

## Introduction

The research area Parasite Chemotherapy comprises activities in drug discovery and development against protozoan parasites, mainly malaria and human African trypanosomiasis (HAT), and for helminths. Besides working on new drugs, the group also collaborates with African partner institutes in the Eastern Africa Network for Trypanosomiasis (EANETT), focusing on sleeping sickness research and control. A third area of study is trypanosome transmission through the tsetse fly vector, to gain more knowledge about parasite-vector interactions and the function of certain surface proteins.

Drug-screening activities increased during the last 2 years. Our group is a main screening centre for the WHO Special Programme for Research and Training in Tropical Diseases (TDR) and the Drugs for Neglected Diseases initiative (DNDi) for in vitro screening against protozoan parasites. The project "Oral diamidines for sleeping sickness" led by the University of North Carolina has changed its name to "Consortium for Parasitic Drug Development" (CPDD) and is inviting projects on chemical classes other than dicationic molecules. A setback in this consortium was the termination of DB289 in phase III clinical trials for first-stage *gambiense* sleeping sickness based on so far unobserved nephrotoxicity. Drug discovery work is now focusing on second-stage sleeping sickness, searching for compounds which have the capacity to enter the brain. We are also collaborating with partners in the EU project "dUTPase as a drug target for malaria", which aims to inhibit the enzyme deoxyuridine triphosphate nucleotidohydrolase in *Plasmodium falciparum* and thus kill the parasite.

The Parasite Chemotherapy group has an additional screening mandate with the Medicines for Malaria Venture (MMV) mainly as collaborators in the project "Next-generation synthetic peroxides" and for exploratory experiments. Further development of the molecule OZ277 was discontinued at the end of December 2006 due to differences in plasma levels between volunteers and patients. The backup programme had already identified other peroxides superior to OZ277 from which a new

clinical candidate was selected in early 2008. In the field of malaria drug discovery and development, we are also engaged in a major collaboration with the NGBS (see below) consortium under the leadership of the Novartis Institute for Tropical Diseases in Singapore. This consortium has its own R&D portfolio and has close links to MMV with funding from the Wellcome Trust. Besides being involved in R&D activities for protozoan parasites, we are carrying out similar drug screening against helminths, mainly schistosomes and food-borne trematodes. The helminth work is under the leadership of J. Keiser, who received an assistant professorship grant from the Swiss National Science Foundation (SNSF). She is mainly testing artemisinins and synthetic peroxides but also compounds which have demonstrated activity against protozoan parasites (see section 6).

EANETT is well established and has incorporated Malawi and Zambia as new member countries. The network supports research collaboration, an annual conference, training workshops and MSc and PhD projects. The network also acts as a platform for clinical trials for new medicines and for new diagnostics.

Fundamental research on trypanosome transmission by tsetse flies has continued. In collaboration with the University of Bern, genetically modified trypanosomes have been used to elucidate the biological role of different surface proteins during development in the vector. The PhD project "Cross-talk between *T. brucei* and the tsetse fly" is a collaboration between the STI, the University of Bern and Yale University in the United States.

## 4.1 Drug discovery of new antiprotozoal compounds

### Drug screening for TDR/WHO

The STI has been a partner of TDR's compound screening and evaluation network for many years. The network allows scientists from academia and industry to submit compounds to be screened against protozoan parasites free of charge. Our mandate is in vitro screening of com-



Group photo of the Parasite Chemotherapy group. (Photo R. Dürri)

pounds supplied by WHO/TDR or through our own collaborations and follow-up of active compounds in mouse models of infection. Antiparasitic activity against several protozoan parasites and a mammalian cell line can be determined using less than 1 mg of a compound. Two types of in vitro assays are used, both in 96-well-plate format: a medium-throughput screen (MTS) and a serial drug dilution assay that determines 50% inhibitory concentration (IC<sub>50</sub>). The MTS uses only one or two compound concentrations to identify active molecules. These are then screened in the serial drug dilution assay to obtain an IC<sub>50</sub> value. Determining cytotoxicity adds information regarding selectivity for the protozoan parasites. We are currently screening about 10,000 compounds annually in the MTS and over 1,500 in the serial drug dilution assays against three to four parasites. Seventy percent of the tested compounds are supplied by WHO/TDR, and 30% are obtained through our own collaborations. We have received compounds from over 20 suppliers, including groups from university institutes and small companies.



Tanja Wenzler reading an in vitro drug assay for African trypanosomes in a fluorescence scanner. (Photo M. Cal)

### Drugs for Neglected Diseases initiative

DNDi was founded in 2003 to promote R&D for drugs for neglected diseases, focusing on the trypanosomiasis and leishmaniasis. In 2005 the STI became a partner, with a screening mandate and a project-independent contract. Our group collaborates in several DNDi projects that screen compounds for antiprotozoal activity in vitro and in rodent models for sleeping sickness. In the project on nitroimidazoles for sleeping sickness and Chagas disease we screened about 600 compounds in vitro and over 50 in our acute *Trypanosoma brucei rhodesiense* mouse model. Twelve compounds cured at least three of four mice at 4 × 50 mg/kg applied intraperitoneally, and an additional 5 compounds at 4 × 100 mg/kg given orally. Two nitroimidazoles cured the chronic mouse model with central nervous system (CNS) infection. The most promising compound is fexinidazole, which is orally bioavailable and cures the CNS mouse model. Fexinidazole was selected by DNDi as a clinical candidate. A phase I clinical trial is planned for early 2009. We also provide training and technology transfer to the members of the

- *Plasmodium berghei* (ANKA strain) in mice
- *P. chabaudi*, *P. vinckei* both in mice
- *P. falciparum* (3D7 strain) in SCID mice with human red cells
- Acute *T. b. brucei* (STIB795), *T. b. rhodesiense* (STIB900) both in mice
- Central nervous system infection *T. b. brucei* (GVR35) in mice
- *T. b. gambiense* (STIB930) in immunosuppressed mice
- *T. evansi*, *T. congolense*, *T. vivax* (animal pathogens) in mice
- *Babesia* spp. in mice
- *Schistosoma mansoni* in mice or hamster

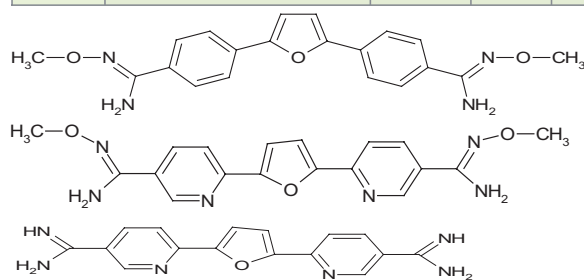
Parasites and the corresponding hosts used for in vivo drug screening at the STI.

Pan-Asian Network for Neglected Diseases, which is coordinated by DNDi.

### Orally available diamidines

The international CPDD is funded by the Bill & Melinda Gates Foundation (BMGF) and was formed in 2000 to conduct research on dicationic molecules active against trypanosomes and leishmanias. The aromatic diamidine pentamidine, a former-generation sleeping sickness drug, is still used clinically, but has the major drawbacks that it is not orally available, is only active in first stage disease, is expensive and has adverse effects. Novel diamidines are being synthesised at the University of Georgia and the University of North Carolina, which also undertakes pharmacokinetic and metabolic studies. Clinical studies are carried out by the Pharmaceutical Medicine unit of the STI. To improve the oral bioavailability of the dicationic diamidines, the chemists have introduced a prodrug approach. This strategy has so far been very successful, leading to the first oral drug (DB289) for first stage sleep-

Identity	Structure	Dose (No. doses x mg/kg)	Cured/ Tested	Mean Survival (days)
DB 289		5x 100 po	3/5	>168
		10x 50 po	1/5	>172
		5x 25 po	0/5	75
DB 868		10x 100 po	5/5	>180
		5x 100 po	4/5	>170
		5x 25 po	0/4	>96
DB 829		10x 20 ip	4/4	>180
		5x 20 ip	1/4	>132



Efficacy of diphenylfuran diamidines in the mouse model with brain infection.

ing sickness to enter clinical trials. The prodrug showed good efficacy in patients with first stage *T. b. gambiense* infection and progressed to phase III trials. Unfortunately, at that point development was discontinued in February 2008 owing to renal toxicity.

So far, we have screened about 1,850 diamidines in vitro and over 1,140 in the acute *T. b. rhodesiense* mouse model. Seventy-two compounds cured at least three of four mice at 4×5 mg/kg applied intraperitoneally, and an additional 25 prodrugs cured at 4×25 mg/kg given orally. These compounds all show superior activity in vivo over the parent compound DB75 (furamidine) and its prodrug DB289 (pafuramidine), respectively, for first stage sleeping sickness. However, the main challenge is to develop a new drug for second stage disease. So far only 5 prodrugs have shown a cure in the CNS mouse model (≥3/5 mice), simulating second stage sleeping sickness in humans: all are derivatives of the lead compound DB289. Interestingly, one dicationic parent drug (DB829) has shown unexpectedly high potency in the CNS mouse model when given intraperitoneally. The parenteral administration is fully acceptable for the severe second stage of sleeping sickness. Further preclinical studies for sleeping sickness are ongoing in an African green monkey model (pharmacokinetics and efficacy) in collaboration with the TRC, Nairobi, Kenya.

Interesting in vitro activity was also found for *Trypanosoma cruzi* and *Leishmania donovani*, but the efficacy in the corresponding rodent model was not that promising. Novel diamidines did show high activity against *Plasmodium falciparum* and several protozoans pathogenic to animals, such as *Babesia divergens* and *Trypanosoma evansi*, in the corresponding mouse model. In a proof-of-concept experiment, goats infected with *T. evansi* were treated with DB75 and DB867 at 1.25 mg/kg on days 32, 34, 36 and 38 post-infection. In all goats parasitaemia disappeared and the animals were cured, whereas the untreated control animals were not able to overcome the infection.

Scientists: R. Brun, M. Kaiser, T. Wenzler  
 Technicians: C. Braghiroli, M. Cal, G. Riccio, S. Schneeberger  
 Students: K. Gillingwater, E. Nally, R. Wüest  
 Collaboration: DNDi, Geneva; Georgia State University, Atlanta (D. Boykin); INBio, Santo Domingo, Heredia, Costa Rica; Institute of Organic Chemistry, Würzburg, Germany (G. Bringmann); Instituto de Química

Médica, CSIC, Madrid, Spain (C. Dardonville); Medivir, Stockholm; Merck-Serono, Geneva; Pentapharm, Basel; Pfizer Inc., Sandwich, UK; Tohoku University, Sendai, Japan (M. Ihara); University of Basel (M. Hamburger, U. Séquin); University of Dublin, Ireland (I. Rozas); University of Dundee, UK (I. Gilbert); University of Geneva (R. Perozzo); University of Graz, Austria (W. Seebacher); University of London (D. Tasdemir); University of North Carolina, Chapel Hill (J.E. Hall, R. Tidwell); WHO/TDR and various academic groups worldwide  
 Funding: BMGF; DNDi; TDR Genomics and Discovery Research; EU

## 4.2 Malaria drug discovery

### MMV

The STI collaborates with MMV and contributes in vitro screening of compounds for antimalarial activity and in vivo efficacy in rodent models. Collaboration with MMV started in the year 2000 in the framework of the “Synthetic peroxide” project dealing with water-soluble trioxolane compounds of the ozonide (OZ) series, which included partners from the University of Nebraska, Omaha (USA), Monash University, Victoria (Australia) and F. Hoffmann-La Roche in Basel. Peroxide #277 (OZ277) was tested in phase II dose-ranging clinical trials at various sites against uncomplicated *Plasmodium falciparum* malaria. In December 2006 MMV decided to discontinue funding of this molecule due to differences in plasma levels between volunteers and patients. The mechanism behind this behaviour seems to be related to the stability of the compound in blood (uninfected and infected). Meanwhile the “Next-generation OZ” project was initiated, which addresses the weakness of OZ277. New lead OZs were indeed identified that showed much better in vitro blood stability. These molecules show a dramatic increase in potency: a single oral dose is curative for *Plasmodium berghei*-infected mice. In 2007 this programme was selected MMV Project of the Year. A highlight of the “Next-generation OZ” project was the selection of OZ439 as a preclinical candidate in spring 2008.

Our MMV mandate also includes exploratory work on novel compounds submitted from various sources, always with the goal of identifying novel chemical entities that could be added to the MMV portfolio.

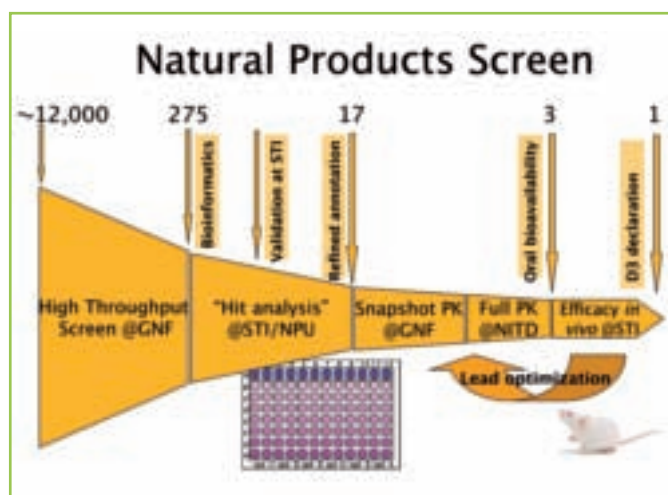
Sergio Wittlin and Jacques Chollet receive the Award “MMV Project of the Year 2006” from His Excellency Mr Yoweri Museveni, the President of Uganda, at the Stakeholder Meeting in Kampala 2007. (Photo MMV)



**NGBS malaria programme**

In June 2006 the new NGBS programme in malaria drug discovery was initiated as a collaboration between the Novartis Institute for Tropical Diseases (NITD), the Genomics Institute of the Novartis Research Foundation (GNF), the Biomedical Primate Research Centre (BPRC) and the Swiss Tropical Institute (STI). This programme aims at solving two fundamental medical needs. The first is a one-dose cure for *P. falciparum* malaria, which would represent a breakthrough in solving compliance problems, and logistical and cost issues. The second basic medical need is to identify targets that enable effective cures for *P. vivax* infections.

The programme has its own pipeline with projects ranging from target identification based on the sequenced genome of *P. falciparum* through lead optimisation to preclinical studies. Our responsibility consists of in vitro screening and in vivo evaluation of the compounds emerging from the different projects. A promising example is the "Natural products" project. Natural product-derived molecules have historically been very successful for developing new anti-infective therapies. This is especially true for antimalarial drugs, for which the most successful compound class, the quinolines and the artemisinins, were developed following identification of the active pharmacophore in plants. The Novartis Natural Products Unit (NPU) in Basel has built a library of over 10,000 compounds extracted from a wide range of natural sources. To identify new antimalarial drugs, this library was screened with a high-throughput cellular proliferation assay at GNF. The hits from that screen were analysed at the STI, and approximately 20 new compounds showed antiplasmodial activity. Already one new compound class that displayed good oral in vivo efficacy in the mouse model, with a favourable pharmacological profile, was translated into a successful medicinal chemistry programme to further improve efficacy.



Pipeline of natural product screen in the NGBS Consortium. Starting with 12,000 compounds only 3 orally bioavailable molecules remain after stringent screening for antimalarial activity and pharmacokinetics.

**Collaboration with Actelion Pharmaceuticals**

A formal collaboration between Actelion and our group began in January 2007. The goal here is to discover a clinical candidate for a new antimalarial drug. The original focus was on plasmepsin inhibitors of the Actelion chemical library. The poor correlation between enzyme assay and whole-cell parasite assay resulted in the discovery of new antimalarial molecules with unknown mechanisms of action. The elucidation of the mode of action of ACT 173890 is now the goal of the PhD thesis of R. Brunner.

Scientists: R. Brun, J. Chollet, M. Rottmann, S. Wittlin  
 Technicians: C. Fischli, S. Keller-Märki, M. Maurer, P. Papastogiannidis J. Santo Tomas, C. Scheurer, C. Snyder  
 Students: O. Abiodun, R. Brunner, S. Stupan, C. Thouvay, L. Tritten  
 Collaboration: Actelion Pharmaceuticals (C. Binkert, W. Fischli, S. Meyer); BPRC, Risjswijk, Netherlands; GNF, San Diego, CA; F. Hoffmann-La Roche, Basel (H. Matile); MMV Headquarters, Geneva (I. Bathurst); Monash University, Victoria (S. and W. Charman); NITD, Singapore; Novartis Pharma, Basel; University of Nebraska Medical Center (J. Vennerstrom)  
 Funding: Actelion Pharmaceuticals; MMV; Wellcome Trust

**4.3 EANETT**

In the year 2000, institutions in four East African countries together with the STI established EANETT, building on the long-standing relationship our group had already had with the Trypanosomiasis Research Centre (TRC) of KARI, Kikuyu, Kenya (formerly Kenya Trypanosomiasis Research Institute) and the Livestock Health Research Institute (LIRI) in Tororo, Uganda. The founding countries of the African network are Kenya, Sudan, Tanzania and Uganda. In 2005 EANETT expanded to Malawi and in 2008 to Zambia.

EANETT was awarded core funding from the SDC for a total of 6 years (2001–2006). In 2007 the network received bridge funding from the SDC to enable the network to find new core support. Each year an annual conference is held for auto-evaluation, planning and as a platform for young scientists to present their research results. EANETT is a functional regional network that has an excellent reputation in Africa and beyond. Links and collaborations have been established with major international organisations as well as with research groups in Europe, Japan and the United States. Co-opted members were incorporated to complement the available expertise in the network. Today EANETT is an ideal platform for clinical and diagnostic trials and for field research.

Over the last 2 years EANETT research activities have focused on surveillance of patients, tsetse fly distribution and transmission potential, isolation of *T. b. gambiense* to determine drug resistance and public health issues. For disease surveillance, thousands of people in old and new sleeping sickness foci were screened, infected people were treated, and villages and health



Board meeting of EANETT in Nairobi May 2008 with the representatives of the six African countries and of the STI (Marcel Kaiser). (Photo EANETT)

facilities were georeferenced. In addition to humans, domestic animals were screened in Uganda, Kenya, Sudan and Tanzania. The distribution of tsetse flies was monitored in selected areas of the network by determining tsetse species and density. At TRC, scientists are still working on establishing a *T. b. gambiense* monkey model. This turned out to be difficult because it took a long time until the animals developed parasitaemia, and parasite loads were very low.

In Uganda, scientists were involved in evaluating a new molecular diagnostic tool for HAT. Loop-mediated isothermal amplification (LAMP) of DNA is a promising new molecular technique that shows high sensitivity and specificity. The test can be carried out with a minimum of equipment.

Malawi health workers in trypanosomiasis case management and laboratory diagnosis received refresher training provided by collaborators of LIRI, Uganda and WHO-Afro Harare. The target group for the training included medical officers, clinical officers, nurses, laboratory technicians and health officers from three endemic foci of Rumphi, Nkotakota and Kasungu. All these foci border a game park, and each has a district hospital in its locality where the HAT cases are diagnosed and managed.

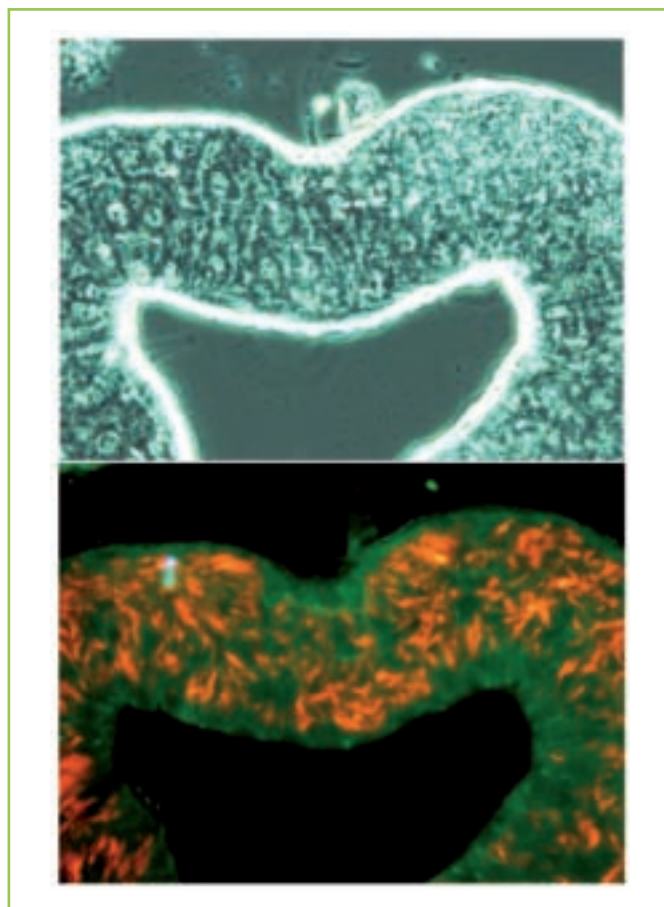
Scientists: R. Brun, M. Kaiser  
 Students: N. Adelbert, O. Balmer, N. Maina  
 Collaboration: LIRI, Tororo, Uganda; Médecins sans Frontières, France and Switzerland; Ministry of Health, Lilongwe, Malawi; National Institute of Medical Research, Tabora Station, Tanzania; Trypanosomiasis Research Centre of KARI, Kikuyu, Kenya; Tropical Medicine Research Institute, Khartoum, Sudan; Tsetse and Trypanosomiasis Research Institute, Tanga, Tanzania.  
 Funding: SDC; WHO

#### 4.4 Tsetse fly transmission studies

Primary collaborations are maintained with the group of I. Roditi at the University of Bern, Institute for Cell Biology, and with the group of S. Aksoy at the Yale School of

Public Health. The objectives of these collaborations are (i) to acquire a deeper understanding of the life cycle of trypanosomes in the tsetse fly, (ii) to study the host-parasite interaction in the vector and (iii) to transmit trypanosomes cyclically through the tsetse vector because this part of the life cycle cannot be imitated in culture.

The procyclins, consisting of EP and GPEET (amino acid repeats), are the major surface glycoproteins expressed by *T. brucei* in the mid-gut of the tsetse fly. While GPEET disappears after 7–9 days, EP remains on the surface. When trypanosomes migrate to the salivary glands, they differentiate into epimastigote forms and express stage-specific *brucei* alanine-rich proteins. The specific roles of EP and GPEET are still under investigation. Surprisingly, even procyclin knockout trypanosomes were able to infect fly salivary glands, but at a lower rate compared with wild-type trypanosomes. Tsetse fly experiments showed that the procyclic stage surface antigen (PSSA2) is not only expressed in procyclic but also in epimastigote trypanosomes and that it is important for an effective salivary gland infection. The procyclins of *T. congolense* are closely related to *T. brucei* procyclins. Besides the two protease-resistant surface molecules and glu-alanine-rich proteins already described, a third protein was discovered. This protein consists of 13 heptapeptide repeats and is expressed continuously during mid-gut infection.



Infected tsetse salivary gland with red fluorescent trypanosomes. (Photo C. Kunz)

We hypothesised that the tsetse innate immune response activates antimicrobial peptides (AMPs) when trypanosomes enter and adapt to the mid-gut. Analyses of the messenger RNA of infected tsetse flies suggests that the two AMPs attacin and defensin are not specifically up-regulated. Nevertheless, barriers severely reduce trypanosome population size during migration to the salivary glands. Investigations into whether these reductions could influence diversity revealed that in fact diversity was not lost in the salivary glands. This finding shows the ability of trypanosomes to acquire and maintain traits such as resistance to human serum or drugs. The STI is the only place in Switzerland that enables extensive research on tsetse flies. For this reason, it constitutes a unique and valuable national resource.

Scientist: **R. Brun**  
 Technician: C. Kunz Renggli  
 Student: M. Oberle  
 Collaboration: Institute for Cell Biology, University of Bern (I. Roditi); Yale School of Public Health, New Haven, CT (S. Aksoy)  
 Funding: Novartis Foundation; Roche Research Foundation; SNSF and Howard Hughes Foundation (both to I. Roditi)

## Publications

- Bakunova SM, Bakunov SA, **Wenzler T**, Barszcz T, Werbovets KA, **Brun R**, Hall JE & Tidwell RR (2007) Synthesis and in vitro antiprotozoal activity of bisbenzofuran cations. *J Med Chem* 50, 5807–5823.
- Barbaras D, **Kaiser M**, **Brun R** & Gademann K (2008) Potent and selective antiplasmodial activity of the cyanobacterial alkaloid nostocarboline and its dimers. *Bioorg Med Chem Lett* 18, 4413–4415.
- Barrett MP, Boykin DW, **Brun R** & Tidwell RR (2007) Human African trypanosomiasis: pharmacological re-engagement with a neglected disease. *Br J Pharmacol* 152, 1155–1171.
- Berger H, Seebacher W, Saf R, **Kaiser M**, **Brun R** & Weis R (2006) Antiprotozoal activities of new bis-chlorophenyl derivatives of bicyclic octanes and aza-nonanes. *Bioorg Med Chem Lett* 16, 5457–5461.
- Berger H, Weis R, **Kaiser M**, **Brun R**, Saf R, Seebacher W. (2008) Novel azabicyclo[3.2.2]nonane derivatives and their activities against *Plasmodium falciparum* K1 and *Trypanosoma brucei rhodesiense*. *Bioorg Med Chem* 16, 6371–78.
- Bolognesi ML, Lizzi F, Perozzo R, **Brun R** & Cavalli A (2008) Synthesis of a small library of 2-phenoxy-1,4-naphthoquinone and 2-phenoxy-1,4-antraquinone derivatives bearing anti-trypanosomal and anti-leishmanial activity. *Bioorg Med Chem Lett* 18, 2272–2276.
- Bringmann G, Kajahn I, Reichert M, Pedersen SE, Faber JH, Gulder T, **Brun R**, Christensen SB, Ponte-Sucré A, Moll H, Heubl G & Mudogo V (2006) Ancistrocladine A and B, the first N,C-coupled naphthyldihydroisoquinoline alkaloids, from a Congolese ancistrocladus species. *J Org Chem* 71, 9348–9356.
- Bringmann G, Gampe CM, Reichert Y, Bruhn T, Faber JH, Mikyna M, Reichert M, Leippe M, **Brun R** & Gelhaus C (2007) Synthesis and pharmacological evaluation of fluorescent and photo-activatable analogues of antiplasmodial naphthylisoquinolines. *J Med Chem* 50, 6104–6115.
- Bringmann G, **Brun R**, **Kaiser M** & Neumann S (2008) Synthesis and antiprotozoal activities of simplified analogs of naphthylisoquinoline alkaloids. *Eur J Med Chem* 43, 32–42.
- Bringmann G, Spuziak J, Faber JH, Gulder T, Kajahn I, Dreyer M, Heubl G, **Brun R** & Mudogo V (2008) Six naphthylisoquinoline alkaloids and a related benzopyranone from a Congolese Ancistrocladus species related to *Ancistrocladus congolensis*. *Phytochemistry* 69, 1065–1075.
- Bringmann G, Mutanyatta-Comar J, Maksimenka K, Wanjohi JM, Heydenreich M, **Brun R**, Müller WE, Peter MG, Midiwo JO & Yenesew A (2008) Joziknipholones A and B: the first dimeric phenylanthraquinones, from the roots of *Bulbine frutescens*. *Chemistry* 14, 1420–1429.
- Brun R** & Balmer O (2006) New developments in human African trypanosomiasis. *Curr Opin Infect Dis* 19, 415–420.
- Calderon AI, Romero LI, Ortega-Barria E, **Brun R**, Correa AMD & Gupta MP (2006) Evaluation of larvicidal and in vitro antiparasitic activities of plants in a biodiversity plot in the Altos de Campana National Park, Panama. *Pharm Biol* 44, 487–498.
- Calis I, Koyunoglu S, Yesilada A, **Brun R**, Ruedi P & Tasdemir D (2006) Antitrypanosomal cycloartane glycosides from *Astragalus baibutensis*. *Chem Biodivers* 3, 923–929.
- Cammerer SB, Jimenez C, Jones S, Gros L, Lorente SO, Rodrigues C, Rodrigues JC, Caldera A, Ruiz Perez LM, da Souza W, **Kaiser M**, **Brun R**, Urbina JA, Gonzalez PD & Gilbert IH (2007) Quinuclidine derivatives as potential antiparasitics. *Antimicrob Agents Chemother* 51, 4049–4061.
- Chackal-Catoen S, Miao Y, Wilson WD, **Wenzler T**, **Brun R** & Boykin DW (2006) Dicationic DNA-targeted antiprotozoal agents: naphthalene replacement of benzimidazole. *Bioorg Med Chem* 14, 7434–7445.
- Coghi P, Vaiana N, Pezzano MG, Rizzi L, **Kaiser M**, **Brun R** & Romeo S (2008) Parallel synthesis and antileishmanial activity of ether-linked phospholipids. *Bioorg Med Chem Lett* 18, 4658–60.
- Corminboeuf O, Dunet G, Hefsi M, Grimont J, Grisostomi C, Meyer S, Binkert C, Bur D, Jones A, Prade L, **Brun R** & Boss C (2006) Inhibitors of Plasmepsin II-potential antimalarial agents. *Bioorg Med Chem Lett* 16, 6194–6199.
- Domenicali PD, Burkard G, Morand S, **Kunz Renggli C**, Roditi I & Vassella E (2006) A Mitogen-activated protein kinase controls differentiation of bloodstream forms of *Trypanosoma brucei*. *Eukaryot Cell* 5, 1126–1135.
- Dong Y, Tang Y, **Chollet J**, Matile H, **Wittlin S**, Charman SA, Charman WN, **Santo Tomas J**, **Scheurer C**, **Snyder C**, Scoreneaux B, Bajpai S, Alexander SA, Wang X, Padmanilayam M, Cheruku SR, **Brun R** & Vennerstrom JL (2006) Effect of functional group polarity on the antimalarial activity of spiro and dispiro-1,2,4-trioxolanes. *Bioorg Med Chem* 14, 6368–6382.
- Dong Y, Creek D, **Chollet J**, Matile H, Charman SA, **Wittlin S**, Wood JK & Vennerstrom JL (2007) Comparative antimalarial activities of six pairs of 1,2,4,5-tetraoxanes (peroxide dimers) and 1,2,4,5,7,8-hexaoxonanes (peroxide trimers). *Antimicrob Agents Chemother* 51, 3033–3035.
- Freitag M, **Kaiser M**, Larsen T, Zohrabi-Kalantari V, Heidler P & Link A (2007) Synthesis and antiplasmodial activity of new N-[3-(4-{3-[(7-chloroquinolin-4-yl)amino]propyl}piperazin-1-yl)propyl]carboxamides. *Bioorg Med Chem* 15, 2782–2788.
- Ganapaty S, Steve TP, Karagianis G, Waterman PG & **Brun R** (2006) Antiprotozoal and cytotoxic naphthalene derivatives from *Diospyros assimilis*. *Phytochemistry* 67, 1950–1956.
- Ge JF, Arai C, **Kaiser M**, **Wittlin S**, **Brun R**, Ihara M. (2008) Synthesis and in vitro antiprotozoal activities of water-soluble, inexpensive 3,7-bis(dialkylamino)phenoxazin-5-ium derivatives. *J Med Chem* 51, 3654–58.
- Gillingwater K**, Buscher P & **Brun R** (2007) Establishment of a panel of reference *Trypanosoma evansi* and *Trypanosoma equiperdum* strains for drug screening. *Vet Parasitol* 148, 114–121.
- Gonzalez JL, Stephens CE, **Wenzler T**, **Brun R**, Tanius FA, Wilson WD, Barszcz T, Werbovets KA & Boykin DW (2007) Synthesis and antiparasitic evaluation of bis-2,5-[4-guanidinophenyl] thiophenes. *Eur J Med Chem* 42, 552–557.
- Haenni S, **Kunz Renggli C**, Fragoso CM, **Oberle M** & Roditi I (2006) The procyclin-associated genes of *Trypanosoma brucei* are not essential for cyclical transmission by tsetse. *Mol Biochem Parasitol* 150, 144–156.
- Hay AE, Ioset JR, Ahua KM, Diallo D, **Brun R** & Hostettmann K (2007) Limonoid orthoacetates and antiprotozoal compounds from the roots of *Pseudocedrela kotschyi*. *J Nat Prod* 70, 9–13.
- Hofer S**, **Brun R**, **Maerki S**, Matile H, **Scheurer C** & **Wittlin S** (2008) In vitro assessment of the pharmacodynamic properties of DB75, piperazine, OZ277 and OZ401 in cultures of *Plasmodium falciparum*. *J Antimicrob Chemother* (epub ahead of print).
- Hu L, Arafa RK, Ismail MA, **Wenzler T**, **Brun R**, Munde M, Wilson WD, Nzimiro S, Samyessudhas S, Werbovets KA & Boykin DW (2008) Azaterphenyl diamidines as antileishmanial agents. *Bioorg Med Chem Lett* 18, 247–251.
- Ismail MA, Arafa RK, **Wenzler T**, **Brun R**, Tanius FA, Wilson WD & Boykin DW (2008) Synthesis and antiprotozoal activity of novel bis-benzamido imidazo[1,2-a]pyridines and 5,6,7,8-tetrahydro-imidazo[1,2-a]pyridines. *Bioorg Med Chem* 16, 683–691.
- Kaiser M**, **Wittlin S**, **Nehrbass-Stuedli A**, Dong Y, Wang X, Hemphill A, Matile H, **Brun R** & Vennerstrom JL (2007) Peroxide bond-dependent antiplasmodial specificity of artemisinin and OZ277 (RBx1160). *Antimicrob Agents Chemother* 51, 2991–2993.

- Karioti A, Skaltsa H, Linden A, Perozzo R, **Brun R** & Tasdemir D (2007) Anthecularin: a novel sesquiterpene lactone from *Anthemis auriculata* with antiprotozoal activity. *J Org Chem* 72, 8103–8106.
- Kuettel S, Zambon A, **Kaiser M**, **Brun R**, Scapozza L & Perozzo R (2007) Synthesis and evaluation of antiparasitic activities of new 4-[5-(4-phenoxyphenyl)-2H-pyrazol-3-yl]morpholine derivatives. *J Med Chem* 50, 5833–5839.
- Leepin A, **Studli A**, **Brun R**, Stephens CE, Boykin DW & Hemphill A (2008) Host cells participate in the in vitro effects of new-generation diamidine analogues against tachyzoites of the intra-cellular apicomplexan parasites *Neospora caninum* and *Toxoplasma gondii*. *Antimicrob Agents Chemother* 52, 1999–2008.
- Lenta BN, Vonthron-Senecheau C, Weniger B, Devkota KP, Ngoupayo J, **Kaiser M**, Naz O, Choudhary MI, Tsamo E & Sewald N (2007) Leishmanicidal and cholinesterase inhibiting activities of phenolic compounds from *Allanblackia monticola* and *Symphonia globulifera*. *Molecules* 12, 1548–1557.
- Lenta BN, Vonthron-Senecheau C, Soh RF, Tantangmo F, Ngouela S, **Kaiser M**, Tsamo E, Anton R & Weniger B (2007) In vitro antiprotozoal activities and cytotoxicity of some selected Cameroonian medicinal plants. *J Ethnopharmacol* 111, 8–12.
- Likefack AC, **Brun R**, Fomena A & Truc P (2006) Comparison of the in vitro drug sensitivity of *Trypanosoma brucei gambiense* strains from West and Central Africa isolated in the periods 1960–1995 and 1999–2004. *Acta Trop* 100, 11–16.
- Maina N**, Maina KJ, Maeser P & **Brun R** (2007) Genotypic and phenotypic characterization of *Trypanosoma brucei gambiense* isolates from Ibba, South Sudan, an area of high melarsoprol treatment failure rate. *Acta Trop* 104, 84–90.
- Maina NW**, **Oberle M**, Otieno C, **Kunz C**, Maeser P, Ndung'u JM & **Brun R** (2007) Isolation and propagation of *Trypanosoma brucei gambiense* from sleeping sickness patients in south Sudan. *Trans R Soc Trop Med Hyg* 101, 540–546.
- Mathis AM, Bridges AS, Ismail MA, Kumar A, Francesconi I, Anbazhagan M, Hu Q, Tanius FA, **Wenzler T**, Sautler J, Wilson WD, **Brun R**, Boykin DW, Tidwell RR & Hall JE (2007) Diphenyl furans and aza analogs: effects of structural modification on in vitro activity, DNA binding, and accumulation and distribution in trypanosomes. *Antimicrob Agents Chemother* 51, 2801–2810.
- Mehner C, Müller D, Krick A, Kehraus S, Löser R, Gütschow M, Maier A, Fiebig HH, **Brun R** & König G (2008) A novel  $\beta$ -amino acid in cytotoxic peptides from the cyanobacterium *Tycho-nema* sp. *Eur J Org Chem* 10, 1732–1739.
- Nwodo NJ, **Brun R** & Osadebe PO (2007) In vitro and in vivo evaluation of the antitrypanosomal activity of fractions of *Holarhena africana*. *J Ethnopharmacol* 113, 556–559.
- Orosio L, Murillo C, Aponte S, Mayagaya V, **Scheurer C**, **Brun R**, Matile H & **Wittlin S** (2007) In vitro susceptibility of *P. falciparum* populations from Colombia and Tanzania to a new synthetic peroxide (OZ277). *Am J Trop Med Hyg* 76, 1024–1026.
- Padmanilayam M, Scoreaux B, Dong Y, **Chollet J**, Matile H, Charman SA, Creek DJ, Charman WN, **Tomas JS**, **Scheurer C**, **Wittlin S**, **Brun R** & Vennerstrom JL (2006) Antimalarial activity of N-alkyl amine, carboxamide, sulfonamide, and urea derivatives of a dispiro-1,2,4-trioxolane piperidine. *Bioorg Med Chem Lett* 16, 5542–5545.
- Patrick DA, Bakunov SA, Bakunova SM, Kumar EV, Lombardy RJ, Jones SK, Bridges AS, Zhironov O, Hall JE, **Wenzler T**, **Brun R** & Tidwell RR (2007) Synthesis and in vitro antiprotozoal activities of dicationic 3,5-diphenylisoxazoles. *J Med Chem* 50, 2468–2485.
- Pontius A, Krick A, Kehraus S, **Brun R**, König GM. (2008) Antiprotozoal activities of heterocyclic-substituted xanthenes from the marine-derived fungus *Chaetomium* sp. *J Nat Prod* 71, 1579–84.
- Posner GH, Chang W, Hess L, Woodard L, Sinihtaj S, Usera AR, Maio W, Rosenthal AS, Kalinda AS, D'Angelo JG, Petersen KS, Stohler R, **Chollet J**, **Santo-Tomas J**, **Snyder C**, **Rottmann M**, **Wittlin S**, **Brun R** & Shapiro TA (2008) Malaria-infected mice are cured by oral administration of new artemisinin derivatives. *J Med Chem* 51, 1035–1042.
- Pudhom K, Kasai K, Terauchi H, Inoue H, **Kaiser M**, **Brun R**, Ihara M & Takasu K (2006) Synthesis of three classes of rhodacyanine dyes and evaluation of their in vitro and in vivo antimalarial activity. *Bioorg Med Chem* 14, 8550–8563.
- Rodenko B, van der Burg AM, Wanner MJ, **Kaiser M**, **Brun R**, Gould M, de Koning HP & Koomen GJ (2007) 2,N6-disubstituted adenosine analogs with antitrypanosomal and antimalarial activities. *Antimicrob Agents Chemother* 51, 3796–3802.
- Rodriguez F, Rozas I, **Kaiser M**, **Brun R**, Nguyen B, Wilson WD, Garcia RN & Dardonville C (2008) New bis(2-aminoimidazole) and bisguanidine DNA minor groove binders with potent in vivo antitrypanosomal and antiplasmodial activity. *J Med Chem* 51, 909–923.
- Roperto S, Borzacchiello G, **Brun R**, Perillo A, Russo V, Urraro C & Roperto F (2008) Multiple glomus tumors of the urinary bladder in a cow associated with bovine papillomavirus type 2 (BPV-2) infection. *Vet Pathol* 45, 39–42.
- Ruda GF, Alibu VP, Mitsos C, Bidet O, **Kaiser M**, **Brun R**, Barrett MP & Gilbert IH (2007) Synthesis and biological evaluation of phosphate prodrugs of 4-phospho-d-erythronohydroxamic acid, an inhibitor of 6-phosphogluconate dehydrogenase. *ChemMedChem* 2, 1169–1180.
- Russo V, Borzacchiello G, **Brun R**, D'Ischia M, Napolitano A, Paciello O, Panzella L, Roperto F, Urraro C & Roperto S (2008) Melanosis of the urinary bladder in a cow. *Vet Pathol* 45, 46–50.
- Schlapper C, Seebacher W, **Kaiser M**, **Brun R**, Saf R & Weis R (2007) Bicyclo[2.2.2]octyl esters of dialkylamino acids as antiprotozoals. *Bioorg Med Chem* 15, 5543–5550.
- Schramm OG, Oeser T, **Kaiser M**, **Brun R** & Müller TJJ (2008) Rapid one-pot synthesis of antiparasitic quinolines based upon the microwave-assisted coupling-isomerization reaction (MACIR). *Synlett* 359–362.
- Sealey-Cardona M, Cammerer S, Jones S, Ruiz-Perez LM, **Brun R**, Gilbert IH, Urbina JA & Gonzalez-Pacanowska D (2007) Kinetic characterization of squalene synthase from *Trypanosoma cruzi*: selective inhibition by quinuclidine derivatives. *Antimicrob Agents Chemother* 51, 2123–2129.
- Seebacher W, Saf R, **Brun R**, **Kaiser M** & Weis R (2006) Isomerization of 4-amino-6,7-diphenylbicyclo[2.2.2]octan-2-ones. *Can J Chem* 84, 1074–1078.
- Seebacher W, Schlapper C, **Brun R**, **Kaiser M**, Saf R & Weis R (2006) Synthesis of new esters and oximes with 4-aminobicyclo[2.2.2]octane structure and evaluation of their antitrypanosomal and antiplasmodial activities. *Eur J Med Chem* 41, 970–972.
- Seebacher W, **Kaiser M**, **Brun R**, Saf R & Weis R (2007) Antiprotozoal activities of epimeric aminobicycles. *Monatsh Chemie* 138, 709–714.
- Seebacher W, **Kaiser M**, **Brun R**, Saf R & Weis R (2007) New 4-amino-2-azabicyclo[3.2.2]nonane derivatives and their antiprotozoal potencies. *Monatsh Chemie* 138, 619–625.
- Senn M, Gunzenhauser S, **Brun R** & Sequin U (2007) Antiprotozoal polyacetylenes from the Tanzanian medicinal plant *Cussonia zimmermannii*. *J Nat Prod* 70, 1565–1569.
- Snyder C**, **Chollet J**, **Santo-Tomas J**, **Scheurer C** & **Wittlin S** (2007) In vitro and in vivo interaction of synthetic peroxide RBx11160 (OZ277) with piperazine in *Plasmodium* models. *Exp Parasitol* 115, 296–300.
- Sparatore A, Basilico N, Casagrande M, Parapini S, Taramelli D, **Brun R**, **Wittlin S** & Sparatore F (2008) Antimalarial activity of novel pyrrolizidinyl derivatives of 4-aminoquinoline. *Bioorg Med Chem Lett* 18, 3737–3740.
- Spoerri I, Chadwick R, **Kunz Renggli C**, Matthews K, Roditi I & Burkard G (2007) Role of the stage-regulated nucleoside transporter TbNT10 in differentiation and adenosine uptake in *Trypanosoma brucei*. *Mol Biochem Parasitol* 154, 110–114.
- Stuart K, **Brun R**, Croft S, Fairlamb A, Gurtler RE, McKerrow J, Reed S & Tarleton R (2008) Kinetoplastids: related protozoan pathogens, different diseases. *J Clin Invest* 118, 1301–1310.
- Stump B, **Kaiser M**, **Brun R**, Krauth-Siegel RL & Diederich F (2007) Betraying the parasite's redox system: diaryl sulfide-based inhibitors of trypanothione reductase: subversive substrates and antitrypanosomal properties. *ChemMedChem* 2, 1708–1712.
- Takasu K, Shimogama T, Satoh C, **Kaiser M**, **Brun R** & Ihara M (2007) Synthesis and antimalarial property of orally active phenoxazinium salts. *J Med Chem* 50, 2281–2284.
- Tang Y, Dong Y, **Wittlin S**, Charman SA, **Chollet J**, Chiu FC, Charman WN, Matile H, Urwyler H, Dorn A, Bajpai S, Wang X, Padmanilayam M, Karle JM, **Brun R** & Vennerstrom JL (2007) Weak base dispiro-1,2,4-trioxolanes: potent antimalarial ozonides. *Bioorg Med Chem Lett* 17, 1260–1265.
- Tasdemir D, **Brun R**, Yardley V, Franzblau SG & Ruedi P (2006) Antitubercular and antiprotozoal activities of primin, a natural benzoquinone: in vitro and in vivo studies. *Chem Biodivers* 3, 1230–1237.
- Tasdemir D, Topaloglu B, Perozzo R, **Brun R**, O'Neill R, Carballeira NM, Zhang X, Tonge PJ, Linden A & Ruedi P (2007) Marine natural products from the Turkish sponge *Agelas oroides* that inhibit the enoyl reductases from *Plasmodium falciparum*, *Mycobacterium tuberculosis* and *Escherichia coli*. *Bioorg Med Chem* 15, 6834–6845.
- Tasdemir D, **Brun R**, Franzblau SG, Sezgin Y & Calis I (2008) Evaluation of antiprotozoal and antimycobacterial activities of the resin glycosides and the other metabolites of *Scrophularia cryptophila*. *Phytomedicine* 15, 209–215.
- Uhlemann AC, **Wittlin S**, Matile H, Bustamante L & Krishna S (2007) Mechanism of antimalarial action of the synthetic trioxolane RBX11160 (OZ277). *Antimicrob Agents Chemother* 51, 667–672.
- Urwyler S, Studer E, **Kunz Renggli C** & Roditi I (2007) A family of stage-specific alanine-rich proteins on the surface of epimastigote forms of *Trypanosoma brucei*. *Mol Microbiol* 63, 218–228.
- Utz S, Roditi I, **Kunz Renggli C**, Almeida IC, Acosta-Serrano A & Bütikofer P (2006) *Trypanosoma congolense* procyclins: unmasking cryptic major surface glycoproteins in procyclic forms. *Eukaryot Cell* 5, 1430–1440.
- Verotta L, Appendino G, Bombardelli E & **Brun R** (2007) In vitro antimalarial activity of hyperforin, a prenylated acylphloroglucinol: a structure-activity study. *Bioorg Med Chem Lett* 17, 1544–1548.
- Wang X, Dong Y, **Wittlin S**, Creek D, **Chollet J**, Charman SA, **Tomas JS**, **Scheurer C**, **Snyder C** & Vennerstrom JL (2007) Spiro- and dispiro-1,2-dioxolanes: contribution of iron(II)-mediated one-electron vs two-electron reduction to the activity of antimalarial peroxides. *J Med Chem* 50, 5840–5847.
- Wang Y, **Utzinger J**, **Saric J**, Li J, Burckhardt D, Dirnhofer S, Nicholson JK, Singer BH, **Brun R** & Holmes E (2008) Global metabolic responses of mice to *Trypanosoma brucei brucei* infection. *Proc Natl Acad Sci USA* 105, 6127–6132.
- Weis R, Berger H, **Kaiser M**, **Brun R**, Saf R & Seebacher W (2008) Synthesis of bicyclic amines and their activities against *Trypanosoma brucei rhodesiense* and *Plasmodium falciparum* K1. *Arch Pharm Res* 31, 688–697.
- Weniger B, Vonthron-Senecheau C, **Kaiser M**, **Brun R** & Anton R (2006) Comparative antiplasmodial, leishmanicidal and antitrypanosomal activities of several biflavonoids. *Phytomedicine* 13, 176–180.
- Zhou L, Alker A, Ruf A, Wang X, Chiu FC, Morizzi J, Charman SA, Charman WN, **Scheurer C**, **Wittlin S**, Dong Y, Hunziker D & Vennerstrom JL (2008) Characterization of the two major CYP450 metabolites of ozonide (1,2,4-trioxolane) OZ277. *Bioorg Med Chem Lett* 18, 1555–1558.