Cell Engineering and Molecular Pharming for Biopharmaceuticals

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Biopharmaceuticals are often produced by recombinant *E. coli* or mammalian cell lines. This is usually achieved by the introduction of a gene or cDNA coding for the protein of interest into a well-characterized strain of producer cells. Naturally, each recombinant production system has its own unique advantages and disadvantages. This paper examines the current practices, developments, and future trends in the production of biopharmaceuticals. Platform technologies for rapid screening and analyses of biosystems are reviewed. Strategies to improve productivity *via* metabolic and integrated engineering are also highlighted.

Cholinesterases Inhibitory Activity of Testosterone and Some of its Metabolites

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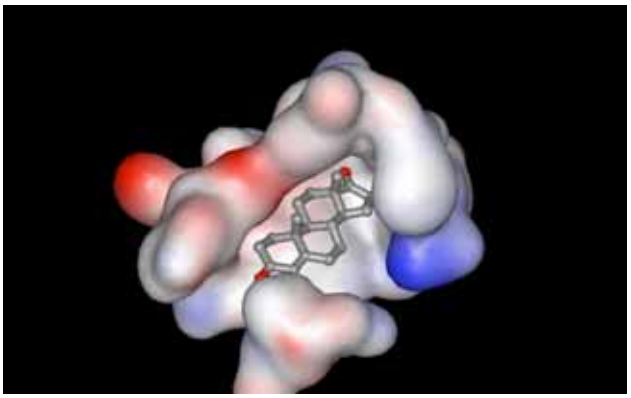
Although many reports demonstrated that the decrease in testosterone levels in elderly men is associated with decline of memory and cognitive functions, the inhibitory effect of testosterone on cholinesterases was not investigated. We report here, the *in vitro* inhibitory effect of testosterone and some of its metabolites on acetylcholinesterase (AChE) and butyrylcholinesterase (BChE). IC_{50}, the type of inhibition, and the kinetic parameters were also determined. Molecular modeling was carried out in order to develop a better understanding of the inhibitor- enzyme interactions.

The results showed that testosterone and some of its derivatives inhibit BChE selectively. The highest inhibition value (IC_{50} = 1.55 μM) was observed for androt-4-en-3,7-dione; the main metabolite of testosterone in the body. The inhibition was found to be noncompetitive, with mainly hydrophobic interactions between the inhibitor and butyrylcholinesterase.



Androst-4-en-3,17-dione at the opening of the gorge of BChE

High Throughput Compound Library Screening for Potential Antiviral Drugs for Dengue Virus

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Dengue infection is re-emerging as a major global disease and is classified as a Category A priority pathogen. Dengue viruses are estimated to infect 50-100 million people annually and are considered to cause one of the most important Arthropod-borne viral diseases in terms of human morbidity and mortality. We have used high performance supercomputer environment: molecular modeling and dynamics, *ab initio* molecular orbital computations to study the Crystallographic models of the dengue viral DEN2 Envelope protein. 500,000 compound compounds were *in silico* screened for potential activity against the dengue fever dimeric and trimeric DEN 2 protein. A particularly promising lead is a plant steroid. The presentation will describe combining the power of high performance computing with wet lab experiments for the discovery of novel antiviral drugs to prevent/treat dengue virus infections by preventing viral entry.

Corneal Delivery and Stability of Connexin43 Antisense Oligonucleotides

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Aim To optimise the delivery of a specific antisense oligodeoxynucleotides (AS ODNs) into the corneal epithelium to knockdown connexin43 (Cx43) using selected colloidal carriers. These proteins are the cause of delayed corneal epithelium healing post corneal trauma and after Excimer laser surgery.

Experimental Fluorescence Resonance Energy Transfer (FRET) combined with Confocal Laser Scanning Microscopy CLSM was used to study the stability of the AS ODN in different colloidal systems, namely two water-in-oil microemulsions (w/o ME), a lamellar liquid crystalline system (LC) and a coarse emulsion as well as for the *in vivo* stability studies. TaqMan probes were used to tag the investigated AsODN. The *in vitro* stability experiments were carried out at 1µM TaqMan concentration. For a FRET control profile DNase was used to deliberately degrade the TaqMan DB1. Both the degraded and intact Tris/1µM TaqMan profiles were used as references for TaqMan stability assessment of the investigated delivery systems. *In vivo* penetration studies as well as monitoring the biological effect (protein knockdown) were conducted using immunolabelling in a rat corneal model.

Results The stability of the AS ODN was significantly improved by incorporation into certain w/o ME and LC systems and to a lesser extent when incorporated into a coarse emulsion. Stability profiles of the AsODN in the ME 5% and ME 10% respectively revealed an intact AsODN in both delivery systems for up to 1 week. On the other hand incorporating the AsODN into a coarse emulsion (EM) formulated with same ingredients used for ME 5% and ME 10% resulted in an almost complete breakdown in 1 week.

Unmodified Cx43 AS ODN [1mM] was successfully delivered to the stratum basale of the corneal epithelium and the connexin protein was down regulated as revealed by immunolabeling using the rat corneal model.

Conclusion. W/o ME systems as well as LC systems could be used to improve stability and optimize delivery of certain AS ODN to the corneal epithelium and as such show a potential as ophthalmic delivery systems for these challenging molecules.

Controlling the Physicochemical Properties of Polysaccharides and Proteins

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Polysaccharides are natural polymers derived from plant or animal origin and have been utilised in food and pharmaceutical applications to perform a number of functions. These include emulsification, encapsulation, stabilization, thickening, gelling and drug delivery etc.. One of the key advantages of polysaccharides in pharmaceutical formulation is that they are inert, biocompatible and safe due to already being accepted as food additives or ingredients. However, unlike synthetic polymers, polysaccharides consist of mixtures of similar, but not identical, molecules which can vary greatly depending on the source, method of extraction and subsequently processing conditions. Key factors which control their performance in a given application are the molecular weight, shape and size. This will determine how the molecules will interact with each other, other molecules and with water. This paper will address how the molecular structures, size and conformations of polysaccharides and proteins can be finely tuned to control their physicochemical properties with emphasis on drug delivery.

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Discovery of Novel, Selective Glucocorticoid Receptor Modulators

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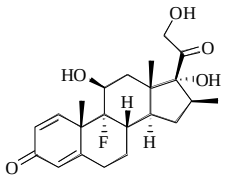
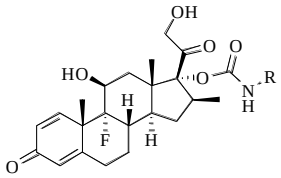
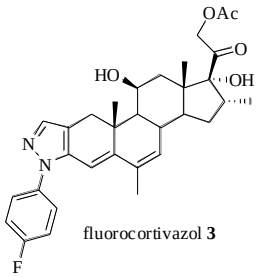
Glucocorticoids (GCs) *e.g.* betamethasone **1** and fluorocortivazol **3** are amongst the most effective agents currently available for the treatment of inflammatory and allergic diseases. However, the desired immunosupressive effects of glucocorticoids are accompanied by a number of side-effects, particularly when the GC is administered over a prolonged period of time. These include weight gain, development or aggravation of diabetes mellitus, onset of hypertension, steroid-induced osteoporosis, and suppression of the hypothalamic-pituitary adrenal axis. Many of these effects may be attributed to the endocrine activity of glucocorticoids and are essentially identical to the syndromes of endogenous corticoid excess (Cushing's syndrome). An unmet medical need of glucocorticoid pharmacology, therefore, is the development of agents with markedly improved therapeutic ratios compared to currently available steroids, particularly upon systemic administration. This may be achieved by the identification of novel glucocorticoid selective ligands that elicit marked anti-inflammatory effects but have an impaired effect on endocrine responses. In a program aimed at discovering such novel GC ligands we have synthesized and evaluated *two* independent series of compounds represented by the general structures **2** and **4**. A detailed overview of the design, synthesis, SAR and *in vivo* activities of these novel GR selective ligands will be described during the course of the presentation.



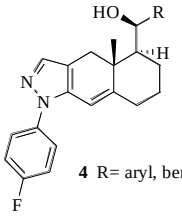
betamethasone **1**



2 R= alkyl, cycloalkyl



fluorocortivazol **3**



4 R= aryl, benzyl, alkyl, alkenyl

Optimizing the Use of Erythropoiesis Agents (ESA) in Renal Anemia

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Erythropoiesis-stimulating agents (ESAs) are effective for management of renal anaemia; however there is still room for further treatment optimization. Particular focus has been placed on ESA selection, route of administration and dosing frequency. In addition, when deciding upon ways to improve treatment strategies, cost is al so an important consideration. Of the three currently available ESAs - epoetin alfa (EA), epoetin beta (ES) and darbepoetin alfa (DA) DA has a longer serum half-life and an increased biological activity.[1]

In order to optimize patient comfort and convenience, intravenous (IV) administration of ESAs is preferred in haemodialysis (HD) patients and subcutaneous (SC) dosing is suggested in pre-dialysis patients.[2] Dosing is more complex in patients receiving recombinant human erythropoietin where dose requirements are increased with IV administration,[2,3] therefore se administration is regarded as more cost effective.[3] DA may offer greater flexibility, as similar efficacy has been shown for IV and se doses.[4,5] Moreover, due to DA's extended half-life, less-frequent dosing is possible.

A prospective, randomized, head-to-head study by Tolman et al evaluated the dose requirements necessary to maintain stable haemoglobin (Hb) after switching patients from three-times weekly (TIW) ES se to DA se once-weekly (QW), or ES se QW. The study found that 20% dose reductions in the DA group occurred compared with 24% dose increases in the ES arm.[7],# In addition, prospective studies have reported 14-31 % decreases in dose-requirements in patients switched from EA or ES to less-frequent dosing with IV DA, while maintaining or improving Hb controI.[5]. Furthermore, a *post hoc* analysis of data from 92 patients at a single haemodialysis unit has shown that switching patients from IV DA to IV ES can increase dose requirements and reduce Hb control.[11]

Dose savings may al so translate into lower costs. Data from a UK dialysis unit found that in patients changed from se EA TIW/SW to IV DA QW/Q2W* the average drug cost per patient per week decreased from f62 to f48, resulting in savings of f75,000 over 1 year.[12] Similarly, drug cost savings of € 570-71 0 per patient per 6 months were observed in an Italian centre following a switch to DA.[13] Furthermore, a Spanish evaluation showed lower drug and nursing costs in patients switched from EA or ES to DA, leading to mean monthly savings of €146.22 per patient.[14]

In conclusion, when considering strategies to optimize ESA use, cost - as well as patient convenience and efficacy is an important factor. Data suggest that treatment strategies using DA may address all of these factors.

For European nephrology patients, 200 IU rHuEPO = 1 µg Aranesp® was used as an appropriate dose-conversion factor when switching nephrology patients from rHuEPO to starting Aranesp®.

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Effect of Preparation Method on Physicochemical Properties of Antifungal Drug-Cyclodextrin Complexes – Comparison between Supercritical Carbon Dioxide and Conventional Methods

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Antifungal drugs are commonly used for the treatment of *Oropharyngeal candidiasis*, which is the first symptom of HIV infection. However, the efficacy and bioavailability of these drugs have been limited by their poor aqueous solubility and dissolution rate. Therefore, the aim of this study was to investigate the effect of different preparation methods (i.e. kneading, coevaporation, sealed-heating, and supercritical carbon dioxide ($SCCO_2$)) for obtaining solid inclusion complexes between *β*-cyclodextrin and two antifungal drugs (econazole, fluconazole). The physicochemical properties of the different products were characterized by differential scanning calorimetry, Fourier transform infrared spectroscopy and powder X-ray diffractometry. For the complexes prepared by the SC CO_2 method, the effects of temperature and pressure were also investigated.

Results suggested the possibility of complex formation between *β*-cyclodextrin and both econazole and fluconazole. However, the inclusion formation was influenced by the method of preparation. SC CO_2 method proved to be an effective technique for preparing solid drug-cyclodextrin systems while avoiding the use of organic solvents. Moreover, temperature of the SC CO_2 played a major role in promoting drug-carrier interactions, whereas pressure had limited effects.

Peptide Based Vaccines: A Structural and Immunological Perspective

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Peptides usually bind MHC class I or II molecules by anchoring specific side chains into specific pockets in the peptide binding groove. Peptides that do not contain these canonical anchor residues are believed to bind with low affinity, that results in loss of stable complex formation and loss of immunogenicity. Our results indicate using conventional computer algorithms to predict MHC binding peptides that a range of potentially effector T cell epitopes are missed. In this regard, we have determined immunologically and by x-ray crystallography at least ten novel families of peptide MHC binding modalities. These include, peptides using alternative anchor pockets, peptides devoid of central anchor amino acids, low affinity peptides, mutations to make high affinity peptides, short peptides, long peptides, looping out peptides, mimic peptides and glycopeptides for anchoring to MHC. We suggest that limitations in the ability to predict MHC binding peptides has hampered the detection of the whole spectrum of immunogenic peptides. The molecular interactions and immunological information elucidated in these non-canonical peptide MHC complexes should uncover additional immunogenic peptides from primary protein sequences and aid in the design of peptide-based vaccines.

Emerging Drugs in Cutaneous Lymphomas

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Cutaneous T-cell lymphomas (CTCL) are a heterogeneous group of lymphoproliferative disorders. In most cases, they are characterised by the accumulation of clonal CD4+ lymphocytes in the skin. Extracutaneous involvement is present in late stages only. Over the last 20 years the treatment of cutaneous T-cell lymphoma has been in a state of continual change. New therapies are constantly emerging as the search continues for more effective and tolerable disease-specific agents that satisfy medical needs. Therapies under investigation include topical and systemic retinoids like Bexarotene an RXR selective retinoid or fusion molecules like denileukin diftitox (Ontak). Other recently identified agents include monoclonal antibodies like the anti-CD4 monoclonal antibody Zanolimumab and histone deacetylase inhibitors (HDACs). HDACs are small-molecule inhibitors of histone deacetylases and represent a promising novel anticancer therapy by epigenetic regulation of gene transcription, which are active against solid tumors and hematologic malignancies. Of these HDACs Vorinostat (suberoylanilide hydroxamic acid) is the first FDA-approved HDAC inhibitor for the treatment of CTCL.

Interesting new insights into CTCL disease biology as well as a number of emerging of novel therapeutic interventions make this an increasingly interesting area for dermatologists and oncologists involved in the treatment of CTCL. This presentation covers much of this new information including the development of new anticancer drugs.

Plasma Expansion by Polyethylene Glycol Modified Albumin (PEG-Alb)

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Systemic inflammatory response conditions are associated with capillary leak and hemodynamic compromise. Resuscitation to reverse the ensuing hypovolemia is, however, plagued by the decreased endothelium reflection coefficient to albumin and other colloids. We developed polyethylene glycol modified albumin (PEG-Alb) as a resuscitative agent. PEG was linked covalently to human albumin at multiple sites on the protein. Based on size exclusion chromatography and osmotic pressure data, the effective volume of PEG-Alb is increased 16-folds compared with unmodified albumin. The relative vascular retention of the PEG-Alb and albumin was assessed in Control and septic rats *via* measurement of transcapillary escape rate (TER; %/hour), T50% (half-life). TER PEG-Alb (%/hr) was significantly lower than TERAlbumin (%/hr) for both Control (8.1±5.6 versus 14.8±7.1; $P<0.01$) and sepsis (14.8±6.6 versus 22.5±7.3; $P<0.001$) rats. The T50% [PEG-Alb] was substantially greater than the corresponding T50% [Albumin] for both Control (29.8±9.8 vs. 7.2±2.0 hours; $P<0.001$) and sepsis (12.9±5.6 vs. 5.1±1.6 hours; $P<0.001$) rats. In different models of shock, , rats treated with PEG-Alb showed better maintenance of blood pressure and less lung injury compared to albumin . The improved plasma expansion achieved with PEG-Alb versus albumin in sepsis is a consequence of the increased intravascular retention of PEG-Alb.

Diversity-Oriented Synthesis: Enantioselective Synthesis of a Pool of Small Molecules as Drug Candidates

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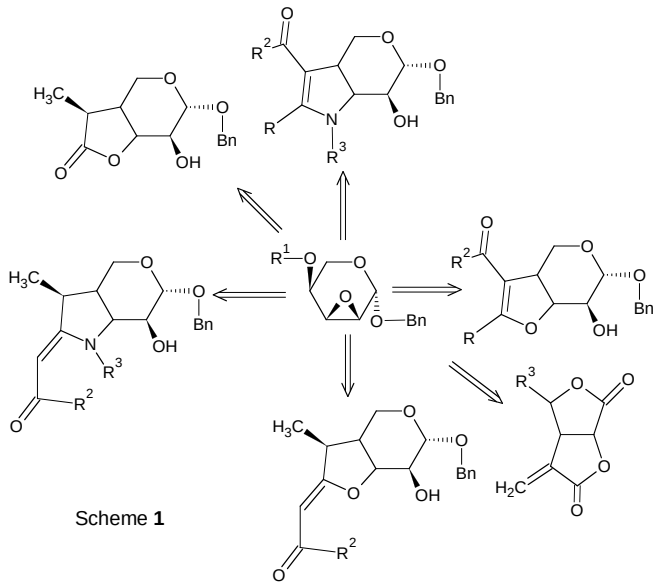
Diversity oriented synthesis, which aims to produce a wealth of structural complexity, may prove to be an effective tools of exploring effective routes toward the link between chemistry and medicine. The key synthesis objective is to generate a collection of structurally complex and diverse compounds capable of modulating any biological pathway or process of interest. It is worth while mentioning that, many of the small molecules known to disrupt protein-protein interactions that are critical for many biological complexity on one side, or agonize/antagonize a biogenic compound on its receptor or enzyme.

In this lecture we will present our efforts in the discovery of **BACE1** inhibitors, a possible drug candidates for Alzheimer disease. Furthermore, the enantioselective synthesis of skeletally diverse compounds (***Scheme 1***) inspired by natural analogues and biologically active compounds for many purposes will be presented.



Scheme **1**

Imino-Aldehydes- A New Tool to Combat Cancer and Viral Diseases

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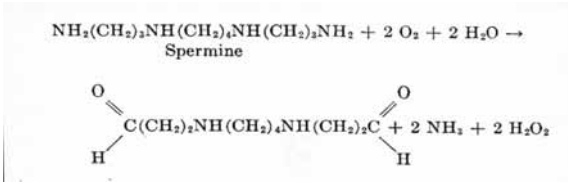
The naturally occurring polyamine-spermine, can be oxidized by serum amine oxidase , to yield imino-dialdehydes.



Similar dialdehydes can be synthesized chemically by treating diamines with β–chloro-propionaldehydes. The oxidation products are cytotoxic and inhibit the growth of cancer cells, as well as bacterial, human, animal and plant viruses.

A synthetic imino-dialdehyde, containing a diamine-hexane moiety, was even more active than the spermine –oxidation product and inactivated influenza and Newcastle disease viruses. The positively charged imino-dialdehydes, cross cellular membranes, interact with cellular nucleic acids and form biologically inactive complexes. Viral vaccines are usually prepared by treating viruses with formalin, which interacts with viral-membrane proteins. Human and/or animal viruses treated with imino-dialdehydes, retain intact membranes and are therefore potent immunogens. Vaccination of animals with influenza and Newcastle disease viruses, inactivated by imino-dialdehydes, gave better results compared with conventionally prepared vaccines.

Cancer cells are rich in polyamines. The injection of amine oxidase into those cells, results in the formation of cytotoxic imino-dialdehydes. This approach can open new avenues in cancer chemotherapy, inactivation of viruses and the preparation of potent vaccines.

Antiulcer and Cardiovascular compounds (Steroids): Structure Activity

U.A. Baltayev

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Some steroid compounds promote activation of regenerator processes in the field of pathologic focus of infection at stomach ulcer and duodenum and help to quickly restore the function of the damaged organ.

According to their ability to impact the bio-synthesis of protein in the human organism, the compounds isolated from plant sources - phytoecdysteroids - are coming very close to the stereoanabolics, but at the same time they don't display any specific for anabolic preparations hormonal effects. Taking the above-mentioned facts into consideration, we have performed the research to define anti-ulcer performance of the phytoecdysteroids, which we have isolated from different plant sources. We have payed special attention to the different structures of phytoecdysteroids. We have come o the conclusion that phytoecdysteroids prevent the formation of destruction of endocrinologic part at the mice. We have noticed different protective performance of different steroids depending on the location of the substitutes.

Anti-oxidant and anti-infarct performance have been also examined. One of the phytoecdysteroids was recommended for the application in the medicine as an anti-infarct preparation.

Identification and Characterization of Novel Antibiotic Targets

Julia E. Bandow, Michael Hecker, Heike Brötz-Oesterhelt and *Harald Labischinski*

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Bacteria are able to rapidly adapt to environmental challenges. We are witnessing an impressive example of bacterial adaptability in the area of antibiotic resistances. Several pathogens have acquired multiple resistances and we are running out of treatment options for patients infected with multidrug-resistant tuberculosis or *Pseudomonas aeruginosa*. In order to develop new effective antibiotics it is crucial to select drug targets which are not negated by pre-existing cross-resistances. We will present how biomolecule profiling like proteomics can support the quest for new antibiotics. Proteomic profiling was used to identify the mechanism of action of antibacterial compounds with novel chemical structure by comparing response patterns to response patterns of known antibiotics. Further, proteomic response profiles gave insights into the molecular pathways and processes affected by antibiotic compounds inhibition and this information was used to generate hypotheses about unprecedented targets and mechanisms.

The use of Gum Arabic Fraction for Treatment of Chronic Renal Failure Patients

Intisar Bashir

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Gum Arabic from Acacia senegal (Wild) has been fractionated by foaming method into two fractions, high protein fraction (fraction H) that represents 30% of the gum and a low protein fraction (fraction L) which represents 70% of the gum. A comparative study between the crude gum and the two fractions carried out in the scope of their physico-chemical properties, GPC analysis and their effects when 25g / day of each was given to chronic renal failure patients (sixty patient divided into three groups each group is composed of twenty subjects) on low protein diet and conservative management for four weeks. Non-protein nitrogenous (NPN) compounds (blood urea nitrogen, creatinine and uric acid), total protein, albumin and electrolytes ($P5^{+}$, Ca^{++}) and the level of haemoglobin and PCV of their blood were monitored.

The effect of supplementation of chronic renal failure patients with crude gum, fraction H and L for four weeks revealed that fraction L had significant effect in decreasing level of blood urea nitrogen, creatinine, uric acid and $P5^{+}$, and also it had significant effect in increasing level of blood Ca^{++} if the patient consumed it for four weeks but it had not significant effect in the level of haemoglobin, PCV, total protein and albumin. Whereas crude gum and fraction H had insignificant effect on the level of blood urea nitrogen, creatinine, uric acid, total protein, albumin, $P5^{+}$, Ca^{++}, haemoglobin and PCV.

Molecular Mechanism of Morphine-6-Glucuronidate Brain Penetration

Noszál Béla

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Morphine-6-glucuronidate (M6G) is an active metabolite of morphine and related opiate agonists with higher analgesic potency and superior side-effect profile. Despite its enhanced water-solubility it can re-enter the central nervous system (CNS) with a reported bioavailability of 100% (!) if administered subcutaneously[3]. This behaviour is even more surprising if its charged and polar moieties are taken into account, perfectly in line with its calculated $\log P_{octanol/water}$ value: -3.77. Such poor lipophilicity is generally considered at least 6 orders of magnitude short of the value (logP>3) sufficient for CNS drugs. Nevertheless, no influx carrier system has been found that would promote its CNS penetration.

In order to explore the molecular bases of this peculiar pharmacokinetics we studied its possible intra- and intermolecular charge compensations, dimerization and hydration by pH-dependent 2D NMR and UV spectroscopies in media identical with those in the brain in terms of relative permittivity and ionic strength. Our results show the various forms of M6G conformation, charge distribution and dimerization in the lipid membrane and CNS tissues, resulting in a plausible interpretation of the pharmacokinetic behaviour.

Case Study of DP Receptor Antagonists: From HTS to Synthesis and Selection of Pre-Clinical Candidate Compounds

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The discovery of potent and selective prostaglandin D2 receptor antagonists (DP) will be presented. We will emphasize on a High-throughput screening (HTS) campaign for the early identification of hits from the Merck sample collection. Parallel and library syntheses were developed to deliver a large number of compounds for the receptor screening assays against the prostanoid family to assess potency and selectivity. Considering the important impact of metabolic bioactivation, we assessed early in the discovery stage the propensity for molecules to cause covalent binding. A semiautomated assay using radiolabeled compounds was designed for the exploration of *in vitro* covalent binding. High-throughput LC-MS methods were used to characterize metabolites and reactive intermediates which may lead to protein labeling. Syntheses of the corresponding radiolabeled compounds were efficiently prepared using newly developed methods in our laboratory. Finally, we will also demonstrate improvements of the pharmacokinetics (ADME) of selected potential pre-clinical candidates.

The Development of Polymer Based Synthetic Vaccines

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Many infectious organisms are recognised by the human immune system by peptide or carbohydrate structures, called epitopes, which appear on the outside surface of the cell or virus. Synthetic vaccines are constructs that endeavour to display multiple copies of these epitopes on a non-infectious synthetic scaffold, priming the immune system to recognise the structures without the need for the use of live or attenuated organisms. This is particularly important for cases where immunisation with the infectious agent, even attenuated forms of it, can cause serious health problems such as autoimmunity, anaphylaxis or re-virulence. Our work involves the attachment of peptide epitopes to novel polymer nanoparticles of known architecture. The star polymers are constructed such that each of the arms consists of various functional groups located in different regions of the polymer chain. This allows for the conjugation of several different peptide epitopes or a mixture of peptides and carbohydrates moieties on the one construct. A preliminary investigation of reliable conjugation techniques that allow the coupling of the epitopes to the polymer structure will be described in this presentation. We have evaluated the use of triazole, amide bond and oxime formation, as conjugation strategies and compared the efficiency of coupling and the stability of the resulting structure in biological media such as Caco-2 cell homogenates and serum.

The Fight Against AIDS: New Avenues for Inhibiting RT, an Old Target

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The Acquired Immunodeficiency Syndrome (AIDS) related to HIV-1 infection is one of the most serious threats to human health and it has been estimated that more than 25 million people were killed since it was first recognized. In the fight against AIDS, first and second generation non-nucleoside reverse transcriptase inhibitors (NNRTIs) are now established as part of highly active antiretroviral therapy (HAART) for treating HIV infection. However, the efficacy of currently available NNRTIs, e.g. nevirapine (NVP, Viramune®), delavirdine (DLV, Rescriptor®) and efavirenz (EFV, Sustiva®, Stocrin®), is impaired by rapid emergence of drug resistance [1]. On the other hand, as patients live longer on HAART therapy and the pool of NNRTI-resistant viruses increases, so does the need for the development of new NNRTIs with antiviral activity against clinically relevant mutant strains [2]. Our research group has been recently involved in a multi-target approach to defeat the HIV-virus, focusing on the inhibition of HIV-1 Reverse Trascriptase according to both classical and non-classical approaches:

A virtual screening procedure, combining receptor-based pharmacophoric models together with docking studies, lead us to the synthesis via a combinatorial approach of new S-DABO derivatives which structure was then refined by means of deep molecular modelling explorations: these studies focused on clinically relevant RT mutant strains and pointed out two interesting candidates for further studies [3].

A combinatorial approach, lead instead to the identification of a new class of compounds (namely 6-vinylpyrimidines) endowed with an unprecedent mechanism of action: these compounds resulted in fact to be the first non-nucleoside RT inhibitors (NNRTIs) competing with the nucleotide substrate. An enzymological and computational study ha been conducted to elucidate their unique mechanism of action [4].

A structure based approach, was used to develop a pharmacoforic model for the connection subdomain of p66. When RT is forming, the first interaction between the two subunits, occurs in a TRP-rich hydrophobic cluster located in the connection subdomain of the two subunits. As a consequence, compounds binding to this region can prevent the formation of the active heterodimeric RT. A virtual screening protocol based on this pharmacoforic model has been applied to a commercial database of around 200.000 compounds, leading to the identification of the first small-molecule HIV-RT dimerization inhibitors [5].

In addition, recent efforts for the identification of novel HIV-integrase inhibitors will be also reported.

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Tumor-Targeted Liposomal Cisplatin (Lipoplatin™) and Oxaliplatin (Lipoxal™) Show Superiority to Free Drugs: From Inception to Phase III

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We have used patented platform technologies for the liposomal encapsulation of cisplatin into tumor-targeted 100-nm diameter liposome nanoparticles in a stable formulation, Lipoplatin™, an extremely promising anticancer drug. Cisplatin is the queen of chemotherapy among 600 FDA-approved drugs with approvals for more than 50% of human cancers, including the critical non-small cell lung cancer. Cisplatin can be combined with radiation therapy and a variety of other cytotoxic drugs such as gemcitabine, taxanes or vinca alkaloids. However, wider use of cisplatin is limited due to severe damage to kidneys, peripheral nerves, bone marrow, gastrointestinal tract, hair follicles and other tissues. Carboplatin (sales 800M) or oxaliplatin (sales 1.9 Billion in 2005 as Eloxatin™) may help reduce the toxic effects of cisplatin, but their use is rather limited by either their own side effects (carboplatin) or limited label (colorectal cancer, Eloxatin). On the other hand, formulation of a liposomally encapsulated cisplatin has been elusive. Regulon has made a major advancement by encapsulating cisplatin in 1999 and solving major hurdles in its production.

One important issue contributing to the therapeutic efficacy of Lipoplatin™ results from its ability to target primary tumors and metastases and to cause greater damage to tumor tissue compared to normal tissue. The liposomal nanoparticles of Lipoplatin™ target tumors and metastases in a very specific fashion using the leakiness of the vasculature of the growing tumor for their preferential extravasation. Tumor uptake of the Lipoplatin™ nanoparticles has been demonstrated in human studies. The nanoparticles are avidly taken up by the tumors either *via* phagocytosis or by direct fusion with the cell membrane. The two mechanisms result in an overall 10- to 400-fold higher intracellular uptake of total platinum in tumor cells compared to cells in normal tissue.

Clinical development is designed to replace cisplatin with Lipoplatin™ in chemotherapy schemes world-wide.

In a Phase I study, Lipoplatin™ substantially reduced the renal toxicity, peripheral neuropathy and ototoxicity as well as the nausea/vomiting caused by cisplatin. Furthermore, Lipoplatin™ has allowed administration of a higher total dose of cisplatin due to highly reduced cumulative toxicity. In a Phase II study in previously-treated pancreatic cancer patients in combination with gemcitabine it produced an astonishing 29% one-year survival compared for example to 24% one-year survival with gemcitabine plus Tarceva as first line treatment. Lipoplatin™ is administered at 120 mg/m^2 every week for 18 treatments compared to 100 mg/m^2 every 21 days for cisplatin. Lipoplatin at this dose can be combined with gemcitabine, 5-FU, paclitaxel, docetaxel or other drugs. Lipoplatin™ is currently under three Phase III evaluation. A Phase III randomized multicenter clinical trial uses weekly 120 mg/m^2 LipoplatinTM in combination with gemcitabine as first line treatment against non-small cell lung cancer (NSCLC) and is being compared to cisplatin plus gemcitabine. This trial showed superiority in the Lipoplatin arm compared to cisplatin arm with lower toxicities. The second Phase III study compares weekly Lipoplatin plus 5-fluorodeoxyuridine (5-FU) versus cisplatin plus 5-FU against head and neck cancers. The third Phase III uses Lipoplatin™ in combination with paclitaxel as first line treatment against NSCLC and is being compared to cisplatin plus paclitaxel. This trial also showed superiority in the Lipoplatin™ arm (partial response of 62%) compared to cisplatin arm (48% PR) with lower toxicities (nil neuro-, nephro- and GI-tract toxicity of Grade III-IV in the Lipoplatin arm). This talk will review the clinical development

of Lipoplatin and discuss the mechanism of lower toxicity *via* the liposomal formulation. It is anticipated that chemotherapy regimens integrating Lipoplatin will allow higher overall survival of patients suffering with non-small cell lung, pancreatic, gastric and other cancers, with low side effects and improvement in quality of life compared to cisplatin regimens. A liposomal formulation of oxaliplatin (Lipoxal™), currently under Phase II evaluation against gastrointestinal cancers, also appears to produce reduced toxicity compared to oxaliplatin. We plan to follow a similar approach to the clinical development of Lipoxal™.

Platinum Coordination Complexes. From DNA Interactions to New Anti-Tumor Drug Design

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Changing the chemical structure of platinum compounds may substantially modulate their mode of binding to DNA, subsequent processing of DNA damage and consequently the mechanism of biological efficacy of these compounds [1]. Such structural modifications may also affect the spectrum of biological activity of the platinum agents, the development of drug resistance, and also their toxicity profile. Hence, a deeper understanding of how new platinum compounds modify DNA and how these modifications are further processed in cells may lead to important insight into "downstream" effects, initiated through differential protein recognition and repair, that may produce unique biological effects. Studies so far performed have implicated multiple systems including several classes of DNA repair, replication, transcription, cell cycle and cell death responses in the processes associated with cellular sensitivity to the platinum drugs. Complete knowledge of how modifications of DNA by anti-tumor platinum compounds and other metal-based drugs affect the components of these pathways should provide a framework for understanding the mechanism of action of platinum drugs and thereby promote a rational basis for the design of new metal-based drugs as well as helping to identify optimal treatment strategies.

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Immune System Modulation with a Homeopathic Medication

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Immunotherapy continues to be investigated intensively in both adjuvant and advanced disease settings. The laboratorial researches with homoeopathic medicines are very scarce; most of them are clinical reports, with varied methodologies, controversial and doubtful results. Even so the number of people that uses this therapeutic system is huge. In Europe, homeopathy is the most frequent complementary and alternative medical therapy. Since 1997, our group has found many important results with a study focused on a homeopathic medicine called Canova (CA). Its manipulation is based on Hahnemann's ancient homoeopathic techniques that use diluted substances that are vigorously shaken (succussed) during the preparation. It is a Brazilian complex homeopathic medicine that represents a new form of immunomodulatory therapy produced from *Aconitum, Thuya, Bryonia, Lachesis* and *Arsenicum.* Previous studies demonstrated that CA activates macrophages (Mϕ) both *in vivo* and *in vitro*. It was observed that the Tumor Necrosis Factor-α (TNFα) *in vitro* production by macrophages is significantly diminished when the medicine is administrated [1]. NADPH oxidase activity was increased as well as that of inducible nitric oxide synthase (iNOS), consequently producing reactive oxygen species (ROS) and nitric oxide (NO) respectively [2]. CA stimulates an increase of the endosomal/lysosomal system as well as the phagocytic activity of Mϕ when interacted with *Saccharomyces cerevisiae* and *Trypanosoma cruzi* epimastigotes [3]. The modulatory effects of CA were also observed both *in vivo* and *in vitro* in experimental infection by *Leishmania amazonensis* and *Paracoccidioides braziliensis,* controlling infection progression and limiting its dissemination [4, 5]. The treatment with CA increased total numbers of leukocytes. Among lymphocytes, T CD4, B and NK cells increased. These results suggested a direct or indirect action of CA on hematopoiesis. Subsequently, the bone marrow cells were studied and all microscopy techniques showed that monocytic lineage (CD11b) and stromal cells (adherent cells) were activated by treatment [6, 7, 8]. Moreover, it is neither toxic nor mutagenic [9].

In 2003, citizens from Botswana came to Brazil, and they took CA to their country. As soon as the Ministry of Health of Botswana and the Federal University of Paraná's Human Research Ethics Committee authorized the project, a prospective study with AIDS patients from the Home Based Care of Gabane, Botswana, Africa, was started. Simultaneously a prospective study began with Brazilian patients, linked to Non Governmental Organizations (NGO) and to a public institution of health in Curitiba, Paraná. The data indicate that the treatment is highly effective in reducing symptomatology and improving quality of life in individuals with HIV by recovering parameters like general pain feeling, appetite, capability to do small efforts and absenteeism among others. The results show a significant change in every evaluated parameter just after the first month of treatment ($p < 0.01$). Furthermore, those changes were sustained after the 18 months period. In Brazil some laboratorial were performed showing improvement in parameters like CD4 and erythrocytes number, as well as diminishing opportunistic diseases.

The global view of changes in expression of genes with known functions can provide a vivid picture of the way in which cell adapts to a changing environment or a challenge. De Oliveira and col. (2007) used GeneChip® MG74Av2 (Affymetrix, Inc., CA, USA) to characterize the changes in gene expression that take place during the process of Mϕ activation by CA. Data from the CEL file obtained from three independent experiments were analyzed by the Robust Multi-Array method - RMA. Statistical analysis of microarray data revealed 147 genes differentially expressed after mice treatment. Interestingly some of them can explain the clinical results obtained in the last years, besides the clinical improvement obtained in several parameters in the prospective studies in Africa and Brazil. The CC-chemokine RANTES (CCL5) along with the receptors CCR2 and CXCR4 were downregulated in cells treated with CA. Several

publications over the last decade have demonstrated the importance of chemokine receptors for the human immunodeficiency virus (HIV) entry. The CXCR4 chemokine receptor has been shown also to be necessary for infection by HIV-1. In fact, CXCR4 acts synergistically with CD4 in an ordered multistep mechanism to allow the binding and entry of the HIV-1 virus. Another CC chemokine receptor, CCR2 was found to act as a co-receptor for HIV infection. The CC-chemokine RANTES (CCL5) can enhance HIV infection of target cells in a manner that is independent of CD4 and any known co-receptor, and even independent of the route of virus entry. Receptors and ligands downregulation by CA may interfere with virus entry by reducing the density of available receptors and co-receptors on the cell surface interfering with propagation of infection. These results indicate a strong biochemical support of the clinical results found in the latest fifty years in patients with many different diseases, mainly HIV/AIDS. Unfortunately, the majority of medical doctors in South America did not publish the clinical findings. Therefore macrophage seems to be a potential therapeutic target for the regulation of immune responses by Canova medication. At last, it is clear to us that the Canova treatment unfortunately is not a cure for HIV/AIDS, but at least helps to improve a lot the quality of life of those individuals infected, and why not say, affected by this terrible virus.

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Development of Potent Atypical Antipsychotics Based on a Novel Neurochemical Approach

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Schizophrenia is a chronic, severe, and disabling brain disease. Approximately 1% of the population develops schizophrenia during their lifetime. The severity of the symptoms and long-lasting, chronic pattern of schizophrenia often cause a high degree of disability. There are no specific characteristics for the diagnosis of schizophrenia, and no single symptom is consistently present in all patients, reflecting the simultaneous imbalance of different CNS neurotransmission systems. The symptoms most commonly associated are positive symptoms and denote the presence of grossly abnormal behavior. Less obvious than the positive symptoms but equally serious are the negative symptoms, namely, absence of normal behavior. Although several new potential antipsychotics have been disclosed over the last few years and a sizeable number of drugs are actually in advanced stages of investigation, the major difficulty that scientists and clinicians have to face in the development of a new antipsychotic is the lack of knowledge about the underlying cause and nature of the disorder. Based on the neurochemical hypotheses (the dopamine, the serotonin and the glutamate hypothesis) which have emerged, and taking into account the challenging pharmacological profile of clozapine, and aripiprazole, our recent and ongoing activities in the development of novel atypical antipsychotic drugs will be discussed.

Artificial Hematopoietic Stem Cell Niche Sustains Growth and Differentiation of Human ES-Derived Early Hematopoietic Progenitors

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Using a novel Microplate Biomaterial Microarray (MBM^{TM}) technology, we have created an artificial hematopoietic stem cell niche that can sustain growth and differentiation of human embryonic stem cells-derived (hES) early hematopoietic progenitors. This hydrogel based *ex vivo* niche allows uploading of human embryoid stem cells, human mesenchymal stem cells (MSC), genes (bcl-2 preventing apoptosis and HoxB4 enhancing hematopoiesis) and extracellular matrices to support growth and differentiation of human ES cells. These experiments were done using NIH-approved hES cell lines H1 and H9 maintained in the undifferentiated state by mitomycin-treated murine feeder layer and serum-free media. Serum-free, feeder-free culture conditions were established and early hematopoietic progenitors grown using SCF, TPO, VEGF and IL-3 with high efficiency. At day 3-5 dual CD34+/CD31+ progenitors were identified, while on day 7-8 CD34+ hematopoietic progenitors were isolated, which formed typical hematopoietic colonies. These progenitors expressed genes related to early hematopoiesis, such as TAL1/SCL, FLT1, GATA2, GATA1, EPOR and TPOR. The early dual endothelio-hematopoietic progenitor (hemangioblast) expressed PECAM-1 and CD34 and showed typical blast-like morphology. Based on mathematical simulations, various micro-niches were designed to establish optimal differentiation conditions for this progenitor using IL-3, IL-6, TPO, EPO, VEGF, SFC, Flt-3 ligand and various extracellular matrices. Specific micro-niches were created for generation of CFU-E, BFU-E, CFU-GM, CFU-GEMM, CFU-M, CFU-G, and CFU-MK progenitors from human ES-derived hemangioblast. Kinetic uploading of TPO, EPO, SCF and VEGF created a niche-sustaining a growth of ES-derived hemangioblast with high efficiency and low apoptosis rate. These niches used pulse –delivery of anti-apoptotic bcl-2 gene and hematopoiesis-enhancing Hoxb4 gene. In the future, this system will allow optimized and upscaled generation of early hematopoietic progenitors from human ES cells, as a first step towards clinical applications of human embryonic stem cells.

A New Paradigm for Cancer Chemotherapy: Intratumoral Drug Delivery

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The safety and efficacy of conventional intravenous chemotherapy is severely limited by drug toxicity. Despite significant advances in cancer diagnosis and biology, CDC estimates for the past 20 years indicate little progress to reduce mortality for breast, lung, and colorectal cancers. Indeed, there has been a >75% increase in lung cancer mortality. Antibody targeting and gene therapy remain exploratory. New clinically practical concepts are needed. Reported here is progress, from laboratory to clinic, for a new treatment paradigm we have pioneered, *intratumoral chemotherapy*. Superdoses of cytotoxic drugs are deposited within tumors to achieve remarkable therapeutic efficacy with minimal systemic toxicity. Although major benefits are seen in animal lung and breast cancer studies with daunorubicin and mitoxantrone, intratumor injection of drug-loaded albumin and DNA nano-mesospheres (0.1-10 microns) is also being studied to prolong local antitumor activity. In collaboration with Dr. S. Celikoglu at Istanbul University, bronchoscopic intratumoral injection of NSC lung cancer with cisplatin, mitoxantrone, and 5-FU, has been clinically investigated for several years with exceptional results; especially for patients presenting with severe bronchial obstruction. We conclude that intratumoral therapy, particularly for preoperative neoadjuvant treatment of lung, breast, and colorectal cancers, will become an important new clinical treatment modality.

Rapid and Label-Free Bio-Detection Based on Localized Plasmon Resonance

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In the life sciences, work is in progress to find simple, rapid, sensitive, and label-free detection schemes for the analysis of biomolecular interactions. Noble metal nanoparticles exhibit a characteristic absorption band in the absorption spectrum, known as “Localized Plasmon Resonance, LPR”. The frequency of the LPR band is highly dependent upon the local environment of a noble metal nanoparticle. As such, the optical properties of a noble metal nanoparticle are sensitive to the refractive index of the surrounding medium and, additionally, the binding event to the functionalized nanoparticle. Furthermore, the sensing sensitivity in term of peak wavelength shift can be increased by using nanoparticles of different shapes while the sensing sensitivity in term of absorbance or intensity change can be increased by lengthening the optical path using optical waveguides. The nanoparticles can also be functionalized to allow the selectivity of the sensor. Since the detection process is based on the change of refractive index of the surrounding medium when an analyte interacts with a molecular recognition group on the nanoparticle surface, label-free and real-time biosensors using noble metal nanoparticles can be realized.

The Carbonic Anhydrases: Potential Therapeutic Targets in Renal Carcinoma

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The zinc enzyme carbonic anhydrase (CA) catalyses the reversible hydration of carbon dioxide to bicarbonate and a proton. Seemingly ubiquitous in nature, it is expressed in 15 different, active mammalian isoforms, some of which play crucial roles in fundamental cellular processes, including the biosynthesis of lipids and nucleotides. Whilst the rate of the uncatalyzed CA reaction may be sufficient to accommodate basal metabolism, it appears that CA activity is required to sustain the higher level of metabolic flux associated with enhanced levels of cell growth such as those encountered in cancer cells (1,2).

In the normal human kidney, CA II, which is among the most widespread and fastest of all enzymes, is expressed in the cytoplasm, CA VB in the mitochondrion, and CA IV and XII on the extra-cellular surface. The CA IX isozyme appears to be virtually specific to cancer cells and is strongly expressed on the cell surface of most renal cell carcinomas, where its presence is considered to be diagnostic. CA IX expression is down-regulated by pVHL and up-regulated by anoxia. It is possible that both, or either, the cytoplasmic and the extra-cellular CA isozymes may facilitate the extrusion of protons from the cell, to create a milieu that is more conducive to cell invasion and metastasis.

Renal cell carcinomas account for about 2 per cent of all adult malignancies, and are characterized both by a tendency to metastasize before giving rise to local signs or symptoms, and by their resistance to chemotherapy and radiotherapy.

We investigated the effects of highly specific CA inhibitors on both the growth and invasive properties of renal cancer cells in culture, and on the growth of human tumors after implantation into immunodeficient mice. We employed two human renal cancer cell lines: one which strongly expresses both the extra-cellular CA IX and XII isozymes, and one which expresses neither. CA inhibitors inhibited the growth and invasion of both cell lines. They also inhibited the growth rate of tumors derived from these human cell lines after implantation into immunodeficient mice.

Our data suggest that the development of specific inhibitors targeted at carbonic anhydrase isozymes may be of value in therapy for renal cell carcinoma.

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New Drug Development Following Characterization of a Novel Atherogenic Lipoprotein

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Electronegative low-density lipoprotein (LDL) is a subclass of LDL that is potentially atherogenic. By subjecting LDL isolated from hypercholesterolemic human plasma (LDL-cholesterol > 160 mg/dL) to ion-exchange chromatography, we divided LDL into 5 subfractions, L1–L5, with increasing electronegativity. While L5 is the most electronegative subfraction, more than 95% of the total LDL belongs to the L1 subgroup, which represents the regular LDL. In cultured human vascular endothelial cells (ECs), L5 induces marked apoptosis, whereas L1 doesn't. L5 also stimulates monocyte-EC adhesion and inhibits differentiation/maturation of endothelial progenitor cells (EPCs). By inhibiting the growth of ECs and EPCs, L5 can effectively impair reendothelialization and compensatory angiogenesis. With the longest dimension of less than 30 nm, L5 is a biological nanoparticle. It is a complex particle that contains hydrophobic triglyceride and cholesteryl esters in the core, and hydrophilic phospholipids, cholesterol, and apolipoproteins on the surface. Its structural characteristics are being determined by cryo-electron microscopy. Basically, its shape and consistency are different from those of L1. As a normal LDL, L1 contains apolipoprotein B100 (Apo-B100) exclusively, whereas L5 contains Apo-A1, CII, CIII, and E, in addition to B100. One most important distinction is that L5 is internalized by ECs and EPCs through lectin-like oxidized LDL receptor-1 (LOX-1), while L1 is internalized by the normal LDL receptor. Transduced by LOX-1, L5's signaling disrupts a variety of fibroblast growth factor 2-regulated pathways to offset cellular homeostasis. It also destroys mitochondrial integrity and dysrgulates transcription of multiple genes in the nucleus. Systems bioinformatics methods are being performed to further identify the functional properties of L5 in live cells. On the basis of the information we have gathered at this time, it appears promising that drugs aimed at different targets can be developed to counteract the adverse effects of L5. These include drugs that can 1) reduce L5 formation or accelerate L5 excretion, 2) inhibit L5 endocytosis by LOX-1 in vascular ECs and differentiating EPCs, 3) protect mitochondria integrity against L5, 4) impede L5 signaling, and 5) protect gene transcription in the nucleus.

In view of L5's cytotoxic effects, we investigated whether its harmful properties could be used for therapeutic purposes. Exposing oral cancer cells to L5 resulted in apoptosis and depletion of mitochondrial membrane potential in a concentration-dependent manner. To avoid potential side effects from damaging ECs while enhancing the cytotoxic effect on cancer cells, the exposed peptide functional groups were modified to link to anti-Her2 monoclonal antibody. The attempt yielded a synergistic anti-cancer effect in the anti-Her2 conjugated L5 hybrid as compared to that of L5 or antibody alone. On the basis of these initial findings, we are constructing a comprehensive hybrid nanoparticle consisting of L5, negatively charged polymers, the anti-Her2 monoclonal antibody, and doxorubicin. Take viruses for example, biological nanoparticles have been used for gene transfer and drug delivery. Endogenous biological nanoparticles, such as L5, harbor intrinsic advantages of low or no immunogenicity and well-documented signaling pathways. Modification of these nanoparticles to become delivery vehicles or reporter systems holds great potentials for advanced applications in disease diagnosis and therapy. Moreover, tracing these nanoparticles in live cells will greatly advance our understanding of basic cell biology.

In conclusion, two types of drugs can be developed following full characterization of L5: Type 1-cardioprotective by counteracting L5; Type 2-immuno-friendly anticancerous by use of smart L5-nanohybrids.

Structure of a Novel Anticancer Peptide CB1a, Based on the N-Terminal Peptide Sequence of Cecropin B

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Several natural antimicrobial peptides including cecropins, magainins and melittins have been found to kill cancer cells. However, their efficacy may not be adequate for their development as anticancer agents. In this study, we used a natural antimicrobial peptide, cecropin B (CB), as a template to generate a novel anticancer peptide. Cecropin B is an amphipathic and polycationic peptide derived from the hemolymph of *Hyalophora cecropia* with well-known antimicrobial and cytolytic properties. The signature *pattern* of cecropins is W-x(0,2)-[KDN]-x-{L}-K-[KRE]-[LI]-E-[RKN] (PROSITE: PS00268), and this signature sequence is located at N-terminus of CB. CB1a was constructed by repeating the N-terminal ten amino acids of CB three times and including a hinge near C-terminus. The circular dichroism spectra show that CB1a is unstructured in aqueous solution, but adopt a helical conformation in membrane-like environment. The solution structure of CB1a in polar solvent was also studied by NMR. CB1a formed a helix-hinge-helix in 20% HFIP solution, and the bent angle between two helical segments was ranging from 60° to 110°. A heparin-binding motif is located in the central part of helix 1. Isothermal titration calorimetry reveals the association constant of CB1a bound to low molecular weight heparin is 1.66×10^5 M^{-1} at physiological ionic strength at 25°C. Binding of CB1a to heparin produces a large conformational change toward a more structural state. CB1a demonstrated promising activity against several cancer cells with low toxicity to the non-cancer cells. The IC_{50} of CB1a on leukemia and stomach carcinoma cells were in the range of 2- to 8-fold lower than those of CB. Besides, CB1a display low hemolytic property on human red blood cell. These properties might make CB1a a good candidate for use as an anticancer agent.

Serotonergic Drug Design – Finding Diamonds in the Rough

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Arguably, serotonin (5-HT) is one of the most studied of the neurotransmitters. The identification of 5-HT as a vasoconstricting agent over 50 years ago marked the beginning of a monumental effort that has extended our knowledge, not only of 5-HT receptors, but of G-protein coupled receptors in general. There are presently fourteen known subtypes of the 5-HT receptor as well as a reuptake transporter. Some of these proteins exist as multiple splice variants. Drug discovery research to date has investigated all of these molecular targets, and there are drugs on the market today that possess some form of 5-HT modulation (either all or in part) as their hypothesized mechanism of action. Most notably, serotonin reuptake inhibitors control depression in many patients and 5-HT1B/1D agonists provide relief for numerous sufferers of migraine. However, with the abundant wealth of information on serotonin and its binding proteins, some argue that the number of successes has been relatively small. Our recent serotonergic drug design efforts have focused on four areas (serotonin transporter, 5-HT1A, 5-HT2C and 5-HT6). Using a variety of medicinal chemistry approaches, researchers were able to solve various problems and identify clinical candidates for all of these mechanistic targets. This presentation will highlight the efforts that led to those successes.

The Heterogeneity of High Density Lipoproteins: Implications for Therapeutic Development

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Despite the effectiveness of statins to lower low density lipoprotein LDL-cholesterol levels, atherosclerosis remains the prime factor responsible for coronary artery disease. Plasma levels of high density lipoprotein HDL is negatively associated with the risk of coronary artery disease CAD, such that the higher the levels of HDL, the lower the likelihood of developing CAD.

Since the atheroprotective effects of HDL are independent of other risk factors, HDL therapy has become a major area for therapeutic development. While generally aimed at increasing HDL levels, it has become increasingly clear that HDL therapy development needs to address other effects such as the functional ability of HDL to reverse cholesterol transport RCT as well as other emerging atheroprotective factors. Such factors include the ability of HDL to act as an anti-oxidant, an anti-inflammatory substance and to restore endothelial dysfunction. This is particularly important in the context of reports demonstrating the discordant functionality of HDL compared to its levels where high levels of HDL can be pro- rather than antiatherogenic under certain metabolic conditions. In humans, the ability of HDL to support cholesterol efflux and maintain endothelial function, or antioxidation function can be diminished despite higher HDL levels. Conversely, the apoA-I Milano mutation leads to lower HDL levels, but is reported to enhance HDL functionality. PLTP deficiency and CETP inhibitors on the other hand increase both HDL levels and their functionality. We infer from these findings that changes in RCT and HDL functionality are as important, if not more so, than changes in concentration, and that these changes may act independently. Thus, any therapeutic approach should take HDL functionality, and not just HDL levels, into account.

Dengue Virus Viremia: Role of NS1 Protein of Virus / Cytotoxic Factor Produced by T-Cells

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Dengue virus (DV) infections are a major cause of morbidity and mortality in the tropical and sub-tropical regions of the world. Symptoms range from mild febrile illness to severe hemorrhagic fever. The pathogenesis is incompletely understood, but immunopathology is thought to play a part, with antibody–dependent enhancement and massive immune activation of T-cells and monocytes / macrophages leading to a disproportionate production of pro-inflammatory cytokines. A unique cytokine , cytotoxic factor (CF) is produced by CD4+ T-cells during dengue virus infection of mice (mCF) and man (hCF) and amount increases in severe cases of DHF. The production of mCF / hCF precedes the clinical illness in mice and man. The mCF / hCF causes pathological lesions in mice such as increased capillary permeability , cerebral oedema and blood leukocyte changes. The Blast 2, PSI homology search of amino terminal sequence of CF showed homology with thiol-oxidoreductase with 2 cytochrome- C heme-binding sites. This is possible that cell death caused by CF and its association with DHF/DSS is may be through release of cyt-C and / or other activators of cell death pathways as permeability- transition is an oxidant- mediated event. Similarly, copious amounts of NS1 protein circulate in DV- infected patients in whom NS1 blood levels have been shown to co-relate with disease severity. Microvascular leakage in such patients has been linked to complement activation by NS1-antibody complexes. The DHF - like pathological lesions produced by mCF / hCF can be prevented by pre-treatment of mice with anti-mCF antibodies. The protective role of hCF auto- antibodies and NS1–auto-antibodies against the development is well demonstrated, thus suggesting a vaccine strategy. The CF and NS1 protein of DV play quite similar role in progression of disease. The CF is a host protein or part of NS1 protein of virus as it is expressed on the surface of DV-infected cells, is secreted into the circulation, the amino- terminal sequence of mCF was aligned with NS1 protein of DV using Clustal W program. The homology observed with NS1 protein raised the necessosity of further studies on production and role of CF as it is present only in dengue.

HIV-1 Nef-SH3 Targeting as a Proof of Concept for the Development of Anti-Viral Protein-Protein Interaction Inhibitors

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Despite considerable progress in the development of antiviral drugs, much remains to be done to improve the efficacy of and tolerance to the so far identified antiviral therapeutics. Emergence of viral resistance to drugs and drug-induced side effects largely incite for further refinement and development of novel drug targets.

Protein-Protein Interaction Inhibition (2P2I) is endowed with great therapeutic potential. Furthermore, targeting viral protein-cellular protein interfaces is expected to combine the advantages of reduced toxicity offered by targeting the viral protein with restrained place for emergence of resistance due to structural constraints imposed by the cellular protein partner. Despite the initial believe that 2P2I was refractory to small molecule intervention, improved knowledge of complex molecular binding surfaces has recently stimulated renewed interest for 2P2I, especially following identification of 'hot spots' and first inhibitory compounds. However, the combination of target complexity and lack of starting compound has thwarted experimental results and created intellectual barriers.

Here, we combined virtual and experimental screening when no previously known inhibitors can be used as starting point in a structure-based research program that target a SH3-binding surface of the Human Immunodeficiency Virus Type I Nef protein. High-throughput docking and application of a pharmacophoric filter, on the one hand, and search for analogy, on the other hand, identified drug-like compounds that were further confirmed to bind Nef in the micromolar range (Isothermal Titration Calorimetry), to target the Nef SH3 binding surface (NMR experiments) and to efficiently compete for functional Nef-SH3 interactions (cell-based assay, GST-pull down). Our results identify the first set of drug-like compounds that functionally target the HIV-1 Nef SH3-binding surface and provide the basis for a powerful discovery process that should help to speed up 2P2I strategies and open avenues for new class of antiviral molecules.

TTR Amyloidosis: From the Molecular Mechanism to Therapy

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Systemic deposition of transthyretin (TTR) amyloid fibrils is always observed in Familial Amyloidotic Polyneuropathy, Senile Systemic Amyloidosis and Familial Amyloidotic Cardiomyopathy patients. It is widely accepted that destabilization of the molecule leads to a cascade of events which result in fibril formation.

Until now the only efficient therapy available is liver transplant when performed in an early phase of the onset of the disease symptoms. This is a very invasive therapy and alternatives are desirable.

Since fibrillogenesis is a multi-step process, it is possible to stop it at different stages, namely through stabilization of the native fold of the potentially amyloidogenic protein, inhibition of fibril formation or fibril disruption.

Based on the proposed mechanism for TTR amyloid fibril formation and the structural information available, we discuss the action of some compounds that have been tested "*in vitro*" as amyloid inhibitors or disrupters.

Enabling Novel Technologies to Expedite Medicinal Chemistry: Combination of Flow Synthesis and Serendipity-Enhancing Technics for Lead Discovery

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Bioanalogous design strategies with novel synthetic technologies for target-family based drug discovery are integrated and applied to several targets, among others for designing of matrix metalloprotease (MMP) inhibitors. About 300 known MMP inhibitors were annotated and organized into a ligand space were a "lead evolution tree" was formed. In that tree, substructural changes leading to significant increase in biological effects were revealed by using the EMIL serendipity-enhancing approach. Subtype-specific privileged fragments were extracted to improve activity and/or selectivity. The compounds with preferred activity profile were correlated with sequence homology as well as binding site similarity within the target family, allowing the identification of substructural modification patterns suitable to produce selective inhibitors. For enhancing synthetic efficiency, flow reactors (such as H-Cube® and X-Cube™) operating on high pressure, high temperature, optionally pre-packed with catalysts, reagents, or scavengers were utilized to perform hydrogenation, coupling and other reactions under normal and supercritical conditions, besides conventional automated HT synthesizers While the presented design approach normally reduced to <100 the number of compounds to be synthesized, the use of high pressure flow reactors (with reaction times < 2 min) enabled to cut down synthetic efforts to days from months.

Prediction of Structural Features of Integral Membrane Proteins

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More than 20% of human genome code for integral membrane proteins (IMPs) which control broad range of cellular functions. Most importantly, IMPs are targets for ~ 60% of pharmaceuticals. Determination of high-resolution three-dimensional structures of IMPs is challenging. In the absence of experimental evidence and taking into account the constraint imposed by lipid bilayer, modeling the structures of IMPs can be viewed as a two-dimensional problem for which prediction of location, basic topology and rotational orientation of helical transmembrane (TM) substructures are required. Predicting the location of the TM regions are usually accomplished based on hydrophobicity and knowledge-based statistical methods.

We have been involved in developing an algorithm for the prediction of IMP structural information based on the lipid bilayer environment. This algorithm necessarily takes into consideration the fact that many of the IMP residues may reside in a lipid environment and the compatibility between residue type and its environment in the 3D structure is calculated. Our results concentrate on seven transmembrane α-helix containing IMPs in which we have validated the method by large-scale modeling of GPCR class of receptors.

The rotational orientations of TM helices of IMPs are often determined by calculating the helical moment vector. Here we describe a method for predicting the rotational orientation of helical TM segments of IMPs based on two new scales derived from the structural analysis of a representative set of non-redundant protein structures taken from protein databank with no bias toward IMPs.

New Glutarimide Alkaloids as Potent Anti-Inflammatory Drugs

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The potential uses of glutarimide alkaloids had been overseen due to the teratogenic effects of thalidomide. However, thalidomide and other derivatives have been used as anti-neoplasic and anti-inflammatory drugs in autoimmune diseases. One of the proposed mechanisms is that the drug is to block Nfkb activation which subsequently induced tumor death and block inflammatory responses. The generation of new structures along with the use of natural glutarimide alkaloids to enhance a specific inhibition of p65 phosphorylation without inducing a teratogenic effect is the aim of our study. Studies on cell viability, proliferation, cell activation, radical production, cytokine secretion, Nfkb, Akt and cell interactions and *in vivo* responses suggests that these new compounds may be more potent that other compounds maintaining the antineoplasic effects described by thalidomide.

From Bench to Bedside: The Clinical Development of Pertuzumab in Women's Cancers

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Translational science requires a partnership between basic science and clinical researchers. The success of this collaboration has been most evident in oncology with the recognition of multiple targetted based therapies with application in lung cancer (erlotinib), breast cancer (trastuzumab), and chronic myelogenous leukemia (imatinib). The HER family of tyrosine kinases continue to be a target for further development and recently, the pan-HER2 inhibitor Pertuzumab has been in clinical development. This session will review the preclinical and clinical development of this agent, with emphasis on its activity in women's cancers.

Relevant Effects of Omalizumab, an Anti-IgE Drug, in Asthma and Other Diseases

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The frequency of allergic diseases has increased in recent decades. Asthma is a high prevalent of these conditions and a leading cause of morbidity. It affects 3-4% of the population in our geographical setting and extrinsic allergens are detected as the disease's etiological agent in around half of these cases.

IgE is one of the molecules involved in the allergic process. Most of the time and resources at asthma units are devoted to corticosteroid-dependent patients. International guidelines for asthma treatment recommend a stepwise therapeutic approach; in the last step, the use of oral corticosteroids is advised when control is not achieved with long-acting ß-2-agonists and high doses of inhaled corticosteroids. No alternatives or complements to oral corticosteroids had been accepted until November 2006, when the latest GINA update included the IgE blocker omalizumab in the last step of asthma treatment.

We will discuss the pathogenesis of the allergic reaction and the key importance of IgE in this process in order to highlight the beneficial effects of a drug able to block the circulation of the free form of this immunoglobulin.

We will also review the most important studies and patents for the efficacy and effectiveness of the drug in the treatment of adults and pediatric patients with asthma and other diseases and communicate our experience in treating corticosteroid asthmatic patients with omalizumab.

Structure Based Discovery of Hsp90 Inhibitors for the Treatment of Cancer

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Heat shock protein (Hsp) 90 is a molecular chaperone that is responsible for the correct folding of a large number of proteins allowing them to achieve their functional conformation. Client proteins of Hsp90 include many key over expressed or mutated oncogenes which are known to be critical for the transformed phenotype observed in tumors. 17-AAG and 17-DMAG are Hsp90 inhibitors derived from the prototypical ansamycin natural product inhibitor geldanamycin, which have shown pre-clinical efficacy in mouse xenograft models, and are now in phase I, II and III clinical trials.

Our own efforts in the Structure Based optimization of a class of Hsp90 inhibitors discovered through a combination of medium throughput screening, virtual screening and our Fragment based SeeDs (Structural exploitation of experimental Drug startpoints) technology, will be described. This has led to the identification of a clinical development candidate NVP-AUY922 currently in phase I clinical trials.

Transdermal Delivery of Arginine Vasopressin with Pheroid™ Technology

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The aim of this study was to investigate *in vitro* transdermal diffusion of a small peptide namely arginine vasopressin (AVP) with the aid of the novel Pheroid™ drug delivery system. Generally, peptides seem unfit for transdermal permeation, but it was thought prudent to explore the suitability of this lipid-based system after success was achieved with entrapment of tuberculostatics, bacteria and viruses. Bestatin (a selective aminopeptidase inhibitor) was employed to circumvent any skin-related degradation of the active. Vertical Franz cell diffusion studies were conducted with female abdominal skin, with AVP at a concentration of 150 µg/ml in the donor phase and Hepes buffer as the receptor phase over a twelve-hour period. To prove entrapment of AVP within the lipid structures of the Pheroids™, fluorescently-labelled samples were monitored by means of confocal laser scanning microscopy (CLSM), which revealed definite entrapment. *In vitro* permeation profiles for AVP exhibitedd a biphasic character, with the majority of permeation occurring during the first two hours. The Pheroid™ delivery system proved to be advantageous when applied as delivery system. The inclusion of bestatin had an enhancing effect on permeation probably due to its protection of AVP.

Identification and Characterization of Skin Biomolecules for Drug Targeting by Vibrational Spectroscopy

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Vibrational spectroscopy allows identification and characterization of different biomolecules in the skin - proteins, lipids, water, nucleic acids and glucose - in a fast and non-destructive manner. In addition to the qualitative characterization *in vitro*, *ex vivo* and *in vivo*, vibrational spectroscopy can make quantitative or semi-quantitative determinations that are manifested in spectra as changed intensities and frequencies of observed bands. By measuring IR- or Raman marker bands of proteins, lipids, water, nucleotides or glucose, the relative ratios and the absolute concentrations of each component can be determined and related to pathogenic changes. Among the disease induced spectral changes, we have found that certain IR spectral regions due to nuclear DNA and amide III appeared to be modified and enhanced with progression to skin malignancy; a water-specific Raman spectral region became gradually enhanced with progression to the degree of inflammation in the skin, detecting even minimal water content changes not palpable in the skin; and a glucose-specific region by ATR-FTIR spectroscopy appeared to be highly sensitive to assess and differentiate glucose activity levels in the skin of normal, pre-diabetic and diabetic subjects. Based on our findings, we suggest that vibrational spectroscopy might be a rapid screening tool with sufficient sensitivity and specificity to identify and characterize skin biomolecules in described diseases for drug targeting by pharmacological community.

An Efficient Solid Phase Synthesis Concept for the Preparation of Various Novel Biologically Active Peptide Mimetics

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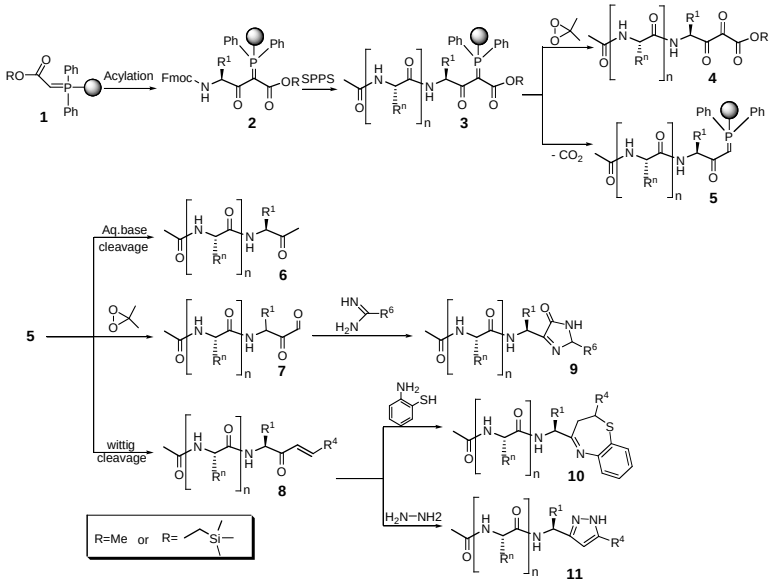
C-acylations of polymer-supported 2-phosphoranylidene acetates **1**(denominated as "linker reagents") are introduced as a flexible entry point to the C-terminal variation of carboxylic acid functionalities. Smooth conditions for the efficient acylation of the phosphoranes with protected amino acids and the subsequent derivatization of the obtained 2-acyl-2-phosphoranylidene acetates **2** were developed.[1] Several alternative cleavage conditions were established: Oxidative cleavage yielded peptidyl 4-amino-2,3-dioxo butanoates (peptidyl-2,3-diketoesters) **4**; saponification on the polymer support led *via* decarboxylation to the peptidyl-3-amino-2-oxo-1-phosphoranylidene propanes **5**. These intermediates could be cleaved by three different methods: oxidatively with DMDO furnishing peptidyl-3-amino-2-oxo-propanals (peptidyl-2-ketoaldehydes) **7**, with aldehydes yielding peptidyl-1-amino-3-buten-4-ones (peptidyl vinyl ketones) **8**, or *via* the basic hydrolysis to peptidyl methylketones **6**. These compounds are excellent intermediates for the synthesis of pharmaceutically important compounds such as 1,3-diamino-2-hydroxy-propane (1,3-Diamino propanol), and for the fast synthesis of peptidyl heterocycles such as imidazolones **9,** thiaazapines **10**, pyrazoles **11**, etc. on the C-terminus.[2]



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Design Studies of Drugs for the Management of Certain Parasitic and Hepatic Viral Infections

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The World Health Organization estimate for the number of humans living(predominantly in low income countries) with parasitic and hepatic B/C viral infections **(HVIs)** is around 2 billion and 500 millions respectively. This grave situation calls for additional comprehensive and sustainable initiatives to study the molecular level genomic and proteomic aspects of the mode of action as well as the mechanism of development of resistance for approved and pipeline drugs for the treatment of such scourges.

The presentation outlines the outcomes of our research efforts in the anti-parasitics domain, including:

1 - **QSAR study** of **pyrantel analogs** using traditional modeling, molecular fingerprinting employing Hologram QSAR (HQSAR®) and 3D QSAR using CoMFA® to explore target binding attributes of the legends. Further, two models were constructed for a series of in-house prepared **levamisole analogs** These models were used to identify potential drug candidates amongst the series.

2 – **Pharmacophore search** for the above-mentioned series of pyrantel analogs . LigandScout® software was used for rigid alignment and GALAHAD® software was used for flexible search (torsional space based) .Features of the generated pharmacophore were analyzed and the two methods were compared regarding model validation and its specificity.

3 – **Docking study** of certain **praziquantel(PZQ)** analogs into schistosomal glutathione-S-transferase enzyme (**GST**),which is considered as potential target for antischistosomal drugs. The utilized software include Molegro® Virtual Docker (MVD) for linear scoring and Surflex® for nonlinear scoring.

In the domain of **HVIs** , the presentation will summarize the results of employing appropriate Computer Aided Drug Design/Discovery (CADD) tools for studying the molecular mechanism of action of an in house prepared library of bridge head fused heterocyclic compounds on specific HCV targets . The studied targets include: human **CD81** large extracellular loop (**LEL**), thus blocking viral entry into human cells, **HCV NS3-4A** Serine protease and **HCV NS5B** Polymerase, thus interfering with viral replication. Further, the ongoing and planned future studies focusing on **HBV** & HCV genotypes that are prevailing in EMRO(WHO) countries will be highlighted.

Discovery of CVT-6883: A Novel A2B Adenosine Receptor Antagonist as a Clinical Candidate for the Treatment of Asthma

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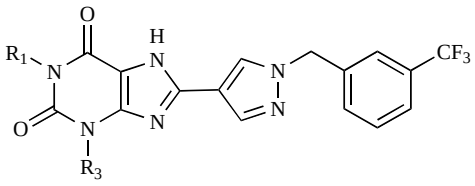
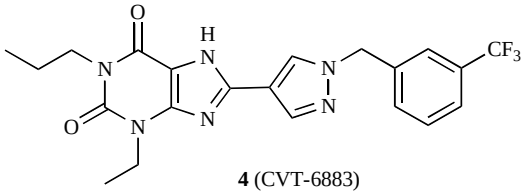
Recently, we have reported a series of new 1,3-symmetrically ($R_1 = R_3$) substituted xanthines (**1**, **2** and **3**) with high affinity and selectivity for the A_{2B}-adenosine receptors (A_{2B}-AdoR). Unfortunately, this class of compounds showed poor pharmacokinetic properties. The 1,3-dimethyl substituted analog 1 that incorporated a 3-CF_3 substituent in the phenyl ring showed greater affinity and selectivity for the A_{2B}-AdoR in comparison to the 1,3- diethyl and dipropyl analogs **2** and **3**. This prompted us to further investigate the effect of differential alkyl substitution at the N_1 and N_3 positions (N1-R ≠ N3-R) on A2BAdoR affinity and selectivity with the overall objective of enhancing affinity and selectivity for the A2B-AdoR as well as improving oral bioavailability. This effort has led to the discovery of compound 4 (CVT-6883) that displayed high affinity and selectivity for the A2B-AdoR. When dosed orally at 2mg/Kg, compound 4 had excellent systemic exposure with t1/2 of 4 hr, Cmax and dAUC >1100 ng/mL and 6500 ng.hr/mL, respectively. In addition, compound 4 showed high potency in inhibiting the accumulation of cAMP induced by NECA in HEK-A2B-AdoR and NIH3T3 cells with KB values 6 and 2 nM, respectively. The synthesis and SAR that lead to the discovery of compound 4 will be presented.





4 (CVT-6883)

1 $R_1 = R_3$ = Me
$K_i(hA_{2B})$ = 1 nM; A_1/A_{2B} = 992; A_{2A}/A_{2B} = 686; A_3/A_{2B} = 686; A_3/A_{2B} = 1000

2 $R_1 = R_3$ = Et
$K_i(hA_{2B})$ = 13 nM; A_1/A_{2B} = 44; A_{2A}/A_{2B} = 34; A_3/A_{2B} = 34

3 $R_1 = R_3$ = propyl
$K_i(hA_{2B})$ = 14 nM; A_1/A_{2B} = 12; A_{2A}/A_{2B} = 28; A_3/A_{2B} = 10

$K_i(hA_{2B})$ = 22 nM; A_1/A_{2B} = 88
A_{2A}/A_{2B} = 149; A_3/A_{2B} = 48

Selective Delivery of Anti-Tumour Agents *via* Matrix Metalloproteinase (MMP)-Cleavable Peptides

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The need for effective delivery systems to treat disease is no greater demonstrated than in cancer, where selective targeting of chemotherapeutic agents that are also toxic to healthy cells remains a formidable challenge. The search for targets or processes with levels of expression or activity in cancer cells that differ from normal cells and tissues is fierce.

The matrix metalloproteinases (MMPs) are a family of zinc-dependent endopeptidases. Within this group, the membrane-type matrix metalloproteinases (MT-MMPs) are highly active in tumours, but are absent or inactive in normal tissues. MT1-MMP is known to be elevated in the majority of human tumours and to be central to tumour invasion and angiogenesis. We have devised a delivery strategy enabling prodrugs of highly potent anti-tumour agents to be targeted to the tumour, whereby the elevated levels of MT1-MMP are utilised to activate them, releasing the potent agent within the tumour microenvironment.

We report the design and synthesis of a series of peptide-based conjugates of a colchicine analogue (the effector), using a combination of solution and solid phase organic chemistries. The effector was conjugated through the peptide *C*-terminus, whereas the *N*-terminus was end-capped to prevent non-specific cleavage by exopeptidases.

Results demonstrate that our lead peptide-based conjugate was stable in plasma and was successfully cleaved in the tumour to release the potent effector. Significant stability in liver tissues was achieved through rational drug design. Furthermore, human preclinical tumour models expressing MT1-MMP have been demonstrated to efficiently cleave this prodrug to release the effector, whilst those negative for MT1-MMP do not. Using LC-MS we have shown that the prodrug is preferentially cleaved in MT1-MMP expressing tumour homogenates relative to plasma and liver, supporting its pharmacological stability. *In vivo* the prodrug demonstrated wide tissue distribution, with activation observed selectively in the tumour and relative stability in plasma and normal tissues. The prodrug resulted in effector levels in the tumour comparable to that achieved by effector alone, but with little or no effector exposure in plasma or normal tissues. Prodrug delivery to the tumour resulted in anti-tumour activity and a significant delay in tumour growth.

In summary, we have demonstrated proof-of-concept for a drug delivery system, whereby prodrugs of potent anti-tumour agents are selectively activated by MT1-MMP within the tumour microenvironment.

Mechanism and Inhibition of Collagenolytic Matrix Metalloproteinases

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Collagen serves as a structural scaffold and a barrier between tissues, and thus collagen catabolism (collagenolysis) is required to be a tightly regulated process in normal physiology. In turn, the destruction or damage of collagen during pathological states plays a role in tumor growth and invasion, cartilage degradation, or atherosclerotic plaque formation and rupture. Only a small number of proteases have been identified capable of efficient processing of triple-helical regions of collagens. Several members of the zinc metalloenzyme family, specifically matrix metalloproteinases (MMPs), possess collagenolytic activity. A mechanistic understanding of the cleavage of intact collagens has been pursued for many years; the results of such studies could lead to the development of truly selective MMP inhibitors. Our laboratory has developed triple-helical peptide (THP) substrates and inhibitors for MMPs, with the goal of using these model systems to dissect collagenolytic behavior. Studies of MMP/THP interactions by biophysical methods [NMR spectroscopy and hydrogen/deuterium exchange mass spectrometry (HDX MS)] in combination with site-specific mutagenesis and kinetic analyses have allowed us to more precisely determinate the roles of MMP regions and residues in the binding, unwinding, and hydrolysis of triple-helical structures. These results have also led to a “conformational entropy shift” hypothesis explaining how MMPs process collagen without input from an external energy source. Ultimately, we are utilizing information about collagenolytic mechanisms to design inhibitors that target proteases implicated in cancer progression (MMP-2, MMP-9, and MT1-MMP) while sparing proteases with host-beneficial functions (MMP-3 and MMP-8).

How Can We Prevent Diabetes in Pharmacological Way?

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Diabetes and its prevention is one of the most important health issues. The data obtained during several years ensured life-style modification as the best way to prevent diabetes. However, pharmacological prevention of diabetes is also possible with the use of drug affecting insulin resistance (metformin, thiazolidinedions TZD, drugs affecting renin-angiotensin system), as well as drugs directly affecting the central obesity - the main risk factor of developing diabetes. Metformin is not registered as the drug to prevent diabetes in glucose intolerant patients although it is use un-label in this indication. TZD – after the success of rosiglitazone in DREAM trial seem the cornerstone of pharmacological prevention of diabetes, but some regards about their cardiovascular safety must be taken into account. Drugs affecting renin-angiotensin system (angiotensin converting enzyme inhibitors - ACE-inhibitors as well as angiotensin receptor blockers - ARB have been proved to prevent diabetes. More recent data and recently published meta-analyses show greater diabetes prevention potential for ARB than for ACE-inhibitors. Some ARBs posses TZD-like properties and are even claimed the separate subgroup of ARBs (telmisartan). The new data from ONTARGET trial coming in March, 2008 will clarify potential differences in diabetes prevention between ACE-inhibitors (ramipril), ARBs (telmisartan), as well as will bring the answer if the double blockade (telmisartan and ramipril) will act synergistically as far as diabetes prevention is concerned. Future directions in pharmacological prevention will probably focus on the new drugs affecting visceral obesity (endocannabinoid receptor antagonists rimonabant, taranabant) - the main risk factor of developing diabetes. Some older drugs to treat obesity recently have been even approved on the OTC (over-the-counter) basis (orlistat). The potential poly-pill for diabetes prevention might be also proposed. The lecture will discuss the modern pharmacological treatment for diabetes prevention as well as the future directions of research.

Prospects for Non-Invasive Delivery of Proteins and Nucleic Acids Across the Skin

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The availability of effective delivery systems is becoming increasingly important as the nature of the therapeutic agents include not only small molecules but macromolecules such as proteins and DNA. Novel delivery systems have already had a major impact on drug therapies and patient well being. For example, oral controlled release systems, injectable sustained release depots, polymeric brain implants, inhaler devices, liposomes, and quick-dissolve tablets were developed to provide patients with reliable and efficient drug therapies. The high interest is reflected in projections for an annual 8% increase in demand for new delivery systems through 2008. There is an especially high demand for non-invasive transdermal technologies, due to the current lack of effective systems that deliver drugs specifically to their site of action without adverse effects.

This will require the development and characterization of delivery and targeting systems suitable for new emerging therapeutic agents such as proteins, peptides, and oligo- and polynucleotides, whose molecular weights are over the generally accepted transdermal delivery limit of 500-1000 Daltons. The development of needle-free methods for delivery of proteins and DNA through permeability barriers such as the intact skin will also greatly facilitate the administration of human and veterinary vaccines and will increase safety and compliance.

Strategies to improve dermal and transdermal delivery include physical and chemical methods. Physical methods utilize i) biolistic devices that 'shoot' liquid or powder particles by pressure into the skin (eg. gene gun, bioject, powderject); microneedles of many types; iii) ultrasound; and iv) electrically driven methods eg. iontophoresis, electroporation. Chemical methods involve i) the use of a wide selection of permeation enhancer compounds; ii) pharmaceutical formulations that improve drug diffusion related parameters; and iii) designing novel micro- and nanoparticles that restructure the skin permeation pathways to facilitate delivery.

This presentation will focus on some recent designs of protein and nucleic acid delivery systems, their potential and limitations. In particular, the design and development of biphasic vesicles and gemini nanoparticles as protein and DNA delivery system, respectively, will be presented.

The structure and properties of biphasic vesicles and examples for delivering proteins and vaccines will be described. Delivery of macromolecules from biphasic vesicles can be explained by a novel mechanism of interaction with the stratum corneum. The achievable delivery efficiency and comparative results with other delivery approaches will also be discussed.

In the second example, the development and application of dicationic (gemini) surfactant-based nanoparticles as DNA delivery systems for cutaneous gene therapy will be presented. Gemini nanoparticles as non-viral carriers have the advantage of having, generally, low toxicity/immunogenicity, as well as having no limitation with regard to the size of DNA that can be delivered. Characterization of the structural and physicochemical properties of these dicationic lipid-based DNA complexes by small-angle x-ray scattering (SAXS), zeta potential and particle size analysis indicate correlation between polymorphic flexibility of the nanoparticles and cellular transfection efficiency.

The successful development of these non-invasive delivery technologies could mean fewer needles for patients receiving protein drugs, patches instead of needles for vaccination and gene therapy, no pain and better targeting of drugs to disease sites.

Mapping Structural and Functional Epitopes for Ligand and Monoclonal Antibody Binding on the Receptor for Urokinase-Type Plasminogen Activator: Identification of Potential Target Sites for Antagonist-Based Cancer Therapy

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During cancer invasion and metastasis the breakdown of extracellular matrix is mediated by several proteolytic enzyme systems including the plasminogen activation system. In this system plasminogen is activated to its active counterpart plasmin by urokinase-type plasminogen activator (uPA). The uPA receptor (uPAR) is a GPI-anchored membrane glycoprotein, which focuses uPA at the cell surface, and *via* its interaction with other ligands such as the extracellular matrix prote*in vitro*nectin (Vn) and certain integrins the receptor also modulates cell adhesion and migration. Increased levels of uPAR expression are often found in the invasive areas surrounding human cancers and correlate with poor prognosis, so this receptor has been recognised for more than a decade as a potential target for development of new anti-invasive cancer therapies.

We have recently solved the 3-dimensional structure of uPAR in complex with a competitive peptide antagonist of uPA-uPAR interaction [1], and this structural information was later extended to include a complex with the amino-terminal fragment of uPA (ATF) by Huai *et al.* [2]. The three homologous domains of uPAR are assembled into a "croissant-like" structure with a large deep central cavity capable of binding either the peptide antagonist or the receptor binding module of ATF, thus leaving the external surface accessible for other protein interactions. Moreover, we have identified the functional binding epitopes on uPAR for uPA and Vn using a comprehensive alanine-scanning library of 244 purified single-site uPAR mutants, which has enabled us to propose a model for the tri-molecular complex of ATF-uPAR-Vn [3,4]. The functional epitopes for several competitive and non-competitive inhibitory monoclonal antibodies directed against uPAR have likewise been determined [5,6].

In conclusion, our data provide new structural and functional information at the molecular level concerning several biologically important binding sites on uPAR. This new insight may potentially direct the future rational development and design of specific uPAR antagonists for use in uPAR-targeted cancer therapy.

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Novel Therapeutic Targets in Esophageal Cancer: Impact of Coexpression of Receptor-Tyrosine-Kinases (RTK) and Chemokine Receptor CXCR4

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Background. Despite curative surgery, prognosis of esophageal cancer is still poor. The aim of our study was to define the (co-)expression pattern of target receptor-tyrosine-kinases (RTK) and to evaluate the role of chemokine receptor CXCR4 in esophageal adenocarcinoma and squamous cell cancer.

Methods. The (co-)expression pattern of *VEGFR1-3*, *PDGFRα/ß* and *EGFR1* was analyzed by RT-PCR in 50 human esophageal cancers (35 adenocarcinomas and 15 squamous cell cancers). In addition, IHC staining was applied for confirmation of expression and analysis of RTK localisation. In 102 consecutive patients undergoing esophageal resection for cancer, the LSAB+ system was used to detect the protein CXCR4. Tumor samples were classified into two groups based on the homogeneous staining intensity (weak and strong CXCR4 expression).

Results. Adenocarcinoma samples revealed a *VEGFR1* (97%), *VEGFR2* (94%), VEGFR3 (77%), *PDGFRα* (91%), *PDGFRß* (86%) and EGFR1 (97%) expression at different intensities. 94% of esophageal adenocarcinomas expressed at least four out of six RTKs. Similarly, squamous cell cancers revealed a *VEGFR1* (100%), *VEGFR2* (100%), VEGFR3 (53%), *PDGFRα* (100%), *PDGFRß* (87%) and EGFR1 (100%) expression at different intensities. All esophageal squamous cell carcinomas expressed at least four out of six RTKs.

With regard to CXCR4 expression, in adenocarcinoma, a rate of 89.1% was detected with a weak intensity in 71.7% compared to strong staining in 29.3%. The overall expression rate for CXCR4 in esophageal squamous cell carcinoma was 94.1%, subdivided into 54.9% with weak and 45.1% with strong staining.

Conclusion. Our results reveal a high rate of receptor-tyrosine-kinases (co-) expression and expression of chemokine receptor CXCR4 in esophageal adenocarcinoma and squamous cell cancer and might therefore encourage an application of multiple-target RTK-inhibitors as well as of CXCR4-antagonists within a multimodal concept as a promising novel approach for innovative treatment strategies.

Unique Autoinhibitory Dimers in the MAP2K MEK6, and MAP4K OSR1

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In new structure determinations of MAP kinase pathway components MEK6 and A putative MAP4K OSR1, it is becoming clear that componentS adopt inactive and processing-inactive structure in the absence of their sustrates or processing enzymes. Appropriate encounters induce conformational changes leading to an active conformation or activation, thus improving pathway specificity. Here we present structural data on the kinase domain of the MAP2K MEK6, the activator of the MAPK p38a. The form of MEK6 has the activating phosphorylation sites mutated to aspartic acid, which in the full length MEK6 induces an active enzyme. The structure observed is that of an elongated inactive dimer in which the active site is not present, and the activation loop is sequested from solvent. The dimer has been validated by low angle x-ray scattering. Second we offer data on the stucture of the stress activated kinase OSR1. This enzyme also adopts and inactive autoinhibitory dimer in which the activation loop is sequestered from solvent. Domain swapping is involved in the dimer formation, the first example of domain swapping in protein kinases. These data offer insight into kinase pathway specificity, and have clear implications for drug discovery.

IIntegrating Gene-Brain-Behaviour Markers for the era of 'Personalized Medicine'

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Personalized Medicine has been driven by the FDA to find the best Markers of treatment prediction. A standardized integrative framework for finding Gene-Brain-Behaviour Markers in brain-related disorders will be presented. Proof of concept successes in Personalized Medicine and exemplars of Gene-Brain-Behaviour Markers will be shown in Depression and Schizophrenia. Exemplar SNPs include BDNF, COMT, 5HTT and 3A which are correlated with an array of EEG/ERP/Autonomic/ Cognitive Markers. These candidate Markers will be discussed with reference to the FDA's Critical Path for qualification and validation of Markers.

Pheroid™ Technology: A Novel Drug Delivery System with Broad Application

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The application of a novel drug delivery system, the Pheroid™, was investigated as a vehicle for the delivery of a wide variety of active pharmaceutical ingredients (APIs). Results indicate that Pheroid™ technology can entrap and deliver most APIs with high efficiency, resulting in enhanced therapeutic efficacy.

According to the WHO, a third of the world's population is infected with *Mycobacterium tuberculosis*, although most individuals are non-symptomatic. The emergence of multidrug (MDR) and extremely drug resistant (XDR) tuberculosis is cause for concern. The formulation of a more effective tuberculosis regime, using Pheroid™ technology, will be illustrated on the hand of both *in vitro* and *in vivo* studies. The enhancement in the bioavailability of especially rifampicin, one of the mainstays of tuberculosis therapy, will be discussed. Results of a comparative phase I study, using a commercial preparation as comparator, illustrate that the use of Pheroid™ technology resulted in faster absorption (shorter T_{max}), increased plasma levels (higher C_{max}), and a longer therapeutic window (area under the curve above minimal effective concentration). Despite increased plasma levels present over longer periods, participating volunteers presented with fewer side effects.

The various patents that have been obtained for a number of applications in countries worldwide will be addressed briefly.

A Synthetic Platelet as Application of a Targeted Controlled-Rate Drug Delivery Vehicle

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We developed a controlled-rate drug delivery vehicle that is amenable to active, rather than passive targeting: individual liposomes with surface-grafted hydrogels. The controlled hydrogel mesh size determines the diffusion of aqueous or lipidic contents by size while the hydrogel itself reduces the liposomes' interactions with blood cells that is the cause of passive targeting. Instead, the surface hydrogel is easy to derivatize by molecules that have reactive SH or NH_2 groups and thus can provide specificity/targeting capabilities. These hydrogel liposomes are stable to both freeze-thawing in aqueous media, as well as to lyophilization.

To create a synthetic platelet, the above construct was derivatized at high substitution levels with a d-amino acid peptidomimetic chosen by vWf binding to a random d-peptide array. *In silico*, the peptidomimetic was docked onto the computer-derived GPIb-vWf interactive surface and its location and binding stability were confirmed. Synthesized, the peptidomimetic inhibited ristocetin-mediated *in vitro* platelet agglutination. On the hydrogel liposome, the peptidomimetic provided platelet microparticle-like behaviour such that 30 min after *iv* injection, this construct reduced mouse tail-bleeding times by 30% compared to buffer, and 60% compared to the non-derivatized hydrogel liposomes.

This study suggests that targeted macromolecular constructs are a new approach to drug delivery.

Structural Basis and Drug-Target Interactions: A Case Study on Avian Influenza (H5N1)

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This study aims at gaining detailed information insight into molecular mechanisms of action of three drug targets of the life cycle of influenza virus A subtype H5N1, hemagglutinin (HA), M2 channel (M2) and neuraminidase (NA) by using MD simulations. At HA target, interest is focused on high pathogenicity (HP) of H5 due to -RRRKK- insertion. Only HPH5 loop binds strongly to furin cavity, serving as a conformation suitable for acylation. Then, the reaction mechanism was investigated by QM/MM approach. First step of acylation was found to be concerted reaction with a formation of tetrahedral intermediate. The second target, M2 complexed with/without inhibitor in hydrated lipid bilayer was studied to understand how drugs inhibit the replication. Two mechanisms of action where drug binds to opening pore and histidine gate, were explained. Investigation was extended to NA. Rotation of -NHAc and -$OCHEt_2$ side chains of oseltamivir, leading directly to rearrangement of catalytic cavity, was found to be a primary source of the lower susceptibility of oseltamivir to N1 than to N2 and N9. In addition, three inhibitiors complexed with N1 were studied to understand the drug-target interactions. The structural properties, position and conformation of peramivir and its side chains are uniformly preferential.

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The Swi2/Snf2-Homolog Protein Fun30 is a Novel ATP-Dependant Chromatin-Remodeler

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Chromatin-remodeling complexes modulate the higher order structure of chromatin and facilitate/hinder the binding of transcription factors. Phylogenetic analysis shows that these complexes share several common features including the presence of a distinct ATPase domain. Based on sequence homology to this domain, we have recently identified new candidate remodeling proteins in the yeast *Saccharomyces cerevisiae*. A homolog to the ATPase domain of the Swi2/Snf2 subunit of the chromatin-remodeling complex SWI/SNF, Fun30 (Function unknown now 30) is one such protein. Here, we demonstrate that Fun30 is a homodimer with a molecular weight of about 250 kDa. We also show that the purified Fun30 stably binds to DNA, nucleosomes, nucleosomal arrays, and can alter the nucleosome structure in an ATP-dependent manner increasing the accessibility of DNA in restriction enzyme digestion assays. In addition, Fun30 can transfer octamers *in trans* to another DNA fragment as well as slide nucleosomes *in cis*. We have also demonstrated that the remodeling of nucleosomes by the Fun30 protein leads to transcription stimulation from nucleosomal templates *in vitro*. These biochemical data show that Fun30 is a novel protein that functions similar to the yeast SWI/SNF complex to remodel chromatin. Interestingly, in an attempt to identify its *in vivo* role, we have observed that the *fun30* null mutant is resistant to DNA damaging agents such as ultraviolet and ionizing radiation as well as to a chemotherapeutic drug, etoposide, suggesting possible involvement of Fun30 in DNA repair/DNA damage processing and/or cancer development. Understanding the role of Fun30 in these pathways may lead to potential therapies that would be beneficial in the treatment of cancer.

Regioselective Synthesis of Symmetric Pyridine-Spaced Sulfated Glucuronic Acid and Study of their Antiproliferative Activity

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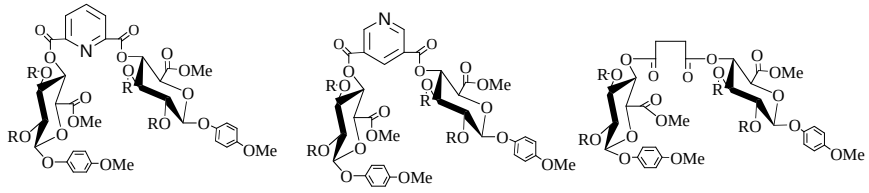
Heparin (HP) is one class of (glycosaminoglycans) GAGs [1], a major class of extracellular complex polysaccharides that represents the primary constituents of every eukaryotic cell surface and the extracellular environment. Heparin is used in therapy as an anticoagulant and antithrombotic drug. However, the anticoagulant properties of heparin involve hemorrhagic risks, when administered to cancer patients, heparin increases survival times but the hemorrhagic risks and non-anticoagulant variants of the polysaccharide endowed with potential antitumor properties are warranted.

Spaced fully sulfated disaccharides in which two units of disaccharide units are separated by aromatic spacer and C6 open chain sugars attached by various linkers to the aromatic spacer, have been reported to have a remarkable antiproliferative activity significantly higher than heparin, which points at the contribution of the spacer to the overall binding.



R = SO_3Na

The ongoing research approach in our lab is dealing with the synthesis of a new type of dimeric sugars possessing symmetrical pyridine bridge *via* an ester linkage. The unique pattern of protecting groups on the sugar moiety would allow us to get a number of sulfated sugars.

Reference:

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Piggy Back Approach: Bisaphthalimides as Muscarinic Agnoists, Cancerostatics, and Antiinfectives

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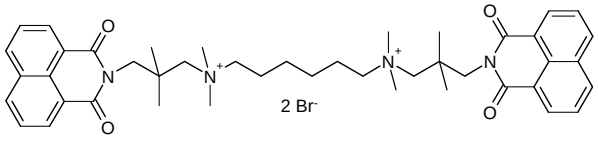
Mono- and bisnaphthalimides such as amonafide and elinafide have been developed as cancerostatic drugs by Brana *et al.* Recently, naphthalimides could be profiled as potent ligands of the muscarinic receptor. Within the frame of a broad screening program for drugs a small library of recently synthezised mono- and bisquartary naphthalimides were tested for their activity against *Trypanosoma brucei*, *Leishmania major*, *Candida albicans*, *Staphylococcus aureus*, *Plasmodium falciparum* and for toxicity against macrophages. Depending on the substitution pattern the mono- and bisnaphthalimides were active against either microorganism in the lower micromolar and nanomolar range of concentration combined with almost no cytotoxicity. Thus, the bisnaphthalimides studied here are perfect lead compounds for further drug development. Interestingly, the structure-activity relationships were different for each purpose indicating that the piggy-back approach can be considered as a worthwhile method for drug development.



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Development of Inhibitors of Hsp90 for Cancer Therapy: The Screening Story

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Inhibition of Hsp90 function has been promoted as a mechanism to degrade oncogenic client proteins involved in tumourigenesis and disease progression; indeed several Hsp90 inhibitors have been shown to lead to client protein degradation and ultimately cell death. Many companies have developed small molecule inhibitors of Hsp90 with first in class inhibitors showing biological and clinical activity at well-tolerated doses.

We have utilised a streamlined screening cascade to rapidly assess compounds for their ability to bind to Hsp90 and inhibit its function *in vitro* and in cell based assays. We have used these assays in conjunction with co-crystal structures of small molecules to drive a structure-based design programme aimed at the discovery and optimization of novel classes of potent Hsp90 inhibitors. During the programme, we have revisited our initial Hsp90 FP assay, using information derived from compounds from our lead series, to provide more sensitivity and hence allow complete dissection of the SAR within these series. We also used this opportunity to transfer the assay to a high throughput format whilst maintaining the quality of the assay. This programme has recently provided compounds for Phase I clinical trials.

The Therapeutic Potential of γ-Secretase Modulators in Treating Alzheimer's Disease

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According to the β-amyloid (Aβ) hypothesis, compounds that inhibit γ-secretase, the pivotal enzyme that generates Aβ from amyloid precursor protein (APP), are potential therapeutics for Alzheimer's disease (AD). Studies in both transgenic and non-transgenic animal models of AD have indicated that γ–secretase inhibitors, administered by oral route, are able to lower brain Aβ concentrations. Initial studies in healthy volunteers and in AD patients have confirmed that γ-secretase inhibitors may lower β-amyloid$_{1-40}$ ($A\beta_{1-40}$) levels in plasma and, to a lesser extent, in cerebrospinal fluid. Unfortunately, animal studies have shown that γ–secretase inhibitors may cause tissue abnormalities in the gastrointestinal tract, thymus and spleen. These toxic effects are likely due to inhibition of the endocelluar cleavage of Notch, a transmembrane receptor involved in regulating cell-fate decisions.

In 2001, some non-steroidal anti-inflammatory drugs (NSAIDs), including ibuprofen, sulindac sulphide and indomethacin, have been found to modulate γ-secretase activity by shifting its cleavage of APP from longer fibrillogenic Aβ species ($A\beta_{1-42}$) in favour of shorter non-amyloidogenic species ($A\beta_{1-38}$). Since these NSAIDs alter the ability of γ-secretase to cleave APP only at the γ cleavage sites, Notch metabolism and signalling are not affected. Thus, *in vivo* γ-secretase modulators are expected to not cause the Notch-dependent toxicities observed with γ-secretase inhibitors. Different long-term studies have shown beneficial neuropathological and behavioural effects of these NSAIDs (mainly ibuprofen) in transgenic animal models of AD. However, due to their low *in vitro* Aβ inhibitory potency and poor *in vivo* brain penetration, it is unclear if the observed *in vivo* effects of selected NSAIDs on Aβ brain pathology and learning depend on their activity on γ-secretase or on other biological targets. Unfortunately, chronic use of NSAIDs may be associated to significant gastrointestinal and renal toxicity and this limits their clinical use in AD. Because the $A\beta_{1-42}$ lowering effects of NSAIDs do not appear to depend on their cyclooxygenase (COX) inhibitory activity, NSAIDs derivatives with optimised $A\beta_{1-42}$-lowering potency and little to no anti-COX activity have been identified. Such agents could represent a new generation of "anti-amyloid" drugs that selectively target production of the highly amyloidogenic $A\beta_{1-42}$ species without inhibiting either COX activity or the physiological proteolytic activity of γ-secretase on important substrates like Notch. The most advanced NSAID derivative with γ-secretase modulation activity and poor anti-COX activity is tarenflurbil, the (*R*) enantiomer of flurbiprofen. A 1-year, placebo-controlled study in 207 AD patients employing two dose regimens of tarenflurbil, (400 mg and 800 mg twice-a-day) indicated that the drug is well tolerated and suggested that the high dose regimen may significantly slow the cognitive and functional decay of mild affected patients. Other NSAID derivatives without anti-COX activity or associated-toxicity have been described by Nicox (NCX-2216, HCT1026), Hoffmann-La Roche (carprofen derivatives) and D-Pharm (phospholipid derivative of indomethacin). Other γ-secretase modulators have been described by Merck Sharp & Dohme, Torrey Pines Therapeutics, Eisai and Chiesi. All these compounds are claimed to reduce *in vivo* $A\beta_{1-42}$ without affecting Notch processing. E2012 has recently described in Phase 1 clinical development. The two ongoing Phase 3 studies with tarenflurbil, will tell us if allosteric modulation of γ-secretase is clinically effective.

Developing Novel Antimicrobial Peptides Active Against Gram-Positive Pathogens

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Lysostaphin is an antimicrobial peptide active against MRSA that consists of an N-terminal catalytic domain and a targeting domain. The mechanism of killing is an M37/M23 endopeptidase activity that cleaves the pentaglycine cross-bridges that are found in the cell wall of *S. aureus*. Lysostaphin producing *S. simulans* strains also encode a lysostaphin immunity factor (Lif) that results in the incorporation of serine residues at positions 3 and 5 of the pentaglycine cross-bridge thus preventing endopeptidase cleavage.

We have developed a novel polypeptide FRET substrate that facilitates the analysis of the endopeptidase activity of lysostaphin. We have validated the FRET assay by demonstrating that site-directed mutants that reduce or abolish the biological activity of lysostaphin similarly inhibit the endopeptidase activity against the FRET substrate. We have also identified the site of lysostaphin cleavage of the pentaglycine target sequence in the polypeptide FRET substrate and generated new information on the affect of serine residues in the pentaglycine target sequence on cleavage. The existence of other members of this M37/M23 protease family that are produced by bacteria that do not contain pentaglycine crossbridges implies a wealth of novel antimicrobial agents that could be revealed by a comparative study of this group of endopeptidases.

The Role of Aldehyde Oxidase in the Metabolism of the Antipsychotic Drug Geodon

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Geodon (ziprasidone) (5-[2-(4-(1.2-benzisothiazol-3-yl) piperazine-1-yl) ethyl]-6-chloroindolin-2-one) is a novel antipsychotic compound developed to target a broad spectrum of antipsychotic efficacy, including positive and negative symptoms in schizophrenia and has been recently approved for the treatment of schizophrenia. It undergoes extensive metabolism in preclinical species and humans after oral administration and only a very small amount of administered dose is excreted as unchanged drug.

In vitro studies using human liver microsomes have shown that the oxidative metabolism of ziprasidone is mediated primarily by CYP3A4. However, co-administration of ziprasidone with ketoconazole, a CYP3A4 inhibitor, showed only a modest increase in its exposure. Therefore, *in vitro* metabolism of ziprasidone was investigated to further understand its clearance mechanisms in preclinical species and humans.

S-Methyl-dihydroziprasidone of the parent drug (M9) is the product of reductive cleavage (dihydroziprasidone) which was not detected either *in vivo* or *in vitro* possibly due to its instability or its rapid metabolism to S-methyl metabolite. The formation of M9 is an important phase II pathway evident in man and accounted for >60% of the administered dose in feces. Therefore, the formation of dihydroziprasidone and S-methyl-dihydroziprasidone and the enzymes involved in their formation were studied in human liver *in vitro* preparations using HPLC/MS/MS and simultaneous radiometric detection.

Peroxisome Proliferator-Activated Receptor Delta Selective Agonists as Potent Anti-Atherogenic Agents *In Vivo*

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Atherosclerosis is a disease of large arterial blood vessel induced by the dysfunction of lipid metabolism and characterized by chronic inflammation progressed through the interaction between lesional lipoproteins and immune cells. The peroxisome proliferator-activated receptors (PPARs) are transcription factors which are activated by their cognate ligands and act as master regulators in mammalian physiology. There are three isoforms, PPARα, PPARγ and PPARδ. Among them, PPARδ has been recently identified as a key regulator in the lipid homeostasis and inflammation. Dysfunction of these elements can lead to atherosclerosis, thus suggesting the beneficial role of PPARδ selective agonist in the treatment of atherosclerosis. PPARδ agonists with lack of selectivity developed so far did not meet the expectation. By using a ligand with PPARδ-selectivity developed in this study, we obtained a significant inhibition of lesion progression on the disease mice model, *apolipoprotein E* deficient mice, through raising HDL and cytokine modulation without any obvious adverse effect on intestinal polyp formation. The results provided the first evidence of anti-atherogenic activity of a PPARδ-selective agonist *in vivo*. In this regard, our results clearly demonstrated that PPARδ is a promising target for drug development to treat a devastating disease, atherosclerosis.

High-Performance Screening of Active Peptide inhibitor of Integrin Receptor by Protein Microarray Chip

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ProteoChip has been developed as a novel protein microarray technology. So far it has been applied in different protein expression profiles and molecular diagnostics and we expect its role to grow in the field of biology. Here, we investigated the application of ProteoChip in new drug discovery. Integrin αvβ3 microarray immobilized on ProteoChip was employed to screen new active peptides against the integrin from multiple hexapeptide sub-libraries of positional scanning synthetic peptide combinatorial library (PS-SPCL). The integrin αvβ3-vitronectin interaction was successfully demonstrated on the integrin microarray in a dose-dependent manner. Novel peptide ligands with high affinity to the integrin were identified from the peptide libraries with this chip-based screening system by competitive inhibition assay in a simultaneous and high-throughput fashion. We have confirmed anti-angiogenic functions of the novel peptides thus screened through *in vitro* and *in vivo* angiogenesis assay. These results provide evidences that ProteoChip is a promising tool for high-throughput screening of lead molecules in new drug development.

Molecular Response to Therapeutic Interferons: Sensitivity and Resistance Mechanisms

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Interferons (IFN) are important proteins that are used in the therapy of a number of diseases, notably hepatitis C, multiple sclerosis, and in several tumor types. IFN is a multi-functional protein that induces functionally diverse and large number of genes particularly during host defense, cell growth control, signaling, and metabolism. Molecular response to rIFN-α2a or rIFN-β, widely used therapeutic forms of IFN, was assessed using whole genome expression microarray in the human IFN-sensitive epithelial cell line, WISH, and in Huh-7 liver cell line. Bioinformatics analysis of the 3′ untranslated regions of IFN-stimulated genes (ISGs) showed that the AU-rich elements (ARE), which are associated with early and transient response genes, exist in approximately 20% of the mRNAs induced by IFN; the ARE transcriptome is ~10% of the entire transcriptome. Subsequently, we analyzed gene expression of such early/transient response genes in HEK293 cell line which is resistant to the antiviral action of IFN using custom-made ARE-cDNA microarray. Also, Daudi and RPMI 1788 B-cell lines were assessed due to their differential response to the anti-proliferative action of IFN-α. These IFN responses were correlated with Stat-1 phosphorylation and 6-16 gene expression. Clustering algorithms generated two informative expressed gene clusters that were selectively associated with cellular sensitivity and resistance to both of the antiviral and anti-proliferative action of IFN. In another model of IFN biology, we have found that the IFN-regulated RNase L but not the cancer mutant R462Q downregulates genes that are involved in cell proliferation and invasion. A gene cluster comprised of small set of genes is able to distinguish responses to IFN which can provide diagnostic and therapeutic monitoring or evaluation of IFN forms.

From Molecular Recognition to Drug Design: Applications of Experimental and Theoretical Enzyme Inhibition Studies

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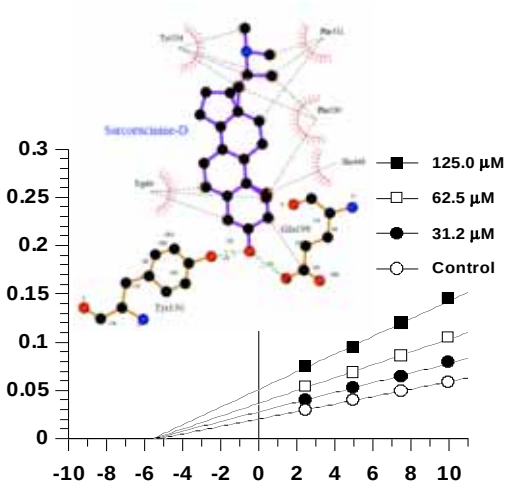
Drug discovery/design has relied very heavily on structural computational chemistry in identifying novel drug compounds, optimizing lead compounds for specific therapeutic targets, and in assisting experimental R&D programs in bringing potential drugs to the market. One of the most important computational chemistry tools to predict the bound conformation of a small molecule to a macromolecular target is molecular docking.



Our research group has been focusing on identification of natural compounds that inhibit various clinically important enzymes, including cholinesterase enzymes. 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 lecture will give an overview of some of our newly-discovered cholinesterase inhibitors. Examples of new interesting potential drug candidates will be shown to demonstrate how theoretical structural computational chemistry could be employed along with experimental enzyme kinetics to investigate the molecular basis of enzyme-inhibitor interactions.

The Sodium Pump and Its Alpha1 and Alpha3 Subunits as Novel Targets to Combat Apoptosis- and/or Multidrug-Resistant Proliferating and Migrating Cancer Cells

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The sodium pump, Na(+)/K(+)-ATPase, could be an important target for the development of anti-cancer drugs as it serves as a versatile signal transducer, it is a key player in cell adhesion and its aberrant expression and activity are implicated in the development and progression of different cancers. Cardiotonic steroids, known ligands of the sodium pump have been widely used for the treatment of heart failure. However, early epidemiological evaluations and subsequent demonstration of anti-cancer activity *in vitro* and *in vivo* have indicated the possibility of developing this class of compound as chemotherapeutic agents in oncology. Their development to date as anti-cancer agents has however been impaired by a narrow therapeutic margin resulting from their potential to induce cardiovascular side-effects. I first propose to discuss (i) sodium pump structure, function, expression in diverse cancers and its chemical targeting and that of its sub-units, (ii) reported *in vitro* and *in vivo* anti-cancer activity of cardiotonic steroids, (iii) managing the toxicity of these compounds and the limitations of existing preclinical models to adequately predict the cardiotoxic potential of new molecules in man and (iv) the potential of chemical modification to reduce the cardiovascular side-effects and improve the anti-cancer activity of new molecules.

I then will show that chemical modifications of 2”-oxovoruscharin (a novel cardenolide extracted from *Calotropis procera*) based on an understanding of the structure activity relationship within the series, has led to the identification of UNBS1450, a molecule characterized by more potent anti-proliferative activity and lower toxicity than classic cardenolides. In aggressive and metastatic orthotopic NSCLC, refractory prostate cancer and glioma models, UNBS1450 is more potent than tested reference compounds, including taxol, irinotecan, oxaliplatin, mitoxantrone and temozolomide

The general mechanism of action associated with UNBS1450-mediated anti-cancer effects relates to the compound’s disorganization of the actin cytoskeleton. UNBS1450 can thus be considered both anti-proliferative (cytotoxic) and anti-migratory given that the actin cytoskeleton is essential to cytokinesis and to cancer cell migration. UNBS1450 also induces non-apoptotic cell death processes (such as lysosome membrane permeabilization and autophagy) and thus may overcome major apoptosis resistance pathways responsible for the failure of therapeutics in certain cancers.

UNBS1450 is currently in preclinical development and should reach Phase I clinical trials in 2008.

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Synthesis of 2',5'-Oligoadenylates having Aromatic Groups and their Biological Activities

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RNase L is activated by binding of unusual 2',5'-kinked oligoadenylates (2-5A) and acts as the effecter enzyme of the 2-5A system, an interferon-induced anti-virus mechanism. Efforts have been made to understand the 2-5A binding mechanism, not only for scientific interests but also for the prospects that the understanding of such mechanism lead to new remedies for viral diseases. In order to prepare more effective 2-5As and to understand the structure-activity relationship of 2-5As, a variety of 2-5A derivatives with base, sugar and/or linkage modifications were synthesized. On the other hand, we have recently elucidated the crystal structure of the 2-5A binding ankyrin repeat domain of human RNase L complexed with 2-5A. On binding 2-5A at the ankyrin-repeat domain, RNase L forms a homodimer and removes the ankyrin-repeat domain from the nuclease domain to become the active form.

For the purpose of new drug discovery and development against infectious diseases, this paper describes synthesis of facile 2',5'-oligoadenylate (2-5A) analogs having aromatic groups and the structure-activity relationship of 2-5As.

Discovery of New Drugs and Innovative Therapeutic Principles for Superior Treatment of Herpes Disease

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The vast majority of the world population is infected with herpes viruses, and associated disease has plagued humanity since ancient times. Herpes simplex viruses (HSVes) are the cause of herpes genitalis (genital herpes) and herpes labialis (cold sores) as well as potentially sight-impairing herpetic eye disease and life-threatening herpes encephalitis or disseminated disease. In 1988, a Nobel Prize was awarded for discovery of principles (anti-metabolites) which led to the milestone development of the anti-viral drug acyclovir in the late 1970's. Until now, herpes disease is treated with nucleoside analogues such as acyclovir (Zovirax), valacyclovir (Valtrex), penciclovir (Fenistil) and famciclovir (Famvir). However, current therapy abrogates or suppresses disease symptoms but does not cure disease.

Despite increased R&D spending, launch of new drugs has been stagnant since the peak years 1996-97 due to established therapeutic standards in key indications and high attrition rates during the critical path of drug development. In the field of anti-herpes drugs, no non-nucleosidic compounds have been launched for 3 decades.

This lecture presents the strategic discovery of new compound classes with potent anti-herpes activity, a novel mechanism of action, a low resistance rate and superior efficacy against HSV in animal models. Well-tolerated members of this drug class significantly reduce time to healing, prevent rebound of disease after cessation of treatment and, most importantly, reduce frequency and severity of recurrent disease. Structural studies are underway and the potential of the innovative therapeutic principle targeting the viral helicase-primase for the treatment of herpes disease in humans, including those resistant to current medications, is reflected by current phase II clinical trials.

Nitric Oxide as a Biomarker of Immunostimulatory Activity of Drugs

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Activation of inducible nitric oxide synthase (iNOS), resulting in enhanced biosynthesis of NO, is under strict control of many cytokines. The crucial role is played by IFN-γ, which induces NO on its own, while other cytokines, such as TNF-α, IL-1, IL-2, IL-17 and chemokines RANTES, MIP-1α/β may provide the major co-stimulatory signal for NO activation. A number of others (e.g. IL-4, TGF-β, G-CSF) possess the NO-down-regulatory function. We have found that distinct members of a novel group of antivirals, acyclic nucleoside phosphonates which are widely employed for treatment of AIDS and hepatitis B, significantly augment production of NO primarily triggered in murine and rat macrophages by IFN-γ. The effect is closely associated with their ability to stimulate secretion of cytokines, e.g. TNF-α, IL-10, RANTES, MIP-1α and others. Highly significant correlation exists between the range of NO production and extent of cytokine stimulation in animal cells. Importantly, the compounds which are active in animal screening design, also exhibit the cytokine-stimulatory effects in human peripheral blood mononuclear cells. Conclusion: The NO is an economical and feasible assay allowing prediction of cytokine-stimulatory properties of drugs in human cell system.

Cancer Diagnostic and Prognostic: European Research Strategies, Recent Sensor Achievements and New Targets

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The development of reliable analytical methods that are easy-to-use and cheap to produce is extremely necessary for many fields of human activity, and especially for diagnostics and prognostics of cancer, which consists of more than 200 distinct diseases affecting over 60 human organs and whose worldwide incidence increased dramatically during last few years. Such methods should:

1. Facilitate early detection and an adequate selection of the treatment of diseases
2. Lead to increased patient survival rates
3. Provide immediate interactive information to health care providers
4. Allow multi-target analyses, automation, and reduced testing costs

Current diagnostic tools, which include various blood marker-based immunoassays, imaging techniques, and biopsy analysis, provide valuable information but have inherent limitations in sensitivity and specificity, involve invasive scoping, and remain expensive. In addition, these tools are effective mostly when the tumour frequency is greater than 1–10%; hence these tests do not provide early detection. The genomic and proteomic molecular tools are also used to profile tumours and produce so-called “molecular signatures”. These signatures include genetic and epigenetic signatures, changes in gene expression, protein profiles and post-translational modification of proteins, but cannot be simply adapted to the clinical cancer testing due to their complexity and requirement for highly-qualified personnel. Recent advances in molecular diagnostics have completely changed the Paradigm of Cancer Patient Care, and in the future, an oncology patient’s care would be composed of the following tests:

1. Predisposition assay
2. Diagnostic/screening assay
3. Disease-recurrence assay
4. Companion diagnostic to stratify responsiveness to therapy

The lecture will be focused on the analysis of recent European research strategies, development of the novel analytical methodologies based on bio/chemical sensors and their potential for point-of-care-testing and search for the new markers/targets for cancer diagnostic and prognostic.

The Immunomodulatory Activities of (S)-Armepavine on Autoimmune Diseases *in Vivo* and *in Vitro*

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T cell-dependent immune responses play important roles in the pathogenesis of systemic lupus erythematosus (SLE). *Nelumbo nucifera* is a remedy for the treatment of diarrhea and inflammation. In the previous study, we have identified a bioactive component (*S*)-armepavine ($C_{19}H_{23}O_3N$; MW313) from *N. nucifera*. The immunomodulatory effects of (*S*)-armepavine on MRL-*lpr/lpr* mice *in vivo* and human T cells *in vitro* were determined in the present study. Treated orally with (*S*)-armepavine could elongated life span of MRL-*lpr/lpr* mice. It seemed to be mediated by inhibition of splenocytes proliferation, suppression of cytokine gene expressions, reduction of glomerular hypercellularity and immune complexes deposition, decrease of autoantibodies production, and impairments of sera cytokines production. The *in vitro* results showed (*S*)-armepavine suppressed inducible T cells kinase and phospholipase Cγ phosphorylation in a phosphoinositide 3-kinase-dependent manner. Through inhibition of downstream pathways including nuclear factor of activated T cells and nuclear factor κB, (*S*)-armepavine attenuated the production of interleukin-2 and interferon-γ to result in suppression of cell proliferation. This study indicates (*S*)-armepavine may be an useful immunosuppressive agent for the management of autoimmune disease like SLE.

A Novel Drug Engineering by Plasma Technique

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Today, cold plasmas are being utilized for ever-increasing number of applications in various industrial fields. For the most suitable therapy, a wide variety of approaches of DDS (Drug Delivery System) have been thus far investigated for oral application but there is no history of drug engineering by plasma techniques. Several selected application works on novel drug engineering through totally dry process using non-polymer forming plasma will be presented, which include (1) preparations of multi-layered tablet applicable to DDS of sustained-release and time-controlled release, (2) preparation of intra-gastric floating DDS (FDDS) for oral controlled-release dosage forms possessing gastric retention capabilities, and (3)development of time- and position-controlled DDS preparation with optimization of chemotherapy, which we newly call a "Patient-Tailored DDS", where plasma operational conditions are selected based on pH measurement of patient's gastrointestinal (GI) tract using a pH sensitive radio telemetry chip passing through the GI tract, as well as monitoring the chip position in the GI tract.

The Asian Gynecologic Oncology Group and Clinical Trial Center of Chang Gung Memorial Hospital

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In Edinburgh, October 2004, during the 10th IGCS Biennial Meeting, a group of gynecologic oncologists met to discuss the idea of constructing an Asian multicenter trial group for gynecological cancer. It was felt that international collaboration between Asian countries is necessary; and although there have been several multicenter trial groups, such as GOG, EORTC in North America and Europe there was no such group in Asia. Every participant supported the idea and the 1st Founding Committee Meeting was held in Bangkok on January 7, 2005. The decision to establish a multicenter trial group named the Asian Gynecologic Oncology Group (AGOG) was confirmed, and the vision, missions and goals were set. The 2nd Founding Committee meeting was held in Taipei on 23 April 2005, where the first version of the Bylaws was drafted. On 15 November 2005 the Bylaws were finalized and the AGOG was officially established in Taoyuan.

The mission of AGOG is to promote understanding, prevention and to improve the quality of care in gynecological cancer in Asia as well as the rest of the world. The goals are (1) to conduct high quality collaborative clinical trials; (2) to undertake basic research; (3) to carry out epidemiological studies; and (4) to answer the most important gynecologic oncology issues in the region. The first AGOG trial was launched after registration on the Australian Clinical Trial Registry (ACTR) website during June 2007. I am sure that AGOG will become more and more important as we continue enthusiastically to conduct high-quality clinical trials aimed at answering the most important gynecologic oncology issues in Asia and abroad.

One of the founder hospital groups was Chang Gung Memorial Hospital (CGMH), established in 1976, and its research grant will give strong support to the AGOG trials. Today it encompasses five hospitals with more than 8,000 beds and serves 30,000 out-patients a day island-wide. We constantly strive to improve our services, teaching and research. Our goal is not only to keep up with the most advanced medical centers of the world but to excel. Reform of infrastructure is under way, including an “Education and Research Park” project to address future challenges.

Faculty members of CGMH are engaged in numerous clinical trials and clinical researches. The superintendent of Linkou Medical Center, Prof. Miin-Fu Chen, has decided to establish in three years a Clinical Trial Center (CTC) in CGMH to foster clinical research by means of the highest ethical and scientific standards in a practical and cost-effective manner to promote new therapeutic discovery and validation. We strive to 1) facilitate routes of communication between industry sponsorship and CGMH investigators and 2) provide the infrastructure for clinical research. By facilitating these key elements, the CTC aims to ensure groundbreaking, efficient clinical investigation leading to improved patient care.

In the 21st century Taiwan aims to impress the world with its vast potential in medicine and biotechnology. National as well as international pharmaceutical companies, government research agencies and private sector laboratories seeking academic medical centers to test new diagnostics and therapies will find that CGMH has positioned itself at the top of this new wave in clinical trials and translational research.

Implication of Estrogen Receptor α in Breast Cancer Development – A New Concept for the Development of Drugs Aimed to Antagonize its Action

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The estrogen receptor alpha (ERα) is a major determinant of hormone dependence in breast cancers. In its activated form (via estrogen binding or a signal transduction pathway), ERα induces the expression of genes implicated in the development of these cancers. Antiestrogens inhibit this induction explaining thereby their antitumor activity.

Search for compounds able to antagonize ERα action remains a topic of prime importance, especially for the treatment of antiestrogen resistant tumors. Conventional approaches aimed to inhibit ERα activation concern the study of ligand-induced conformational changes. Our approach distinguishes from these investigations: we evaluate the biological activity of small synthetic peptides susceptible to modulate exposition of regulatory motifs onto the receptor through interaction with the latter or related co-regulators (cooperation with Y. Jacquot, Université Pierre et Marie Curie, Paris). Data reported hereunder demonstrate the potential value of this approach in the design of new drugs.

In the N-terminal part of the ligand binding domain of ERα, we have identified a multifunctional motif, P295LMIKRSKKNSLALSLT311, that seems to play a role of prime importance for gene transcription (i.e. nuclear localization signal, phosphorylation, acetylation, calmodulin binding...). This "platform" has a repressive activity : its deletion by mutation led to the emergence of high, constitutive, transcription of a reporter gene (levels amount to those induced by estradiol). Moreover, a synthetic peptide corresponding to the P295-T311 motif (ERα17p) was found to produce estrogenic responses in various ERα-positive breast cancer cell lines while it was inactive in ER-negative counterparts (induction of reporter genes, ERα down regulation, growth stimulation...) most probably *via* a competitive mechanism.

In MCF-7 cells, ERα17p decreases the capacity of ERα to bind estradiol while it is unable to compete with the hormone for ERα binding. This decrease, usually found with ligands, is relevant to a conformational change preceding most often the proteasomal degradation of the receptor, a property also provoked by ERα17p. However, in contrast to estradiol, ERα17p acts without phosphorylation of serine-118, an early step of the activation process of the receptor. We believe that the peptide acts at a downstream step (bypass).

By modeling, Y. Jacquot has shown that the P295-T311 sequence interacts with a domain of the receptor implicated in the binding of co-activators. This intramolecular interaction would most likely antagonize the recruitment of such co-activators. Most recent findings support this view : ERα17p destabilizes the association of Hsp-70 to ERα, a chaperone that maintains the receptor in an inactive conformation, inappropriate for co-activator binding. Hence, displacement of Hsp-70 by ERα17p may favor recruitment of co-regulators.

ERα17p corresponding to a regulatory motif of ERα, one may propose that proteasomal degradation of the latter would generate a peptide with similar properties. If so, abrogation of the ability of such a peptide to act may have a high therapeutic impact. To this purpose, we attempt to produce ERα17p antagonists (peptides able to complex ERα17p; design and synthesis by Y. Jacquot).

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Peptide-Targeted Chemotherapy Against Nasopharyngeal Carcinoma

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In the last five decades, surgery, radiotherapy and chemotherapy have been used to treat different types of cancers. For localized cancers, surgical intervention and radiotherapy have been applied and obtained a great achievement. However, for the advanced cases especially the patients with long distant metastasis, chemotherapy has been intensively used with certain degree of success.

Since the conventional chemotherapy and radiotherapy are not selective for malignant cells, they are limited by serious side effect that arises from toxicities to sensitive normal cells. To overcome this disadvantage, in the last several years, in our laboratory, we have developed a novel technique to improve the efficacy of chemotherapy. It is called peptide-targeted chemotherapy. To explain this technique and its efficacy, I would like to give an example to explain its feasibility. I will use the peptide-targeted chemotherapy against nasopharyngeal carcinoma (NPC) as the example.

NPC is one of the common cancers among Chinese living in South China, Taiwan and Singapore. The etiological factors have not been clearly identified yet, but hereditary and other environmental factors, such as consumption of salted fish and Chinese herbs in Hong Kong and long term exposure to the sulfuric acid vapor in Taiwan have been suspected to be related to NPC induction; in addition, Epstein-Barr virus (EBV) has been proposed to be closely associated with NPC progression. Over the past 3 decades, the outcome of localized NPC has steadily improved to more then 90% of 5 year survival rate with radiotherapy, surgery and chemotherapy. However, the 5 year survival for advanced cases is still below 50%. There is a great need for developing new modality of therapy such as high dose chemotherapy with stem cell rescue and tumor targeting therapy.

In our laboratory we have established 10 NPC cell lines previously. We used the NPC cell lines to select a 12-mer specific peptide which can bind specifically to the surface of NPC cells by phage-displayed random peptide library. This peptide has met several criteria for targeted drug delivery into a NPC solid tumor. *In vitro* experiment the peptide can bind specifically to the cell surfaces of most NPC cell lines and biopsy specimens; the peptide-linked liposome containing fluorescent substance is capable of binding to and translocation across cell membranes; *in vivo*, this specific peptide can bind and accumulate in the xenograft in SCID mice, but not in normal organs; similarly, the peptide-linked liposome carried doxorubicin not only can cause marked cytotoxicity of NPC cells *in vitro*, *in vivo*, it can also suppress markedly the xenograft growth in SCID mice without systemic side effect. In conclusion, the novel peptide we identified can be used for targeted chemotherapy very effectively and without systemic side effect. Application of this peptide-targeted therapy against NPC may let this cancer becomes a curable disease.

A Biomarker Concept for Anticancer Drug Development

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A major reason for the efficiency of clinically used anticancer agents such cisplatin is that these agents induce tumor cell death by complex mechanisms, involving both nuclear and cytoplasmic targets (Mandic *et al.*, J Biol Chem. 278, 9100, 2003; Berndtsson *et al.*, Int J Cancer, 120: 175, 2007). Many of the currently used anticancer agents were identified using cell based assays. It is in fact difficult to identify agents with complex modes of action using *in vitro* HTP screening.

We have developed a cell based assay for identification of apoptosis-inducing drugs. The assay measures accumulation of a caspase-cleavage product of CK18 (M30-Apoptosense). Using this assay compounds have been identified that induce apoptosis in p53-defective cells (Erdal et al, PNAS 102, 192, 2005). We have screened for agents that induce the lysosomal cell death pathway and agents that induce apoptosis in cells overexpressing AKT. These data will be discussed.

Cell based assays use monolayer cultures of tumor cells that poorly reflect the *in vivo* situation. We have sucessfully adapted multicellular spheroids for screening using the principle of spheroid formation in hanging drops. Finally, we have developed the caspase-cleaved CK18 marker for clinical trials (Hägg Olofsson *et al.*, Clin Cancer Res. 13: 3198, 2007). This system makes it possible to perform PK/PD studies in mouse xenograft models and in early clinical trials.

Prospects for the Use of NK-Cells in Immunotherapy Against Human Cancer

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Current insights into the molecular specificities that regulate natural killer (NK) cell function suggest that it might be possible to design NK-cell-based immunotherapeutic strategies against human cancer. Here, we describe evidence for NK-cell targeting of human tumours and address crucial questions that, in our opinion, require consideration for the development of successful NK-cell-based therapies. Appropriately used, we predict that NK-cells will find a role, both directly and in combination with other treatment modalities, in future cancer treatment.

High-Content Screening Based on Electric Cell-Substrate Impedance Sensing

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There is a great demand for cell-based *in vitro* screening systems for drug target validation and generation of resources for cell therapies. Our assay is focused on the formation of contacts between cells and the extracellular matrix (ECM) or the basement membrane (BM), which is relevant for cell differentiation processes relevant in cancer therapy and tissue repair. Cell interactions are measured by electric cell-substrate impedance sensing (ECIS). Cells are plated on a chip with integrated electrodes. By applying alternating current and measuring the voltage across a electrode, the impedance can be calculated and broken down into a series resistance and capacitance (Giaever and Keese, Proc Natl Acad Sci USA (1991) 88, 7896-7900). When cells attach and spread upon the electrode, their insulating membranes block and constrain the current flow, resulting in measured variations in the impedance. From these impedance changes, cellular behavior can be determined. We examined the applicability of this system with different cell types. The first experiments asked for the effect of nidogen on cell attachment. Nidogen is critical for BM formation in various organs, as it was shown in knockout mice before (Bader *et al.* (2005) Mol Cell Biol 25, 6846-6856), strongly dependent on the tissue type and microenvironment (Nischt *et al.* (2007) J Inv Dermatol 127, 545-554). We tested +/+ and -/- mouse fibroblasts and human epidermal keratinocytes expressing recombinant nidogen after tetracycline induction. After plating of the cells, attachment was followed for 24 to 48 hours by impedance measurement, accompanied by light microscopy. Secondly, we investigated the attachment of mesenchymal stem cells (MSCs), which can be differentiated into bone or fat tissue, respectively (Kern *et al.* (2006) Stem Cells 24, 1294-1301). We were interested to see how these processes change the response of the cells to the electrical stimuli. With our measurements, we were able to discriminate between cell types, cell densities, and differentiation status. Therefore, this noninvasive cell-based assay most probably not only will help us to improve biological resources for advanced cell therapies. Simulation of the homing process of stem cells in an *in vitro* assay also is a promising setup for large-scale gain-of-function or loss-of-function screenings in functional genomics.

Synthesis and Antimicrobial Activity of Some Novel Oxadiazole Derivatives

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A series of 5-{3'-oxo-6'-(substituted aryl)-2',3',4',5'-tetrahydropyridazin-2'-yl methyl}-2-substituted 1,3,4-oxadiazole has been synthesized. Appropriate aromatic hydrocarbon reacts with succinic anhydride in presence of $AlCl_3$ to yield β-Aroyl propionic acid. The corresponding acid is cyclised with hydrazine hydrate to give 6-(substituted aryl)-2,3,4,5-tetrahydro-3-pyridazinone.This intermediate after reaction with ethyl bromo acetate, hydrazinolysed into 3-oxo-6-(substituted aryl)-2, 3, 4, 5-tetrahydropyridazinyl acetohydrazide.The resulting product was converted into 5-{3'-oxo-6'-(substituted aryl)-2',3',4',5'-tetrahydropyridazin-2'-yl methyl}-2-substituted 1,3,4-oxadiazole. All the final compounds was structurally elucidated on the basis of IR, ^{1}H-NMR, mass spectral data and elemental analysis and screened for antibacterial, antifungal and antitubercular activity. All the compounds were evaluated for their antibacterial activity against *E. Coli, S.aureus, Micrococcus luteus* and *Klebsiella pneumonia* by using cup plate technique in the nutrient agar at 100μg/ml concentration. DMSO was used as a control.Most of the compounds have significant activity against these bacteria comparable to standard drugs, ampicillin and chloramphenicol.Antitubercular activity was determined using the BACTEC 460 system. Stock solutions of test compounds was prepared in DMSO.MIC of rifampin was calculated by established procedures. All the synthesized compounds were screened at 6.25 μg/ml. One compound emerged as highly active analogue in this series with 91% inhibition against *M .tuberculosis* H37 Rv comparable with that of standard rifampicin and isoniazid.All the final compounds were evaluated for antifungal activity against *C. albicans* and *C. neoformans* by using cup-plate method in the sabouraud agar media The zone of inhibition (mm) of each compound was determined and compared with standard drug fluconazole. Most of the compounds were found to be active against the microorganism.

QSAR molecular modelling are in progress to find out the conformation of drugs possessing antimicrobial activity especially antitubercular activity.

From Rational Drug Design to Disease Modifying Drugs for Alzheimer´s Disease

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Alzheimer's disease (AD) is a neurodegenerative disorder that currently affects nearly 2% of the population in industrialized countries. Treating AD is the biggest unmet medical need in neurology. Current drugs are safe and improves symptoms, but do not have profound disease-modifying effects. Therapeutic strategies aimed at lowering levels of Aβ peptides as well as reducing Aβ deposition in the brain constitute a very promising approach to search for disease-modifying effects.

Recently, *in vitro* and *in vivo* evidence points out to an important secondary non-cholinergic function of acetylcholinesterase (AChE). It binds through its peripheral site to β-amyloid, acting as a pathological chaperone with subsequent formation of amyloid fibrils. The potential to interfere with the pathology of β-amyloid targeting a well-known drugable enzyme, the AChE, is open. Peripheral or dual binding site inhibitors of AChE may simultaneously alleviate the cognitive deficit in AD patients and, more importantly, act as disease-modifying agents delaying amyloid plaque formation.

As part of a rational drug design program, using molecular modelling drug design techniques, directed to find dual AChE inhibitors, several families of compounds have been synthesized as potent AChE inhibitors. From these series, several drug candidates were selected based on their potent and selective inhibition of AChE (sub-nano molar activity) and their interference with the β-amyloid aggregation *in vitro* (IC_{50} in the micro molar range). To gain insight into the precise mechanism by which NP0361 might act, *in vitro* Aβ aggregation experiments have been performed showing that this compound indeed inhibits AChE-induced Aβ aggregation with the highest potency reported so far. Moreover, NP-61 is able to significantly reduce Aβ production in several APP-transfected cell lines and.

First *in vivo* data confirm our initial hypothesis. Oral treatment with NP-61 for 3 months is able to reverse the cognitive impairment (Morris Water Maze) and to reduce plaque load in the brains of hAPP transgenic mice (Swedish and London mutation). These results suggest that NP-61, a potent beta-amyloid modulator, is able to reverse the AD-like neurodegenerative phenotype in transgenic mice, indicating a promising disease-modifying agent for clinical application. Currently, the phase I is ongoing without any report of adverse events.

Following the Critical Path: Development of Multi-Analyte Profiles for Sensitive, Early Detection of Toxicity

Ralph L. McDade

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Sensitive and specific toxicity testing provides for more efficient drug development processes. Rules-Based Medicine, Austin, Texas, USA provides a biomarker patterning service using comprehensive, quantitative immunoassay panels that provide robust data for drug development applications. One common application is the assessment of compound toxicity using Multi-Analyte Profiles (MAPs) developed specifically for this purpose. Several projects will be described with emphasis on one project involving the USFDA and the Critical Path Initiative. This example, describing a rat kidney-specific MAP, will demonstrate the utility of this simple, rapid, cost-effective biomarker approach.

Novel Building Blocks and Templates for GPCR and Kinase Research

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In the presentation we will describe the creation of various series of novel building blocks based on druggable scaffolds (ChemKits™) and privileged templates (ChemCores™). These entities have shown broad application in GPCR and Kinase based drug discovery when used for SAR studies and library synthesis in high throughput and medicinal chemistry research. The building blocks can be used either as capping reagents or starting points for further expansion into chemical libraries. Scaffold designs have been incorporated into the design of the compounds to mimic drug like properties. Introduction of planar or spirocyclic templates and exploration of a wide range of substitution patterns on various rings allow the for creation of novel pharmacophores. We will also demonstrate the incorporation of these chemical moieties in the development of new potential drug like candidates for infectious diseases.

A Comprehensive Map of Chromosomal Aberrations in the MCF-7 Breast Cancer Cell Line Genome: Technology, Informatics, and Significance

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Recurrent chromosomal aberrations in cancer involving fusion events relevant for clinical treatment decisions are best understood in leukemias, lymphomas, and sarcomas. However, the recent discovery of highly recurrent gene fusions in prostate cancer suggests that carcinomas also contain clinically important highly recurrent fusions which are not detectable using current methods. To address this deficiency, as part of a pilot stage of The Cancer Genome Atlas (TCAG) project (http://www.genome.gov/25521889), we have developed a prototype implementation of the End Tag Profiling (ETP) method for mapping chromosomal aberrations in cancer genomes at a kilobasepair level of resolution using next-generation sequencing technologies. In its final fully developed version, the ETP method will be sufficiently affordable for wide application in small laboratories requiring only a low-footprint next-generation sequencing machine and supported by the turnkey Genboree informatic system accessible over the internet. An application of the prototype implementation of the ETP method on the genome of the MCF-7 breast cancer cell line revealed chromosomal rearrangements resulting in 398 aberrant fusions of chromosomal segments. One of the rearrangements we rediscovered involved the ZNF217 gene and was previously demonstrated to confer doxorubicin resistance in cell transfection assays. We report on the development of the ETP method and progress in characterizing remaining rearrangements detected in the genome of MCF7 for their functional and clinical relevance.

Multi-Component Peptide-Based Carriers for Non-Viral DNA Delivery

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From the many studies addressing non-viral gene delivery, a number of promising approaches have emerged. Delivery using receptor-mediated internalisation or lipid-based cell penetration has been widely studied but recently, cell penetrating peptides have attracted a growing interest. For the uptake and delivery of exogenous DNA to occur optimally, the DNA requires packaging into condensed particles that can cross the plasma membrane and translocate to the nucleus. The physical characteristics of the DNA, its shape, surface charge and extent of condensation, can markedly affect both uptake and subsequent gene expression.

While peptides have been identified that can facilitate gene delivery by increasing membrane uptake, release from endosomes or translocation to the nucleus, few studies have addressed their function and importance in gene delivery when used in combination. In this presentation, we will describe our recent work characterising the role of various peptide entities in DNA uptake and gene expression in mammalian cells. A library of peptide-based carriers consisting of various combinations of the TAT peptide, the nuclear localisation signal from SV40 Large T protein, the fusogenic peptide from the hemagglutinin protein and a polylysine dendrimer, was constructed and evaluated for DNA uptake, and transgene expression. The results demonstrate how various components can be used alone and in combination to deliver DNA to cells. Moreover, we have been able to determine the mechanism of uptake and subsequent intracellular compartmentalisation. These studies provide a foundation for future development of modular gene delivery systems.

Cardiac Imidazoline Receptors, a New Therapeutic Target

Suhayla Mukaddam-Daher, Ahmed Menaouar, Pierre-Alexandre Paquette, Rouwayda El-Ayoubi, Safa Bentaeibi and *Jolanta Gutkowska*

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Brainstem imidazoline receptors (I_1-receptors) have been implicated in blood pressure regulation through inhibition of peripheral sympathetic activity. We have shown that chronic *in vivo* activation of I_1-receptors in hypertension goes beyond blood pressure reduction to include protection from left ventricular hypertrophy. Biochemical and molecular investigations revealed that I_1-receptor activation by moxonidine in hypertensive rats is associated with transient increase in the production of atrial natriuretic peptide (ANP), a cardiac diuretic, natriuretic, and vasodilator hormone with anti-proliferative and anti-inflammatory properties. Moxonidine inhibited cardiac DNA synthesis and resulted in transient apoptosis, evidenced by increased DNA fragmentation, Bax/Bcl-2 ratio, and caspase-3 activity, that returned to corresponding normotensive levels by 4 weeks of treatment. In addition, moxonidine inhibited cardiac iNOS, cytokines, and downstream NFκB phosphorylation, revealing anti-inflammatory actions. We have enough evidence to propose that in addition to centrally mediated effects, cardioprotection by moxonidine involves I_1-receptors that we have identified in the heart and shown that they are responsive to the cardiovascular environment, and that upon activation can stimulate the release of ANP without contribution of the central nervous system. Thus, cardiac I-receptors may be target for treatment. Current experiments investigate the cellular targets and the intracellular pathways implicated in the effects of cardiac I_1-receptor activation.

Synthesis of Bioactive Natural Products

John Nielsen

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Natural products continue to serve as excellent leads for the discovery and development of new drugs * and at the same time, provide both synthetic challenges for organic chemists. The isolation and characterization of new natural products are still rather sluggish * and still today, frequently their structural complexity hamper practical applications and commercialization.

Combinatorial chemistry emerged about 15 years ago as the chemist perception of 'Nature' evolving through synthesis, selection, replication and survival. We have established ourselves on the interface between natural products and combinatorial chemistry. During this lecture, examples from our research program including new quorum sensing inhibitors will be presented. This will also include a novel and highly efficient synthetic pathway towards sulfonamide analogues of the natural signal molecules.

Finally, we have investigated potential routes towards isobenzofuranones natural products and their analogues. By serendipity, we discovered of a simple and general method for the synthesis of *-enaminoketoesters, which are versatile and useful synthons in organic synthesis and the newly established reaction pathways breaks limitations previously associated with the synthesis of heteroaromatics such as pyrazoles and pyrimidines but also imminium-substituted butenolides or rare amino acids.

Regulatory Mechanism of Assembly of Transcription Factors on DNA

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Transcriptional regulatory factors fulfill their functions as a stereospecific multiprotein assembly on an enhancer DNA. Stabilization of the assembly is strictly regulated by protein-protein interactions in an allosteric manner. We solved crystal structures of some types of multiprotein-DNA complexes using X-ray crystallography and further analysed regulatory mechanism of the assembly from dynamical aspects of molecules using NMR relaxation measurements and functional aspects using some molecular biological assays for various point mutants. We will discuss a suggested transcriptional regulatory mechanism and also some analogous feature of functional regulation between the transcriptional regulatory factors and other kinds of proteins.

Discovery of the RNA Synthetic Activity of Glutamate Dehydrogenase and Application in Drug Metabolism

G.O. Osuji

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Glutamate dehydrogenase (GHD) catalyzes the reversible deamination of glutamate. However, it polymerizes nucleoside triphosphates to RNA independent of a template because they possess binding and polymerization groups. GDH synthesizes RNAs in response to xenobiotics. The GDH-synthesized RNAs regulate mRNA abundance. But the coordination of genes, drugs and diseases by the RNA has not been studied. The coordination of the abundance of the mRNAs encoding the drug-metabolizing enzymes was studied by northern hybridization using the GDH-synthesized RNAs as probes. Administration of ATP upregulated the mRNAs encoding superoxide dismutase (phase I), GSH S-transferase (phase II), and ABC transporters (phase III). Administration of CTP upregulated the mRNAs encoding alternative oxidase (phase 1), and H^+-ATP pump (phase III) but no phase II enzymes. ATP plus UTP plus GTP administration upregulated the mRNAs encoding cytP-450 (phase I), UDP-glucosyltransferase (phase II), and ABC transporters (phase III). The control, GTP, and UTP administrations did not upregulate any of the mRNAs. There were extensive sequence similarities among the GDH-synthesized RNAs homologous to the mRNAs encoding the drug metabolizing enzymes. Therefore, GDH coordinated the drug metabolizing enzymes as a pathway at the molecular level. ATP and the other nucleotides are antihypertensives, antineoplastics, antiarrhythmics, antimetabolites etc. GDH catalyzes the biological processes connecting genes, drugs and diseases.

NAD-Based Anticancer Therapeutics

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Nicotinamide adenine dinucleotide (NAD) plays an important role in biology and medicine. Enzymes' NAD binding site has sufficient variation to allow the development of agents highly selective for an enzyme of particular interest. Numerous protein kinase inhibitors that mimic ATP and bind selectively at the ATP binding domain of kinases have been developed, indicating that a similar approach with NAD mimics is feasible. Indeed, we have demonstrated that NAD analogues show high selectivity in inhibition of IMP-dehydrogenase, alcohol dehydrogenase, NAD-kinase, or M. tuberculosis enoyl-ACP reductase. IMPDH emerged as an important therapeutic target in cancer. In the treatment of chronic myelogenous leukemia (CML) imatinib is a phenomenal success, however, the drug does not affect a small population of leukemic stem cells (less than 1% of all tumor cells) and resistance develops. As the cells emerge from the progenitor stem cell they are destroyed by imatinib, which needs to be taken every day for a life time. Little is known about the differences between normal and cancer stem cells that would allow for designing drugs that specifically target the malignant cancer stem cells. However, cancer stem cells, especially leukemic stem cells, can be selectively disarmed by induction of apoptosis and/or differentiation.Recently it was foun that induction of differentiation severely limited the cancer stem cell's ability to form new tumors.

Delivering DNA and Protein Antigens – A Role for Cationic Liposomes

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Cationic lipids and cationic liposomes are more frequently reported for their use as gene delivery systems than for applications as vaccine adjuvants. However, issues with biological recognition of cationic lipids, whilst a problem in gene therapy could indeed mean they offer a realistic opportunity for vaccine adjuvants – not only for DNA vaccines but also for protein-based vaccines. The potential of cationic surfactant vesicle based formulations using two agents; the cationic amine containing [*N*-(*N*′,*N*′-dimethylaminoethane)-carbamoyl] cholesterol (DC-Chol) or dimethyl dioctadecylammonium bromide (DDA) to delivery DNA-based and protein antigens for the hepatitis B surface antigen (HBsAg) has been studied and will be discussed within this presentation. The synthetic mycobacterial cord factor, trehalose 6,6'-dibehenate (TDB) has been used as an adjuvant. However DDA-TDB vesicles can be subject to instabilities and short shelf-lives. We have demonstrated that the addition of 1-monopalmitoyl glycerol and cholesterol to these DDA-TDB systems can facilitate both their formulation and application, whilst only marginally altering their strong immunogenicity (a reduction in HBsAg specific IL-2). Overall, DDA formulations incorporating TDB showed markedly increased antigen specific splenocyte proliferation and elicited cytokine production concomitant with a strong T cell driven response, delineating formulations that may be useful for further evaluation of their clinical potential.

Identification, Optimization and In Vivo Activity of a Branched Antimicrobial Peptide Selective for Gram-Negative Bacteria

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The growing emergency of multi-drug resistant bacteria is a global concern, therefore, the demand for new antibiotics urges researchers and pharmaceutical companies to consider new antimicrobial agents. Among these, antimicrobial peptides turned out to be particularly interesting, in consideration of their peculiar mechanism of action, which is specifically targeted to bacterial membrane.

By selecting a large combinatorial library we identified a non-natural peptide sequence, showing a strong antimicrobial activity especially against Gram-negative bacteria (Pini *et al.*, 2005, Antimicrob Agents Chemother). This peptide (QKKIRVRLSA) (M6) was synthesized in a tetra-branched form which we had previously demonstrated to induce general peptide resistance to proteolysis (Bracci *et al.*, 2003, J Biol Chem; Falciani *et al.*, 2007, Chem Biol Drug Des), rendering peptide molecules more compatible with an *in vivo* use.

Here we report results about M6 characterization, focusing on the last *in vivo* results of sepsis models that demonstrated peptide capability to prevent animal death and to neutralize sepsis symptoms. These results, in addition to the characteristics of secondary structure, side and acute toxicity, immunogenicity, poor haemolysis and mechanism of action (Pini *et al.*, 2007, J Pep Sci), make this branched peptide a strong candidate for the development of a new antibacterial drug.

Engineered Antibody CDRs as Novel Antimicrobial, Antiviral and Antitumor Drugs

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A killer decapeptide (KP) has been generated by alanine substitution of the first aminoacid of the most candidacidal fragment (P6), including seven aminoacids of the framework region and the first three residues of the light chain CDR1, of a recombinant antiidiotypic antibody (Ab) representing the internal image of a yeast killer toxin characterized by the wide spectrum of microbicidal activity against eukaryotic and prokaryotic microorganisms expressing specific cell-wall receptors mainly constituted by β-glucans *(1)*. KP has shown an increased antifungal, antibacterial and antiparasitic activity *in vitro* that was neutralized by adsorption with β-glucans and a strong therapeutic effect in well established murine models of vaginal and systemic candidiasis, cryptococcosis and paracoccidioidomycosis (*2,3-5*). Based on the recognized sequence homology of P6 with critical segments of the gp160 precursor and the light chain variable region of an Ab (HC63) that prevents the hemagglutinin low pH fusogenic transition, KP proved to inhibit *in vitro*, *ex vivo* and/or *in vivo* the replication of HIV-1 and Influenza A virus by downregulation of CCR5 co-receptor and/or physical block of the gp120-receptor interaction and inhibition of (glyco)proteins respectively (*6,7*).

CDR-related synthetic peptides of murine and human monoclonal Abs directed to: i) a cell-wall protein epitope of *C. albicans* Als3 and enolase, as well as the nuclear pore complex Nup88 (mAb C7), ii) a synthetic peptide containing the surface antigen of hepatitis B virus and the T-helper-cell epitope from the circumsporozoite protein of *Plasmodium falciparum*, sharing H1 and H2 with mAb C7 (mAb pc42) and, iii) the difucosyl human blood group A substance sharing no CDR homology with mAb C7 and mAb pc42, showed differential activities *in vitro*, *ex vivo* and/or *in vivo* against *C. albicans*, HIV-1 and B16F10 melanoma cells, conceivably involving different mechanisms of action (*8-11*). Engineered peptides, obtained by mAb CDRs alanine substitution, showed further differential increased/unaltered/decreased antimicrobial, antiviral and/or antitumor activities.

Irrespective of the specificity of the native Abs, small sized synthetic (engineered) CDRs may represent an unlimited source for the discovery and drug design of novel antimicrobial, antiviral and antitumor drugs.

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Steroid Sulfatase Inhibitors: From Concept to Clinic

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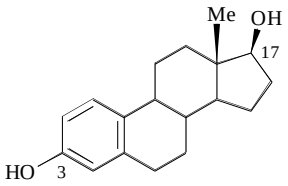
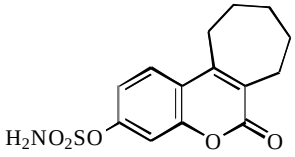
Many breast tumours are hormone-dependent (17ß-estradiol) with estrogens playing a key role in their growth and development. There is increasing evidence that inhibition of steroid sulfatase (STS), which converts oestrone (E1) sulphate to E1 and also dehydroepiandro- sterone (DHEA) sulfate to DHEA, will attenuate estrogenic stimulation in hormone dependent breast cancer (HDBC). We designed E1-3-*O*-sulfamate (EMATE) as the first potent, orally active, irreversible active site-directed STS inhibitor (1) and this compound reached multiple phase I/II clinical trials for a non-oncology indication. We subsequently synthesised non-steroidal compounds with even superior potency to EMATE and a series of tricyclic sulfamate candidates led to the clinical drug candidate STX64 [2].



17ß-estradiol



STX64

In vivo, STX64 was non-estrogenic, inhibited STS potently and caused regression of E1S-stimulated growth in an NMU-induced tumour model and in nude mouse tumour xenografts. STX64 had a very high rodent oral bioavailability of 95%, attributed to the protection of STX64 from metabolic degradation through sequestration into red blood cells by binding to carbonic anhydrase II (hCAII). We solved the crystal structure of STX64 bound to hCAII [3]. STX64 is the first STS inhibitor to enter clinical trial and, in postmenopausal women with HDBC, is well tolerated orally and very potent, as measured in peripheral blood lymphocytes or even directly in tumour tissue samples, with *ca* 100% targeted enzyme inhibition even at doses as low as 5-20mg and with an elimination half-life of ca 30h. The recently concluded Phase I clinical trial in women with locally advanced or metastatic breast cancer, who had already been heavily pre-treated with other agents including tamoxifen and aromatase inhibitors, showed evidence of 5/8 evaluable women exhibiting stable disease for up to 7 months, indicating that inhibition of STS constitutes a promising novel form of anti-endocrine therapy for the treatment of HDBC [4]. Further clinical trials are in progress during 2007/8. Extension of the concept to dual sulfatase-aromatase inhibition *via* a single molecule [5] and the potential of other novel sulfamate-based agents as anti-angiogenic agents targeted against *hormone-independent* cancers [6] and now in preclinical development, will also be discussed

Steroid sulfatase is an attractive novel oncology target for clinical intervention. The aryl sulfamate pharmacophore is a powerful new motif for anticancer drug design.

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Discovery of mu Opioids with Altered Receptor Regulation

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Increasing evidence indicates that different ligands can elicit different receptor regulation pathways. For example, the μ opioid agonists morphine, methadone, and fentanyl each promote μ opioid receptor (μOR) coupling to G proteins, but they differ in their ability to direct receptor trafficking. This may be due to differences in agonist-induced receptor conformations, resulting in different degrees of phosphorylation, arrestin recruitment and vesicular trafficking. Such differences in μOR regulation and trafficking are physiologically relevant as mice lacking β arrestin2 display enhanced antinociception, decreased tolerance, and greatly diminished side effects (constipation and respiratory suppression) following morphine treatment. Therefore, a μ opioid agonist conferring non-conventional receptor conformations may yield novel analgesics with reduced potential to produce unwanted side effects.

Currently, there are no selective pharmaceutical or biochemical inhibitors of G-protein coupled receptor (GPCR) desensitization nor are there specific inhibitors of G-protein receptor kinases (GRKs) or βarrestins. A therapeutic approach in which β arrestins or GRKs were individually inhibited would be expected to produce unwanted alterations of the function of other GPCRs. Furthermore, since arrestins regulate >1000 different GPCRs, it will be exceedingly difficult to produce receptor selective effects using this approach. An alternate approach would be to selectively target μOR regulation by designing ligands that confer μOR conformations that allow for signaling yet disrupt receptor regulation.

Here, we describe the discovery of a series of μ opioid agonists derived from salvinorin A, a psychoactive natural product, which have altered receptor regulation. Our structure-activity relationship studies have identified agonists that activate the μOR yet do not promote receptor phosphorylation, recruit β arrestins or internalize the receptor. In addition, our studies have identified another compound which retains agonist affinity yet now induces receptor β arrestin translocation and receptor internalization. The discovery of two compounds with nearly identical chemical structure and similar binding affinity and efficacies which elicit differential signaling at the cellular level would suggest that not only receptor conformation but also ligand structure contribute to signaling events. Considering the important role μ opioid receptor regulation plays in determining physiological responsiveness to opioid narcotics, μ opioids derived from salvinorin A may offer a unique template for the development of functionally selective μ opioid receptor ligands with the ability to produce analgesia while limiting adverse effects.

Smart Flexible Nanovectors

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Injectable drug-delivery nanovectors, such as liposomes, are used in nanomedicine for cancer therapy. These vectors must be large enough to evade the body's defences but sufficiently small to avoid blockages of even the capillaries, thus nanosized by definition. Nanovectors can extravasate into the tumor stroma through the submicrometric fenestrations of the angiogenic vasculature, a phenomenon called enhanced permeation and retention. Such a mechanism, as well as multiple antibodies, are used to target the nanovectors to epitopes on cancer cells. Finally, the nanovectors are activated and release their cytotoxic action when irradiated by external energy or by environmental conditions, such as metabolic markers or the acidity levels that accompany inflammatory states, infections and neoplastic processes [1]. But controlling both targeting and drug delivery, under the body's defences, remains a complex task.

In this note, we will introduce a new concept, of smart flexible -a property that could be crucial for smart drug delivery but still ignored in the literature- nanovectors, based on smart adhesion [2,3]. Targeting, in addition to the classical strategies, is enhanced by designing the nanovector in order to activate an adhesion force larger than the drag force only in the capillaries. A hierachical architecture is used to model a real vasculature. During adhesion, the smart nanovector considerably changes its shape in a controllable way and, in case, can implode due to buckling [4]. Such a mechanism will smartly deliver drug in a controllable way, ideally aborting the tumor invasion [5].

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Improved In Vitro Model to Assess Drugs Release Characteristics of Products for their Absorption from Human Gastrointestinal Tract

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Drug release assessment, commonly also known as dissolution testing, is an integral part of pharmaceutical products development and establishing their quality, in particular for solid oral dosage forms such as tablets and capsules. This test is based on the principle that for a drug to be absorbed from the gastrointestinal (GI) tract following oral ingestion, it must first be released from the product and then should dissolve in the aqueous-based milieu of GI tract. At present, the *in vitro* model (test) to evaluate drug release and dissolution are based on solubility determination using specially designed apparatuses, generally described in pharmacopeias such as USP, Eur. Ph., BP. The most commonly used apparatuses, for such drug release testing are known as Paddle and Basket, named based on their stirrers designs. Although, such tests and apparatuses have been employed for many years, their use has always been questioned due to poor reproducibility and bio-relevancy of the results obtained. Recent studies have demonstrated that the observed problems appear to be related to anomalies of *in vitro-in vivo* environments, in particular regarding stirring and mixing. Thus a new approach, based on a revised stirring device, has been suggested which provides an improved *in vitro* model. This presentation will summarize description of current approaches with potential artefacts along with results using the newly proposed approach, reflecting its superiority. The presentation will also focus on evidence demonstrating significant reduction in testing burden for product development and evaluation for drug products manufacturers. These efficiencies are achieved by utilizing a single dissolution test, vs multiple tests using current approaches, for evaluating a variety of product parameters such as release characteristics, content uniformity and potency.

Adverse Drug Reactions Among Hospitalized Patients in Nephrology Wards- An Intensive Monitoring Study

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Background: There are only few studies conducted to evaluate the occurrence of ADRs in patients in nephrology wards.

Purpose: To study the incidence and pattern of ADRs among hospitalized patients in nephrology department and to estimate the direct cost attributable to ADRs.

Methodology: The present prospective intensive monitoring study was conducted in the nephrology department of Kasturba hospital (1475 bedded, tertiary care hospital in South India) for duration of eight months. All the hospitalized patients were intensively monitored for the identifying ADRs. ADRs reported were analysed for various parameters like patient demographics, reaction and drug characteristics, severity, and direct cost attributed to ADR.

Results: Out of 259 hospitalized patients, 58 patients developed 94 ADRs with an overall incidence of 22.39%. In 3.86% of patients admitted during study period, ADR was the reason for hospital admission. The incidence of ADRs was higher (35.48%) in age group of >60 years and in females (30.13%). Drug class most commonly implicated in the ADRs was immunosuppressive agents (45.74%) The system most commonly affected by ADR was endocrine and metabolic (22.34%). In 50% of the ADRs the suspected drug was withdrawn. Majority (58.51%) of the ADRs was of moderate severity. Total cost attributed to ADRs was estimated to be INR 92,019 (2190 USD). No significant difference was observed in the incidences of ADRs based on the stage of renal failure.

Conclusion: ADRs occurred commonly in hospitalized patients in nephrology ward. Vigilant monitoring of drugs most commonly implicated in ADRs in nephrology ward is of utmost importance.

Pharmacogenomics of Antiretrovirals

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HIV infection is a serious but treatable disease, yet current treatment is limited by development of resistance and high rates of adverse drug reactions.

Antiretroviral therapy is especially suitable for pharmacogenomic investigation as both drug exposure and treatment response can be reliably measured. Increasing knowledge about genes implicated in pharmacokinetics, mode of action, efficacy and toxicity of drugs has already provided relevant results for clinical practice:

The strong association of the abacavir hypersensitivity reaction with *HLA-B*5701* permits testing patients for the presence of the allele, and if present avoiding the drug and therefore preventing the reaction.

Persons with the alleles *CYP2B6*6, *11, *18, *27, *28, *29* present higher efavirenz and nevirapine "area under the curve", and have increased risk of neuropsychological toxicity.

Detailed knowledge of genetics of cellular receptors and co-receptors of HIV has allowed the development of maraviroc, a CCR5 antagonist, which prevents entry of the virus in cells. Moreover, testing the genome of HIV permits ascertain if the virus is susceptible to the drug or not.

It is expected that larger-scale comprehensive genome approaches will profoundly improve the landscape of knowledge of HIV therapy in the future.

Role and Importance of Hepatic and Extrahepatic Drug Metabolizing Enzymes in the Bioavailability of Anti-Cancer Agents in Cancer Cells

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Like for cytotoxic chemotherapies, drug resistance to newly developed anticancer drugs, such as the protein kinase inhibitors, is increasingly recognized as a major problem. One mechanism of resistance of the tumor is to lower the intracellular drug bioavailability. Indeed, the cancer cells can modify the uptake, the biotransformation and the efflux of the active molecules.

Drug metabolizing enzymes (DME) are key players in the disposition of drugs in cancer cells. They can be extrahepatic and specific to cell subclone. Whereas drug discovery mainly focuses on hepatic biotransformation and systemic disposition, tumor localized drug availability can be strongly altered by the activity of extrahepatic metabolizing enzymes over-expressed in tumors. In fact, due to hypomethylation and instability of DNA in cancer cells, some DME, e.g. CYP1B1 and CYP2W1, have been found to be over-expressed in a wide range of tumors whereas they are absent in the liver.

Today, there are many Tumor Expressed Metabolizing Enzymes (TEME), which need to be evaluated in their capability to transform new anti-cancer drugs and candidates. Whereas this mechanism of resistance is not really new, the list of TEME is growing and deserves investigation in drug discovery. Some TEME have been discovered only recently by the human genome project. Today, TEME are mainly CYP1A1, 1B1, 2F1, 2J2, 2R1, 2S1, 2U1, 2W1, 4B1, 4F3A/B, 4F12, 4Z1.

Interestingly, the use of TEME to activate pro-drugs, specifically in the target cells, has been proposed by some authors. On the other hand, inhibition of some TEME, e.g. CYP4F, known for their endogenous role in cell metabolism, could decrease cancer cell growth or clonal evolution. In this case, TEME inhibitors could possibly be used in the approach of anticancer drug cocktail.

After reviewing the TEME isozymes and their impact on drug disposition and efficacy, the aim of this lecture is to underscore what should be taken into account in the discovery of anticancer agents today.

Global Metabolomic Analysis in Drug Discovery and Development

John Ryals

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Small molecule biomarkers are the most common approach to diagnose and monitor human disease. Detection of endogenous biochemicals (i.e., the metabolome) and xenobiotics in a systematic and statistical manner also provides solutions for compound profiling and optimization, drug discovery, and drug safety. Metabolon's analytical mass spectrometry platform and proprietary data analysis software provides a global, unbiased method for the detection and identification of small molecules across a wide array of sample types. Metabolon's mission is to provide high quality metabolomic analysis that extracts, identifies and quantitates most of the small molecules in biological samples. Metabolon has completed over 100 metabolomic studies with clients in areas such as toxicology, molecular target identification, consumer good claims, and biomarker discovery. An overview of the technology and numerous case study examples will be provided.

Development of Small Molecule and Antibody Microarrays Designed for Drug Discovery

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Fast and cost-efficient high-throughput methods enable to detect small molecules that bind to druggable ligand-binding sites in proteins could make a valuable contribution to reducing the total cost of drug development programs. We have developed the small molecule microarray method based on the non-covalent immobilization of chemical compounds on a new 3D support, and the near-infrared fluorescence detection of the bound molecules in microspots. This method has several advantages over existing ones because (i) no fastidious chemical protection/deprotection steps are required; (ii) all moieties of the printed molecules can be recognized by target proteins; (iii) near-infrared fluorophores provide high detection sensitivity; (iv) several leads with different mechanisms of action can be identified in a given compound library in a single assay.

Screening of discrete chemical compound libraries to identify potential anti-bacterials has demonstrated the effectiveness of the method. Competition assays between compounds immobilized in microspots and known antibiotics in the liquid phase has made it possible to distinguish between the potential leads, which target the catalytic site of penicillin-binding proteins, and molecules that bind to non-druggable binding sites. High-affinity molecules have been selected that are bound with similar efficiency to wild type and mutant proteins isolated from antibiotic-resistant strains. Several molecules exhibited remarkable antibacterial activity against gram-positive and gram-negative bacteria. These inhibitors could be used to create improved antibacterial agents.

The support developed has also been used to fabricate antibody microarrays designed to assess the effect of various small molecules on protein expression and protein phosphorylation in breast cancer cells. Both sample preparation and binding conditions have been optimized to increase the presentation of low-abundance phosphorylated and non-phosphorylated proteins, and to decrease the cross-reactivity between mAbs and non-target proteins. We have analyzed the phosphorylation status of signal transduction pathways in MDA MB-231, MDA MB-468, SR-3, T47-D and MCF-7 cell lines treated with several potential anticancer compounds. In particular, we studied the global regulation of key protein kinases, transcriptional factors and cell cycle proteins in breast cancer cells treated by resveratrol, a natural anticancer molecule. This analysis allowed us to identify possible targets for resveratrol, a prerequisite to designing more specific, high-affinity anticancer agents.

The data obtained highlight the advantages of miniaturized arrays in the design and discovery of drugs to target various types of disease.

Squalamine, a New Antibiotic Extracted from Marine Environment: How About its Mechanism of Action with Respect to Gram-Negative Bacteria?

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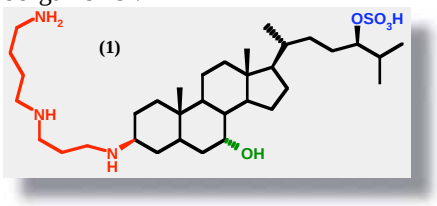
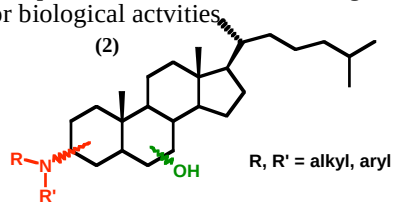
Extensive use of antibiotics has raised a serious public health problem due to infections caused by multidrug-resistant bacterial pathogens. Consequently, there is a pressing need to develop new antibiotics to keep pace with bacterial resistance. Recently, a new aminosterol called squalamine (**1**) has been isolated from tissues of the dogfish shark, ***Squalus acanthias.*** This unusual natural product has attracted considerable interest because of its potent antimicrobial activities against a broad spectrum of microorganisms[1].

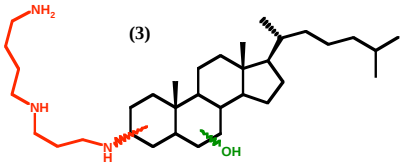


The squalamine

Squalus acanthias

However, the feasibility of obtaining large quantities of this steroidal antibiotic, from natural sources or by synthesis, appears questionable. In order to understand the structure-activity relatationships of such compounds, we have recently developed the synthesis[2] of structural analogues of squalamine (**2**) and (**3**) that mimic not only the structure of squalamine but also its extraordinary antimicrobial properties[3]. The results revealed the importance of each functional group (polyamine, hydroxyl or sulfate group) of the natural molecules for biological actvities.





In addition, we have envisioned the possibility that squalamine, an amphipathic compound, might be able to disrupt the organization of the Gram-negative bacterial membranes. The antibacterial action manifested *via* permeabilization of the outer membrane of *Escherichia coli* bacteria will be discussed.

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Focused Proteomics on Membrane Microdomain-Associated Signal Transduction in Fertilized Amphibian Eggs and Human Bladder Carcinoma Cells

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Membrane microdomain or lipid/membrane raft has been implicated in several aspects of cellular functions such as immunity, neuronal network, and pathogenesis. Here we report on the structure and function of membrane microdomains of African clawed frog *Xenopus laevis* and human bladder carcinoma cells. In *Xenopus* system, we have been interested in signal transduction pathways for sperm-egg interaction and subsequent egg activation, which should be occurred successfully for accomplishment of fertilization. In bladder carcinoma cell system, our interest is to understand how malignant, transformed cells can survive under stressful conditions (e.g. serum deprivation), where normal cells cannot survive. By applying focused proteomics strategies on membrane microdomains, we could have identified several signaling molecules that would act both in fertilized eggs and carcinoma cells. We will discuss their non-genomic as well as genomic function in these cellular functions, merit of our focused proteomics approach to pick-up valuable cellular targets for pharmacogenomics, and future trends in the field.

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Novel Strategies for the Treatment of Asthma and COPD - Future Challenges of Drug Therapy

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The paper will illustrate future challenges of drug therapy for asthma and chronic obstructive pulmonary disease (COPD). New therapeutics are desperately needed, especially those that target the underlying causes and prevent disease progression. Although asthma and COPD have distinct etiologies, both are associated with reduced airflow caused by excess infiltration of inflammatory cells into healthy lung tissues. Phosphodiesterase-4 (PDE4 Cyclic AMP (cAMP) is a key second messenger in all cells. The selective inhibition of this family generates profound, functional effects and PDE4 inhibitors are currently under development to provide potential, novel therapeutics for the treatment of inflammatory diseases, such as asthma and COPD. Drug discovery efforts have yielded many different classes of selectin inhibitors, including soluble protein ligands, antibodies, oligosaccharides and small molecules. Although many selectin inhibitors have shown activity in preclinical models, L-selectin monoclonal antibody has shown promise in reducing airway recruitment of eosinophils after intravenous administration. Further clinical development of an inhaled formulation is underway. Other drugs include the adenosine receptor antagonists theophylline and doxofylline (both used as bronchodilators in respiratory disorders such as asthma). Many pharmaceutical companies and institutions are addressing the huge potential for the development of selective adenosine receptor agonists and antagonists and new compounds approaching the market for asthma and COPD therapy.

Using Sugars in Drug Discovery

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Sugars are critical molecules that regulate biological processes in the human body and play fundamental roles in diseases as well as in drug action. Nature has used sugars to modulate the biological activity of natural products. For instance, erythronolides are inactive while erythromycin and megalomicin both with attached sugars have anti-infective properties, wherein the addition of two (erythromycin) or three (megalomicin) sugars present distinctly unique mechanistic profiles. An additional example of where sugars modulate therapeutic activity yet this time in oncology is with the anthracyclines. In these cases, even slight changes in sugar configuration can minimize cardiotoxicity and improve therapeutic index. Despite the clear importance of sugars in drug activity, the utilization of sugar attachment and manipulation has found limited appeal in the drug industry.

Centrose is exploring unusual sugars to produce new drugs that target specific disease mechanisms. Using breakthroughs in the understanding of non-natural sugar chemistry and the biology behind it, Centrose is using its CarboConnect platform to rapidly develop broad new classes of human therapeutics. CarboConnect offers a commercially relevant approach to capture the therapeutic power of sugars.

Anti-RNA Virus Activity of Polyoxometalates

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The polyoxometalates (POM) are negatively charged clusters of inorganic substances principally comprised of oxide anions and early transition-metal cations. They have been shown to exhibit antiviral activity against several RNA and DNA viruses including the orthomyxoviruses, paramyxoviruses, flaviviruses, coronaviruses, retroviruses and herpesviruses. Human acute respiratory diseases are mostly caused by orthomyxoviruses, paramyxoviruses and coronaviruses, and although some antiviral agents have been developed for clinical use, their efficacy is specific for each causative virus. It is very difficult to identify specific viral agents from clinical manifestations and we need some broadly effective anti-respiratory virus agents for the general physicians' use. We examined several types of polyoxotungstates for anti-RNA virus activities and found some types of the compounds have indeed broad antiviral activities against RNA viruses which cause acute respiratory infections. The structure-activity relationship of POM was analyzed and the substitution of tungsten ions by the other metal ions sometimes increased antiviral activity. The mechanism of antiviral action of POM has been searched by examining RS viruses resistant to the agents for the amino acid sequence of some functional proteins. POM are safe and promising antiviral agents against the broad spectrum of acute respiratory infections and we are under investigation for development towards their clinical use.

Vaccines against Group A Streptococcal Infection and Human Papillomavirus Type-16 Associated Cervical Cancer

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Infection with group A *Streptococcus* (GAS, *Streptococcus pyogenes*) is responsible for many human diseases including streptococcal pharyngitis, streptococcal toxic shock syndrome, rheumatic fever and rheumatic heart disease. Lipopetide synthetic vaccines against GAS represent thus a new approach in a drug delivery. The lipid core peptide (LCP) system is a lipopeptide vaccine delivery system that incorporates lipidic adjuvant, carrier, and peptide epitopes into a single molecular entity.

The self-adjuvanting GAS vaccines, composed of a universal helper T-cell epitope (P25), a target GAS B-cell epitope (J14), and a lipid moiety were developed. Systemic J14-specific IgG antibodies were detected following subcutaneous immunization of BALB/c (H-2^d) mice with each construct without the need for an additional adjuvant. The effect of changing the order of P25, J14, and lipid moiety attachment, or incorporation of P25 and J14 into a LCP system on antibody titres was assessed. The point of lipid moiety attachment had the greatest influence on systemic J14-specific IgG antibody titres. Overall, the best vaccines featured a C-terminal lipid moiety, conjugated through a lysine residue to P25 at the N-terminus, and J14 on the lysine side-chain.

Carbohydrates provide multiple attachment points for peptides, and the conjugation of multiple copies of a single peptide to a carrier has been demonstrated to produce higher antibody responses compared to the administration of a single peptide epitope. Therefore the conjugation of a drug with lipid and sugar units represents one of the most important strategies being investigated. Our novel drug delivery system combines lipoamino acids (adjuvant), carbohydrates (carrier) and peptide (antigens) to induce antibody responses without co-administration of adjuvants. Glucose and galactose core based vaccines bearing peptide antigens containing a portion of carboxy-terminal C-repeat region of the GAS cell surface M protein (J8 or J14) and incorporating LCP system were synthesized by stepwise solid-phase synthesis. *In vivo* experiments performed in female B10.BR (H-2^k) mice proved that these liposaccharide vaccines elicit serum specific IgG antibody response.

Novel vaccine candidates against HPV-16 associated cervical cancer were also developed using a modified LCP system. Three complexes were synthesised, each containing four copies of the HPV-16 $E7_{44-62}$ peptide. Two of the vaccine candidates featured four mannose residues to target dendritic cells, which have mannose-specific receptors. Immunological activity was assessed by immunising C57BL/6 mice which were then challenged with TC-1 tumour cells. Mice immunised with the HPV-16 $E7_{44-62}$ LCP vaccine appeared to have smaller tumours compared to mice immunised with control immunogens and with vaccine containing acetylated mannose displaying no tumour growth. These results suggest that the use of mannose as a dendritic cell targeting molecule increases the CTL response to the HPV-16 $E7_{44-62}$ peptide when incorporated into the LCP system.

Which ABC-Transporters Should we Target in Leukemia?

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ABC-transporters are a large family of proteins involved in active transport across biological membranes. Some members of this family cause drug resistance in malignant diseases *via* ATP dependent drug efflux from malignant cells. This phenomenon was intensively analyzed in leukemia.

ABCB1 (P-g) and ABCG2 (BCRP) were shown to be associated with poor response to chemotherapy in adult acute myeloid leukemia (AML). Both proteins confer resistance to a wide range of chemotherapeutic drugs *in vitro*. Therefore, they represent possible therapeutic targets. In pediatric AML, this is the case for ABCG2 but not for ABCB1. In acute lymphoblastic leukemia (ALL), both proteins appear less relevant with the probable exception of ABCB1 in adult patients.

ABCC3 (MRP3) has a strong prognostic impact in AML and ALL independent of age group. However, ABCC3 does not cause much drug resistance *in vitro*. Therefore, it remains to be elucidated whether its correlation with poor response to therapy is causative or just an epiphenomenon.ABCA3 might be an additional cause of drug resistance in AML. It is associated with *in vitro* drug resistance and response to therapy but both aspects need further studies for verification. Specific inhibitors of ABC-transporters can sensitize leukemic cells to chemotherapy. For some types of leukemia it would be desirable to develop drugs that inhibit a set of ABC-transporters.

Crosslinked Micelles *via* the RAFT Process: Towards a More Biocompatible Gene Delivery System

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Oligonucleotides (ONs) are short single strands of DNA are promising as clinical therapeutic regimen for genetic, neoplastic and infectious diseases [1]. However, several major obstacles still limit the successful delivery of ASONs. ASONs can easily be damaged by nucleases which are ubiquitous in the body. Furthermore, equivalent to conventional drugs the ultimate goal of drug treatment is the targeted delivery to a specific site. For ASONs to convey their full potential they are required to find particular targets in the human body.

To date, various methods have been studied, e.g. chemical modifications, using viral or non-viral systems. Currently viral systems are widely used as gene delivery vectors due to their ability to promote efficiently DNA delivery and DNA expression [2]. However, virus-based vectors as gene delivery systems can introduce high risks in toxicity and immunogenicity. Furthermore, the technique is usually not cost-efficient. Polymeric gene delivery systems have been widely explored as a viable alternative to viral systems [3]. Polycationic polymers have been largely used due to their ability to condense DNA for more efficient uptake. However, the use of cationic polymers is partly hampered due to their toxicity [4].

A solution to reduce toxic side effects is the synthesis of nano-sized core-shell particles. The core of the particle encapsulates the oligonucletide while the shell ensures biocompatibility or contains biologically active moieties, which can target specific sites in the body. A versatile way to build up core-shell particles is by self-assembling amphiphilic copolymers [5].

A versatile way of preparing a variety of block copolymers and therefore stable core-shell particles is the RAFT (reversible addition fragmentation transfer) process [6]. By variation of the ratio of both blocks the structure and size of the self-aggregate could be influenced leading to micelles or vesicles of varying diameters. An easy and promising approach to target a more robust delivery system consists in crosslinking of micelles to stabilize the aggregates against disintegration upon dilution or changes of the environment [7].

Here, we employ the RAFT process [8] to synthesize nanoparticles for gene delivery. Starting of with cross-linked core-shell nanoparticles containing cationic polymers, we also explore alternatives to cationic polymers, which can potentially lead to fully non-toxic gene carriers [9].

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Protein C Inhibitor Regulates Breast Cancer Cell Growth, Metastasis and Angiogenesis

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Protein C inhibitor (PCI) regulates the anticoagulant protein C pathway by inhibiting activated protein C, and also inhibits urinary plasminogen activator, a mediator of tumor cell invasion. In the present study, we evaluated the effect of human PCI and its inactive derivatives on tumor growth and metastasis of human breast cancer (MDA-231) cells, and on angiogenesis *in vivo*. The invasiveness of MDA-231 cells was inhibited by recombinant intact PCI, but not by the reactive site-modified PCI (R354APCI) or by the N-terminal fragment of protease-cleaved PCI (NTPCI). The *in vitro* invasiveness of MDA-231 cells expressing intact PCI (MDA-PCI) was significantly decreased as compared to MDA-231 cells expressing Mock (MDA-Mock), R354APCI (MDA-R354APCI) or NTPCI (MDA-NTPCI). The *in vivo* growth and metastatic potential of MDA-PCI, MDA-R354APCI and MDA-NTPCI cells in severe combined immunodeficient (SCID) mice were significantly decreased as compared to MDA-Mock cells. Angiogenesis was also significantly decreased in Matrigel implant containing MDA-PCI, MDA-R354APCI or MDA-NTPCI cells as compared to the cells containing MDA-Mock cells. *In vivo* angiogenesis in rat cornea and *in vitro* tube formation were also inhibited by recombinant intact PCI, R354APCI and NTPCI. Furthermore, the anti-angiogenic activity of PCI was strong as cleaved antithrombin, and slightly stronger than that of plasminogen activator inhibitor-1 and pigment epithelium-derived factor. These data suggest that, in addition to a reactive site-dependent mechanism, PCI also regulate tumor growth and metastasis independently of its protease inhibitory activity by inhibiting angiogenesis.

Development of Gaseotransmitters (Nitric Oxide, Carbon Monoxide, Hydrogen Sulfide) as Pharmaceutical Agents: Past Challenges, Future Opportunities

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The explosion of scientific knowledge in the area of endogenous gaseous transmitters (late 1980's-mid 1990's: nitric oxide [NO], 1990's-2000's: carbon monoxide [CO] and 2000's: hydrogen sulfide [H_2S]) opened the door for innovative drug development. These gases are produced by the body by highly specific enzyme systems (NO by NO synthases from L-arginine; CO by heme oxygenases from heme; H_2S by cystathione-γ-lyase and cystathione-β-synthetase from cysteine) to serve cardiovascular and anti-inflammatory regulatory actions. The pharmacological exploitation of NO gas began with the discovery that inhaled NO gas selectively dilates pulmonary blood vessels in the well-ventilated parts of the lung, thereby improving oxygenation. Inhaled NO (INOmax) is now an approved drug for the therapy of primary pulmonary hypertension of the newborn. Published clinical data also indicate the potential benefit of inhaled NO in preventing the development of chronic lung disease in premature babies. Preclinical data suggest the benefit of inhaled NO in a multitude of additional diseases (from vascular restenosis, myocardial infarction, sickle cell disease. Various forms of inflammation.) The mechanism of NO's action involves multiple pathways and extends beyond the activation of soluble guanylyl cyclase. With respect to CO, multiple sets of preclinical data indicate its therapeutic potential not only as a vasodilator molecule, but also as an anti-inflammatory and cytoprotective agent. Potential uses of inhaled CO include transplantation, vascular restenosis, postoperative ileus and neuroinflammation. Clinical trials with therapeutic administration of CO are on-going. The mechanism of CO's action involves guanylyl cyclase, as well as heat shock proteins, kinase pathways, mitochondrial alterations and changes in the regulation of certain receptors. With respect to H_2S, preclinical studies have described its unique effects in modulating cellular metabolism and inducing a suspended animation-like state in rodents. These effects, as well as vascular actions and modulation of gene expression play a complex role in the therapeutic benefit of H_2S, as evidenced in preclinical models of lethal hypoxia and lethal hemorrhage, vascular injury, myocardial infarction and gastric ulceration.

In some cases, aqueous solutions of biological gases were shown therapeutic benefit. Peritoneal lavage with CO-saturated physiological solutions is effective in murine models of ileus. The aqueous formulation of H_2S, as a parenteral injectable drug candidate (IK-1001), exerts cardioprotective effects and is in Phase I clinical trials. Small molecules releasing NO, CO or H_2S have also been synthesized. The pharmacological exploitation of NO releasing small molecules has began over a decade ago with the medical introduction of nitroglycerin, followed by various organic nitrates. It was only discovered much later that these compounds release NO, mediating their therapeutic effects. In case of CO, several classes of 'CO-Releasing Molecules" were synthesized, with preclinical efficacy in cardiovascular diseases and inflammation. In certain cases, anti-inflammatory molecules (e.g. nonsteroidals) were coupled with specialized functional groups releasing NO or H_2S, in order to improve their characteristics.

Many of the classical paradigms of drug development had to be revised during the pharmaceutical development of gaseotransmitters. The SAR and MOA work is unique when the active principle is an endogenous small molecule consisting of a few atoms, which exerts complex biological actions on multiple targets. The GMP synthesis of a gaseous drug requires a specialized approach. The pharmacokinetic and ADME work involves specialized methods, frequently necessitating the monitoring of 'biomarkers' (e.g. met-hemoglobin for NO or carboxy-hemoglobin for CO). As all three biological gases have had a previous 'life' as environmental toxins, environmental guidelines are in place which limit exposure levels. This can present challenges in planning clinical trials. The logistics of the delivery of biological gases to the medical units, the possibility of exhalation of the biological gases and the monitoring of the safety of bystanding medical personnel also requires specialized approaches.

Frontiers in Medicinal Chemistry. Time for Another Big Bang?

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The number of new drugs reaching the market has dramatically decreased over the past decade, despite the highly interdisciplinary nature of medicinal chemistry, as well as the numerous technological advances aimed at supporting the medicinal chemist to more rapidly access drug like compounds. Maybe the time has come for another “big bang” in the field of medicinal chemistry, in order to solve the “pipeline issue” faced by many drug discovery companies. Innovation will be of key importance to “make it happen”. This talk will mainly address three important aspects of innovation that may lead to the formation of a completely new chemical universe. The lecture will thus focus on how innovative drug discovery tools together with innovative drug targets and a more innovative drug discovery process could lead the medicinal chemists to push back the frontiers of Medicinal Chemistry.

The MAPPIT Toolbox: Strategies to Analyze Molecular Interactions in Intact Cells

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MAPPIT (Mammalian Protein-Protein Interaction Trap) is a cytokine receptor-based two-hybrid method that operates in intact mammalian cells (1). Modification-independent and tyrosine phosphorylation-dependent interactions can be studied in their normal physiological context (2,3,4). Interactor hunts for novel protein-protein interactions can be performed using either a FACS-based protocol using complex cDNA libraries (5) or using an array format.

Several variations on the basic concept were developed:

In **Reverse MAPPIT**, disruption of a designated protein-protein interaction leads to a positive read-out, allowing easy screening for disruptors in intact human cells (6,7). Proof-of-concept experiments were performed using polypeptides as well as small organic molecules.

In the **three-hybrid MASPIT modus**, interactions between small molecules (e.g. metabolites, drugs) and proteins can also be studied (8).

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Proteasome Inhibition Therapies and Bone Metabolism in Multiple Myeloma

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Bone disease is one of the most debilitating manifestations of multiple myeloma (MM). A complex interdependence exists between myeloma bone disease and tumor growth, creating a vicious circle of extensive bone destruction and myeloma progression. Proteasome inhibition has been implicated in the regulation of bone metabolism through the reduction of *receptor activator of nuclear factor-κB ligand* (RANKL)-mediated osteoclast differentiation *via* the inhibition of nuclear factor-kappa B (NFκB) signaling. Furthermore, proteasome inhibitors have recently been shown to promote bone formation *in vitro* and *in vivo*. Preclinical studies have demonstrated that proteasome inhibitors, including bortezomib, which is the first such agent discovered, stimulates osteoblast differentiation while inhibiting osteoclast formation and bone resorption in MM. Clinical studies confirm these observations. Bortezomib counteracts the abnormal balance of osteoclast regulators (RANKL and osteoprotegerin), leading to osteoclast inhibition and decreased bone destruction, as measured by a reduction in markers of bone resorption (C- or N-telopeptide of collagen type I and tartrate resistant acid phosphatase isoform 5b) in MM patients. In addition, bortezomib stimulates osteoblast function, possibly through the reduction of dickkopf-1, a *Wnt* signaling inhibitor, leading to increased bone formation, as indicated by an elevation in bone-specific alkaline phosphatase and osteocalcin. The effect of bortezomib on bone disease is thought to be direct, and not merely a consequence of the antimyeloma properties of the agent, making it an attractive substance for further investigation as it may combine potent antimyeloma activity with beneficial effects on bone.

Identification of Drug Candidates for Prostate Cancer Using Computational and Experimental Methods

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Prostate cancer is the most common malignancy and age-related cause of cancer among male deaths worldwide. Although the exact cause of prostate cancer is not known, 75% of cases are reported due to problems associated with androgen biosynthesis, specifically testosterone production. There are several methods in use to treat prostate cancer; however, none are proved to be effective against androgen biosynthesis causing the reappearance of prostate cancer. Recently, it has been shown that direct blocking of androgen biosynthesis using inhibitors against cytochrome P450 monooxygenase 17αhydroxylase/17,20-lyase (CYP 17) can completely terminate the progress of prostate cancer. CPY 17 catalyzes the hydroxylation of progesterone and pregnenolone into the corresponding 17 α-products, as well as the cleavage of the C_{17}-C_{20} bond to yield androstenedione and dehydro-epiandrosterone (DHEA) in the androgen biosynthesis pathway.

A combination of structure-based drug design and experimental approaches is used to design inhibitors against CYP 17. The structure-based approaches include the design of novel molecular entities that create strong interactions with important atoms in the active site of CYP 17 that contains a heme group. The designed drugs as well as molecules available in several databases are further screened using docking studies to analyze the structural effectiveness considering their binding and docking energies. Based on the computational studies, we identified three different groups of molecules according to their molecular structure: steroidal, steroidomimetic and aliphatic compounds. It is shown with *in silico* tests that steroidomimetic compounds show stronger binding interactions compared to steroidal and aliphatic compounds. The effect of selected inhibitors has been tested using *in vitro* assays. These include development of protein expression, purification and enzyme assay. We are currently testing the effect of these drugs by CYP 17 assays. Selected molecules that inhibit CYP 17 activity will be further subjected to toxicity assays. Conclusive results are expected within a few months. The most promising molecules based on the data obtained from computations and experiments will be tested *in vivo* using mice in order to investigate the pharmacokinetic behaviour of these molecules.

"Engineered Heparins": Novel β-Secretase Inhibitors as Potential Therapeutics for Alzheimers Disease

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Apart from their anticoagulant properties, heparins have known potential as novel agents for treating a number of disease processes including cancer, inflammation and neurodegenerative disorders. We have developed approaches for producing chemically modified heparins in order to access the chemical space offered by these molecules (Yates et al, *J Med Chem* 47, 277-280, 2004). Novel compounds with optimised activities and reduced off-target effects can be produced and provide new opportunities for drug discovery. One area on which we have focussed is Alzheimers disease. Cleavage of amyloid β-protein precursor protein (APP) by the protease beta-secretase (BACE1) is a key step in Aβ peptide processing. We recently described a novel role for heparan sulfate polysaccharides in AD pathology as one of the first naturally occurring inhibitors of β-secretase (BACE1) (Scholefield *et al*., *J. Cell. Biol*, 163, 97-107, 2003).

We are currently evaluating "engineered" (chemically modified) heparin analogues as novel BACE1 inhibitors *in vitro* and *in vivo*. To this end we have developed and extensively tested a number of engineered heparin analogues for their ability to inhibit BACE1 and also their activity as anticoagulants and as inhibitors of other proteases related to BACE1 (Patey et al, *J Med Chem* 49, 6129-6132, 2006). Several lead compounds have been identified that are effective BACE1 inhibitors, but have negligible activity as anti-coagulants or on other proteases related to BACE1. They are effective at lowering Aβ production and do not show cytotoxicity in organotypic brain cultures. We have gone on to test these novel compounds in the Tg2576 transgenic mouse model of AD. Initial assessments have been made of the bioavailability, pharmacodynamics and toxicity of both high and low molecular weight types of these compounds *in vivo*. We have tested the efficacy of the compounds by measuring brain Aβ levels as well as by behavioural testing. These data provide crucial new insights into the *in vivo* efficacy of selective engineered heparins as BACE1 inhibitors, and could underpin the development of new therapeutic strategies for human AD and other neurodegenerative disorders.

Apoptosis as a Target for Peptide and Protein Therapies

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The cell death program called apoptosis is central in many aspects of normal and pathological tissue physiology. An excess of apoptosis is associated with neurodegenerative and autoimmune diseases and restrictions of this program is often implicated in cancer progression and resistance to therapies[1]. The proteins of the bcl-2 family are instrumental in the active phase of apoptosis. This family is subdivided into anti- (Bcl-2, Bcl-Xl …) and pro-apoptotic (Bax, Bak…) proteins, which share some common structural characteristics and have opposite roles in apoptosis[2]. We will present the mechanisms by which these proteins control apoptosis as well as the domains involved in the regulation of their functions and/or their interactions. Some functional domains have proved valuable in the designing of new therapeutical tools destined to combat excessive apoptosis or induce apoptosis. We will discuss the nature and the structure of these regions as well as newly discovered domains that could have a putative therapeutic interest. We will describe the first *in vitro* and *in vivo* experiments, which support the use of these proteins and/or peptides derived from the Bcl-2 family in cancer and neurodegenerative models.

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Transcorneal Permeation of NSAIDs Drug Delivery in a Corneal Device

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The cornea provides natural barriers to drug transport of topical drugs. In the last years several studies have been made for estimating ocular bioavailability "*ex vivo*" using corneal perfusion chambers.

Nonsteroidal anti-inflammatory drugs (NSAIDs), like diclofenac and flurbiprofen, have been found as viable alternatives to steroids in treating postoperative ocular inflammation, chronic non-infectious inflammation and prevention of intraoperative miosis during cataract surgery. This therapy requires, due to low permeation rates and a rapid pre-corneal loss, a frequent application or a highly concentrated eye drop formulations. With the aim to avoid this, different kind of drug delivery systems were developed.

The present research was focus on the study of corneal permeation of two anti-inflammatory drugs: diclofenac and flurbiprofen (as a models of hydrophilic and lipophilic drugs, respectively) loaded to ciclodextrins or polyesters nanoparticles in order to determine differences in their corneal permeation against free drug or commercial eye drops.

These studies were carried out in a corneal device designed and constructed in our laboratory and validated with sodium fluorescein, dye widely used in ophthalmology. Albino New Zealand rabbits weighting 1.8 to 2.2 kg, were used. After rabbits were sacrificed, the corneas were removed and immediately clamped in the chamber. The apparent corneal permeability coefficient of samples was calculated showing low permeation values for free drugs in comparison with ciclodextrins and nanoparticles.

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Aldehyde Oxidase in Drug Metabolism: Simple Preclinical Tests to Avoid Potential for Clinical Pharmacokinetic Failure

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Aldehyde Oxidase (AO) is a soluble cytosolic enzyme that contains molybdopterin, flavin, and an iron-sulfur as prosthetic groups. The enzyme is present in various tissues with high hepatic levels. It is best known for its metabolism of aldehydes to carboxylic acids. It is also known to oxidatively metabolize iminium ions and nitrogen-containing aromatic heterocycles to amides, and reductively metabolize N-O and N-S bonds. As AO is a cytosolic enzyme, its potential role in metabolism is not detected in the hepatic microsomal lability screen. Furthermore, the enzyme shows significant interspecies variability in substrate specificity. Consequently, when AO is involved in metabolism of a new chemical entity (NCE), in vivo pharmacokinetic results in preclinical species may not extrapolate to humans, resulting in potential clinical failure. When NCEs contain aromatic nitrogen heterocycles, secondary lability screens using hepatic cytosol from various species (rat, monkey, guinea pig and human) should be conducted to explore a potential role for AO in metabolism and species selection for pharmacokinetic analysis. Additionally, the identification of metabolites in plasma from preclinical and clinical pharmacokinetic studies can provide clues to the role of AO in metabolism. A case study within Pfizer's preclinical and clinical drug discovery and development program will be presented.

Therapeutic Proteins: New Achievements for Stabilization and Delivery

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Therapeutic peptide and protein represent an important field of investigation since their easy availability by the genetic engineering. However they suffer from instability in blood and formulation, immunogenicity and rapid excretion.

Among the methods to overcome these drawbacks several approaches were developed and among these the entrapment into liposomes or particles, the production of properly designed muteins or the conjugation with low or high mass molecules.

The last approach, based on the covalent linking of poly(ethylene glycol) PEG, an amphyphylic very flexible polymer already approved by FDA for internal use, was the winning procedure since it already allowed for marketing several protein drugs (1-2). The polymer yields a water cloud around the protein surface that protects from approaching of antibodies or proteolytic enzymes, while the increase in weigh reduces the kidney elimination.

In these conjugates PEG is linked by stable bonds to the protein amino acid residues, lysine in particular, but to histidine, cysteine or glutamine also, or to the amino terminal amino acid, by stable bounds, although releasable ones were proposed.

Presently the research is very active in developing specific reagents or methods, in order to obtain selective protein PEGylation among the many possible isomers that are usually obtained by a random reaction.

The different solutions and the still open approaches in conjugation will be discussed, with particular emphasis to the enzymatic method of conjugation based in particular on the use of transglutaminase. A new procedure for the identification of the PEG conjugation site in the protein will be presented also.

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High Throughput X-Ray Crystallography: Feeding the Drug Discovery Pipeline

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Protein tyrosine kinases (PTK) are a large family of important signalling proteins that play a fundamental physiological role within the cell. Evolution has combined these kinases with a broad range of regulatory and effector domains. Dysregulated activity of members of this family has been implicated in pathological processes such as cancer and immune related disorders that cause significant morbidity and mortality within the population. Accordingly, kinases offer a smorgasbord of drug discovery opportunities. However, due to the high degree of functional and hence sequence conservation within the family, they also present a very significant challenge for drug discovery. To meet these challenges, we have established an integrated and multi-disciplinary approach to develop a suite of rationally-designed, highly-specific, high affinity small molecule inhibitors against a selected number of PTK's with an ultimate goal to produce a portfolio of phase I therapeutics. To achieve this aim, we have combined the state-of the art drug discovery platform technologies of an industry partner, Cytopia Pty Ltd, a Melbourne-based drug development company, with the expertise of the Protein Crystallography Unit at Monash University.

Our drug discovery pipeline includes members of the Janus kinase (JAK) family and the PDGR (Platelet-derived growth factor) receptor family. The pipeline comprises a number of discrete stages with protein crystallography as the centrepiece of the drug discovery process. Highlights of the Monash University/Cytopia collaboration include the recently published crystal structures of the PTK domains of JAK2 and c-FMS, a member of the PDGFR family. We are currently using a high-throughput crystallography approach to solve the co-crystal structures of the JAK2 and c-FMS PTK domains in complex with a range of high-affinity compounds developed by Cytopia using *in vitro*, *in vivo* and *in silico* techniques. Greater understanding of the specific binding modes from this crystallographic data aids in the design of more specific, higher-affinity compounds that can re-fed back into the drug discovery pipeline. This structure-based approach has proven to be extremely powerful, as it has facilitated the accelerated development of potent and selective compounds with greater pharmacokinetic properties.

I will present an overview of the targeted drug discovery design platform that forms the basis of the Rossjohn lab at Monash University/Cytopia collaboration. Since our first structural report of a JAK2 PTK domain in an active form and in complex with a pan-JAK kinase inhibitor (Lucet *et al.*, 2006), we have determined the crystal structures of several JAK2 PTK/Inhibitor complexes, including complexes with many of Cytopia's lead compounds that target the V617F mutation causative of haematological disorders such as *Polycythemia vera* in humans. Specific focus also will be given to the recent crystal structure of the c-FMS PTK domain (Walter *et al.*, 2007). This structure describes the autoinhibited conformation of c-FMS *via* its N-terminal juxtamembrane domain (JM). The structure revealed the crucial inhibitory role of the JM that prevents the activation loop from adopting an active conformation. Moreover the structure provides an understanding of c-Fms inhibition by Gleevec as well as providing a platform for the development of more selective inhibitors that target the inactive conformation of c-Fms kinase.

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Endotoxin-Stimulated Responses and Therapeutic Herbal Medicines Development by a Systems Biology Approach

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Sepsis, the systemic response to severe infection, and the resulting multiorgan failure it induces are major contributors to intensive care unit morbidity and mortality. The purposes of the study are integrated in the systems biology, including global genomics, proteomics and bioinformatics profiles, combining with molecular biological technologies in endotoxin-treated rat endothelial cells, to establish the systemic pathophysiological platform and the specific detecting biomarkers. Moreover, the expressions of altered genes, proteins, and their involvement in the hypothetical signaling pathway can provide further understanding of inflammation associated responses and developing therapeutic strategies. Methods included rat cDNA microarray, 2-DE and MALDI-TOF MS/MS, cytokine protein array, bioinformatics software as well as biochemical analyses. Western blotting, qRT-PCR, siRNA, microRNA, ELISA and cell biology tools were used to validate the gene and protein network. It was observed that endotoxin could promote some signaling or metabolic pathways as well as pathophysiologic phenomena of proliferation, atherogenesis, inflammation, and apoptosis through activated nuclear factor κB pathway in endothelial cells. Interestingly, endothelial cells also activated the mediators of anti-inflammation, antiapoptosis, and anti-oxidation to protect themselves. Carpesium nepalense, Murdannia bracteata and other several herbs, which target on nuclear factor κB pathway, might be a potential therapeutic agent for systemic inflammation.

New Theranostic Approach To Diagnose and Treat Traumatic Brain Injury

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Theranostic describes the parallel use of a new Therapy and Diagnostic test for a human disease or disorder so as to facilitate drug development and clinical trials and to achieve optimal clinical outcomes in a large population of patients. New therapeutic development traditionally has a high triage rate and more than 50% of drugs in clinical trials also fail. Some argue such lockjaw can be overcome by guiding the new therapeutic development and clinical trials with a disease-relevant diagnostic test or tests. One example is the use of LDL, HDL and total triglyceride levels to track how a new atherosclerosis drug will fare when compared with existing drugs. Major pharmaceutical companies and biotech companies have been trying for years to tackle acute brain injury (traumatic brain injury and ischemic stroke) without success[1]. Enabled by recent technological advances in proteomics, we identified novel brain injury biomarkers that have elevated levels in biofluids such as cerebrospinal fluid or blood after traumatic brain injury[2,3]. Sensitive and selective sandwich ELISAs for such biomarkers have not only allowed us to quantify the extent of brain injury, but also have the ability to monitor the neuroprotecitve effects of a new drugs *in vivo*[4]

Importantly, in recognizing the emerging role of theranostic approach, FDA has recently drafted a "*Drug-Diagnostic Co-Development Concept Paper*" with the goal of setting guidelines for prospective co-development of a drug or biological therapy (drugs) and a device test in a scientifically robust and efficient way.

Lastly, another advantage of the theranostic approach is its built-in ability to achieve a post-marketing personalized medicine paradigm (i.e., drug treatment could be tailored according to a patient's diagnostic biomarker profile over time.

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Thio-sugars as Emerging Targets for Carbohydrate Therapeutics Discovery

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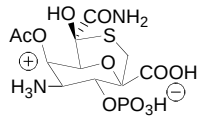
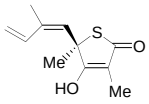
Given the fundamental differences in the chemical character of sulfur in comparison to oxygen, thio-sugars constitute a new lead in the development of carbohydrate therapeutics as new potential anticancer and anti HIV agents [1]. As the new concept of incorporating the sulfur linkage into an S-di, S-tri- and S-oligosaccharide analogs and sulfur as heteroatom progresses, the validity of the synthetic approaches and their stereoselectivity play a critical role in their synthesis. Our stereoselective approach to specific class of S-linked thio-sugars was first developed in 1995 [2] and continue [3-7] with new classes of specific RNA polymerase inhibitors such as tagetitoxin. [8] The second target is thiolactomycin [9] as a reversible inhibitor of the β-ketoacylsynthase (KAS) of bacterial fatty acid synthase (FAS and FAS II). Both thio-derivatives serve key regulatory functions and are excellent possible targets for drug design.

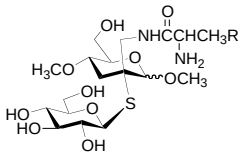
Our new concept of incorporating peptide link into nonhydrolyzable thio-sugars moiety at different positions leads us to the development of the lead compound ZJW-13. The thio-carbo peptide (ZJW-13) and its new functional analogs are being tested as potential agents to inhibit HIV-induced cell killing and virus production in CEM or MT-2 cells [10].



Tagetitoxin



Thiolactomycin



ZJW-13

This presentation will summarize recent developments in the biological and chemical characterization of new analogs of thiolactomycin and tagetitoxin and thioanhydro-sugars, thio-disaccharides, from three major families. Progress toward the design and discovery of RNA polymerase and KAS specific inhibitors will be discussed as well.

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Identification and Characterization of Therapeutic Targets for Prostate Cancer

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The prostate gland is a well-known androgen-dependent tissue, and dihydrotestosterone (DHT) plays an important role in its development as well as in prostatic diseases including cancer. Serial analysis of gene expression (SAGE) is a powerful strategy for understanding global patterns of gene expression in tissues and their regulation. By sequencing 4,294,186 SAGE tags, we have investigated the transcriptomes of 15 tissues in order to identify tissue-specific transcripts in the prostate, as well as to target the DHT-responsive transcripts specifically regulated there. Methionine adenosyltransferase II alpha (MAT2A) and heat shock 70KD protein 5 (HSPA5) were among the genes specifically expressed in prostate that were upregulated by DHT. Furthermore, knockdown of MAT2A or HSPA5 by siRNA decreased the DNA content of human prostate cancer cells and increased the proportion of cells in apoptosis. Stress induction of HSPA5 plays a major role in the protein response which contributes to tumour growth and confers drug resistance upon cancer cells. Moreover, HSPA5 is expressed on the surface of prostate cancer cells. MAT2A promotes polyamine biosynthesis which is crucial for growth and proliferation of mammalian cells. The present results point to the importance of these genes in prostate cancer cell growth.

Vascular Surface Semicarbazide-Sensitive Amine Oxidase (SSAO/VAP-1) as a Novel Target for Treatment of Chronic Vascular Disorders Associated with Protein Misfolding and Inflammation

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Semicarbazide-sensitive amine oxidase (SSAO) is located on the surfaces of vascular endothelial and smooth muscle cells. It catalyzes the deamination of endogenous substrates methylamine and aminoacetone leading to production of toxic formaldehyde and methylglyoxal, respectively, as well as hydrogen peroxide and ammonia. The enzyme is also independently known as a vascular adhesion protein (VAP-1), which regulates leucocytes trafficking and related to inflammation. This intriguing protein with dual functions has been found increased in diabetes mellitus, heart and Alzheimer's patients in different labs. SSAO-mediated deamination has also been shown to play a role in lipopolysacharide (LPS)-induced pulmonary inflammation in transgenic mice over-expressing human SSAO as well as in an animal model of inflammatory bowel disease. Blocking SSAO activity significantly reduces such inflammation. SSAO/VAP-1 is up-regulated in response to inflammation, which in turn catalyzes the production of more toxic products and thus induce a toxic vicious cycle silently progresses in chronic vascular disorders. Atomic microscopy, dynamic light scattering and circular dichroism spectroscopy scan reveal that indeed aldehydes derived from SSAO-mediated deamination can induce β-amyloid misfolding, oligomerization and fibrillogenesis, which are common pathologic features associated with Alzheimer's disease, diabetic complications, and aging process. SSAO/VAP-1 may be a novel target for treatment of these disorders.

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In Silico Pathway Analysis: The Final Frontier towards Completely Rational Drug Design

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I overview currently available technologies and methods for pathway analysis and how they can be applied in drug discovery. I will cover the following aspects of pathway analysis: the software architecture and infrastructure, algorithms for automatic pathway building, statistical analysis of high-throughput data, and pathway visualization. The emphasis will be done on the currently available technologies and how they are applied in drug discovery today. In particular, I will describe how pathway analysis can be used for drug target selection and prioritization, estimating biomarkers reliability, validation of drug efficacy and evaluating potential side-effects. I will also describe the potential advantages of pathway analysis for design of promiscuous drugs and selective drug mixtures.

In the final part of the talk I will specify the challenges and unmet needs existing in pathway analysis such as reduction of graph complexity for visualizations of large biological networks, maintenance and utilization of multiple focused ontologies for gene set enrichment analysis, availability of comprehensive pathway collection for human tissues, and accepting universal protein identifiers for data exchange.

Novel Aldehyde Dehydrogenase-2 Inhibitors as a Potential Treatment of Alcohol Addiction

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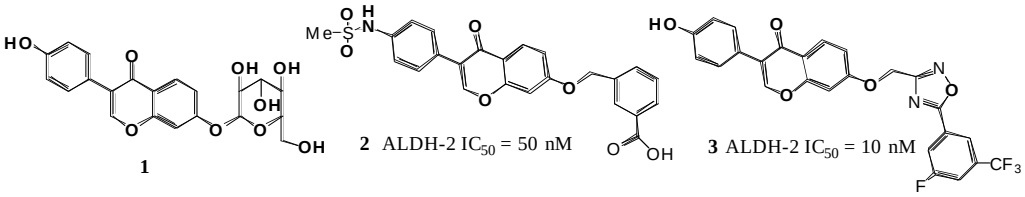
Daidzin **1**, inhibits aldehyde dehydrogenase-2 (ALDH-2, IC_{50} = 40 nM) and is the active principle of an ancient Chinese herbal medicine "kudzu root" that has been used to treat alcohol addiction for over a 1000 years. Although "kudzu root" has some effect in humans, the low oral bioavailability and short half-life of the principle daidzin (F < 1% and $t_{1/2}$ = 0.18 h, rat) has led us to optimize its pharmaceutical properties. In particular, we found replacements for the polar glucose group that led to two distinct series of molecules illustrated by the meta-benzyl acid derivative **2** and the 5-phenyloxadiazolyl compound **3** that retained similar inhibition of the ALDH-2 enzyme and resulted in an increased oral bioavailability and half-life. Both **2** and **3** inhibit alcohol consumption in rodent models in a dose-dependent manner at doses well below those for acamprosate and comparable to those for naltrexone, approved agents for alcohol addiction. The docking poses for **2** and **3** will be presented based on an X-ray of daidzin-ALDH-2 complex.



A Novel Class of Mechanism-Based Drugs for the Treatment of Alzheimer's Disease

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AD is characterized by chronic and progressive loss of neurons in discrete areas of the brain, causing debilitating symptoms such as dementia, loss of memory, and eventually, premature death. Given the poor performance of existing therapies, there is an increasing need to develop alternative drugs that modify the disease process. We have discovered *a novel class of mechanism-based drugs for the treatment of Alzheimer's disease*. These drugs specifically target the transcription factor Sp1 and thus provide the ability to influence the expression of genes associated with amyloidogenesis. Our studies using rodent and primate brains, show that Sp1 is differentially expressed and found in neurons which are abundant in the Amyloid Precursor Protein (APP) and its amyloidogenic Aβ cleavage product. Further published studies by us have shown that the expression of APP is dependent on Sp1 (Basha *et al.*, 2005) and others have showed the importance of Sp1 for BACE1 expression (Christensen *et al.*, 2004). We have discovered a class of drugs that are capable of inducing degradation of Sp1 in the brain consequently lowering APP and Aβ levels. These small molecules accomplishes what cumbersome and non-drugable gene knock-down or knock-out approaches can and belong to a group of drugs already approved for human use. These disease-modifying small molecules are highly promising as novel treatments for AD.

The Effect of Liver Disease on Cytochrome P450 Mediated Metabolism

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It is common for practicing physicians to be routinely faced with the problem of optimizing efficacy and toxicity in individual patients with liver disease. The functions of the liver, including metabolic activity, are dependent on its functional integrity. Consequently, any factor or condition that affects the integrity of the liver has the potential to alter these functions. Unfortunately, there is no reliable "gold standard" to guide such therapeutic decisions. Currently, the Child-Pugh score (CPS) is the most widely used index for the functional capacity of the liver; however it is limited by its sensitivity to the higher end of the liver disease spectrum. We have applied the Pittsburgh drug cocktail strategy to discriminate *in vivo* activity between different phenotypic measures for individual drug metabolizing enzymes as a quantitative approach to evaluate hepatic function in humans. We have shown that liver disease severity has a differential effect on the metabolic activity of specific cytochrome P450 (CYP) enzymes, with CYP2C19 being the most sensitive followed by 1A2, 2E1 and 2D6 in decreasing order of sensitivity.

We hypothesized that a "sequential progressive model of hepatic dysfunction" may characterize the differential quantitative change in liver function and may be a predictor of liver cirrhosis progression as well as provide a guide to appropriate modification of doses of drugs that are substrates for the different enzymes. In this model, it is acknowledged that different aspects of hepatic function are modified in the presence of liver disease but suggest that the order of progression of alteration of each function follows a defined sequence. In order to further evaluate this model as a potentially useful assessment of liver function, we conducted a sequential evaluation of change in CYPs activity in subjects with liver disease over a time frame of 18 months. Interestingly, preliminary data has shown that CYP2E1 activity decreased over time despite the absence of clinical evidence of hepatic function deterioration by the Child-Pugh score. This finding might suggest that the activity of CYP enzymes may be a better marker of liver disease progression and/or regression than clinical scores. We have also extended our work that so far focused on intra-hepatic disease to include extra-hepatic cholestatic disease. Our intent was to identify subgroups of liver disease patients whose disease process can be more clearly defined to better characterize not one phenotype, but multiple phenotypes. Our preliminary data has shown a trend of increased *in vivo* activity of CYP2E1 in cholestatic liver disease in comparison to the decrease in intra-hepatic disease. We are currently pursuing this research project with a larger sample size and broader liver disease etiologies.