

**COST ACTION B22**  
**DRUG DISCOVERY & DEVELOPMENT FOR PARASITIC DISEASES**  
**3rd ANNUAL CONGRESS**  
**1-4 October 2006**  
**National Hellenic Research Foundation**  
**48 Vassileos Constantinou Avenue**  
**Athens, Greece**

The 3rd COST B22 Annual Congress was held at the National Hellenic Research Foundation in Athens, Greece, 1-4 October 2006 and was attended by one hundred and seventy participants. The organising committee was very glad to welcome scientists from all over the world, since all five continents were represented at the congress. The majority of the participants came mostly from the 21 European countries which have signed the memorandum of understanding of COST Action B22 but there was also active representation from South Africa, Congo, Brazil, China, Taiwan, India, U.S.A., Canada and Australia. In addition, eight scientists from developing countries received a scholarship from the IOCD (International Organization for Chemical Sciences in Development) and were able to attend the conference.

In recent years the large volume of information accumulated in the fields of parasite biology and biochemistry enabled the rational design of antiparasitic agents. In addition, the challenge of identifying good drug targets relies on integration of disparate data from high-throughput technologies such as genome analysis, microarrays, proteomics, structural genomics and metabolic networks. Furthermore, medicinal chemistry plays a pivotal role in the drug discovery process, linking and exploiting the associated biological sciences. Thus, we organised the 3rd COST B22 Annual Congress in 8 thematic sessions, with 7 state-of-the art lectures, 40 oral presentations and 62 poster presentations, highlighting a variety of emerging fields and novel applications. The posters were very centrally located and could be viewed by the participants not only during the coffee and lunch breaks but also during the Poster Session on October 3rd (9.00-10.30 a.m.).

The congress was opened on October 1st with welcome addresses by the local organizer, the President of the National Hellenic Research Foundation, Prof. Dimitrios A. Kyriakidis, the ESF-COST office Scientific Officer of Biomedicine and Molecular BioSciences, Prof. Mihail Pascu, and by the Chairman of COST Action B22, Prof. Fred R. Opperdoes.

Subsequently, the Chairman of the Scientific Council of the European Research Council, Professor Fotis C. Kafatos (Imperial College London, UK) delivered the Plenary Lecture of the congress, entitled "Innate immune responses of Anopheles to Plasmodium: Opportunities for novel antimalarial interventions?" Prof. Kafatos' talk focused on *Anopheles gambiae*, the most important vector of the human *Plasmodium malariae* parasite in Africa and by employing comparative genomic studies including transcriptomic profiles and genetic screens, information was obtained on the immune system as a whole and its functional modules. More specifically, the modules of phagocytosis, local responses of the midgut epithelium during its invasion by the parasite and humoral melanization responses were studied in more depth, which could serve as revealing opportunities for future development of novel antimalarial interventions.

The first Session, entitled "Drug discovery and development for parasitic diseases" began on Monday October 2nd. Dr Mary Moran (The George Institute for International Health, Australia) was invited to deliver a keynote lecture on different funding models of R&D for neglected diseases. Dr Moran highlighted the successful results of public-private R&D collaborations in terms of both the developing country

health value they deliver and their cost-effectiveness to donors. Furthermore, the formal Product Development Public-Private Partnership organisations (PDPs) are by far the most successful approach according to Dr Moran's research results.

Dr Ian Gilbert (U. of Dundee, UK) described their studies on the unique substrate specificities of P2 transporters of *Trypanosoma brucei*, that led to the design and synthesis of selective antiparasitic compounds bearing the melamine motif that don't cross-react with the mammalian purine transporters. Dr Davioud-Charvet (University of Heidelberg, Germany) reported the development of prodrugs consisting of an optimised chloroquine analogue and a glutathione reductase inhibitor linked via a labile bond. The compounds are released within the parasite where they are able to act synergistically via two different mechanisms. The compound optimisation led to the second generation of antimalarials with more potent activities. Dr Paul O'Neil from the University of Liverpool, UK, described their studies on the mechanism of action of different classes of antimalarial compounds that contain an endoperoxide moiety. By using single-cell imaging in living malaria parasites and isobologram analysis with the iron chelator desferrioxamine they have demonstrated that the analogues were irreversibly bound to a yet unidentified protein in the parasite cytosol. He reported on new synthetic fluorescent probes that will be used for the proteomic analysis of the endoperoxide targets.

Dr Rentala Madhubala from the Jawaharlal Nehru University, India, reported on the activity of 3-aminooxy-1-aminopropane (APA), a ornithine decarboxylase inhibitor as antileishmanial compound. Overexpression of ornithine decarboxylase (ODC) caused not only resistance to APA but also resistance to pentostam. Dr Narimantas from the Institute of Biochemistry in Lithuania reported on the cross-reaction of quinoidal and nitroaromatic compounds, which are inhibitors of malarial glutathione reductase and trypanothione reductase, respectively with the mammalian thioredoxin reductase. Enzymatic analysis studies will lead to compound optimisation for reduced mammalian cell cytotoxicity.

The second session dealt with "Selective targets for antiparasitic compounds." Prof. Mike Gelb from University of Washington, U.S.A.. was invited to discuss about farnesyltransferase inhibitors as anti-malarial agents and the progress toward the pre-clinical development of these compounds including SAR studies, pharmacokinetic studies and efficacy in rodents. In addition, Prof. Gelb discussed the development of lanosterol 14-demethylase inhibitors as clinical candidates for Chagas disease. The talk of Prof. Gelb illustrated how pre-clinical drug development can be achieved in an academic environment. Prof Fred Opperdoes from Christian de Duve Institute of Cellular Pathology, Belgium reported on the studies on trypanosomal peroxins. The parasite orthologues were identified and their role in the glycosome biogenesis was investigated by RNAi-dependent knockdown of expression. The interesting feature of these enzymes is their little homology with their human counterparts that makes them promising drug targets.

Dr Silvia Moreno from University of Illinois, U.S.A described their studies on the identification and characterisation of the first bifunctional farnesyl diphosphate/geranylgeranyl diphosphate synthase described in *Toxoplasma gondii*. This mitochondrial enzyme can be readily inhibited by bisphosphonates making it a promising target for the chemotherapy of toxoplasmosis. Dr R Luise Krauth-Siegel from the University of Heidelberg, Germany reported on the elucidation of the function of the two types of peroxidases in *T. brucei*. Site directed mutagenesis highlighted the role of the three residues (Cys47, Gln82 and Trp137) essential for catalysis in the peroxidase III, but also pointed out the role of an extra residue, the Cys95, that is not present in the classical glutathione peroxidases. Crystallographic

analysis of these peroxidases will give information on the specific sites that will be considered as potential drug targets.

Dr Ingrid B Mueller from the Bernhard Nocht Institute for Tropical Medicine of Hamburg, Germany discussed their studies on the polyamine synthesis on *P.falciparum*, and its exploitation as drug target. By using specific inhibitors of the enzymes ODC, AdoMetDC and SpdSyn and gene depletion experiments, the importance of the pathway to the survival of the parasite was shown. Dr Mueller also pointed out the complete dependence of the parasite on the spermidine biosynthesis by the corresponding synthase thus, making the pathway a valid target for drug development. Dr G. Coombs University of Glasgow, UK reported on the metacaspases of trypanosomatids, which are distant orthologues of caspases and are not present in mammals. In *T. brucei* five such enzymes are found, but in *L. major* a single one is transcribed in both stages. These enzymes do not seem to have a direct role in apoptosis processes but rather on the cell cycle, making them possible drug targets. The theme of the third session was "Antimalarial drug targets and evolution of resistance."

The keynote speaker, Ian Hastings from the Liverpool School of Tropical Medicine, UK discussed and analysed the complexity, causes and sources of the spread of drug resistance in the plasmodium. The model takes in account the sequential accumulation of several mutations during drug treatment in the mammalian host. Interestingly not all of these mutations persist during the life cycle of the parasite, but some of them are restricted probably in the vector stage. He also stressed on how the sequential accumulation of several mutations of the parasite and the persistence of drugs at sub-therapeutic levels in the human host are important drivers of the resistance. The model he presented will be particularly useful for the design of an effective combination therapy. The session then continued with oral presentations on new antimalarial drug targets with the aim to overcome the resistance problem. Dr Timothy Egan from the University of Cape Town, South Africa, reported on the exploitation of the unique pathway of haemozoin formation as a drug target for antimalarials. This target will be of importance especially in resistant parasites. Studies on structure-activity relationships using -haematin inhibitors gave valuable results on the identification and mode of action of active chloroquine analogues.

Another interesting work was presented by Dr Carsten Wrenger from the Bernhard Nocht Institute for Tropical Medicine, Germany. Dr Wrenger presented the biochemical evidence of a B1 and B6 synthetic pathway in malaria parasite that is absent in the mammalian host, thus making it a potential antiparasitic drug target. Dr Angus Bell from Trinity College, Ireland discussed aminopeptidase inhibitors as antimalarial drugs including their mode of action. Dr Yaakov Pollack from Ben-Gurion University of the Negev, Israel reported on the expression of the var genes in *P. falciparum*. The Var proteins are located on the infected erythrocyte membrane and play a role on the parasite virulence. The study was focused on the control mechanism of expression of the var gene family and the possibility to design inhibitors that will be valuable drugs against cerebral and placental malaria.

In the fourth session entitled "Rational design and drug delivery systems," Prof. Don Hilvert from ETH, Switzerland presented the interesting story of the intracellular shikimate pathway as a promising target for the development of antiparasitic drugs and herbicides and utilised chemical and biological approaches to elucidate the role of chorismate mutases which are key enzymes in the shikimate pathway. The session continued with three oral presentations on drug design. Dr Giuseppe Campiani from the European Research Centre for Drug Discovery and Development, Italy described the rational design and synthesis of novel antimalarials based on a polyaromatic scaffold. Dr Maria P. Costi from the University of Modena, Italy reported on the use of Autodock 3.0, a structure-based drug design program, for the assessment of the

binding mode of inhibitors to the known enzyme pteridine reductase. This approach led to the rational selection of the best scored inhibitors for synthesis and in vitro assays. Dr Thomas Mavromoustakos from the National Hellenic Research Foundation, Greece reported on the application of COMFA and COMSIA 3D-QSAR studies on antileishmanial ring-substituted ether phospholipids which can be utilised in the rational design of new analogues with improved pharmacological profile.

The two final presentations of this session dealt with drug delivery systems for antiparasitic drugs and more specifically Dr Marinos Pitsikalis from the University of Athens, Greece described double hydrophilic block copolymers carrying zwitterionic groups as encapsulating carriers of antileishmanial ether phospholipids while, Dr. E. M. Cruz from INETI, Portugal dealt with liposomes as delivery systems for antiparasitic dinitroanilines. These formulations have been tested in a visceral model of *L. donovani* and in a cutaneous model of *L. major* and they have proved to reduce by 70% the parasite growth.

The fifth session explored the subject of "Nature as a source of antiparasitic drugs." The keynote lecture was given by Prof. Ernesto Fattorusso from the University of Napoli, who discussed the exciting story of Plakortins, 1,2-dioxane derivatives isolated from the Caribbean sponge *Plakortis simplex* which exhibit potent antimalarial activity. SAR-studies with semi-synthetic derivatives probed the stereochemical features important for activity, while extensive computational analysis including molecular mechanics, molecular dynamics and quantummechanical calculations gave insight into the mechanism of action of plakortins.

Four oral presentations on naturally occurring antiparasitic compounds followed, and in more detail, Dr Richard K. Haynes from The Hong Kong University, R.O.C., reported on artemisinin antimalarials and more specifically about their thermal and chemical stability. Dr Nancy Terryn from Department Plant Systems Biology, Belgium, described a project on genomic approaches for the production of artemisinin in plants, by integrating genome-wide microarrays, proteomics and bioinformatics in order to identify the genes involved in artemisinin biosynthesis and regulation. Dr H. Ginsburg from The Hebrew University of Jerusalem, Israel reported on structure-activity relationship studies of the antimalarial peptide Dermaseptin S4. More precisely by shortening the peptide, increasing the hydrophobicity and maintaining the lysyl residues they managed to enhance the antimalarial activity without lysing the erythrocyte membrane.

The session ended with the presentation of Alicia Ponte-Sucre from the University of Würzburg, Germany on synthetic derivatives of the naturally occurring naphthylisoquinoline alkaloids derived from Ancistrocladaceae and Dioncophyllaceae lianas as antileishmanial drugs. The analogues proved to be more selective to the parasite and the presented evidence demonstrate the importance of the quaternary nitrogen atom for this selectivity.

The sixth session was devoted to "Genomic and proteomic approaches for drug discovery." Prof. Barbara Papadopoulou from the Laval University, Canada was invited to discuss the unique features of regulation of gene expression in *Leishmania*. She reported on the large-scale identification of stage-specific genes expressed in *Leishmania* using genome-wide approach consisting of protein pre-fractionation followed by proteomic analysis and DNA microarrays. They have thus identified both proteins and mRNAs specifically expressed in the different stages of *leishmania*. Interestingly there is poor, or absence of correlation of mRNA and protein expression in the amastigote and promastigote stage of the parasite, meaning that translational regulation is very important. Moreover proteomic analysis revealed extensive posttranslational modifications in stage specific proteins. Dr Papadopoulou analysed a mechanism of posttranscriptional regulation in the amastigote stage that

is mediated by class I and II retroelements that are found within 3' untranslated regions. These mechanisms could be good targets for new antiparasitic drugs.

Subsequently, Dr Sho Tone Lee from the Academia Sinica, Taipei, R.O.C., reported on the screening for genes and biochemical pathways involved in arsenite resistance in *Leishmania* using a genome-wide screening approach. The results reveal the importance of genes involved in energy generation, protein phosphorylation and detoxification of oxidative stress in the survival of resistant variants., Dr Frederick Buckner, University of Washington, U.S.A. described the studies on the identification of ligand-bound structures for different biomedically important protozoan proteins. The procedure includes identification of protein targets using genome information and protein crystallization in the presence of the relevant ligands.

The next speaker was Dr Georgios Tsiotis from the University of Crete, Greece, who presented exciting data on the proteomic analysis of *Coxiella burnetii* Phase II stage. The analysis was performed by 2D PAGE and the identification by MALDI-TOF MS. The study seems to be very useful in identifying proteins expressed during the infectious process that could serve as drug targets. Dr. Despina Smirlis from the Hellenic Pasteur Institute, Greece reported on the role of the Leishmanial histone H1 in the virulence of the parasite. With an elegant methodology she found that overexpression of H1 dramatically affects the differentiation of promastigotes to amastigotes. The hypothesis that H1 interferes with cytoskeletal proteins is under investigation.

The seventh session entitled "Antiparasitic drugs: the resistance challenge" began with the keynote lecture by Marc Ouellette from Laval University, Canada. He reported on RNA and protein expression profiling as a means for finding novel drug targets and identifying resistance mechanisms in *Leishmania*. For this purpose full genome DNA microarray and proteomic analysis have been applied for the study of the mode of action and resistance mechanisms to anti-leishmanial drugs. He described the exciting results on resistant field isolates that have been used for studying the alteration in drug-induced programmed cell death.

Subsequently, Prof. Rentala Madhubala from Jawaharlal Nehru University was invited to speak about their investigations on the mechanism of resistance to sodium antimony gluconate (SAG) in *Leishmania* parasites isolated from SAG unresponsive patients. The resistance of clinical isolates of *Leishmania donovani* to SAG has become a critical issue in India since this is the mainstay of treatment in Indian visceral leishmaniasis. She showed that MRPA gene amplification is also taking place in some field isolates. Also polyamine and glutathione biosynthesis enzymes are found to be overexpressed in many field isolates, suggesting that they participate in the expression of resistance.

This session then continued with interesting new findings on the targeted metabolic stimulation of the host resistance in mebendazole-tolerant nematodes, presented by Dr Igor G Bondarenko Chydenius Clinic, Finland. Dr Vincent Delespau from the Institute of Tropical Medicine of Antwerp, Belgium, presented their studies on the uptake of diamidine compounds in *T. brucei* and *T. congolense*, and on stage-specific in vitro activity of reference drugs against *Leishmania donovani* field strains. The results showed that there is no common mechanism for the uptake of different diamidines and therefore various resistance mechanisms can be developed. Dr M. Vermeersch from the University of Antwerp, Belgium presented interesting results on the differentiation rate of field strains of promastigotes to intracellular amastigotes. Using in vitro inhibition and ultrastructural studies it was shown that this process may take up to 96 h. This is particularly important for the development of in vitro drug testing models.

The eighth and final session entitled "New insights in antiparasitic drug development" encompassed several short communications on antiparasitic drug development and more specifically, Dr Amanda M. Mathis from University of North Carolina, U.S.A. reported on the in vitro accumulation and distribution of diphenyl furans in relation to their anti-trypanosomal activity. The uptake of the analogues is comparable but the site of accumulation within the parasite is different. It was presented that the most potent compounds accumulated in the kinetoplast.

Dr Vanessa Yardley from the London School of Hygiene & Tropical Medicine, UK, reported on the clinical development of the anticancer drug Tretazicar as a new treatment for leishmaniasis. The interesting feature about this compound is that it is a prodrug which can be selectively activated by the parasite by nitroreduction. It shows activity against *Trypanosoma cruzi* as well as *Leishmania infantum*. Dr D'Alessandro Sarah from the University of Milan, Italy reported on the evaluation of the antimalarial activity of Artemisone which is 10 times more potent than dihydroartemisinin and also less cytotoxic. More precisely, it is less inhibitory against endothelial cell proliferation and erythroid cell differentiation in vitro and less neurotoxic.

Dr Ioulia Smonou from the University of Crete, Greece described the enzymatic synthesis of chiral valuable intermediates for the preparation of antiparasitic compounds. Finally, Dr Ana Domingos from INETI, Portugal reported on chabaupain-1 which is present in infected but not in healthy *Anopheles* extracts and thus, could be a very interesting target for malaria therapy.

The congress was closed by the chairman of the COST B22 action, Prof. Fred Opperdoes, who invited all the participants to the next annual conference, which will take place in Dundee, UK. Athens, 26 October 2006.