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LECTURES

KEYNOTE LECTURE

Antiviral drugs for AIDS: current state of the art

Erik De Clercq

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Antiviral drug development is now at a pace antibodies were some 30 years ago [De Clercq, E. Nature Rev. Drug Discovery 6: 941 (2007)]. There are at present some 60 antiviral drugs that have been approved for the treatment of virus infections. Almost half of these antiviral preparations are used for the treatment of human immunodeficiency virus (HIV) infection, the other half being used for the treatment of hepatitis B virus (HBV), herpes simplex virus (HSV), varicella-zoster virus (VZV), cytomegalovirus (CMV), influenza virus and hepatitis C virus (HCV) infections [De Clercq, E. Nature Rev. Drug Discovery 6: 1001-1018 (2007)]. For HIV infections (i.e. AIDS), twenty-five compounds are currently licensed for clinical use. They fall into the following categories: nucleoside reverse transcriptase inhibitors (NRTIs: zidovudine, didanosine, zalcitabine, stavudine, lamivudine, abacavir, emtricitabine), nucleotide reverse transcriptase inhibitors (NtRTIs: tenofovir disoproxil fumarate), non-nucleoside reverse transcriptase inhibitors (NNRTIs, nevirapine, delavirdine, efavirenz, etravirine), protease inhibitors (PIs: saquinavir, ritonavir, indinavir, nelfinavir, amprenavir, lopinavir, atazanavir, fosamprenavir, tipranavir, darunavir), viral entry inhibitors [co-receptor inhibitors (CRIs: maraviroc), fusion inhibitors (FIs: enfuvirtude)] and integrase inhibitors (INIs: raltegravir). Another three anti-HIV compounds are expected to be approved within the next year(s): the NNRTI rilpivirine, the CRI vicriviroc and the INI elvitegravir. As a rule, anti-HIV drugs should be used in combination, so as to achieve a synergistic antiviral action and to reduce the risk of virus-drug resistance development. Numerous anti-HIV drug combinations could be concocted, the only triple-drug combination pill (to be taken once daily) presently available being Atripla® containing a fixed dose of tenofovir disoproxil fumarate, emtricitabine and efavirenz [De Clercq, E. Future Virol. 1: 709-715 (2006); De Clercq, E. Biochem. Pharmacol. 73: 911-922 (2007)].

SESSION 1:
International Activities and General Topics

Global North-South Networks for Drug Discovery: Promoting Innovation in Developing Countries

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TDR is an intergovernmental agency, with the vision to foster an effective global research effort on infectious diseases of poverty, in which disease endemic countries play a pivotal role. Some of the achievements of TDR in the past 30 years includes the training of over 1,500 researchers in developing countries, the strengthening of many research institutions that are today recognized as centres of excellence for tropical diseases, the development of over half of the drugs used today for tropical diseases in partnership with industry, as well as helping to establish independent public private partnerships such as Medicines for Malaria Venture (MMV) and Foundation for Innovative New Diagnostics (FIND).

As part of ongoing activities, TDR is implementing an innovative drug discovery platform for infectious tropical diseases (malaria, tuberculosis, African Sleeping Sickness, Chagas disease, Leishmaniasis, schistosomiasis, filariasis as well as onchocerciasis) that is based on networks and partnerships with industry and academia in developed and developing countries. These networks include: compound screening, medicinal chemistry, drug metabolism and pharmacokinetics as well as drug target networks. This drug discovery platform is now supporting the promotion of R&D innovation in developing countries, for example, the establishment of the African Network for Drug and Diagnostics Innovation (ANDI). ANDI aims to contribute to a sustainable African-led R&D innovation by strengthening and utilizing existing capacity and infrastructure to promote collaborative efforts for the discovery, development and delivery of affordable new tools including those based on natural products and traditional medicines. The first meeting of ANDI is scheduled for the October 6-8, 2008 at Abuja Nigeria.

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ICS-UNIDO programmes in the field of Drug Design and Discovery

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ICS programmes are based on the Centre' mandate: the transfer of knowledge in applied sciences to developing countries, and promotion and assistance in the development selection, transfer and use of applied technologies. ICS consists of three main areas; 1) Pure and Applied Chemistry; 2) Earth, Environmental and Marine Sciences and Technologies; 3) High Technology and New Materials.

Within the Area of Pure & Applied Chemistry, the sub-programme on Combinatorial Chemistry and Molecular Design has been carried out since 1997. Combinatorial chemistry and molecular design constitute a modern approach to the research and development of new molecules (drugs, agro-chemicals, polymers, catalysts…) which is being more and more applied in R & D as well as in industries including SMEs. In this sub-programme the focus is on capacity building, training courses, conferences, fellowship programmes and promotion and implementation of research projects involving centres of excellence worldwide and institutions and researchers from developing countries and countries in transition.

ICS-UNIDO has already established long-term collaboration in the field with important centres in DCs as well as recognized research centres in Europe. Among the important cooperation activities included in the Work Programme 2008 are those with the Thai Universities of Chulalongkorn and Naresuan, with the support and cooperation of the Thai Government. This initiative comprises several subprojects focussed on capacity building, research or new initiatives on resistant HIV viruses and bird-flu viruses as well as setting up an Academia-Industry Consortium. Similar initiatives are being promoted with other Asian countries (India, Malaysia), Latin American countries (Uruguay, Mexico, Argentina, Brazil) and African countries (Ivory Coast, Cameroon, Ghana, South Africa) as well as with Russia, CEE and NIS countries.

Examples of the ongoing cooperative research programmes of ICS-UNIDO in the field of CC/MD will be presented with particular focus on design and development of new potential drugs for the treatment of diseases widespread in developing countries such as malaria, AIDS, hepatitis, bird flu and others.

Few examples of molecular design on the modelling of degradable polymers and of reactions of biofuels formation will be also presented.

The Roles of Electrostatics in Drug Design

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The electrostatic force is the strongest and most directional of the non-covalent interactions between molecules. It thus plays a key role in the analysis of such topics as the delivery of drugs and the specificity and tightness of their binding to target molecules. The physics of electrostatics (ES) is well understood, and a variety of powerful computational methods have been applied to ES problems. Nevertheless, obtaining quantitatively correct results from calculations is still a difficult matter.

Rather than attempting to review the huge field of ES, this talk will briefly discuss three current topics that show the promise of ES for drug design and delivery.

1. Computer simulations on models can provide insight into the origins of specificity and promiscuity in ligand binding Radhakrishnan and Tidor (J. Phys. Chem. B 2007, 111, 13419-435, 2007.) point out that drug binding can be too specific. An effective drug must bind with high specificity to its intended target. However, when the target can mutate or evolve - as in the case of viruses - too great specificity can mean ready loss of efficacy. The capacity to tune the specificity of drug binding could thus be very valuable. The authors create model receptor and ligand molecules in which the shapes stay constant but the pattern of charges can be varied widely. They quantified the following insights into the modulation of specificity.

- hydrophobic ligands are more promiscuous than charged ones
- flexibility in the ligand and the bound state may further increase the specificity of charged or polar ligands
- larger ligands are more specific than smaller ones.

2. Charged (cationic) peptides such as octa-arginine can mediate the transduction of larger peptides or other drugs into cells. (Rothbard et al. Adv. Drug Del. Rev. 57, 495-504, 2007). The cell membrane isolates the interior of the cell from foreign substances - often including drugs. It is energetically expensive to pass ions through the hydrophobic membrane core, and it is therefore counterintuitive that cationic peptides such as octa-arginine can readily pass through membranes and pull small cargoes along. The authors present a mechanistic picture of this process, which appears to work without the involvement of cell surface receptors. Arginine-rich peptides can also be incorporated into vesicles that can serve as drug delivery vehicles (Holowka et al. 2007, Nature Materials 6, 52-57).

3. Many electrostatic effects, such as bond breaking, electron transfer, and proton tunneling, can only be analyzed using quantum mechanical (*ab initio)* calculations. Cavelier and Amzel (Proteins, 43, 420-432, 2001) present an *ab initio* analysis of the 2-electron reduction of quinones carried out by quinone reductase (QR). QR plays an important protective role in cells, but can also activate anti-tumor drugs in cells. Results include understanding of the dynamics of a charge relay system, and of details of the evolving charge pattern in the NADP(H) cofactor. The method holds promise for the use of generalized charge complementarity in the design of drugs.

Virtual Screening – Problems and Success Stories

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Several computer-aided techniques for automated database searches and docking developed over the past ten to fifteen years. Chemical database filters, pharmacophore searches, and docking and scoring provide a powerful and flexible set of computational techniques for virtual screening. In the most effective application, cascades of different steps reduce very rapidly the number of potential ligands of a certain target from hundred thousands or even millions of structures to a manageable size, e.g. by first applying simple filters, followed by pharmacophore generation and topological or 3D pharmacophore searches. If a 3D structure of the biological target is available from protein crystallography or NMR studies, or can be modeled by homology, the last step is flexible docking, followed by scoring and a careful visual inspection of the obtained results. In this manner, virtual screening became a routine technique in the search for new leads, as illustrated by many successful applications.

The presentation will discuss some problems in protein and ligand pre-processing, e.g. the definition of ionisation states and tautomeric forms, and present several success stories of virtual screening for new lead structures.

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Progress on the incorporation of cage amino acids into non-natural peptides

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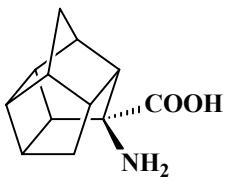
Computational studies[i,ii] in our laboratory indicated that the cage skeletons (**1** and **2**) have the tendency to impose a 3_{10}-helix as well as an α_L-helix on the polypeptide chain. Non-natural residues (such as **1** and **2**) are useful tools for the study of the conformational preferences of their models, the design of peptide analogues with improved pharmacokinetic profiles and the development of pharmacophore models.[iii] Synthesis of trishomocubane amino acid analogues suitable for peptide synthesis would enable us to verify the computational predictions and to contribute to this very active field of research.

This investigation relies on the synthesis of the sterically demanding amino acids through hydrolysis of the bis-Boc-protected hydantoin **3**. The NMR elucidation[iv] of some of the amino acid derivatives will be presented.



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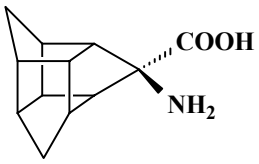

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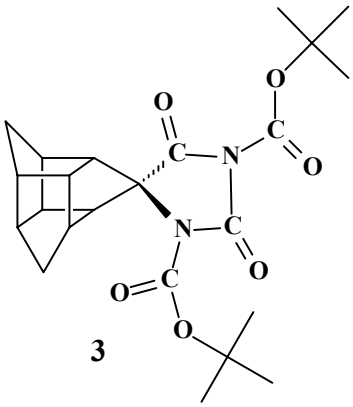

3

Progress to incorporate the amino acids **1** and **2** into non-natural peptides will also be reported.

Medicines for Malaria Venture (MMV)

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A nonprofit organization created to discover, develop and deliver new antimalarial drugs through effective public-private partnerships. Our vision is a world in which affordable drugs will help eliminate the devastating effects of malaria and help protect the billions of people, especially children and pregnant women, at risk of this terrible disease. Our mission is to bring the public, private and philanthropic sector partners together to fund and manage the discovery, development and registration of new medicines for the treatment and prevention of malaria in disease-endemic countries.

Why is MMV needed?

- Malaria kills between one and two million people annually.
- The majority of malaria's victims are children under five and pregnant women.
- Each year 300-500 million new clinical cases of malaria are reported in official statistics; only drugs can be used to cure these potentially fatal infections. While the true number is not known recent estimates suggest that actual numbers may be larger.
- 75-90% of malaria cases are found in Sub-Saharan Africa, but this could change if climactic conditions change. Warming and flooding are favorable to the spread of the main tropical mosquito species that principally transmits malaria.

The way forward

- Today the potential of drug research within MMV's pipeline is exceptionally promising
- There is no technical reason why we should not aspire to much more than what we have as standard drugs today from single dose cures to very safe intermittent treatments (surrogate vaccines) for those at heightened risk of malaria.
- It is now critical that as a global community we do not repeat the well documented mistakes of the failed eradication era (the 1950-60s), by thinking that all that is needed is already on hand.

A balance between continuous innovation and evidence-based use of malaria control and treatment measures provides the only rational way forward to achieving MMV's vision.

Public-private partnerships: the formula MMV chose to fulfill its mission.

Established as a Foundation in Switzerland, MMV was officially launched on 3 November 1999 as a public-private partnerships the model that has become one of the preferred ways to ensure that progress can be made in addressing healthcare issues which neither the public nor the private sector can solve on their own. MMV is among the first of these public-private partnerships established to tackle a major global disease. The initiative arose from discussions between the World Health Organization (WHO) and the representative body of the pharmaceutical industry, the International Federation of Pharmaceutical Manufacturers Associations (IFPMA). Early partners in these exploratory discussions were the Global Forum for Health Research, the Rockefeller Foundation, the World Bank, the Swiss Agency for Development and Cooperation, the Association of the British Pharmaceutical Industry and the Wellcome Trust.

The combination of the pharmaceutical industry, with its knowledge and expertise in drug discovery and development, and the public sector, with its depth of expertise in basic biology, clinical medicine, field experience and above all its public remit, constitutes the rationale for MMV.

SESSION 2:
Antiparasitic Drug Discovery

Structure-Based Approaches for Drug Design of new Antimalarials

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Design and synthesis of new classes of derivatives bearing antitrypanosomal and antileishmanial activity

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Trypanosomatids are the causative agents of various lethal parasitic diseases such as Chagas' disease (*Trypanosoma cruzi*), African sleeping sickness (*Trypanosoma brucei* with the two sub-species *T. b. gambiense* and *T. b. rhodesiense*), and leishmaniases (*Leishmania donovani, L. major*, and *L. tropica*).[1] To confront these diseases, two different strategies, which are nowadays well-known within the medicinal chemistry community, have been adopted by us: i) structure-based drug design; ii) combinatorial library design. Following the first approach, we computationally designed a quinazoline-based series of new derivatives, which were then synthesized and tested against the *T. cruzi* trypanothione reductase enzyme. The biological profile of the new molecules was also assessed using whole-cell assays. Following the second approach, a combinatorial library was generated based on the 1,4-naphtoquinone and 1,4-anthraquinone natural scaffolds. We synthesized 16 new compounds, which incorporated a selection of aromatic groups that would mimic a structural element of triclosan, a general biocide able to kill both procyclic forms and bloodstream forms of *T. brucei*.[2] The compounds were then tested by means of whole-parasite assays. In this case, the molecular target is initially unknown, and *a posteriori*, by means of reverse-chemical-genetics strategies like the chemical proteomics approach, the target will be tentatively fished out from parasitic cell extracts. The results of both approaches will be reported and discuss in this presentation.

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Molecular Modeling of the Antimalarial Artemisinin

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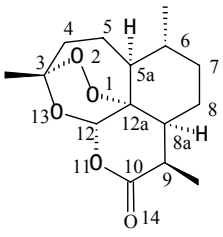
Malaria is an infectious disease caused by the unicellular parasite *Plasmodium sp*. Currently, the malaria parasite is becoming resistant to the traditional pharmacological alternatives, which are ineffective. Artemisinin is the most recent advance in the chemotherapy of malaria. Artemisinin is a sesquiterpene lactone with an endoperoxide function that is essential to its antimalarial activity. Endoperoxides are supposed to act on heme leading to reduction of the peroxide bond and production of radicals that may be responsible for the parasite's death. In the present communication we will show results of AM1, PM3 and DFT B3LYP/6-31+G(d,p) calculations for a set of radical anions and neutral species supposed to be formed during the rearrangement of artemisinin from the two radicals (C-centered and O-centered) that are supposed to play a relevant role in the mechanism of action of artemisinin. The B3LYP results show that the primary and the secondary radicals centered on C_4, generated by homolytic cleavage of the C_3-C_4 bond and by 1,5 hydrogen shift, respectively, are more stable than radicals centered on oxygen. The calculations show that the activation barriers for rearrangement are low, leading to exothermic processes.

Additionally, we have undertaken a systematic study of several interaction arrangements between artemisinin and heme. Density Functional Theory calculations were employed to calculate interaction energies, electronic states and geometrical arrangements for the complex between the heme group and artemisinin. The results showed that the interaction between the heme group and artemisinin at long distances occurs through a complex where the iron atom retains its electronic features, leading to a quintet state as the most stable one. However, for interaction at short distances, due to artemisinin reduction by the heme group, the most stable complex has a septet spin state. These results suggest that a thermodynamically favorable interaction between artemisinin and heme may happen.



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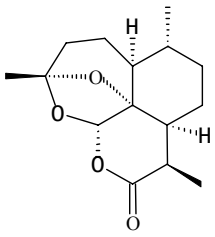

2

Examples of the Design and synthesis of some selective inhibitors of the glycolysis in the Trypanosomatidea

Nyasse Barthelemy

University of Yaounde - Cameroon

The parasitic protozoa Trypanosoma and Leishmania are the causative agents of the highly disabling and often fatal diseases, such as African sleeping sickness, Chagas' disease and leishmaniasis. Millions of people are at risk in the areas of Africa, South America and Asia where these diseases are endemic. Unfortunately, current drugs are far from satisfactory as they often possess toxic side effects, are ineffective against certain disease forms and may require expensive administration procedures; in addition, resistance to these drugs is becoming an increasingly common problem.[1]

In the last two decades, experimental approaches such as gene cloning, protein crystallography and RNA interference have been introduced to define and validate potential new drug targets in Trypanosomatidae. Glycolysis appears to provide an excellent therapeutic target because it is essential to bloodstream form Trypanosomatidae as the only catabolic source of ATP.[1, 2]

Furthermore, due to the evolutionary distance between Trypanosomatidae and humans, [3] the parasite enzymes within the pathway possess distinct properties that differentiate them from their mammalian counterparts, and which could be exploited in the design of parasite specific drugs.[4] The unique organization of the glycolytic pathway in trypanosomatids, with most of the enzymes present in peroxisome-like organelles called glycosomes, is correlated with exploitable differences. Thus, the compartmentalization has resulted in different kinetic properties and activity regulation mechanisms that correspond to differences in enzyme structure.[4] Phosphofructokinase (PFK), pyruvate kinase (PyK) and Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) are validated as such enzymes which could, therefore, be targeted.[2, 4–7]

Here, we report the design and the synthesis of inhibitors targeted against *Trypanosoma brucei* phosphofructokinase (PFK) and Glyceraldehyde-3-phosphate dehydrogenase (GAPDH). As concerned the first enzyme, stepwise synthesis and inhibitor design from a rational starting point identified furanose sugar amino amides as a novel class of inhibitors for this enzyme. The synthesis of GAPDH Inhibitors was inspired by both substrate structure and pharmacophoric moieties present in two known naturally occurring GAPDH inhibitors, pentalenolactone[8] and koningic acid[9]. Trypanocidal activity also showed potency in the low micromolar range and confirms these inhibitors as promising candidates for the development towards the design of anti-trypanosomal drugs.

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STRUCTURAL INSIGHT INTO SELECTIVE INHIBITION OF *SCHISTOSOMA MANSONI* PURINE NUCLEOSIDE PHOSPHORYLASE (*SM*PNP)

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Purine nucleoside phosphorylase (PNP, EC 2.4.2.1) is a key enzyme in the purine salvage pathway which has been largely studied as a target for the treatment of T-cell proliferative diseases such as T-cell leukemias or lymphomas, for the prevention of transplant rejection, and in the treatment of T-cell autoimmune diseases such as rheumatoid arthritis and systemic lupus erythematosus in humans.[1-3] More recently, it has been suggested that selective inhibitors of PNP could also be important for the treatment neglected diseases,[1, 4-6] such as schistossomiasis, the second most important neglected disease in world, infecting about 200 million people in 74 countries. This hypotesis is supported by the fact that *Schistosoma mansoni* worms are unable to synthesize purine nucleotides *de novo*, thus depending exclusevely on purine salvage pathway for reproduction and survival.[6-7]

Aiming at developing selective inhibitors of *Sm*PNP, kinetic studies of 12 ground-state inhibitors were carried through a standard spectrophotometric assay, employing 10 μM of inosine (substrate) for *Sm*PNP and 64 μM for *Homo sapiens* PNP (*Hs*PNP), at pH 7.4, and 50 mM of phosphate buffer. Readouts, performed at 293 nm, show that on one hand all inhibitors have similar competitive inhibitory profile towards SmPNP, on the other hand deazaguanine derivatives with aromatic moieties at position 9 have no selectivity towards *Sm*PNP in comparison to *Hs*PNP, whereas 9-ribose substituted compounds show 3-6 fold selectivity ratio towards *Sm*PNP.

In order to shed some light over the structural features that are responsible for this, the crystallographic structure of *Sm*PNP in complex with guanosine (*Sm*PNP-GUA) was crystallized and its X-ray crystallographic structure solved and refined to 2.05Å resolution using CCP4 software (R=0.18, R_{free}=0.25). The interaction profile of guanosine in the *Sm*PNP active site shows that purine moiety binds exactly as in *Hs*PNP, however the ribose is H- bonded to Tyr^{202}, what can not be possible in the human counterpart that shows a Phe in the equivalent position. 9-ribose substituted compounds have high similarity to guanosine and thus should bind equally. Therefore, it is reasonable to assume that ribose containing inhibitors also H-bond to Tyr^{202}, thus selectively inhibiting *Sm*PNP. The reduced potency of these compounds might be a consequence of the ribose puckering in *Sm*PNP active site. This information shall guide the design of more selective and potent inhibitors of *Sm*PNP

Acknowledgements: CNPq, FAPESB, FAPESP.

SESSION 3:
Antiparasitic Drug Discovery

Uncovering false positives on a virtual screening search for cruzain inhibitors

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Identification and avoidance of potential false positive results, such as screening hits that are unsuitable as lead structures, have been recognized as a big concern in every virtual screening (VS) or high throughput screening (HTS) program.[1,2] These undesired results could be due to many reasons,[3] being one of them the presence of promiscuous inhibitors among the tested compounds. [4] Organic molecules able to form micromolar or submicromolar aggregates in aqueous buffers are classified as promiscuous inhibitors[5] due to the capacity of these aggregates to inhibit nonspecifically enzymes at in vitro assays. For this reason, these molecules are rarely suitable for drug development and their early detection can avoid worthless work.[2] Promiscuous inhibitor prediction studies, based on chemical properties, have been carried out with some degree of success.[6,7] Despite these early positive results, the widespread application validity of these empirical models is still being discussed considering that they have been applied only to a limited size test series, and also they seem to be still unreliable for filtering databases with up to a million compounds. Additionally, it has been stressed that more studies should address the issue of false positives and the appearance of promiscuous inhibitors in HTS/VS programs.[2,3,6,7] Therefore, a demand for higher quality data to feed knowledge-based tools which will, one day, be able to reliably identify these undesirable compounds is quite clear.

In the present work[8], in the attempt to find specific inhibitors for cruzain (the recombinant form of cruzipaine, a major *Trypanosoma cruzi* protease present in every stage of the parasite life cycle[9]) it has been observed that some compounds showed typical promiscuous properties, such as poor specificity, micromolar activity, and no structure activity relationship. In this report the procedures applied to select these potential cruzain inhibitors using virtual screening applied to ZINC data base as well as the methods used for identification and confirmation of promiscuous inhibitory activity have been described. Physical–chemical and pharmacophore model filters were used to reduce the database size. The selected compounds were docked into the cruzain active site. Six hit compounds were tested as inhibitors. Although the six compounds have been engineered to be nucleophilically attacked by the catalytic cysteine of cruzain through the inclusion of chemically reactive groups (ex. nitriles, semicarbazones, and unsaturated ketones) into the pharmacophoric features used to select compounds, three of them were, in fact, promiscuous inhibitors, regarding cruzain. This high proportion (50%) of promiscuous acting compounds shows that this kind of artifact cannot only be prevalent at in vitro assays but also present a real concern in both HTS and VS programs.

MECHANISM OF ACTION OF ARTEMISININ AND DEVELOPMENT OF A HIGH THROUGHPUT SCREEN FOR ANTIMALARIALS

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A lack of understanding of the mechanisms of action of artemisinin has hindered the development of the endoperoxide class of compound. Here we report that these drugs exert their effect by interfering with the plasmodial hemoglobin catabolic pathway and inhibition of heme polymerization. We observed inhibition of digestive vacuole proteolytic activity of malarial parasite by artemisinin as well as accumulation of hemoglobin in the parasites treated with artemisinin. In aprotic solvent, heme and artemisinin react to give in a high (>50%) yield of a heme-artemisinin adduct. This adduct designated as 'hemart' mimics heme in binding to Pf-histidine rich protein II (HRP II) but lacks the ability to self polymerize. Instead, it inhibits basal heme polymerization as also heme polymerizations triggered by (a) PfHRP II, (b) Monooleoyl glycerol and (c) whole /Plasmodium *falciparum* / extract. Our results suggest that artemisinin reacts with heme before it antagonises heme polymerization. The heme artemisinin adducts stall all mechanisms of heme polymerization adopted by the malaria parasite. The resulting accumulation of free heme to toxic levels could be one of the mechanisms for antimalarial action. The fact that HRP-II bound can be displayed many known antimalrial drug was probed and has been developed as a high throughput screen for antimalarial activity.

Drug Design and Discovery for Chagas' Disease

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Trypanosoma cruzi is the hemoflagellated protozoan parasite that causes American trypanosomiasis (Chagas' disease), which is an endemic disease widespread from southern United States to southern Argentina. In addition, Chagas' disease is a well-recognized opportunistic infection in AIDS patients. It has been estimated that close to 18 million people are infected and over 40 million are at risk of infection by *T. cruzi*. Chemotherapy for Chagas' disease is still unsatisfactory. The two drugs available for Chagas' disease treatment, nifurtimox (now discontinued), and benznidazole are associated to long-term treatment and severe side effects.

One pathway that has been particularly useful for the identification of new targets against *T. cruzi* is the isoprenoid pathway. Enzymes studied so far that are involved in the synthesis of sterols and farnesyl diphosphate, and in protein prenylation, have been reported to be excellent drug targets against pathogenic parasites such as farnesyl pyrophosphate synthase (FPPS). Another interesting pathway is trypanothione biosynthesis. Trypanothione is the bisconjugated product between the tripeptide glutathione and the polyamine spermidine: N^1,N^8-bis-(L-□-glutamyl-L-cysteinylglycyl)spermidine. The uniqueness of this metabolite and its biosynthetic pathway in parasites of the order Kinetoplastida, confer to the involved enzymes of its biosynthesis a great usefulness as a molecular target. Because of the absence of this metabolite in the host mammalian cells, there is an opportunity to design highly selective antiparasitic drugs against leishmaniasis and trypanosomiases without toxic effects.

Bearing in mind our hypothesis that the isoprenoid pathway and trypanothione biosynthesis constitute major targets for the treatment of Chagas' disease and other tropical diseases, we have investigated the effect of bisphosphonates (pyrophosphate analogues) derived from fatty acids against *T. cruzi* FPPS and SPPS, and *in vitro* against *T. cruzi*, and perform structure-activity relationship studies to assist drug design. In addition, we have studied the effect of aryloxyethyl thiocyanate derivatives against *T. cruzi* squalene synthase (TcSQS), and also against *T. cruzi* cells taken 4-phenoxyphenoxyethyl thiocyanate as a lead drug. Finally, we have designed, synthesized and evaluated a series of phosphinopeptides structurally related to glutathione as antiproliferative agents against *T. cruzi*. The rationale for the synthesis of these compounds was supported on the basis that the presence of the phosphinic acid moiety would mimic the tetrahedral transition state of trypanothione synthase (TryS). It has been recently confirmed that the mentioned phosphinopeptides inhibited the enzymatic activity of TryS.

Medical Structural Genomics of Pathogenic Protozoa: A genome-wide search for protein ligands and ligand-bound structures for drug discovery

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Ten major pathogenic protozoa form a serious threat to the well-being of humanity and cause a global disease burden to hundreds of millions of people. They are: *Plasmodium falciparum, Plasmodium vivax, Trypanosoma brucei, Trypanosoma cruzi, Leishmania major, Leishmania infantum, Toxoplasma gondii, Entamoeba histolytica, Giardia lamblia, and Cryptosporidium parvum*. There is a desperate need for new medicines to treat these protozoa infected patients and for use as prophylaxis. Current drugs have major side effects and are increasingly becoming ineffective due to drug resistance. In this bleak situation, the genome sequences for these parasites, which are becoming available just now, function as new resources for developing novel medicines in the battle against the related diseases.

The Medical Structural Genomics of Pathogenic Protozoa (MSGPP) project aims to explore the genomic sequences of the ten protozoa and arrive at a large number of protein structures to guide structure-based drug development effort. High-throughput structure determination pipeline has been established in the MSGPP consortium. This includes: a) Target selection; b) Protein expression and purification; c) Crystal growth and optimization; d) X-ray diffraction data collection; and e) Structure determination. A central informatics unit tracks and updates all activities in the pipeline. In addition to these traditional structural genomics setups, a special emphasis of the goals of MSGPP is to obtain ligand-bound crystal structures of protozoan proteins. Therefore, we have incorporated ligand selection, synthesis, and screening capabilities into the MSGPP program. We will report and discuss the latest results from our structural genomics pipeline, with emphasis on ligand screening methods and obtaining ligand-bound structures of selected protein targets.

SESSION 4:
Antiviral Drug Discovery

Structure-based Development of Antiviral Therapeutics for HIV-1 Infection and AIDS

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Upon the discovery of the first human pathogenic retrovirus HTLV-1, virtually no attempt was made to explore antiretroviral therapy, since it was believed that once target cells were infected by cellular-genome-integrating retrovirus and antiretroviral drugs would do nothing to the progress of the retrovirus-associated diseases. The first three dideoxynucleoside reverse transcriptase inhibitors (zidovudine or AZT, didanosine, and zalcitabine) made changes to this "central dogma". After these first drugs proved to be efficacious in patients with HIV infection, a number of antiviral agents were added to our armamentarium in the fight against HIV infection. Combination chemotherapy using such antiretroviral agents or HAART has had a major impact on the morbidity and mortality of patients with HIV infection. However, we have faced multiple major problems, which represent the challenges different than we faced in the development of the first drugs. They include (i) drug-related toxicities, (ii) emergence of drug-resistant HIV variants, (iii) only partial restoration of immunologic functions, (iv) paradoxical flame-up of inflammation, and (v) increased cost of antiviral therapy. Nevertheless, extensive knowledge of the molecular, biochemical, and structural interactions of antiretroviral agents and their targeting viral components has been acquired. We are obviously at a new forefront in the therapy of HIV infection.

One new area in the development of antiretroviral agents is predictive modeling, which maximizes our chances of success. I will discuss an approach of combining site-directed mutagenesis-based data and molecular modeling, which represent a novel strategy for gaining structural insights for drug design of novel compounds. One of recently FDA-approved HIV therapeutic is darunavir, also developed at the NCI, was designed based on such structural approach. We most recently discovered that darunavir and a group of newly designed and synthesized agents block the dimerization process of HIV protease, an essential step in the replication cycle of HIV. Further improved approaches to explore new treatment modalities should be continued in the hope that with new and more potent antiviral agents, we will certainly be able to control HIV diseases more efficiently and effectively. The early success in the treatment of HIV-1 infection we have seen with structure-based development of anti-HIV-1 drugs should serve as a precedent for developing therapeutics for the "neglected diseases" in developing countries.

DENGUE VIRUS NONSTRUCTURAL PROTEINS NS2B/NS3 - MOLECULAR TARGETS FOR DRUG DISCOVERY

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Dengue fever (DF) and dengue hemorrhagic fever/dengue shock syndrome (DHF and DSS) are caused by four serotypes of dengue virus that belongs to the *Flaviviridae* family. Yearly incidence rates in tropical regions have increased dramatically over the past few years making dengue diseases the most prevalent mosquito-borne viral illnesses of humans worldwide. Fatality rates of DSS can become as high as 10% depending on health care quality and patient disposition. Several vaccines are currently being evaluated in clinical trials. However, it is unlikely at present that these vaccines will be routinely applicable for prevention and/or treatment in the near future making development of small-molecule drugs mandatory as treatment options for dengue virus diseases.

The 69 kDa nonstructural protein NS3 is an important component involved in polyprotein processing and viral replication and contains multiple enzymatic functions representing attractive targets for the structure-based design of antiviral inhibitors. The N-terminal domain of NS3 consists of serine protease with a catalytic triad reminiscent of proteases of the chymotrypsin family. Activation of the protease requires the presence of a short hydrophilic region of the NS2B cofactor.

With the objective to assist ongoing efforts for drug development against this important target, our laboratory has characterized structure-activity relations within the dengue virus NS2B-NS3 two-component protease. The mechanism of NS3 protease activation by the NS2B cofactor was investigated by introducing site-specific mutations in a conserved core sequence of NS2B which had significant impact on enzyme activity. We have demonstrated prominent inhibition of the NS3 protease by short cleavage peptides derived from native polyprotein junctions and substrate specificity studies have identified major determinants of enzyme activity.

Recently, we have addressed the question of biochemical diversity among the 4 dengue virus serotypes. These results will be discussed with a view to the design of rationale-based inhibitors directed against viral polyprotein processing.

Design of Inhibitor for Dengue Virus Type 2 NS2B/3 Serine Protease

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Keywords: DEN2 serine protease / 4-hydroxypanduratin / nicotinic acid

Dengue is a serious disease that is endemic in over 100 countries, with more than 2.5 billion people at risk for epidemic transmission. About 100 million cases of Dengue Fever (DF) and 500 000 cases of Dengue Haemorrhagic Fever (DHF) have been reported globally and this figure has been on the rise in the recent years. There is currently no available vaccine or drug for treatment of dengue.

Studies have shown the NS2B/NS3 component of the protease to be involved in the virus replication process by activating the cleavage in the non-structural region of the viral polyprotein at NS2A/NS2B, NS2B/NS3, NS3/NS4A and NS4B/NS5 junctions (Arias et al., 1993; Teo et al., 1997; Yusof et al., 2000). Bioassay-guided screening of natural product extracts gave leads into some compounds which inhibited the activity of NS2B/3 DEN2 serine protease, both competitively and non-competitively. Based on these leads, docking studies were carried out and a compound was designed to mimic the interactions observed between the protease and the natural ligand. A synthesis of the newly designed ligand was then carried out. Biological screening of this compound and some of its intermediates gave some positive indications as to the potential of this "new" class of compounds as anti-viral agents for the NS2B/3 DEN2 serine protease.

Characterization of new target against HIV-AIDS: the Tat-P/CAF interaction

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HIV-1 replication can only be controlled by current therapies that require life-long regular assumption of the so called “drug cocktails”. This, added to the drug resistance effect originated from the high variability in viral proteins prompts to develop new generations of drugs directed towards new targets besides those addressed by existing therapies. An alternative to the traditional targeting of viral enzymatic functions is to tackle protein-protein interactions engaged between human and viral partners needed for viral replication. The very low mutation rate of human proteins may help to overcome drug resistance. One of such interactions is the binding between HIV-1 Tat acetylated at Lys50 and the Bromodomain of the cellular acetyltransferase P/CAF, which is essential to promote transcriptional activation of the integrated provirus.

Starting from a NMR derived structure of an acetylated Tat peptide we have used modelling and simulation techniques to construct a model of full length Tat acetylated at Lys50 bound the P/CAF Bromodomain. Our model sowed large differences respect to the initial structure.

Residues completely solvent exposed in the original NMR structure came to form a crucial part of the protein-protein interface. These apparently contrasting results were validated by functional assays and FRET experiments on key mutated residues guessed from the simulation, providing a solid support to our predictions. Our model identify crucial interaction points in the HIV-1 Tat - P/CAF complex that can be used as a new scaffold for rational design of new generations of antiviral compounds.

Rational Design of C_2 -Symmetric Pyrrolidines as HIV-1 Protease Inhibitors

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Aspartic proteases play an important role in various physiological but also patho-physiological processes including e.g. Alzheimer's disease, viral (HIV) as well as parasitic infections (malaria). In case of the HI-virus, HIV-protease has proven to be an invaluable drug target due to its essential role in the virus' replication process. Although very potent HIV-1 protease inhibitors have already been successfully launched to the market, the continuously increasing drug resistance towards existing drugs calls for the design of new inhibitors possessing either a novel binding mode or a new mechanism of action.
In a rational, structure-based approach, a new class of inhibitors bearing a 3,4-disubstituted pyrrolidine moiety was developed. In order to exploit the C_2-symmetry of HIV-protease, we have exclusively designed and synthesized symmetric inhibitors. The X-ray structure of the enzyme-inhibitor complex revealed that the endocyclic protonated amino functionality establishes strong electrostatic interactions as well as hydrogen bonds to the carboxylates of the aspartic residues present in the catalytic dyad. Starting from the initial lead which showed affinity in the low micromolar range, the activity of this new class of HIV protease inhibitors could be significantly optimized by means of rational structure-based design up to the two-digit nanomolar range for the final inhibitor.
Encouraged by these results, point mutation studies addressing drug-resistant variants were carried out. The novel pyrrolidine-based inhibitors also showed remarkable nanomolar affinity against these mutants. Overall, the efficient enantioselective synthesis, the unique binding mode, and the high affinity against mutants make this class of compounds promising candidates for the development of new anti-HIV drugs.

Design and *in silico* screening of combinatorial library of oseltamivir analogs inhibiting neuraminidase of avian influenza virus H5N1

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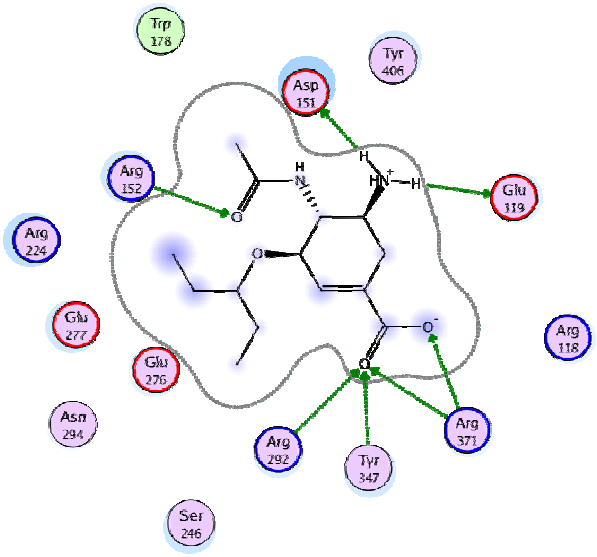
Neuraminidase is an important target for design of specific antiviral agents in prophylaxis and treatment of infections by avian influenza virus. We have applied computer-assisted combinatorial techniques to design, focus and *in silico* screen a virtual library of analogs of oseltamivir (Tamiflu), the clinically used inhibitor of viral neuraminidase subtype N1. A naturally occurring compound - shikimic acid - precursor of the synthesis of oseltamivir, was considered as the starting material. Influenza A neuraminidase N1 contains a cavity adjacent to the active site that closes upon ligand binding. A library of oseltamivir analogs was designed, focused and in silico screened with the goal to find new potent inhibitors of the neuraminidase that fill this cavity. The initial diverse virtual library of oseltamivir analogs was focused by fragment-based and analog-based methods. X-ray crystallographic structure of neuraminidase N1 complexed with oseltamivir was used in the structure-based focusing and virtual screening of the focused library. A target-specific PLP1 type scoring function fitted for a training set of 14 carbocyclic inhibitors of the N1 neuraminidase and validated on 3 other inhibitors was used to screen the library for virtual hits and rank order the analogs by predicted inhibitory activities. The analogs with predicted potencies higher than oseltamivir were analyzed in terms of the frequency of occurrence of individual building blocks. A highly focused virtual combinatorial subset was thus defined from the most successful blocks. This subset contains analogs with predicted inhibitory activities against N1 neuraminidase in the subnanomolar range. The results of this computational library design are useful as a rational guide for synthetic and medicinal chemists who are developing new drugs against the H5N1 avian influenza virus and its emerging drug-resistant forms.



SESSION 5:
Antibiotic Drug Discovery

Tuberculosis, nitric oxide and truncated hemoglobins: understanding the molecular basis of NO detoxification

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Mycobacterium tuberculosis, the causative agent of human tuberculosis, is forced into latency by nitric oxide produced by macrophages during infection. In response to nitrosative stress, *M. tuberculosis* has evolved a defense mechanism that relies on the oxygenated form of truncated hemoglobin N (trHbN), formally acting as NO-dioxygenase, yielding the harmless nitrate ion. This process is catalyzed very efficiently, as the enzyme activity is limited by ligand diffusion. X-ray crystal structures have shown that trHbN hosts a two-branched protein matrix tunnel system, proposed to control diatomic ligand migration to the heme. By using extended molecular dynamics simulations we have explored the mechanism that regulates ligand diffusion, the role played by residues that assist binding of O_2 and NO to the heme group, and the egression of the nitrate anion. Our results suggest that O_2 migration in deoxy-trHbN is restricted to a short branch of the tunnel, and that O_2 binding drives both local and global conformational and dynamical fluctuations that promote NO migration through the tunnel long branch. Access of NO to the heme cavity is dynamically regulated by the TyrB10-GlnE11 pair, which acts as a molecular switch that controls opening of the ligand diffusion tunnel controlled by an adjacent phenylalanine residue (PheE15). In addition, our results have shown that the nitrate anion escapes from the heme cavity through an egression pathway other than those used for the entry of reactants, and its release is promoted by hydration of the heme cavity. Overall, the results provide a detailed understanding of the molecular basis of the NO detoxification mechanism used by trHbN to guarantee an efficient NO detoxification and thus warrant survival of the microorganism under stress conditions.

Identification and Prioritization of Novel Inhibitors of *Mycobacterium tuberculosis* Thymidylate Kinase using Virtual screening, Molecular docking and Structure Interaction Fingerprints

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Tuberculosis is an increasing threat, owing to the spread of AIDS and to the development of resistance of the causative organism, *Mycobacterium tuberculosis*, to the currently available drugs. *Mycobacterium tuberculosis* Thymidylate Kinase or Thymidine Monophosphate Kinase (TMPKmt) is an important enzyme of the folate cycle; inhibition of TMPKmt inhibits growth and causes cell death. Therefore, the identification of selective inhibitors targeted against TMPKmt has become an attractive area of research. For the purpose of finding novel TMPKmt inhibitors and providing new idea for drug-design, we have employed an integrated pharmacophore and structure-based virtual screening using a small molecule compound database, subsequently followed by Structure Interaction Fingerprints (SIFt) generation to prioritize the virtual screening leads. In the process potential leads are identified after a thorough examination by a combination of methods: (i) visual examination of how well they dock into the TMPKmt binding site, and detailed analysis of their docking scores (ii) comparative investigation of the binding mode of the hits with that of the so far reported inhibitors and (iii) examination of how the hits retain interactions with the important amino acid residues of the TMPKmt binding site. In addition, clustering of the structure interaction fingerprints permits the easy separation of active from inactive binding modes. The structural models of the ligands in the TMPKmt binding site will facilitate further medicinal chemistry efforts in search and rational design of more potent anti-tubercular agents.

Inhibition of Glutamine Synthetase as a Potential Chemotherapy for *Mycobacterium Tuberculosis* Infection

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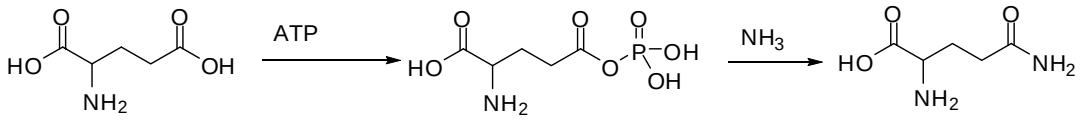
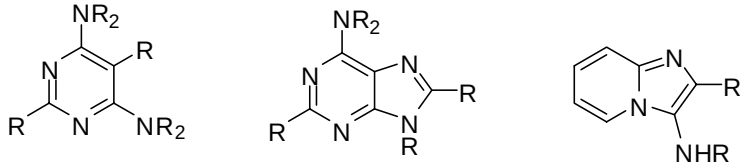
It would not be overstating the severity of the situation to state that emerging antibiotic resistance has the potential to become a threat to our current way of life.

As a result of the maturity of the current antibiotic classes and the resultant emergence of bacterial resistance, radically new approaches to antimicrobial chemotherapy are required. The inhibition of glutamine synthetase is one such approach, the enzyme being central to nitrogen metabolism in all cells. It is apparent that downward manipulation of glutamine levels will have a negative effect on messaging and DNA / RNA biosynthesis. A mechanism for differentiation between mammalian and bacterial enzymes is, however, critical to this approach.



The current work demonstrates that the bacterial enzyme is fundamentally different in that it changes ATP counter ion specificity dependent on metabolic flux through adenylation of a tyrosine residue. The adenylation of the enzyme results in alteration of the mechanism of phosphoryl transfer to glutamic acid and a change in the spatial characteristics of the ATP metal ion complex. This can be used as a basis for selective inhibition.

To this end, a collection of pyrimidines, purines and imidazopyridines were prepared. The potential for selective inhibition of either deadenylated or adenylated enzyme has been demonstrated. A subset of inhibitory compounds were submitted to the BACTEC assay against tuberculosis itself. A substantial number of these compounds proved inhibitory against the bacterium at levels below the cytotoxicity levels (CHO cells).



The compounds demonstrating inhibitory characteristics against drug sensitive TB were examined against two strains of excessively drug resistant (XDRTB) bacteria. There was strong evidence of strain independent inhibition of XDRTB.

SESSION 6:
Anticancer, Antifungal, Antialzheimer and other Drug Discovery

Targeted Therapy in Cancer: Myth or Reality?

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The burden of cancer in developing countries is impressive. According to WHO estimates, cancer killed 7.6 million people in 2005, three quarters of whom were in low and middle income countries.

Cancers share a restricted set of characteristics crucial to the tumor phenotype: proliferation in the absence of external growth stimuli, avoidance of apoptosis and no limits to replication, escape from external growth-suppressive forces and the immune response, an inflammatory micro-environment with new blood vessel formation, and an ability to invade normal tissues. In the last 20 years, the molecular determinants of these behaviors are becoming increasingly well understood. This has changed the current paradigm underlying diagnosis and therapy of cancer.

The "molecular target paradigm" represents the dominant approach to discover novel anti-cancer drugs. This paradigm is focused on the molecular determinants of aberrant cancer behavior. Conceptually, this paradigm starts with the identification and molecular characterization of proteins that are mutated or over-expressed in cancer cells, and that are believed to play a key role in cancer cell biology. High throughput screening and modern medicinal chemistry, along with sophisticated techniques like computational chemistry and modeling, lead to rapidly identifying hits and then leads that specifically and potently inhibit the activity of proteins mutated or over-expressed in cancer. Recombinant approaches have also been successfully used to generate molecular-targeted biologicals that specifically hit proteins aberrantly expressed in cancer cells.

The aim of the presentation will be to discuss the paradigm shift from empiricism to molecular-targeted therapies and the contribution of this new paradigm to the translation of basic molecular knowledge of cancer into new therapies for cancer patients.

Molecular Modeling Studies on AChE Inhibitor Carbamates to Design and Synthesize Antialzheimer Agents

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Virtual screening is gaining importance in the current scenario of drug discovery research. The development of new predictive models is the most important step in the process. In an attempt to develop a model for designing novel antialzheimer agents using AChE as the target, the systematic QSAR studies (CoMFA, advance CoMFA and CoMSIA) have been carried out on a series of carbamates as AChE inhibitors. The total set of 78 molecules was divided into training and test sets of 52 and 26 molecules, respectively.

Statistically significant 3D QSAR models were developed on training set molecules using CoMFA and CoMSIA and validated against test set compounds. The highly predictive models (CoMFA q^2=0.733, r^2=0.967, predictive r^2=0.732, CoMSIA q^2=0.641, r^2=0.936, predictive r^2=0.812) well explained the variance in binding affinities both for the training and the test set compounds. The generated models suggest that steric, electrostatic and hydrophobic interactions play an important role in describing the variation in binding affinity. In particular the carbamoyl nitrogen should be more electropositive, substitutions on this nitrogen should have high steric bulk and hydrophobicity while the amino nitrogen should be electronegative in order to have better activity. These studies have provided important insights into structural variations leading to the development of novel AChE inhibitors which may be useful in the development of novel molecules for the treatment of Alzheimer's disease.

DESIGN, SYNTHESIS AND EVALUATION OF NEW GLYCOSIDASE INHIBITORS

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Glycosidases are the enzymes that catalyze glycosyl transfer reactions that assemble, trim and shape bioactive glycoprotein and glycolipid conjugates. These enzymes play a fundamental role in biochemistry and metabolism of carbohydrates. They are believed to act through protonation of the anomeric hydroxyl moiety involving a flattened, half-chair oxocarbenium ion intermediate. Therefore, designing of a chemical entity which on protonation could mimic either the charge or shape (or both) of the transition state can act as a reversible inhibitor of that particular substrate and these entities are termed as glycosidase inhibitors. Developments of such chemical entities are emerging as potential drugs for the treatment of carbohydrate mediated diseases such as diabetes, cancer, HIV and influenza.

Carbohydrate analogues in which one or more of the oxygen atoms have been replaced by nitrogen, also known as “aza-sugars”, have emerged as important glycosidase inhibitors recently. As a part of our broad research program aimed at the design, synthesis and evaluation of new glycosidase inhibitors of the azasugar class, we have developed a new synthetic strategy for the construction of several azasugars and their biological activities are evaluated. Conformationally restricted azasugars and glyconolactones have also been designed and synthesized and evaluated as specific inhibitors. Details of the concept, design, synthesis and evaluation of several glycosidase inhibitors will be discussed.

Quantitative Structure Activity Relationship (QSAR) Study of Natural Estrogen-Like Isoflavonoids from Thai Medicinal Plants

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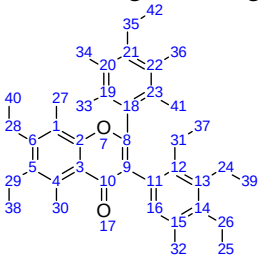
In searching for new phytoestrogens from Thai medicinal plants, *Dalbergia parviflora* R. (Leguminosae) and *Belamcanda chinensis* L. (Iridaceae) were found to contain a number of potential compounds with estrogenic activities. Seventy compounds were isolated from the heartwood of *D. parviflora*, used traditionally as a blood tonic to normalize menstruation in Thais, and sixteen compounds were from the rhizome part of *B. chinensis* which has been used for the regulation of menstrual disorders. The isolates were all characterized for their structures by spectroscopic methods and assessed for their estrogen-like activity by using breast cancer cell lines of MCF-7 and T-47D. Subsequently, this set of estrogenic molecules was studied by means of both Quantitative Structure-Activity Relationships (QSAR) and *in silico* modeling of receptor binding, based on the proliferation assays expressed as equivalent concentrations causing 10% increase in the cell growth (EqE_{10}).

From the 2D-QSAR analysis, the genetic function approximation (GFA) algorithm of Cerius[2] could generate optimized set of QSAR correlation equations. Twenty five best equations were further analyzed by removal of outliers and leave-one-out cross validation and 6 best QSAR models for the MCF-7 biological activity were recorded. It was found that that the estrogenic potencies of the studied compounds depended mainly upon the presence/absence of hydroxyl groups attached to the carbons C_{13} and which is reflected by the magnitudes of the atomic charges at these positions as well as the distance between the hydroxyl groups at the positions C_6 and C_{14}.

For the approach of *in silico* modeling of estrogen receptor binding, all the molecules with estrogenic activities measured in MCF7 and T47D cells proliferation assays were docked into human α and β estrogen receptors (ER_α and ER_β) using the LigFit tool of the Cerius[2]. It was found that, out of 9 scoring functions available in the virtual screening module of Cerius[2], the LigScore function led to the best correlation between receptor affinities representing simultaneous binding to both ER_α and ER_β receptors and the estrogenic activities measured in MCF7 and T47D cells. Descriptors $LigScoreER_\alpha$ and $LigScoreER_\beta$ considered independently did not lead to statistically significant QSAR models. Thus, it was suggested that simultaneous and possibly competitive interaction of the estrogenic compounds with the ER_α and ER_β receptors, in which the molecular size and presence of hydroxyl groups at positions 6 and 13, 14, 15 of the molecular scaffold play a dominant role, might represent the key mechanism determining the estrogenic potency of phytochemicals.



The structure of estrogenic compounds with atomic position numbering of the estrogen core and essential substituents.

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Combinatorial approaches in cancer early detection

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With the anticipated growth in cancer cases as population's age, the early detection has become critical and represents a key driving force to improve and expand health services. Availability of efficient and cost effective screening devices for cancer early detection are urgently needed to improve quality of life. Mass screening for early detection of cancer will reduce the ever increasing social costs associated to handling patients with diseases which are at advanced stage for failure of existing diagnostic procedures or non adequateness for broad use. One of the most important factors in the survival of cancer is detection at an early stage, and tumour biomarkers are important molecular signatures of the phenotype of a cell that aid in early cancer detection and risk assessment. Many conventional biomarkers have been found overexpressed in neoplastic tissues and some are used for diagnostic application. However, mainly due to the cancer heterogeneity, no single marker has shown to be fully satisfactory in terms of sensibility and specificity for early cancer detection. This still unmet medical need could be fulfilled by combining non overlapping biomarkers and developing nanosized technological platforms for their simultaneous detection, with adequate sensitivity and specificity for clinical use at significantly lower costs per assay than traditional methods. Xeptagen is exploiting a novel class of cancer biomarkers (biomarker-IgM immune-complexes **Cancer Epid Biomarkers** 2007;31:402, **Clin Chim Acta.** 2007 383:147, **Int J Cancer** 2006 119:735, **Int J Cancer** 2005 117:579 **Cancer** 2005 103:2558, **Int J Biol Markers**. 2005 20:204) for the generation of nanosensing devices for the simultaneous detection of different types of neoplastic diseases at the same time in order to achieve an early diagnosis and monitoring of disease progression so as to increase the possibilities and effectiveness of the existing therapies. This novel class of biomarkers has shown higher diagnostic accuracy than conventional biomarkers in detecting cancer at the early stage and with a self-built capability to amplify detection which makes biochip fabrication easier, cheaper and faster. Our goal is to develop diagnostic devices that will make the identification of many cancers possible within minutes and directly at the point-of-care, thus doing away with the conventional techniques, where samples are sent to a laboratory and put through labour-intensive processes that may take several hours to achieve a result. Detailed results related to the design, development and clinical validation of a biochip for hepatocellular carcinoma will be presented, with particular attention to the deposition technique of the antibody probes, Thermal Ink Jet printing, which was successfully employed to realize the multispecies spot array.

Computer Aided Drug Design and Discovery Studies of Antitumoral Active Heterocyclic Compounds in Turkey

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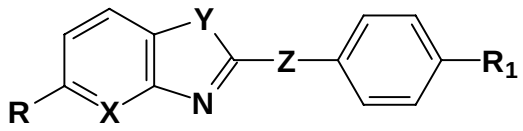
Drug design studies in Turkey has been studied by using the computer aided rational drug design methods including quantitative structure-activity relationships and molecular modelling studies in the recent years.

Our group have been working on the rational design of antitumoral active new heterocyclic compounds possessing benzoxazole, benzimidazole, benzothiazole and oxazolopyridine fused ring systems in their structures by testing their DNA Topoisomerase I and II inhibitory activities.

Some of our studies include the COMFA (Comparative Molecular Field Analysis) and CoMSIA methods as the 3D-QSAR application for the lead optimisation to the training set of compounds given in Figure 1 using the Sybyl 6.6 Software in SGI workstation.

We have been also working with the three-dimensional pharmacophore hypotheses generated by program Catalyst/HipHop from Accelrys in SGI workstation. Molecules shown at Figure 1 were edited using the Catalyst 2D/3D visualizer which Catalyst automatically generated conformational models for each compound using the Poling Algortihm.



R = H, Cl, CH_3, NO_2, NH_2, $COOCH_3$

$\mathbf{R_1}$= H, Cl, Br, F, NO_2, NH_2, CH_3, C_2H_5, $C(CH_3)_3$ OCH_3, $NHCH_3$, $NHCOCH_3$

X = CH, N

Y = O, NH, S

Z = –, CH_2, C_2H_4, CH_2O, CH_2S, CH_2NH

Figure 1

The 3D-QSAR analysis results obtained from the computer aided methods like CoMFA, CoMSIA and pharmacophore analysis have been discussed.

SESSION 7:
Computational Methods in Drug Discovery

Molecular Mechanisms of Antibiotic Resistance Investigated by Molecular Simulation

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Multiscale Molecular Strategies for Drug Delivery

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Nanotechnology, as generally accepted, is concerned with the structures, properties, and processes involving materials having organizational features on the spatial scale of 1-300 nm. At this scale, devices may lead to dramatically enhanced performance, sensitivity, and reliability with considerably decreased size, weight, and cost. Indeed, these scales can lead to new phenomena, providing opportunities for new levels of sensing, manipulation, and control. However, being much smaller than the wavelength of visible light, but much larger than simple molecules, it is difficult to characterize the structure and to control the processes involving nanomaterials. And because it is difficult to see what we are doing at the nanoscale, it is essential to develop theoretical and computational approaches sufficiently fast and accurate that the structure and property of the materials can be predicted for various conditions as a function of time. A particular advantage of using theory is that the properties of new materials can be predicted in advance of experiments. This allows the system to be adjusted and refined (or designed) so as to obtain the optimal properties before the arduous experimental task of synthesis and characterization. However, there are significant challenges in using theory to predict accurate properties for nanoscale materials, especially when biomacromolecules are involved. Indeed, despite the tremendous advances made in the modeling of the structural, thermal, mechanical and transport properties of materials at the macroscopic level (i.e., finite element analysis of complex structures or continuum simulations), there remains a remarkable uncertainty about how to predict many critical properties related to final performance. The main problem lies in the fact that most of these properties depend on the interactions and chemistry taking place at the atomic level, involving electronic and atomic descriptions at the level of nanometers in the length scale, and picoseconds in the timescale. Conversely, the material designer needs answers from macroscopic modeling (the "finite element paradigm") of components having scales of the order of centimeters, and of phenomena taking place in a time range of milliseconds or much larger. Thus, to achieve a dramatic advancement in the skill of designing innovative, highly-performing materials, it is mandatory that we link the chemistry (micro) to the macroscopic (finite elements or continuum) modeling.

The actual computational modeling of biological macromolecules, mainly based on molecular dynamics (MD) simulations, commonly revolves around structure representations in atomic or near-atomic detail, with a classical description of physical interactions. Such models have been quite successful in complementing experimental data with structural, dynamic, and energetic information, but involve substantial computational resources for larger systems, or when long time scales have to be considered. In particular, structure-activity calculation applications, the formation and interaction of supramolecular assemblies, and the prediction of kinetic and transport phenomena become prohibitive, if feasible at all, with models at atomic details. Thus, we need to develop some computational strategy to link the atomic length and time scales of MD and the macroscopic length and time scales (microns to mm and □s to s) of finite element analysis (FEA). Only by establishing this connection from microscale to macroscale it is possible to build first principles methods for describing the properties of new materials and systems. Our aim is to reach the domain of materials science and engineering by building from fundamental principles of physics and chemistry. Thus, for fundamental predictions to play a direct role in materials innovation and design, it is mandatory to bridge the micro-macro gap.

Considering the dimensions of the biomolecules and the relaxation characteristic time of bio phenomena and compared with the dimension of nanomaterials, it turns out that a list of potential drug nanocarriers can be prepared. Micelles, vesicles, multifunctional dendritic polymers, bioconjugates, nanocapsules and nanospheres are potential drug carriers for controlled drug release. In this scenario, we will discuss two important examples in which we tried to fill this gap in the case of controlled drug delivery: (i) the study and design of nanocarriers based on polymers for targeted-drug delivery and (ii) the controlled-release of proteins from silicon membrane nanochannels.

From mathematical chemistry to drug design

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The complexity of biological systems is overpowering. To attempt to follow, in details, the interaction between a drug (a microsystem) and an organism (a macrosystem) is very difficult, if not an unachievable task. Modelling of some sort is therefore essential in drug research. There are many different modeling approaches to this problem. We will describe one approach to model the bioactivity of a drug (a chemical). This will be the QSAR modelling. QSAR stands for quantitative structure-activity relationship. The QSAR models are based on the empirical observation that these exists a connection between the strcture of a molecule and its biological response. We will outline one approach to building up the a QSAR model. QSAR models are usually constructed for sets of structurally related molecules whose bioactivities are ordinarily recorded as single numbers. This requires that the structure of a molecule must also be numerically encoded in some way. Two sets of numbers are then for example statistically analysed by way of a suitable algebraic expression. The final QSAR model is thus a regression model and one has to be careful about its statistical stability.

There are a number of QSAR models available in the literature. We will present the classification of the QSAR models, based on the method of encoding the structure of molecules, into four groups: (i) empirical models, (ii) quantum-chemical models, (iii) non-empirical models and (iv) computer-graphics models. Here we will be concerned with the non-empirical models based on the use of molecular descriptors derived using mathematical techniques in the framework of (chemical) graph theory, which is nowadays a very active part of mathematical chemistry. To illustrate the discussed approach, we will present the use of the procedure based on molecular descriptors to predict the ability of flavone derivatives to inhibit cAMP phospodiesterase

SESSION 8:
Computational Methods in Drug Discovery II

Exploring complex protein-ligand recognition mechanisms with coarse metadynamics

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The Metadynamics method has been shown to be a valuable tool to study the mechanism of molecular recognition in atomistic detail [1-4]. However it requires an a priori knowledge of all slow degrees of freedom relevant to the docking/undocking mechanism and its computational cost scales exponentially with their number.
Recently we have investigated a combination of docking/clustering with a metadynamics performed with a sub-set of the necessary degrees of freedom (coarse metadynamics) [5].
The proposed protocol clearly enhances the predictive power of standard docking techniques joined to geometrical cluster analysis. At the same time it is computationally cheaper with respect to the plethora of enhanced sampling techniques nowadays available. Moreover, the applied procedure intrinsically provides the full putative undocking mechanism, including the TS structure. This knowledge could be used to optimize the ligand to increase its affinity and/or selectivity toward a certain biological counterpart.

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The two faces of systems biology

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Genome biology and high throughput methods created an intense need for a unified conceptual framework for the biomedical sciences, that allows one to approach diverse data. This has led to the foundation of systems biology, which is a broad conceptual paradigm, rather then a set of methods of tools.

Complex systems consist of units or components of known behaviour Typically, complex systems contain a large number or components belonging to relatively few types, some of the components serve as input units, where data, information, energy or relevant material substances enter the system from the environment; other components serve as output units, where data etc. leave the system and may have an influence on the environment. Input and output units make up the interface between the system and its environment. The components interact along certain pathways or links which leads to a structured flow or transport of data, information, energy or material substances within the system. The connectivity structure or topology is a key factor and its changes are often interpreted as part of adaptation, or "learning" of the system.

All complex systems exhibit the phenomenon of emergence, the appearance of new features that could not have been predicted from the the individual components or interaction types. Systems have an inherent tendency to adapt, to optimise or at least to improve their behaviour with respect to external conditions. This is the evolution of the system. System biology today has to approaches:

- A *bottom-up* approach starts from the basic components and their interactions, and develops methods, models and theories in order to predict the behaviour of the entire system. Lab research is often based on explicit methods, an numercal methods are used to the best models to high throughput data
- A *top-down* or *holistic* approach starts from the phenomenological behaviour of the entire system and develops models that can reproduce the system's behaviour in terms of subsystems and interactions

Biological systems have intriguing properties, such as robustness, modularity, feedback loops and topological modules that makes them highly evolvable yet resilient to attcks in complex environments.

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Virtual and Experimental High Throughput Screening Are Complementary

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Virtual screening (VS) is usually considered as "in silico" analogue of in vitro HTS. Both technologies require the selection of the target, the development of the assay/protocol and the definition of the screening library in a suitable physical/virtual format. Hits are typically identified in a highly automated process generating huge amounts of data that should be stored, treated and analyzed. HTS and VS have also a common conceptual framework having the limited accuracy that is compensated by the number of compounds investigated. In addition to true actives and inactives both high throughput technologies surely miss some active compounds (false negatives) but pick up inactives (false positives).
Recent efforts on the integration of these technologies involve (i) sequential, (ii) iterative, (iii) fully integrated, (iv) parallel, and (v) independent screening strategies. There is only limited experience with the first three strategy. In most application HTS and VS are performed independently using distinct physical and virtual compound collections. Since missed and false hits have a serious impact on the applicability domain of these technologies we investigate them comparatively using the strictly parallel strategy using the very same set of compounds for both experimental and virtual screening.
Our goal was to analyze the screening results of several libraries obtained both in silico and in vitro on three different kinase targets. Similar enrichment observed for HTS and VS suggests that virtual screening protocols could identify active ligands from large libraries even in real screening situations. We found that the hit rates in VS are typically higher than that obtained by HTS. Direct comparison of complete hit lists and clusters of hits revealed that the overlap between the results of the two approaches is limited but acceptable. The large number of false positives and false negatives in virtual screening, however, suggests VS to be a complementary rather than competitive approach to HTS.

Concerns, Recent Outbreak and Molecular Insight into Avian Influenza H5N1

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Avian influenza virus A (H5N1) has resulted disease outbreaks throughout the world, causing a large-scale death in poultry, and the subsequent infection and death of over 200 humans. An important and growing problem of resistance to available anti-influenza drugs, oseltamivir and adamantane analogs, have called the need for understanding in details how drug interacts with its enzyme target. Molecular basis of drug binding is the primary information needed to design and develop more potent inhibitors against both wild-type and mutant influenza strains. This study aims at gaining detailed information insight into molecular mechanisms of action of three drug targets of the life cycle of influenza virus H5N1: hemagglutinin (HA), M2 ion channel (M2) and neuraminidase (NA), using molecular dynamics simulations.

At HA target, interest is focused on the high pathogenicity (HP) of the H5 due to the –RRRKK– insertion. The different cleavage loops of HA bound to furin were examined. Only the cleavage loop of HP-H5 was found to bind strongly to the furin cavity, serving as a conformation suitable for the proteolytic reaction. This provides a clear answer to the question of why inserted H5 is better cleaved by furin, explaining the high pathogenicity of avian influenza H5N1. Then, the reaction mechanism of acylation in furin was investigated using QM/MM methods. First step of acylation was found to be concerted reaction with a formation of tetrahedral intermediate.

The second target, the M2 protein complexed with/without inhibitor in a fully hydrated lipid bilayer was studied to understand of how drugs inhibit the proton transport in channel. Five different protonation states of the selectivity filter residue, His37, were taken into account. Less water was found in the rimantadine-M2 complexes indicating that water was considerably better inhibited by rimantadine than amantadine, agreed well with the IC_{50}^{exp} values. Moreover, amantadine resistance to the M2 channel due to the single mutations (A30T, S31N and L26I) was studied. The data suggested that resistance toward amantadine is likely caused by a decrease in the binding of amantadine to His37 according to an increase in binding to water.

For NA, the rotation of the –NHAc and $–OCHEt_2$ groups of oseltamivir, leading directly to rearrangement of the catalytic cavity, was found to be a primary source of the lower susceptibility of oseltamivir to N1 than N2 and N9. In addition, three inhibitiors (oseltamivir, zanamivir and peramivir) bound to N1 were studied to understand the drug-target interactions. The structural properties, position and conformation of peramivir and its side chains are uniformly preferential, *i.e.*, its conformation fits very well with the N1 active site and, thus, it shows the highest binding free energy to N1. Additionally, we attempted to obtain the molecular details on the oseltamivir resistance to H274Y N1 mutant. Reduction of the hydrophobicity and size of pocket in the area around an ethyl moiety at the bulky group of oseltamivir were found to be the source of the resistance. These changes were primarily due to the dramatic rotation of the hydrophilic $–COO^-$ group of Glu276 toward the ethyl moiety. The finding is in contrast to what previously proposed.

Knowing new zeolite-drugs interactions by computational studies

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Medical and pharmaceutical applications of natural zeolites have enjoyed considerable attention over the last decade. It is known that purified natural clinoptilolite from Tasajeras deposit in Cuba, NZ, does not cause damage to humans [1,2]. It has been used as raw material in the pharmaceutical industry for the therapy of some pathologies, both in animals and in humans, using different pharmaceutical forms [2]. The possible interaction between organic molecules of pharmaceutical interest and clinoptilolite has been studied using experimental and theoretical tools [3-5]. Aspirin, metronidazole and sulfamethoxazole has been the evaluated drugs based on their wide use and because they produce side effects associated with gastrointestinal disturbances [6,7], which could be attenuated by the clinoptilolite. Regarding experimental studies, the results suggest a poor adsorption of these drugs on the natural clinoptilolite and their ion exchanged modified forms in good agreement with the calculations [4,5]. In order to develop drug support systems for slow release, a new potential application of purified natural clinoptilolite has been explored, which involves the modification of the zeolite surface with surfactants [8]. The obtained composites can co-adsorb organic molecules, as sulfamethoxazole and metronidazole, due to the variation of the hydrophilic character of the zeolite.

In the present work, different combined systems formed by surfactants, drugs, water and a clinoptilolite channel model have been studied using semiempirical calculations. We modelled the interaction of each organic molecule with the external surface of clinoptilolite model. The results indicate that the cationic surfactant is well adsorbed on the clinoptilolite model unlike the anionic surfactant. The most polar drug, metronidazole, is the best adsorbed on the zeolite model, followed by aspirin and sulfamethoxazole. Taking into account this fact, we also model another system formed by surfactant–drug–water (S–D–W) in order to reproduce the interaction of the different drugs with a cationic surfactant in solution and to evaluate the role of the surfactant in the drug adsorption process. In this system the order of the drug adsorption is opposite to that obtained for the zeolite alone: the adsorption of hydrophobic molecules like sulfamethoxazole is favoured. Moreover, for aspirin and sulfamethoxazole the adsorption enthalpies are higher in the S–D–W system. This fact suggests that the presence of surfactant on the external surface of clinoptilolite could improve the adsorption of some drugs on this zeolite.

However, the interaction of the zeolite with cationic surfactant and drugs is very complex and involves many different particles. As first step in some complex system, we have modelled the aggregation process of the cationic surfactant benzalkoniumchloride (BC-R12) in water using molecular dynamics in order to know the size and shape of the micelles. The energy of micelles with different aggregation number (Nagg) in water have been calculated and compared with the analogue system where molecules are randomly distributed. Our result suggest that micelles are more stable than the random systems and the smallest micelle, Nagg=6, is the most stable. At 5ps, micelles of intermediate size are reorganized and appear smaller aggregates formed by 7 or 10 molecules. We find that the alkyl chain of the BC-R12 molecule folding back on itself; this effect was observed both in the random and micelle distributions.

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SESSION 8:
From Traditional Natural Medicine to New Generation Drugs

Finding combinatorial drugs from traditional medicines

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To overcome the "more investments, less drugs" challenge in drug development, more and more attention is paid to multicomponent therapeutics, which attempts to incorporate two or more active ingredients (most of which are clinically used drugs) in one capsule to hit the multiple targets implicated in the complex diseases. This drug discovery strategy will take many advantages over the prevalent single-component paradigm. However, to implement the new strategy, we still have to cope with some challenges, such as the explosive increase of drug combination quantities, the unpredictable pharmacokinetic properties of multiple components and the potential risks of drug-drug interactions. In spite of the rapid technical progresses in high-throughput screening, high-content screening and systems biology and various "omics", we still need a long time to perfect the related techniques. Since traditional medicines, in particular traditional Chinese medicine (TCM), have accumulated rich experiences in combinatorial use of natural medicines (for instance, more than 100,000 formulae have been documented in TCM). We speculate that maybe we can start with traditional medicines to find modern drug combinations. This tactic is preliminarily supported by the finding that a certain part of TCM components have counterparts of modern Western drugs or candidates and the synergistic effects of some TCM formulae can be understood in terms of the Western-medicine-justified activities. In addition, starting with traditional medicines will take the advantages of controlling the pharmacokinetics and drug-drug interactions of multiple components, because most combinatorial modes of TCM combinations have been used clinically for hundreds years and by thousands of patients (if not millions). To further evaluate the potential of this tactic, we will focus on anti-dementia TCM formulae to examine whether some clues can be derived from these prescriptions to help find anti-dementia combinatorial drugs.

Acknowledgements
This study was supported by the National Basic Research Program of China (grant 2003CB114400) and the National Natural Science Foundation of China (grant 30570383).

Nature Based Drug Discovery System (NADI) and Its Application to Avian Flu Chemotherapeutic Design

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http://www.pha.usm.my

It is a well known fact that 63% of drugs in the market today originally derived from natural sources. At one stage of time, many pharmaceutical companies felt that it was too costly to screen plants for useful drugs given the low success rate, especially once advances in science had made it easier and cheaper to design synthetic drugs which are more easily patentable. However, in recent years, the pharmaceutical industry's productivity continues to be declining, the state of affairs incidentally coincide with the industry embracing exciting new technologies such as computational and combinatorial chemistry as well as bioinformatics techniques.

The development of bioactive compounds from natural products in drug discovery involves screening of natural products derived from plants and microorganisms and testing them for activity in animal models. This, however, is a slow and labour-intensive process. In this talk, I will be presenting a concept in drug discovery that combined the theoretical methods of computational chemistry and natural product research to discover new drug entities. A new integrated system, NADI which means "pulse of life" in Malay language is a Natural Based Drug Discovery Intelligent resource that has been developed with intended aim as a one-stop center for *in silico* drug discovery from natural products. It consists of NADI-CHEM, a Natural Product 3D Chemical Structure Database, which has many cheminformatics features such as structure and sub-structure search as well as a neural network based machine learning program to distinguish drugs from non-drugs. In addition to this, a 3D Drug Receptor, NADI-RA Database, and Herbal Monograph, NADI-Herb are also included to provide extensive information for natural-based drug discovery. NADI is also complemented with NAPIMM (Natural Product Molecular Modelling System) that could undertake virtual high throughput screening at a rapid rate through utilization of Grid computing.

NaDI's application in natural product drug discovery will also be demonstrated through our current study of avian flu and the design of avian nueraminidase inhibitors. The study involved virtual screening of 3500 compounds from NADI using NAPIMM (docking and redocking of MD snapshots of neuraminidase) which was used as a based for generating pharmacophore hypothesis or active molecules. It is hoped that a comprehensive utilisation of NADI/NAPIMM would identify natural products with novel indications, which could be developed into novel lead compounds which eventually could be further developed as therapeutic agent for the disease.

Indian Ayurvedic System of Medicine: Scope for New Drug Design and Discovery

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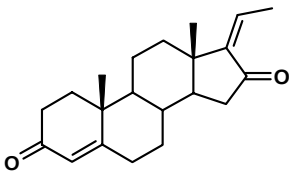
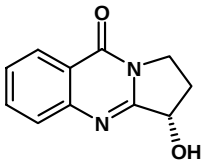
Ayurveda is an ancient science which deals with the healing of the human body and mind to achieve optimum balance between the body, mind and consciousness. Specific plant species of Indian origin have been used in Indian ayurvedic system of medicine. We strongly believe that a systematic scientific study of those plant extracts using modern techniques will provide an avenue for new drug discovery. Following are the representative bioactive compounds isolated from the plant species used in Indian ayurvedic system of medicine. A concise account of Indian ayurvedic system of medicine and its scope for new drug development will be presented with the help of case studies.



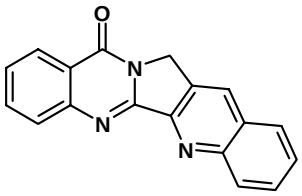
Z-Guggulsterone

(Cholesterol lowering agent)



(-)-Vasicinone

(Anti-asthma)



Luotonin A

(Human DNA topoisomerase inhibitor)

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Lessons learned from plants-based drug discovery in Madagascar

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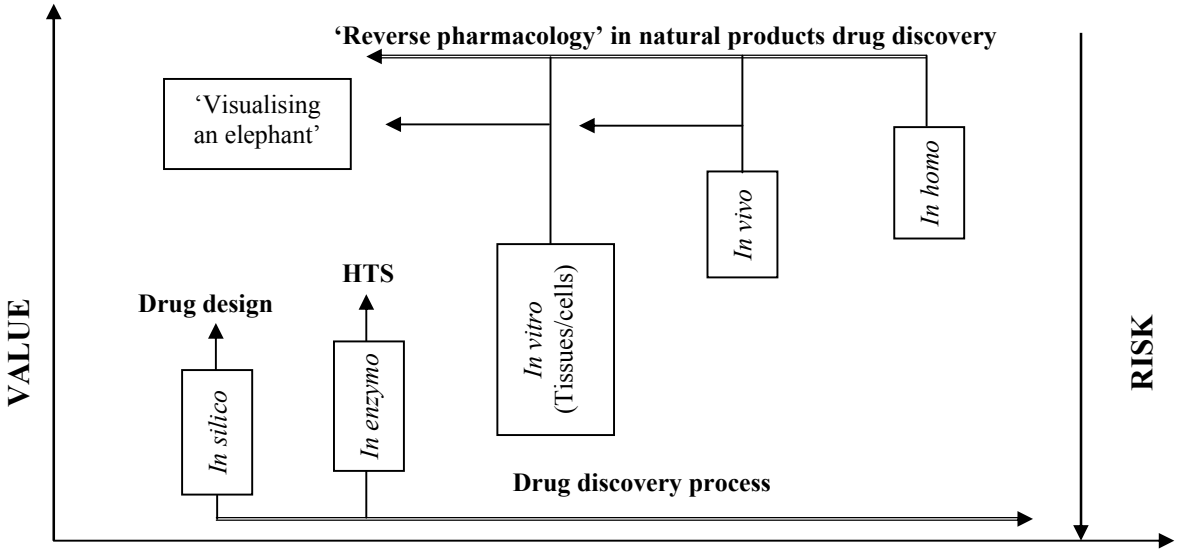
We currently use two approaches to select plants for the search of biologically active compounds. The current approach of our Institute is based on the deeply dependant relationship and interactions between plants and the whole related ecosystem comprising humans. Bioactive compounds that have arisen from this approach include the malaria chemosensitizers malagashanine and hervelines A-D, as well as tazopsine which was found to be selectively active in the malaria liver stages (1). The second approach as required by Western partners is random collecting in which plants are collected in what ever is available within a certain biodiversity-rich area to generate a large number which may provide a great chemical diversity. Lessons learned form these programmes include: (i) ethnobotany-based collecting *versus* biodiversity-based collecting, (ii) single active compounds *versus* multi-component extracts, (iii) one single solvent extract *versus* several solvents/pre-fractionated extracts, (iv) choice of test concentrations, (v) *in vitro* or mechanism-based screening *versus in vivo* screening/*in homo* observational studies, (vi) effect of plant processing on the biological activities, (vii) known bioactive compounds, what next? Particularly, why there are many medicinal plants used to treat malaria all over the world, and so far, only two drugs have emerged, quinine and artemisinin (2)? We have proposed a new scheme outlined in the figure below to validate the ethnomedical knowledge. At this point, we borrowed the terms 'reverse pharmacology' (3) to name this scheme and apply it to natural products drug discovery. This scheme is driven by the cross-pollination and recombination of ideas and experiences to generate new possibilities.



Relevant results will be presented.

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Modern High Throughput Technologies in Drug Design and Discovery: Solutions and Trends for Developing Countries

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In this presentation the concepts of Combinatorial Chemistry and Technologies, High Throughput Screening and Molecular Design (CC/CT/HTS/MD) will be theoretically described, commenting upon various terms and methodologies, and upon their usefulness. Particular attention will be given to the evolution of high throughput, modern approaches in the last 15 years and to the different trends which have emerged, or disappeared, in the past decades. Currently popular approaches will be privileged, and will be illustrated with the help of examples highlighting their usefulness and cost-effectiveness in the Drug Discovery process; and a set of suggestions for laboratories in developing countries entering the CC/CT/HTS/MD field will be formulated.

Namely, we will discuss the use of virtual high throughput screening (HTS) in the identification of novel, drug-like and patentable hits and leads, using either the target-based and/or the ligand-based design approaches; the use of fragment screening, including biophysical/structural approaches such as X-ray, NMR and surface plasmon resonance (SPR) to high ligand efficiency hits and leads; the use of an innovative scaffold hopping approach to rapidly diversify and improve the drug-like character of hits and leads, while also generating IP-protectable novel compounds; and the results of a metrics process applied to lead discovery by a major pharmaceutical company. All these examples will be analyzed with an eye to the best possible solutions for developing countries, and synergies among these and various other emerging trends will be discussed.

POSTERS

SESSION I

I-1

Computational Approaches for Analyzing the Interaction of Renieramycins and DNA

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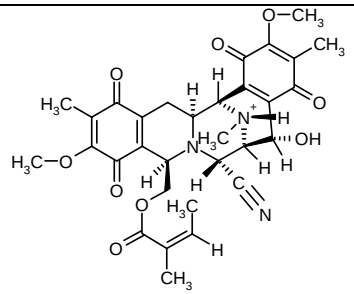
Renieramycins (RM) are potential anticancer agents consisting of tetrahydroisoquinoline subunits and an active α-carbinolamine (N-C-OH) group. The activity of RM is associated with covalent adduct formation between C21 of RM and N2 of guanine in the minor groove of a DNA duplex. This reaction occurs specifically at a d(TGA) sequence. We hypothesize that the adduct is preceded by formation of a high affinity non-covalent complex. Therefore, in this work, the structures of non-covalent renieramycin O (RMO)-DNA complexes were investigated using molecular modeling. Initial structures of RMO-DNA complexes with different RMO binding orientations were generated using NASDAC 1.01 and energy minimized using AMBER 8.0. The results indicated two energetically favorable RMO-DNA complexes (Figure 1) with a distance between C21 of RMO and N2 of guanine of about 4 Å. This suggests that these complexes are non-covalent precursors to the bioactive covalent adducts. Therefore, the predicted RMO-DNA complexes may contribute to the design of RM derivatives with higher DNA affinity and more potent anticancer activity.



Renieramycin O (RMO)

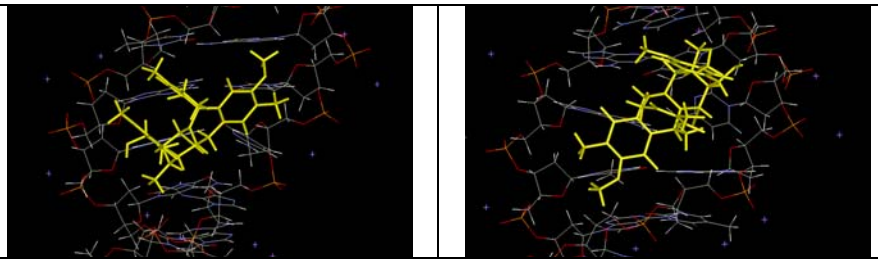


Figure 1

I-2

Iminosugars as Glycosidase Inhibitors: Synthesis and Biological Study

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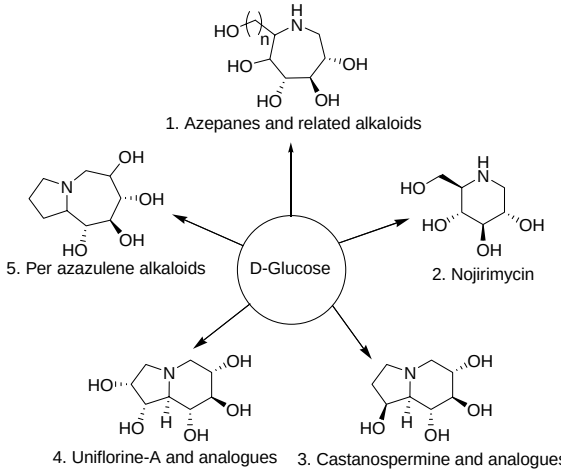
Glycosidase is a class of enzymes that modify glycoconjugates by hydrolyzing glycosidic linkages - a process essential for normal cell growth, regulation and development. Any disorder in this process leads to genetic diseases such as diabetics, obesity and also viral infection including AIDS. The iminosugars being reversible competitive glycosidase inhibitors can neutralize the disorder by binding on to the specific enzymes and thereby can act as potential therapeutic agents in the treatment of glycosidase mediated diseases. In recent years, attention has been increasingly focused on the structure-activity relationship of iminosugars, in particular in seven membered hydroxy-azepanes **1**, six membered piperidine alkaloids namely nojirimycin **2** and indolizidine alkaloids such as castanospermine **3**, Uniflorine-A **4**, and per azazulene alkaloids **5**. Azepanes are also potentially useful as DNA minor groove binding ligands (MGBL). The hydroxyl groups in azepanes adopt different conformations due to the flexibility of the seven membered ring (compared with five or six-membered rings) thereby increasing the probability of forming hydrogen bonds with nitrogen base, thus showing the ability to point into the minor groove of DNA. The development of new azasugars thus opened a dynamic research field at the interface between glycobiology and synthetic organic chemistry. During the course of our investigations in the area of iminosugars, we have reported syntheses and glycosidase inhibitory activities of a number of nojirimycin, castanospermine and azepane analogues. Our recent results in this area will be presented.



(a) K S. Ajish Kumar, Vinod D Chaudhari, and Dilip D. Dhavale**;** *Organic and Biomolecular Chemistry,* **2008, *1*,** 703. (b) Vinod P. Vyavahare, Chaitali Chakraborty, Viswanath Maity, Subrata Chattopadhyay, Vedavcati G. Puranik, and Dilip D. Dhavale**;** *Journal of Medicinal Chemistry,* **2007,** *50,* 5519-5523. (c) K. S. Ajish Kumar, Vinod D. Chaudhari, Vedavati. G. Puranik, Dilip D. Dhavale; *European Journal of Organic Chemistry*, **2007**, 4895. (d) Omprakash P. Bande, Vrushali H. Jadhav, Vedavati G. Puranik, and Dilip D. Dhavale* *Tetrahedron: Asymmetry,* **2007**, *18,* 1176-1182. (e) Karanjule, N. S.; Markad, S. D.; Dhavale. D. D.; *J. Org. Chem*, **2006**, *71*, 6273-6276 (f) Markad, S. D.; Karanjule, N. S.; Sharma, T.; Sabharwal, S. G.; Dhavale, D. D.; *Organic and Biomolecular Chemistry*, **2006**, *4*, 2549-2555 and references cited therein.

I-3

A QSAR Study of 2-arylamino-4-cyclohexylmethoxy-5-nitroso-6-aminopyrimidine inhibitors of cyclin-dependent kinase 2

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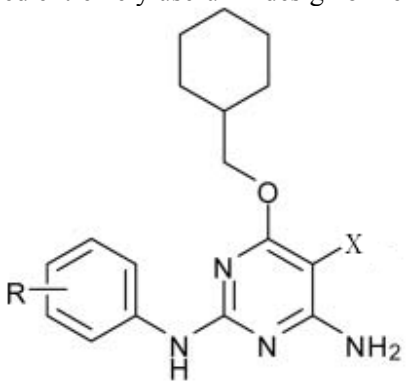
The cyclin-dependent kinases (CDKs) are a group of serine/threonine kinases involved in the regulation of the cell cycle [1]. Binding of a cyclin with its associated kinase activates the cell from one phase of the cell cycle to another. Overactive cyclins or CDKs have been associated with several cancers. Thus, kinases have increasingly become important targets and the hunt for kinase inhibitors has captured a great deal of attention in drug discovery over the years. The QSAR (quantitative structure-activity relationship) approach has proved extremely useful in design of new drugs.



(1)

For the development of QSAR models on 2-arylamino-4-cyclohexylmethoxy-5-nitroso-6-aminopyrimidine derivatives (Eq 1), the antitumor activity by inhibiting CDK2 was utilized. Experimental IC50 values were taken from literature.[2] Dielectric energy, shadow_xyfrac, indicator parameters (Ix), N_Count and Jurs_Rasa were used as molecular properties for QSAR study. Negative logarithm of CDK2 inhibitory activity of 2-arylamino-4-cyclohexylmethoxy-5-nitroso-6-aminopyrimidine derivatives (pIC50) were used for developing QSAR model to get linear relationship with independent variables.

References

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I-4

Antimalarial Drug Design Promises from African Plants

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Malaria caused by *Plasmodium falciparum* is lethal and responsible for major losses and death in Sub-Saharan Africa. About 80% of the world population resides in developing countries and over 80% of people in developing countries utilize plants to meet their primary health care needs. The long-established use of quinine and the more recent introduction of artemisinin and its derivatives as highly effective antimalarials demonstrates that plant species are an important resource for the discovery of new antimalarial agents. Furthermore, many plant species across Nigeria, Madagascar, Kenya, Mali and other African countries continue to be used in traditional medicines for the treatment of malaria and many people depend on such remedies as they cannot afford effective antimalarial drug needed to treat chloroquine-resistant *Plasmodium falciparum* infections. A selection of natural products of these plant origin ranging from alkaloids, terpenes, quinines, methanol and miscellaneous compounds can provide lead compounds for conventional drug development.

In this work, we conducted literature mining and analysis of major plants of African descent fingered with antimalarial properties and potency with a view to cataloging them as possible sources for new lead compounds and consequently to stimulate further clinical researches for new drugs. Among the in-silico analysis, we seek to employ pharmacogenomics techniques involving sequence retrieval from notable databases to perform BLAST searches. Percent identity or percent similarity is engaged to quantify the similarity among the biomolecule sequences for possible new lead compounds for drug discovery and design. However, this study also reveal that some promising African antimalarial plants are yet to be sequenced, as no sequence data was traceable to them in genomic databases. To this end, we recommend that urgent sequencing is needed to assist in exploring the wider horizon provided by some of these naturally occurring African plants with potency in malaria treatment.

Keywords: Antimalarial, Alkaloids, Lead Compound, Drug design, Pharmacogenomics

I-5

Resistance to First-Line Anti-Tuberculosis Drugs in Bogota, Colombia

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Introduction:
Tuberculosis remains a serious health problem in Colombia with an incidence of 25 cases per 100.000 habitants. Over the years it hasn't been observed an increase in the resistance to anti-tuberculosis drugs, being the multidrug-resistant (MDR) rate under 3%.
Objective:
To investigate anti-tuberculosis (TB) drug resistance rates and multidrug-resistant TB (MDR-TB) in 163 isolates obtained of 156 patients in Bogotá – Colombia.
Methods:
This retrospective study included 166 isolates obtained of 156 patients since 1995. The isolates were obtained from different specimens: sputum, bronchial washes, lymphatic nodules, urine, biopsies, necropsies, etc. Susceptibility was tested in all isolates for first line antimicrobial agents according to standard proportional method. Spoligotyping method was realized to 21 resistance strains.

Results:
Ethambutol was the most sensitive drug corresponding to 160/166 isolates (96.4%). The resistance pattern is as follows: Streptomycin 19/166 (11.4%), Isoniazid 12/166 (7.2%), Rifampicin 6/166 (6%) and Ethambutol 6/166 (3.6%). Multi-Drug Resistant tuberculosis was observed in 6/156 (3.8%) patients. We found 2 MDR patients (5 isolates) with Beijing genotype SIT190, another resistance isolates were of LAM, Haarlem and T1.
CONCLUSION:
In this short study, the presence of Beijing genotype creates an urgent need of a proper nationwide survey to evaluate the true picture of resistance.

I-6

Antileishmanial drug screening

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Infection with pathogenic *Leishmania* results in a spectrum of human diseases, termed leishmaniasis which has an annual incidence of 2 million cases in 88 countries. *Leishmania* have a digenetic life cycle, first residing in the gut of phlebotomine sand flies where they replicate as procyclic promastigotes. During a blood meal, the parasites are transmitted and engulfed by vertebrate mononuclear phagocytic system cells, where they will then transform into the amastigote stage and divide within the acidified phagolysosomes. No effective vaccines are available against *Leishmania* infection as yet and treatment relies mainly on chemotherapy. In order to generate the adequate armory of drugs to treat visceral leishmaniasis, new and effective drug targets are required to combat the dreadful form of this disease. Enzymes or metabolites present in the parasite but absent from their mammalian host are considered as ideal targets for rational drug design. Thus pteridine reductase 1 (PTR1, EC 1.5.1.33) of *Leishmania* is an excellent target due to the unusual salvage of pterin from the host. We have focused on a virulent clinical isolate of *L. donovani* when investigating the role of pteridine metabolism for antiparasite chemotherapy. We have overexpressed *L. donovani* PTR1 in *E. coli* host and purified the recombinant protein. We have also overexpressed PTR1 tagged at the N-terminal with green fluorescent protein (GFP) in *Leishmania* cells. The promastigotes/amastigotes thus developed showing GFP fluorescence, were used for antileishmanial drug screening using flow cytometry to test a collection of pteridine analogs for activity, in an effort to identify compounds targeting this enzyme specifically. With this analysis we have obtained a good impression of the activity in vitro and in vivo of two compounds. Molecular modeling and docking studies with these two compounds revealed tight binding to pteridine reductase and identify the important interactions necessary to assist the structure based development of novel pteridine reductase inhibitors. Mechanism of cell death upon exposure to these compounds has also been analyzed.

I-7

Discovery of new lead compounds from Peruvian medicinal plants for the treatment of tuberculosis, leishmaniasis, and Chagas disease

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A great part of the Peruvian population still relies on traditional medicine and, especially, the use of medicinal plants to meet their health care needs. Peru is considered a megadiverse country and its flora accounts for around 20000 species, of which 30% are endemic. Aproximately, 1400 plants are currently used in Peruvian traditional medicine, however, only a small percentage of them has been investigated.
Our group evaluated the antitrypanosomal activities of 278 plant extracts in a colorimetric, intracellular assay, using VERO cells as a host for *Trypanosoma cruzi* amastigotes. The ethanol extract of *Costus arabicus* was the most active sample, causing a 67% parasite growth inhibition at a concentration of 10 □g/ml. Bioassay-guided isolation studies on *C. arabicus* are currently in progress.
Over 200 plant extracts have been tested in a colorimetric MTT assay against axenic amastigotes of *Leishmania amazonensis.* One of the most active samples was the ethanol extract of the stem bark of *Himatanthus sucuuba*. Bioassay-guided studies on this plant showed that the compounds responsible for the leishmanicidal activity were the spirolactone iridoids plumericin and isoplumericin (IC_{50}= 0.21 and 0.28 □M, respectively).
A colorimetric Tetrazolium Microplate well Assay (TEMA) was used to evaluate the antituberculosis activity of crude extracts against *Mycobacterium tuberculosis* H_{37}Rv. Fifty-nine (6.04%) extracts showed MIC values ≤ 100 □g/ml. The most active corresponded to the extract from the stems and bark of *Clavija procera* with a MIC of 12.5 □g/ml. Bioassay-guided fractionation of the crude extract led to the isolation of the triterpene aegicerin. This compound (MIC = 1.6 □g/ml) was more active than isoniazid (MIC > 4 □g/ml) against the multidrug-resistant *Mycobacterium tuberculosis* strain.

I-8

Building a new model for pharmaceutical - P-MAPA - a novel immunomodulator against virus, bacterial, and protozoan infections

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The discovery and development of pharmaceuticals is a high-risk, long-maturation endeavor requiring the participation of many groups and research institutions. This process can be facilitated by the existence of a social and economic environment that stimulates the creation of technology-based products by offering an economic reward for the usefulness and novelty created. Such is the case in developed countries, where the pharmaceutical industry is one of the most important if not the most important type of organization mobilizing scientific, material and human resources for long-term projects of medicine research and development. In this social model, molecules and active principles developed by the industry itself or acquired from third parties, after a long process of development, are transformed into medicines and made available for consumption through market mechanisms. Such entrepreneurial agents and the incentive mechanisms associated with these economic environments are often absent in developing countries, as their pharmaceutical industry is often directed toward importing medicines or producing copies of products from developed countries. Human and material resources that could allow the creation of medicines exist in universities and research centers in developing countries. However, they are dispersed, and seldom organize themselves for multidisciplinary, long-term projects such as the research and development of pharmaceuticals. In order to overcome this gap and evolve its projects, Farmabrasilis, a non-profit research network congregating Brazilian, Chilean, American, and European scientists.
(www.farmabrasilis.org.br) based in Brazil, is bent on building a pharmaceutical research and development environment, unique in South America. This environment has been created and is maintained by the interaction and teamwork of researchers, scientists, volunteers and organizations from the civil society, pooled around Farmabrasilis in long-term projects for the development of medicines for the benefit of excluded populations. The results are now being delivered to the public in the form of a range of medicaments and technologies: P-MAPA, a state-of-the-art imunomodulator for infectious diseases and other important medicaments now in an advanced stage of research. Furthermore, Farmabrasilis in collaboration with individuals and groups around the world is now preparing to make available the P-MAPA immunomodulator to combat infectious diseases, caused by intracellular pathogens, particularly in poor countries, where the problem is compounded by the spread of HIV-AIDS. It's already passed all pre-clinical studies as well as Phase I clinical trials. Experiments in animal models and preliminary clinical trials in humans have shown that P-MAPA is able to reestablish the immunocompetence when the immune system is impaired by infectious diseases and cancer. If this is borne out by Phase II and Phase III clinical trials, this medicament is expected to have an extensive impact on the treatment of infectious diseases in populations of whole countries. The Farmabrasili´s product licensing policy includes the possibility of royalty-exempt transference of technology to public or autonomous local production centers in the case of neglected diseases and poor populations.

I-9

A Phase 2/3, randomized, Double-Blind, Comparative trial of Azithromycin Plus Chloroquine versus Mefloquine for the Treatment of Uncomplicated *Plasmodium falciparum* Malaria in Africa

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Rationale: Options for effective safe therapy for symptomatic, uncomplicated malaria caused by *P. falciparum* are becoming increasingly limited as resistance to standard regimens increases throughout the world. The development of new therapies that are safe, effective and easily administered is critical.

Objectives: The primary objective is to confirm the hypothesis that Azithromycin plus Chloroquine is non- inferior to Mefloquine for the treatment of symptomatic, uncomplicated malaria due to *P. falciparum.*

Methodology: Phase 2/3, double-blind, comparative study in which subjects were randomized to treatment with azithromycin plus chloroquine or mefloquine after informed consent was obtained and inclusion/exclusion criteria were determined to have been met. Duration of dosing was 3 days. Each subject would be asked to participate for 42 days. Subjects were randomized to one of two active arms. Males and females aged 18 years and above with symptomatic, uncomplicated *P. falciparum* malaria in. Approximately two hundred and thirty six (236) subjects from 2 or more locations in Africa. Five African countries were involved, i.e. Zambia, Uganda, Ghana, Mali and Kenya

Results: Determination of non-inferiority of azithromycin 1g plus chloroquine was based on a 95.04% CI for the difference in cure rates using a normal approximation to the binomial distribution, accounting for the Interim Analysis performed on two occasions. There was no adjustment for centers. Subjects treated with azithromycin 1g plus chloroquine had a parasitological clearance rate of 98.06% through Day 28 compared with 99.03% for subjects treated with mefloquine.

I-10

Pharmacokinetic & Pharmacodynamic Drug Interaction Study Following Concurrent Administration of Andrographis Paniculata, and Selected Antiretroviral Agents

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2 Department of Pharmacology & Toxicology, NIPRD, Abuja, Nigeria
3Department of Microbiology & Biotechnology, NIPRD, Abuja, Nigeria
4Institute for Human Virology Nigeria

Introduction: (AP), is a member of the plant family *Acanthaceae*, and has been used as a medicinal herb for centuries to treat a variety of ailments ranging from GI tract and upper respiratory infections, fever, to a host of other chronic and infectious diseases. It is a prominent component of many Ayurvedic formulas and Traditional Chinese Medicine (TCM. Early scientific studies have confirmed that *Andrographis* has a surprisingly broad range of pharmacological effects, prominent among which is a direct antimicrobial action. However, the weight of clinical evidence now shows that the real value of this ancient herb is as a stimulant for the immune system. It improves non-specific immune response, stimulating powerful immune responses in living creatures. The immune response may be specific, directed at a microbial invader already present in the body, or generally, strengthening the immune system in preparation against future infections. Preclinical animal studies conducted at the National Institute for Pharmaceutical Research and Development (NIPRD), Abuja, Nigeria, show that CD4+ counts were increased by over 118% following a one month treatment at a dose of 50-200mg/kgbody weight of AP extract, thus exhibiting a positive immune enhancing effect. This plant thus can be developed as an immune booster.
Study Objective: The study aims to evaluate the possibility of a pharmacodynamic drug interaction following the concurrent administration of AP extract and selected antiretroviral agents.
Methods: Wistar rats of weight range 150-200 were procured from the animal facility center of the department of Pharmacology and toxicology in NIPRD and University of Jos, Nigeria. The animals were left to acclimatize, after which they were divided into six groups of twenty rats each. Group I = Stavudine 0.5mg+AP 200mg/kgbw, Group II = Lamuvudine 2.14mg/kg +AP 200mg/kgb, Group III = Nevirapine 2.8mg/kgbw +AP 200mg/kgbw, Group IV = Combination (1&II&III), Group V = AP 200mg/kgbw, Group VI = Control
First week the animals were treated 12hourly for seven days after which 5 rats from each group were sacrificed and blood samples taken for haematological analysis and CD4+ count. The organs brain, kidney, liver, heart, spleen, testes/ovaries and stomach were taken out and kept in formalin for histopathological studies. 2nd 3rd and 4th weeks, the animals were treated on same drugs once daily and weekly samples were collected as in week one. Feed and water intake were monitored daily and the weight gain weekly.
Results and Observations: There was insignificant, time-dependent increase in body weight of animals in all the treatment groups. There was insignificant, time-dependent increase in water intake of animals in all the treatment groups. There was insignificant, time-dependent decrease in water intake of animals in all the treatment groups. There was time-dependent increase in values of CD4 count across all the treated groups; this was however significantly ($p<0.05$) different from the controls in the groups that received stavudine-AP as well as the group that received lamivudine-AP respectively in week 3. There was no significant difference in the RBC values of all the treated groups through the duration of study. There was time-dependent increase in the values of WBC in all the groups. This increase was however significantly ($p<0.05$) different from the control in the groups that received stavudine-AP in the third week. There was a time-dependent but insignificant increase in the platelet count in groups that received stavudine-AP, nevirapine-AP, AP alone and lamivudine-AP. There seems to be a beneficial pharmacodynamic interaction between AP and the nucleoside reverse transcriptase inhibitors.

I-11

Challenges of Drug Design and Development in Nigeria

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The African continent is burdened by the problem of infectious diseases of poverty including malaria, HIV and tuberculosis despite concerted efforts at controlling them. Drug treatment of the diseases has not been effective enough due to several factors such as resistance, availability, cost, apathy and adulteration. There is therefore a need for continuous search for new drugs and identification of drug targets and biomarkers

Scientific breakthrough and better understanding of biology of cells, tissues, disease pathways and drug capacities have given hope for a revolution in drug design and development. The process of drug discovery involves multi- and interdisciplinary approaches. Advances in biotechnology, genomics, DNA sequencing, microarray methods etc have contributed immensely to providing leads for drug design

Plants have been the basis of many traditional medicine systems throughout the world for thousands of years and continue to provide mankind with new remedies. Plant-based
medicines initially dispensed in the form of crude drugs and herbal formulations serve as the basis of novel drug discovery. Nigeria and other tropical African countries are a very rich source of ethno medicine that provides a value resource for potential natural products that can be used for drug development. Less than 5% of the plant resources in Nigeria has been screened for biological and pharmacological activities.

In the developing world more money is reported to be spent on R&D though the number of new drug submission is not commensurate. In Nigeria records show that government is not committed to spending money on R&D. The private sector through pharmaceutical industries commits less than 2 million dollars annually. It is estimated that about 1 billion dollars will be required to develop a drug to the clinical stage and could take about 15 years. Sometimes the drug might not be acceptable due to certain factors such as efficacy, toxicity, cost etc. This cost is beyond the reach of African countries most of whom have very low GDPs.

The bane of drug development in Nigeria is the lack of infrastructure, human capacity, commitment, patience and proper regulatory guidelines.

I-12

Effect of herbal and synthetic protoscolicidal agents on protoscoleces of hydatid cyst

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Hydatidosis, is one of the most important zoonotic parasitic diseases. The choice treatment is surgery in which some chemical agents are used to prevent secondary hydatidosis. However these protoscolicidals often cause some side effects such as cholangitis and the fibrosis of the biliary tract.
According to the lack of high effectivity of common protoscolicidal agents and their side effects on human, the garlic choloroformic extract could be a suitable replacement as a natural and herbal agent. Garlic chloroformic extract has been shown a good protoscolicidal effect within 30 minutes (Sadjjadi et al, 2004:
In vitro screening of different allium sativum extracts on hydatid cysts protoscoleces. XXIst International Congress of Hydatidology, 16-21 Aug. 2004, Nairobi, Kenya. P.111). But spending such long time would not be applied during surgery. Therefore, the shorten times which is usually used before surgery, was applied to find out the protosclicidal effect of this agent.

In this regard, the effect of garlic chloroformic extract on the protoscoleces was compared with common protoscolicidal agents such as; cetrimide 0.5%, silver nitrate 0.5% and hypertonic sodium chloride 20% in limited times of exposure i.e. less than five minutes.

Sheep liver and lung with hydatid cysts from Shiraz slaughterhouse, were carried to parasitology laboratory of Medical School. A total of 3000-4000 protoscoleces were separated in a strile condition and exposed to 1 ml of cetrimide 0.5%, hypertonic sodium chloride 20%, silver nitrate 0.5%, normal saline and garlic chloroformic extract (200 mg/ml) with the exposure time of 1,2 and 5 minutes, their viability were assessed by aqaous eosin 0.1%.

In vitro observations, showed that the protoscolicidal effect of garlic chloroformic extract (200 mg/ml) in one minute was higher than silver nitrate 0.5% and sodium chloride 20% (P=0), but was similar to cetrimide 0.5% (P=0.36). Also at the time of two minutes, the protoscolicidal effect of garlic chloroformic extract (200 mg/ml) had significant difference with silver nitrate 0.5% and hypertonic sodium chloride 20% (P=0, P=0.003). In five minutes exposure, there was no difference between garlic chloroformic extract 200 mg/ml and sodium chloride 20% (P=0.36), however the difference between these agents and silver nitrate 0.5% was significant (P=0).
The findings indicate that garlic chloroformic extract in short time of exposure has a high protoscolicidal effect and could be a suitable replacement for other common protoscolicidal agents.

I-13

Probable interaction of therapeutic monoclonal antibody 13.1 with the ookinete surface protein Pb28 from P. berghei: A molecular modeling and docking study

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Background: Malaria affects 300-600 million people and takes 1-3 million lives annually. Proteins expressed on the surface of sexual stages of *Plasmodium* are considered promising vaccine candidate proteins. Vaccines against these proteins induce antibodies in the vertebrate host, which inhibit parasite development in mosquito midgut and consequently prevent the transmission of parasite to another human host. One such family of ookinete surface proteins is P28 family. These proteins along with P25 proteins have their role in parasite protection inside mosquito midgut. Despite having such an important function no x-ray structure of this family is available till date. The purpose of this study was to structurally characterize Pb28 protein from P. berghei anka strain and to study the interaction of Pb28 protein with scFv of TBmAb (transmission blocking monoclonal antibody) 13.1.
Methodology: Pb28 protein was modelled with the help of Modeller and refined using ModLoop program specially loop B of EGF domain II. Evaluation and validation was done with the help of Procheck, WhatIF and ProsaWeb. Single chain variable fragment of TBmAb was modelled with the help of WAM (web antibody modeling) server and refined with the help of ModLoop. Pb28 and TBmAb 13.1 were docked using ZDOCK and RDOCK softwares.
Conclusion: results obtained indicate that Pb28 protein has four EGF domains arranged in triangular fashion with maximum root mean square deviations in B-Loop of EGF domain II and EFG domain III. With the help of docking we are able to show that the B loop of the EGF domain II of Pb28 protein interacts with scFv of TBmAb 13.1. Model of Pb28 protein and 13.1 TBmAb may help in designing transmission blocking vaccine against malaria in the absence of experimentally determined structures of this surface protein and TBmAb antibody. Predicted antigen-antibody (scFv only) complex may suggest a mechanism of transmission blocking.

I-14

Protein kinases as potential targets for antimalarial chemotherapy

Abdirahman Abdi

Christian Doerig and the INSERM U609 team

The vast phylogenetic distance between Apicomplexa and model eukaryotes such as yeast and mammalian cells is reflected by profound divergences in the properties of the *Plasmodium* protein kinases. A genome-wide *in silico* kinome analysis (Ward et al., *BMC Genomics*, 2004) revealed the presence (i) of 65 genes encoding PKs in the *P. falciparum* genome, many (but not all) of which can be classified in PK groups identified in other eukaryotes, and (ii) a novel, Apicomplexan-specific PK family with 20 members. This study confirmed our previous experimental data pointing to the absence of typical MAPK pathways in *Plasmodium*, a first among eukaryotes. A collaboration with J. Endicott (Oxford) on the 3D structure of a divergent plasmodial PK revealed that the ATP-binding pocket is different from that of mammalian PKs, in line with its vastly divergent susceptibility to known kinase inhibitors. This suggests that specific inhibition *Plasmodium* PKs is a realistic goal.

We are now addressing the question of the function played by the PKs in the parasite's life cycle. Using reverse genetics, we demonstrated essentiality of several PKs for erythrocytic schizogony, and also identified PKs that are not required for asexual growth but are essential for transmission to the mosquito. These studies validate specific PKs as targets for schizonticidal or transmission-blocking intervention.

In collaboration with academic and industrial laboratories, we use recombinant enzymes in the screening of chemical libraries.

I-15

Neuraminidase Inhibitors Identification by Pharmacophore Modelling and Mapping from Malaysian Medicinal Plants

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Three-dimensional pharmacophore hypotheses were built from a training set of 17 compounds selected with great diversity in molecular structure and bioactivity, for discovering new potent neuraminidase inhibitors (NAIs) to fight against avian influenza virus. A three-point pharmacophore of selective NAIs was derived from the training set, using the Catalyst program. Among the ten common-featured models generated by program Catalyst/HipHop, a hypothesis including two hydrogen-bond acceptors (HBA), two hydrogen-bond donors (HBD) and a negative ionizable (NI) features was considered to be important in evaluating the NAIs activity. Active NAIs mapped well onto all the HBA, HBD and NI features of the hypothesis. On the other hand, the less active compound was shown to be difficult to achieve the energetically favorable conformation which is found in the active molecules in order to fit the 3D common-feature pharmacophore models. The present studies on NAIs demonstrate that two HBA, two HBD and a NI sites located on the molecule seem to be essential for NAI activity. The hypothesis (Hypo 1) was used to screen the NADI-CHEM natural products (Malaysian medicinal plants) database and allowed to identify potential compounds as anti-bird flu agent. Since betalains are the most active compounds screened, therefore this type of compounds would be compound of interest with betalamic acid as a starting materials for semi-synthesis of the the anti-bird flu agent.

Keywords: Hypothesis, pharmacophore, neuraminidase inhibitors, training set, HipHop, hydrogen bond acceptor, hydrogen bond donor, negative ionizable, NADI-CHEM, natural products, betalains, betalamic acid

I-16

Synthesis of 16-Dehydropregnenolone Acetate (16DPA) Using Solasodine Isolated From Solanum khasianum var. chatterjeeanum Linn. (Family: Solanaceae)

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Aims: The use of solasodine as starting material for the synthesis of steroid hormones was strongly stimulated by the possibility to isolate large amounts (ton scale) of glycoalkaloids from various plants of the family solanaceae, dioscoreaceae. So many procedures are available to isolate the glycoalkaloid in pure form and its conversion into16DPA. But none of these approaches were successful. The main objective is to Synthesis 16DPA from Solasodine, a Steroidal glycoalkaloid (tetra cyclic-triterpenoid) isolated from *Solanum khasianum var. chatterjeeanum* Linn. (Solanaceae). 16DPA acts as a starting material for the synthesis of antifertility and anti-inflammatory agents. So many procedures are available for the synthesis of DPA from different plant materials but Clarke plant is being used for this purpose with the aim of producing solasodine by cheapest method along with better conversion rate of solasodine into 16DPA.

Methods and Materials:

Materials:

For Extraction / Isolation Process: Fresh fruits (without seed) / Dried finely ground powder of fruits of *Solanum khasianum var. chatterjeeanum Linn.* plants, Toluene, Conc. Hydrochloric Acid, Sodium Hydroxide, Acetone,

For synthesis of 16DPA: Solasodine, Pyridine, Acetic Anhydride, Glacial Acetic Acid, Chromium trioxide (CrO_3),Sodium bisulphite ($NaHSO_3$) ,Dilute Hydrochloric Acid

Methods: First the compound Solasodine was isolated from the fresh fruits from *Solanum khasianum var. chatterjeeanum* Linn. using toluene-acid mixture (1:1). Then it was purified, crystallized and prepared for the synthesis of 16-DPA. First solasodine was converted into O, N-diacetylsolasodine using reflux with acetic anhydride. Then O, N-diacetylsolasodine was converted into O, N-diacetyl pseudosolasodine using reflux with glacial acetic acid. Finally O, N-diacetyl pseudosolasodine was converted into 16-DPA by CrO_3 oxidation. After getting 16-DPA, it was purified and crystallized from acetone/hexane. All the structures were confirmed by TLC, M.P., FTIR, ^{13}C NMR.

Results: Identification of Solasodine and 16DPA both were done. The Melting point of solasodine and 16DPA were 198-200^{0}C and 272.5^{0}C. The Rf values for the compounds were 0.5 and 0.23 respectively. The percentage of conversion was 70% (Soalsodine-400mg and 16DPA was 280 mg respectively. The final product 16DPA was white, crystalline.

Conclusions: The interesting finding of our study was that Solasodine was successfully converted into 16DPA. This compound acts as a starting material (novel precursor for antifertility like Norethynodrel and anti-inflammatory corticosteroidal drugs like Betamethasone.16DPA acts as a hypoglycaemic agent too. The Percentage of conversion was about 70% from Solasodine.

Key-words: *Tetra cyclic- triterpenoid, Solasodine HCl, Anti leukemic, Solanaceae, ARA-C.*

I-17

Treatment challenges of Leishmaniasis in Ethiopia

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Visceral leishmaniasis, also known as kala-azar, is a systemic parasitic disease caused by the *leishmania donovani* species complex. Co-infection with human immunodeficiency virus (HIV) and *leishmania donovani* visceral leishmaniasis (VL) in Africa is an emerging, poorly understood disease. Because of combination of demographic and geographic factors, increased risks of both HIV transmission and visceral leishmaniasis have posed serious public health challenges in Ethiopia. Coinciding with HIV infection, the number of visceral leishmaniasis cases in Ethiopia has increased in the last decades. In study conducted in patients coinfected with HIV and VL treated, the risk factors for VL relapse, death and the effect of antiretroviral therapy (ART) were evaluated and VL relapse among those receiving the ART was associated with a baseline $CD4^+$ cell count <100 cells/µl. Failure to clear parasites after VL treatment was usually followed by symptomatic VL relapse. The risk of treatment failure is high, whatever antileishmanial drug is used. Patients who relapsed showed poor $CD4^+$ cell count recovery while receiving ART. Indeed, ART was partially protective against VL relapse. Concordant HIV and VL is a major acquired immunodeficiency (AIDS) defining illness with high relapse and mortality rates. ART seems to reduce the risk of relapse by ~50%. High rates of treatment failure, possibly leading to development of resistant parasites, indicate that combination therapy, instead of monotherapy, should be used to treat HIV-VL coinfection. Sodium stibilogluconate (SSG) monotherapy has an unacceptable mortality rate in HIV-VL coinfected patients and theoretically, may induce an increase in HIV-1 virus replication. Given the problem associated with the handful of currently available drugs for VL, new and improved treatments to relapse or complement existing therapy are needed urgently. Moreover, an increased and sustained commitment from all implementing and funding partners therefore remains an urgent priority. Current diagnostic techniques are invasive and complicated and require trained staff. Treatments are toxic, expensive and difficult to administer. These limitations have the improvements of access to treatment. Thus, the availability of safer drugs with affordable price for patients with HIV-VL coinfection (e.g. miltefosine, paromomycin, liposomal amphotercin B) is highly demanding.

I-18

A novel jacaranone derived glucosidic ester from Jacaranda glabra

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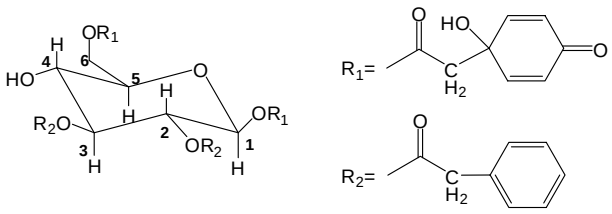
Plants from the genus *Jacaranda* (Bignoniaceae) are native to the New World but also widely cultivated in the Old World. The genus contains 49 species and is interesting from both biological and chemical perspective. *Jacaranda* species have been used in traditional medicine for the treatment of protozoa-caused diseases as well as of skin illnesses[1, 2]. Recent studies on anti-leishmania activity and hypotensive effects have been performed with crude extracts[3, 4].

In our current project on the validation of anti-protozoal activity of plants traditionally used in Ecuador, the dichloromethane extract of the leaves of *Jacaranda glabra* (DC.) Bureau & Schumann showed promising activity against *Plasmodium falciparum* K1 strain. Activity guided isolation yielded a novel glucosidic ester (**1**) containing quinolacetic acid ($\mathbf{R_1}$) and phenylacetic acid ($\mathbf{R_2}$) moieties. The compound was identified by NMR experiments and MS techniques.

It showed activity against *Pl. f.* K1 strain (IC_{50} 1.13 µg/mL) and low cytotoxicity on L-6 cells (IC_{50} 15.5 µg/mL).



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Acknowledgements: This research is part of a dissertation funded by the Austrian Exchange Service (ÖAD).

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I-19

MRP-like ABC transporters modulate Glucantime activity in Leishmania

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One of the major causes of drug resistance and chemotherapeutic failure in anti-infective therapies is the active movement of drugs across membranes through ATP-binding cassette (ABC) transporters. Herein, we show that Glucantime´s, but not Amphotericin B´s efficacy in decreasing the infection rate of *Leishmania*-infected macrophages is strongly enhanced when used in combination with Glibenclamide, a specific blocker of multidrug resistance-associated protein (MRP)-type ABC transporters. Glibenclamide labels an intracellular tubular system that begins at the anterior end of the cell and runs along the parasite. Fluorescent microscopy studies revealed that this intracellular tubular system is simultaneously labeled by the acidic marker LysoTracker-red, but not by the nuclear marker 4',6-diamidino-2-phenylindole, the mitochondrial marker MitoTracker-red, or the endocytic marker FM 4-64. These results demonstrate for the first time that Glibenclamide labels *Leishmania major* organelles associated with the lysosomal multivesicular system. The Glibenclamide labeled compartments may be involved in sequestering Glucantime from the cytosol; therefore, inhibition by Glibenclamide of the ABC transporters located at these organelles membranes may effectively increase the cytoplasmic concentration of Glucantime and consequently its leishmanicidal activity. In contrast, Amphotericin B toxicity occurs through its binding to sterols located at the parasite cell membrane. Since the Glibenclamide-Glucantime combination is especially efficient in reducing the infection rate of infected macrophages, and since combination therapy is a way to increase the efficacy of individual drugs and decrease both duration of therapy, and potential development of resistance, the herein described results could thus be of fundamental impact for leishmaniasis therapy.

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I-20

VirulentPred: a machine learning based prediction method for virulent proteins in bacterial pathogens to aid discovery of novel drug targets.

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Prediction of bacterial virulent protein sequences facilitates identificationand characterization of novel virulence-associated factors, finding novel drug/vaccine targets,proteins indispensable to pathogenicity, and understanding the complex virulence mechanism inpathogens.

In the present study, we have developed a bacterial virulent protein prediction method based on an artificial intelligencebi-layered cascade Support Vector Machine (SVM). The first layer SVM classifiers were individually trained andoptimized with different protein sequence features like amino acid composition,dipeptide composition (occurrences of the possible pairs of $i^{th}$and $i+1^{th}$amino acid residues), higherorder dipeptide composition (pairs of $i^{th}$and $i+2^{nd}$residues) and Position Specific Iterated BLAST(PSI-BLAST) generated Position Specific Scoring Matrices (PSSM). In addition, a similarity-searchbased module was also developed using a dataset of virulent and non-virulent proteins as BLASTdatabase. A five-fold cross-validation technique was used for the evaluation of various predictionstrategies in this study. The results from the first layer (SVM scores and PSI-BLAST result) werecascaded to the second layer SVM classifier to train and generate the final classifier. The cascadeSVM classifier was able to accomplish an accuracy of 81.8%, covering 86% area in the ReceiverOperator Characteristic (ROC) plot, better than that of either of the individual classifiers of first layerbased on single or multiple sequence features.

Conclusion: Till date**,** artificial intelligence based methods have been successfully used in several prediction problems involving complex biological data.VirulentPred is a SVM based method to predict bacterial virulent proteins sequences,which can be used to screen virulent proteins in proteomes. Together with experimentally verifiedvirulent proteins, several putative, non-annotated and hypothetical protein sequences have beenpredicted to be high scoring virulent proteins by the present prediction method. VirulentPred is availableas a freely accessible World Wide Web server – VirulentPred, at http://bioinfo.icgeb.res.in/virulent/

I-21

In vivo antioxidant and potential antitumor activity of aqueous ethanol extract of leaves of senna alata (l.) Roxb (ceasalpiniaceae) on bearing carcinomatous cells

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The study was designed to investigate the subacute toxicity, *in vivo* antioxidant and antitumor activity of aqueous ethanol extract of *Senna alata* on bearing carcinomaous cells. The results of the evaluation of the toxicity on albinos *wistars* rats showed no death of rats. However the of animals increased significantly after 26 days of administration of the extract. The liver enzymes activity alanine amino transferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) did not vary significantly as well as the concentration of glutathione, creatinine of the treated rats both in the liver homogenate and serum. The study was extended to the evaluation of *in vivo* antitumor activity of extract of *S. alata* on bearing carcinomatous cells on *nude* mice. The results showed that after treatment with the extract at 100 mg $kg^{-1}$and 200 mg kg^{-1} body weight, the levels of MDA decreased significantly ($3.44 \pm 0.76 – 1.97 \pm 0.48$) while the concentration of glutathione and the activities of CAT and SOD increased significantly. The results of phytochemical analysis of aqueous-EtOH extract of *S. alata* indicated the presence of tannins, polyphenols, steroids, glycosides, flavonoids, anthraquinone and saponins. The results suggest that the aqueous ethanol extract of *S. alata* is not toxic and exhibits significant antitumor and antioxidant effects on bearing carcinomatous cells.

Key words: *Antitumor, antioxidant, oxidative stress, carcinomatous cells*

I-22

Molecular modelling on the acylation process in furin complexed with the cleavage site of hemagglutinin H5

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The first step of the acylation reaction of furin in which the cleavage site of the highly pathogenic avian influenza virus subtype H5N1 (HPH5) acts as its substrate was investigated using combined QM/MM approaches. Only one transition state is detected along the reaction pathway indicating a concerted reaction in which the proton transfer from Ser368 to His194 is simultaneously accompanied by the nucleophilic addition to the substrate. The energy profiles for the formation of a tetrahedral intermediate between the catalytic furin residue (Ser368) and the HPH5 reacting center (S1-Arg) were calculated using the PM3/CHARMM method with correction of the gas-phase QM energy by the B3LYP/6-31+G* level of theory. As the reaction proceeds, the positively and negatively charged components of the tetrahedral intermediate complex are extensively stabilized by the electrostatic contributions from the protein environment rather than from the neutral components of the enzyme-substrate complex. Asp153 provides the highest degree of stabilization to the enzymatic reaction and in particular stabilizes the protonated His194 through two strong hydrogen bonds. Asn295 and Ser368 build up the oxyanion hole for stabilizing the negatively charged carbon oxygen of tetrahedral intermediate, which is also suggested by the observation of two formed hydrogen bonds.

I-23

Molecular Dynamics Study of Regulation of NF-κB Transcription Factor Complex Activity by Specific Lysine Acetylation

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Nuclear Factor-κB (NF-κB) is a DNA sequence-specific regulator of many important biological processes that are activated under stress-related signals as immune, inflammatory and anti-apoptotic responses, cellular growth and development. The most abundant and biologically active form of NF-κB is the p50/p65 heterodimer. Histone acetyltransferases and/or histone deacetylases can modulate NF-κB activity by acetylating and/or deacetylating specific lysine residues. Among all the possible acetylation sites on both p50 and p65 subunits, it is known that single acetylation at K221 of p65 subunit raises the affinity for its cognate DNA, whereas double acetylation at K122 and K123 decreases it. This work casts light onto the molecular mechanisms that underlie the change of DNA affinity of p50/p65 heterodimer upon acetylation of lysines 122 and 123 or 221 by using Molecular Dynamics simulations. Results show that double acetylation at positions 122 and 123 cause the collapse of the interaction network between p65 subunit and DNA, whereas the single acetylation at position 221 induces conformational changes of the DNA double helix, especially by lowering the buckling and the staggering of base pairs with respect to the unacetylated complex.

Keywords: lysine acetylation | Modulation of NF-κB activity | DNA curvature | MD simulations | post-translational modifications

I-24

Binding free energy simulations of inhibitors of avian influenza A virus neuraminidase

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The outbreak of avian influenza A (H5N1) virus has raised a global concern for both the animal as well as human health. Recently, drug resistance in H5N1 infections has been widely reported due to neuraminidase mutations. Consequently, understanding of inhibitor-target interactions at the molecular level represents the main goal of our study. Molecular dynamics (MD) simulations were carried out for the neuraminidase subtype N1 complexed with nine different inhibitors: oseltamivir-, zanamivir- and peramivir- carboxylate, phosphonate and sulphonate. Binding free energies were calculated using the Linear Interaction Energy (LIE) method. Good agreement with experimental results was obtained. 20 ns MD trajectories were extensive analyzed in terms of important interactions between inhibitors and the enzyme target. Calculations of binding free energies for selected compound moieties provided even deeper insight into the source of inhibitory activity. These results constitute new valuable information to assist further drug development towards inhibition of the H5N1 avian influenza virus.

I-25

Computational analysis of HIV-1 reverse transcriptase inhibitors as a molecular basis for drug development

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As it is known that the understanding of the basic properties of enzyme-inhibitor complexes leads directly to enhancing the capability in drug design and drug discovery. HIV-1 reverse transcriptase (RT) is an important target for drugs used in the treatment of AIDS. Drugs known as non-nucleoside RT inhibitors (NNRTIs) appear to alter the structural and dynamical properties of RT which in turn inhibits RT's ability to transcribe. To examine detailed information on structure, solvation, dynamic and thermodynamics properties, molecular dynamics simulations have been performed for the three known NNRTI inhibitors, efavirenz (EFV), emivirine (EMV) and nevirapine (NVP), embedded in the catalytic site of HIV-1 RT. In terms of hydrogen bonding, EFV and EMV bind with surrounding residues of HIV-1 RT while for NVP system such binding was not found. The complexation energies of RT with the inhibitors were calculated to identify residues that are important for binding. From the calculated results, L100, K101, K103, V106, Y181 and Y188 show the largest contributions to the ligand-enzyme interaction energies. The obtained results support clinical data which reveal that these residues are the most frequent residues that mutation against NNRTIs takes place. Taking into account the binding affinities calculated by molecular mechanics/Poisson-Boltzmann surface accessibility (MM-PBSA), $\Delta G_{binding}$ values were observed in the following order: EFV > EMV > NVP. This agrees very well with the experimental IC_{50} values. In addition, the distribution and binding of water molecules, in terms of hydrogen bonding to the donor atoms of the inhibitors were investigated and discussed, referring to those which were found in crystal structure.

Keywords: HIV-1 RT, NNRTIs, Drug, Molecular dynamics Simulations

I-26

Molecular Studies and Drug Design for NS2B/NS3 protease of Dengue Virus and West Nile Virus

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Dengue Virus (DV) and West Nile Virus (WNV) are causes of severe public-health problems in many countries around the world. Nowadays, neither effective human vaccine nor drug is available against both viruses. Therefore, NS2B/NS3 protease, which is an essential enzyme for both viruses, is currently focused as the promising enzyme target for drug development. In this work, several computer aided-drug design methods were applied to study the NS2B/NS3 protease of WNV and DV. 3D-QSAR was used for a set of tetrapeptidic inhibitors of WNV NS2B/NS3 protease. The CoMFA model gives q^2 = 0.720 and r^2 = 0.964 and the CoMSIA model yields comparable statistic values; q^2 = 0.576 and r^2 = 0.961. Contour plots of CoMFA and CoMSIA indicate the important of P1 subsite for interacting with the enzyme. 3D structure of the DV's enzyme was built using the X-ray structure of WNV NS2B/NS3 protease-inhibitor complex (2FP7.pdb) as a template. The DV model and 2FP7.pdb (WNV-X) were subjected to molecular dynamics (MD) simulations for 10 ns. MD simulations reveal the important of NS2B for interacting with both NS3 protease and P2 subsite of inhibitor. Residues in each pocket interacting with each subsite of inhibitor were addressed. The final MD snapshot of DV model and WNV-X were employed for GRID field calculations. Results show bigger N^+ and OH probes at the S1 pocket of DV model than those of WNV-X implying the importance of the electrostatic or H-bond interaction at the S1 pocket with the P1 subsite of inhibitor of DV than that of WNV. On the other hand, the Dry probe at the S1 pocket of WNV-X is larger than that of DV model indicating the necessary of hydrophobic interaction at this pocket for WNV. In addition, the suitable MD snapshot of DV model was selected, by means of clustering analysis, for molecular docking calculations. Molecular docking results reveals that Tyr161_NS3 of DV and the S1 pocket are important for interacting with inhibitors. All these results provide basic knowlegement for better understanding in the enzyme-inhibitor interaction, which are helpful for further drug development against WNV and DV infections.

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I-27

Computer-Aided Drug Design of HIV-1 Integrase Inhibitors: Three-Dimensional Quantitative Structure Activity Relationship Study

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HIV-1 integrase (IN), one of three essential enzymes in HIV life cycle, is an attractive target for development of anti-AIDS drug. HIV-1 IN catalyses an insertion of viral DNA into host cell receptors. Nowadays, there is only one HIV-1 IN drug available on the market. This is owing to many IN inhibitors show low activities including lack of selectivity. Therefore, it is necessary to develop new IN inhibitors with high potent and high selectivity. In this study, three-dimensional quantitative structure-activity relationship (3D-QSAR) using comparative molecular field analysis (CoMFA) [1] and comparative molecular similarity indices analysis (CoMSIA) [2] techniques were applied to investigate the relationship between structures of diverse classes of IN inhibitors and their biological activities.

The in vitro biological data for strand transfer mechanism of diverse structural classes of IN inhibitors were used in this study. Structures of 86 compounds; 64 and 22 compounds for training and test sets, respectively, were geometry optimized at HF/3-21G level of theory. Using the optimized geometries and Gasteiger-Masili charges, models of comparative statistical significance of three different fitting methods were obtained.

The multi-fit fitting method yields the best predictive CoMFA model (r^2_{cv} =0.672, r^2 =0.915, F value =124.516, r^2_{pred} =0.635 with 5 components). The steric and electrostatic contributions are 62.7% and 37.3%, respectively. CoMFA results indicate a strong correlation between inhibitory activities of these IN inhibitors and their steric as well as electrostatic fields. The CoMSIA model gives r^2_{cv} of 0.593 which is less than CoMFA model. The steric, electrostatic, hydrophobic, hydrogen bond donor and hydrogen bond acceptor contributions are 12.5%, 21.7%, 23.0%, 21.9%, and 20.9%, respectively. In addition to steric and electrostatic fields, CoMSIA indicates that hydrophobic and hydrogen bond fields are necessary parameters for improving activities of HIV-1 IN inhibitors.

These CoMFA and CoMSIA studies of diverse structural classes of HIV-1 IN inhibitors show good predictive abilities. The results provide better understanding of structural requirement of IN inhibitors. The detailed information would be helpful for further designing of high potential HIV-1 IN drugs.

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I-28

Anti-Leishmanial Activity of Betulin Derivatives

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Betulin (lup-20(29)-ene-3β,28-diol) is an abundant naturally occurring triterpene, and it is found predominantly in bushes and trees forming the principal extractive (up to 30% of dry weight) of the bark of birch trees. Betulin and its derivatives such as betulinic acid have many interesting pharmacological properties, such as cytotoxic activity against many tumour cell lines and anti-HIV activity with a new mechanism of action. Several synthetic betulin derivatives that have been chemically modified at the positions C-3 and C-28 of the lupane skeleton were produced, and the anti-leishmanial inhibition activity of compounds was evaluated at 50 μM against *Leishmania donovani* and *Leishmania tropica*. Betulonic acid had the best anti-leishmanial activity with remarkable 98% inhibition at 50 μM giving a GI_{50} value of 14.6 μM. In conclusion, carbonyl or carboxyl groups seem to have beneficial effect in anti-leishmanial inhibition activity, and these compounds represent important leads for further optimization.

SESSION II

II-1

Microwave Catalysis

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In this contribution we propose a novel physical mechanism for microwave catalysis based on rotationally excited reactive species and verify its validity through a computer simulation of a realistic chemical reaction – neutral ester hydrolysis. This non-equilibrium system is formally described by introducing rotational temperature, which is higher than the translational temperature. A Born-Oppenheimer surface was constructed on the density functional theory level and applied to a modified Monte Carlo scheme. The simulation gave a reduced activation free energy when the rotational temperature was higher than the translational temperature, which constitutes a catalytic effect. For example, our calculation predicts that with rotational and translational temperatures of 310 and 300 K, respectively, the reaction should proceed 4.5 times faster than when both temperatures are 300 K. Moreover, this microwave catalytic effect is less pronounced at higher temperatures, which may have serious implications for the interaction of microwaves with living organisms in the context of widespread mobile telephony.

II-2

Synthesis of Anacardic Acids and Analogs with Potential Application in the Treatment of Tropical Diseases

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Chagas' disease, caused by *Trypanosoma cruzi*, is endemic in 15 countries in Latin America, including Brazil, and it is estimated that about 16 million people are infected by this protozoan. The enzyme glyceraldehyde-3-phosphato-dehydrogenase (gGAPDH) is one of the nine enzymes involved in the *T. cruzi* glycolysis and experimentally it has been proved that the inhibition of this metabolic pathway causes the elimination of trypanosomes in the bloodstream.[1]

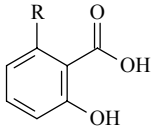
Schistosomiasis is a tropical disease which affects about 300 million people around the world. The lack of effective treatments for schistosomiasis has been stimulating the search for new attractive biological targets as well as promising molecules for drug research. Parasite enzymes are attractive targets for drug discovery. The enzyme purine nucleoside phosphorylase (PNP) from *Schistosoma mansoni* is an important target for the development of new chemotherapeutic agents.[2]

Several natural products have been evaluated against *T. cruzi* gGAPDH enzyme,[3] and among some other active compounds, a mixture of anacardic acids, isolated from cashew-nut shells, presented interesting inhibitory activity.[4] These compounds are reported to exhibit a variety of biological activities, including molluscicide[5] and inhibition of glycerol-3-phosphate dehydrogenase enzyme.[6]

In this work we have described the inhibitory activity of a small library of natural and synthetic anacardic acids against gGAPDH and PNP enzymes. Because of the easy attainment of the starting material and especially for the possibility of preparing a series of anacardic acids and analogues from a common intermediary, we employed the methodology described by Yamagiwa et al.[7]

The most active compounds were 6-*n*-pentadecylsalicilic (**1**) and 6-*n*-dodecylsalicilic acids (**2**). Kinetic studies have shown that these inhibitors bind noncompetitively to the gGAPDH enzyme with an IC_{50} of 28 and 55 □M, respectively.

For PNP enzyme, compounds **1** and **2** showed an IC_{50} of 10 and 30 □M, respectively. Molecular modeling performed through docking protocol demonstrates that the alkyl side chain would be located in the hydrophobic region of the enzyme active site. The carboxylic acid would perform a hydrogen bonding with amino acid residue Met-221, and the methoxy group with the Ser-35 and Tyr-90. In order to further investigate these results and generate useful data for computer-aided molecular design, modeling studies are being performed.



1: R=$C_{15}H_{31}$
2: R=$C_{12}H_{25}$

The authors gratefully acknowledge the financial support from FAPESP, CAPES and CNPq.

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II-3

2D-QSAR and Activity Predictions of 1-(2,3-dihydro-1H-inden-1-yl)-3-(1H-indazol-4-yl)urea derivatives as TRPV1 Antagonists for Pain Relief

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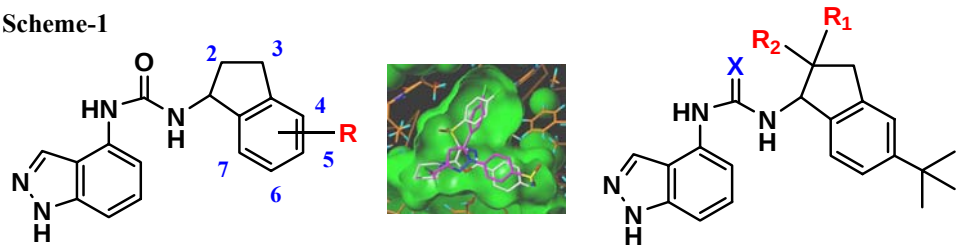
Scheme-1



R : H, N, F, Cl, Br, CF_3, Me, OMe, ter-Bu, Piperidino, 4-CF_3-piperidino, Cyclopropyl, Pyrrolidino, Morpholino.

R_1 & R_2 : H, F
X : O, S, N-CN

Vanilloid receptor TRPV1 is a cation channel that can be activated by a wide range of noxious stimuli, including capsaicin, acid, and heat. Antagonist blockade of TRPV1 activation is under investigation by several pharmaceutical companies in an effort to identify novel agents for pain management.

In this study, prediction and evaluation of activities and quantum chemical descriptors with 2D-QSAR of previously described[1] *3-dihydro-1H-inden-1-yl)-3-(1H-indazol-4-yl)urea derivatives* as TRPV1 Antagonists are reported in Scheme-1. For 2D-QSAR prediction and geometry optimization of these compounds have been based on CHARMm, semiemprical AM1 and PM3 methods with the help of ACCELRYS and GAUSSIAN 03 softwares. Furthermore, both the analgesic activity and drug-like properties of TRPV1 antagonists are discussed based on comparison of theoretical and experimantal log1/IC_{50} values.

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II-4

Quantum-chemical study of weak intermolecular complexes:an useful tool for determinationt and modeling tuhe drugs

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Nowadays there are at disposal to research microscopic molecular study the effective and massive theoretical computational methods based on approximate solution of Schroedinger equation (as an example is package program of J. Pople at al. named Gaussian 03 (2003 y.)). This – being the basing eq. of both quantitative and qualitative microscopic considerations- describes all properties of molecular systems. Until now, we can calculate, with a reasonable accuracy, the molecular systems up to hundreds atoms like carbon, nitrogen, oxygen and hydrogen. These, however, are the main constituents of all biomolecules like proteins, sacharides or nucleic acids, for example..

In the present contribution we outline our results on some π-EDA complexes: thermodynamics of their formation as well as electric and IR (e.g. INS- inelastic neutron scattering spectra-which are experimentally an very efficient and new method) characteristics are being calculated . Comparison with available experimental data reveals a good correspondence [1 - 3].. EDA complexes are also of importance to antibacterial activity of some drugs[4] as well as for stacking contribution to stability of secondary structure of DNA[5], e.g.

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II-5

Characterization of the Binding Sites of Heat Shock (CLPQY) Protease of Plasmodium Falciparum

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II-6

SKPDB: a database of comparative protein strucuture models for shikimate pathway enzymes of microorganisms

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The SKPDB (ShiKimate Pathway DataBase) is a relational database applied to the study of shikimate pathway enzymes in microrganisms. The main interest in the study of shikimate pathway enzymes is that its not present in humans, which make them selective targets for drug design, decreasing the impact of drugs in humans. In the database, there are currently 3185 proteins from enzymes of the shikimate pathway of microorganisms. It can be accessed on the web at http://laboheme.df.ibilce.unesp.br/skpdb/index.html or http://www.biocristalografia.df.ibilce.unesp.br/tools/index.php.

Extensive information is recorded for each enzyme, including a deitaled description of the enzyme, about sequence, structure studies, functional studies, and references. All structure files are available for downloading. The current database still is in its version pilot, the data is updated regularly with the addition of new data. The models were constructed using MODELLER (a comparative protein modelling program for modelling protein structures). The overall stereochemical quality of the models were assessed by the program PROCHECK. The Root Mean Square Deviation (RMSD) between Cα – Cα atoms distance was superposed using the program SUPERPOSE from MODELLER. The G-factor values were calculated using PROCHECK. The Verify-3D measures the compatibility of a protein model with its sequence, using a 3D profile. The modeled structures can be viewed visualization tools with Jmol. The SKPDB is a powerful tool for use in protein-ligand docking analysis.

II-7

Design and Synthesis of novel potential drugs based on thioxo-pyridines

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The importance of sulfur-containing heterocyclic compounds for biomedical has led to increase the number of synthetic methods available for the preparation of different heterocyclic compounds. In particular, thioxo-pyridines have attracted attention as reactive compounds with cardiovascular, hepatoprotective and antioxidant activity. The preparation of the 3,4-dihydropyridine-2(1*H*)-thione ring is a synthetic challenge. In this sense, we took advantages of new technologies for high-throughput parallel synthesis like the microwave-assisted organic synthesis. This is a high-speed technique with a higher product yields which have recently attracted the interest of the drug discovery and medicinal chemistry communities.

In the present work we report the synthesis of a new series of 4-aryl substituted 1,4,5,6-tetrahydro-2-methyl-6-thioxopyridine-3-carboxylates from 4-aryl substituted 1,4,5,6-tetrahydro-2-methyl-6-oxopyridine-3-carboxylates by employing the Lawesson's reagent (LR = 2,4-bis-4-(methoxyphenyl)-1,3,2,4-dithiadiphosphetane 2,4-disulphide) under conventional and microwave irradiation conditions. The key issue here is the observed chemoselectivity of the LR when a nucleophilic centre, other than a carbonyl group, is present in the molecule. Quantum chemical calculations were done in order to explain the above behavior.

II-8

QSAR MODEL FOR CRYPTOLEPINE an Indoloquinoline alkaloid from West African plants

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The urgent need of new antimalarials to diversify the use of artemisinin and its derivatives, the growing number of deaths from malaria especially in African countries, rich of fauna and flora diversity, justify fully that African Researchers orient an important part of their capacity to address this development issue through financially affordable solutions.
The lack of information on the mechanism of action of Cryptolepine (CLPs) from cryptolepis sanguinolenta and sida acuta, makes worthy carring out QSAR study regarding activity and cytotoxicity. In this work we are starting a QSAR model of activity from about 20 compounds (training set) and 7 others as test set. This one and 2D QSAR seem encouraging for deeper insight in CLPs activity.
From a same kind of study about cytotoxicity it will be possible to define a combined model of selectivity.

II-9

Binding Free-Energy Calculations of MurD Ligase Inhibitors Using Linear Interaction Energy Method

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The growing occurrence of bacterial resistance to most of the available antibiotics has underlined an urgent need for the discovery of novel efficacious antibacterial agents. The biosynthesis of bacterial peptidoglycan, where MurD enzyme is involved in the intracellular phase of UDP-MurNAc-pentapeptide formation, represents a collection of highly selective targets for novel antibacterial drug design. Structural studies of *N*-sulfonyl-glutamic acid inhibitors of MurD enabled the possibility of examining the binding modes of this class of compounds providing valuable information to the lead optimization phase of the drug discovery cycle.

Binding free energies were calculated for a series of MurD *N*-sulphonyl-Glu inhibitors using Linear Interaction Energy (LIE) method. Analysis of the interaction energy during the 20ns MD trajectories revealed non-polar van der Waals interactions as the driving force for the binding of these inhibitors. Considering this observation excellent agreement with the experimental free energies was attained. Calculations of binding free energies for selected compound moieties provided even deeper insight into the source of inhibitory activity. These results constitute new valuable information to assist further lead optimization process.

II-10

From Mono to Bifunctional Binding of Cisplatin to DNA: Characterizing the Sequence-Dependent DNA Structural Changes with QM/MM Methods

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Keywords: Cisplatin-DNA interactions, MD and QM/MM.

Cisplatin, *cis*-[$Pt(NH_3)_2Cl_2$] -a widely used anticancer drug- displays its activity primarily by modifying genomic DNA through a mechanism involving activation by hydrolysis prior to the N7 bonding on two adjacent purines that bends and unwinds DNA, causing a distortion that triggers the apoptotic pathway. Cisplatin-d(GpG) is the mayor adduct -65% of DNA injuries-, followed by ~25% of d(ApG) 1,2 intrastrand cross-links. No d(GpA) adducts have been observed, a fact initially attributed to steric hindrance between Cisplatin NH_3 ligands and the exocyclic NH_2 in A. Finding the explanation incomplete compelled some of us to study how B-DNA local environments modulate G/A intrinsic trend to react within representative Cisplatin targets. DFT studies on G/A platination by Friesner *et al.* using bare nucleobases clearly supported Cisplatin kinetic/thermodynamic preference to G over A, but still lacked in the discrimination among 5'/3' purine's positions. Last year, Mantri *et al.* have calculated the approximated activation free energies for the d(pApG) and d(pGpA) closure showing that the bifunctional adducts formation is ~9 Kcal/mol more favorable for the AG sequence. However, the diaqua-substituted derivatives of Cisplatin was used for the bifunctional adduct formation (nowadays, the monoaquo-substituted derivatives of Cisplatin is considered the most important active compound under physiological conditions). Also, the unconstrained optimization of the dinucleotides without considering DNA context did not allow them to conclude about the thermodynamic and structural differences. This point out the need of conducting studies on purine's platination using more realistic models of DNA, able to include the influence of the macromolecular context in near physiological environment.

In the present work three B-DNA 6 bp structures embedding Cisplatin GG, AG and GA intrastrand targets have been generated under near physiological conditions (37 ºC, 1 atm, electroneutrality by Na^+ counterions) with Molecular Dynamics simulations (AMBER force field, TIP3P water in a octahedral box under PBC). 5'G/3'G monoadducts (5'G/3'G in GG; 3'G in AG; 5'G in GA) corresponding to reaction with mono- and diaquated Cisplatin derivatives have been generated on representative structures and optimized by QM/MM at the platinated region within each physiological DNA frame. The relaxed part of each structure included the drug moiety and the bonded G -described through a quantum DFT approach up to the center of the respective N-C glycosyl linkage- together with all the classical atoms comprised in a 8 Å regions from the former (~615 atoms). A detailed analysis of the electronic structure over the 4 central nucleobases in the 5'G/3'G sequences has also been performed by QM/MM single-point calculations considering a single strand quantum window. This made possible to assess the nature of the electron density reorganization accompanying 5'G/3'G platination. To characterize bifunctional adducts formation, structural changes from the calculated 5'G/3'G in the GG monofunctional adducts were analyzed using the available experimental structures (PDB ID: 1a84, 1aio and 1au5). Results reveal a more favorable bifunctional closure starting from the 5'G monofunctional adduct in the GG sequences.

II-11

Development of a coarse-grained model of DNA and bulk water to tackle the simulation of DNA-drug interactions at the mesoscopic scale

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Keywords: Coarse-grained models, DNA, Bulk water, Molecular Dynamics.

All-atoms molecular dynamics (MD) simulations are a very powerful tool to predict structural, dynamical, and thermodynamical properties of biological molecules. Nevertheless the current computational power constrains this analysis to time scales of a few hundreds of nanoseconds, too short to follow several important biological processes, such as ligand-biomolecules recognition, protein-protein interactions, transcription regulation, signaling, complex self-assembly, etc. In addition, the number of degrees of freedom of biological systems is very large, and an appropriate phase space exploration of large length scales biological molecules is not feasible. To bridge the gap between times scales of practicable simulations and those of biologically relevant motions and also fill the lack between a microscopic representations of biomolecules to mesoscopic length scales, several simplified methods have been proposed. One type of such methods are based on a coarse-grained (CG) representation of the all-atoms system in which the potential energy is expressed in terms of harmonic springs between spatially close effective centroids representing functional groups or residues in biomolecules. Several properties calculated with these approaches agree well with experimental and/or MD data with significantly less computational efforts.

While adequate representations of DNA exist at the atomic (all-atoms) and continuum level, there is a relative lack of models capable of describing its behavior at mesoscopic length scales. In addition, the need of a coarse-grain model of DNA that preserves the molecular recognition and specificity between DNA strands and between DNA and physiological conditions compelled us to develop a mesoscale model of DNA that reduces the complexity of a nucleotide to six interactions sites. This model preserves the 5'-3' structural polarity of the DNA chains and the molecular nature of the Watson-Crick's hydrogen bonds. As most of the degrees of freedom of a biological system, and hence the computational demand to simulate it, are generally associated with the environment (solvent, ions concentration, etc.), we also present a coarse-grained model to describe bulk water (WAT4 model) in which five water molecules are replaced with four effectives centroids. Three duplex DNA sequences, each containing 24 base pairs (bp), namely $d\{pA_{24}\}\bullet d\{pT_{24}\}$, $d\{pC_{24}\}\bullet d\{pG_{24}\}$, and a 24 bp extension of the Dickerson's dodecamer $d\{pCpGpCpGpApA\mathbf{pCpGpCpGpApApTpTpCpGpCpG}pTpTpCpGpCpG\}_2$ were simulated with MD methods using all-atoms and the coarse-grained representations. For DNA, parameters compatibles with amber type force fields are being adjusted to reproduce the structural behavior of the double helix. Preliminary results about the DNA structural stability and the transitions from the A to the B form are presented. In the WAT4 model the parameters that are also compatibles with the amber force field are being tuned to reproduce water properties like fluidity, and radial distribution function of water molecules. These models provide a scaffold for a more realistic representation of Drug-DNA interactions within a biological environment.

II-12

Drug design & discovery in resource limited settings- the Pacific experience

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Traditional health practices have involved a large number of plants being used for treatment of human disorders. World Health Organization estimates that 80% of people living in developing countries use traditional medicine with medicinal plants forming an important component of traditional medicine. This gives an impression of about 3.3 billion people using medicinal plants on a routine basis. Pacific wide practice of relying on traditional healers and their use of natural products has been documented since the early colonial times. Compared to other traditional medicine systems, Pacific indigenous practitioners have relied on fresh products from plants. Besides many common and incurable ailments, traditional medicine offers a glimmer of hope for emerging health problems. Much of this knowledge had been passed on to next generation by older practitioners in the absence of written vernacular records.

Antimicrobial resistance among organisms responsible for many serious diseases is one such area. We conducted a study to standardize the testing method for antimicrobial activity among natural products of the Pacific Islands. Antimicrobial sensitivity was done for extracts of 10 herbal plants using fresh and dry plant material, different solvents for the reconstitution of plant extracts and different agar diffusion methods. These plant extracts were selected after consultation with WAINIMATE (traditional women healer group in Fiji). Of the 10 plant extracts tested; all showed activity against one or more of the organisms, and five of them showed strong activity. We also found that methanol is better solvent than di-metyl suphoxide (DMSO) for reconstitution of crude extracts. Dry extracts showed stronger activity than fresh ones, & well diffusion method was more useful than disk impregnation method.

Conclusion: Traditional herbal medicines have been used in Pacific for thousands of years. Although their benefits have long been observed, most of these plants have ever been scientifically tested for their effects. Anti microbial activity exhibited by almost all the plants proved their use by traditional healers for various infectious conditions. Therefore, in addition to standardize the method for antimicrobial testing, this study has sought to verify the effects as believed by the indigenous population of Fiji. Further analyses of extracts to identify active ingredients have been a limiting factor in exploiting commercial benefits of these products. Besides antimicrobial properties, a number of other plant products have been used for there beneficial effects on various ailments, ranging from common cold to life threatening conditions. Identification of active ingredients from these extracts after proving their efficacy has the potential to be of huge economic advantage to the indigenous populations in the pacific.

II-13

Comparative Study on the Incorporation Efficiency of Zanthoxylum tingoassuiba Essential Oil in Multi-and Unilamellar Vesicles: Potential Antimicrobial Applications

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Essential oils have a high content of terpenes easily prone to oxidation and resinification. In addition, oils have low solubility in aqueous media, limiting their pharmaceutical applications. In order to circumvent those disadvantages, microencapsulation has been proposed as an alternative to improve stability, solubility and biological activity of essential oils. In recent years, liposomes have been extensively studied as a delivery system which can improve the activity and safety of many molecules. Further, liposomes are regarded as suitable carriers because they can serve as a depot system for the sustained release of an associated compound and enhance the molecules targeting to cells. Pursuing our interest in medicinal products obtained from Rutaceae plants, in this work we have studied the preparation and characterization of multilamelar liposomes entrapping essential oil (EO) from Zanthoxylum tingoassuiba. Essential oil from Z. tingoassuiba loaded into multilamellar liposomes was successfully produced through thin film hydration method with mean diameter of 9.4μm. The liposome-incorporated EO showed good sphericity and narrower size distribution than empty liposomes. Results of GC-MS and UV-VIS spectrophotometer revealed that EO incorporation efficiency in liposomes was approximately 50%. A qualitative analysis by Thin Layer Chromatography revealed that essential oil was successfully incorporated into liposomes with no exclusion of the essential oil components. Differential scanning calorimetry (DSC) was applied to further investigate interactions of essential oil with a liposome. Changes in the liposome DSC endotherms occurring in the presence of essential oil suggest that it is situated in lipid bilayer of liposomes. The antimicrobial activity of incorporated and free EO against some ATCC bacteria, mushroom and multiresistent clinical isolates of S. aureus was evaluated. Free EO inhibited the growth of all microorganisms tested. Results obtained clearly indicate that essential oil from Z.tingoassuiba has been successfully incorporated into multilamellar liposomes. Liposomes can be useful in enhancing the solubility and antimicrobial activity.

II-14

Design of Ligands with High Affinity to the Cellular Form of the Prion Protein

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Prion diseases, also known as transmissible spongiform encephalopathies (TSE), are fatal neurodegenerative disorders of the central nervous system. The main molecular mechanism underlining TSE is based on the aberrant misfolding of the cellular form of the prion protein (PrP^C) into its pathological counterpart denominated PrP^{Sc}. To date there are no identified therapies. One therapeutic strategy against this disease is focused on the stabilization of the PrP^C in order to prevent its conversion to PrP^{Sc} [1]. Designing ligands targeting PrP^C with a high binding affinity might augment its stability and prevent its misfolding. Here, we have set-up two computational protocols aiming at addressing this issue.

Protocol **1**

i. Identification of putative binding sites of PrP^C. Although this approach follows already established procedures [2], this task is challenging due to the lack of ligand-specific binding pockets along PrP^C structure.
ii. Docking of known active compounds against PrP^{Sc} putative replication pathway [3, 4] in the PrP^C binding sites identified in (i).
iii. Molecular dynamics simulations to investigate the stability of the ligand-PrP^C adducts predicted in (ii).
iv. Calculation of the binding affinity of these ligands to PrP^C by means of enhanced sampling simulation techniques [5].

The obtained results are in agreement with experimental data [3]. We are now using this protocol for the design of new compounds with a high affinity to PrP^C, which may eventually show an improved activity against PrP^{Sc} conversion and replication. Such compounds will be synthesized and subsequently tested in cellular models of prion replication.

Protocol **2**

We have screened different ChemBridge® databases including about 280,000 small organic molecules (all commercially available), with specific properties such as drug-likeness, oral bioavailability and blood-brain barrier permeability [6].

Using ligand-based searching techniques we identified ~100 compounds chemically similar to previous reported active molecules [3, 4]. Furthermore, these molecules have been submitted to protocol **1**. Candidate compounds, that will exhibit a high affinity to PrP^C *in silico*, will be tested in cellular models of prion replication.

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Acknowledgements

Funding by ***IIT-SISSA*** is gratefully acknowledged

II-15

3D QSAR Studies of Gossypol-like Inhibitors of *Plasmodium falciparum* Lactate Dehydrogenase as Potential Antimalarial Drugs

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Gossypol [*2,2'-bis-(Formyl-1,6,7-trihydroxy-5-isopropyl-3-methylnaphthalene)*], a polyphenolic, binaphthyl disesquiterpene extracted from seeds of the cotton plant, *Gossypium hirsitum*, was shown to be lethal to the parasite *Plasmodium falciparum* by inhibiting the lactate dehydrogenase (*pfLDH*) responsible for the oxidation of NADH to NAD^+[1]. This is a vital process for the survival of this parasitic protozoan, largely responsible for the endemic malaria disease in most parts of the world. Several quantum chemical calculations have earlier been performed by our group in unpublished works which we do not present here. The work presented here is the second stage of our investigation of the gossypols and hemi-gossypols as potent inhibitors of the *pfLDH*. In this study, we investigated gossypol[2] and hemi-gossypol[1, 3] derivatives from two series of experimental data, that have been proven to bind effectively at the active site of the *pfLDH* near the Arginine 171. Using the 3D QSAR CoMFA approach, as implemented in the ALMOND software, we have established a good correlation between the structure and the desired activity. Calculation of Quantum Chemical Molecular descriptors leading to the LUMO-HOMO investigations of the hydrogen transfer processes are also performed with Gaussian 03W and are in line with experimental data. This proved that the structures being investigated could serve as leads in our search for new anti-malarial drugs.

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II-16

3D-QSAR model of β-secretase inhibitors as potential anti Alzheimer drugs

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Beta-amyloid peptides are produced in a β-secretase (BACE) catalyzed process and play an important role in the degeneration of the Alzheimer's disease. Therefore extensive screening procedures have been done in order to identify BACE inhibitors which could play an important role for the treatment, if not the cure of the disease itself.
A 3D-QSAR (quantitative structure activity relationship) approach has been successfully applied to build a predictivity model correlating structural properties of BACE inhibitors and their respective IC50 values. It is an alignment independent, GRID derived QSAR generated with the Almond algorithm. The initial data set included all structural classes of known bace inhibitors, corresponding to 79 compounds with IC50 values spanning from very high to very low activities.
The model has been developed with *in silico* screening approach based on ligand docking and energy minimization techniques yielding active conformers, subsequently analyzed in order to obtain suitable molecular descriptors which would account for the structural properties of the compounds, i. e. the molecular interaction fields (MIF). Finally multivariate analysis based on PLS has been performed to regress the so obtained data with the compound's due activity.
The generated model proved to be robust and predictive in both cross-validation and external validation approaches, performing a r^2 of 0,88 and a q^2 of 0,718 in the LOO (leave one out) validation method whereas the external validation corroborated the model's validity in correctly predicting the IC50 value of 12 additional inhibitors.

II-17

Peptidomimetic Antimalarials, Inhibitors of Plasmepsin II

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The study of highly active inhibitors (Ki 0.9 693 nM) of plasmodium falciparum aspartic protease Plasmepsin II in order to design new ones more active and selective to Cathepsin D with improved ADMET properties.

The study to construct the complexation model "protein ligand interaction" leads to a correlation coefficient of R2 = 0.86 (for 19 ligands) with a significance test (F test) of 108.32 between the complexation free energy ΔΔG and the inhibitory activity pKi (pKi = logKi).

In a second part it will be interesting to establish a similar correlation on the "Cathepsin D – ligand interaction". From those models and the general QSAR, new inhibitors with improved activity, selectivity and ADMET will be designed.

II-18

A Structure-Activity Relationship (SAR) Study on Analgesic Activity of Cannabinoid Compounds Using Chemometric Methods

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Keywords: cannabinoid compounds, analgesic activity, classification methods

Cannabis sativa is one of the first plants that have been used as a medicine, in religious ceremonies and recreationally. The first accounts of its use for these purposes stretching back 5000 years [1]. This plant is the unique source of a set of more than 60 oxygen containing aromatic hydrocarbon compounds known collectively as cannabinoids. It also contains a number of other compounds of potential interest, including at least 120 different terpenes and 21 flavonoids [2]. From these constituents, only two of these ones have many findings about their pharmacology: (1) Δ^9-tetrahydrocannabinol (Δ^9-THC), which has psychoactivity, and (2) cannabidiol, which is psychoinactive [2].

The rational search for new drugs is a very efficient strategy to obtain more specific and potent compounds without side effects. Some methods used for this strategy include studies based on structure-activity relationships (SAR) and quantitative structure-activity relationships (QSAR). The main goal of applying these methods is to transform the chemical structure of a compound into a set of numbers (parameters, properties or variables) that correlate them with the biological activity, establishing a qualitative/quantitative relationship between calculated molecular properties and biological activity [3]. The main goal of this work is to investigate the therapeutical aspect of cannabinoid compounds according to their analgesic potency by establishing the relationship between structure and activity for a set of 27 cannabinoid molecules. For this purpose we made use of two classification methods, Stepwise Discriminant Analysis (SDA) and Soft Independent Modeling Class Analogy (SIMCA), in order to predict the activity of new cannabinoid compounds and to validate previous results obtained with PCA and HCA analyses where five descriptors were selected: R3 (charge density on substituent at position C3), Q1 (charge on atom C1), A (surface area), log P (logarithm of the partition coefficient) and MR (molecular refractivity) [4]. After the PCA and HCA analyses, the supervised methods (SDA and SIMCA) were used to classify future samples and to build a model for prediction studies. The classification rules achieved were validated by means of a cross-validation procedure.

It is possible to observe in the three discriminant functions obtained with SDA that the variables R_3, log P, Q_1, MR and A have an important role in the SDA equations. The best SIMCA model found was the one built with the same variables used in SDA method. The results obtained with the SDA and SIMCA methods agree perfectly with our previous model. Comparing these results with PCA and HCA analyses we can note that all methods classify the 27 cannabinoid compounds studied in three groups exactly in the same way: active, moderately active and inactive.

CNPq, FAPESP, ICS-UNIDO

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II-19

Application of QSAR Studies for Searching Porphyrin Derivative Compounds as New Active Photosensitizer

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The presence of photodynamic activity as new treatment against cancer has created an urgent need to discover new photosensitizer agents. Quantitative structure activity relationship (QSAR) studies can be applied here to develop model that can correlate the structural features of porphyrin compounds with their PDT activity. The model development process began with the generation of molecular descriptors from three dimensional representations of 36 compounds in the data set. The best QSAR model developed using multiple linear regression analysis (MLRA) has r^2 value of 0.8697 and r^2 *(CV)* value of 0.7080. This model has high predictive power to predict the activity of unknown compounds in the external test set which is consists of 62 compounds.

II-20

Theoretical approach to depict the HP1γ- Suv39H1 interaction. Looking for a new target against HIV-1 infection

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The Hetrochromatin Protein 1 (HP1) is a well conserved nuclear non histone protein. This protein is present in almost all eukaryotic organisms from yeast to mammals. HP1 family mediates protein-protein interactions working as molecular adaptors. In such way HP1 plays many roles in the cell biology including gene regulation and chromatin condensation. Recently, it was shown that the interaction between the human proteins HP1γ isoform gamma), the methyltransferase Suv39H1 and the histone H3 is one of major cellular determinants responsible for chromatin mediated transcriptional silencing/activation of HIV-1. The inhibition of these interactions offers possibilities o new drugs targets to interfere with the viral replication process. The biologically active structure of HP1 is a homodimer. Each subunit is formed by two highly homologous domains at N and C terminal regions, which are respectively named chromo (CHROMatine MOdifier) and chromo-shadow (CS) domains, and an approx. 35 residues long region linking them. In particular, CS domain mediates homodimerization as well as the binding to a canonical motive PXVXL present in most of the proteins recognized by this domain. Suv39H1 binds to the CS though a 39 amino acid long sequence placed at N terminal region of the protein but does not contain the canonical motive and the specific recognized motive is still unknown. So, the fist step toward the design of a drug to prevent such interaction should be the identification of the interacting residues.

The present work shows preliminary *in silico* results of docking calculations performed in order to identify the specific motive in Suv39H1 recognized by HP1γ CS. Because up to date there is no resolved structure of HP1γ, we have used the structure of its high homologous isoform β, which shares nearly 80% identity, to build the homology model of the HP1γ,. To represent the structure of Suv39H1 at the binding site pocket, we have built small peptides containing various sequences to be tested for the binding affinity to the HP1γ,.

II-21

Characterizing Δ-[Ru(bpy)$_2$dppz]$^{2+}$ DNA probe intercalative behaviour at GG, GC and CG steps

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Ruthenium (II) complexes presenting the *dppz* (bypirido[3,2-a:2',3'-c]phenazine) ligand, are able to intercalate between DNA base pairs with high binding affinity (K_b>10^6 M^{-1}).[1,2] They also present the ability to emit when intercalated into DNA but are largely quenched in water solution, this effect is called *light switch*.[3] These features focus the attention on the usage of these complexes in diagnostic applications which target nucleic acids. It is know that many diseases are related to particular changes or mutations at DNA base pairs. So, two important aspects in the design of a drug or probe targeting DNA are the selectivity and affinity for a specific sequence. That sort of questions can be studied throw a theoretical approach by modeling and simulating the DNA-probe interactions at molecular level.

In the present study we present molecular mechanics and molecular dynamics calculations performed to get insight into the specific interactions between a Ru(II) complex and the base pairs at the intercalation site pocket. Results were analyzed from a structural and energetic point of view. The modeled system included the Δ-[Ru(bpy)$_2$dppz]$^{2+}$ complex intercalated into different steps of a DNA dodecamer within a salvation sphere of water and counter ions. Effects in changing the DNA sequence were explored comparing the behavior of tree sequences: 5'-CGCTT**GG**TTGCG-3', 5'-CGCTT**CG**TTGCG-3' and 5'-CGCTT**GC**TTGCG-3'.

Important sequence dependence behavior was observed in both structural and energetic properties. Some correlations with experimental data were done.

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II-22

Comparison of Two QSRR Studies of Substituent Effects in Flavones Using Multiple Linear Regression

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The methodology of relating chemical structures of solutes to chromatographic retention parameters aimed at determination of the most informative structural descriptors is recognized as quantitative structure-retention relationship (QSRR). In order to study the effects of various substituents in different positions in compounds derived from the same "parent compound" (flavone), the so-called *de novo* non-parameter approach has been applied. The assumption of additive substituent effects on retention was employed for estimation of the influence of OH- and OCH_3-groups in the most often substitution positions 3, 5, 7, 3', 4', 5', 6 and 8 on the retention in two separate groups of 21 and 38 flavone aglycones using the same approach and identical chromatographic conditions. The first group was tested using 15 indicator variables as independent ones describing the type and position of substituents and the logarithm of the retention factor (log k) of each solute obtained with isocratic elution with methanol or acetonitrile and 5 % acetic acid as dependent variables. In the second study, 19 independent variables were correlated to chromatographic retention data for 38 flavones. The effect of each substituent in each position in both models was evaluated by multiple linear regression. Satisfactory correlation coefficients were obtained in the range from 0, 9784 to 0, 9736. The regression coefficients confirm that incorporation of an OH-group does not necessarily reduce retention, and OCH_3-groups, depending on the position, can either cause a decrease or increase in retention. It is important to point out that the model still shows good correlation by the increase of the number of indicator variables. This structure retention approach is evident for either assumption of retention of new flavones, or simulation the possible substitution pattern of an unknown flavone derivative based on its retention.

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Homology Modelling of Cav1.2 Calcium Channel

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Cav1.2 calcium channel belong to L-type voltage gated channel (together with Na^+ and K^+ channels) which play a crucial role for pharmacological targets for treatment of cardiovascular disease. The access to experimental data about their structures by X-ray crystallography or NMR-spectroscopy is rather limited due to the fact that the geometries of these channels are to some extent determined by the surrounding matrix, the cellular membrane and the water molecules in the intra- and extracellular moiety. Up to now theoretical methods only allow to give some information about the molecular structures such ion channel. In particular homology modeling and molecular dynamics simulations are helpful tools in the investigation of this type of proteins.

Homology Modeling based on the crystal structure of KscA and NaChBac channel have been applied to create models which can be used for docking of verapamil molecules in order to identify the interaction of various verapamil molecules with corresponding Cav1.2 calcium channel. Development of the model and subsequent refinement based on the theoretical concept and mutation data lead not only action of ion channeals but given also some extending information about drug-channel interaction to a better understanding mechanism.

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Interaction with π-System in Crystal Structures of Transition Metal Complexes: New Types of Noncovalent Interactions

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By analyzing crystal structures of metal complexes from CSD and PDB and by quantum chemical calculations recognized noncovalent interactions:

(a) Cation-π interactions (that can be considered as XH/π hydrogen bonds of coordinated ligands) (b) C-H···π interactions with chelate ring (c) Chelate-phenyl stacking interactions. Interactions of cationic transition metal complexes with aromatic rings, triple and double bonds were predicted by DFT calculations. In these interactions ligands coordinated to the metal interact with aromatic ring and they are metal-ligand aromatic cation-π (MLACπ) interactions. They can be consider also as X-H···π hydrogen bond (MLXH/π) .Stacking interactions are preferred , while CH/π interactions are formed when stacking interactions are prevented. Geometry and energy of CH/π and geometry of stacking interactions similar to interactions of benzene ring indicating aromatic character of chelate rings.

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Bioassay-guided isolation of antimalarial protoberberines and aporphine alkaloids from *Annickia kummeriae*

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Malaria has remained a serious endemic disease in sub-Saharan Africa, Asia, Latin America and Oceania, with almost 40% of the world's population living in areas whereby the disease is endemic. Malaria causes mortality of about 2.7 million people each year. Because of the worsening problems of drug resistance, there has been an urgent need for the discovery of a new chemical class of antimalarial agents. In our on-going research program on antimalarial natural products we have screened a number of Tanzanian medicinal plants for their antimalarial activities. Among these, an extract from the leaves methanolic extract of *Annickia kummeriae* possessed significantly high antimalarial activity against *Plasmodium falciparum* (K1, a multidrug-resistant strain), with a 50% inhibitory concentration (IC_{50}) of 0.15 µg/ml and low cytotoxicity activity against rat myoblast L-6 cells with IC_{50} value of 15.6 µg/ml (selectivity index (SI) values of 104). Therefore, bioassay-guided chromatographic fractionation was carried out to identify fractions with even higher activity and with favourable SI and, thereafter, specific constituents from these fractions. This led to the isolation of six antimalarial alkaloids which were identified using spectroscopic methods as palmatine (**1**) (IC_{50} = 0.075 µg/ml), jatrorrhizine (**2**) (IC_{50} = 0.24 µg/ml), columbamine (**3**) (IC_{50} = 0.16 µg/ml), (-)-tetrahydropalmatine (**4**), lysicamine (**5**) (IC_{50} = 3.01 µg/ml), and trivalvone (**6**) (IC_{50} = 1.83 µg/ml). In this paper, detailed bioassay-directed chromatographic isolation of these antimalarial compounds and spectroscopic elucidation of their chemical structures will be presented and discussed.

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Genetic Algorithms for Molecular Docking Studies of HIV-1 Protease Inhibitors

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Keywords: docking methods, genetic algorithms, HIV-1 protease inhibitors

With the increasing amount of molecular biological structures available, docking approaches have been very important and useful tools in structure-based rational drug discovery and design. In this work, we analyzed the performance of a real coded "steady-state" genetic algorithm with a multi-solution strategy for the protein-ligand docking problem. The proposed method is the Modified Restricted Tournament Selection (MRTS) technique that has as objective to favour the preservation of good quality solutions in the population. The implemented method was tested in re-docking and cross-docking studies of five HIV-1 protease inhibitors with high conformational flexibility. The docking methodology was able to re-dock successfully all flexible ligands with a success ratio >97% and a mean RMSD lower than 0.8 Å with respect to the corresponding experimental structures. In the cross-docking experiments we observed a strong dependence of the mean success ratio with respect to the protein structure used as the reference. The results obtained indicate that the use of multiple-solution techniques for the preservation of useful diversity can be a powerful tool for highly flexible ligand docking. These strategies increase the probability of finding structures close to the experimentally determined ones, and permit the determination and posterior investigation of distinct ligand-receptor binding modes.

Currently, the test set of ligands has been extended to ten HIV-1 protease inhibitors and we are applying the concept of complexation energy:

$$E_{int} = E_c - E_f$$

where E_{int} is the non-bonding ligand-receptor interaction energy, E_c is the calculated total molecular mechanics energy of the ligand-protein complex and E_f is the total energy of the free ligand. The relationship between the complexation energy and the experimental affinity for each ligand tested is being investigated. Furthermore, the inclusion of the receptor side chain flexibility of residues surrounding the active site pocket is also being studied.

CNPq, FAPERJ, ICS-UNIDO