Kinetic study on the Decomposition of 2-Chloroethylnitrososulfamides (CENS) in Organic Media

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Anticancer 2-chloroehtylnitrosoureas (CENUs) have an established place in the clinical treatment of human malignancies. The mechanism of action of these drugs has been extensively studied and the therapeutic efficacies of CENUs are known to be related to their spontaneous decompositions to generate both electrophilic species which alkylate DNA and isocyanates which carbamoylate proteins especially DNA repair proteins, contributing substantially to toxic side effects. On the basis of these observations, we have previously developed a new family of alkylating agents structurally related to 2-chloroethylnitrosoureas (CENU) but devoid of any carbamoylating activity: 2-chloroethylnitrososulfamides (CENS).

To understand the details of the mechanism of action of these valuable biological compounds in different media we investigated their kinetics of decomposition in organic media using ^{1}H and ^{15}N NMR spectroscopy. Our first observation showed that the kinetics is complex and acts very slowly comparatively to the aqueous and biological media, as described in our previous works [1,2]. The use of LC-MS as separative and analytical technique shows several products which have been tentatively identified and a general mechanism has been proposed.

References:

1. Seridi, A.; Kadri, M.; Abdaoui, M.; Winum, J-Y.; Montero, J-L.; *Bioorg. Med. Chem. Lett* **2006**, 16, 1021-1027.

2. Seridi, A.; Winum, J -Y.; Kadri, M.; Abdaoui, M.; Montero, J-L.; *Arch. Pharm. Chem. Life Sci.* **2006**, 339, 521 - 526.

3. Gnewuch, C.T.; Sosnovsky, G. *Chem. Rev.* **1997**, *97*, 829-1013.

4. Korfmacher, W. A. *Drug. Discov. Today.***2005,** 10,1357-1367.

Anticancerous Properties of Phorbol 12-Myristate 13-Acetate (PMA) in Human Thyroid Cancer Cells

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Phorbol 12-myristate 13-acetate (PMA) is a natural compound that is known to affect a variety of basal cellular processes, including cell proliferation, differentiation, apoptosis, cell spreading and cell migration. PMA has been shown to promote antiproliferative and antimigratory effects in many types of cancer cells (1,2). The effect of PMA varies depending on cell type, the relative expression and distribution of different phorbol ester receptors and their downstream effectors. The most common phorbol ester receptor is protein kinase C (PKC). We have shown previously some implications for the role of PKC in thyroid cancer cell signaling (3). The aim of this study was to examine the effect of PMA on proliferation, the cell cycle, cell cycle regulators and migration of human thyroid cancer cells. The effect of PMA on proliferation was investigated with CellTiter analysis, 3H-thymidine incorporation and cell counting. Stimulating the anaplastic FRO thyroid cancer cells with PMA induced a strong antiproliferative effect in a concentration-dependent manner. Migration studies revealed that PMA effectively inhibit the FRO cell migration toward serum. In ML-1 follicular thyroid cancer cells, PMA also attenuated proliferation and migration. Fluorescence activated cell sorter (FACS) analysis revealed that PMA increased the fraction of cells in G1 phase of the cell cycle, whereas the fractions in S and G2 phases decreased. Moreover, PMA evoked a significant increase in the levels of the cell cycle regulators p21Waf1/Cip1 and p27Kip1. The levels of the cyclin dependent kinases, cdk4 and cdk6, decreased and thereby also the phosphorylation of the Rb-protein. When cells treated with PMA were washed and replated they continued to grow, indicating that PMA does not induce apoptosis. No effect on proliferation and migration was observed when the cells were treated with the inactive phorbol ester analog 4α-phorbol and a diacylglycerol (DAG) analog 1,2-dioctanoyl-sn-glycerol (DOG). Furthermore, we investigated the distribution of different PKC isoformes in cells treated with PMA for 10 min. PKCα, -βI and -δ isoforms translocated to the membrane. When cells were treated with PMA for 24 h the phosphorylation of MAPK (ERK 1/2) decreased. Overall the results from this study indicate that PMA is an effective inhibitor of thyroid cancer cell proliferation and migration, probably by a mechanism involving PKC-MAPK (ERK 1/2) signaling pathway.

A New Humanized Mouse Model for Testing HIV/AIDS Therapies and Microbicides

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With the goal of utilizing a novel humanized mouse model (RAG-hu) that permits multilineage human hematopoiesis for HIV/AIDS therapies, neonatal $Rag2^{-/-}\gamma_c^{-/-}$ mice were transplanted with human CD34 hematopoietic stem cells. Human cell engraftment was seen in peripheral blood, thymus, spleen, liver, lymph nodes, and bone marrow. There was generation of cells of the human adaptive immune system including human macrophages, B cells, T cells and dendritic cells. To assess virus susceptibility, RAG-hu mice were infected by HIV-1. Viral infection was assessed by PCR, co-culture, and in situ hybridization. Our results showed that both X4 and R5 viruses are capable of infecting RAG-hu mice and that viremia lasts for more than 1 year. Moreover, HIV-1 infection leads to systemic CD4 T cell depletion thus mimicking key aspects of AIDS pathogenesis. We also achieved successful mucosal viral transmission by infecting these mice *via* vaginal and rectal routes with both R5 and X4 tropic HIV-1. The ability to infect these humanized mice with HIV-1 by natural routes and showing viral pathogenesis now permits testing of anti-retroviral therapeutics and microbicides using a small animal model that harbors human cells.

High Throughput Compound Library Screening and Herbal Supplements to Mitigate Dengue Fever

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The prevalence and severity of dengue fever have greatly increased in recent years, so that about 40% of the world's population is at risk. In Asia, dengue fever is rivaling malaria as the leading cause of death in children. Our *in silico* and *in vitro* research concentrates on two promising areas: NS2B/3, a viral protease complex without which the protein components needed for viral reproduction cannot be activated, and the viral envelope (E) protein. The E protein undergoes major conformational change in order to create a channel through which the viral RNA enters the host cell, so inhibiting this process will prevent infection. Several promising protease inhibitors have been isolated from herbal products and *in vitro* tested for their activity against dengue virus including experiments in human tissue cultures. *In silico* screening of 500,000 compound compounds were performed for potential activity against the dengue virus dimeric and trimeric DEN 2 E protein. A particularly promising lead is a plant steroid. The poster will describe combining the power of high performance computing with wet lab experiments to prevent/ treat dengue virus infections by preventing viral entry and replication.

HPLC-DAD-MS/MS-ESI Screening of Phenolics with Potential Bioactivity in *Pieris brassicae* L. Reared on *Brassica rapa* var *rapa* L.

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In this research, we proceeded to the study of *Pieris brassicae* (Lepidoptera: Pieridae), an insect whose larvae constitutes a frequent pest of some Brassica species. With this study we intended to take advantage of the damage caused, using *P. brassicae* as a source of compounds with interest for the health. The phenolic profiles of *P. brassicae* at different development stages (larvae, exuviae and butterfly), its excrements and its host plant Brassica rapa var. rapa L. were determined by HPLC-DAD-MS/MS-ESI. Twenty five acylated and nonacylated flavonoid glycosides and ferulic and sinapic acids were identified in host plant, from which only twelve compounds were found in the excrements. In addition, the excrements showed the presence of sulphate flavonoids and other flavonoid glycosides not detected in the leaves of the host plant. In the larvae kept without food for twelve hours, only three compounds common to the plant material and two others also present in the excrements were characterized. The results indicate that deacylation, deglycosylation and sulphating steps are involved in the metabolic process of *P. brassicae* and that, besides the larvae, its excrements may constitute a promising source of bioactive complex compounds, hard to be isolated or synthesized in the laboratory. These two insect materials already displayed strong antioxidant capacity in distinct assays.

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Uniformly Sized, Molecularly Imprinted Polymers for Enantioselectivity of Pseudoephedrine by Precipitation Polymerization

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Molecularly imprinting is a convenient and powerful technique for preparing polymeric materials with artificial receptor like binding sites for various substances. Imprinting polymers prepared by this technique can be applied to a wide variety of areas requiring specific binding such as selective detection, separation and purification. Usually non-aqueous bulk polymerization methods have been utilized to obtain MIPs. The disadvantage of the method is that the obtained polymers had to be crushed, ground and sieved to produce packing materials, as a result, the irregular particles generally exhibit low separation efficiency for target molecules.

The aim of this research is to synthesize MIPs by the method of precipitation polymerization and to use the syntheses MIPs for enantioselectivity of a pseudoephedrine (chiral drug). The control of particle size is suitable for different application such as selectivity and separation. Uniform molecularly imprinted microspheres and nanoparticles were prepared by precipitation polymerization. This method is based on the precipitation of the polymeric chains out of the solvent in the form of particles as the growth is more and more insoluble in an organic continuous medium. In this case particles are prevented from coalescence by the rigidity obtained from the cross linking of the polymer so there is no need for any extra stabilizer and no need to crush the ground.

We prepare uniformly - sized MIPs for pseudoephedrine by precipitation polymerization method using methacrylicacid and acrylamide as functional monomer and EGDMA as across-linker in different solvent such as acetonitrile, chloroform and THF.

References:

1. Kosuke Araki, *Analytica Chimica Acta*, 469 (2002) 173-181.
2. Jun Haginaka, *Jornal of Chromatography A*, 948 (2002) 77-84.
3. Jia-Ping Lai, *J. Anal. Bioanal. Chem.*, 216 (2007).
4. N. Perez-Moral, *J. Analytica Chimica Acta*, (2003).
5. Haruyo Sambe, *Journal of Chromatography A*, 1134 (2006) 88-94.

Discovery of a Drug that Blocks the Trojan Horse-like Strategy Used by Pathogenic *Helicobacter pylori* and its Associated Bacteriophage

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Aim: Non-invasive *Helicobacter pylori* (*Hp*) is coated by ITAM-like antigen effectors, which allow escape from the host immune response. Here, we discover a new antimicrobial drug that blocks the "Trojan Horse" strategy of *Hp* by its ITAM-like antigen effectors.

Results: We show (1) that complexes of *Hp* and Viro-Adembeads-associated bacteriophage enhanced IL6/IL-8 production 3-5 fold, but the bacteria or the bacteriophage alone did not elicit any cytokine production (2) *Hp*-specific bacteriophage was associated with *Hp* periplasmic virulence factors including the molecular chaperones ITAM-like-HSP60/-HSP70, ITAM-like- UreaseB, ITAM-like-CagA, Flagellin/FlaA, and hemolysin secretion precursor *in vitro*. These periplasmic ITAM-like antigens were tyrosine-phosphorylated by various active PTKs including Src and/or Syk, Fyn/Jak2, PDGFR, and IGF-1R *in vitro*. The phosphorylation of these ITAM-like antigens and hemolysin secretion precursor may be essentially involved in *Hp*-infectious diseases. (3) Bacteriophage-associated ITAM-like HSP60, bound to angiotensin II receptor, and was involved in early intracellular [Ca^{2+}] mobilization and IL-6 production, in a Fyn/Jak2 and OipA/CagA-dependent manner. Late in infection, non-phosphorylated CagA which is associated with OipA, was also involved in changes in cell morphology, and these changes may be carcinogenic. (4) Anti-pY-ITAM polyclonal antibody (pAb) blocked the tyrosine-phosphorylation of all these periplasmic-ITAM-like antigens by various PTKs and in the *Hp*-induced early intracellular [Ca^{2+}]i in a IgG- and dose-dependent fashion. The tyrosine-phosphorylation of *Hp*-periplasmic-ITAM-like-HSP70 by Syk alone under 0.2M-NaCl or by IGF-1R and PDGFR, is suppressed by anti-pY-ITAM pAb in IgG- and dose-dependent manner. (5) *Hp* infected liver tissue sections were stained by anti-*Hp*-HSP60/DnaK mAb and anti-pY-ITAM pAb in IgG-dependent fashion. Moreover, anti-pY-ITAM pAb specifically stained the nuclei of cells from *Hp*-infected bile duct cancer patient isolates.

Conclusion: Anti-pY-ITAM pAb specifically suppress the tyrosine-phosphorylation of the ITAM-like antigen effectors and the attachment of the surface ITAM-like antigens associated with bacteriophage to host-receptor. To block non-phosphorylated ITAM-like CagA antigen which may be specifically associated with mouse IgG, another antibody, anti-non-pY-ITAM mAb may be needed. Our findings provide new insights into the concept and molecular basis of the pathogenesis of infectious bacterial diseases. Moreover, they may be helpful in diagnosing and preventing disease, and for helping to develop new vaccines against various PTK-associated, microbial ITAM-like antigen mediated diseases and cancers.

Therapy Against Microbial-Encoded ITAM-Like Motifs May Treat and/or Prevent a Number of Serious Infections Caused by Microbial Pathogens

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Background: The immuno-receptor tyrosine-based activation motif (ITAM: E/D-xx-Y-xx-L/I-x_{6-8}-Y-xx-L/I) is critically involved in immune receptor activation via Src- and Syk/Zap-ITAMs interactions. Although some viruses are known to possess ITAM, the role of ITAM-like motifs in viral infectious diseases remains to be defined.

Aim: To examine whether there are functional ITAM-like motifs (E/DxxYxxI/L, YxxI/L, FxxI/L) in serious pathogenic viruses.

Methods: We investigated the genomes of influenza A (H5N1, H1N1, H9N2, H3N2, H7N1, and H7N7) virus and SARS corona virus, human coronavairus 229E, human herpesvirus 8, Simian Virus 40, human immunodeficiency virus (HIV), and *staphylococcus aureus* phage phiP68 for ITAM-like sequences. We used *Helicobacter pylori (Hp)* as a model for ITAM-like motifs interactions with anti-pY-ITAM like peptide polyclonal antibody (anti-pY-ITAM pAb). To capture *Hp* specific bacteriphage, we employed Viro- Adembeads which were designed for simple and rapid capture and culture of viruses, including HIV, influenza A (H1N1), and cytomegalovirus (CMV).

Results: We found that *Hp* and its bacteriophage-associated ITAM-like antigen effectors including molecular chaperones HSP60/HSP70, CagA and Urease B were all tyrosine-phosphorylated by Src and/or Syk, Fyn/Jak2, IGF-1, and PDGF$\alpha\beta$ R PTKs. Anti-pY-ITAM pAb, in an IgG- and dose-dependent fashion, blocked tyrosine- phosphorylation of all *Hp* periplasmic ITAM-like antigens and *Hp*-induced early intracellular $[Ca^{2+}]i$. We also found the following: (1) hemagglutinin (HA) and neuraminidase (NA) amino acid sequences in the super-virulent H5N1 influenza A virus possess ITAM-like motifs (ExxYx_9DxxYxxDxxxYxxL) and (DxxxYxxLx_{49}YxxL) that are potential sites for tyrosine phosphorylation. (2) the ITAM-like motif's region of HA and NA has high homology with RSVSPEPI(p)YATIDDL peptide antigen, suggesting that they may associate with IgG-Fc. (3) the HA and NA proteins also contain the putative ubiquitiniation di-lysine (KK) motifs. (4) the Severe Acute Respiratory Syndrome (SARS) corona virus encodes putative membrane targeting proteins with ITAM-like motifs and the di-lysine motif in ORF1a and ORF1b as well as the replicase 1AB gene. (5) other ITAM-like motifs containing microorganism include human herpesvirus 8, SV40, *Staphylococcus aureus* phage phiP68, human immunodeficiency virus (HIV), Epstein-Barr virus, *Mycobacterium tuberculosis*, *Helicobacter bilis* and *Helicobacter hepatitis*, and (6) of special importance, the HA proteins from the non-virulent A/HongKong/1073/99 (H9N2), A/Finland/338/95(H3N2), A/Netherlands/33/03(H7N7), and A/chicken/TX/02 (H5N3) influenza A viruses do not contain these motifs.

Conclusions: The presence of functional ITAM-like proteins within various microbes including influenza A (H5N1, H1N1) may impair the normal host immune response, enhance the Src and/or Syk, Fyn/Jak2, IGF-1R or PDGF $\alpha\beta$R PTKs signaling response via microbial ITAM-like proteins, and contribute to the pathogenesis of serious diseases. We speculate that influenza A (H5N1, H1N1) virus infection depends on ITAM-like HA and NA in a tyrosine-phosphorylation dependent fashion and that anti-pY-ITAM pAb may be an effective drug to prevent the Trojan Horse-like strategy used by these microbes. Therapy or vaccines directed against PTK-associated microbial ITAM-like antigen maybe useful for treatment or prevention of diseases such as SARS or N5H1 influenza A infection.

Inhibition of Src for Potential Use in Cancer Chemotherapy

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Colorectal cancer is one of the principal causes of cancer-related death in the West. c-Src protein levels are elevated in colon cancer cells compared to non-malignant cells. In addition, Src signalling and transduction are directly involved in cell growth, the cell cycle, malignant transformation and cell migration, providing multiple opportunities for therapy.

Two approaches have been applied to the design of non-kinase inhibitors of c-Src. UCS15A is a natural Src inhibitor, and here a novel difluoro analogue has been synthesized and shown to activate Src in a HCT116 colon cancer cell line.

In the second approach, *in silico* screening of large compound libraries was conducted focusing on potential inhibition of Src-SH3 protein-protein interactions. The ZINC database (2.7 million compounds) was docked using GOLD into the proline binding site of the active Src crystal structure (PDB code 1Y57). Post-docking filtering tools to select for oral bioavailability were performed giving rise to a set of lead compounds. The top 16 were screened using a fluorescence polarization assay. A benzoquinoline derivative showed *in vitro* inhibition of the protein-protein interaction between a small peptide ligand and the Src-SH3 domain.

Anti-HIV Prodrugs Conjugating a Reverse Transcriptase Inhibitor with Integrase Inhibitors by a Spontaneously Cleavable Linker: Synthesis and Evaluation of Antiviral Activity

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Based on the prodrug concept as well as the combination of two different classes of anti-HIV agents, we designed and synthesized a series of anti-HIV double-drugs consisting of a nucleoside reverse transcriptase inhibitor (NRTI) conjugated with integrase inhibitor (INI) through a spontaneously cleavable linker in an effort to enhance the antiviral activity. These conjugates combined in their structure a dideoxy-didehydro-nucleoside (ddN) such as d4T and an INI such as α,γ-diketo acid (DKA) analogues of L-708,906 and L-731,988 linked through an appropriate self-immolative spacer. Among these novel bi-substrate inhibitors, several conjugates exhibited antiviral activity but, for some of them, their cytotoxicity was increased compared to the parent drugs (d4T, DKA) or even some precursors. These compounds are nevertheless interesting candidates for further investigations.

A Novel Therapeutic Use of Fibrin Sealant to Promote Saphenous Graft Wound Healing in Patients Undergoing Cardiac Surgery: A Randomised Study

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Background and Objectives: The efficacy of fibrin sealant and its extensive use in surgical field is already well known. Although its primary uses are mainly demonstrated for haemostasis, adhesive and untreated coagulopathy, its use in prevention of wound infection is still in the infancy. This randomised controlled trial was carried out to investigate the role of topical application of fibrin sealant in promoting wound healing on saphenous vein graft site in patients undergoing cardiac surgery.

Materials and Methods: The fibrin sealant used consisted of two lyophilised, proteinaceous components of fibrinogen and thrombin involved in the blood coagulation cascade.

Two hundred elective patients undergoing coronary artery bypass graft surgery were recruited into the study, and randomised blindly (n=100 in each group) into the treatment group versus placebo. The treatment arm group received a topical application of fibrin sealant on saphenous vein harvest site prior to skin closure. Early post-operative evaluation is done in terms of serious adverse events, post-operative cardiovascular complications and red cell transfusion required.

Post-operative wound assessment was done daily during in-hospital stay, then weekly basis by telephone interview up to 6 weeks follow-up appointment. A numerical-based novel wound scoring system was used to ensure consistent assessment depending on characteristics of the wound and treatment received.

Appendix 1: Wound scoring table will be included in the poster

Results: The saphenous wound complications are markedly reduced in patients receiving fibrin sealant intraoperatively ($p<0.05$). Wound complication score was higher by 37% during in-hospital stay, compared to post-discharge.

Conclusion: The multiple intraoperative use of fibrin sealant as haemostatic agent as well as in preventing wound infection should be acknowledged to improve clinical outcome and enhance cost benefits in patients healthcare.

Nanoconfinement: A Strategy to Manipulate the Crystallization Behavior of Drugs

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Control of the crystallization behavior of polymorphic drugs is essential for their application. Unstable crystalline forms and amorphous drugs are of interest since their solubility and bioavailability are better compared to stable crystalline forms. We show that it is possible to stabilize such states by confining drugs in nanoporous media. A series of controlled porous glasses (CPGs) with average pore diameters d between 4nm and 100nm was infiltrated with the commercial drug acetaminophen. The host-guest-systems are studied by differential scanning calorimetry and x-ray scattering. The results for CPGs with larger pore diameters ($20nm<d<100nm$) clearly show that the metastable crystalline form III of acetaminophen melts and persists for long times [1], which was never observed for bulk samples. Melting temperature and heat of melting of form III are estimated. Experiments on acetaminophen in CPGs with 4nm pores indicate that the amorphous state is stabilized. Amorphous acetaminophen in 4nm pores is unable to crystallize even if the samples are stored at suitable crystallization temperatures for weeks. These results underline that nanoconfinement is an interesting strategy to manipulate the crystallization behavior of drugs. Thermodynamic effects in nano-sized systems as well as changes in crystallization kinetics and nucleation behavior are considered to explain the reported observations.

Reference:

1. M. Beiner, G.T. Rengarajan, S. Pankaj, D. Enke, M. Steinhart, *Nano Letters* 7 (2007) 1381.

Intelligent Decision Support System-Based Biomarker Discovery

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The successful treatment of cancer depends on early and accurate detection. Molecular diagnosis is expected to greatly improve current diagnostic process. Biomarkers are molecular signatures that can be used to identify the presence or absence of a particular disease state and to measure the physiological effects of therapeutic intervention in the treatment of disease. Life sciences researchers do not have complete information about the molecular markers that are responsible for causing most cancers. The high throughput experimental methods, including microarrays, provide an excellent tool for parallel measurement of expression of all the biological molecules such as genes, proteins and metabolites. Though many methods already exist for the determination of markers and tumor diagnosis using high throughput data, more precise and accurate method for feature selection as well as tumor classification are still needed. In this poster we will present a general overview of new strategy for gene expression-based biomarker discovery. By applying our strategy to data from tissue samples, we were able to identify the patterns of gene expression -biomarkers- unique to prostate cancer. We hold a patent on the biomarkers for prostate cancer and we have applied the same technology to identify colon cancer biomarkers, for which a patent is pending.

Use of the Cryopreserved Human Hepatocyte Sandwich-Culture Model to Measure Hepatic Metabolism and Biliary Efflux

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Drug clearance is a key parameter in drug discovery and development, which includes, but is not limited to, hepatic metabolic and non-metabolic pathways. While hepatic *in vitro* metabolic pathways have been extensively studied in both academia and industry, our lab has developed and also reported on a convenient *in vitro* model that can measure hepatic uptake and biliary excretion using cryopreserved human hepatocytes in a sandwich culture configuration. To date, this model has been used to track parent compound only, due to the lack of understanding for metabolic activity in sandwich cultured hepatocytes. In this presentation, our results show the apparent intrinsic clearance ($CL_{int,app}$) of the CYP3A4 substrate midazolam in sandwich-cultured (SC) hepatocytes on day 5 and non-cultured hepatocyte suspensions was similar (5.0 and 5.3 μL/min/10^6 cells, respectively). Furthermore, the metabolic turnover of midazolam to 1'-OH-midazolam was 90% abolished by the CYP3A inhibitor ketoconazole at 3 μM in both hepatocyte systems. Gene chip analysis also indicated, along with several other CYPs and transporters, a similar degree of CYP3A4 mRNA expression between non-cultured hepatocyte suspensions and day 5 SC hepatocytes. Ongoing studies with two internal Pfizer compounds in cryopreserved sandwich-cultured hepatocytes show that parent compounds and their corresponding multiple metabolites can be detected in hepatocytes and bile canaliculi. The metabolites formed in SC hepatocytes are identical to those found in fecal samples from healthy volunteers after oral administration of parent drug. For one compound, the mean *in vitro* biliary excretion of parent and metabolites M3, M4 and M6 are 0, 48, 32 and 39, respectively, compared to percents of administered dose *in vivo* of 0, 73, 3.4 and 11. In summary, these findings suggest that the cryopreserved human hepatocyte sandwich-culture model may have further potential to estimate the biliary clearance of both parent and metabolites.

Improvement of Fluvastatin Controlled Release Through the Use of New Biocompatible Aliphatic Polyesters

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Fluvastatin is a totally synthesized 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitor. HMG-CoA reductase is the rate-limiting enzyme in cholesterol biosynthesis. The nonlinear pharmacokinetic profile of the drug substance leads to greater than expected, systemic drug concentrations which may lead to increased risk of adverse events at doses greater than 20mg. Therefore, controlled release formulations would be considered necessary in order to improve tolerability at higher doses while maintaining the drug's efficacy. However, the high solubility of the sodium salt (50g/L) is the limiting factor in achieving the optimal release rate, usually leading to extend "burst" effects and significant deviations from zero order kinetics.

In the present study, controlled release formulations based on poly(propylene-co-ethylene succinate) (PPSu-co-PBSu) copolymers were developed and evaluated. The biocompatibility of both polymers and their copolymers was investigated by measuring the viability of HUVEC cells using different polymer concentrations. The release studies showed an optimal controlled release profile up to 12 h for the matrix of PPSu-co-PBSu 50/50 w/w copolymer, while the systems proved to be suitable matrices for controlling the release rate of the drug substance.

New Paradigm in Anti-Viral Therapy

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Carbohydrate chains of mammalian cells are known to be receptors for influenza virus and many other viruses and bacteria. For example, human influenza viruses H1, H3 and B isolated after 1970 demonstrated remarkable ability to bind the same receptor, trisaccharide 6-sialyl-*N*-acetylglucosamine (6'SLN). The corresponding counter-receptors of the microorganisms, lectins, ensure *adhesion* to these carbohydrates. The general principle of *anti-adhesion* therapy is blocking of a lectin binding site with a receptor analog for prevention of adhesion or detachment of already bound microorganism. Anti-adhesion principle promise to be a prospective alternative for substitution of antibiotics and other conventional therapeutics, due to the known structure of the active substance and absence of "drug pressure" effect on microorganism evolution. Experimental data evidence that natural receptors, oligosaccharides, are low-potent blockers. In contrast, mimetics are more potent as lectin blockers, however they are expected to cause drug pressure resulting in selection of resistant strains. An evident way to improve affinity of natural oligosaccharides is design of their multivalent forms. Particularly, polymers with pendant trisaccharide 6'SLN proved to be tremendous influenza blockers both *in vitro* and *in vivo*; however they remain rather research tools than real drugs due to intrinsic inconsistency of true polymers. We present an approach for design of non-polymeric multimerics, namely self-assembling glycopeptides. The carbohydrate part of glycopeptide is responsible for binding with influenza virus lectin, whereas very simple, glycine-based peptide part is responsible for assembling into the so-called tectomers (non-covalent, or supramolecular polymers).The tectomers displayed 1000-fold or more potency as blockers for wide range H1, H3 and B influenza viruses comparing to monomeric 6'SLN. We also present a more advanced version, when glycopeptide-*prodrug* remains to be small molecule in absence of influenza virus, being however capable of forming tectomers in a virus-provoked fashion.

Cytarabine Prodrugs for Transdermal Delivery

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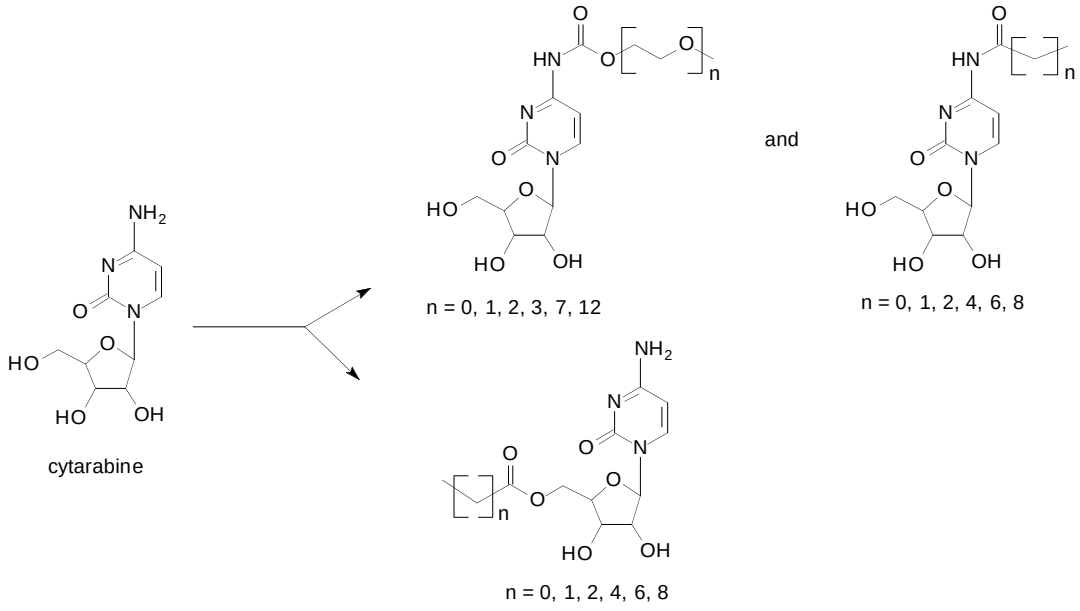
Cytarabine (Ara-C) is a pyrimidine nucleoside drug used in the treatment leukemia. The clinical utility is, however, limited by the action of deaminases which converts it to the inactive arabinofuranosyl uracil (Ara-U) metabolite. As a consequence Ara-C has a very short half-life and is only given as continuous infusion to achieve therapeutic efficacy. Transdermal delivery has the potential for sustained drug release useful for drugs with a short half-life. The skin, although an ideal site for drug administration, is, however, also a major barrier to this process. To efficiently penetrate the skin a drug must possess the required physicochemical properties.

Selected N4-carbamides, N4-amides and 5'-esters of cytarabine were synthesized with the aim of determining their physicochemical properties, such as aqueous solubility and lipophilicity and to measure their *in vitro* transdermal flux through excised human skin. These prodrugs were obtained by coupling the parent drug to methoxypolyoxyethylene glycol moieties of various lengths *via a* carbamate spacer or by direct amidation or esterification as illustrated below.



Novel Prodrugs of Lamivudine and Zidovudine for Transdermal Delivery in the Treatment of HIV/AIDS

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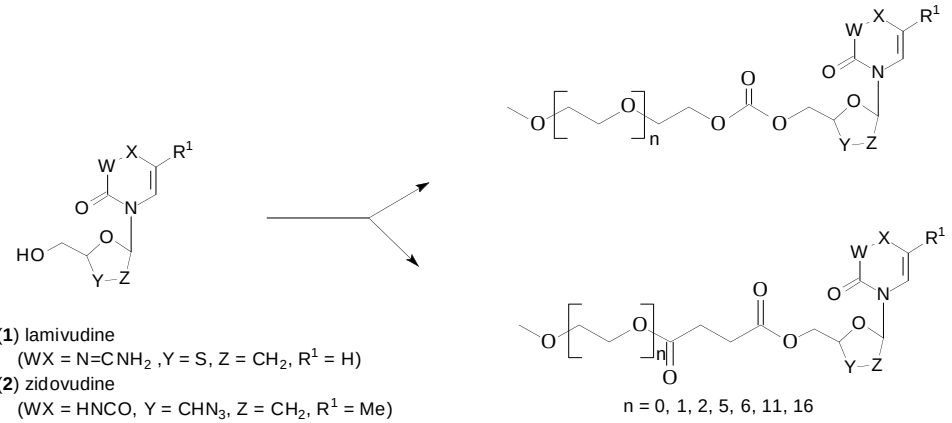
The adverse effects such abdominal pain, nausea, vomiting, diarrhoea, etc. associated with the oral delivery of ARV drugs in the treatment of HIV/AIDS encouraged the development of new strategies. Prodrugs of the antiretroviral medicines applied transdermally can be of great value, especially for patients who have difficulty taking medicines orally like children and those with concomitant conditions such as oesophageal infection or cancer. Although an ideal site for drug administration the skin is, however, also a major barrier to this process. Effective drug therapies must therefore overcome the challenge of finding a technology to administer, measure and deliver the required quantity of drug into or through the skin.

Novel prodrugs of two ARV drugs of the NRTI family, i.e. lamivudine (3TC) (**1**) and zidovudine (AZT) (**2**), were synthesized with the aim of determining their physicochemical properties such as aqueous solubility and lipophilicity as well as to measure their *in vitro* transdermal flux through excised human skin. These prodrugs of tripartate-type were obtained in a two-step process by coupling the parent drugs to methoxypolyoxyethylene glycol moieties of various lengths *via* carbonate or succinate spacers as illustrated below.



(**1**) lamivudine
(WX = N=CNH_2 ,Y = S, Z = CH_2, R^1 = H)
(**2**) zidovudine
(WX = HNCO, Y = CHN_3, Z = CH_2, R^1 = Me)

n = 0, 1, 2, 5, 6, 11, 16

PEGylation - A Key Technology to Improve Solubility and Pharmacokinetic of Peptides, Proteins and other Biopharmaceuticals for Superior Drug Delivery

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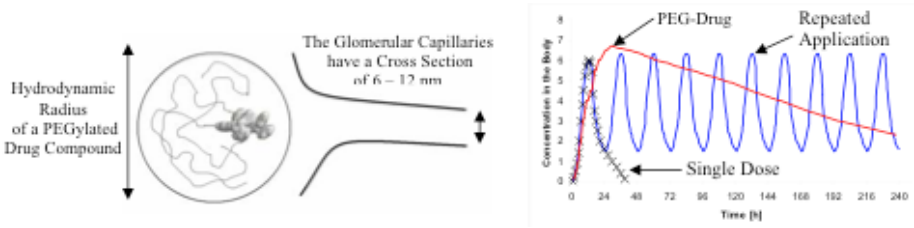
The big advantages of proteins, antibodies, siRNA, and other natural products in their usage as drugs is their high specifity combined with low side reactions. They interact with the dedicated target only and do not have activities at any other place in the body. A significant drawback is their low stability under physiological conditions, as they are easily attacked by the immune system of the body, i.e by antibodies and proteolytic degradation enzymes, and have therefore often poor pharmokinetic properties.

PEGylation, i.e. attaching PolyEthylene Glycol chains (PEG) has mainly two effects to the active component:

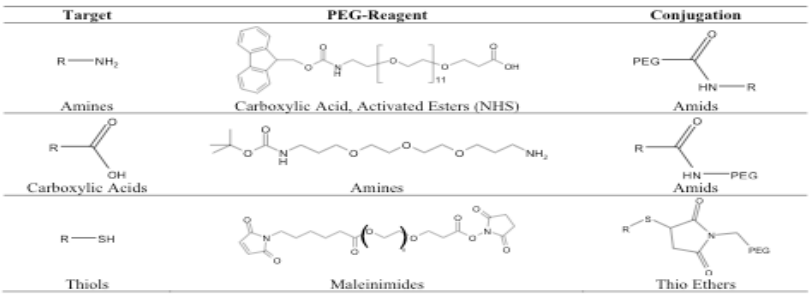
The PEG shields the compound against attacks by the immune system, in particular by antibodies and degradation enzymes. The half life in the body is significantly improved.

Attaching PEG compounds to proteins increases the hydrodynamic radius of the whole component to an order of magnitude, which is larger than the normal cross section of the glomerular capillaries. The renal clearance is therefore reduced and the compound stays longer in the body.



A PEGylated drug stays much longer in the body, lower doses of application are required, which leads to a much higher acceptance, less toxic side reactions and an over all much more convenient pharmacokinetic behaviour.

The most common functional groups used for conjugation are amines, carboxylic acids and thiols. However, many more reactive groups can be used to attach PEGs to a target compound.



References:

1. Duncan, Ruth, The Dawning Era of Polymer Therapeutics, Nature Reviews Drug Discovery 2 (2003) 347-360.

2. Veronese, Francesco M. Peptide and protein PEGylation: a review of problems and solutions, Biomaterials 22 (2001) 405-417.

3. Chapman, Andrew, P. PEGylated antibodies and antibody fragments for improved therapy: a review; Advanced Drug Delivery Reviews 54 (2002) 531-545.

4. Bailon, P. *et al*. Rational design of a potent, long lasting form of interferon: a 40 kDa branched polyethylene glycol-conjugated interferon alpha-2a for the treatment of hepatitis C. Bioconjug. Chem. 12 (2001) 195-202.

5. Roberts, M.J., Bentley, M.D., Harris, J.M.; Chemistry for peptide and protein PEGylation; Advanced Drug Delivery Reviews 54 (2002) 459-476.

6. Haag, R., Kratz, F.; Polymer Therapeutics: Concepts and applications; Angew. Chem., 118 (2006) 1218-1237; Angew. Chem. Int. Ed. 45 (2006) 1198-1215.

Hepatoprotective Effect of Diltiazem and Silymarin

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We previously reported that *d-cis* diltiazem but not *l-cis* diltiazem was associated with a hepatoprotective effect, suggesting that diltiazem is stereospecific in protecting against lipid peroxidation using isolated microsomes. In the present work we extend our studies to examine the hepatoprotective effect in Chang and PLC hepatocytes. We investigated the antioxidant properties of *d-cis* diltiazem and the combination of diltiazem and silymarin. Intracellular free radical levels were assessed using DCF fluorescence following exposure of cells to an oxidative stress of 400 μM H_2O_2. Diltiazem (2.5 μM and 10 μM) significantly reduced the DCF fluorescence signal as did Silymarin (1 μg/mL). The combination of diltiazem and silymarin further protected cells against oxidative stress ($p<0.001$) compared to use of either drug alone. Both drugs statistically enhanced cell growth ($p<0.01$), ATP levels and reduced the pro-apoptotic protein Bax with the combination providing a further beneficial effect ($p<0.001$). We conclude that low dose diltiazem and/or the combination of diltiazem and silymarin provide a hepatoprotective effect against free radical damage due to oxidative stress.

Local Interpretation of Machine-Learning Models using Attribute Gradients, Applied to Ames Mutagenicity Data

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A method for local interpretation of QSAR-data using machine-learning models is presented. This work shows that it is indeed possible to interpret machine-learning methods in a neighborhood to a prediction. Furthermore, the locally most important variable does not comply with the over-all most important variable if the relationship modeled is non linear. QSAR models are today applied to many endpoints where the common knowledge is poor. It is however rare that the relationships behind these endpoints are of linear nature and that researchers could come up with a simple and understandable model which covers the full activity domain. This calls for local interpretation of global models that is handle non-linear relationships. In the present work this is achieved by computation of the decision function gradient in any data point. Since each explanatory variable gives a contribution to the prediction and its gradient, the variable with the largest or smallest gradient contribution is the variable that has the highest importance in the data point, and is therefore the most important variable in the local setting. The method described has been verified using two sets of simulated data and Ames mutagenicity data where our results are well in line with other results in the literature.

Molecular Understanding of Histone Deacetylase Inhibitors (HDACIs) Efficiency in Cancer Cells

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Cancer cells are much more sensitive to Histone Deacetylase Inhibitors (HDACIs) then normal cells. Several mechanisms, including the capacity of the normal cells to repair their damaged DNA, have been proposed to explain this selectivity. However, none of the mechanisms proposed so far can explain why other anticancer drugs that damaged DNA do not show this preference for cancer cells. We have shown that pre-treating cancer cells with either trichostatin A (TSA) or vorinostat (SAHA), two HDACIs, increased the killing efficiency of conventional anticancer drugs. No sensitizing effect was observed when the HDACIs were added after the conventional anticancer drugs suggesting that the HDACIs pry open the chromatin structure to enhance the efficiency of the drugs. Using micrococcal nuclease digestions and PCR-Stop assays coupled to RT-PCR we now show that HDACIs facilitate chromatin accessibility at specific loci targeted by anticancer drugs in human cancer cells. Moreover, the increased accessibility to chromatin DNA is even more pronounced in cells synchronized in S and G_0 phases of the cell cycle. These data indicate that intrinsic chromatin properties are likely to contribute to HDACIs selectivity for cancer cells and should be considered in the development of mechanism based clinical trials.

An Effective Purification Method for Cucurbit[*n*]uril; a Molecular Host and Drug Delivery Vehicle

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Cucurbit[*n*]uril are empty barrel shaped macromolecules known that can encapsulate small molecules [1]. Cucurbit[*n*]uril can act as slow-release drug delivery vehicles, protecting the drugs encapsulated in its cavity by limiting access to reactive intermediates. The electronegative repulsion from the electron rich portals and bulk of the cage limits nucleophilic attack [2,3].

It is important to have pure cucurbiturils of a particular size for each targeted drug delivery system, and for other applications. Cucurbiturils are synthesised as a mixture of Q[5], Q[6], Q[7], Q[8] and Q[5@10][4]. Existing purification methods are applicable mainly for unsubstituted cucurbiturils[5]. We are developing a purification method that is applicable to both substituted and unsubstituted cucurbiturils, which exploits the size of the cavity and the affinity of this cavity to particular molecular shape and dipoles or ions.

References:

1. Jason Lagona, Pritam Mukhopadhyay, Sriparna Chakrabarti, and Lyle Isaacs, *Angew. Chem. Int. Ed*.,(2005), 44(31), 4844-4870.

2. Bali, Mark S.; Damian P.; Coe, Andrew J.; Day, Anthony I.; Collins, J. Grant. *Dalton Transactions* (2006), 45, 5337-5344

3. Poster presentation: - Yunjie Zhao, Anthony I. Day, Damian Buck, Mark Bali and Grant Collins, *Cucurbit[n]uril for drug delivery.*

4. I. Day, R. J. Blanch, A. P. Arnold, S. Lorenzo, G. R. Lewis, and I. Dance, *Angew. Chem. Int. Edit.*, 2001, **41**, 275.

5. Day. A; Arnold, A.P; Blanch, R. J.; Snushall, B. *J.Org.Chem* **2001**, 66, 8094-8100.

Controlled Release of Sulfosalicylic Acid from Polypyrrole/ Poly(acrylic acid) Hydrogel by Electrical Stimulation

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Transdermal drug delivery system is a system that delivers a drug into a body at a desired site and rate. The conductive polymer-hydrogel blend between polypyrrole (PPy) doped with anionic drug and poly(acrylic acid) (PAA) were developed as a matrix/carrier of drug for the transdermal drug delivery in which the characteristic releases depend on the electrical field applied. The PAA films and their blend films were prepared by solution casting using ethylene glycol dimethacrylate (EGDMA) as a crosslinking agent. A mechanical blending of PPy particles and PAA matrix was then carried out. Drug diffusion coefficients of PPy/PAA hydrogel blended and the non-blended ones were investigated and determined by using a modified Franz-diffusion cell with an acetate buffer, pH 5.5, at 37 ^{0}C, for a period of 48 hours to determine the effects of crosslinking ratio and electric field strength. Amounts of the released drug were measured by UV-Visible spectrophotometry. The diffusion coefficients of drug were determined through the Higuchi equation *via* different conditions, with and without an electric field. Moreover, thermal properties and electrical conductivity of the polypyrrole and drug-loaded polypyrrole were investigated by means of the thermogravimetric analysis and by using a two-point probe meter, respectively.

Combined Ligand-Based and Target-Based Approach to Design NR2B/NMDA Receptor Antagonists

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NMDA subtype of ionotropic glutamate receptors (iGluRs) are macromolecular complexes comprised of distinct functional regions. A large class of compounds are able to bind to the different receptor sites, such as amino terminal domain (ATD), and determine their modulation. To date, various chemical families of selective antagonists of NR2B subunit of NMDA receptor have been described as neuroprotective agents and also for treating of neuropathic pain .

In a view to help in the design of such compounds, the NR2B binding-pocket and the main chemical features for NR2B/NMDA noncompetitive antagonists were investigated by means a combined ligand-based and target-based approach consisting of i) pharmacophore modelling, ii) dynamic studies and ii) binding-pocket docking of selected representative molecules. Moreover, starting from these studies new ligands bearing the required structural features were designed, synthesized and their biological effects were also evaluated.

Acknowledgement: MiUR

Reference:

1. Gitto R, De Luca L, Ferro S *et al.* (ACS-EFMC) Frontiers in CNS and Oncology Medicinal Chemistry, Siena October 7-9, 2007, P79.

A Semi-Total Synthesis of Guggulsterones, Natural Hypolipidemic Drugs, from Various Steroids as Antagonists for the Farnesoid X Receptor

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Bile acids are the major products of hepatic cholesterol catabolism and are natural ligands for the Farnesoid X receptor. Bile acids play very critical roles in the regulation of intestinal lipid absorption, bile flow, and biliary lipid secretion in mammals. Also, bile acids induce bile salt export pump but inhibit cholesterol 7α-hydroxylase (CYP7A1) gene transcription in the liver. CYP7A1 catalyzes the rate-determining step in bile acid biosynthesis and is feedback inhibited by bile acids returning to the liver via enterohepatic circulation. So, FXR has become a challenging target for the discovery of new drugs to treat hyperlipidemia, obesity, and hypercholesterolemia. Guggulsterones are identified as active ingredients of guggulipid, traditional hypolipidemic drugs, extracted from *Commiphora mukul*. Guggulipid has been widely used in Ayurvedic medicine, medicine of the ancient Indian, to treat obesity and hyperlipidemia in India. Study of Urizar *et al.* showed that the molecular mechanism by which lowers cholesterol is through antagonism of FXR by negative-feedback loop regulation. We succeeded in synthesis of *E*-guggulsterone in 87% overall yield from 16α, 17α-epoxypregnenolone in just two steps. we also attempted to synthesize *E*-guggulsterone selectively from other steroids. Furthermore, *Z*-guggulsterone was easily prepared from *E*-guggulsterone with photochemical, thermochemical, and acid-catalytic reactions.

Peptide Inhibitors of DNA Replication in *Staphylococcus aureus*

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During the last decades bacteria have developed resistance towards numerous antibiotics. A good drug target has a conserved function in a wide spectrum of pathogens and is essential for bacterial proliferation. Drug targets that lack homologues in eukaryotes are favored because inhibitors would be predicted to have low toxicity in humans. This is fulfilled by the replication machinery. The aim of this work is to characterize protein-protein interactions of the replisome of *S. aureus* and to identify peptides that interfere with these protein-protein interactions.

We have identified the protein-protein interactions in the replisome of *S. aureus* by use of a bacterial two-hybrid system (BTHS). A reverse BTHS has been developed to directly select for compounds that disrupts selected interactions. The data demonstrates that we can use the R-BTH system to screen for compounds that disrupts several essential protein-protein interactions.

We have adopted the SICLOPPS (split intein-mediated circular ligation of peptides and proteins) technology for intracellular synthesis of cyclic peptides. A library has been constructed by insertion of 6 random amino acids between the C- and N-terminal parts of the split intein. We have currently identified several cyclic peptides able to disrupt specific protein-protein interactions in the replisome of *S. aureus*.

Anticoagulant Actions of Structure-Diverse Sulfated Phenols

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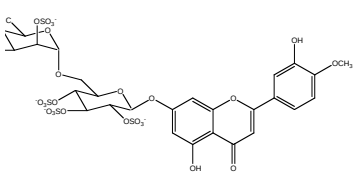
Anticoagulant and antithrombotic activities are among the most widely studied properties of sulfated macromolecules [1]. In light of recent anticoagulant results concerning two glycosylated flavonoids [2] sulfated diosmin (**1**) and sulfated hesperidin (**2**), the sulfation of other glycosylated phenolic compounds was accomplished and the sulfated derivatives **3-6** of rutin, trihydroxyethylrutin, esculin and chlorogenic acid, respectively, were obtained. Sulfation was carried out with triethylamine-sulphur trioxide adduct, in dimethylacetamide at 65ºC, and the purification step optimised by dialysis.

The sulfated derivatives **1-6** were investigated for their *in vitro* anticoagulant actions. Four different concentrations of each compound were assessed, showing at 1mM significant prolongation on the activated partial thromboplastin time (APTT), less on the prothrombin time (PT), and no effect on the thrombin time (TT).



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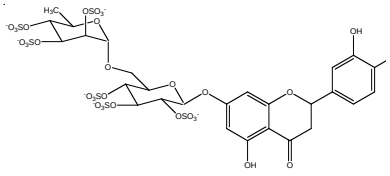

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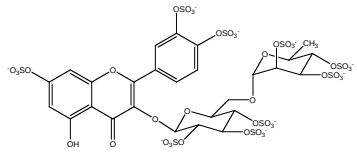

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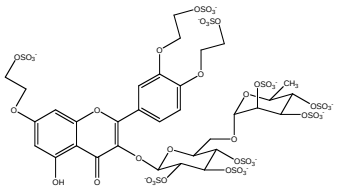

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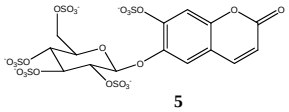

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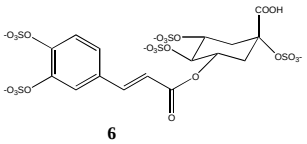

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Acknowledgments: FCT, I&D 226/2003; FEDER; POCI, FCT - SFRH/BD/22962/2005.

References:

1. Gunnarsson GT, Desai UR *Bioorg Med Chem Lett* 2003, 13, 679-683.

2. Sousa ME, Correia-da-Silva M, Pinto M *Drugs Fut* 2006, *31*, P119, 105.

Theoretical Analysis of Metabolic Pathways in Cancer Development - The Effect of Zn^{2+} Concentration Changes on Prostate Cell Metabolism

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Metabolic changes are increasingly seen as crucially important in cancer development and provide possible treatment and biomarker targets. Glycolysis-citrate-lipogenesis pathway is understood as a major source of synthetic and bioenergetic requirements that are essential for growth and proliferation of tumour cells. Prostate cells present a unique metabolic system with altered glycosilation and TCA cycle relative to other mammalian cells caused by an extremely high level of zinc. Furthermore, the malignant process in prostate cancer involves a required metabolic transformation following their lost ability to accumulate zinc. Better insight in this process is one of the crucial steps in our improved understanding of prostate cancer. The potential of mathematical models in describing biological systems is well understood and there are several software tools that allow system level modelling of networks. This poster will present detailed computer model of TCA and glycosylation under different conditions. The interactions of all metabolites in the model are described by kinetic equations. The components of the model are kinetic parameters and state variables, which indicate the state of a system in time. This theoretical model provides better understanding of the effects of changes in zinc concentration on prostate cells metabolic transformations during carcinogenesis.

Synthesis and Biological Evaluation of Novel Antifolates Towards Filariasis

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Brugia malayi causes filariasis in humans, which is one of the major neglected tropical infectious diseases. Treatment of filarial infections is difficult as the repertoire of effective drugs is very limited. Dihydrofolate reductase (DHFR) is well-known target enzyme for antibacterial and anticancer drug design due to its crucial role in DNA synthesis. Use of DHFR inhibitors has already been in practice against protozoan parasitic systems but is yet to be explored against metazoan species. Hence the present work was aimed at synthesis and evaluation of the efficacy of the DHFR inhibitors as potential anti-filarial agents.

Novel molecules with triazine or biguanide moieties, which are structural features present in known DHFR inhibitors, but also incorporating other novel potential pharmacophoric features were designed. These molecules were further studied computationally for ADMET profiles, and selected molecules were synthesized and characterized. These molecules were screened for pharmacological activity against *Brugia malayi* microfilariae *in vitro*, with some encouraging results. This provides possible new leads against human lymphatic filariasis, using the folate metabolic pathway.

Viability Modification of *Listeria monocytogenes* by the Effect of Phenolic Compounds Combination

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Despite the efforts to eradicate the contaminant organisms from foods, *Listeria monocytogenes* continues being a problem. It is a microorganism frequently detected in fresh and processed foods. It may be transferred to food producing negative effect on the human health.

Phenolic compounds have been proposed to have a variety of biological effects on human health, including anti-oxidant, anti-inflammatory, anti-allergic and antimicrobial activities. We investigate the effect of phenolic flavonoid and non-flavonoid compounds combination to test the synergistic effectiveness in the inhibition of the *Listeria monocytogenes* growth. Among the seven non-flavonoid compounds mixture tested gallic-protocatechuic and gallic-caffeic acids (100 and 200 mg/l) produce the most synergistic antimicrobial effect showing cellular death at the higher concentration. The combination of the flavonoids quercetin-rutin (from four tested mixtures) is the only that produce cellular death from 100 mg/l increasing this effect at higher concentration.

The synergistic antimicrobial effect of the cited phenolic compounds combination on *Listeria monocytogenes* compared with the results that we obtained on *Escherichia coli*, permit to conclude that these effects are independent of the Gram-positive or Gram-negative microorganism character and that could be an interesting alternative to be used as natural preservative against pathogenic microorganisms.

Palladium Catalyzed Multicomponent Reactions: A Facile One-Pot Coupling Approach to α-Amino Acid and Peptide Derivatives

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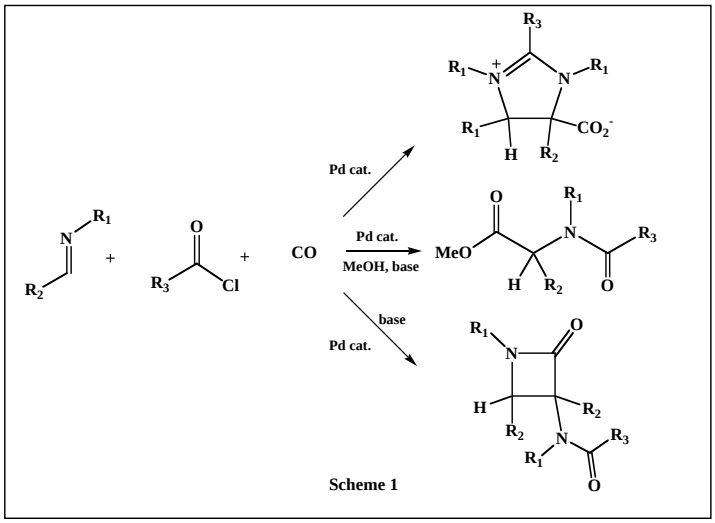
Peptides and amino acid based products are important components of a large variety of biologically and pharmaceutically relevant molecules. Herein, we describe the development of a novel one pot palladium catalyzed method to prepare Münchnones, and the extension of this method to generate a number of heterocycles and peptide based products as new core structures for drug discovery

The palladium catalyzed coupling of imines ($R^1N{=}C(H)R^2$), carbon monoxide (CO) and acid chlorides (R^3COCl) to generate 1,3-oxazolium-5-oxides (commonly referred to as Münchnones) is described. This catalytic method is further developed to generate new compounds with the basic skeletons of imidazoles, β-lactams, and α-amino acid derivatives (Scheme 1).

The described process provides an efficient alternative to the classic multi-step synthesis of these compounds, and allows the large scale access to these products from readily available and very flexible building blocks.



Scheme 1

Pd(0)-Mediated Rapid *C*-Methylations for the Synthesis of ^{11}C-Incorporated PET Tracers

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With the aim of creating novel methodologies to realize the incorporation of a short-lived radioactive nuclide into an organic carbon framework for the study of positron emission tomography (PET), we have developed the Stille type Pd(0)-mediated rapid cross-coupling using methyl iodide and aryltributyl-stannane (excess) in the presence of a tri-*o*-tolylphosphine-bound coordinatively unsaturated Pd(0) complex, a Cu(I) salt, and K_2CO_3 in DMF[1)]. Actually, rapid cross-coupling between sp^2_{aryl} and sp^3 carbon (sp^2_{aryl}-sp^3 type) has been applied to the synthesis of 15*R*-[^{11}C]TIC methyl ester to image a novel CNS-type prostacyclin receptor (IP_2) in human brain[1)].

This novel method of *C*-[^{11}C]methylation offers a number of benefits. For one thing, the *C*-$^{11}CH_3$ group is metabolically highly stable in comparison with *N*-$^{11}CH_3$, *O*-$^{11}CH_3$, and *S*-$^{11}CH_3$ ones thus the resulting image is very reliable. We here report the extension study on the Suzuki-Miyaura type rapid cross-coupling using organoborons by complementary use with organostannanes, which will be useful for the introduction of fluoromethyl group into carbon frameworks[2)].

References:

1. M. Suzuki, H. Doi, T. Hosoya, B. Långström, Y. Watanabe, *Trends Anal. Chem*., *23*, 595-607 (2004).

2. Patent application.

Peptide-Like and Lipid-Type Compounds on the Basis of Unsaturated Cyclic Amino Acids - Carbonyldihydropyridines

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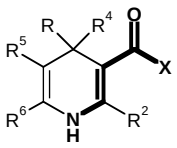
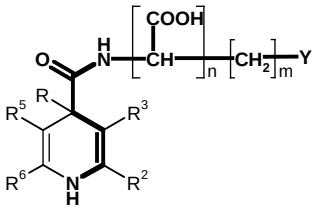
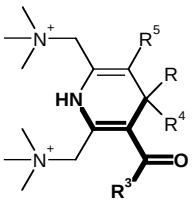
Synthesis and studies of novel unsaturated cyclic amino acids and their derivatives were performed in the field of 1,4-dihydropyridines **1**, 1,4-dihydroisonicotinic acid derivatives **2**, dihydropyridones. They demonstrated peptide-like modulatory and regulatory properties, such as anti-neurodeficiency, anti-apoptotic, anti-inflammatory, anti-diabetic, as well as immunoprotective and immunomodulatory actions, stimulation of hormone biosynthesis. These effects were long-lasting and manifested at low doses.



1, X = O^-, OH, NHR^3, OAlk
R^3 = Alk, Ar, $ArCH_2$



2, n = 0, m = 1,2,3; Y = COOH
n = 1, m = 1,2; Y = COOH
n = 0, m = 2; Y = SO_3H



3

Cyclic unsaturated amino acid derivatives **1**-**3** have also specific properties:

1. acidity due to >NH deprotonation;
2. dehydrogenation to heteroaromatic form, so antioxidants, antimutagens were obtained, compound *Diethone* was developed and used as radioprotector;
3. amphiphilic lipid-type compounds **3** possess gene transfection properties.

Low toxic anti-hypertensive and anti-anginal drug *Riodipine (Foridone)* has been developed and registered. Multi-component one-pot reactions and stereospecific chemoenzymatic transformations were performed.

Peptide-like properties open new vistas to obtain novel biologically active compounds and drugs.

Transdermal Drug Delivery: Current Status and Solutions to Problems

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Optimum therapeutic outcomes require not only proper drug development, but also effective drug delivery. The human skin is a readily accessible surface for drug delivery. Only a small number of drug products are currently available *via* transdermal delivery. In many cases, a drug's physical properties, including molecular size and polarity, have limited its capacity to be delivered transdermally. Similarly, the biological properties of drug molecules, including dermal irritation and insufficient bioavailability, have been problematic.

Transdermal drug delivery offers several important advantages over more traditional dosage forms. Some of the greatest disadvantages to transdermal drug delivery is the possibility that local irritation will develop at the site of application and the skin's low permeability limits the number of drugs that can be delivered in this manner.

Bearing in mind that the basic functions of the skin are protection and containment, it would seem exceptionally difficult to target the skin for drug delivery. Many of the recent developments in transdermal drug delivery target the more hydrophilic compounds that were previously undeliverable *via* this method. With a greater understanding of the structure and function of the skin, and how to alter these properties, more and more new drug products are being developed for transdermal delivery.

Modeling of Individual Water Molecules Preferentially Bound to Proteins

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Precise volume, surface and hydration properties of low-molecular compounds (e.g. inhibitors, drugs) and macromolecules (proteins, protein-ligand complexes) in aqueous solution are required for numerous purposes, including understanding behavior and interactions of hydrated proteins as crucial prerequisites for flexibility, dynamics and functionality of these molecules, and the construction of tailor-made proteins and complexes in context with drug-design and other pivotal projects.

Volume, surface and hydration parameters can be obtained by sophisticated calculative approaches considering manifold peculiar interactions with the solvent [1, 2]. Sequence and crystallographic data of proteins and ligands may be used as database, to predict molecular volume, exact surface topography ('molecular dot surface'), and the presumable position of individual water molecules on the protein surface (obtained by applying our hydration algorithms for atomic or amino acid coordinates) [3-5].

The present paper summarizes typical examples of calculations, in particular volume, surface and hydration properties of several proteins (e.g., proteases, receptors, virus capsids), highlights the visualization of hydrophilic and hydrophobic AA patches on the surface, docking maneuvers, localization of individual waters and water clusters, position of crevices, channels and contact areas, fine structure of active centers of enzymes and ligand binding sites, influence of rugosity effects, etc.

References:

1. H. Durchschlag and P. Zipper, in: Analytical Ultracentrifugation: Techniques and Methods (D.J. Scott *et al.*, eds.) RSC, Cambridge, 2005, pp. 389-431.
2. H. Durchschlag and P. Zipper, Bussei Kenkyu (Japan) **87** (2006) 68-69.
3. H. Durchschlag and P. Zipper, Biophys. Chem. **93** (2001) 141-157.
4. H. Durchschlag and P. Zipper, J. Phys.: Condens. Matter **14** (2002) 2439-2452.
5. H. Durchschlag and P. Zipper, Eur. Biophys. J. **32** (2003) 487-502.

Apoptotic Cell Death and Cell Cycle Events Mediate the Action of 6-Hydroxycoumarin-3-Carboxylatosilver in Human Malignant Hepatic Cells

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Interest in coumarins as potential anti-cancer agents arose from initial reports, where favorable responses were achieved in patients with advanced malignancies[1]. Our research group has shown that selected coumarins act by altering biochemical signals associated with cellular differentiation and death[2,3]. Furthermore, several reports have highlighted the use of transition metal complexes of coumarin as both anti-microbial and anti-cancer agents[4].

Previously, our research group has focused considerable attention on determining the anti-proliferative effects of novel metal-based coumarins. From these studies, 6-hydroxycoumarin-3-carboxylatosilver (6-OH-C-COO-Ag) has been shown to reduce the viability of human cancer-derived cells, while leaving cells derived from normal tissue, viable. Additionally, this agent displayed an IC_{50} value approximately three times lower than that observed for cisplatin, but unlike cisplatin, was non-genotoxic[5,6]. Given the apparent cyto-selective and potent anti-proliferative nature of 6-OH-C-COO-Ag, we proposed to extend these findings by probing the mechanism underlying the action of this drug. Here we investigated the effect on genomic DNA, and showed the induction of ladder pattern characteristic of apoptotic cell death. This was subsequently confirmed using morphological analysis, along with selected biochemical studies showing activation of caspase-3 & 9, a disruption of cell cycle events, and cleavage of poly(ADP-ribose)-polymerase protein (PARP). Additional studies are currently underway to elucidate the absorption of this novel anti-cancer chemotherapeutic through tight junctions of human colonic cells (Caco-2), using Trans Epithelial Electrical Resistance (TEER) [7]. In conclusion, this and related compounds would appear to offer significant potential as chemotherapeutic agents for the successful treatment and management of cancer in man.

References:

1. Egan, D. *et al.*, Drug Metab. Rev., 1990, 22, 503-529.
2. Finn, G. *et al.*, E. J. Pharm., 2003, 81, 159-167.
3. Finn, G. *et al.*, Cancer Lett., 2004, 205**,** 69-79.
4. Irena, K. *et al.*, E. J. Med. Chem. 2001, 18(2), 161-165.
5. Thati, B. *et al.*, Cancer Lett., 2007, 248, 321-331.
5. Thati, B. *et al.*, Cancer Letters, 2007, 250, 128-139.
6. Hubatsch, I. *et al.*, Nature Protocols, 2007, 2, 2111-2119.

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Molecular Docking and Analysis of Certain Potent Anti-angiogenic Vascular Endothelial Growth Factor Receptor-2 (VEGFR2) Inhibitors

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Induction of angiogenesis is essential for the continued growth of solid tumors. One critical component of tumor-induced angiogenesis involves the stimulation of VEGFR2 by VEGF. Hence VEGF and the tyrosine kinase domain of VEGFR2 are potential targets for the treatment of cancer.

The crystal structures of human tyrosine kinase domain of VEGFR2 in complex with different inhibitors are available in the PDB. We were interested in studying the pocket of these inhibitors as blocking this pocket prevent stimulation of VEGFR2 by VEGF. This study was achieved by docking different inhibitors from then two were approved by the FDA, Sunitinib and Sorafenib and two are under phase II clinical investigation, Pazopanib and Axitinib into the tyrosine kinase domain of the receptor.

This study provides a detailed information about the side-chain interaction in that pocket (it is the largest one of the tyrosine kinase domain) which assists in designing molecules of higher affinity to the receptor than the available inhibitors. It can also help in studying the resistance to tumor cells to some of the existing inhibitors that could result from mutation of any of the amino acids forming the pocket.

Melt Sonocrystalization of Flurbiprofen: Physicochemical and Biopharmaceutical Evaluation

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Aim: To investigate the suitability of melt sonocrystallization technique to modify the undesirable properties of non-steroidal anti-inflammatory drug, flurbiprofen (Fbp), such as poor flowability, solubility, and dissolution rate.

Methods: Flurbiprofen melt was poured in deionized water at 25℃ and sonicated for 4 min at amplitude of 60% and cycle of 40s on and 10s off. The product obtained was evaluated by SEM, image analysis, DSC, FT-IR, XRPD. Flow properties, intrinsic dissolution rate, solubility and bioavailability were also evaluated.

Results: The particle size of the treated Fbp is significantly reduced. Thermal behaviour and FT-IR spectra of untreated and treated Fbp are not significantly different. Low intensities peaks in the X-ray diffraction are noticed. On the other hand, there is significant enhancement in flow properties of treated Fbp as indicated by the value of angle of repose and the flow constants calculated from Kawakita equation. There is increase in the solubility of treated Fbp by about 35%. The intrinsic dissolution rate constant increased by 2-fold. The dissolution rate studies revealed that 90% drug is released within 20 min for treated Fbp compared with 60% release for untreated drug. Evaluation the bioavailability of the treated Fbp is currently investigated.

Conclusion: Melt sonocrystallization technique is a promising technique that may afford powder with improved flow and tablet functionality , dissolution and bioavailability.

Synthesis and Evaluation of Colon Delivery System of Flurbiprofen Prodrugs

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Aim: To investigate the potential of flurbiprofen-aspartic acid (Fbp-AS) and flurbiprofen-cyclodextrin (Fbp-CD) prodrugs as colon delivery system for treatment of inflammatory bowel diseases.

Methods: An anti-inflammatory drug flurbiprofen (Fbp) was conjugated onto the hydroxyl group of cyclodextrin (CD) or amino group of aspartic acid (AS). Prodrugs hydrolysis in buffer and in rat gastrointestinal tract homogenates was investigated. The effect of esterase and amidase enzymes on the *in vitro* hydrolysis of the proposed prodrugs was also examined. Additionally, the effect of oral administration of Fbp -CD prodrug on the experimentally induced colitis in rats was evaluated.

Results: No significant hydrolysis of Fbp-AS or Fbp-CD prodrugs in buffers having pH of 1.2, 4.6, 6.8 and 7.5 was observed. Fbp-AS prodrug showed no significant hydrolysis in gastrointestinal rat homogenates or in presence of amidase enzyme. On the other hand, Fbp-CD prodrug hydrolyzed in rat colon homogenate at pH 7.2.

Oral administration of Fbp-CD prodrug to rats after induction of colitis significantly attenuated the severity of the colonic injury and reduced the score of the macroscopic damage. Determination of level of reduced glutathione in colonic tissues is currently investigated.

Conclusion: The formulation of prodrug of flurbiprofen-cyclodextrin may provide a versatile mean for construction of colon targeted delivery.

New Asymmetric *meso*-Tetraphenylporphyrins Peripherally Diverse Substituted with Pyridyl or Dimethoxy Groups as Promising Photosensitizers in Photodynamic Therapy

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Because of the amazing photophysical properties of porphyrin derivatives they could represent interesting potential PDT photosensitizers.

The present report is concerning with innovative formulations for some new, so-called *second generation* photosensitizers, based on pyridyl and methoxy systems, which have been obtained and photophysically characterized. These pyridyl dimethoxyphenylporphyrins, were prepared through Adler-Longo method by a multicomponent reaction, in which 3,4-dimethoxybenzaldehyde and isonicotinaldehyde, in a molar ratio of 3:1, were condensed with pyrrole, in propionic acid medium. The mixture of products contained four new mixed asymmetrically porphyrins with up to three 3,4-dimethoxyphenyl groups at the *meso*-positions, the remaining *meso*-positions being occupied by pyridyl rings. The porphyrins were isolated, purified and characterized by HPLC, TLC, UV-vis, fluorescence, MS, ^{1}H-NMR and ^{13}C-NMR analysis.

The new obtained porphyrins have the advantages of strong absorption around 651-652 nm, and fluorescence quantum yields (Φ_f) values of up to 0.3.

Knowing that the medium in cancerous tissues is often more acidic than in normal tissues, the capacity of these porphyrins to exist simultaneously in aggregated and protonated forms was investigated by UV-vis spectroscopy in acidic media. The type of generated porphyrin aggregations were investigated by atomic force microscopy (AFM).

Retinoids Induce apoE Gene Upregulation in Macrophages

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Aim: Retinoids are key regulators of inflammation by mechanisms that are not completely understood. We postulated that retinoids influence apoE expression in macrophages and searched for the molecular mechanism involved**.**

Methods: Modulation of apoE expression in RAW 264.7 murine macrophages treated 24h with 1μM 9-*cis* retinoic acid (RA) and in peritoneal macrophages (MPM) of C57BL/6 mice treated with vitamin A was tested by quantitative PCR. To identify the region involved in apoE gene regulation, transient transfection experiments were performed using plasmids encoding the proximal apoE promoter in the presence or absence of multienhancer 2 (ME2) or its deletion mutants, fused to the luciferase gene.

Results: RA increased endogenous apoE gene expression in RAW 264.7 cells and vitamin A increased apoE expression in MPM. Transient transfections showed that apoE promoter activity was not affected by RA, consistent with the lack of binding sites for RXR in this region. In the presence of ME2, the promoter activity was significantly increased by RA. Analysis of a series of ME2 deletion mutants indicated that a RXR binding site is between nucleotides 321-407.

Conclusion: Retinoids upregulate apoE expression in macrophages, suggesting their potential therapeutic use for the regulation of lipid metabolism and prevention of atherosclerosis.

Computational Mapping of Druggable Sites in Proteins

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Protein association regulates a vast number of biological phenomena, and a very significant fraction of drugs in use nowadays have as their target a protein:protein interface. Bioavailable drugs are usually small molecules, which are only able to bind a small region of the usually large protein interface. To efficiently disrupt protein associations the drugs must bind and occlude the interface hot-spots. These regions are constituted by a small number of amino-acids, but contribute with the majority of the total binding free energy.

In this work we have developed an improved Molecular Mechanics Poisson-Boltzmann Surface Area (MM-PBSA) approach[1, 2] to study a set of several protein:protein complexes (see examples in 3-6), and to map the potential drugabble sites in their interfaces. A critical evaluation of the accuracy and limitations of the computational method to map druggable sites has been made. Validation against experimental data highlights the usefulness and reliability of the methodology.

References:

1. Kolmann P. A. *et al., Acc. Chem. Res.* 2000, 33, 889.
2. Moreira I. S., Fernandes P. A., Ramos M. J., *J. Comput. Chem*, 2007, 28, 644.
3. Moreira I. S., Fernandes P. A., Ramos M. J., *J. Phys. Chem.B.* 2006, 110, 10962.
4. Moreira I. S., Fernandes P. A., Ramos M. J., *Proteins*, 2006, 63, 811.
5. Moreira I. S., Fernandes P. A., Ramos M. J., *J. Phys. Chem. B* 2007, 111, 2697.
6. Moreira I. S., Fernandes P. A., Ramos M. J., *J. Chem. Theory Comput.* 2007, 3, 885.

Preparation and Characterization of Radioactive Dirhenium Decacarbonyl-Loaded PLLA Nanoparticles for Radionuclide Intra-Tumoral Therapy

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We describe the elaboration of biocompatible radioactive rhenium loaded nanoparticles for radionuclide anti-cancer therapy. Dirhenium decacarbonyl [$Re_2(CO)_{10}$] has been encapsulated in poly(L-lactide) (PLLA) based nanoparticles by an oil-in-water emulsion-solvent evaporation method. The nanoparticles size ranged between 330 nm and 1500 nm and the maximum rhenium loading was 24% by nanoparticles weight as determined by neutron activation analysis. The rhenium distribution within nanoparticles has shown to be homogenous as confirmed by energy dispersive X-ray spectrometry.

To render them radioactive, these nanoparticles were bombarded with a neutron flux of $1.45X10^{13}$ $n/cm^2/s$ during 1 hour. After neutron bombardment, the nanoparticles remained spherical and separated but slightly misshaped. These applied neutron activation conditions yielded a specific activity of about 32.5 GBq per gram of nanoparticles. DSC results showed a decrease in the PLLA melting point and melting enthalpy in loaded nanoparticles indicating a decrease in polymer crystallinity. In addition, the polymer molecular weights (Mn, Mw) decreased after irradiation indicating an irradiation-induced PLLA chain scission in a random way. FTIR spectra showed that irradiated nanoparticles retained the chemical identity of the used $Re_2(CO)_{10}$ and PLLA although the reduction in polymer crystallinity and molecular weight. These nanoparticles represent a novel interesting candidate for local intra-tumoral radiotherapy.

Discovery of BNC105P, A Clinical Candidate for the Treatment of Cancer: A Case Study in the Application of the Diversity-Oriented Synthesis Technology, MultiCore®

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Drug-discovery efforts invariably involve the identification of a start-point molecule (library screening hit, *in silico* screening hit or literature compound) that exhibits some affinity for a target or some relevant pharmacology, but which must undergo substantial optimisation in order represent a viable therapeutic. Poor control over the properties of the start-point molecule and/or the lack of a tractable synthesis process, are often key limitations to such programs. MultiCore® is a diversity-oriented synthesis tool that allows fluid movement, synthetically, across a range of both similar and dissimilar scaffolds that exhibit a drug-like bias. This tool provides for novel strategies for optimisation, where-by the pharmacophoric features of a compromised start-point molecule can be "transplanted" onto a range of different, drug-like scaffolds and more effectively optimised.

Our discovery of BNC105P, a clinical candidate for the treatment of cancer, serves as a case study for the practical application of MultiCore®. Using the natural product, combrestatin A4 as a start-point compound, we have applied MultiCore® to the identification of new and improved agents on patently distinct scaffolds. In particular, BNC105P, exhibits 20 fold greater potency, 100 fold greater selectivity and a 20 fold greater therapeutic index compared to CA4.

Allosteric Inhibitors of Myosin Function

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Myosins participate in a wide range of motile processes. Small chemical compounds that selectively inhibit individual myosin isoforms, possess a considerably strong therapeutic potential, since aberrant motile activities are in many respects causative for the emergence of particular diseases. Essential requirements for an active agent are beside its high specificity for a particular type of myosin, which is directly involved in pathogenic processes, its ability to transform the myosin into a state of weak actin interaction.

Small chemical compounds that inhibit myosin function due to an increased accumulation of the strong actin binding states are inappropriate drugs because they lead to strong side/adverse reactions. We performed structural analysis of a novel allosteric binding site around the strut-regions that shows larger myosin isoform-specific differences and thus a great potential for the development of highly specific agents for the therapeutic treatment of a wide range of diseases including cancer, cardiovascular diseases, neurodegenerative diseases, and Apicomplexa mediated infections. This work has led to the identification of promising lead compounds that keep myosin in a state of weak actin interaction; amongst them flavinoids and halogenated alkaloids.

In silico Study of the Protein Kinase Inhibitory Profiling and Antihyperlipidemic and Antitumor Activity of Various Saffron Extracts and Flavonoids Using Molecular Docking Techniques

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Saffron extracts demonstrated to have antitumor, antioxidant and hypolipaemic effects, due to its secondary metabolites and their derivatives. However, there has been no information on the inhibitory action of these metabolites on various protein kinases and other protein-targets, playing significant role in the biosynthesis of cholesterol. In this *in silico* study we explored the ability of saffron extracts: crocin, crocetin, dimethylcrocetin, picrocrocin, safranal, and the flavonoid compounds quercetin and flavone, to act as potent inhibitors of protein kinases and other target-proteins involved in the signal transduction pathway. These natural products have been screened virtually against a large Protein Drug Target Database, comprising almost 1,000 target-proteins. For each compound, at least 20 target-proteins were found to be inhibited in a specific order of binding capacity. Molecular docking studies revealed that most of these compounds inhibit the cancer-related target-proteins: bcr-abl, c-kit, ret-protein tyrosine kinase, Akt2/PKBβ, PKCθ and HMG-CoA-R (target for statin drugs). Other targets found were: thymidine kinase, casein kinase-II, protein kinase-ck2, dihydroorotate dehydrogenase, cAMP-dependent protein kinase, dihydrofolate reductase, calmodulin, elongation factor-Tu, thymidylate synthetase, RNA triphosphatase and tubulin. These docking studies indicated that tested compounds may play a role in the blockade of proteins being able to counteract cancer or hyperlipidemia.

Molecular Docking Studies Reveal the Inhibition Mechanism of D-Homo-Aza-Steroids on PKC and PI3K

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Protein Kinase C (PKC) and phosphoinositide 3-kinase (PI3-K) enzymes, involved with cell signaling mechanism of various cancers, are important target proteins for drug-design. The aim of this study was to generate small molecule inhibitors, being able to specifically target these proteins. To this end we have synthesized two *D*-homo-aza-modified steroidal lactamic derivatives of androsterone and estrone, proved to exert variform activity against human cancer and normal cells. In an attempt to elucidate the underlying mechanism of action of these steroidal compounds, we carried out molecular docking calculations on PI3-K p110-gamma isoform and five isozymes of PKC, to investigate their effects on these target proteins, exploring binding modes and chemical interactions and to finally see if they might make potent inhibitors. Docking studies revealed that all compounds found to be positioned inside the binding cavity of the target proteins, in the same position occupied by their co-crystallized bound inhibitors, exhibiting significant influence on the catalytic domain of the proteins, which inhibit with different binding affinities according to isozyme they are bound to. This *in silico* approach is informative in pointing out the mechanism of the interaction in a molecular level *via* interference with PKC and PI3-K signal transduction pathways.

New Inhibitors of D-Alanine:D-Alanine Ligase (DdlB) Discovered by Structure-Based Virtual Screening of the NCI Diversity Set

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The emergence of bacterial resistance to antibiotic therapy has become a global health threat. To overcome this problem, new antibacterial agents directed toward novel targets have to be developed. The best known and most validated target for antibacterial therapy is the system of enzymes responsible for the construction of peptidoglycan. D-Alanine-D-alanine ligase (Ddl) catalyzes the biosynthesis of an essential bacterial peptidoglycan precursor D-alanyl-D-alanine and it represents an important target for development of new antibacterial drugs.

Structure-based virtual screening dedicated to discovery of new enzyme inhibitors involves computational fitting the structures of compounds to the active site of an enzyme (docking) and scoring and ranking each compound. Only the highest ranked compounds are then tested for activity in a biological or biochemical assay. In this study, the virtual screening of the National Cancer Institute "Diversity Set" against the DdlB was performed with the AutoDock 4.0 program. Several new and structurally diverse hits were discovered that have IC_{50} values in low micromolar range and also have *in vitro* antibacterial activities against both Gram-positive and Gram-negative bacteria. These inhibitors are structurally distinct from both ADP and D-alanine, so we expect that they inhibit Ddl by a novel binding mode.

Efficacy of Microparticulated BDS Dosage Forms in the Treatment of Experimentally Induced Colitis in Rats

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The aim of this work was to determine the efficacy of the microparticulate systems (MP`s) containing budesonide (BDS) in the treatment of experimentally induced colitis in rats. Eudragit coated BDS miroparticles (E-MPB) were compared with uncoated chitosan-Ca-alginate BDS MP`s (MPB) and BDS suspension, respectively. MP`s were prepared using one step spray-drying process[1]. To induce the model of chronic inflammation in the rat colon, we followed the method described by Morris *et al.* 2]. Male Wistar rats (average weight 230ˆ250 g; 12ˆ15 weeks; *n*= 5/group) were used. The effect of administered samples was evaluated by determination of colon/body weight ratio [3], assessment of macroscopic ulceration and histological evaluation[4] and clinical activity score system[5].

Macroscopic ulceration and histological evaluation and clinical activity score points were 6±0 and 2.2±0.12 for control (non treated) group respectively, 5±0.55 and 2.2±0.23 for BDS suspension, 4.33±0.41 and 2.07±0.26 for MPB and 3.67±0.52 and 1.63±0.49 for EMPB, respectively. All results indicate increased efficacy of coated BDS loaded MP's in the treatment of induced colitis in rats. The increased efficacy of these drug delivery systems is probably due to the fact that the coating has successfully sustained release of BDS in buffers at pH 2.0 and 6.8, while providing improved dissolution of BDS at the site of action and prolonged residence time because of the drug delivery system mucoadhesivity.

Acknowledgments:

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References:

1. K. Goracinova, Formulation and preparation of spray-dried alginate-Ca microparticles Patent No.: MP/MK/05/01/FF/BE/01/IP (2005).

2. Morris G.P., Beck P.L., Herridge M.S., Depew W.T., Szewczuk M.Y., Wallace J.L., Hapten-induced model of chronic inflammation and ulceration in the rat colon. *Gastroenterol* 1989; 96, 795-803.

3. Yue G., Sun F.F., Dunn C., Yin K., Wong P.Y.-K., The 21-aminosteroid tirilazad mesylate can ameliorate inflammatory bowel disease in rats. *J Pharmacol Exp Ther* 1996; 276, 265-270.

4. Rodriguez M., Antonez J.A., Taboada C., Seijo B., Torres D., Colon-specific delivery of budesonide from microencapsulated cellulosic cores: evaluation of the efficacy against colonic inflammation in rats, *J Pharm Pharmaco*l 2001; 53 (9), 1207-1215.

5. Lamprecht A., Ubrich N., Yamamoto H., Schäfer U., Takeuchi H., Maincent P., Kawashima Y., Lehr C.M., Biodegradable nanoparticles for targeted drug delivery in treatment of inflammatory bowel disease, *J Pharmacol Exp Therap* 2001; 299 (2), 775-781.

Chemical Structure-Aqueous Solubility Relationship of Amiodarone

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Amiodarone (AMI) is a poorly water soluble antiarrhythmic agent. The aqueous solubility of AMI (S_{int}) is difficult to be measured, because in water, AMI forms transparent aggregates. In this study, S_{int} of ^{125}I-AMI and those of its derivatives was determined by cosolvency. The results are useful for improving AMI pharmacokinetics by using more water soluble derivative.

Mixtures of phosphate buffer pH 7.4 and methanol were added to previously drugs-coated vials. The reaction was allowed to reach the equilibrium. The aggregates were separated by centrifugation at 25°C, and drugs concentrations in supernatant were measured.

In the range of 10-90 nM, the relationship between the apparent solubility of AMI ($S_{w.app}$) and S_w followed a log-linear model $\log S_{w.app} = S_w + \sigma * f_c$. The formation constant (k) of AMI aggregates was estimated by self-association kinetics. The mean value (n=7) at 25°C for AMI were: S_{int}= 1.86±0.44 nM; $k=(0.14\pm0.09).10^9$ l/mol. Aqueous solubility of dealkylated AMI analogues was 50-fold higher than that of AMI. However, this parameter increases 4000-time, when n-butyl side chain of AMI is hydroxylated.

AMI exhibits very high self-association ability, and its aqueous solubility is in the range of nM. In order to increase the aqueous solubility of AMI, polar group should be introduced into its benzofuran-diiodophenyl moiety.

A Novel Human-cell Based Pyrogen Test

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All products intended for injection need to be tested for pyrogenic contaminants. There are two pharmacopoeia-approved methods to do this: the LAL test and the Rabbit Pyrogen Test (RPT). Both currently approved tests have limitations. There are therefore global interests in finding a suitable cell based pyrogen test to replace the RPT. Our aim is to develop such a method. Our test is based on the human leukaemic cell line HL-60. The cells must be differentiated before use. Differentiated HL-60 cells react very quickly when exposed to pyrogens by producing reactive oxygen species (ROS). The time profile of ROS production is measured by chemiluminescence.

The differentiated HL-60 cells were exposed to endotoxin, lipoteichoic acid (LTA), peptidoglucan (PGN), Gram positive bacteria, fungi and yeasts. The test showed excellent sensitivity towards both gram-positive (LTA 25 ng/mL), PGN (500ng/mL) and gram-negative pyrogens (LPS 10 pg/mL). Furthermore it detects small pyrogenic concentrations of yeasts and other fungal contaminants.

We have developed a new pyrogen assay based on the HL-60 cell line. The assay provides a better sensitivity towards all tested substances in respect to the RPT. The assay is fast and cost efficient, and therefore provides an alternative to the much disputed use of laboratory animals in routine drug testing.

A New Era of Medicine “Genomic Medicine”: Connecting Genes, Drugs and Diseases

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When the Human Genome Project completed the first draft of all human genes, the stage was set for great advances in medicine and biotechnology applications. First, scientists would more quickly identify the genes that foster common diseases and people would start having their genomes analyzed early in life to reveal their risks. Then, armed with that information, we would all adopt lifestyles-maybe even take medicines-tailored to our own needs "personalized genomics or medicine". This enthusiasm was even enhanced by the promises that new combinations of genomic, proteomic and bioinformatics research will provide deeper insights into disease mechanisms, novel markers for diagnostics, new molecular targets for therapeutic intervention and for new drug discovery. The field is being driven forward both by innovative biotechnology companies and by academicians who are introducing the technology required for the parallel identification of individual proteins. This presentation will shed some light on current innovative approaches in functional genomics and proteomics, their biotechnology applications and prospects for developing countries.

Antineoplastic Effects of α-Terpineol, Linalyl Acetate and dl-Camphor, Components of Lebanese Sage Essential Oil

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Salvia is one of the most widely used plants in traditional medicine. The essential oil of Lebanese sage is known to have antitumor activity. Our objective was to characterize the antitumor activity of three components of the sage essential oil, α-terpineol, linalyl acetate and camphor. Cytotoxicity was measured using a well-characterized ten human tumour cell line panel, chronic lymphocytic leukaemia (CLL) and normal mononuclear cells (PBMCs) using the Fluorometric Microculture Cytotoxicity Assay. An integrated drug and gene expression database was used in data analysis. α-terpineol and linalyl acetate showed similar activity patterns in the cell line panel, suggesting similar mechanisms of action. Camphor demonstrated low activity in all cell types tested. α-terpineol and linalyl acetate showed highest activity in the small cell lung carcinoma and myeloma cell lines. They were more active against CLL cells compared to PBMCs which may indicate some tumor selectivity. Only limited influence on efficacy was observed of some classical mechanisms of multidrug-resistance. High correlations between the activity patterns of α-terpineol/linalyl acetate and the tyrosine kinase inhibitor PKC412 and the antialcoholic disulfiram in the cell line panel, suggests a potential similarity in mechanistic pathways, which is also supported by the gene correlation analysis.

A Method for Automated Molecular Optimization by Inverse QSAR Applied to Ames Mutagenicity Data

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A method for automated molecular optimization by Inverse QSAR is presented and applied to mutagenicity data. In the present work a new method that utilizes a local optimization, of a compound or drug-scaffold, where all possible solutions in a defined chemical subspace are generated. The concept of redesigning molecules by using inferences from QSAR models is not new, but the complexity of many problems adressed by QSAR models often renders highly complex models where a simple interpretation is very rare. The approaches used leaves a tough task to the chemists in finding new substituents or compounds that will meet the refined requirements from the QSAR models. The procedure of finding new substituents to drug-scaffolds is therefore highly dependent on the skill and expertise of the chemists and is also very time consuming. This method automates this process by relating the local importance of the molecular fragments from the QSAR model. After that mapping the substructure with the highest importance is replaced. For the generated core structures all permutations of the core and the original endgroups are computed. These new compounds are then predicted using the model to identify the new molecules that match the requirements. This result can then presented to the chemist for further analysis and synthesis.

Blazein (A New Steroid) Isolated from Mushroom (*Agaricus blazei Murill)* Induce Apoptosis in Human Lung Cancer LU99 and Stomach Cancer KATO III Cells

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Blazein was isolated from mushroom (*Agaricus blazei Murill*) and identified by Mass, and ^{1}H-NMR to be blazein. Effect of blazein on DNA of human various cancer cells have been investigated. The fragmentations of DNA by blazein to oligonucreosomal-sized fragments, a characteristic of apoptosis, were observed in the human lung LU 99 and stomach KATO III cancer cells. The DNA fragmentations by blazein were observed from 2 days (KATO III cells) or 3 days (LU 99 cells) after addition of blazein to the culture cells. These findings suggest that growth inhibition by blaszein results from the induction of apoptosis by the compound.

A Microcell Hybrid Based "Elimination Test" in Anticancer Drug Design

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The biological complexity of neoplasms requires multiple targets in antitumor drug design. One of the complexity sources are the deletions that provide selective growth advantage and are found in virtually all carcinomas. We have developed a functional model system called "elimination test (Et) based on the transfer of single human chromosomes into tumor cells of human or murine origin, *via* microcell fusion. (see Kost-Alimova M & Imreh S, Modeling non-random deletions in cancer, Seminars in Cancer Biology, 17, 19-31, 2007, for a rev). Microcell hybrid derived SCID mouse tumor panels were analysed for the elimination of specific genomic segments after 1-4 passages. On human chromosome 3, we gradually narrowed down a common eliminated region on 3p21.3 (CER1) that contains 33 genes, six of them discovered and cloned by us. Seven may be considered as tumor inhibitory gene candidates and we have published results that confirm the suppressor function for 3 of them: LF, LIMD1 and TMEM7. Evolutionarily sorted in close proximity, they interefere with E2F meditated RB (LIMD1) and NFkB mediated p53 (LF, TMEM7) senescence pathways. Analysis of eliminated regions on human chr 1, 3, 8, 13 and 17 may provide further suppressor segments with multiple anticancer candidates.

Protein-Protein Binding Sites Prediction

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Protein-protein binding sites are functionally important regions of proteins which can be detected by searching for similar shapes and properties in related protein structures. An algorithm is described which uses conservation of function and 3D structure in protein surfaces, as opposed to sequence, to detect protein-protein binding sites. This algorithm explores this approach using structurally related proteins to find conserved regions on protein surfaces.

Proteins in which protein-protein binding sites are sought were compared with systematically analyzed related proteins and the predicted binding sites were found to correspond well with the known binding sites. Comparison of this method with an algorithm using a support vector machine approach for predicting protein-protein binding sites shows topological conservation to be an important characteristic that distinguishes binding sites from the remainder of protein surfaces. It was found that patches that are conserved at least once are likely to be involved in protein-protein binding sites.

Our algorithm differs from others in that only the structure of a protein and a couple of its structural neighbors is needed. Our algorithm can also be used to reduce the search space of docking algorithms and can provide new targets for potential inhibitors of protein-protein interactions.

Polymeric Matrix System for Transdermal Delivery of Tramadol Hydrochloride

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Tramadol, a centrally-acting potent analgesic with weak opioid agonist activity (mainly M1 metabolite) and less side effects than other analgesics, has a half-life of ~6hours, therefore, requires frequent dosing. It is freely soluble in water; hence, judicious selection of retarding formulations is necessary. Transdermal tramadol delivery reduces dose and frequency of administration, side effects; by-passes first pass effect, therefore, decreases abuse liability; and hence, improve patient compliance.

Polymeric films were prepared using single or binary blends of polymers aiming to optimize physico-chemical and biological properties. Physical evaluation comprised: appearance; water-uptake; mechanical properties and bioadhesion using Chatillon® apparatus; pH; rheology of film-forming solution using Brookfield viscometer. *In vitro* release was evaluated using USP dissolution tester. *Ex-vivo* permeation through rat skin was performed using Franz cell. Chitosan film showed excellent appearance, suitable strength, best flexibility and bioadhesion, fast release and permeation. Binary blends (1:1) of chitosan with other polymers improved their bioadhesion and flexibility and, modified other properties. Chitosan-Eudragit®NE30D film showed relatively rapid yet extended release and permeation and, seems to attain our targeted sustained release formula achieving rapid but long-lasting analgesia using the cost-effective, easily-available, non-toxic polymers. This ameliorates patient's quality of life by a non-invasive, easy-to-use and cheap drug delivery system.

Detection and Identification of Banned Chemicals in Dietary Food Supplements

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A set of herbal dietary supplements, marketed as natural products for the enhancement of sexual function, was purchased from a supermarket and analyzed by LC-MS and High Pressure Liquid Chromatography Photodiode Array (HPLC-PDA). Three samples were found to contain compounds related to synthetic phosphodiesterase-5 (PDE-5) inhibitors. Two of the analyzed samples (**C5** and **C6**) were found to contain high concentrations of seldinafel 9.0 % (mass %) and 5.7 %, respectively. In addition, sample **C8** was found to contain 0.15% of tadalafil. Based on the results shown in this study, samples **C5**, **C6**, and **C8** can cause a sudden increase in blood pressure due to the high concentration levels of PDE-5 inhibitors. The pure active ingredients can be extracted from the analyzed herbal samples at the optimal conditions shown in this study.

Hydrogen Bonding Interactions as a New Sustain Release Mechanism

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The commonly used devices for sustain release formulations are based on matrix systems or diffusion controlled membranes, while the release mechanism corresponds to erosion, relaxation and/or diffusion phenomena. The optimal release kinetic is the zero order. However, usually this cannot be achieved for soluble drug substances due to the "burst effect" phenomena which lead to deviations from the optimal release (first order kinetics).

In the present study a new sustain release mechanism is investigated for the drug substance Fluvastatin. This mechanism is based on the formation of intermolecular complexes between the drug substance and the carrier, and gradual dissociation of these complexes which results to the controlled release of the drug substance.

The characterization of the complexes showed that they are based on the formation of a hydrogen bond which was identified by FT-IR, DSC, UV-vis and viscometric techniques.

The release mechanism was investigated *in vitro* while a high correlation with the hydrogen bond was observed.

Finally, the *in vivo* behavior of the above system was investigated by bioavailability studies under fasting and fed conditions, covering the variable conditions of the Gastrointestinal track, which verified the achievement of a controlled drug release during administration to healthy volunteers.

Unique Properties of DNA Interstrand Cross-Links of Antitumor Oxaliplatin

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The different antitumor and other biological effects of the third generation antitumor platinum drug oxaliplatin [(1*R*,2*R*-diamminocyclohexane) oxalatoplatinum(II)] in comparison with those of conventional cisplatin [*cis*-diamminedichloridoplatinum(II)] are often explained by the ability of oxaliplatin to form DNA adducts of different conformation. This work describes the structural and biochemical characteristics of the interstrand cross-links of oxaliplatin.[1] We find that: 1) DNA bending, unwinding, and delocalization of the conformational alteration induced by the cross-link of oxaliplatin are greater than those observed with the cross-link of cisplatin; 2) the affinity of high-mobility-group proteins (which are known to mediate the antitumor activity of platinum complexes) for the interstrand cross-links of oxaliplatin is markedly lower than for those of cisplatin; and 3) the chirality at the carrier 1,2-diaminocyclohexane ligand can affect structural properties of the interstrand cross-links. The significance of this study is also reinforced by the fact that, in general, interstrand cross-links formed by various compounds of biological significance result in greater cytotoxicity than is expected for intrastrand DNA lesions. Therefore, we suggest that the unique properties of the interstrand cross-links of oxaliplatin are at least partly responsible for this drug‘s unique antitumor effects.

Reference:

1. J. Kasparkova, M. Vojtiskova, G. Natile, V. Brabec, Chem. Eur. J., 2007, in press (doi: 10.1002/chem.200701352).

Searching for New Cholinesterases Inhibiting Drug Candidates from Natural Sources

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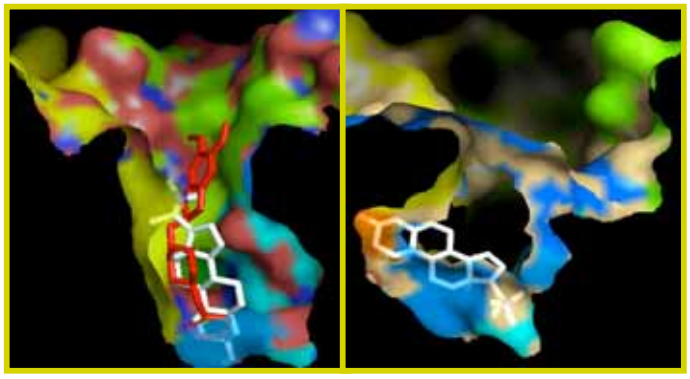
The growing interest in plant-based drugs is demonstrated by the increased consumption of medicinal and botanical plants, which has doubled in the last 10 years. This is mainly due to the unorthodox and often unanticipated chemical structures of natural products that offer novel leads for clinically useful drugs.



During the last decade, our research group has been focusing on identification of natural compounds that can inhibit various clinically important enzymes, including cholinesterases. Cholinesterase family of enzymes consists of acetylcholinesterase (AChE) and butyrylcholinesterase (BChE). Inhibition of AChE and BChE is an attractive target for rational drug discovery for the treatment of neurodegenerative disorders including Alzheimer's, Parkinson's and myasthenia gravis diseases.

This poster will discuss some of our recent discoveries in the field of cholinesterases inhibition. Molecular mechanisms of action some selective examples of the new natural drug candidates will also be highlighted.

Peptide Regulation of the Functional Activity of the Retina

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Tissue specific peptides are the most interesting physiologically active substances. Retinal peptide, obtained on the basis of targeted chemical synthesis, is one of such substances. This peptide was constructed basing on the data from the analysis of the complex medication, which was obtained from animal retina (Retinalamin), this medication being included in the State Pharmacopoeia of the Russian Federation. The new peptide was synthesized from amino acids, which are present in Retinalamin in the highest concentrations.

Purpose: to evaluate the retinoprotective effect of synthesized retinal peptide.

Methods: Campbell rats with genetically determined retinal degeneration, obtained from the laboratory of Surgery Department of University College London, were used as an experimental model. Electrophysiological and histological studies were performed to evaluate the effect of the peptide on the functional activity and morphological structure of the retina. 120 animals born from 42 female rats were evaluated. The experiment consisted of two stages. During the first stage female rats from the experimental group (n=25) received the synthesized retinal peptide intramuscularly in the dose of 1.0 μg per animal during 3 weeks before mating and during the whole period of pregnancy. Control female rats (n=17) received sterile 0.9% NaCl solution by the same schedule. The second stage of the experiment was performed using the offspring of these female rats. Rat pups of the experimental group (n=70) received the peptide intramuscularly beginning from the 5th day of their postnatal life in the dose of 0.5 μg up to the 35th day, and 1.0 μg up to the 81st day, in 0.2 ml per animal. The injections were performed 5 days a week, with a week interval. Control rat pups (n=50) received 0.2 ml NaCl solution by the same schedule. Retinoprotective effect of the peptide was evaluated according to the results of electrophysiological and morphological studies of the offspring.

Results: By the 23rd day the difference in total bioelectric activity in both groups was not statistically distinguishable. However, beginning from the 35th day of control rats' life a sharp decrease in the retinal bioelectric activity was observed, and by the 53rd day none of the control animals showed any ERG. Experimental rats showed high level of ERG amplitude after the 41st day of their postnatal life, this amplitude beginning to decrease only on the 70th day of life. The results of electrophysiological study were confirmed by morphologic data. That is, the control group showed utter destruction of all retinal layers by the 41st day of animals' life, while experimental animals had all their retinal layers preserved. Thus, the peptide has prolonged the period of time, during which the morphological structure of the retina stayed intact.

Conclusion: It was found, that this method of administration of the peptide enables the two-fold prolongation of the period, during which the retina is capable of functional activity, as compared to the control. Thus, the synthesized retinal peptide reveals a protective effect on eye retina of Campbell rats with genetically predetermined retinal degeneration. Therefore, the pronounced retinoprotective and antioxidant effect of the retinal peptide, as well as the absence of any common toxicity of the substance (by the results of previous studies - Ocular Pharmacology and Therapeutics, 2004) point out the good prospects of the clinical application of the peptide in the treatment of retinitis pigmentosa.

Rational for Focused Diversity™ Approach to Discovery of Signaling and Developmental Pathway Inhibitors. A Case Study for PI3K and Hh Modulators

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Hedgehog (Hh), PI3K/Akt, BMP/TGFb, FGF, WNT, Notch and some other signaling pathways constitute the stem cell signaling network, playing a key role in a variety of processes, such as embryogenesis, maintenance of adult tissue homeostasis, tissue repair during chronic persistent inflammation, and carcinogenesis. The alterations of these pathways have been proven in numerous human malignancies. The various members of these pathways define stem cell fate and offer new potential targets for therapeutic intervention.

ChemDiv has collaborated with laboratories of Prof. Kinzler and Prof. Beachy at Johns Hopkins University and Johns Hopkins School of Medicine, Prof. Chen at Stanford University to hatch and iteratively improve the rational for ChemDiv's Focused Diversity™ strategy with array of chemical library tools in this area to discover novel orally available leads.

The strategy is aiming at specific elements of primary discovery chemical libraries to identified targets along the pathway modulating, inhibiting, or attenuating 7TM, kinase and protein-protein interaction with orthogonally selected SAR clusters of small molecules. Each cluster contains where possible ligands known from the literature or proprietary data, their analogs and products of bioisosteric morphing, pharmacophore, hopping, docking and arsenal of other applicable methods of ligand and target structure-based design. This design allows for simultaneous hit finding and provides 1st pass to target-function assessment early in the discovery process.

The pathway driven approach to selecting maximally informative functional cell -based assays for hit identification dictates the efficient size of ca. 5-10K compound space for iterative screening. Exhaustive hit confirmation analysis including selectivity with additional biochemical and functional assays, elements of *in vitro* ADME/Tox , ranking of chemistry series based on their med chem tractability, elements of SAR and IP potential determines synthetic approach with secondary discovery libraries.

Hit to lead process is streamlined towards confirmation of concept of the mechanism, as well as improving lead series profile for functional animal model testing.

Selection of an appropriate animal model is done to enable a fast go /no-go decision for a POC lead program.

The efficiency and success of Focused Diversity™ strategy and pathway driven discovery process will be illustrated by ChemDiv's novel orally bioavailable antagonists of Hh/Smo and other Hh pathway modulators; selective orally bioavailable inhibitors of PI3K/Akt pathway.

Aspirin: The Anti-Inflammatory Antifungal

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Oxylipin research in fungi exposed new targets for antifungal action. The presence of aspirin-sensitive 3-hydroxy oxylipins in yeasts was first reported in the early 1990's. Since then these oxidized fatty acids were found to be widely distributed in yeasts. A link between 3-hydroxy oxylipin production, mitochondria and aspirin sensitivity exists. Research suggests that (i) 3- hydroxy oxylipins in yeasts are produced by mitochondria through the fatty acid synthesis type 2 (FAS II) and/or incomplete beta-oxidation, (ii) aspirin inhibits mitochondrial beta-oxidation and 3- hydroxy oxylipin production, (iii) yeast sexual stages, which are more dependent on mitochondrial activity are also characterized by higher 3-hydroxy oxylipin-levels compared to asexual stages, (iv) yeast sexual developmental stages as well as cell adherence/flocculation are more sensitive to aspirin than corresponding asexual growth stages and (v) mitochondrion-dependent asexual yeast cells with a strict aerobic respiring metabolism are more sensitive to aspirin than those that can also produce energy through an alternative anaerobic glycolytic fermentative pathway where mitochondria are not involved in the energetic pathway. This review interprets a wide network of studies that expose aspirin as a novel antifungal.

Reference:

1. Kock *et al.* (2007). Oxylipin studies expose aspirin as antifungal. FEMS Yeast Research (http://www.blackwell-synergy.com/doi/full/10.1111/j.1567-1364.2007.00273.x).

General Strategy for Synthesis of Oligosaccharide Combinatorial Library to Develop for Oligosaccharide Array

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There are three important bio-functional chains, nucleic acid (DNA and RNA), protein (peptides) and oligosaccharide for retaining the homeostasis of a life. Until now both of the DNA array and peptide array were well developed and have already applied for drug design and discovery. On the other hand, oligosaccharide array have been still developing. Though, existing oligosaccharide array were built by using enzymatically prepared oligosaccharide combinatorial library, there are no existing a perfect oligosaccharide array which is built by fully chemically synthesized oligosaccharide combinatorial library. If any kinds of oligosaccharides whichever you designed are placed on the array plate, you can clearly understand the detail of the structure dependency of recognition molecule and also the perfect oligosaccharide array will identify the difference of similar diseases or toxins. To develop the perfect oligosaccharide array, the synthesis of oligosaccharide combinatorial library is indispensable. But until now, there is no general method for synthesis of oligosaccharide combinatorial library.

Recently, I have developed the new method for synthesis of oligosaccharide so called "UCP method"[1,2]. By using this UCP method, exhaustive oligosaccharide combinatorial library have been successfully synthesized. This newly established UCP method will be the general method for synthesis of oligosaccharide combinatorial library.

References:

1. Komba S., Kitaoka M. and Kasumi T. A new method of carbohydrate synthesis in both solution and solid phases using a special hydroxy protecting group. *Eur. J. Org. Chem*. **2005**, *24*, 5313-5329.

2. Komba S., Kitaoka M. and Kasumi T. A new oligosaccharide synthesis using special hydroxy protecting group. *Tetrahedron Lett*. **2004**, *45*(13), 2759-2762.

Greater Inhibition of Connective Tissue Growth Factor Synthesis By a MAP Kinase Inhibitor Compared to Amino Guanidine in Cultured Human Retinal Pigment Epithelial Cells

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Purpose: The antifibrotic effect of amino guanidine (AG), an inhibitor of advanced glycation, in the diabetic animal model has been shown to be mediated by Connective Tissue Growth Factor (CTGF). Since human retinal pigment epithelial (hRPE) cells, in the presence of hyperglycemia, have been implicated in the pathogenesis of proliferative diabetic retinopathy (PDR) and retinal fibrosis, and CTGF acts via MAP kinase mediated pathways, we compared the effect of AG and the MAP kinase inhibitor, PD 98059 (PD) on CTGF synthesis in presence of high glucose in cultured hRPE cells.

Methods: hRPE cell cultures were established from four human eyes and were grown to confluence in media containing 14% fetal bovine serum (FBS). The hRPE cells were then washed with minimum essential media prior to exposure to experimental reagents for 24 to 48 hours. The proliferative effect of the reagents on the hRPE cells was determined by cell counting using the trypan blue exclusion method (T) and by measurement of thymidine incorporation (3H-thy). CTGF synthesis was measured by immunoprecipation and immunohistochemical analysis of 14C-methionine-CTGF (14-C-CTGF) using COOH-terminal specific anti-CTG. Data were analyzed by Student 't' test.

Results: Increasing concentrations of FBS stimulated hRPE proliferation as measured by 3H-thy as well as T, while increasing concentrations of glucose (2-20mM) alone had no significant effect. As hypothesized, AG and PD inhibited this FBS (10%) stimulated cell proliferation. In addition, glucose (2-20 mM) stimulated 14C-CTGF syntheses in a dose-dependent manner. This increased synthesis was not observed in the presence of an osmotic control, mannitol. Both AG (100 mM) and PD (50 μM) inhibited this glucose (20mM) stimulated 14C-CTGF synthesis; although PD caused greater inhibition than AG (6275±1707 vs. 8317±2514, CPM±SEM, $p \leq 0.05$, n=4).

Conclusion: We conclude that cultured hRPE cells represent an excellent in vitro model for the study of proliferative eye disease. Since glucose increases CTGF expression (a potent stimulator of hRPE proliferation) in this model and PD98059, a MAP kinase inhibitor, more than AG inhibits this synthesis, we postulate that inhibition of the MAP kinase signaling pathway may be a useful therapeutic strategy in preventing pathological cell proliferation and fibrotic membrane formation in patients with PDR and secondary retinal fibrosis.

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A New Inhibitor of Serine-Threonine Protein Phosphatase, PP2A, Suppresses Growth and Induces Differentiation of Medulloblastoma Cells

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We have shown previously that the nuclear receptor co-repressor (N-CoR) is over-expressed in cell lines and in primary tumor cells of glioblastoma multiforme (GBM), the most common and aggressive brain cancer of adults [Park et al, Cell Cycle 6:467-70: (2007)]. A complex of N-CoR, histone deacetylases, and retinoic acid receptor represses transcription of genes required for GBM stem cell differentiation resulting in increased tumor cell proliferation. We found that okadaic acid, an inhibitor of protein phosphatase PP2A, inhibits GBM cell growth by blocking N-CoR function through enhancing the protein phosphorylation. We have developed new inhibitors of PP2A that mimic the effects of okadaic acid on GBM cell growth. Medulloblastomas, the most common brain cancers of children, like GBMs, contain many undifferentiated tumor stem cells. We have now found that the medulloblastoma line, DAOY, like GBM cells, over-expresses N-CoR. Our lead inhibitor, LB-1, suppresses proliferation of DAOY cells at low micro-molar concentrations. This inhibition is accompanied by rapid loss of N-CoR expression, cell differentiation, and apoptosis. The findings raise the possibility that phosphatase ligands, such as LB-1, may be useful agents for the treatment of adult and pediatric brain tumors and other tumor types over-expressing N-CoR.

On the *in vitro* Oscillatory Chiral Inversion of Profen Drugs and its Implications

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Spontaneous chiral inversion of profen drugs taking place *in vivo* has been reported in a large number of publications. In article [1], a question was raised as to the very essence of its nature, considering coincidence vs. principle.

Our papers on the *in vitro* oscillatory chiral inversion of profen drugs [2-6] are the first ones in the field. The experimental results contained therein point to the following important issues:

(i) the differentiated dynamics of this phenomenon, depending on the molecular structure of the profens involved

(ii) the necessary physicochemical preconditions for oscillatory behavior

(iii) the molecular mechanism of this process, which results in temporal oscillation and spatial patterns

(iv) the virtual inability to quantify scalemic mixtures of profens by means of chromatography

(v) the pharmacological implications of the phenomenon

In this paper we are going to address all the above issues (i) - (v).

References:

1. V. Wsol, L. Skalova, B. Szotakova, *Curr Drug Metab*. **2004**, *5*, 517-533.

2. M. Sajewicz, R. Piętka, A. Pieniak, T. Kowalska, *Acta Chromatogr*. **2005**, *15*, 131.

3. M. Sajewicz, R. Piętka, A. Pieniak, T. Kowalska, *J. Chromatogr. Sci*. **2005**, *43*, 542.

4. M. Sajewicz, R. Piętka, A. Pieniak, T. Kowalska, *J. Liq. Chromatogr. Relat. Technol*. **2006**, *29*, 2059.

5. M. Sajewicz, R. Piętka, G. Drabik, T. Kowalska, *J. Liq. Chromatogr. Relat. Technol*. **2006**, *29*, 2071.

6. M. Sajewicz, M. Gontarska, D. Kronenbach, Ł. Wojtal, G. Grygierczyk, T. Kowalska, *Acta Chromatogr*. **2006**, *18*, 226.

Elimination of Matrix Effects in Analyses of Trace Metabolites in Body Fluids by Capillary Electrophoresis

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Capillary electrophoresis belongs to analytical methods that are frequently used for determination of drugs and their metabolites in body fluids. Capillary zone electrophoresis (CZE) is a fast and sensitive method; however, varying composition of the matrix can strongly affect parameters used for qualitative and quantitative evaluation. Capillary isotachophoresis (CITP) is a robust method that can cope with complex matrices; however, its sensitivity is lower compared with CZE. Matrix effects in CZE analyses can be eliminated off-line by sample pretreatment or on-line by proper selection of the separation medium. Sensitivity of CITP can simply be improved by on-line combination with CZE. Vice versa, the CITP-CZE hyphenation contributes to elimination of matrix effects, and due to higher column hold-up also to sensitivity enhancement of CZE.

The proper way to eliminate matrix effects in analyses of trace anionic components including drug metabolites in body fluids by a single CZE method is demonstrated in slightly acidic and basic media, and parameters that should be followed are presented. Further sensitivity and reproducibility enhancement of these analyses is demonstrated by results obtained by CITP-CZE combination.

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Design of Depot Forms of Anti-HIV Agents Based on Nucleoside -Phosphonate Derivatives

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Intensive efforts are underway to develop chemotherapeutic agents effective against HIV. Among the diversity of compounds active against HIV, AZT is the most extensively studied. The most limitations of AZT treatment of HIV infected patients are due to clinical toxicity, dependence on host cell kinases, and a short half-life in plasma. In attempts to overcome these problems, a "prodrug" strategy has been intensely developed.

Herein, we present alkyl- and aminocarbonyl phosphonate derivatives, new classes of AZT prodrugs. As phosphorus substituents, hydrophobic, hydrophilic, and positively charged groups were selected. We evaluated their stability in human blood serum, activity in HIV-1 infected cell culture, and behavior in animal models. Some of the compounds displayed anti-HIV activity similar or higher relative to 5'-hydrogenephosphonate of AZT (Nicavir®), anti-HIV drug approved for the treatment of HIV patients in Russia. Pharmacokinetic parameters of 5'-(amino)carbonylphosphonate AZT (**I**) were determined and compared with that of AZT and nicavir. Acute toxicity in mice was twice and four times lower than that of AZT, respectively. The bioavailability of **I** was only 1.5 times lower than nicavir. To conclude, **I** is the most promising depot form of AZT among the synthesized compounds.

Preclinical Experiments on Culevit Infusion Developed on the Basis of a Passive Antitumor Defence System and Investigation of Mechanism

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While other research groups, realizing the inefficiency of the immune system against cancer, studied immune escape or response modifiers we thought that the failure of tumours to develop in the majority of the population during their lifetime despite ineffective immune surveillance indicates the existence of other defence system(s) whose agents should lie in the circulatory system (CS). It is well known and used for tumour detection (as in PET) that the uptake of most substances (amino acids, monosacharides, nucleobases, etc.) occurring in CS is strictly regulated by normal cells, whereas it is unregulated and elevated by tumour cells. Considering that many molecules in the living system have more than one role we supposed that some of the accumulated substances might be the agents of a defence system capable of killing emergent cancer cells. We substantiated this hypothesis by experimentally selecting 16 substances of the CS (of 89 investigated) whose mixture had toxic effects *in vivo* and *in vitro* on all tumour cell lines investigated, but not on normal cells or animals - as expected for agents of a defence system (called by us a Passive Antitumor Defence system, PADS). Knowing the 16 agents of the PADS the possibility arose of practical usage as medication. The composition has been protected by patent in many countries. On the basis of these patents two products (Culevit® tablets and Culevit® cream) have been developed by Immunal Ltd. (Budapest). The development of an infusion is at the preclinical phase in the National Institute of Oncology (Hungary) where significant inhibitory effects of Culevit infusion have been observed in the case of all tumours (P-388, S-180, B-16, MXT, Colon-26, He/De, Ne/De, HL-60) investigated, in accordance with previous results and *in vitro* experiments despite the fact that the infusion was administered only as an injection (8 times a day for 10 days i.p.). Culevit infusion compared to cytostatics (5-FU, Cisplatin) had slightly better inhibitory effect on Colon-26. It is important to note that the infusion significantly potentiated the effects of both cytostatics and vice versa when they were used simultaneously. It appeared that the components of Culevit, as the agents of PADS, could also kill multidrug resistant cells (AT3B-1, MCF7/ADR) and the infusion abrogated the resistance towards 5-FU of B16 melanoma. No lethality, toxic effects, adverse clinical symptoms or body weight loss were observed in toxicity studies of Culevit infusion. On the basis of all the preclinical experiments it can be concluded that Culevit infusion, beside having significant inhibitory effect on tumours, can improve the efficacy and reduce the side effects of chemotherapy and radiation and can decrease the possibility of relapse.

Universally labelled ^{13}C-glucose was used to investigate the mechanism of action of Culevit on HeLa cells. ^{13}C NMR spectra demonstrated that the cells really accumulated the components of Culevit and the treatment decreased the production of lactate. ^{31}P NMR spectroscopy showed that the treatment inhibited glycolysis at the glyceraldehyde-3-phosphate dehydrogenase (GAPDH) step because the concentrations of fructose-1,6-bisphosphate, dihydroxyacetone phosphate and glyceraldehyde-3-phosphate all increased. According to the literature, inhibition and subsequent nuclear translocation of GAPDH causes apoptosis. We demonstrated that the treatment induced activation of p53 and fragmentation of nucleosomal DNA. Further investigation of the apoptotic mechanism is in progress in several laboratories including our own.

Can the Acute Toxicity of Antidepressants be Predicted Using the Protozoan *Tetrahymena pyriformis?*

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Safety in overdose is an important consideration in the development of antidepressants, as it is quite common for depressed patients to use their medication in suicide attempts. Although currently used antidepressants are of relatively similar efficacy, they differ greatly in terms of their toxicity in acute overdose situations. Traditionally, the preclinical acute toxicity of drugs has been tested using the LD_{50} first described by Trevan in 1927. To reduce the use of animals, a number of *in vitro* tests are now under investigation to determine the acute toxicity of drugs, and in this study, we investigate whether the fresh water protozoa *Tetrahymena pyriformis* would be of value in predicting the acute toxicity of a range of currently marketed antidepressants. The cytotoxic effects of a range of marketed antidepressants were studied using the MTT assay. The results showed that the IC_{50} values did not correlate with the FTI (Fatal Toxicity Index) values in humans. Thus it can be concluded that the *Tetrahymena pyriformis* assay is a poor predictor of acute toxicity in humans, and that in the absence of credible *in vitro* alternatives, that animals will continue to be the method for predicting the acute toxicity of antidepressants.

α-Arylation of a Protected Glycine in Water: 3-Phenylglycine Derivatives as Inhibitors of the Tuberculosis Enzyme, Glutamine Synthetase

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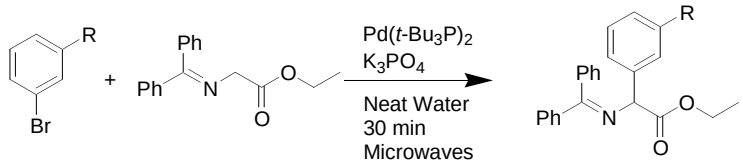
In a medicinal chemistry project aiming at the design and synthesis of novel inhibitors towards the *Mycobacterium tuberculosis* enzyme glutamine synthetase, we became interested in α-arylated amino acids. Although, these derivatives are generally synthesized by Strecker-type chemistry, a few methods have recently been developed for direct α-arylation of amino acids using palladium catalysis.[1,2] The reported protocols were carried out under inert conditions and in most cases the reactions require long reaction times. Thus, we therefore decided to investigate this reaction class under non-inert conditions using high-density microwave irradiation as the energy source. We hereby report the development of a rapid palladium-catalyzed protocol in neat water.[3]



Yield 36-78%

References:

1. Lee, S.; Beare, N. A.; Hartwig, J. F. *J Am Chem Soc* **2001**, *123*, 8410-8411.

2. Gaertzen, O.; Buchwald, S. L. *J Org Chem* **2002**, *67*, 465-475.

3. Lagerlund, O.; Odell, L., R.; Mowbray, S., L.; Nilsson, M., T.; Krajewski, W., W.; Nordqvist, A.; Karlén, A.; Larhed, M. *Comb Chem High Throughput Screen* **2007**, *10*, 783-789.

Narciclasine Displays Potent and Selective Anti-Tumor Effects by Impairing Cancer Cell Migration Through a Phosphocofilin-Mediated Increase of Actin Stress Fibers

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Among the compounds isolated from Amaryllidaceae, the isocarbostyrils have long been under scrutiny as promising anti-cancer agents, and notably two narciclasine (NCL) and pancratistatin are in the NCI database. However, little is known of the mechanism of action of this family of compounds. NCL was originally described as anti-mitotic and displaying colchicine-like effects (Ceriotti *et al.*, Nature 1967). More recently McLachlan *et al* (2005) demonstrated that pancratistatin, whose chemical structure is very close to that of NCL, induces rapid apoptosis in SHSY-5Y neuroblastoma cells. We have recently shown that NCL at 1µM *in vitro* induces marked apoptosis-mediated cytotoxicity in certain human cancer cells but not in normal fibroblasts, by triggering the activation of initiator caspases in the death receptor pathway (capase-8 & 10) at least in human MCF-7 breast and PC3 prostate carcinoma cells (Dumont *et al.*, Neoplasia 2007).

In the studies reported here, NCL inhibited cell growth with mean IC_{50} values of 30nM for 6 human tumor cell lines investigated, 100nM for 3 distinct human umbilical vascular endothelial cell lines and 7.5µM for 3 distinct normal human fibroblast cell lines. At 0.1 & 1.0µM, NCL failed to induce cell death and/or apoptosis in human glioblastoma (GBM) U373 cells, although it inhibited cell growth with an IC_{50} of 40nM. At concentrations of 10 & 100nM, NCL markedly impaired the migration of GBM, prostate and breast cancer cells, a feature paralleled by compound-induced loss of tumor cell polarity. NCL also induced rapid increases in F-actin concentrations (principally at the cortical cell location) in U373 and PC3 cells, an effect not observed with normal fibroblasts at the same concentration. Moreover, the increase in F-actin was associated with a dramatic modification in serine/threonine phosphoprotein expression. Given that altered cofilin expression controlled by phosphorylation is associated with the initiation and progression of conditions involving actin dynamics, such as cancer, the effects of NCL on cofilin phosphorylation status were assessed. NCL treatment significantly and rapidly increased cofilin phosphorylation levels *in vitro* and through this mediate increases in actin stress fibers responsible for the reduced migration seen in cancer cells. NCL has also be shown to be active in murine (P388 lymphoma) and human models of cancer (A549 NSCLC & PC3 prostate) in mice following repeat i.v. or p.o. dose regimens. In conclusion, NCL could represent an interesting chemical scaffold to derive novel isocarbostyril derivatives for combating apoptosis-resistant migrating or metastasizing cancer cells.

An *In vitro* Evaluation of the Efficacy of Anti-Malaria Drugs by Pheroid™ Technology

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Approximately 300 million people are affected by malaria. Malaria is now mainly confined to Africa, Asia and Latin America. Inadequate health structures, poor socio-economic conditions and the increase in resistance to anti-malarial drugs aggravate the situation. The need to develop drug delivery systems that minimize the likelihood of drug resistant microorganisms requires a multidisciplinary approach. Novel Pheroid™ Technology may be applicable and its effectiveness in combination with anti-malarial drugs was investigated.

The efficacy of existing anti-malarial drugs in combination with Pheroid™ formulations was investigated using a human erythrocyte *in vitro* infection model and a chloroquine resistant strain (RBy1) of *P. falciparum* with selected concentration ranges of chloroquine phosphate, mefloquine, artemether and artesunate. Two distinctively different Pheroid™ formulas, vesicles and microsponges were used. The parasiteamia levels were determined microscopically and the comparative anti-parasitic effect was determined by calculating the total comparative growth inhibitory effect.

The comparative efficacy of chloroquine phosphate in Pheroid™ vesicles was significantly enhanced by 1544.62%. The efficacy of mefloquine, artemether and artesunate in Pheroid™ sponges were enhanced by 314.32%, 254.86% and 238.7% respectively. This study holds merit for further investigation of the effects of Pheroid™ technology on anti-malarial treatment with *in vivo* methods.

Improvement of Drug Virtual Screen by GA/GP: Docking Studies on Tubulin Inhibitors as Anticancer Agents

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Conventional procedures for drug design have been very expensive and time-consuming. Due to the enormous progress in information technology during recent years, it is expected to shorten the required research time spent in the early stage of development through computer calculation. CADD (Computer Aided Drug Design) is one of the most powerful concepts applied to satisfy such demand. Upon docking simulations, it is allowed to find out the binding sites and orientations between target proteins and drug molecules in several days. This is not only to save the time and the cost used in drug development, but also for us able to understand the structural implications used for further design. However, it is still currently difficult to formularize efficient software to carry out the docking simulations as a standard procedure leading to definite results with high accuracy. Therefore, we are in attempt to propose a new category of programming, for which the standard effectiveness for docking procedure can be anticipated in the near future.

To initiate such computer simulations, many factors have to be taken into consideration. The first is to decide which algorithms should be applied to perform the job. GA (Genetic Algorithms) and GP (Genetic Programming) seem to be excellent candidates to solve this problem. The next concern is the determination of scoring function, which is appropriate for either GA or GP to generate their scores. As being the best commercially available scoring function with high accuracy and flexibility, XSCORE is used to satisfy this purpose. Therefore our study has been concentrated in the search of binding site(s) between protein and the drug molecule through docking simulations by applying the aforementioned special algorithms and scoring function. Target protein is at first regarded as a rigid body, whereas the drug molecule is allowed to be entirely flexible. According to our results, GA and GP indeed improve the search of docking sites between the target protein and the drug molecules in both accuracy and efficiency. Partially flexible protein regions, depending on each individual interaction chosen for experiments, were then added into the docking system. Successful examinations with known protein structures and drugs provide evidence for the possibility of applying our new software in accelerating the design of new lead compounds.

We try to apply our virtual screen system on tubulin inhibitors. In order to find some lead compounds like taxol- a drug used to treat breast cancer, ovarian cancer, and AIDS-related Kaposi's sarcoma. It is also used together with another drug to treat non-small cell lung cancer. Taxol is also being studied in the treatment of other types of cancer. It belongs to the family of drugs called mitotic inhibitors. We test the accuracy and sensitivity by using known drug-tubulin crystal structure and other drug-protein complex structure. It is easy to get correct three type of tubulin inhibitor-tubulin complex by docking with our GA/GP virtual screen system.

The Copolymer-1 Glatiramer Acetate (Copaxone) Inhibits Natural Killer (NK) Cells Lysis of Tumor Cells but Enhances Their Cytolysis of Syngeneic or Allogeneic Immature and Mature Monocyte-Derived Dendritic Cells

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We describe here a novel mechanism for the therapeutic effect of the drug Glatiramer acetate (GA; Copaxone). This drug is used for preventing the relapse in patients with Multiple Sclerosis (MS), and in the mice model Experimental Allergic Encephalomyelitis (EAE). However, its mechanism of action (MOA) is not clearly known. Injection of GA into EAE mice resulted in reduced EAE clinical score, particularly 17 days after induction of the disease and three rounds of GA treatment. At this time point, spleens were collected from these mice and were examined for natural killer (NK) cell activity. The results indicate that EAE mice treated with GA had significantly lower NK cell activity than vehicle treated mice. However, neither the number of NK1.1 nor the expression of the activation molecule CD69 was affected in these mice. Incubating GA with the mixture of human NK cells plus tumor cells inhibited the lytic activity of the cytolytic $CD56^{dim}CD16^{+}$ and IL-2-activated subsets of NK cells but not the regulatory $CD56^{bright}CD16^{-}$ NK cell subset. However, GA did not affect the proliferation of $CD56^{bright}CD16^{-}$ or $CD56^{dim}CD16^{+}$ and neither affected the expression of CD69 molecule on the surface of these cells. Surprisingly, GA enhanced the cytolysis of IL-2-activated NK cells toward syngeneic or allogeneic immature and mature monocyte-derived dendritic cells (DCs). This is the first demonstration that a compound or a drug enhances the cytolysis of mature DCs by NK cells, a phenomenon that may have important application for the treatment of autoimmune diseases, such as MS. The results may also impact the clinical utilization of GA in bone marrow transplantation and in suppressing graft versus host (GvH) disease, among many other disorders.

Effect of Moist Heat Sterilisation on Pyrogenicity of Whole Bacteria and Cell Wall Components from *Staphylococcus aureus*

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In the monocytic cell line Mono Mac 6 pyrogens induce interleukin-6 secretion dose dependently. The aim of this study is to examine the pyrogenic activity of *Staphylococcus aureus* (*S. aureus*) and its cell wall components lipoteichoic acid (LTA) and peptidoglycan after moist heat sterilisation procedures. The pyrogenic activity is determined as the ability of the substances to induce interleukin-6 secretion in Mono Mac 6 cells.

Moist heat sterilisation of *S. aureus* at 121°C and 134°C does not reduce the pyrogenic activity of *S. aureus*. On the contrary moist heat sterilisation procedures at 134°C significantly increase the pyrogenic activity compared to untreated *S. aureus*. This is confirmed in the rabbit pyrogen test.

The pyrogenic activities of LTA and peptidoglycan are not eliminated at moist heat sterilisation 121°C 15min or 134°C 3min. Increased processing times eliminate the pyrogenic activity of LTA at both temperatures, whereas the activity of peptidoglycan is not removed after 160min where only 2-log reductions are obtained.

In conclusion terminal heat sterilisation procedures cannot inactivate the pyrogenic activity of *S. aureus*, LTA and peptidoglycan. Increased processing times eliminate the pyrogenic activity of LTA, but not of peptidoglycan. The heat resistance of the pyrogenicity of *S. aureus* cannot be ascribed to LTA or peptidoglycan alone.

Probing the HIV Reverse-Transcriptase Enzyme: Synthesis and Biological Evaluation of Novel Bifunctional NRTI/NNRTI HIV-1 Reverse-Transcriptase Inhibitors

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HIV reverse transcriptase (RT) is a multifunctional enzyme that catalyzes as a RNA-dependent DNA polymerase, as well as a DNA-dependent DNA polymerase, thus effectively replicating the genomic single-stranded RNA of HIV into double-stranded DNA (dsDNA). It has therefore long been recognized that reverse transcriptase is a key step in the HIV life cycle, making it a primary target for the development of new anti-HIV drugs used in the treatment of AIDS.[1]

Combination chemotherapy of different anti-HIV agents has been widely used to treat HIV patients in an attempt to delay the emergence of drug-resistant mutants. Certain combinations of nucleoside (NRTI's) and non-nucleoside inhibitors (NNRTI's) lead to synergistic inhibition of *in vitro* HIV replication, thus resulting in greater efficacy.[1] Thus, combining these HIV inhibitors into a single molecular entity is a strategy that is growing in popularity in HIV chemotherapy research. The high levels of resistance elicited by both nucleoside and non-nucleoside inhibitors has prompted the design of double-drugs (bifunctional drugs) combining these two entities with the aim of addressing the emergence of resistance.[2] The strategy involves combining the two inhibitors (Figure 1) *via* a non-cleavable linker, with the aim of improving the physicochemical characteristics of the individual compounds as well as probing the synergy between the substrate and allosteric binding sites of the enzyme.

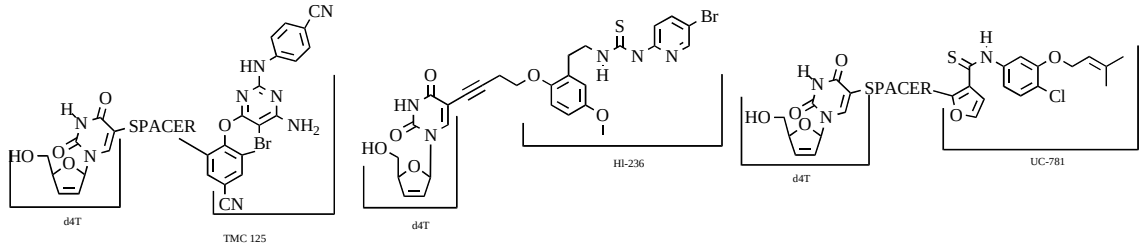

Fig. (1). Bifunctional HIV-1 reverse-transcriptase inhibitors synthesized.

This presentation aims to extrapolate crucial aspects of drug design (modeling), synthesis and biological evaluation of a series (Fig. **1**) of such bifunctional HIV-1 reverse-transcriptase inhibitors.[3,4,5]

References

1. Spence, R. A.; Kati, W. M.; Anderson, K. S.; Johnson, K. A. *Science*, **1995**, *267*(5200), 988-993.
2. Muhanji, C. I.; Hunter, R. *Current Medicinal Chemistry*, **2007**, *14*, 1207-1220.
3. Arnott, G. A.; Hunter, R.; Mbeki, L.; Mohamed, E. *Tetrahedron Letters*, **2005**, *46*, 4023-4026.
4. Hunter, R.; Mohamed, E. *Afinidad*, **2007**, in press.
5. Hunter, R.; Muhanji, C. I.; Anderson, K. S. *et al. Bioorganic and Medicinal Chemistry Letters*, **2007**, *17*, 2614-2617.

Neurokinin 1 Receptor Antagonist Aprepitant as an Antitumor Drug

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Aprepitant is a selective high-affinity antagonist of human substance P (SP)/Neurokinin-1 (NK-1) receptors. Until now this drug has been used as anxyolitic, antidepressant and antiemetic. It has been demonstrated that SP induces cell proliferation and NK-1 receptor antagonists different to Aprepitant inhibit growth in several human cancer cell lines, where NK-1 receptors are overexpressed. The purpose of this study is to demonstrate the antitumor action of Aprepitant. We performed an in vitro study of the growth inhibition capacity of the NK-1 receptor antagonist Aprepitant against glioma, neuroblastoma, insert here melanoma, retinoblastoma and (pancreas, larynx and breast carcinoma) cell lines. Coulter counter was used to determine viable cell numbers followed by application of the MTS colorimetric method. Furthermore, a DAPI method was applied to demonstrate apoptosis. We have demonstrated: Aprepitant at (5-70 μM) concentration elicits growth cell inhibition in a concentration dependent manner in these tumor cells. Maximum inhibition (100%) was observed when the Aprepitant was administered at a concentration of ≥ 70μM in all cancer cell lines studied and cell death was by apoptosis. These new findings reported here for the first time indicate that Aprepitant is a new and promising antitumor drug in the treatment of cancer.

Endogastric Capsule for E-Cadherin Gene (CDH1) Promoter Hypermethylation Assessment in DNA from Gastric Juice of Patients with Gastric Cancer

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We investigated whether an endogastric capsule (EC) may be a valuable tool for collecting DNA from exfoliated cells from the gastric mucosa and for carrying out an analysis of promoter methylation status of the E-cadherin (CDH1) gene in poorly differentiated diffuse gastric cancer (DGC). Material and methods: Consecutive patients with a confirmed diagnosis of poorly differentiated DGC underwent collection of gastric juice by EC. Subjects without cancer and premalignant lesions were also accrued as controls. Exfoliated normal or cancer cells from gastric juice were demonstrated by touch deposition on slides .Samples of gastric juice were processed for DNA isolation and amplification and used for analysis of CDH1 promoter hypermethylation. The procedure successfully allowed the analysis of CDH1 promoter hypermethylation in 20 patients and 14 controls. This pilot study showed feasibility of the procedure and a significantly different CDH1 promoter hypermethylation status between DGC patients and controls : 90% of all patients with DGC showed hypermethylation while all patients without cancer resulted negative . The EC may represent an innovative and noninvasive tool for the analysis of a specific epigenetic change in patients with gastric cancer. This method may represent a cost-effective tool for early detection of sporadic as well as hereditary DGC in CDH1 germline mutations carriers.

Reference:

1. Muretto P. *et al.* Annals of Oncology, 6 November 2007 .

Acetylcholinesterase Reactivator – Oxime HI-6 – Antidote Against Nerve Agents

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Oxime HI-6 (1-(2-(hydroxyiminomethyl)pyridinium)-3-(4-carbamoylpyridinium)-2-oxapropane) belongs to the most promising acetylcholinestrase reactivators – antidotes used against nerve agents (sarin, cyclosarin, tabun, VX, etc.). According to the present knowledge, its reactivation potency is the highest compared to other commercial oximes (pralidoxime, obidoxime, trimedoxime, MMB4).[1,2] Thanks to its promising reactivation potency, the development of this compound and its further large-scale production were done at our department within last four years.

In this presentation, we would like to summarize our results to show what we have done in this topic. We will describe preparation of twelve different HI-6 salts (sulfate, chloride, acetate, bromide, phosphate, mesylate, tartrate, iodide, malonate, salicylate, maleinate, tosylate), their quick TLC and HPLC analysis and solubility testing. Furthermore, chloride (Cl) and dimethanesulfonate (DMS) salts of the HI-6 were tested *in vitro* and *in vivo* to compare their reactivation differences.

References:

1. Lundy PM, Raveh L, Amitai G. Development of the bisquaternary oxime HI-6 toward clinical use in the treatment of organophosphate nerve agent poisoning. *Toxicological Reviews.* 2006; 25: 231-243.

2. Kuca K, Jun D, Bajgar J. Currently used cholinesterase reactivators against nerve agent intoxication: comparison of their effectivity in vitro. *Drug & Chemical Toxicology.* 2007; 30: 31-40.

Synthesis and Anti-*Helicobacter pylori* Activity of (*Z*)-2-(Nitroimidazolylmethylene)-2(3*H*)-Benzofuranone Derivatives

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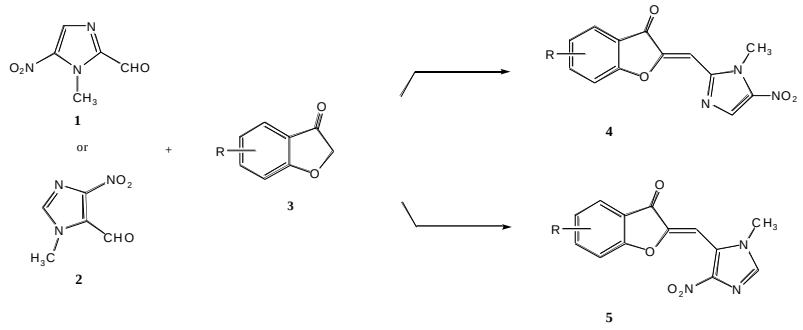
It is well known that *Helicobacter pylori*, an Gram-negative bacterium is considered the major causative agent of several gastric pathologies, such as chronic active gastritis, peptic ulcer disease and gastric cancer. Clinical evidence shows that the eradication therapy of this microorganism can significantly reduce the risk of ulcer relapse and may help prevent mucosa-associated lymphoid tissue (MALT)-type gastric carcinoma and other gastric cancers.

The use of nitroheterocyclic drugs (such as 5-nitroimidazoles) as antibacterial, antiprotozoal, and anti-cancer agents is well-established [1]. Furthermore, a series of (*Z*)-2-benzylidene-6,7-di- hydroxy-3(2*H*)-benzofuranones have shown antibacterial activity by inhibiting the chorismate synthase, a key enzyme in the shikimic acid pathway which is essential for the synthesis of aromatic amino acids in bacteria [2].

The title compounds were prepared according to the scheme shown below. 3(2*H*)-Benzofuranones **3** are key intermediates for the production of the desired compounds. Condensation of latter **3** with corresponding carbaldehyde **1** in acetic acid, in the presence of catalytic amount of sulfuric acid afforded compound **4**, while title compound **5** was prepared by a different method in acetic anhydride in the presence of anhydrous sodium acetate [3].

All the synthesized compounds were screened against clinical species of *H. pylori* and shown excellent activity in comparison with the reference drug.



References:

1. Edwards, D. I. *J. Antimicrob. Chemother.* **1993**, *31*, 9.

2. M. G. Thomas, C. Lawson, N. M. Allanson, B. W. Leslie, J. R. Bottomley, A. McBride, O. A. Olusanya, *Bioorg. Med. Chem. Lett.* **2003**, *13*, 423.

3. N. Hadj-esfandiari, L. Navidpour, H. Shadnia, M. Amini, N. Samadi, M. A. Faramarzi, A. Shafiee, *Bioorg. Med. Che. Lett.* **2007**, *17*, 6354.

Electric Field Drive Transdermal Drug Delivery of Salicylic Acid -Loaded Polyacrylamide Hydrogels

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The release mechanisms and the diffusion coefficients of salicylic acid -loaded polyacrylamide hydrogels were investigated experimentally by using a modified Franz-diffusion cell at 37^0C to determine the effects of crosslinking ratio and electric field strength. A significant amount of salicylic acid is released within 48 hours from the hydrogels of various crosslinking ratios, with and without electric field. The release characteristic follows the Q vs. $t^{1/2}$ linear relationship. Diffusion coefficient initially increases with increasing electric field strength and reaches the maximum value at electric field strength of 0.1 V; beyond that it decreases with electric field strength and becomes saturated at electric field strength of 5 V. The diffusion coefficient increases at low electric field strength (less 0.1 V) as a result of the electrophoresis of the salicylic acid, the expansion of pore size, and the induced pathway in pigskin. For electric field strength higher than 0.1 V, the decrease in the diffusion coefficient is due to the reduction of the polyacrylamide pore size. The diffusion coefficient obeys the scaling behavior D/D_o=(drug size/pore size)^m, with the scaling exponent m equal to 0.93 and 0.42 at electric fields of 0 and 0.1 V, respectively.

Phenolic Compounds from Siberian Conifer Trees - Multi-Purpose Drug Substances and Functionalized Scaffolds for NCEs Design

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Plant phenolic compounds represent a challenging group of natural biology active compounds exhibiting anti-estrogen, anti-oxidant, immunomodulating and other types of biology activity making possible wide uses of such compounds in the treatment of tumours, inflammation, microbial infections, cardiovascular and other diseases. Next to pioneering works by B. Holmbom *et al.* we have systematically studied the profiles of phenolic compound contents in Siberian conifer trees (larix, spure, pine) and developed efficient schemes for extraction the members of lignans, flavonoids and stilbenes including individual optically active isomers of secoisolariciresinol, hydroxymatairesinol, dihydroquercetin, pinosylvin and others suitable for the use as the API in pharmaceutical products. The results of these works and characteristics of phenolic compounds together with chemical schemes for their site directed functionalizations towards a variety of NCEs will be presented in the poster. This work was supported by the Russian Academy of Science (6st Program of Division of Chemistry and Material Sciences).

Combination Therapy of Personalized Peptide Vaccination and Low-Dose Estramustine Phosphate for Metastatic Hormone Refractory Prostate Cancer Patients

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The prognosis of patients with metastatic hormone refractory prostate cancer (HRPC) remains poor. No effective standard treatments are currently available for patients with metastatic HRPC. The development of novel therapeutic modalities for the treatment of HRPC is necessary. Many tumor antigens recognized by human leukocyte-associated antigens (HLA) class I-restricted cytotoxic T lymphocytes (CTLs) have been identified in the past decade, and new approaches to HRPC with tumor vaccines have been investigated. Our approach in the immunotherapy for HRPC patients is a pre-vaccination measurement of peptide-specific CTL precursors in the circulation of cancer patients reactive to more than 30 kinds of vaccine candidates with the ability to induce CTLs, followed by administration of only reactive peptides (personalized peptide vaccination). We showed that additive anti-tumor effects could be achieved by a combination of peptide vaccination and a low dose of (280 mg/day) estramustine phosphate (EMP). From February 2001 to July 2004, 58 men with metastatic HRPC received the combination therapy of personalized peptide vaccination and a low dose of EMP. Conducted immune monitorings for those patients were included peptide-specific CTL precursor analysis by interferon-γ production and peptide-reactive immunoglobulin G (IgG) by an enzyme-linked immunosorbent assay. Clinical responses and survival times were also evaluated. The combination therapy was well tolerated with no major adverse effects. Increased levels of CTL precursors and IgG responses to the vaccinated peptides were observed in 29 of 37 (78%) patients and in 36 of 41 (88%) patients tested, respectively. A prostate-specific antigen decline of at least 50 percent occurred in 24% of patients. The median survival time was 17 months (95 percent confidence interval, 12 to 25 months). Cox proportional hazards analysis showed that a low number of lymphocytes (p = 0.0075, odds ratio 2.700), a negative immunological activity response after the vaccination (p = 0.0185, odds ratio 2.658) and poor performance status (p = 0.0347, odds ratio 2.569) were independent predictors of disease death. These encouraging results show the need for further evaluation of the combination of personalized peptide vaccination and a low dose of EMP for metastatic HRPC patients.

Evaluation of Various P1' Substituents in Tertiary Alcohol Containing and Elongated HIV-1 Protease Inhibitors

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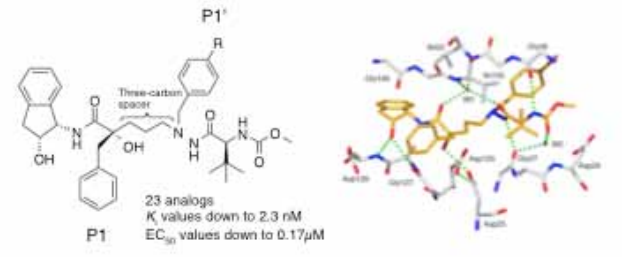
HIV protease inhibitors have become an important component of the Highly Active Antiretroviral Treatment (HAART) since they were introduced on the market. In general HIV protease inhibitors contain a secondary alcohol based transition-state mimic. Ekegren *et al.* have previously reported a novel class of HIV protease inhibitors with a shielded tertiary alcohol in the transitions-state mimicking scaffold. 1-3 Using the same tertiary alcohol concept, a new series of elongated HIV protease inhibitors with a three carbon spacer was developed. Compared to previous studies of masked HIV protease inhibitors we have also extended the SAR of the P1' group by synthesizing 23 analogs, investigating a variety of small to large P1' side chains with different polarity and hydrogen bonding potential. We were able to identify three new para-P1' substituted groups, a pyrimidine-, an imidazole- and an oxadiazole, as good alternatives to the established pyridines for achieving improved antiviral cell activity. We were also able to identify inhibitors with medium to high membrane permeability in the Caco-2 assay and with excellent stability when incubated with human liver microsomes. The binding mode of the elongated inhibitors was e.g. studied by the p-bromo-benzyl P1' inhibitor-enzyme X-ray structure. 4



References:

1. Ekegren, J. K.; Unge, T.; Safa, M. Z.; Wallberg, H.; Samuelsson, B.; Hallberg, A. A new class of HIV1 protease inhibitors containing a tertiary alcohol in the transition-state mimicking scaffold. J. Med. Chem. 2005, 48, 8098-8102.
2. Ekegren, J. K.; Gising, J.; Wallberg, H.; Larhed, M.; Samuelsson, B.; Hallberg, A. Variations of the P2 group in HIV-1 protease inhibitors containing a tertiary alcohol in the transition-state mimicking scaffold. Org. Biomol. Chem. 2006, 4, 3040-3043.
3. Ekegren, J. K.; Ginman, N.; Johansson, A.; Wallberg, H.; Larhed, M.; Samuelsson, B.; Unge, T.; Hallberg, A. Microwave-Accelerated Synthesis of P1'-Extended HIV-1 Protease Inhibitors Encompassing a Tertiary Alcohol in the Transition-State Mimicking Scaffold. J. Med. Chem. 2006, 49, 1828-1832.
4. Wu, X.; Öhrngren, P.; Ekegren, J. K.; Unge, J.; Unge, T.; Wallberg, H.; Samuelsson, B.; Hallberg, A.; Larhed, M. Two-Carbon-Elongated HIV-1 Protease Inhibitors with a Tertiary-Alcohol-Containing Transition-State Mimic. J. Med. Chem. 2008, ASAP.

Novel Anti-Parasitic Targets from Human Pathogenic Amoebas: *Entamoeba histolytica*, *Acanthamoeba Polyphaga* and *Naegleria fowleri*

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In this review we have searched the presence of drug targets in some species of human pathogenic parasites, mainly the amoebas *Entamoeba histolytica, Acanthamoeba polyphaga* and *Naegleria fowleri*. We started with the analysis of the concepts of essentiality and validity of the targets and continued with the description of the main characteristics of pathogenicity of these amoebas. Then we proceeded to the study of the targets arranged mainly in seven groups corresponding to: **a**) thiols and enzymes of redox metabolism, present only in trypanosomatids, *Entamoeba* and *Naegleria*, like the trypanothione/ trypanothione reductase, that maintains the reducing environment within the cell **b**) antioxidant enzymes to regulate the oxidative stress produced by the phagocytic cells of the host or by the parasite metabolism, like the NADPH-dependent trypanothione peroxidase, in connection with trypanothione/trypanothione reductase which may be is present in *Naegleria fowleri,* and peroxiredoxin in *E. histolytica*, **c**) enzymes for the synthesis of trypanothione like the ornithine decarboxylase, spermidine synthase and trypanothione synthetase, **d**) enzymes which are secreted by these parasites to invade the human host, like the proteinases, phospholipases and pore forming peptides **e**) glycolytic enzymes from *Entamoeba* and *Naegleria*, like the PPi-dependent phospho-fructokinase, different to the enzyme from the host **f**) some of the proteins assembling the secretory vesicles with the cell membrane, like the synaptobrevins and finally **g**) encystment pathways and cyst-wall assembly proteins. Some of the above new targets will need to be studied in a more profound detail to continue with the crystallographic studies of the enzymes from the parasites for a rational drug design. At present, as far as we know, there is no one example of this type being conducted on these three amoebas, as it has been the case for various targets from the trypanosomatids.

Synthesis of Some New Hydrazino-Thiazolic Compounds

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Thiazole heterocycle is known to be present in various molecules having a biological activity (antimicrobial, antifungal, anti-inflammatory, etc.)

From these considerations, our aim in the present study was to obtain some new thiazolic compounds and to prospect their antimicrobial activity.

In the synthesis of this type of compounds, we started from the thiosemicarbazone of 2-phenyl-4-methyl-5-acetyl-thiazole which were condensed with various halocarbonyls: α-chloroacetylacetone, α or γ chloro-acetylacetate, monochloracetone, 1,3 dichloracetone, bromoacetophenone and 5-bromoacetyl-2-phenyl-4-methyl-thiazole.

The structures of the new compounds were confirmed by elemental analysis and spectral data (^{1}HNMR, MS).

Investigation of the P1' Substituent - Synthesis and Evaluation of Inhibitors of Malarial Aspartic Proteases

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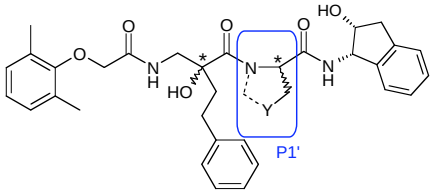
Ten aspartic proteases are encoded in the most lethal malaria species, *Plasmodium falciparum*. Three of these, plasmepsin I, II and IV, have all proved to be involved in the haemoglobin degradation in the parasite food vacuole.[1] Thus, it is likely that a promiscuous ligand that inhibits all three isoenzymes is needed for reducing parasitic growth successfully.

The structural starting point has been the efficacious inhibitor KNI-10006 synthesised by Kiso *et al.*[2] Our aim is to improve the membrane permeability by changing the transition state mimicking secondary hydroxyl into a tertiary hydroxyl group while retaining the plasmepsin inhibiting capacity. This structural modification has proved to be successful in the case of HIV-1 protease inhibitors.[3] Another challenge is to amend the selectivity profile towards the human aspartic protease cathepsin D (Cat D).

Our strategy has been to vary the P1' side chain in a series of compounds, by employing cyclic (proline and dimethylthioproline) or acyclic (phenylalanine) α-amino acids. Two of the present stereocentra have been altered to elucidate the preferred stereochemistry for inhibition of the food vacuole plasmepsins.

The compounds presented herein have different inhibitory effect on the plasmepsins and show low affinity to Cat D. We believe that they can be valuable research tools in the ongoing battle towards malaria.



General structure of the investigated compounds comprising variation of the P1' position and with two tunable (*) stereocentra.

References:

1. Bonilla, J.A., Moura, P.A., Bonilla, T.D., Yowell, C.A., Fidock, D.A., and Dame, J.B. *Int J Parasitol.* **2007**, *37*, 317-327.
2. Kiso, A., Hidaka, K., Kimura T., Hatashi, Y., Nezami, A., Freier, E., Kiso, Y. *J. Peptide Sci.* **2004**, *10*, 641-647.
3. Ekegren, J. K., Unge, T., Safa, M. Z., Wallberg, H., Samuelsson, B., Hallberg, A. *J. Med. Chem.* **2005**, *48*, 8098-8102.

Synthesis and Physicochemical Characterization of Some Thiazolium 2-Aryl Ammonium Quaternary Salts

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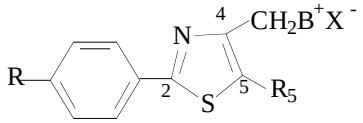
New ammonium quaternary compounds having at the basis 2-aryl thyazolic nucleus diversely substituted at 2,4, and 5 positions have been synthesized.

The basic component is represented by tertiary amines in which the nitrogen is either included into a heterocycle (pyridinic, chynolinic), or it is a derivative of N-methyl (the nitrogen being included into a hydrogenate heterocycle pyperidine, morpholine); in this way, it is possibly to investigate the influence of p-π conjugation within the nucleophyle substitution reaction, which intermediates the formation of new compounds.

The benzenic nucleus in the 2nd position can be substituted or not. The selected substitutes reject electrons (methyl), or attract them (chlorine) indicating the extent of electronic movement influence upon the antibacterial and/or antifungal effects.

To elucidate the structure of the resulting compounds UV, IR, Ramman, mass and MR spectroscopy were performed.

The corroboration of the obtained data does confirm the structures suggested by us:



R: H, H_3C, Cl
R_5: H, Br
B: 1-Methyl-pyperidine, 4-Methyl-morpholine, Pyridine, Beta-Pycoline, Chinoline
X: Cl, I

The formed compounds were submitted to an antibacterial and antifungal screening revealing that the chinolinium salt was the most active.

An Anticancer Peptide Histonine Derived from Histonine H2A and its Mechanism of Action

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Histonin is a novel cell-penetrating anticancer peptide derived from histone H2A. In this study, we analyzed the effects of this 21-amino acid peptide on a variety of cancer cell lines and its antitumor activity. Histonin displayed selective cytotoxicity against 62 cancer cell lines, with IC_{50} values (concentration of peptide at 50% cytotoxicity) in the 6 to 24 μg/ml range. Histonin bound to cell surface gangliosides and sialic acids for penetration into cancer cells. Histonin penetrated cancer cell membranes selectively without damaging them and accumulated primarily in the nuclei. The penetration of histonine into cancer cell was mediated by gangliosides and sialic acids present on the surfaces of cancer cells. Once inside the cells, histonin bound to intracellular macromolecules, such as DNA, RNA, and heat shock proteins, and induced mitochondria-dependent apoptosis. *In vivo* analysis of histonin revealed that it reduced about 90% of the volume of a fibrosarcoma mass (a cancerous tumor of the fibrous connective tissue) in p53-deficient mice and killed almost all of the tumor cells. Moreover, histonin displayed remarkable tumor suppression activity in a mouse tumor xenograft model. The high selectivity of histonin for cancer cells as well as its ability to reduce the mass of established tumors and to suppress tumor formation suggests that histonin has a great potential as a novel therapeutic agent for the treatment of cancers.

The Absorption, Distribution, Excretion and Pharmacokinetics of Carbon-14 Labeled Pyronaridine Tetraphosphate in Sprague-Dawley Rats

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Malaria is transmitted by a female anopheles mosquito and causes about 1.5 to 2.7 million deaths per year. The high incidence of malaria in spite of the availability of several drugs is mainly attributed to the development of a resistance by these parasites to the available antimalarial drugs. Hence, the development of new antimalarial drugs is inevitable. Pyronaridine tetraphosphate (PNDP) is a new highly active antimalarial drug first developed in China. It is highly effective in treating malaria-infected patients in the regions of a chloroquine resistance. PNDP is a blood schizonticidal drug, active against the erythrocytic stage of malarial parasites. And it has already undergone extensive trials in humans against both *Plasmodium falciparum* and *Plasmodium viva*, and was found to be highly effective. The first objective of the present investigation was to synthesize carbon-14 labeled pyronaridine tetraphosphate (^{14}C-PNDP) by both a classical method and a microwave irradiation technique. Microwave irradiation technique was preferred because the classical method posed certain disadvantages such as a long reaction time, low yield and the usage of a large amount of starting materials. The application of a microwave irradiation facilitated in the use of high temperatures resulting in a decreased reaction time and employed a far less amount of the starting material when compared to the classical method. The second objective of this investigation was to determine the absorption, distribution, excretion and pharmacokinetics of the isotope labeled pyronaridine tetraphosphate in Sprague-Dawley rats. The details of this work will be presented.

The Effect of Some New Chromanon-1,3,4-Oxadiazolines on Inflammation-Induced Nitric Oxide Synthesis

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Flavones are a class of compounds widely present in nature. An excessive production of nitric oxide following the inductions of inducible nitric oxide synthase (iNOS) is involved in the pathogenesis of inflammation. It was found that some flavones block the activation of iNOS gene, by blocking the nuclear translocation of p65 and subsequent nuclear factor-kappaB inactivation. The literature reports some data regarding the importance of the azole heterocycles for the antimicrobial and antiphlogistic activity.

The aim of the study was to evaluate the effect of some newly synthesized polyheterocyclic systems containing the chromone and 1,3,4-oxadiazoline nucleus, on inflammation-induced nitric oxide synthesis. We used 6 groups of Wistar-Bratislawa male rats. The inflammation was induced by i.m. administration of turpentine oil. The positive control group of inflammation, and those treated with the 2-(2'-phenyl-7'-oxymethyl-croman-4'-on)-4-N-acetyl-5-aryl-Δ_2-1,3,4-oxadiazolines synthetized were compared with a group treated with diclofenac, a selective NOS2 inhibitor. After 24 hours from turpentine administration blood samples were harvested for the *in vitro* phagocytosis test, total leukocyte count, differential leukocyte count expressed as percentage, and serum nitrite/nitrates determination (Griess).

All tested compounds reduced nitric oxide synthesis, total leukocyte count, phagocytes percentages, and phagocytes activity.

References:

1. Alcaraz L.E., Blanco S.E., Puig O.N., Thomas F., Ferretti F.H. - Antibacterial Activity of Flavonoids Against Methicillin-Resistant *Staphylococcus aureus* strains, *J. Theor.Biol*., 2000**,** 205:231-240;

2. Timberlake C.F., Henry B.S. - Plants Flavonoids in Biology and Medecine II: Biochemical Cellular and Medicinal Properties, Eds V. Cody. E. Middleton. J.B. Harborne, A. Beretz, A.R. Liss, New York, 1988**,** 107-121;

3. Rada F., Tiperciuc B., Chirtoc I., Ionescu M., Oniga O., Ghiran D. - The Synthesis and the Study of the Antimicrobial Activity of Some Cromananon-Oxymethyl-Δ^2-1,3,4-oxadiazoline, Revista de Medicina şi Farmacie Tg. Mureş-Orvosi es Gyogyszereszeti Szemie, 50, Supl.II., pp191-195, 2004;

4. Tiperciuc B., Pârvu A., Palage M., Oniga O., Ghiran D. - The Synthesis and the Study of the Anti-Inflammatory Activity of Some 3-N-Acetyl-2-R-[2'-Aryl-4'Methyl-Thiazole-5'-yl]-Δ_2-1,3,4-Oxadiazoline, *Farmacia*, 5, 77-84, 1999;

The Effect of Pegylated Antisense Acetylcholinesterase on Hematopoiesis

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To determine whether the efficacy of entry and action of antisense oligonucleotides (AS-ODN) on hematopoietic stem cells *in vitro* could be improved by the addition of polyethylene glycol (PEG), a molecule of PEG was bound to AS- or sense-acetylcholinesterase (AS-ACHE or S-ACHE). The introduction of 0.1-0.5 μM PEG-AS-ACHE or 0.5 μM AS-ACHE into methylcellulose bone marrow (BM) cultures produced a doubling in number of colony-forming unit-granulocyte-erythrocyte-macrophage-megakaryocyte (CFU-GEMM) and a 5-fold increase in cell number of the PEG-ODN. Further increase in concentration of the PEG-ODN reduced colony numbers. PEG-AS-ACHE induced higher colony numbers and greatly increased megakaryocyte (MK) formation when compared with PEG and AS-ACHE added separately to the culture. In addition, differentials of the CFU-GEMMs indicated there was a direct relationship between MK number and PEG-AS-ACHE concentration. Under these culture conditions, 5 μM PEG alone gave control values of CFU-GEMM. On addition of FITC-PEG-AS-ACHE to the cell cultures, using confocal microscopy, the nuclei of both early and mature MKs were labeled specifically, whereas all other cellular nuclei were negative to the stain. The use of PEG-AS-ODN, affording specific delivery of AS-ODN to target cells, increased cell proliferation, and enhanced ODN uptake, may be of potential importance in stem cell expansion for BM transplantation and gene therapy.

NK1 / NK3 Dual Antagonists: Finding a Lead for a Schizophrenia Drug Discovery Programme

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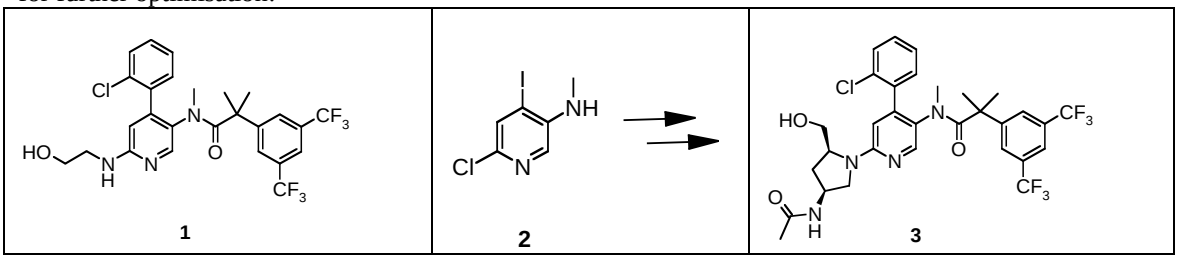
Schizophrenia affects 1% of the population and is characterised by hallucinations and delusions (positive symptoms) as well as anhedonia and social withdrawal (negative symptoms). The introduction of the first antipsychotic drug, chlorpromazine, in 1952 revolutionised the treatment of the disease. However, it soon became apparent that this and other antipsychotic drugs had serious side effects, a high non-responder rate, and did not substantially improve negative symptoms. Despite 50 years of research, a therapy that alleviates positive as well as negative symptoms, and that has a benign side effect profile, remains elusive.

The NK3 receptor antagonists osanetant and talnetant are novel investigational drugs which were shown to improve psychotic (positive) symptoms in schizophrenic patients, but had little effect on negative symptoms.[1] The NK1 receptor antagonists aprepitant, L-759274, and CP-122,721 were shown to be clinically efficacious in treating depression,[2] the symptoms of which are similar to the negative symptoms in schizophrenic patients. Both the NK3 receptor antagonists and the NK1 receptor antagonists did not show the side effects associated with established antipsychotic drugs. These clinical results provide a strong rationale for dual NK1 / NK3 receptor antagonists as a novel treatment for schizophrenia, addressing both positive and negative symptoms, while having an improved side effect profile.[3]

During our NK1 receptor antagonist project,[4] we discovered compound **1** with high NK1 receptor affinity, and some affinity for the NK3 receptor. Recognising an opportunity to optimise **1** into a lead for a schizophrenia drug discovery programme, we prepared a series of 100 similar compounds from a novel building block, **2**. A single compound from this series, **3**, met our requirements of high and reasonably balanced affinities for both the NK1 and the NK3 receptor, and was further characterised. PK and PD experiments demonstrated high exposure as well as target occupancy and confirmed **3** to be a viable lead for further optimisation.



References:

1. W. Spooren, C. Riemer, H. Meltzer, *Nature Drug Disc.* **2005**, *4*, 967.
2. S. McLean, *Curr. Pharm. Des.* **2005**, *11*, 1529.
3. C. A. Rizzo, J. C. Anthes, M. R. Corboz, R. W. Chapman, N.-Y. Shih, G. A. Reichard, K. J. Ng, J. A. Hey, *Curr. Top. Med. Chem.* **2003**, *3*, 1410.
4. T. Hoffmann, M. Boes, H. Stadler, P. Schnider, W. Hunkeler, T. Godel, G. Galley, T. M. Ballard, G. A. Higgins, S. M. Poli, A. J. Sleight, *Bioorg. Med. Chem. Lett.* **2006**, *16*(5), 1362.

Prenylation on Xanthonic and Flavonic Scaffolds: A Key Approach for Antitumor Activity

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Focusing on prenylated derivatives associated with xanthonic and flavonic scaffolds interesting bioactivities were revealed [1, 2]. If the synthesis of xanthones and flavonoids has been largely considered [3,4] the synthetic strategy for prenylation has been neglected.

In this communication we present synthetic methodologies for prenylation, applied very efficiently for the first time to xanthonic and flavonic building blocks, employing reactions catalyzed by *clays*, assisted by microwave (MW) and also combining clays and MW. Some prenylated derivatives evaluated for PKC modulation and inhibitory activity of growth of human tumor cell lines showed an increased performance and selectivity for MCF-7 cell line (breast), compared with simple oxygenated precursors, as well as estrogenic/antiestrogenic activities.

Acknowledgements: FCT, I&D 226/94; FEDER; POCI

References:

1. M. M. M. Pinto, *et al. Curr. Med. Chem.* **2005**, *12*(21), 2517-2538.
2. B. Botta, *et al. Curr. Med. Chem.* **2005**, *12*, 713-739.
3. M. E. Sousa, *et al. Curr. Med. Chem.* **2005**, *12*(21), 2447-2479.
4. *The Flavonoids*, Chapman and Hall, London, 1975.

Design and Synthesis of Isozyme Selective Protein Kinase C Inhibitors

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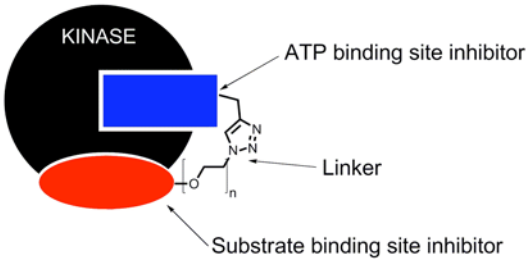
The Protein Kinase C (PKC) family consists of 11 homologous isoforms and dysfunctioning of single isozymes is related to diseases like tumour formation and diabetes. Due to the high similarity between isoforms the development of specific inhibitors is difficult. Our design of isozyme selective PKC inhibitors was based on bisubstrate inhibitors, in which an ATP competitive inhibitor is linked to a substrate binding site inhibitor. Inhibitor substrates were selected using dynamic peptide microarrays (Pamgene International B.V.*) that were phosphorylated with several PKC-isozymes. Substrates selectively phosphorylated by one single isoform, were synthesized as pseudosubstrate analogues suitable for bisubstrate formation. Mimetics of well known kinase inhibitor staurosporine were synthesized and used as ATP-binding site inhibitor and connected to all pseudosubstrates with the Huisgen 1,3-cycloaddition. Inhibition screening experiments have been carried on the dynamic peptide microarrays.



Post-Training Administration of Corticosterone Enhances Consolidation of Contextual Fear Memory and Hippocampal long-Term Potentiation in Rats

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This study was planned to study the effect of corticosterone on consolidation of contextual fear memory and long-term potentitaion in rats. In Experiment 1, Adult male rats were trained in a contextual fear conditioning system using a moderate (0.4 mA) intensity and received different doses of corticosterone immediately after training. Testing 24 h later, it revealed that corticosterone enhanced memory-consolidation. The most effective dose was 3 mg/kg. In Experiment 2, long-term potentitaion (LTP) was examined in rats that memory consolidation has been enhanced with corticosterone. For this, the rats were trained as above and received corticosterone at dose of 3 mg/kg immediately after training. Immediately after retention test (24h later), for LTP induction, 3 episodes of high frequency stimuli, 30 s apart, were delivered to the perforant path (PP), each consisting of 10 stimuli at 250 Hz. Population spikes (PS) of the population excitatory post synaptic potentials in the dentate gyrus (DG) was recorded 10 min before, and for 120 min after tetanization with 10 min intervals. Data indicated that corticosterone-treated animals display enhanced hippocampal neuronal excitability and LTP. The above findings suggest that glucocorticoids enhance memory consolidation through enhancing LTP in the DG of the hippocampus.

Synthesis, Biodistribution and Pharmacological Studies of Iozapine: A Potential Ligand for Brain Imaging

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The neurotransmitter dopamine plays an important role in the development of several neurological and psychiatric disorders such as schizophrenia, Huntington's disease, Parkinson's disease. Schizophrenia is a mental disorder characterized by chaotic jumbling and fragmentation of sensation and thought processes. The dopamine hypothesis is one of the several proposed in the last 50 years to understand the biology of schizophrenia, which links the positive psychotic symptoms with hyperactivity of dopaminergic neurons in the mesolombic region of the brain. The present paper shows the synthesis of Iozapine, radiolabeling, bidistribution studies and the relative competition profiles of iozapine and clozapine in regarding displacing [^{3}H]-clozapine, and radioligands for the 5-hydroxytryptamine$_2$ (5-HT$_2$), muscarinic, and dopamine D_1 and D_2 receptors in beef cortical or striatal tissue. Iozapine differed from clozapine chiefly in that it required concentrations more than an order of magnitude greater than those of clozapine to displace the ligand for muscarinic receptors. This characteristic of iozapine may be advantageous in brain imaging studies. In addition, it is possible that iozapine may have therapeutic benefits relative to clozapine in the treatment of schizophrenia.

A Novel Strategy of Anti Cancer Therapy: Targeting Specifically the GTPase RhoA and Development of a new Strategy for siRNA Therapy: *In vitro* and *In vivo* Assays

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We present here a new strategy of cancer therapy, which brings new developments in biotechnology and a new formulation allowing the intra venous administration of siRNA. Over expression of RhoA in cancer indicates a poor prognosis, due to increased tumour cell proliferation and invasion and tumour angiogenesis. We also showed that in breast cancer, the increase in RhoA is significantly associated with lymph node invasion. Therefore, the inhibition of RhoA may be a good strategy in breast cancer therapy.

We firstly showed that *in vitro* anti-RhoA siRNAs inhibited breast cancer proliferation and invasion more effectively than conventional blockers of Rho-mediated signalling pathways, such as geranyl-geranyl transferase inhibitors (GGTis): ie 40 ± 7 % inhibition by GGTi and 95 ± 4 % by siRNA anti RhoA for both cell proliferation and invasion of aggressive cancer cell line MDA-MB 231. It is suggested that is it is due to the specificity of siRNA action as GGtis inhibited both RhoA and RhoB which is considered as an anti oncogen. In addition siRNa anti RhoA inhibited angiogenesis whatever the angiogenic factor (bFGF, VEGF etc…) but it was without effect on unstimulated endothelial cells. Therefore using siRNA it was demonstrated the clinical relevance to specifically target RhoA in aggressive breast cancer. Therefore it was tempting to use siRNA nor only for target validation but also for therapeutical purpose.

In a second step, we presented the efficacy and lack of toxicity of intravenous administration of anti RhoA siRNA encapsulated in chitosan-coated polyiso-hexylcyanoacrylate (PIHCA) nanoparticles, in xenografted MDA-MB-231 tumours. The siRNA was administered every 3 days at a dose of 150 or 1500 µg/Kg body weight in nude mice. This treatment inhibited the growth of tumours by 90% in the 150 µg and even more in the 1500 µg group. Necrotic areas were observed in tumours from animals treated with 1500 µg/Kg anti-RhoA siRNA, due to angiogenesis inhibition. This therapy was found to be devoid of toxic effects, as evidenced by similarities between control and treated animals for the following parameters: body weight gain, biochemical markers of hepatic, renal, and pancreatic function, and macroscopic appearance of organs after 30 days of treatment. Due to its efficacy and the absence of toxicity, it is suggested that this strategy of siRNA anti RhoA holds significant promise for the treatment of aggressive cancers. In addition, our strategy represents also a novel formula to inhibit specific expression of a gene in cancer therapy or other diseases.

Antiproliferative Effects and Enhancement of the Growth Inhibitory Action of 4-Hydroxytamoxifen in Estrogen Receptor Positive (ER+) Breast Cancer Cell Line Induced by Prenylated Xanthones

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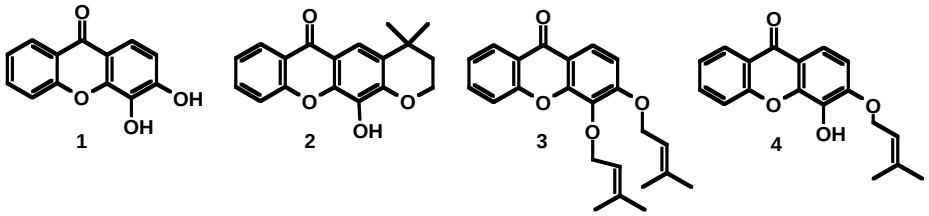
In order to improve inhibitory effects of 3,4-dihydroxyxanthone (**1**) on the growth of human breast tumor cell lines [1,2], the synthesis of new prenylated derivatives was planned. The effects of xanthones **2-4** on the *in vitro* growth of ER (+) MCF-7 and ER (−) MDA-MB-231 cells were investigated. Prenylated compounds comprise more selective derivatives to ER(+) MCF-7 cells, with prenylxanthone **2** being the most potent (GI_{50} 5 μM).

Compound **2** showed a dose-dependent inhibitory effect either on complete and steroid-free RPMI medium suggesting estrogenic independence. The growth inhibitory action of the antiestrogen 4-hydroxytamoxifen in ER(+) MCF-7 cell line was strongly enhanced by compound **2**. This is the first report of additive interactions between xanthones and antiestrogens.



Acknowledgements: FCT, I&D 226/94; FEDER; POCI

References:

1. Pedro M *et al*. *Bioorg Med Chem* **2002,** 10, 3725.

2. Castanheiro RAP *et al*. *Bioorg Med Chem* **2007**, 15, 6080.

A Residue Specific Sight of Anthrax Lethal Factor – MKKS Interaction and Selectivity Towards Structure-Driven Drug Design

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Anthrax toxin (ATx), which is produced by the bacterium *Bacillus anthracis*, possesses a key role to the Anthrax disease. It consists of three distinct proteins, one of which is the anthrax Lethal Factor (LF), a gluzincin Zn-dependent highly specific metalloprotease (~90 kDa) [1]. LF cleaves most isoforms of the Mitogen-Activated Protein Kinase Kinase (MAPKK or MKK) family close to their amino termini, leading to the inhibition of one or more signalling pathways [2]. Recently, the crystal structure determination of complexed and uncomplexed LF has promoted studies on the molecular basis of their enzymatic activity and a platform for rational design of domain-selective inhibitors [3]. Since there is not enough data available on the enzyme-substrate interactions available so far, we applied Molecular Dynamics Simulations in order to study the factors govern the complex LF-substrate interactions at atomic level. A novel *fragment- and knowledge-based docking approach* [4] has been applied in order to pose the peptide substrates (MKKs) into LF catalytic site. This is molecular modeling study of an LF-peptide substrate complex that monitors at atomic level the structural basis of LF's peptidase activity and offers novel insights into subsites that are distant (>20 Å) from the obligatory binding site (Zn(II) metal ion) and were not identified in the crystal structures of LF-inhibitor complexes.

Further comparison and analysis of Molecular Dynamics simulation data of eight LF-MKKs complexes provide valuable information in the quest for understanding the structural and physicochemical determinants of enzyme substrate binding and specificity. LF catalytic site residues, critically involved in enzyme-peptide substrate binding through electrostatic or water-mediated interaction have been elucidated and could be targeted for further design of bioactive molecules with enhanced binding activity against LF metallopeptdase [5].

References:

1. Pannifer, A.D.; Wong, T.Y.; Schwarzenbacher, R.; Renatus, M.; Petosa, C.; Bienkowska, J.; Lacy, D.B.; Collier, R.J.; Park, S.; Leppla, S.H.; Hanna, P.; Liddington, R.C. *Nature*, **2001,** 414, 229.

2. Duesbery, N.S.; Webb, C.P.; Leppla, S.H.; Gordon, V.M.; Klimpel, K.R.; Copeland, T.D.; Ahn, N.G.; Oskarsson, M.K.; Fukasawa, K.; Paull, K.D.; Vande Woude, G.F. *Science* **1998,** 280, 734-737.

3. Dalkas G.A., Papakyriakou A., Vlamis-Gardikas A., Spyroulias G.A., *Mini Rev Med Chem*. **2008,** 0000.

4. Papakyriakou A., Spyroulias G.A., Sturrock E.D., Manessi-Zoupa E. and Cordopatis P. *Biochemistry* **2007**, 46, 8753-65.

5. Dalkas G.A., Papakyriakou A., Vlamis-Gardikas A., Spyroulias G.A., *submitted.*

Novel Insights into Angiotensin-Converting Enzyme Domain Selectivity & Structure-Based Drug Design

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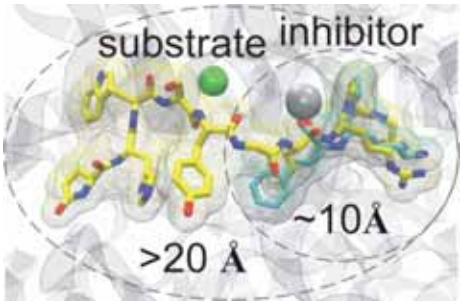
Human Angiotensin-I Converting Enzyme (ACE) is a central component of the renin-angiotensin system and a major target for cardiovascular therapies. The somatic form of the enzyme (sACE) comprises two homologous domains (N and C), each bearing a zinc active site with similar but distinct substrate and inhibitor specificities. On the basis of the recently determined crystal structures of both ACE domains [1,2], we have studied their complexes with Angiotensin-I (AI), Bradykinin (BK) and Gonadotropin-Releasing Hormone (GnRH), which is cleaved releasing both the protected NH_2- and COOH-terminal tripeptides. This is the first molecular modeling study of an ACE-peptide substrate complex that examines the structural basis of ACE's endopeptidase activity and in concert with the data for AI and BK offers novel insights into subsites that are distant from the obligatory binding site and were not identified in the crystal structures. Especially, data which indicate that a bridging interaction between positively charged ACE residues (Arg500) and buried chloride, when compared with structural information of ACE-inhibitors complexes: (a) promotes our understanding of how the two domains differ in their function and specificity, (b) elucidate the endo-/exo-peptidase ACE activity *in vitro* and (c) provides an extension of the pharmacophore model used for structure-based drug design up to the S_7 subsite of the enzyme [3], towards new inhibitors with enhanced biological and pharmaceutical value.



References:

1. Natesh, R., Schwager, S.L.U., Sturrock, E.D., and Acharya, K.R. (2003) Nature 421, 551-554.

2. Corradi, H.R., Schwager, S.L.U., Nchinda, A.T., Sturrock, E.D., and Acharya, K.R. (2006) J. Mol. Biol. 357, 964-974.

3. Papakyriakou A., Spyroulias G.A., Sturrock E.D., Manessi-Zoupa E. and Cordopatis P. (2007) Biochemistry, 46, 8753-65.

Nanoparticle-Based Delivery of Aminoglycoside Antibiotics

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Tobramycin and carbohydrates were covalently linked and formulated into nanoparticles using a microemulsion technique resulting in particles of 30-50 nm in diameter. Release studies indicated two populations of aminoglycosides, one freely and one tightly associated with the nanoparticle matrix. The MIC values were comparable among nanoparticle-bound tobramycin and non-formulated tobramycin against *Pseudomonas aeruginosa*. To test the efficacy of tobramycin-nanoparticles in lung infections in mice, 3 studies were performed using *Pseudomonas* inoculations of $0.64x10^7$, $1.46x10^7$, and $0.84x10^7$ CFU/ml, and with intravenous injection of drug. Nanoparticles were either untargeted or targeted to macrophages *via* the attached peptide CLVGFY. Mouse body temperatures were measured for 24 hours, at which time the animals were sacrificed to determine CFUs in their lungs. In all 3 studies, the non-targeted nanoparticles showed comparable efficacy to free tobramycin, indicating that the nanoparticles were not removed from circulation such that tobramycin was excreted more rapidly than the free drug, or did not distribute to a compartment such that access of tobramycin to the site of infection was lost. More importantly, all the mice in the group that were treated with macrophage-targeted nanoparticles showed the largest reduction in CFU in the lungs. This signifies the potential of targeted nanoparticle-based antibiotics in improving therapeutic efficacy.

Comparison the Effects of Aqueous Extract of Glycyrrhiza Glabra, Dexamethasone and Stress on Acute and Chronic Pains in Mice

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Our previous investigation showed that Glycyrrhiza Glabra (GG) modulates pain in mice. The aim of this work was to examine the role of GG on acute and chronic pain and compares its effect with Dexamethasone (DEX) and stress (ST) using formalin test in mice. In this study male albino mice (25-30 gr.) in 7 groups (n=49) were used. GG (200, 500 and 1000 mg/kg), DEX (0.5, 1 and 2 mg/kg) and vehicle were injected 30 min before test. Stress was applied by 1 min swimming in cold water (18 – 22°). Acute (5 min) and chronic pains (5-40 min) were assessed after injection of formalin 5% (25µl) in right paw using standard scores. Results indicated that GG, DEX and ST have analgesic effects both on acute and chronic pains ($P<0.01$ in comparison with control group). Finding above showed that GG extract, DEX and ST have modulator effects on both acute and chronic pain formalin test. Further research is required to determine the mechanisms by which GG extract has an inhibitory effect on pain sensation.

Biochemical Analysis of Two Isoforms of the Human Rad51 Protein

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In the homologous-recombinational repair (HRR) of the double strand DNA breaks (DSBs), which are frequently induced by ionizing radiation and replication error, the Rad51 protein is an essential enzyme. The Rad51 protein catalyzes homologous-pairing and strand-exchange reactions during the HRR. In humans, two Rad51 isoforms have been reported. The conventional Rad51 (HsRad51) protein has a Lys residue at position 313. On the other hand, the HsRad51-Q313 protein, in which the Lys313 residue is replaced by Gln, was reported, probably a polymorphic variant. In this study, we purified the HsRad51-K313 and HsRad51-Q313 isoforms, as recombinant proteins, and tested their biochemical activities *in vitro*. We found that the HsRad51-Q313 protein exhibited significantly enhanced strand-exchange activity under conditions with Ca^{2+}, as compared to the HsRad51-K313 protein. Interestingly, the difference in their strand-exchange activitues was not observed without Ca^{2+}. A double-stranded DNA unwinding assay, which is sensitive assay for the Rad51-filament formation, revealed that the HsRad51-Q313 protein clearly showed enhanced DNA unwinding activity, probably due to its enhanced filament-formation ability. These implicate that the surface around position 313 may be a potential target for drug design to regulate the recombinase activity of the Rad51 protein, which is frequently perturbed in tumor cells.

In Silico Protein-Protein Interaction Peptide Inhibitors Design for Cyclin-Dependent Kinases (CDKs)

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Both CDK5 and CDK2 belong to a large family of heterodimeric serine/threoine protein kinases. In spite of the very similar 3-dimensional structures as reflected in their sequence identity of 60% between these kinases, they are respectively involved in the pathology of distinct diseases, for instance, neurodegenerative diseases (Alzheimer's disease) and cancer. Presently, all the existing inhibitors target the ATP-binding pocket of the catalytic site of the kinases; unfortunately, selectivity of these inhibitors is far from ideal and remains a challenging area, probably due to the conservation of the amino acids lining the CDK ATP-binding pocket. CDK5 and CDK2 are respectively regulated by different activating proteins, which share little sequence similarity and are subject to different regulatory strategies of the CDKs. Recently, important differences in the interactions between the kinases and their activators were revealed. These results, therefore, lead us to the ideas of locating new binding site of inhibitors and designing new types of peptide inhibitors with new inhibition mechanism to effectively and selectively stop the activition of the kinases.

Cryopreservation of Differentiated HL-60 Cells - Development of a Novel *in vitro* Pyrogen Testing Kit

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Introduction*:* In order to insure safe administration of parental drugs all products must be tested by one of two validated pharmacopoeia methods. The LAL test or the Rabbit Pyrogen Test. We have recently developed a new pyrogen assay based on the HL-60 cell line. The assay has proven to provide a better sensitivity towards all tested substances in respect to the rabbit pyrogen test, and the assay is capable of detecting a broad variety of pyrogens.

The HL-60 cells must be differentiated before they can be used in the assay. This process requires facilities for cell culturing and in many occasions this may limit the use of the test. To eliminate this limitation we have developed a rate controlled process for cryopreservation of the differentiated cells.

Results: After washing the thawed cells can be used directly in the assay. The cells maintain their ability to produce reactive oxygen species (ROS) upon challenge with pyrogens and this property is stable for at least 2 months.

Conclusion: Cryopreservation of differentiated HL-60 cells is an efficient method to supply differentiated cells to laboratories without cell culturing facilities. Furthermore the process makes a kit for pyrogen testing obtainable

The Effect of Some New Synthesis Flavonoids on Inflammation-Induced Oxidative Stress

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Reactive oxygen species (ROS) play a key role in enhancing the inflammation through the activation of NF-kappaB and AP-1 transcription factors, and nuclear histone acetylation and deacetylation in various inflammatory diseases. Flavonoids have been suggested to exert human health benefits by anti-oxidant and anti-inflammatory mechanisms. The literature reports some data regarding the importance of the 1,3,4-oxadiazole and Δ_2-1,3,4-oxadiazoline nucleus for the antibacterial, antifungal and antiflogistic activity. In this study, we investigated whether some newly synthesized heterocyclic systems containing the chromone nucleus, influence the inflammation-induced oxidative stress. Six groups of Wistar-Bratislawa male rats were used. The inflammation was induced by i.m. administration of turpentine oil. The positive control group of inflammation, and those treated with the 7-Arylidenhydrazinocarbonyl-methylen-oxy-flavones and 2-(2'-phenyl-7'-oxymethyl-croman-4'-on)-4-N-acetyl-5-aryl-Δ_2-1,3,4-oxadiazolines synthetized were compared with a group treated with diclofenac, a selective NOS_2 inhibitor. After 24 hours from turpentine administration blood samples were harvested for measuring total oxidation status (TOS) and measuring total antioxidant response (TAR). According to the results we concluded that the tested compounds reduced the oxidative stress by decreasing TOS and increasing TAC. The anti-oxidative effect was better than that of diclofenac.

References:

1. Rahman I, Biswas SK, Kirkham PA.- Regulation of Inflammation and Redox Signaling by Dietary Polyphenols, Biochem Pharmacol., 72(11):1439-52, 2006.

2. Harris GK, Qian Y, Leonard SS, Sbarra DC, Shi X. - Luteolin and Chrysin Differentially Inhibit Cyclooxygenase-2 Expression and Scavenge Reactive Oxygen Species but Similarly Inhibit Prostaglandin-E2 Formation in RAW 264.7 Cells, J Nutr.,136(6):1517-21, 2006.

3. Rada F., Tiperciuc B., Chirtoc I., Ionescu M., Oniga O., Ghiran D. - The Synthesis and the Study of the Antimicrobial Activity of Some Cromananon-Oxymethyl-Δ^2-1,3,4-Oxadiazoline, Revista de Medicina şi Farmacie Tg. Mureş-Orvosi es Gyogyszereszeti Szemie, 50, Supl.II., pp191-195, 2004.

4. Tiperciuc B., Pârvu A., Palage M., Oniga O., Ghiran D. - The Synthesis and the Study of the Anti-Inflammatory Activity of Some 3-N-Acetyl-2-R-[2'-Aryl-4'Methyl-Thiazole-5'-yl]-Δ_2-1,3,4-Oxadiazoline, *Farmacia*, 5, 77-84, 1999;

Pharmacogenetic Approaches in Psychiatric Patients

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Although polymorphic enzyme CYP2D6 represents only 2% of total CYP450 protein in the liver, it is involved in the metabolism of approximately 20-25% of commonly prescribed drugs among them antidepressants and antipsychotics. To date, more than 60 variant alleles have been described (www.cypalleles.ki.se), which can be grouped in functional alleles (like *1, *2, *35) encoding normal activity, null alleles (like *3, *4, *5) encoding no activity, and decreased activity alleles (like *9, *10, *41) encoding enzymes with residual metabolic capacity. Consequently, four phenotypes can be manifested: extensive metabolizer (EM; normal metabolism: 2 active alleles), intermediate metabolizer (IM; only 1 active allele), poor metabolizer (PM; 2 inactive alleles) and ultra-rapid metabolizer (UMs; gene duplication). However, CYP2D6 phenotypes show large differences in distribution among ethnic groups: Caucasians PM 5-10%, UM 1-10%; East Asians PM 1%, UM 0-2%; African Americans PM 1-23%, UM 2%; North Africa and Middle East PM 2%, UM 10-29%; and Mexican Americans PM 3%, UM 1%.

In the light of these data, the aim of the study was to assess the prevalence of null alleles, genotypes and phenotypes in a group of psychiatric patients suffering from schizophrenia (n=86) in comparison with healthy individuals (n=145), and to study the association of CYP2D6 allele and genotype distribution with adverse drug effects. The method of multiplex allele specific PCR identifying *3,*4,*5,*6,*7 and *8 alleles was used. Only CYP2D6*3,*4 and *6 mutant alleles were found in all study subjects. A significant difference was observed between schizophrenic patients and controls in allele frequency ($p=0.002$), genotype distribution ($p=0.016$), and phenotype prevalence ($p=0.018$). The odds ratio of 2.542 for 2D6*4 suggested a significant association between this allele and schizophrenia, significantly contributing to PM phenotype (odds ratio=5.020). Results of the association study revealed a significant difference in allele prevalence ($p=0.002$), genotype ($p=0.029$), and phenotype ($p=0.002$) distribution between patients without and with adverse drug effects. A relative risk of 2.626 and 5.333 for 2D6*4 and 2D6*6, respectively, and of 7.08 for PM phenotype suggested a significant association between hereditary susceptibility to a particular type of drug metabolism and drug side effects. Although referring to a limited number of patients, our results confirmed the clinical use of the CYP2D6 genotype profile to be useful in predicting the effect of psychoactive drugs, as already recommended, and it should precede therapy initiation to achieve optimal therapeutic effect in patients administered drugs that are substrates of this highly polymorphic enzyme.

Design and Synthesis of Transition State Inhibitors for Glycosyltransferases

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Glycosyltransferases (GT's) are indispensable to cellular life in eukaryotes by producing glycan linkages with a unique contribution to the development and function of physiological systems in the context of living organisms. Also connections between GT's and mammalian disease processes are being made recently. Therefore, inhibitors of these enzymes have a great therapeutic potential.

A new generation of inhibitors based on carbohydrate mimetics has been recently emerged. The model compounds of this type are structurally altered analogs of carbohydrates designed to mimic the shape and functionalities of the natural substrates in the ground and/or transition state (TS), respectively, with the aim of modulating their biological activity. In the case of inhibitors of GT's, carbohydrate mimetics are often used to imitate the TS of the enzymatic reactions promising a better inhibition than the natural carbohydrate substrate.

This contribution is based on the determination of the TS structure for GT's and design of TS analogues based on molecular modeling and introduces the synthesis of the first simple precursor of this type - namely benzyl 2-thio-β-D-fructofuranoside 1-phosphate.

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Orally Active TNF-Alpha Synthesis Inhibitors: A Role in the Treatment of Neurodegeneration?

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Unregulated activation of macrophages and microglia cause the overproduction of cytokines, particularly TNF-alpha, which are associated with rheumatoid arthritis, Crohns disease, Alzheimer's and Parkinson's disease. In arthritis, sequestration of circulating TNF-alpha protein has proven a viable clinical approach, epitomized by agents like Remicade and Enbrel (protein based anti-TNF-alpha agents). However, large protein based treatments do not easily translate to the brain. We preferred to reduce the biosynthesis of TNF-alpha protein by novel agents with high brain bioavailability. Thalidomide reduces the translation of TNF-alpha mRNA into protein. We have instituted thiocarbonyl groups to thalidomide to generate novel thiothalidomides. The effects of thalidomide and analogs on bacterial lipopolysaccharide (LPS)-induced overproduction of TNF-alpha in rodents have been assessed. Animals were administered vehicle/drugs prior to a single challenge of LPS, 4 hr after LPS exposure the animals were sacrificed and TNF-alpha gene expression and protein levels were analyzed. LPS caused marked increases in TNF-alpha mRNA and protein. When compared with thalidomide several thiothalidomide agents were more effective at lowering the levels of TNF-alpha mRNA (hippocampus) and protein in plasma and CSF. Thus novel thiothalidomide agents may present a novel approach to treating the neuroinflammatory component that drives many common neurodegenerative diseases.

Effect of Hydro-Alcoholic Extract of *Valeriana Officinalis* on Anxiety Related Behavior in Mice: An Interaction with Serotonergic and Noradrenergic Systems

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This study was designed to evaluate the effects in different doses of the hydro-alcoholic extracts of *Valeriana Officinalis* VO on anxiety reaction in mice and interaction of these effects with Serotonergic and Noradrenergic systems. 80 male albino mice (25–30 gr) and Elevated plus Maze (EPM) model for assessment of anxiety were used. Hydro-alcoholic extracts of VO (200 and 250 mg/kg) with or without Ketanserin (KET) 1 mg/kg or Propranolol (PRO) 1 mg/kg or saline (1ml/kg) were injected IP, 30 min before of test. At the first for increasing activity animals have put inside the black wall box for 5 min. Then animal transfer to the EPM and evaluation their anxiety reaction that including of number entrances and time spent in open arm. Results indicated that extract of VO in doses of 200 and 250 reduced of anxiety reaction ($P<0.05$) and injection of KET or PRO modulate of these effects significantly ($P<0.05$). It is concluded that the extract of VO plays an important role in fear and anxiety and there is interaction between these effects and Serotonergic and Noradrenergic systems.

Effects of Central Application of Carbenoxolone on Ischemic Damage in Transient Model of Focal Cerebral Ischemia

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Using a potent gap junction blocker carbenoxolone (CBX), we investigated the contribution of gap junctional communication to cell death in cortex and striatum regions in transient model of focal cerebral ischemia. Transient focal cerebral ischemia was induced in rats by 60 min middle cerebral artery occlusion (MCAO), followed by 23 h reperfusion. CBX administered into the right ventricle at doses 1, 12, 25, 50 and 100, μg/kg at the beginning of MCAO. Cortical, striatal infarct volume and motor dysfunction were assessed 24 h after MCAO. Administration of CBX at doses of 1, 12, 25 and/or 50 μg/kg significantly reduced cortical infarct volumes by 35%، 49%, 41% and 43%, respectively ($P<0.001$). The higher dose of CBX (≤100 μg/kg, icv) had no effect or even exacerbated ischemic injuries. CBX only at dose of 25 μg/kg significantly reduced striatal infract volume and neurological dysfunction ($P<0.01$). These results also provide evidence that gap junctional communication may involve in the pathophysiology of secondary brain damage after transient focal cerebral ischemia.

Towards Potent Inhibitors of the MDM2-P53 Protein-Protein Interaction

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The p53 tumor suppressor acts as "the guardian of the genome" by reacting to cellular stress, which may be caused by hypoxia, DNA damage, or oncogenic signalling. The activity of p53 is tightly regulated by the MDM2 protein, which is transcribed in response to p53 activation. In normal cells the balance between active p53 and inactive MDM2-bound p53 is maintained in a negative feedback loop. Inhibition of the MDM2-p53 protein-protein complex by small molecule inhibitors is expected to reactivate normal p53 pathways in cells overexpressing MDM2, consequently exerting an anti-cancer effect.

Our previous studies, using structure-based design approaches, have resulted in the identification of novel small molecule inhibitors of the MDM2-p53 interaction, based on an isoindolinone scaffold. ^{1}H-^{15}N HSQC NMR structural studies indicated a plausible binding mode, which was used to design improved inhibitors. Further NMR studies have indicated that alternative binding modes are likely, and additional structural modifications have been explored, with the aim of defining the main interactions between these isoindolinones and MDM2. The cellular activities of key compounds have been determined and show dose dependent induction of p53 regulated genes in a variety of model systems.

Synthesis of BACE-1 Inhibitors with a Tertiary Hydroxyl Group as the Central Core

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Alzheimer's disease is a neurodegenerative disorder characterized by an increasing accumulation of plaque in the brain. The major component of the plaque found in Alzheimer patients is the peptide fragment amyloid-beta (Aβ). BACE-1 (β-secretas), a human aspartic protease, is believed to be responsible for the *N*-terminal cleavage of a large transmembrane protein resulting in the Aβ fragment after subsequent proteolysis by γ-secretas. An attractive approach to slow down or halt the progress of Alzheimer's disease, supported by knock out studies, is to inhibit the BACE-1 enzyme.

Previously reported BACE-1 protease inhibitors often contain a transition state isostere utilizing a secondary hydroxyl to provide key interactions with the enzyme. The aim with this study was to synthesize and evaluate BACE-1 inhibitors containing a tertiary alcohol in the transition state mimic (Figure 1). Introduction of a masked tertiary hydroxyl group might improve both membrane permeability and the ability to cross the blood-brain barrier.

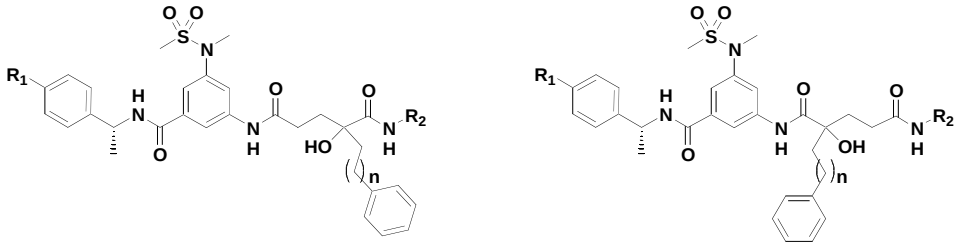

Figure 1. Generic structures of synthesized BACE-1 inhibitors.

Correlation of Activity and Sequence Similarity in Kinase Targets

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In a project, knowledge from similar targets can provide a rationale for a lead-finding strategy. If it would be possible to link sequence information to data describing activity of compound classes, this could be employed directly and cheaply at the start of a project. Here we describe an analysis of kinase sequence and kinase compound activity data. We investigate whether correlations can be found, and discuss the possible use of such information in a project.

Investigation of the Effect of Silymarin on the Liver in Experimental Sepsis

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Aim: The aim of this study was To investigate the effect of silymarin (extract of *Silybum marianum*) on the liver of septic rats was at early stage of sepsis.

Material and Methods: Polymicrobial sepsis was induced by the cecal ligation and perforation (CLP) technique. Sepsis and sepsis + silymarin treated groups received vehicle or silymarin (50 mg/kg, orally). The rats were decapitated 6 h after the CLP procedure. Protein, glutathione, lipid peroxidation levels, tissue factor activity and some enzyme activities were determined and polyacrylamid gel electrophoresis was carried on liver samples. TNF-α, IL-1β and IL-6 were determined in blood samples.

Results: Glutathione levels significantly increased and tissue factor activity significantly decreased in both sepsis and sepsis+slymarin treated groups when compared with control. Superoxide dismutase activity significantly decreased in sepsis+silymarin treated group when compared control and sepsis groups. No significant differences were found between protein bands in electrophoresis. Blood proinflammatory cytokine levels significantly decreased in silymarin treated sepsis group when compared with sepsis group.

Conclusions: The effect of silymarin did not become definite since oxidative damage was not obvious in liver consequently it can be suggested that some protective mechanisms related with glutathione in liver may be involved in early stage sepsis.

Stability indicating HPTLC Determination of-Carvedilol in Pharmaceutical Dosage Form

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A simple, selective, precise and stability-indicating high-performance thin-layer chromatographic method of analysis of carvediiol in pharmaceutical dosage form was developed and validated. The method employed TLC aluminium plates precoated with silica gel 60F-254 as the stationary phase. The solvent system consisted of toluene-2-propanol-ammonia (7.5:2.5:0.1, v/v/v). This system was found to give compact spots for carvediiol (R, value of 0.50). Carvediiol was subjected to acid and alkali hydrolysis, oxidation, photochemical degradation and thermal degradation. Also, the degraded product was well separated from the pure drug. Densitometric analysis of Carvedilol was carried out in the absorbance mode at 240 nm. The linear regression analysis data for the calibration plots showed good linear relationship with coefficient of regression value, r^2 = 0.9986 in the concentration range 64-192 ng per spot. The value of correlation coefficient, slope and intercept were 0.9992, 11.38 and 193.57, respectively. The method was validated for precision, recovery, ruggedness and robustness. The limits of detection and quantitation were 1.6 and 6.4 ng per spot, respectively. The drug undergoes degradation under acidic and basic conditions. All the peaks of degraded product were resolved from the active pharmaceutical ingredient with significantly different R, values. The samples degraded with hydrogen peroxide, thermal and photochemical degradation showed no additional peak. This indicates that the drug is susceptible to acid-base hydrolysis degradation. Statistical analysis proves that the method is reproducible and selective for the estimation of said drug. As the method could effectively separate the drug from its degradation product, it can be employed as a stability-indicating one.

Cucurbit[n]uril for Drug Delivery

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Cucurbit[*n*]uril Q[*n*] (eg fig 1) are a family of macrocyclic molecules with hydrophobic cavities and electronegative carbonyl rimmed portals[1,2].

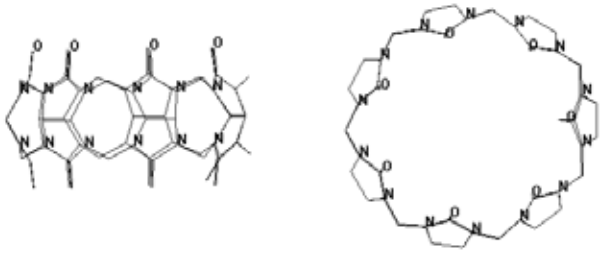

Fig. 1 Q[7]

Cucurbiturils can encapsulate a number of smaller molecules, including multinuclear Pt(II) anticancer drugs[3], increasing their bio-life-times and reducing toxic side effects. EdPzMN Pt(II) a new Pt drug is encapsulated in Q[7] and Q[8] (fig. 2 left to right), showing an increased folding in Q[8] which leads to an increased protection of the Pt(II) metallo centre. The encapsulation and mechanism of protection is discussed.

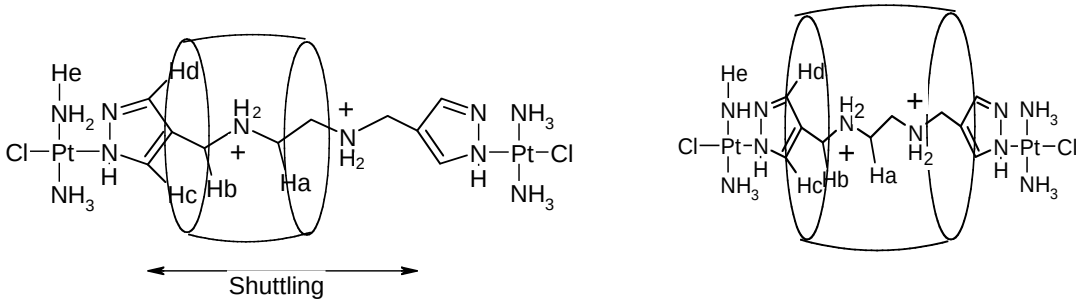

Fig. 2 Encapsulation of EdPzMN with Q[7] (left) and Q[8] (right).

References:

1. I. Day, R. J. Blanch, A. P. Arnold, S. Lorenzo, G. R. Lewis, and I. Dance, *Angrew. Chem. Int. Edit.*, 2001, **41**, 275.

2. J. Kim, I. S. Jung, S. Y. Kim, E. Lee, J. K. Kang, S. Sakamoto, K. Yamaguchi, and K. Kim, *J. Am. Chem. Soc.*, 2000, **122**, 540.

3. N. J. Wheate, Collins, J. G., *Coordination Chemistry Reviews*, 2003, **241**, 133.

Novel Group of Biological Response Modifiers: Inhibitors of Endoplasmic Ca^{2+}-ATPase

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A crucial role in the maintaining Ca^{2+} homeostasis within the cell is played by a family of sarco/endoplasmic reticulum (ER) Ca^{2+}-ATPases (SERCA). Inhibition of SERCA pumps leads to a release of Ca^{2+} from ER stores and an influx of Ca^{2+} from extracellular space. Changes in intracellular free Ca^{2+} levels are known to modulate cellular signaling and gene expression. The most widely used selective SERCA inhibitor is a sesquiterpene lactone of guaianolide type thapsigargin (TG) isolated from the Mediterranean plant *Thapsia garganica* L. We have found that TG is a potent stimulator of NO production in rat peritoneal macrophages, the effect being more pronounced in the presence of lipopolysaccharide. The effect is obviously due to the ability of TG to stimulate production of IFN-γ. Similar effects have been found for the TG structural analogue trilobolide isolated from *Laser trilobum* L., and for other SERCA inhibitors such as cyclopiazonic acid (CPA) and 2,5-di(*t*-butyl)-1,4-benzohydroquinone (DBHQ). Thapsigargin and trilobolide stimulate secretion of IFN-γ also in human peripheral blood mononuclear cells. As far as IFN-γ is recognized for its antiinfectious therapeutic effectiveness, further preclinical research of these agents is warranted.