

The conformers obtained (144 for each tautomer) have been docked to paclitaxel as in 1JFF model and then complexes have been tightly optimized. The stability of complexes has been estimated as the π-π stacking interactions among imidazole ring of histidine and the

3'-benzamide and the 2-benzoyl residue of paclitaxel. Both the intramolecular hydrogen bond in N-acetyl-N-methyl histidine and the intermolecular hydrogen bonding stabilizing complexes have been observed. An analysis of the complexation energy [δ(ΔH) = -3,7 to -7,1 kcal/mol] showed that the stability of complexes in a disctinct way depends on the stability of the histidine conformer.

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17:35 Poster II-149

ENERGY OF PACLITAXEL BINDING TO PROTEIN: EVALUATION OF THE CONTRIBUTION OF PROTONATED 229 HIS OF β-TUBULINE BY COMPUTATIONAL METHODS

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The amino acid histidine (His) is unique, as it can exists in the neutral and positively charged form at the physiological pH. Its imidazole residue with the pK_a of 6.5 may or may not adopt proton in the process which thermodynamics depends on the protein environment. As such, it can interact with other

residues (or functional groups of ligands) forming hydrogen bonds with both proton donor and proton acceptor residues. Therefore, the imidazole ring of histidine plays an important role in enzymatic catalysis as well as in the interactions having remarkable contribution toward protein stability.
The chemoterapeutic drug paclitaxel (Taxol®) interacts with the specific site on β-tubuline and its location is well defined with electron crystallography. Unfortunately, despite the enormous advance in structural information about the amino acids involved with paclitaxel binding, there is the lack of relevant energetic data. Our model was constructed to ascertain the special role of the 229 His residue in anchoring and positioning the ligand in this binding site. Thus our data using imidazolium cation as the central unit of the model, maximize the ability for ligand bounding.
Quantum chemical calculations (PM3) were performed to investigate the effect on the geometry of complex and both hydrogen-bonds and stacking interactions of protonated imidazole as N-acetyl-N-methylamide of histidine (amaHis) when surrounded by two hydrogen bond acceptors (1-hydroxyl and 3'-carbonyl) and two phenyl rings of paclitaxel ligand side chains. This work tries to provide a better molecular understanding of the complex energetics estimated to be of -20.3 kcal/mole for this specific part only. We have also estimated the potential energy surface (PES) of amaHis. The PES indicates that 229 His has only one deep minimum of energy, which plays an important role in molecular recognition of paclitaxel molecule by acting probably as the strongest preliminary attractor during the docking process.
The optimized model reliably reproduces the features of electron crystallography structure and moreover, calculations provide interesting new structure details. Finally, the calculated atomic charges, binding energy and molecular orbitals provide a detailed picture of the chemical interactions and hydrogen bonding in part of the active site that would facilitate further inquiry into the mechanism of complex formation with other antimitotic, taxol-like drugs.

17:35 Poster II-150

THE INFLUENCE OF LECITHIN ON RELEASE OF PARACETAMOL FROM SUPPOSITORIES IN IN VITRO STUDIES

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Various methods can be used for the release studies of drugs from suppositories, including beaker method, basket method, membrane diffusion method, dialysis method and flow-through cell method [1]. Among those the pharmacopoeial (Ph. Eur.) flow-through cell is probably the most useful although published data on its feasibility are rather scarce.

The aim of this study was to investigate the influence of lecithin on the release rate of paracetamol from suppositories.

The suppository was placed in the cell with a temperature sensor, the apparatus was thermostated at 37 ± 0.1 °C, the flow rate of an acceptor medium (phosphate buffer pH 7.3) was 1.7 ml/min. Samples were collected at: 0.5; 1; 1.5; 2; 3; 4 and 5 h. The concentration of drug in the dissolution medium was analysed using spectrophotometry and first derivate spectrophotometry.

The test was carried out on six suppositories from each formulation. The preliminary test was performed for two commercially available products, with and without lecithin. Much slower paracetamol release was found from the product A containing lecithin than from the product B containing another surfactant: after 4 h the dose released was 16 % and 98 %, respectively. In the second step of investigation comparison between two different formulations containing 125 and 500 mg of paracetamol confirmed that the addition of 2% lecithin decreased the rate of drug release from suppositories. It was observed that for the suppositories containing 500 mg paracetamol, with and without Lipoid S-100, after 4 h the release of the drug was 37 % and 97 %, respectively. For 125 mg paracetamol the dose released after 0.5 h was 24 % (with lecithin) and 45 % (without lecithin).

The flow-through cell method shows the differences in release profiles of paracetamol depending on the presence of lecithin, an excipients often used in commercial preparations. It is demonstrated that lecithin significantly decreases drug release rate from fatty base suppositories.

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17:35 Poster II-151

GC METHOD FOR QUANTITATIVE DETERMINATION OF RESIDUAL 2-(2-CHLOROETOXY)ETHANOL (CEE) AND N-METHYL-2-PYRROLIDINONE (NMP) IN PHARMACEUTICAL ACTIVE SUBSTANCE

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The European Pharmacopoeia describes a general procedure for Identification and Control of Residual Solvents in a drug substances [1]. Implementation of this general method is a

subject of major concern in the pharmaceutical industry. Some problems have been overcome, for instance quantitative determination of hight-boiling solvents such as 2-(2-chloroetoxy)ethanol (CEE) and *N*-methyl-2-pyrrolidinone (NMP).

A gas chromatographic method with direct-injection for quantitative determination of residual CEE and NMP in pharmaceutical active substance has been developed. The separation was obtained on a DB-624 column (60 m x 0.32 mm i.d. x 1.0 μm coating thickness). A dimethylformamide is proposed as sample solvent to obtain good selectivity and sensitivity.

Validation was performed within the requirement of ICH validation guidelines Q2A and Q2B [2, 3]. Precision, intermediate precision, linearity, accuracy, limits of detection and quantitation and robustness were determined, and excellent results were obtained.

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17:35 Poster II-152

PYRIDO-1,2-THIAZINES AND THEIR IN VITRO ANITBACTERIAL EVALUATION

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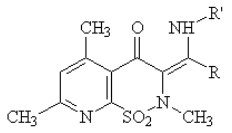
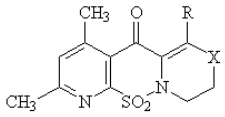
A series of pyrido-1,2-thiazine derivatives of enamine type **(E)-1** and the related triheterocycles **2**, **3** were prepared and tested *invitro* in a microbiological evaluation in our laboratories. It is worth noting that triheterocycles **2** and **3** belong to the new systems we described in 2004 [1] and here we present the first information about their pharmacological (microbiological) properties.



(E)-1: R = CH_3 or C_6H_5
R' = aminoalkyl, hydroxyalkyl, *p*-Cl-C_6H_4-CH_2, tetrahydrofurfuryl



2: R = CH_3 or C_6H_5; X = O
3: R = C_6H_5; X = NH

The **(E)**-configuration of enamines **1** and the structures of triheterocycles **2, 3** were assigned on the bases of IR, ^{1}H NMR, and X-ray data (in some cases) [1]. In this context it should be noted that triheterocycle **3** can be considered as an **(Z)**-analogue of **(E)**-enamines **1**.

Eleven of new and described recently compounds **1** - **3** were tested against *Mycobacterium fortuitum* and *Staphylococcus aureus*. Activity against *M. fortuitum* correlates closely with that against *M. tuberculosis* and may be also used as a measure of anti-*Mycobacterium tuberculosis* activity because of the potential hazards of using *M. tuberculosis* [2].

Under microbiological evaluation, the compounds demonstrated no activity at the MIC_{50} and MIC_{90} levels, even at the maximal employed concentration (250 mg/mL), or their limited solubility prohibited an accurate determination.

However, to our complete surprise, most of the compounds tested helped to stimulate the growth of both *M. fortuitum* and *S. aureus.* The best stimulants enhanced the growth of the microorganisms within the range 10-50% at different concentrations of individual compounds (from 15.6 m g/mL to 250 mg/mL). It should be noted that for some of these compounds the effect of enhanced bacterial replication at a level of 10% was observed even at sub-m g/ml concentrations (0.03-0.45 mg/mL). However it is difficult to say if the stimulation observed at low concentration was a consequence of enhanced bacterial replication by the preparations or by a lack of activity of the compounds which allowed natural growth of the microorganisms.

On the basis of above data it may be concluded that the pyridothiazines of **(E)-1** enamine type and derivatives of the new triheterocyclic systems **2** and **3** do not seem to be any specific antibacterial groups.

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17:35 Poster II-153

GENISTEIN, ITS NEW ANALOGS AND COMPLEXES - THE ANTIPROLIFERATIVE EFFECTS ON HL-60 LEUKEMIA CELL LINE

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Genistein, a naturally occurring isoflavonoid, has estrogenic activity and is used as a natural substitute for estrogen replacement therapy in postmenopausal women. Genistein displays antitumor, antimetastatic and antiangiogenic properties, described in various experimental in vitro and in vivo models. We observed that genistein and its new analogs: IGF-027, IFG-043 and polysaccharide complexes: schisophillan x genistein (SG) and xyloglucan x genistein (XG) significantly inhibited the growth of human leukemia cell line HL-60. The ID50 value of genistein was 2.95 mg/ml. The analogs of genistein had stronger antiproliverative activity than genistein (ID50 value of IFG-027 was 0.35 mg/mL, of IGF-043 was 0.41 mg/mL), whereas the complexes had similar antiproliferative properties like genistein (ID50 value of SG - 2.4 m/ml, of XG - 2.15 mg/mL). We studied also the influence of genistein, its analogs and complexes on the cell cycle of HL-60 cell line. The concentration 0.1 mg/mL of this agents was too low to exert some effects on the cell cycle. In concentration 1 mg/mL only IFG-027 and IFG-043 decreased the number of cells in S and G2/M phase and also increased the number of apoptotic cells. Genistein in the highest concentration studied (10 mg/mL) stopped the cells in G2/M phase and increased apoptosis. Additionally decrease in the number of cells in S and G0/G1 phase was observed. The analogs of genistein (in concentration 10 mg/mL) decreased number of cells in S, G0/G1 and G2/M phase and also led the cells to apoptosis. SG complex has weak influence on the cell cycle of HL-60 cells. This agent in concentration 10 mg/ml stopped the cells in G2/M phase and increased the number of cells in G0/G1 phase. XG complex didn't exerts the effects on the cell cycle of HL-60 cells. Then we studied the influence of this compounds (in concentration 1, 5, 10 mg/mL) on the apoptosis and necrosis of HL-60 cells. Genistein, IFG-027, IFG-043 and SG increased number of cells in late apoptosis and necrosis (IFG-027 and IFG-043 showed this effects also in the lowest concentration used (1 mg/mL)). Only IFG-027 and IFG-043 increased the number of cells in the early apoptosis. XG complex didn't exert effects on apoptosis (late and early) and necrosis in concentrations used. The analogs of genistein (IFG-27, IFG-043) have higher antiproliferative and proapoptotic activity but exert different influence on cell cycle than genistein. This two compounds could be considered as lead compounds for further development.

This work was supported by the Foundation for Development of Pharmaceutical Sciences (grant 8/FB/2004, Poland)

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COMBINATION OF: IMATINIB, PRI-2191 AND CYTOSTATICS - THE ANTIPROLIFERATIVE EFFECTS ON HL-60 LEUKEMIA CELL LINE

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One of the main problems in chemotherapy of patients with cancer is administration of drugs in high doses usually resulting in side effects. Drug administration protocols include different cytotoxic drugs used in various combinations. Consequently, it is of major interest to identify compounds combinations that can enhance their individual activity. The purpose of the work was to analyze the influence of imatinib, PRI-2191 (analog of calcitriol) and cytostatics (cisplatin, idarubicin and docetaxel) combination on the inhibition of proliferation of human leukemia cell line HL-60.This compounds take action by means of different mechanisms. Therefore we carried out simultaneous treatment of HL-60 cells with these drugs. We observed that PRI-2191 strengthened the effect of imatinib and cytostatics. Imatinib combined with cytostatics had an antagonistic effect (combination with cisplatin or idarubicin) or additive effect (combination with docetaxel). Moreover, we observed the synergistic effect of interaction in triple combinations of cytostatics used together with imatinib and PRI-2191. Many anti-cancer therapies exert their therapeutic effect by inducing apoptosis in some tumor cells. Therefore we studied the influence of compounds combination on the cell cycle and apoptosis of HL-60 cells. Imatinib and cytostatics alone increased the number of cells in apoptosis. While PRI-2191 alone stopped the cells in G0/G1 phase and decreased the number of cells in S phase. Imatinib, cisplatin or idarubicin in combination with PRI-2191 increased number of cells in G0/G1 phase and decreased number of cells in S phase and in apoptosis in comparison with cytostatic alone. The combination of docetaxel and PRI-2191 decreased number of cells in G2/M and S phase and stopped the cells in G0/G1 phase and in apoptosis. The triple combin-

ation of imatinib and PRI-2191 together with cisplatin or together with idarubicin decreased number of cells in S phase and increase in G0/G1 phase. Imatinib with PRI-2191 and docetaxel stopped cells in G0/G1 phase and in apoptosis, and decreased number of cells in G2/M and S phase. In summary, applied combinations act on the cell cycle stronger than compounds used individually. Moreover, the calcitriol analog-PRI-2191 proved to be able to abolish the antagonistic interaction between imatinib and cytostatics. We suppose, that calcitriol analogs may be of potential use in anti-cancer therapy when are combined with different types of drugs.

This work was supported by the Foundation for Development of Pharmaceutical Sciences(grant 9/FB/2004, Poland)

17:35 Poster II-155

PREPARATION OF NEW DERIVATIVES OF 2-ARYLSULFONYLIMINO-6-ALKYLPERHYD ROPYRIDO[4,3-d]PIPERYMIDINE WITH POTENTIAL PHARMACOLOGICAL ACTIVITY.

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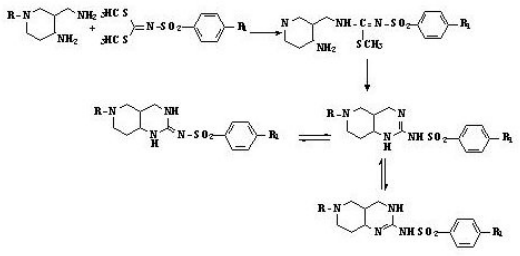
Synthetic derivatives of pyrimidine are a significant group of medicines that is characterized by varied pharmacological activity, hypoglycaemics among others.

When searching new compounds with potential pharmacological activity 2-arylsulfonylimino-6-alkylperhydropirydo[4,3-d]pyrymidines derivatives were obtained. These compounds were synthesized by condensation of dimethyl *N*-arylsulfonyliminodithioic acid esters with respective 1-alkyl-3-amine-4-amine- methylpiperydines, isolation of intermadiate products (isothiourea derivatives) and their thermal cyclization to 2-arylsulfonylimino-6-alkylperhydropirydo[4,3-d]pyrimidines .

Cyclic derivatives obtained in that way can exist in three tautomeric forms (see scheme)



R = Cl $R_1 = C_2H_5$

R = CH_3 $R_1 = C_2H_5$

R = CH_3 $R_1 = C_3H_7$

The structure of new compounds was confirmed by elemental analysis, as well by the ^{1}H NMR, ^{13}C NMR and MS spectra.

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NEW AMINOCARBONYL DERIVATIVES OF 1-ARYLIMIDAZOLIDINE-2 WITH POTENTIAL PHARMACOLOGICAL ACTIVITY.

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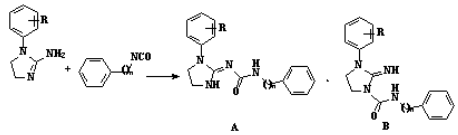
The aminocarbonyl derivatives of 1-aryl-2-imidazolidine-2 both chain or fused were found to have significant antinociceptive activity connected with activation of the MOP (mu opioid protein) receptor [1-3]. Recent results on the chain (imidazol-2-yl)urea derivatives are implementing the pharmacophorie model with new features, which have to be taken under consideration in planning further synthesis.

Series A compounds exhibited significant antinociceptive activity in the "writhing test", reversed by small dose (5mg kg^{-1} i.p.) of naloxon.



R = H, 2-CH_3, 4-CH_3, 2-OCH_3, 4-OCH_3, 2-Cl, 3-Cl, 4-Cl

n = 1, 2

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

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[2] D. Matosiuk, S. Fidecka, L. Antkiewicz-Michaluk, J. Lipkowski, I. Dybała, A.E.Kozioł, *Eur. J. Med.. Chem.***2002**, *37,* 761.

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

17:35 Poster II-157

2-(4,5-DIHYDROIMIDAZOL-2-YL)BENZIMIDAZOLES AS SELECTIVE IMIDAZOLINE I_2 / ADRENERGIC A_2 RECEPTOR LIGANDS

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In our search for novel selective I_2 / a_2 ligands we have prepared 13 benzimidazole derivatives shown in figure 1. 2-(4,5-Dihydroimidazol-2-yl)benzimidazoles **1**, **2**, **4** have been identified as selective imidazoline I_2 / a_2-adrenoceptor ligands. 4-Methyl derivative **2** displays I_2 affinity at nanomolar concentration (K_i = 4.4 nM) and a high I_2/a_2 selectivity ratio = 4226. Analysis of the structure - activity relationships indicates that for this class of imidazoline ligands the presence of a weakly acidic NH group of benzimidazole ring and the basic NH group of imidazoline ring connected by a two atom C-C bridge is an important motif responsible for binding to I_2 receptors. Moreover, an evidence has been obtained that pKa value of imidazoline ligands can influence their a_2-adrenoceptor activity.

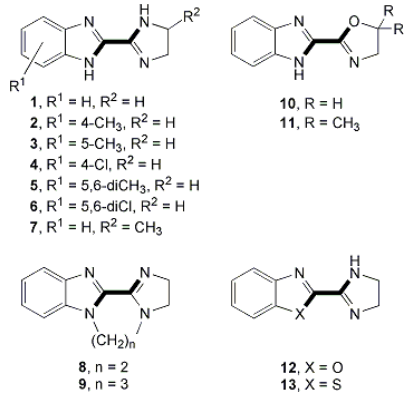

Figure 1.
Structures of benzimidazole derivatives **1** - **13**.

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THE STRUCTURE OF AZO-ANALOGS OF 8-STYRYLXANTHINES - X-RAY AND MOLECULAR MODELLING STUDIES

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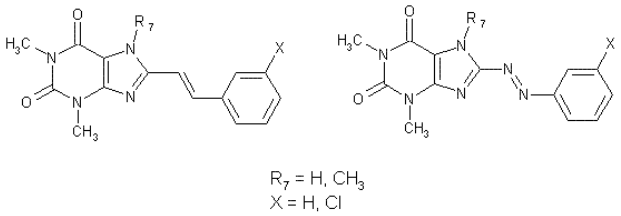

Phenylazoxanthines are less potent than corresponding styrylxanthine derivatives. In styrylxanthines, methylation in the N7 position increases A_{2A}-AR affinity, while decreasing A_1-affinity. In azo analogs, A_{2A}-affinity is also increased by 7-methylation of the xanthine structure (from 19 to 27-fold), but A_1-affinity is increased as well, although to smaller extent (about 3-fold). As observed for styrylcaffeines, a *meta*-chloro substituent on the phenyl ring increased A_{2A}-affinity about 2-fold, virtually without having any effect on A_1-affinity, thus increasing A_{2A}-selectivity. In azo analogs group the presence of chloro- or bromo-substituents makes no differneces in affinity.

With the aim to find the nature of those differnces we did an x-ray analysis of 8-fenylazo-7-methylxanthine. Than we made molecular modelling calculations of this derivative and other azo-anologs as well as corresponding styrylxanthines in 2 data bases.

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CRITERIA FOR QUALITY ASSESSMENT IN APPLICATIONS FOR MARKETING AUTHORISATION OF HERBAL MEDICINAL PRODUCTS

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Every medicinal product for human use that is to be placed on the European Community market must be granted a marketing authorisation delivered by a competent authority. Marketing authorization herbal medicinal products is done in Poland according to national law and Ministry of Health regulations.

Quality is the basis of reproducible efficacy and safety of the medicinal products.

The quality of herbal medicinal products is determined by the quality of the starting plant material, adherence to Good Agriculture and Collection Practice, product development, in-process controls (e.g. raw material testing, in-process testing, stability testing, etc), Good Manufacturing Practice controls, by validated manufacturing process and by specifications applied to them throughout development and manufacturing.

Control strategy for herbal substance, herbal preparation and herbal medicinal product include testing of identification,

purity and impurities (microbial levels, aflatoxins, heavy metals, pesticides, and fumigants), content of the active substances or markers determination, profile and stability data.

The rigorous standardization is defined as content of a constituent or a group of substances with known therapeutic activity or markers of the herbal substances and herbal medicinal products. It is a necessary step for the industry in order to prepared the registration dossier, validate preclinical and clinical data to register the product as a medical product and control its quality after marketing authorization during the shelf. The proper level of standardization is possible to attain by using the combination of different analytical techniques (GC, HPLC) for the known compounds or for the unknown components.

The general quality aspects of medicinal products cover European Pharmacopoeia, Polish Pharmacopoeia and CPMP-ICH, CPMP guidelines (International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use and Committee for Proprietary Medicinal Products).

The particularly guidelines of herbal medicinal products are provided by HMPC guidelines of the Committee for Herbal Medicinal Products being part of the European Medicines Agency (EMEA), body of the European Union with headquarters in London.

The format of chemical, pharmaceutical and biological documentation of a registration application and list of references to quality guidelines for herbal medicinal product is defined in:

The Rules governing Medicinal Product in the European Community:

The Notice to Applicants Volume 2B, CTD-Module 2.3 Quality Overall Summary-herbal and Module3 Quality-herbal, edition July 2003.

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ACCUMULATION OF ZINC BY THE LENTINUS EDODES (BERK.) MYCELIUM CULTIVATED IN SUBMERGED CULTURE.

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Zinc is one of trace elements of fundamental importance to human health. Inadequate intake of this metal can effect any of over 200 enzymes. The structural function of zinc is its role in the structure of proteins and membranes. Zinc finger proteins have been found to regulate gene expression by acting as transcription factors. Zinc also plays a role in cell signaling, has been found to influence nerve impulse transmission and hormone release. Adequate zinc intake is essential in maintaining the integrity of the immune system. HIV infected individuals are particularly susceptible to zinc deficiency.

Many species of mushrooms have been found to be highly potent immune system enhancers. *Lentinus edodes* is one of medicinal mushrooms from which extracted highly purified polysaccharide fraction (lentinan) is an approved drug, used in cancer treatment as well as in AIDS research. Hot water and alcohol extracts from *L. edodes* mushroom mycelium and culture media (LEM, LAP) demonstrate strong immunostymulating and antioxidant activity. The chemical composition of cultivated in submerged culture mycelial biomass, which can be used to obtain pharmacologically active extracts, depends on the nutritional conditions. The present study involves the optimization of medium constituents for both - *L. edodes* mycelium growth and high zinc content in mycelial biomass, possibly in form of a highly bioavailable organic compounds. High zinc content in mycelial biomass possibly would enhance immunostymulating and antioxidant activity of mushroom extracts.

The effect of Zn(II) on the mycelial growth and chemical composition of *Lentinus edodes* submerged cultured mycelial biomass was investigated in shake flask culture. Culture media were enriched in zinc by addition of $ZnSO_4$ in concentration range from 0 to 90 ppm, added to the medium before inoculation. The Zn(II) content in cultivated mycelial biomass was determined by use of the atomic absorption spectrometry (AAS) on GBC Avanta Ultra Z spectrometer. Zinc concentration expressed in mg% of mycelial dry mass rose from 90 mg%, estimated for mycelium cultivated in not enriched in Zn (II) medium, to 400 mg% for mycelium cultured in medium supplemented in 30 ppm of zinc. The higher than 30 ppm concentration of Zn(II) in medium affects lower zinc content in mycelial dry mass. Zinc concentration in medium strongly affects on the mycelial growth. Productivity of the mycelium rose proportionally to the increasing concentration of Zn(II) in medium. The highest mycelial growth was recorded for media enriched in Zn(II) in concentration of 30 ppm. Higher than 30 ppm concentration of Zn (II) in medium acts depressive on the mycelial growth. Optimal pH of medium for zinc accumulation was estimated by cultivation of *Lentinus edodes* mycelia in media of pH ranging from 3.5 to 7, enriched in 30 ppm of zinc(II). The optimal pH of medium for zinc accumulation was 7. Proportionally to the increasing concentration of zinc(II) in medium rose the percentage of this metal adsorbed on the cell surface, easy to remove by washing of the mycelium with the 0.05 molar EDTA solution. The value of the adsorbed on the cell surface zinc percentage changed in range from 30% to 70% for concentrations of Zn(II) in medium rising from 0 to 90 ppm.

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ARGININE-TERMINATED ANTIMICROBIAL PEPTIDES WITH DENDRIMERIC STRUCTURE

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Recently, antimicrobial properties of dendrimeric peptides designed as branched analogs of natural linear peptides of various defence systems have been discovered [1]. Biological tests against Gram-positive (S. *aureus*), Gram-negative (*E. coli*) and *C. albicans* as well as cytotoxicity studies showed that better properties have compounds with higher degree of branching ("dendrimeric effect") [2]. In order to follow their interactions with biological membranes a library of dendrimeric peptides including amino acids of L- and D-chirality was synthesized by stepwise solid phase synthesis (AA1: L- or D-Lys, AA2: L- or D-Arg, or Z-L-Arg , Z-D-Arg).

Antimicrobial tests showed higher potency of Z-protected derivatives. On the other side, isomeric compounds differ by configuration of amino acids expressed similar activity. This strongly suggested that so called "carpet mechanism" is the dominating interaction between microbial membranes and dendrimeric peptides.

This project was supported by grant No. 3T09B 115 28 from the Ministry of Education and Science.

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17:35 Poster II-162

CONJUGATED LINOLEIC ACIDS PREPARATIONS AS EFFECTIVE NUTRACEUTICS

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Nutraceutics became important components of successful therapies. Recently particular attention has been focused on linoleic acid isomers (CLA) containing conjugated double bonds.

Conjugated linoleic acids (CLA) represent a family of octadecadienoic acids which constitute a mixture of geometric and position isomers of linoleic acid in which the double bond occurs in the positions ranging from 6,8 to 9,11 and to 10,12. The 9c,11t $C_{18:2}$ acid is the predominant isomer.

The content of the 9c,11t $C_{18:2}$ isomer in the cow milk fat, goat milk fat and sheep milk fat were found to oscillate within the ranges 0.65-1.8%, 0.3-1.2% and 1.2-2.3%, respectively. The sheep milk fat shows the highest content of the isomer.

Sheep milk fat containing 2% of the 9c,11t $C_{18:2}$ conjugated linoleic acid (CLA) isomer was selected to study the antiproliferative activity of this isomer. Sheep milk fatty acids containing 2.0% of the 9c,11t $C_{18:2}$ isomer, and the acids enriched in this isomer up to 12.1%, (N-CLA), were used in the studies.

The product (N-CLA) contained 15.0% of the 9c,11t $C_{18:2}$ isomer and about 8.0% of the 11t $C_{18:1}$. The sheep milk fatty acids composition and the product enriched in CLA (N-CLA), and also a commercial CLA preparation, were studied in antiproliferative activity against few lines of human cancer.

The effect of CLA-enriched preparation of sheep milk fatty acids, N-CLA, revealed higher antiproliferative activity than standard pure CLA and sheep milk fatty acids.

The present study was carried out in the framework of the Research Projects 3 T09 B 059 26 financed by the Ministry of Education and Science.

17:35 Poster II-163

VIRTUAL SCREENING - SOFTWARE TOOLS AND SUCCESSFUL APPLICATIONS

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Traditional *in vitro* high throughput screening (HTS) is the prevailing although expensive method to discover new leads for drug development. Therefore virtual high throughput screening (vHTS) can be an attractive alternative. Among many applications published recently there are several examples of novel drugs discovery by virtual screening.

The screening of large databases for possible lead compounds has recently become a routine procedure [1]. In this way a large number of compounds can be evaluated against target in a rapid and automated manner. In this process smaller sets of pre-filtered, top-scored molecules are selected as candidates for biological assays in HTS. Combination of vHTS and HTS is actually very significant to drug discovery.

Software tools for virtual screening can be grouped in four classes [2]:

- Structure-based virtual screening
- Ligand-based virtual screening
- Similarity-based virtual screening
- Pharmacophore-based virtual screening.

An attempt to classify and summarize the most important software tools for virtual screening methods will be presented. Moreover several successful examples of novel bioactive compounds discovery by vHTS during the past three years will be provided.

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17:35 Poster II-164

SYNTHESIS OF URIDINE DERIVATIVES OF 2-DEOXY SUGARS AND THEIR BIOLOGICAL ACTIVITY

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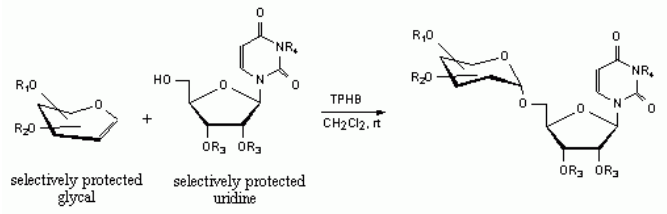
Glycosyltransferases (GTS) are enzymes responsible for processing variety of biomacromolecules, generating higher saccharides such as glycolipids, glycoproteins and other glycoconjugates. They regulate many cellular functions and therefore are important targets in medicinal chemistry. Inhibitors of GTS have already found application in various therapeutic categories [1].

GST catalyse sugar unit transfer from a sugar nucleotide donor to an unprotected sugar acceptor. We report herein the synthesis of several analogues of sugar nucleotides, which were designed to act as inhibitors by binding in the active site of the enzyme in competition with natural donor substrates.

In order to construct analogues of uridine diphospho sugars we have chosen glycal chemistry. First, uridine and glycals were selectively protected. Afterwards addition of uridine derivative to a glycal, catalysed by triphenylphosphine hydrobromide, was performed [2]. In this way we have synthesized, in totally stereoselective manner, several uridine derivatives of 2-deoxy sugars in high yields.



Biological activity studies with these inhibitors were divided into two parts. First, using the neutral red cytotoxicity assay, we established the optimal doses of inhibitors when the viability of swine kidney cells (SK6) was higher than 50%. In the next experiments, we examined the effect of concentration of inhibitors on penetration and propagation of classical swine fever virus (CSFV). The best results were observed for IW3 inhibitor. Even low doses of this inhibitor (20 μg/mL), when the viability of SK6 cells was higher than 90%, inhibition the propagation of CSFV virus and the viral yield was decreased by over 80%.

Acknowledgement

Financial support from the Polish State Committee for Scientific Research (Grant No. 3 T09 A 01226) is gratefully acknowledged.

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17:35 Poster II-166

ANTI-PROLIFERATIVE EFFECTS OF INOSITOL HEXAPHOSPHATE AND VERAPAMIL ON HUMAN COLON CANCER CACO-2 AND HT-29 CELLS

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Inositol hexaphosphate (IP6), a natural dietary ingredient has revealed anti-carcinogenic effect in various *in vivo* and *in vitro* models including colon cancer. Several studies have suggested that calcium antagonists, such as verapamil (VP) had inhibitory influence on cell proliferation or might potentiate the effects of numerous chemotherapeutic drugs on malignant cells. The purpose of this study was to evaluate the growth inhibitory activity of IP6 in combination with VP in comparison to that of IP6 alone and VP alone using two colon carcinoma-derived cell lines Caco-2 and HT-29. In combination treatment, IP6 (0.5, 1 and 5 mM) and VP (0.1, 0.5 mM) were added simultaneously and sequentially with VP added 2 h prior to IP6 to the cell cultures. The cells were cultivated for 72 h in RPMI 1640 medium at 37 °C in a humidified atmosphere with 5% CO_2. Cellular growth was quantified with the use of CyQUANT Cell Proliferation Assay Kit based on cellular DNA content determination. Cell morphology following VP and IP6 treatment was evaluated using an Olympus IX 50 light microscope under 100x magnification. Histochemical assessment of nuclear morphological features associated with apoptosis/necrosis was performed on the basis of staining characteristics of VP-and IP6-treated cells with hematoxyline/eozine. Additionally, necrotic cells were characterized by trypan blue uptake. The activity of caspase-3, the chief executor of apoptosis, in colonic cells was estimated with the use of the Colorimetric Caspase-3 Assay Kit (Sigma) with Ac-DEVD-pNA as a substrate. The results of this study showed that VP alone reduced colon cancer growth and HT-29 cells appeared to be more susceptible to growth inhibition compared to Caco-2 cells. Statistically significant inhibition of Caco-2 cells proliferation was observed at VP doses ≥ 0.5 mM, whereas its growth inhibitory activity in regard to HT-29 cells was observed at 0.1 mM VP. The 1 mM VP appeared to be highly cytotoxic, resulting in a reduction of cell proliferation by 100-fold. IP6 at 5 mM induced significant growth suppression of both Caco-2 and HT-29 cells and its lower doses were ineffective. The joint administration of 0.5 mM VP and 1 mM IP6 enhanced growth inhibitory effect of IP6 compared to 1 mM IP6 alone on HT-29 but not Caco-2 cells. Substantial growth suppression of HT-29 cells was achieved when either 0.5 or 1 mM VP was administered prior to the addition of 1 and 5 mM IP6. Although some of nuclear changes associated with apoptosis such as chromatin condensation and nuclear fragmentation appeared sporadically after cells treatment with 0.5 and 1 mM VP, caspase-3 activation in cells of both lines was not observed following drug treatment, indicating that VP did not induce cell apoptotic death. The percentage of apoptotic cells under treatment with IP6 was dependent on its concentration, however, microscopic examination of trypan blue-stained cells treated with VP and IP6 revealed prevalence of morphological changes typical for necrosis.

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SEARCH FOR NEW ANTICONVULSANT AGENTS, DERIVATIVES OF γ-BUTYROLACTONE AND γ-HYDROXYBUTYRIC ACID

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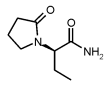
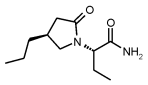
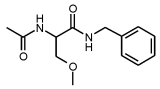
On account of complex and unclear mechanisms underlying the onset and development of epilepsy, in search for new anticonvulsant agents, various chemical structures and many targets are taken into consideration. It has been proved that γ-hydroxybutyric acid (GHB) as well as its cyclic analogue, γ-butyrolactone (GBL), both influence the gabaergic transmission, which plays a pivotal role in physiological and pathological processes in the central nervous system (CNS), including epilepsy. In recent years several α-substituted butyramides have been approved as the antiepileptic drugs (levetiracetam, breviracetam), and other related structures are in early clinical development (harkoseride) [1, 2].


LEVETIRACETAM


BREVIRACETAM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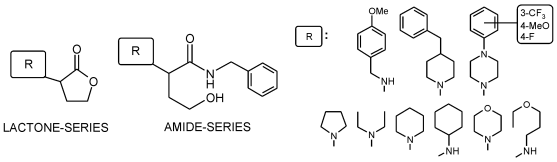
This work is a part of physicochemical and pharmacological studies of *N*-substituted amides of α-arylalkylamino-GHB

with expected anticonvulsant activity and gabaergic mechanism of action [3]. With the aim to determine the influence of the α-arylalkylamine moiety on anticonvulsant activity, two series of compounds, derivatives of α-substituted GBL and GHB were synthesized. These compounds contain aryalkylamine substituents with modified lipophilic phenyl ring or alkylamine groups (aliphatic or cyclic) in the α-position.



Preliminary anticonvulsant *in vivo* tests of these compounds, *i.e*. the maximal electroshock test, the subcutaneous metrazole induced seizures, and the rotorod toxicity assay on mice were employed.

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Acknowledgements: We thank Dr J.P. Stables for anticonvulsant testing and to the Anticonvulsant Screening Project, National Institute of Neurological Disorders and Stroke, Bethesda, USA.

17:35 Poster II-168

ANTIPROLIFERATIVE ACTIVITY IN VITRO OF DIASTEREOMERIC ANALOGUES OF VITAMIN D3 AGAINST NORMAL AND CANCER CELL LINES

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The analogues of 1,25-dihydroxyvitamin D_3 with reversed configuration at C-1 or C-24 and *E* or *Z* geometry of double bond at C-22 in the side-chain or at C-5 in the triene system were examined for their antiproliferative activity *in vitro* against a spectrum of various human and mouse cancer cell lines.

Calcitriol and tacalcitol (used as a control compounds) as well as analogues coded PRI-2201, PRI-2202 and PRI-2205 revealed strong antiproliferative activity against human HL-60 leukaemia cells, breast MCF-7 and T47D cancer cells, squamous SCC-25 cancer cells and mouse WEHI-3 leukaemia cell line. Interestingly, new analogs, as well as the control tacalcitol were more active against HL-60/MX2 (subline resistant to mitoxantrone) than calcitriol.

The novel analogues similarly to calcitriol appeared to be less active against A549, FaDu, K562, CCRF/CEM, 16/C, MDA-MB-231, ASPC-1, LNCaP (inhibition of proliferation lower than 50%) and not active against other tested human cancer cell lines (antiproliferative activity lower than 20%).

The mechanism of the observed *in vitro* antiproliferative effect of calcitriol, tacalcitol and PRI-2201 may be related to their effect on the cell differentiation (the accumulation of cells in G0/G1 phase). The appearance of antigen CD14 and CD11b expression after exposure of HL-60 cells to calcitriol or tacalcitol and PRI-2201 confirmed their cell differentiating effect. Analogues PRI-2202 and PRI-2205 were less potent in induction of G0/G1 phase accumulation, rather increases percent of apoptotic cells.

This research was supported by KBN (Polish State Committee for Scientific Research) Grant No. 3P05A08725.

17:35 Poster II-169

EFFECTS OF hGH-RH ANALOGUES ON GH RELEASE IN PITUITARY CELL CULTURE

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The major problem of the therapy based on hGH-RH or hGH-RH-(1-29)-NH_2 is their susceptibility to enzyme cleavage. To overcome this problem several hGH-RH-(1-29)-NH_2 analogues have been obtained by these authors [1]. Analogues containing homoarginine (Har) or Orn instead of Arg and Lys were completely resistant to trypsin. Two of these analogues, namely [Dat^1,$Har^{11,12,20,29}$,Ala^{15},Nle^{27},Asp^{28}]-hGH-RH-(1-29)-NH_2 (**1**) and]-hGH-RH-(1-29)-[Dat^1,$Har^{11,20,29}$,$Orn^{12,21}$,Ala^{15},Nle^{27},Asp^{28}]NH_2 (**2**) were about 50 times as potent as hGH-RH-(1-29)-NH_2 itself *in vivo*.

To get insight into the process of releasing GH we examined stimulation of cultured pituitary cells with **1** and **2**. The level of GH was measured with RIA methods after 30, 60, 120, and

240 minutes using hGH-RH-(1-29)-NH_2 and its analogues in doses 1, 10 and 100 nM. The profile of GH release indicated that analogues were more active then hGH-RH-(1-29)-NH_2. The maximal effects of the analogues was after 60 minutes of incubation, and was still noticeably higher after 240 minutes.

These results indicate that duration of cell stimulation may be one of the reasons of incrased activity *in vivo*. It is also implies that the used procedure can be useful for preliminary evaluation of new GH releasing substances.

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17:35 Poster II-170

GENETIC POLYMORPHISM OF DEXTROMETORPHAN OXIDATION IN POLISH POPULATION

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Genetically determined individual differences in the ability of oxidation of certain drugs have raised recently a considerable interest in view of their clinical importance. Determination of oxidation phenotype is used to obtain the best results of pharmacotherapy and to explain a lower efficiency of some drugs in particular patients. It has also been proved that the frequency of drugs side effects occurance and predisposition to some disease may depend on oxidation phenotype. In addition, investigation of oxidation polymorphism is used in pharmacotherapy in the prevention of drugs interactions.

The aim of the study was to identify the oxidation phenotype in a Polish population. The oxidation phenotype was determined with dextrometorphan (DM) as the indicatory drug. The study included 104 healthy Polish volunteers. DM (40 mg) was given orally to healthy adults and 10-hour urine samples were collected. DM and the metabolite dextrorphan (DT) were analysed by the HPLC methods. Phenotyping was performed using the metabolic ratio (MR) calculated as:

MR = 0-10 h urinary output of DM/ 0-10 h urinary output of DT.

Taking into consideration the metabolic ratio we can distinguish extensive (EM) and poor (PM) metabolizers in human population. Studies of populations indicate that 3-10% of Caucasian breed represents the slow oxidation phenotype (PM).
In our studies of the Polish population the frequency of slow oxidation phenotype is similar to the results in other European populations.

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GENETIC DETERMINANTS IN ISCHAEMIC HEART DISEASE

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The role of genetic factors in the pathogenesis of ischaemic heart disease has recently raised a considerable interest among researchers. Numerous investigations aim at finding variants of genes which might be responsible for increasing the risk of this illness. Many studies investigate polymorphic variants of genes whose protein products contribute to the genesis and development of atherosclerosis of coronary arteries, thrombogenesis and fibrinolysis and other processes significant for the progression of ischaemic heart disease.

Genes whose polymorphisms are potentially connected with a given illness are called genes candidates. In ischaemic heart disease the most often analyzed genes are those connected with metabolism of lipids, the coagulation and fibrinolytic system and the renin-angiotensin-aldosterone system. Factors of inflammation (cytokines, TNF), proliferation of smooth muscles cells and vasoactivation are also important.

The analysis of genetic multifunctional basis of the disease is rather difficult. Manifestation of the illness is connected with accumulation of several genetic determinants, while the clinical picture is additionally modified by environmental factors. Studies of genetic etiopathogenesis of ischaemic heart disease may result in effective prevention and treatment in particular patients.

The present paper is a review of current knowledge about the relations between gene polymorphism and predisposition for ischaemic heart disease.

17:35 Poster II-172

SYNTHESIS AND AFFINITY FOR SEROTONIN RECEPTOR 5HT$_{1A}$ AND SEROTONIN TRANSPORTER OF SOME 6-NITROQUIPAZINE ANALOGUES.

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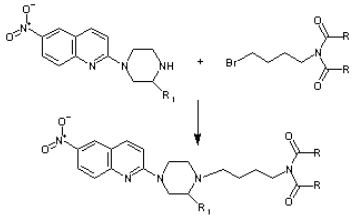
The aim of our work was syntheses of 6-nitroquipazine and 3'-methyl-6-nitroquipazine analogues conected by butyl chain with four imids (ftalimide, glutarimide, 3,3-dimethylglutarimide and 3,3-tetramethyleneglutarimide) as a potential antidepresant drugs.



Six final products were examined for inhibition of serotonin transporter and as a antagonists of 5HT$_{1A}$. For comparison imipramin and serotonin (5HT) were used as a standard compaunds. The best results were obserwed for 3'-methyl-6-nitroquipazine conected with 3,3-dimethylglutarimide.

17:35 Poster II-173

FLUORIMETRIC DETECTION OF ALDEHYDE DEHYDROGENASE ACTIVITY IN HUMAN SALIVA IN DIAGNOSTIC OF CANCERS OF ORAL CAVITY

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Aldehyde dehydrogenase isozyme (ALDH3A1) is a dimeric enzyme oxidizing mainly long- and medium-chain aliphatic, as well as aromatic, aldehydes, but exhibiting very low affinity toward acetaldehyde. ALDH3A1 isozyme, is responsible for detoxication of many drugs, including an alkylating agent cyclophosphamide (CP) widely utilized in cancer chemotherapy, usually in combination with other drugs [1, 2, 3].

It has been recently shown that ALDH3A1 is the only aldehyde dehydrogenase isozyme present in human saliva [4]. It is also well documented that ALDH3A1 can be induced several-hundred-fold in some neoplastic states of the different cancers e.g. liver, breast, and colon [5].

We have applied the fluorimetric method to study aldehyde dehydrogenase isozyme (ALDH3A1) activity in saliva of cancerous patients. A highly fluorogenic aldehyde, 6-methoxy-2-naphthaldedyde (MONAL-62) was used as an indicator of the aldehyde dehydrogenase isozyme (ALDH3A1) in human saliva [6, 7].

Cancerous patients' saliva was collected and examined over a prolonged time, both prior to and after tumor removal. In most cases studied thus far, ALDH3A1 activity was initially elevated, but in consecutive assays after surgery significantly decreased. This phenomenon could indicate that this isozyme was induced in malignant tissue, resulting in increasing it's total activity in saliva.

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17:35 Poster II-174

REACTIONS OF 3-(MORPHOLIN-4-YL)PROPIONIC ACID HYDRAZIDE WITH ISOTHIOCYANATES

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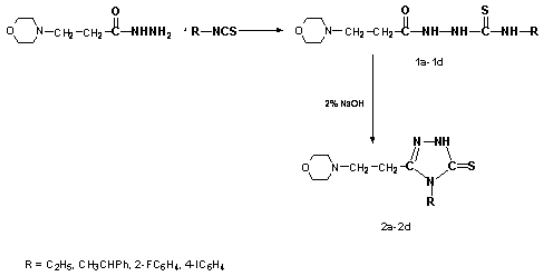
Organic compounds containing aromatic heterocyclic rings have received considerable attention among medicinal chemists because many of them play a role in various biochemical

processes. 1,2,4-Triazole and morpholine derivatives belong to an aromatic heterocyclic group exhibiting a wide range of biological activities, such as antimicrobial and anti-inflammatory.

In general, 3,4-disubstituted-1,2,4-triazole could be prepared by the intramolecular dehydrative cyclization of 1,4-disubstituted thiosemicarbazides.

In our experiments the hydrazide of 3-(morpholin-4-yl)propionic acid was used as a starting material for the preparation of thiosemicarbazide derivatives **1a**-**1d**. Next, cyclization of these compounds led to new compounds **2a**-**2d** with a promising pharmacological activity.



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

17:35 Poster II-175

SYNTHESIS OF 1-AMINOMETHYL DERIVATIVES OF 3-BENZYL - 4 - ETHYL - 1,2,4 - TRIAZOLINE - 5 - THIONE

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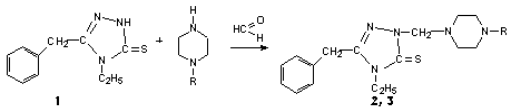
Depending on the nature of substituents, some derivatives 1,2,4-triazoline-5-thione show various biological activities, such as antiinflammatory, antifungal, analgesic and antibacterial. In previous paper we have described antibacterial activity against Gram-negative bacteria of aminomethyl derivatives of 1,2,4-triazoline-5-thione [1]. This work is a continuation of the research on the new compounds with promissing biological activities. Thus, 3-benzyl-4-ethyl-1,2,4-triazoline-5-thione (**1**) were subjected to the reaction with piperazine derivatives and next transformed into the corresponding 1-aminomethyl-3-benzyl-4-ethyl-1,2,4-triazoline-5-thione derivatives (**2**, **3**). The aminomethylation reactions were carried out in ethanolic solution, in the presence of a small amount of formalin, by using *N*-phenyl piperazine and 1-(4-methoxyphenyl) piperazine. The conditions of the reactions were established experimentally.



R= C_6H_5, 4-$OCH_3C_6H_4$

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

[1]. Pitucha M., Wujec M., Dobosz M., Kosikowska U., Malm A.: *Acta Pol. Pharm*., **2005**, *62*, 443.

17:35 Poster II-176

AMINO ANALOGUES OF DNA MINOR GROOVE BINDER PENTAMIDINE - SYNTHESIS AND STRUCTURAL STUDIES

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Pneumocystis pneumonia (PCP), caused by *Pneumocystis carinii,* is the opportunistic infection in people with weakened immune system. Available therapies (for example those using pentamidine) have many adverse effects and are limited by toxicity. Therefore we have designed and synthesized new pentamidine analogs with amino groups (Fig. 1).

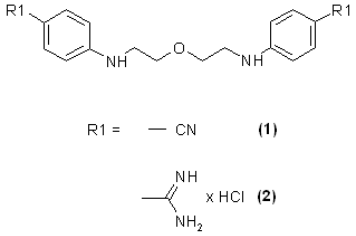

Fig. 1. Synthesized compounds.

We analyzed the solid state ^{13}C CP/MAS NMR spectra of obtained compounds and discussed their conformations, intermolecular interactions and polymorphism.

Comparison of melting points between $d(CGCGAATTCGCG)_2$ DNA fragment and its complex with 1,5-*bis*[(4-amidinophenyl)amino]-3-oxapentane dihydrochloride **(2)** proves that this compound could bind to minor grove of DNA.

17:35 Poster II-177

SYNTHESIS, IN VITRO AND IN VIVO ACTIVITY OF THE ARYLPIPERAZINYLPROPYL DERIVATIVES OF THE IMIDAZO[2,1-f]THEOPHYLLINE

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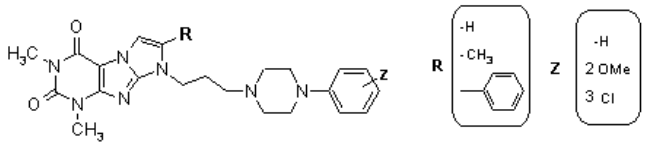
Long chain arylpiperazines have been found as serotonin receptor ligands in particular 5-HT_{1A} and 5-HT_{2A} ones. Their general chemical structure contains: an alkyl chain (2-4 methylene units) attached to the N4 atom of the piperazine moiety, and terminal fragment: amide or an imide. The significance of the terminal part in ligand-receptor interactions has been the subject of many structure-activity relationships studies [1]. In our earlier study a series of arylpiperazinylalkyl derivatives with a complex terminal part based on the purine moiety had been synthesized. For compounds with pirymido[2,1-f]theophylline fragment high or very high receptor affinity and diversified pharmacological profile were observed [2-4]. The most potent for serotonin receptors were compounds with double bonds at anneleted six member ring at 7,8 position of the theophylline [2-4]. On the basis of the above data we have synthesised the new tricyclic theophylline with five member ring (Fig 1) to study the influence of ring size and the presence of double bond, the kind of the substituent at N4 position of arylpiperazine moiety on serotonin receptors activity.

Fig 1.



The newly synthesized compounds in a form of water-soluble hydrochlorides have been tested *in vitro* for their 5-HT_{1A} and 5-HT_{2A} receptor affinities. The investigated compounds are potent 5-HT_{1A} receptor ligands with K_i within range on 5.6-96.5 nM and demonstrate lack of affinity for 5-HT_{2A} subtype. With respect to the potential multireceptor profile, binding affinity for dopaminergic D_2 receptor was evaluated. The most potent ligands were tested *in vivo* to evaluate their functional CNS activity.

This study is supported by Polish Ministry of Scientific Research and Information Technology, grant No 2P 05F04226.

1. Lopez-Rodriguez, M. L.; Ayala, D.; Benhamu, B.; Morcillo, M. J.; Viso, A. *Curr. Med.Chem*. **2002**, *9*, 443-469.

2. Chojnacka-Wójcik, E.; Kłodzińska, A.; Drabczyńska, A.; Pawłowski, M.; Charakchieva-Minol, S.; Chłoń, G.; Gorczyca, M. *Eur. J. Med. Chem*. **1995**, *30*, 587-592.

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17:35 Poster II-178

ARYLPIPERAZINYLBUTYL DERIVATIVES OF SOME IMIDAZO[2,1-f]THEOPHYLLINE AS CNS RECEPTOR LIGANDS.

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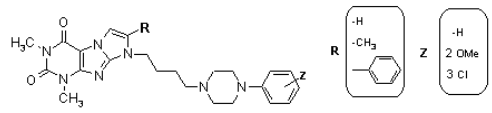
Arylpiperazines with an amide moiety are one of the most frequently investigated classes of 5-HT_{1A}/5-HT_{2A} receptor ligands. Although the terminal amide fragment significantly affects binding of 1-arylpiperazine derivatives for serotonin receptors, its role is not clear yet [1]. Structure-activity relationship studies showed that the length of the alkyl chain is great importance for 5-HT_{1A}/5-HT_{2A} receptor affinities. As a general trend, maximum affinity for 5-HT receptors is reached with 3-4 methylene units [2].

In our earlier attempt to find new 5-HT_{1A}/5-HT_{2A} receptor ligands, series of the imidazo[2,1-f]theophylline derivatives with the arylpiperazinylpropyl substituent at N8 position had been synthesized. These compounds have been tested *in vitro* for their 5-HT_{1A} and 5-HT_{2A} receptor affinities and are potent 5-HT_{1A} receptor ligands with K_i within the range on 5.6-96.5 nM and demonstrate lack of affinity for 5-HT_{2A} subtype [3]. In this work the series of the imidazo[2,1-f]theophylline derivatives with arylpiperazinylbutyl substituent at N8 position have been synthesized to study the influence of the length of the spacer between the imidazo[2,1-f]theophylline and the arylpiperazine moiety on serotonin receptors activity.



The newly synthesized compounds in a form of water-soluble hydrochlorides have been tested *in vitro* for their 5-HT_{1A} and 5-HT_{2A} receptor affinities. Pharmacological *in vivo* studies directing to CNS receptor profile of the synthesized compounds are in progress.

1. Mokrosz M. J.; Duszyńska B.; Bojarski A. J.; Mokrosz J. L.: *Bioorganic & Medicinal Chemistry*, **1995,***3***,** 533-538.
2. Lopez-Rodriguez, M. L.; Ayala, D.; Benhamu, B.; Morcillo, M. J.; Viso, A. *Curr. Med. Chem*. **2002**, *9,* 443-469.
3. Zagórska A., Pawłowski M., Dybała M., Nowak G.: Joint Meeting on Medicinal Chemistry, June 20-23, 2005, Vienna, Austria, poster.

17:35 Poster II-179

HPLC AS A METHOD FOR ANALYTICAL CONTROL OF SYNTHESIS AND DETERMINATION OF PRAMIPEXOLE

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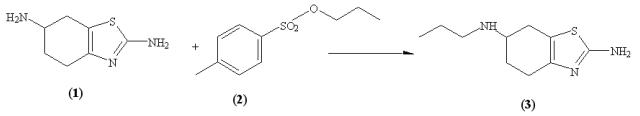
Pramipexole is a new generation dopamine receptor agonist, used as antiparkinson and antidepressant drug. It shows significant lower side effects compared to previous generation drugs (e.g. imipramine).

Pramipexole, namely)-(-)-2-amino-6-propylamino-4,5,6,7-tetrahydrobenzotiazol (*Se* (**3**) is obtained by direct alkylation of (*S*)-(-)-2,6-tiamino-4,5,6,7-tetrahydrobenzotiazole (**1**) with n-propyl tosylate (**2**) and isolated as monohydrate of dihydrochloride.



The scope of analytical development covered methods for determination of raw materials and intermediates, in process control and certification of final active substances.

Efficient method for routine control of synthetic process was necessary for process optimalization. Reversed phase HPLC technique was applied. Satisfying results were obtained by gradient technique. The same method was used for determination of purity and assay of final product for certification.

17:35 Poster II-180

SYNTHESIS OF NEW DERIVATIVES OF OLEANOLIC ACID AS POTENTIAL TRANSDERMAL PENETRATION PROMOTERS

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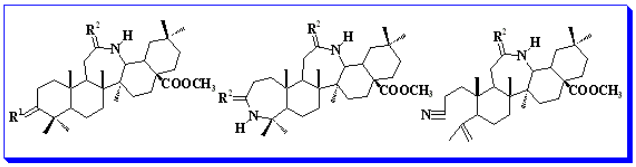
Some new derivatives of oleanolic acid were obtained, in which an atom of nitrogen was involved both in A and C ring and was an element of hydroxyimino or nitrile function, and also in heterocyclic ring (lactam system). In order to obtain these compounds, methyl 3- acethyloleanolate was subjected to a number of transformations which led to the derivatives **1** - **8** (oxidation, synthesis of oximes, Beckmann rearrangement, oxygen atom substitution, hydrolysis):



1: R^1 = O, R^2 = O
2: R^1 = N-OH, R^2 = O
3: R^1 = O, R^2 = S
4: R^1 = N-OH, R^2 = S
5: R^2 = O
6: R^2 = S
7: R^2 = S
8: R^2 = S

As it is known from our previous experiments [1, 2], the derivatives of oleanolic acid that contain seven-membered ring with a nitrogen atom are very good transdermal penetration promoters of therapeutic preparations. The effectiveness of these compounds is comparable to, and many a time more effective than that of *N*-dodecylcaprolactame, known as Azon®, one of the most effective accelerator of transdermal transport. The element that is probably responsible for this activity of this compound is the lactam system, present in molecules of derivatives **1** - **8** as well. It is supposed that the azacompounds **5** and **6** will be one of the most active transdermal penetration enhancers obtained to the present time.

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[2] L. Zaprutko, D. Partyka, B. Bednarczyk - Cwynar, "Triterpenoids. Part 21: Oleanolic Acid Derivatives as Percutaneous Transport Promoters"; *Bioorg. Med. Chem. Lett*. **2004**, *14*, 4723.

17:35 Poster II-181

CYCLIC OPIATE PEPTIDE ANALOGUES CONTAINING A CARBONYL BRIDGE

Ewa Witkowska[1], Agnieszka Zieleniak[1], Katarzyna Filip[1], Małgorzata Ciszewska[1], Nga N. Chung[2], Peter W. Schiller[2], Jan Izdebski[1]

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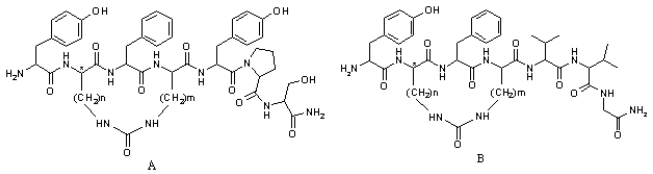
Recently we described synthesis and biological activity of several cyclic opioid analogues which contained *N*-terminal sequence of enkephalin and the common sequences for dermorphin and deltorfin [1,2,3]. These peptides, containing only the "messeage sequences" of natural peptide showed very high dual agonist potency in the GPI and MVD assays.

In this report we present synthesis and biological activity of peptides containing also "address sequence" for dermorphin (A) and deltorfin (B). Also, enkephalin sequence was elongated by alkylurea unit.



A B

References:

[1] D. Pawlak, M. Sidor, J. Wójcik, M. Pachulska, N.N. Chung, P.W. Schiler, J. Izdebski, *J. Peptide Sci.,***2001**, *7*, 128-140.

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[3] K. Filip, M. Oleszczuk, J. Wójcik, N.N. Chung, P.W. Schiller, D. Pawlak, A. Zieleniak, A. Parcińska, E. Witkowska, J. Izdebski, *J. Peptide Sci.*, **2005**, *11*, 347-352.

This work was supported by the Polish State Committee for Scientific Research (Grant No. 3T09A 02328)

17:35 Poster II-182

THEORETICAL ANALYSIS OF BIOLOGICALY ACTIVE CONFORMATIONS OF SOME BIS-AMIDINES

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To prevent *Pneumocystic carinii* pneumonia (PCP) in case of patients, with weakened immune systems (ex. AIDS, cancer, inherited immune deficiencies, or organ transplants), a *bis*-amidine aromatic compound - pentamidine (1,5-bis(4-amidinophenoxy)pentane) is used as a drug of second choice. The acute toxicity originally associated with pentamidine, have prompted investigations for new compounds with greater potency combined with lower toxicity. The binding affinity of *bis*-amidines to poly(dA)-poly(dT) DNA fragment correlated well with their *in vitro* activity against PCP.

The DNA binding affinity could be estimated by measurements of DNA phase transition temperature T_m. The higher this temperature is, the more stable DNA - ligand complex is formed.

Our aim was to compare the theoretically estimated binding energies of *bis*-amidines-DNA complexes with experimental differences of T_m temperature between free and bounded DNA. Optimized at DFT level structures were also compared with crystallographic data.

The results discussed in this work were obtained using resources of Interdisciplinary Centre for Mathematical and Computational Modelling Warsaw University.

17:35 Poster II-183

ANALOGUES OF DISTAMYCIN - SYNTHESIS AND BIOLOGICAL ACTIVITY

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The natural antibiotics, netropsin and distamycin, showed high selectivity of binding to the A-T rich regions of DNA, as well as both the antineoplastic and antiviral activity [1]. The model of binding of netropsin and distamycin with B-DNA became the inspiration to searches of compounds with similar DNA-ligand interaction.

New analogues of distamycin with modification of *N*-pyrrole rings were synthesized. In these investigations we concentrated on connecting benzene segments with some heterocyclic fragments. We chose three different aromatic diamines and four aromatic acid chlorides (Fig.1) and planned the synthesis of distamycin analogues.

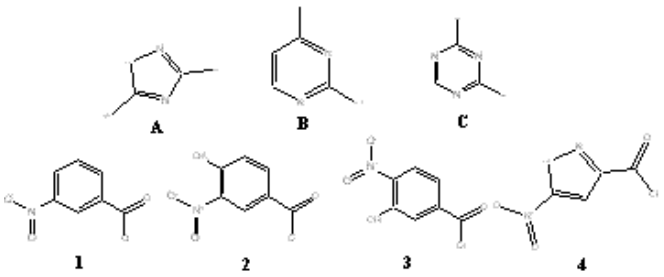

Fig.1. Heterocyclic aromatic diamines (**A**, **B**, **C**) and aromatic acid chloride (**1-4**)

The free amine groups were acylated by acid chloride in stechiometry 1:2, to obtain twelve new compounds. All of them were investigated and showed antiproliferative and cytotoxic effects in the standard cell line of mammalian tumour MCF-7.

This work was founded by grant KBN No. 2P05F 017 27.

[1] Ch. Bailly, Sequence-specific recognition and modification of double-helical DNA by minor-groove binding conjugates structurally related to netropsin and distamycin, in: *Advances in DNA Sequence-Specific Agents*, **1998,***3*, 97-156.

17:35 Poster II-184

SYNTHESIS OF GLIMEPIRIDE - CRYSTAL STRUCTURE ANALYSIS STEP BY STEP

Katarzyna Korczak[1], Adam Andrzej Pietraszko[2], Katarzyna Badowska-Rosłonek[1], Hanna M. Beczkowicz[1]

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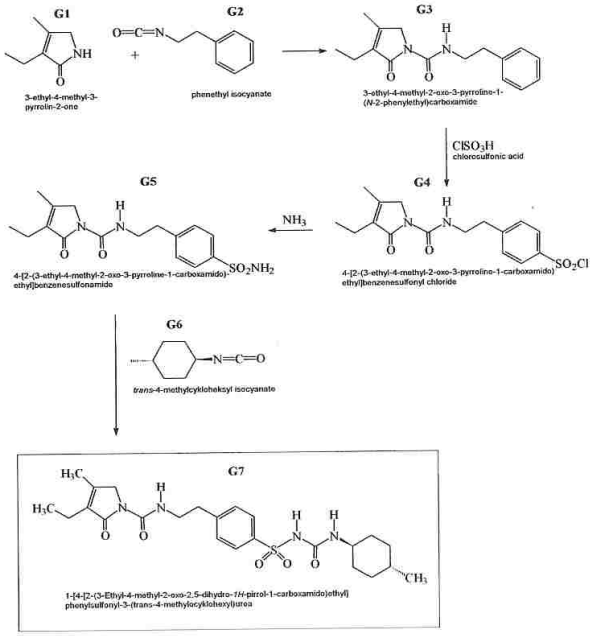
The chemical formua of glimepiride is 1-{4-[2-(3-ethyl-4-methyl-2-oxo-2,5-dihydro- 1*H*-pirrol-1-carboxamido)ethyl]phenylsulphonyl}-3- *trans*-(4-methylocykloheksyl)urea. Glimepiride is a new oral antidiabetic drug in the sulfonylurea class having a prolonged effect. Glimepiride synthesis is outlined in Scheme [1].



The single crystal structure of Glimepiride (**G7**) was determined by W. Grell & col. [3] (code: TOHBUN01 in database CSD). An aim of our work was the determination of unknown crystal structure of compounds **G3** and **G5**. The crystal structures of the **G3** and **G5** were determined using single crystal X-ray diffraction method. We have found that commercial **G3** contained contamination, which was identified as *N*,*N'*-bis(2-phenylethyl)urea (**X3**) [4]. Conformation of molecules and their packing in the crystal structures (Figure 1 and 2 shows the projestion of molecules) will be discussed .

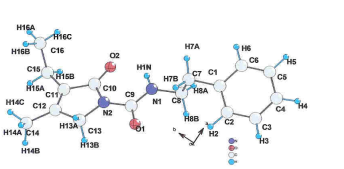


Fig. 1

G3 $C_{16}H_{20}N_2O_2$
3-ethyl-4-methyl-2-oxo-3-pyrroline-1 (N-2-phenylethyl) carboxamide

Monoclinic P21/n
a = 7.132Å
b = 25.758Å
c = 8.433Å
β = 99.24°

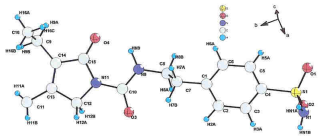


Fig. 2

G5 $C_{16}H_{21}N_3O_4S$
4-[2-(3-ethyl-4-methyl-2-oxo-3-pyrroline-1-carboxamido)-ethyl] benzenesulfonamide

Monoclinic P21/n
a = 8.234Å
b = 14.291Å
c = 14.823Å
β = 93.486°

References:

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Mark, P. Luger, H. Nar, H. Wittenben, P. Muller, *J. Med.Chem*., 1998, *41*, 5219.

17:35 Poster II-185

INFLUENCE OF POLYMER MATRIX STRUCTURE ON CONTROLLED RELEASE OF IDARUBICIN IN A CSF AND SALINE SOLUTION.

Janusz Kasperczyk[1,2], Katarzyna Stokłosa[1], Piotr Dobrzynski[2], Waldemar Przybyszewski[3], Marek Jurkowski[3], Krystyna Trzepietowska-Stępień[1], Adam Wilczok[1], Maria Sokół[3]

1. *Śląska Akademia Medyczna, ul. Narcyzów 1, Sosnowiec 41-200, Poland* **2.** *Centrum Chemii Polimerów PAN, Curie-Skłodowskej 34, Zabrze 41-800, Poland* **3.** *Centrum Onkologii-Instytut im. M.Skłodowskiej-Curie Oddział w Gliwicach (I.O.), Al. Wybrzeża AK15, Gliwice 44-101, Poland*

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In this study, the use of biodegradable copolymers such as poly(glycolide-co-lactide), poly(lactide-co-ε-caprolactone) and poly(glycolide-co-ε-caprolactone) for incorporation of idarubicin to polymer matrices with variable molar ratio of glycolydyl, lactydyl and ε-oxycaproyl units is investigated. The effect of different microstructure of polymer chain, on *in vitro* idarubicin release from matrices in two types of fluids: artificial cerebrospinal fluid and saline solution and *in vivo* idarubicin release from matrices in rat implantable with glioma was studied.

Figure 1. Cumulative release of idarubicin from biodegradable copolymers in a CFS observed for 356 days.

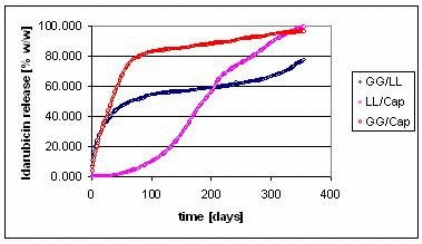
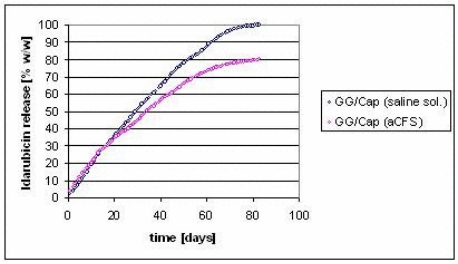


Figure 2. Cumulative release of idarubicin from biodegradable copolymer in saline and aCSF solutions observed for about 80 days.



From three types of copolymers the most stable idarubicin release for poly(glycolide-co-ε-caprolactone) with random chains is observed. No burst effect for all biodegradable aliphatic polyesters was confirmed. As expected, type of co-polyesters, especially molar ratio of sequences and type of fluids amount of drug release and time of degradation were observed. Microstructure analysis of poly(glycolide-co-ε-caprolactone) matrices with idarubicin determined by ^{1}H NMR. Regular degradation rate by monitoring of such parameters as degree of randomness, average length of sequences and transestrification coefficient of the second mode were confirmed. Presence of idarubicin and metabolites in rat urine and serum weren't observed using NMR *in vivo* and HPLC measurements. Besides in urine change of glucose, glicine, alanine, histidine, lactate, acetate, citrate, succinate levels weren't also noticed. The result suggest that lack of systemic toxic effects of idarubicin device can be achieved using random poly(glycolide-co-ε-caprolactone) matrix with anthracycline. In this case side effects of chemiotherapy with idarubicin such as inflammation of gastrointestinal mucosa, nausea, chills and cardiotoxicity might be eliminated. In future bioresorbable copolyester devices with idarubicin could be effective and nontoxic therapy of brain gliomas used.

This study was supported by a grant (No. 3/FB/2004) from Foundation of Pharmacetical Sciences Development.

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DETERMINATION OF SELECTED MEDICINES REDUCING THE LEVEL OF CHOLESTEROL BY CHROMATOGRAPHIC METHODS.

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Sclerosis and its consequences as the appearing circulatory failures, mainly manifested as disorder of the coronary, celebral and peripheral circulation are at present dominant wholesome problems of modern society. Among the numerous factors causing sclerosis an important reason are disturbances of lipides and lipoproteins. They are characterized by increasing content of cholesterol and/or triglicerides in plasma.

Medicines - derivatives of lovastatin are inhibitors of reductase of hydroxylmethylglutaryl - coenzyme A, an enzyme which catalysed the early stage of synthesis of cholesterol.

Conditions were elaborated for the identification of hiperlipidemia compound - simvastatin, lovastatin, calcium salt of atorvastatin, sodium salt of fluvastatin and sodium salt of pravastatin and three preparations (tablets or capsules), containing the above mentioned compounds - Lipitor - tablets, Lescol - capsules and Pravastatin - tablets.

The elaborated conditions were shown as a good ones for quantitative determination of compounds of this group by gas chromatographic method present in substances and pharmaceuticals. Six compounds were selected for GC assays. The method of internal standard was applied.

For identification of a mixture of the derivatives of lovastatin (statins) the capillary column CP-Sil 5 CB (HP-1), flame - ionization detector and two differences programmes were used.

For determination of substances from this group the capillary column CP-Sil 5, FID detector and temperatures were used: column temperature - 260 °C (295 °C only for calcium salt of atorvastatin); detector temperature - 320 °C and injector temperature - 300 °C.

The statistical data, indicate satisfactory precision and accuracy of the GC method, both for the compounds and pharmaceuticals.

To compare the obtained results, another set of experiments was performed using a HPLC method, which is routinely applied to studying compounds from the group of statins in the same substances and pharmaceutical preparations.

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MOLECULAR PROPERTIES OF SULCONAZOLE AND ECONAZOLE RELEVANT TO BIOAVAILABILITY

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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 medicinal products various exemption procedures and rules were implemented. One of them is substitution of *iv-vivo* bioequivalence study by *in-vitro* dissolution similarity determination. This makes a base for so called Biopharmaceutical 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 a promising tool for fast chemical substance classification within BCS. Moreover the BCS so far is limited to the orally administrated form and there is a need to extent this approach on product applied superficially. Improvement of the solubility by modification of the structure can lead to orally administrated forms.

The water solubility determination can be efficiently made based on free enthalpy of solvation, 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 have focused on the important from therapeutical view-point antimycotic medicinal products containing the imidazole ring. Here we calculated free enthalpies of solvation (ΔG_{solv}) by water molecules at the Hartee-Fock 6-31G* level using SCI-PCM model. For econazole and suconazole these values are ΔG_{solv} = 0.51 kcal/mole and ΔG_{solv} = -2.99 kcal/mole, respectively. This surprisingly indicates that for sulconazole better water solubility is expected, what reflects experimental results. Another molecular property calculated was electrostatic potential at isodensity surface, Again for sulconazole this value falls within the range from -54.28 to 36.41 kcal, while for econazole from -52.52 to 32.60 kcal, what discriminates both compounds wit respect to water solubility.

17:35 Poster II-188

MOLECULAR DETERMINANTS FOR METABOLIC STABILITY OF NICOTINIC AND PICOLINIC ACID DERIVATIVES

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We have synthetized a number of nicotinic and picolinic acid derivatives with expected anti-seizure activity. One of the important issues in this therapeutical group are pharmacokinetic properties and metabolic stability due to inter- and intra-subject variability. From experimental studies on metabolic stability it appears that more susceptible for liver and kidney metabolism are picolinic acid derivatives. For four derivatives: *N*-(2-fluorobenzyl)-2-pyridinecarboxoamide (**1**), *N*-benzylpyridinecarboxoamide (**2**), *N*-benzylnicotinamide (**3**), *N*-benzyl-4-(trifluoromethyl)nicotinamide (**4**), Energy of formation reaction calculated for each compound as a difference between total energy of product and substrates were calculated at the Hartree-Fock 6-31G* level of approximation. These values are -2.37 kcal/mole (**1**), 6.86 kcal/mole (**2**), 3.20 kcal/mole (**3**) and 0.74 kcal/mole (**4**). It appeared that energy of formation is not a suitable discriminative property to judge stability difference. Thus we calculate the energy of amide bond for studied compounds: $E_{C\text{-}NH}$ = 257.85 kcal/mole (**1**), $E_{C\text{-}NH}$ = 245.83 kcal/mole (**2**), $E_{C\text{-}NH}$ = 249.10 kcal/mole (**3**)

and E_{C-NH} = 253.53 kcal/mole (**4**).

For compounds (**1**) and (**2**) the values of E_{C-NH} well correlate with the experimental stability data. The results for compounds (**3**) and (**4**) suggest that they should be more susceptible to metabolism, but in fact they are stable. It might mean that for compound (**1**) and (**2**) the appropriate enzymatic systems exist, while for compounds (**3**) and (**4**) active enzymes in liver and kidney are not present.

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INVESTIGATIONS ON THE SYNTHESIS AND PROPERTIES OF 2-(ALKYL,ARYL)-1,4,5-TRIOXO-1,2,3,4,5,6-HEXAHYDROPYRIDO- [3,4-D]-PYRIDAZINES DERIVATIVES WITH POTENTIAL BIOLOGOCAL ACTIVITY.

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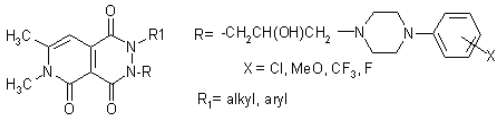
The studies of pyrido[3,4-d]pyridazine derivatives are a continuation of the former investigations of N-arylpiperazinylalkyl 1,4-dioxo(1,4,5-trioxo)-1,2,3,4-tetra(and 1,2,3,4,5,6-hexa)hydropyrido[3,4-d]pyridazines, which in pharmacological screening turned out to reduce spontaneous and amphetamine induced motility and display analgesic and antiserotonic activity. The continuation of the research in this field was due to the positive results of preliminary tests, in which some of the derivatives acquired through condensation of pyrido[3,4-d]pyridazine with 1,2,3-triazepine ring, and resulting formation of the triheterocyclic pyrido[2,3,4-ef]pyridazine[3,4-e]1,2,4-triazepine, appeared to have strong analgesic activity and no toxic effects (LD 50>2000 mg/kg). Our hitherto studies (chemical and biological) proved that many derivatives developed from bicyclic arrangements possess hipotensic, analgesic, anxiolytic and tuberculostatic activities. Exceptionally interesting and significant were the results of the tests for their antimycobacterial activity (TAACF) conducted at Colorado State University. These studies were based mainly on the chemical substances obtained in our department and encourage us to continue the synthesis of drugs from this group.



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2. H. Śladowska, B. Filipek, *Boll. Chim*., 2004, 143, no 5.

This investigation was supported by grand No 1521.

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THERMOSENSITIVE TRI-BLOCK-CO-POLYMERS POLY- (HPMAm-MONO/DI LACTATE)-PEG-POLY(HPMAm-MONO/DI LACTATE) FOR THE CONTROLLED RELEASE OF OPIOID PEPTIDES

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Many drugs, especially proteins and peptides, when administered by conventional method (*e.g.* oral administration in the form of tablets or capsules and intravenous or intramuscular injections), do not cause the desired therapeutic activity. Therefore, the therapeutic applicability of these pharmaceutically active proteins/peptides is limited because of lack of suitable delivery systems. At present a number of delivery systems for peptides/proteins is under investigation, among which liposomes, polymeric nanoparticles and microspheres, as well as implants from biodegradable polymers and hydrogels.

ABA triblock copolymers (block A: poly(*N*-(2-hydroxypropyl)methacrylamide mono/dilactate), block B: PEG) have thermosensitive behavior. These polymers are soluble in water below the Lower Critical Solution Temperature (LCST) of the thermosensitive A block and gellify above this temperature. The LCST of this polymer can be modulated by HPMA-mono/di lactate ratio.

The aim of this study was to design an injectable formulation with these polymers from which opioid peptides are released for a period of around 10 days.

Thermosensitive polymers (with different length of PEG) were synthesized using radical polymerization and characterized by DSC, rheology and Static Light Scatering measurements. Hydrogels with different water contents and loaded with two opoid peptides (biphalin, β-casomorphin) were prepared by bringing aqueous solutions of the ABA block copolymers above the LCST of the thermosensitive blocks. The released peptides were quantified using HPLC.

The obtained results show that hydrogels based on thermosensitive poly(HPMAm-mono/di lactate)-PEG4000-poly(HPMAm-mono/di lactate) triblock copolymer showed a sustained release of the peptides for 5 till 10 days. These systems are therefore suitable candidates for further in vivo evaluations.

Acknowledgements

The investigations were supported by a research grant cooperation Polish-Netherlands scholarship.

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SYNTHESIS AND ANTIPROLIFERATION ACTIVITY OF THE PRODUCTS OBTAINED IN THE REACTION OF N^3-SUBSTITUTED AMIDRAZONES FROM 1,2-CYCLOHEXANE-DICARBOXYLIC ANHYDRIDE.

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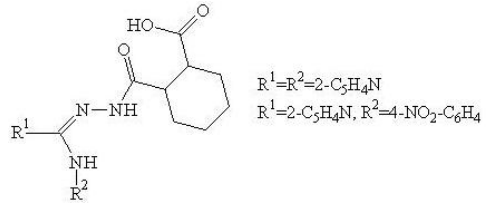
In the reaction of N^3-substituted amidrazones with *cis*-1,2-cyclohexanedicarboxilic acid anhydride N^1-carbonyl-(2-carboxycyclohexane-1-yl)-N^3-substituted-2-picolinamidrazones were obtained.



The chemical structures were confirmed by IR, ^{1}H NMR, EJ-MS and elemental analysis. Their purity was confirmed by chromatografic methods.

The antiproliferative activity of tested compounds will be assessed in glioma C6 cell culture. Tumor cells will be plated on 96-well microplates (NUNC) at density 0.5 x 10^4 cells/mL. Next day culture medium will be changed and cells will be exposed to 10 and 100 mM concentrations of tested compounds. Cell proliferation will be assessed after 96 h by means of MTT (Cell proliferation kit I, Roche Diagnostics, Germany) method in which the yellow tetrazolium salt (MTT) is metabolised by viable cells to purple formazan crystals. Microplates will be incubated for 3 h with MMT solution (5 mg/mL). Formazan crystals will be solubilized overnigth in SDS buffer and the product will be quantified spectrophotometrically by measuring absorbance at 570 nm wavelength using E-max Microplate Reader (Molecular Devices Corporation, Menlo Park, CA, USA).

Zakończenie Konferencji
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Thursday, 18 May

Śniadanie
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Wyjazd
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