Natural products active against African trypanosomes: a step towards new drugs

Sara Hoet,* Frederik Opperdoes, Reto Brun and Joëlle Quetin-Leclercq

*Laboratoire de Pharmacognosie, Unité d’Analyse Chimique et Physico-Chimique des Médicaments, Université Catholique de Louvain, UCL 72.30-CHAM, Av. E. Mounier 72, 1200 Brussels, Belgium. E-mail: sara.hoet@cham.ucl.ac.be
b Research Unit for Tropical Diseases, Christian de Duve Institute of Cellular Pathology and Laboratory of Biochemistry, Université Catholique de Louvain, Av. Hippocrate 74–75, 1200 Brussels, Belgium

Reto Brun received his PhD in Biology in 1973 from the University of Basel. From 1974 to 1976 he was a post-doctoral fellow at the University of California at Irvine in the lab of Prof. Stuart M. Krassner working on transformation processes of Leishmania. During the following 20 years he studied various aspects of African trypanosomes at the Swiss Tropical Institute (STI) in Basel and during many short stays as a visiting scientist in Africa. The development of in vitro assays for trypanosomes and other protozoan parasites offered the possibility of doing drug discovery work for tropical diseases. Today Reto Brun is head of the Parasite Chemotherapy Unit at STI and professor for biology at the University of Basel. His lab is a reference screening center for TDR/WHO Drug Discovery Research and also for the Medicines for Malaria Venture (MMV) Foundation. His research interests are drug discovery and development work for malaria and African sleeping sickness and the control of the latter disease in collaboration with African national institutions.

Joëlle Quetin-Leclercq is a pharmacist and received her PhD in pharmaceutical sciences in 1989 from the University of Liege where she mainly worked on alkaloids from Strychnos. She moved to the Université Catholique de Louvain in 1995 where she is now professor of pharmacognosy and drug analysis, director of the laboratory of pharmacognosy and head of the analytical chemistry and drug analysis unit. Her main research interests deal with plants used in African traditional medicine: analysis of their activity, isolation and structure determination of their active molecules, quantification in plants or crude extracts and quality control, in collaboration with several African partners.
1 Introduction

African trypanosomiasis is a vector-borne parasitic disease which is causing major health and economic problems in rural sub-Saharan Africa. The etiologic agents of the disease are flagellated protozoa that belong to the genus *Trypanosoma*: *Trypanosoma brucei rhodesiense* and *T. b. gambiense* cause human sleeping sickness and *T. b. brucei* (which is morphologically and biochemically indistinguishable from the two other subspecies), *T. congolense* and *T. vivax* cause nagana in livestock (cattle, sheep and goats). The parasites are transmitted between vertebrate hosts by the tsetse fly (*Glossina spp.*).1,2 Two other important livestock trypanosome species are *T. evansi* and *T. equiperdum* causing surra and dourine which are transmitted by biting flies or during coitus, respectively.3

In the 1960s, human African trypanosomiasis (HAT) was brought under control thanks to a combination of measures including treatment of patients, active case finding and vector control. However, since the 1970's, the disease has re-emerged dramatically. Sleeping sickness is again endemic in over 30 African countries threatening over 60 million people and has reached epidemic proportions in some countries, such as Angola, southern Sudan, Uganda and the Democratic Republic of Congo. Almost 45,000 cases of HAT were reported in 1999 but the World Health Organization estimates that the actual number of cases is between 300,000 and 500,000, since only 3–4 million people at risk of infection are under surveillance with regular examination or access to health centres.4

HAT exists in two clinical forms depending on the parasite involved: a chronic form caused by *T. b. gambiense* affecting countries in west and central Africa and an acute form, caused by *T. b. rhodesiense* in east and southern Africa. Infection with either subspecies is fatal, if left untreated, in a matter of weeks (*T. b. rhodesiense*) or within months or years (*T. b. gambiense*).2 Animal infection with *T. b. brucei* produces, in some species, no apparent symptoms (e.g. goats, pigs and antelopes) while in others (e.g. cattle and dogs) it can be fatal. Cattle infected with *T. congolense* or *T. vivax* are chronically ill, show reduction in milk production, in weight gain and reproduction and eventually die. Nagana is thus a serious obstacle to the cattle industry in Africa.5

African trypanosomes undergo life cycles which alternate between a vertebrate host (blood and other body fluids, and tissues) and the tsetse fly (gut and salivary glands) (Fig. 1).6 To survive in these different environments, various metabolic and morphological changes are necessary which involve the mitochondrial system and the surface membrane. During a blood meal on an infected mammal, the vector ingests bloodstream trypanomastigotes. The parasites multiply in the fly and go through several developmental stages (procyclics, epimastigotes and metacyclic trypanosomes) in a period of three to four weeks. When the blood-sucking fly bites another mammal, it injects metacyclic trypanosomes which transform into bloodstream trypanomastigotes in the skin, and subsequently disseminate into the bloodstream via the lymphatic system. The parasites proliferate in waves, evading the host's immune system by continuously changing their antigenic coat of variant surface glycoproteins.2

This first haemolymphatic stage of the disease, which appears one to three weeks after inoculation, is characterized by non-specific symptoms such as irregular bouts of fever, headaches, joint pains and itching which are very often misinterpreted as influenza or malaria. After weeks or months depending on the parasite involved, trypanosomes cross the blood–brain barrier to invade the central nervous system resulting in a chronic meningo-encephalitis eventually leading to encephalopathy. During this second stage of HAT, characteristic symptoms appear: headaches, neurological symptoms, personality and behavior alterations, poor coordination, changes in the sleep cycle (giving the disease its name) and body wasting, leading eventually to a terminal somnolent state and finally to death if left untreated. An early diagnosis as well as early treatment are thus important for a better management of the second stage of the disease.5,7

Current chemotherapeutic options are very limited and far from ideal. There are only four approved drugs for HAT, three of which were developed more than half a century ago: suramin, pentamidine, melarsoprol and eflornithine (Fig. 2). Other molecules such as homidium, isometamidium, and diminazene aceturate are used in animal infections. Only melarsoprol and eflornithine, which are able to cross the blood–brain barrier, can be used for the second stage, with eflornithine only effective in *T. b. gambiense* infection. The mechanisms of action of these molecules remain poorly understood except for eflornithine, which inhibits the polyamine biosynthesis pathway. All these drugs have to be administered by injection over a long period of time thus requiring medical facilities and specialized staff which often do not exist in rural areas. Adverse effects are severe, sometimes life-threatening. There are also increasing reports of treatment failures, especially with melarsoprol. The availability of these drugs has not always been guaranteed as drug companies periodically abandon production because of lack of profitability. Therefore, there is an urgent need for new molecules against sleeping sickness which are safe, effective, cheap and easy-to-administer and for new leads with novel mechanisms of action.8–11

Nature with its numerous plants, microorganisms and marine organisms is a potential source of such new drugs since it contains a countless quantity of molecules with a great variety of structures and pharmacological activities.12 Throughout the ages, mineral, plant and animal products have been the main sources of medicines for man. It is estimated that two thirds of the world population still rely on traditional medical remedies, mainly plants, because of limited availability or affordability of pharmaceutical medicines.13 Several well established human
antiprotozoal drugs have their origins in nature, such as quinine, an alkaloid from Cinchona sp. (Rubiaceae) and artemisinin, a sesquiterpene lactone from Artemisia annua (Asteraceae) used to treat malaria, or emetine, an alkaloid from Cephaelis ipecacuanha (Rubiaceae) used to treat amoebiasis. Additionally, these antiprotozoal plant-derived compounds have been used as leads to develop other semi-synthetic or synthetic drugs with better efficacy, safety or pharmacokinetic profiles. The diversity of natural products with antiprotozoal activities has been illustrated in several reviews which cover molecules that are mainly active on the etiological agents of malaria, leishmaniasis or Chagas disease. This review includes the natural products that are active on the trypanosomes responsible for sleeping sickness and nagana.

Publications reporting the activity of plant extracts on African trypanosomes are not abundant compared to other protozoal diseases such as malaria. There are even less publications on this subject with regard to isolated natural products. The majority of these compounds have been tested for their in vitro activity on bloodstream trypanastigotes of African trypanosomes. Additionally, the in vitro cytotoxicity for mammalian cells of several of these natural metabolites was at the same time assessed allowing the determination of the selectivity index (SI = ratio of the IC50 value obtained for mammalian cells divided by the IC50 on trypanosomes). Even if this in vitro selectivity index does not allow extrapolation to the in vivo situation, this parameter is valuable for selecting compounds with a selective activity against trypanosomes that are worth investigating, for example in animal models.

Among the natural compounds isolated from different alkaloids, those with a piperidine, pyridine, tropane, quinolizidine, indole, or purine skeleton were found, in general, to be inactive on T. b. brucei and T. congolense bloodstream forms and non cytotoxic for the human myeloid cell line HL-60. However, a number of isoquinoline and quinoline alkaloids exhibited IC50 values below 10 µM. T. congolense was always less susceptible to these compounds than T. b. brucei. The quinoline alkaloids from Cinchona bark (Rubiaceae) (quinidine 1, cinchonine 2, quinine 3, cinchonidin 4) had significant trypanocidal activity with IC50 values of 0.8, 1.2, 4.9, 7.1 µM, respectively, on T. b. brucei. For 1 and 2, the selectivity indices were greater than 200, indicating the potential of these alkaloids for further drug development. Emetine 5, an isoquinoline alkaloid from Cephaelis ipecacuanha (Rubiaceae) which has been used in the treatment of amoebiasis, was very trypanocidal (IC50 = 0.039 µM and 0.43 µM for T. b. brucei and T. congolense) but without any selectivity. Berberine 6 and sanguinarine 7, two quaternary benzylisoquinoline alkaloids found in a number of plant families, and berbamine 8, a bisbenzylisoquinoline (BBIQ), were trypanocidal with little or no selectivity (IC50 = 0.5, 1.9, 2.6 µM on T. b. brucei respectively with SI = 51, 0.7, 7). DNA intercalation in combination with the inhibition of protein synthesis could be responsible for the observed trypanocidal and cytotoxic effects of these different alkaloids.

Out of the 12 BBIQ alkaloids tested by Camacho et al. on T. b. brucei bloodstream trypanastigotes, eight had an IC50 between 1 and 2 µM, but none were as active as pentamidine.

2 Natural products with antitrypanosomal activity

2.1 Alkaloids

Several alkaloids have been tested on trypanosomes in vitro. In a study of 34 different alkaloids, those with a piperidine, pyridine, tropine, quinolizidine, indole, or purine skeleton were found, in general, to be inactive on T. b. brucei and T. congolense bloodstream forms and non cytotoxic for the human myeloid cell line HL-60. However, a number of isoquinoline and quinoline alkaloids exhibited IC50 values below 10 µM. T. congolense was always less susceptible to these compounds than T. b. brucei. The quinoline alkaloids from Cinchona bark (Rubiaceae) (quinidine 1, cinchonine 2, quinine 3, cinchonidin 4) had significant trypanocidal activity with IC50 values of 0.8, 1.2, 4.9, 7.1 µM, respectively, on T. b. brucei. For 1 and 2, the selectivity indices were greater than 200, indicating the potential of these alkaloids for further drug development. Emetine 5, an isoquinoline alkaloid from Cephaelis ipecacuanha (Rubiaceae) which has been used in the treatment of amoebiasis, was very trypanocidal (IC50 = 0.039 µM and 0.43 µM for T. b. brucei and T. congolense) but without any selectivity. Berberine 6 and sanguinarine 7, two quaternary benzylisoquinoline alkaloids found in a number of plant families, and berbamine 8, a bisbenzylisoquinoline (BBIQ), were trypanocidal with little or no selectivity (IC50 = 0.5, 1.9, 2.6 µM on T. b. brucei respectively with SI = 51, 0.7, 7). DNA intercalation in combination with the inhibition of protein synthesis could be responsible for the observed trypanocidal and cytotoxic effects of these different alkaloids.

Out of the 12 BBIQ alkaloids tested by Camacho et al. on T. b. brucei bloodstream trypanastigotes, eight had an IC50 between 1 and 2 µM, but none were as active as pentamidine.
Thalisopidine 9 displayed the strongest trypanocidal activity (IC₅₀ = 1.1 µM). Berbamine 8 was active on T. b. brucei in the same concentration range as reported in the previous study. Phaeanthine 10, isolated from the leaves of Triclisia patens (Menispermaceae) was active on T. b. brucei in vitro (IC₅₀ = 1.7 µM) but in another study it was shown to be inactive in mice infected with the same parasite.²²,²³ No clear structure–activity relationship could be extracted from this study of several BBIQ alkaloids.

Naphthylisoquinoline (NIQ) alkaloids are axially chiral natural biaryls isolated from African plants belonging to the small Ancistrocladaceae and Dioncophyllaceae families which have already shown promising antiprotozoal properties, in particular antiplasmodial activity. Among the monomeric NIQ alkaloids tested up to now, dioncophyllines A 11, B 12 and E 13 were the most active with IC₅₀ values of 2–3 µM on T. b. brucei or T. b. rhodesiense bloodstream forms.²⁴,²⁵ Ancistroealaines A 14 and B 15, isolated from the stem bark of Ancistrocladus eulaeensis (Ancistrocladaceae) display a lower activity on T. b. rhodesiense (IC₅₀ of 8 and 5 µM respectively) but exhibited cytotoxic effects on mammalian L6 cells (rat skeletal myoblasts) at much higher concentrations (IC₅₀ >215 µM for 14 and = 220 µM for 15).²⁶ Dioncopeltine A 16, with its additional alcohol function on the methyl group of the naphthalene ring, has a distinctly lower activity on T. b. brucei bloodstream trypanomastigotes (IC₅₀ = 22 µM) and habropetaline A 17, isolated from the root of Triphyophyllum peltatum (Dioncophyllaceae) was inactive.²⁴,²⁷ The dimeric NIQ, ancistrogrii ftine A 18, obtained from the twigs of Ancistrocladus griffithii (Ancistrocladaceae), had trypanocidal activity (IC₅₀ = 1.2 µM on T. b. rhodesiense) but unfortunately showed some cytotoxicity (IC₅₀ = 7.4 µM on L6 cells).²⁸ The anti-HIV dimeric NIQ, michellamine B 19, was, on the contrary, devoid of any antitrypanosomal activity.²⁴ Bringmann et al. also tested compounds corresponding to the two bicyclic parts of NIQ alkaloids, as well as molecules related to the central biaryl core to try to elucidate the structural prerequisites for good antitrypano-
somalous activity. Among all compounds tested, the natural, genuine alkaloids themselves, showed the highest activities.\textsuperscript{24} NIQ alkaloids could thus constitute an interesting class of antitrypanosomal compounds worth further optimization.\textsuperscript{24–30}

Out of the three oxoaporphines (O-methylmoschatoline 20, lycisamine 21, lirioidene 22) isolated by activity-directed fractionation from the stem bark of Unonopsis buchtienii (Annonaceae), only 20 showed some activity on the mammalian stage of *T. b. brucei* (IC\textsubscript{50} = 20 \mu M) but without selectivity.\textsuperscript{31} In a study which compared the *in vitro* antitrypanosomal activity of seven aporphines, only the three alkaloids isolated from *Cassysba filiformis* (Lauraceae) were active on *T. b. brucei* bloodstream trypomastigotes: actinodaphnine 23, cassytheine 24 and dincertine 25 had IC\textsubscript{50} of 3.2, 6.0, and 14.6 \mu M, respectively, but were also toxic to HeLa cells (SI < 5). The antitrypanosomal activity of these aporphines, which intercalate into DNA, seemed to be related to their ability to stabilize the DNA helix against heat denaturation and to inhibit the catalytic activity of topoisomerase I.\textsuperscript{32} Bulbocapnine 26 was inactive *in vitro* and *in vivo* on *T. b. brucei*, providing some structure–activity relationships.\textsuperscript{33,34}

The plants of the Amaryllidaceae family are often toxic and contain a special class of isoquinoline alkaloids, especially in the bulbs.\textsuperscript{35–38} Pancraceine 27, an alkaloid, with a 5,11-methanomorphanidine ring, obtained from the fresh bulb of Narcissus angustifolius subsp. transcarpathicus was active on the mammalian stage of *T. b. rhodesiense* with an IC\textsubscript{50} of 2.4 \mu M and was not cytotoxic for L6 cells at concentrations up to 300 \mu M.\textsuperscript{39} Nangustine 28, a regioisomer of pancraceine, was also devoid of cytotoxicity which was almost 14 times less active (IC\textsubscript{50} = 33.4 \mu M) on the trypanosomes.\textsuperscript{36} The antitrypanosomal activity of some Amaryllidaceae alkaloids of the crinane type were evaluated on *T. b. rhodesiense* bloodstream forms: haemantidine 29 was active with an IC\textsubscript{50} of 3.5 \mu M, oxomari- tidine 30 was weakly active (IC\textsubscript{50} = 9.8 \mu M), and maritidine 31 was inactive.\textsuperscript{34,35} Galanthine 32, an alkaloid of the lycorane type had the same activity on trypanosomes as 30.\textsuperscript{35}

Several alkaloids isolated from marine organisms such as sponges, ascidians and tunicates have been evaluated for their antitrypanosomal activity.\textsuperscript{37–39} Lepadins D 33, E 34 and F 35 isolated from a tunicate species from the genus Didemnum, possess an unusual decachrysinoline skeleton and show significant and selective antitrypanosomal activity *in vitro*. Compounds 35 and 34, two diastereoisomers, displayed an IC\textsubscript{50} on *T. b. rhodesiense* bloodstream trypanomastigotes inferior to 1 \mu M and were respectively 80 to 43 times less toxic on L6 mammalian cells. Compound 33 was weakly active with an IC\textsubscript{50} on trypanosomes of 19 \mu M. The presence of the 2-E-octenoic acid ester function at C\textsubscript{3} in 34 and 35 in place of the secondary hydroxyl function increased the antitrypanosomal activity significantly.\textsuperscript{37} Fascaplysin 36, a quaternary indole alkaloid isolated from the sponge *Hytios erecta*, was also active on the mammalian stage of *T. b. rhodesiense* (IC\textsubscript{50} = 0.6 \mu M) but was cytotoxic to L6 cells.\textsuperscript{38} Two pyridoacridone alkaloids, ascidi- demin 37 and 2-bromoascididemin 38 are other marine metabolites. Compound 37 is a pentacyclic DNA intercalating agent with marked cytotoxic activities. The two compounds were more cytotoxic to macrophages (IC\textsubscript{50}= 6 and 0.7 \mu M for 37 and 38 respectively) than to *T. b. rhodesiense* bloodstream forms (IC\textsubscript{50} = 14 and 5 \mu M, respectively).\textsuperscript{39} Several related synthetic derivatives were also tested for their antitrypanosomal activities. The two most active compounds 39 and 40 were respectively 2000 and 770 times more trypanocidal than the parent compound 37 (IC\textsubscript{50} = 0.007 and 0.018 \mu M, respectively, while IC\textsubscript{50} for melarsoprol, the reference drug, = 0.003 \mu M) but the cytotoxicity did not vary (IC\textsubscript{50} = 6–7 \mu M). The authors speculate that the mechanisms of action of these derivatives involve DNA intercalation and DNA oxidative damage.\textsuperscript{39}

The indoloquinoline alkaloids, cryptolepine 41 and neo- cryptolepine 42, isolated from *Cryptolepis sanguinolenta* (Periplocaceae) were shown to be potent antiplasmodial compounds. When tested on *T. b. brucei* bloodstream forms, their IC\textsubscript{50} were 3 and 4 \mu M, respectively. However these compounds, especially 41, are cytotoxic, due to DNA interaction and inhibition of topoisomerase II (IC\textsubscript{50} on a human embryonic lung cell line MRC-5 = 1.5 and 11 \mu M for 41 and 42, respectively). The synthesis of neo-cryptolepine derivatives such as 2-nitrocryptolepine 43 has led to compounds with an IC\textsubscript{50} on *T. b. brucei* of 0.7 \mu M whereas the IC\textsubscript{50} on MRC-5 cells was greater than 32 \mu M constituting a new lead structure with antitrypanosomal potential.\textsuperscript{40}

Tryptanthrin 44 (indolo[2,1-b]quinazoline-6,12-dione) is unusual in that its synthesis was described 50 years before it was discovered in a number of plant species. Scowill *et al.*
studied the in vitro activities of 44, 4-azatryptanthrin 45, and a series of substituted derivatives against bloodstream forms of T. b. brucei. The unsubstituted compounds were weakly active (IC50 = 23 and 40 μM for 44 and 45 respectively). The anttrypanosomal activity was markedly improved by the presence of an electron-withdrawing group (halogen or nitro) at position 8 of the (aza)tryptanthrin ring system: the most active compound 46 (IC50 = 0.4 μM). In a study of a series of camptothecin analogs, the unsubstituted compounds were weakly active against bloodstream forms of T. b. brucei (IC50 = 1.6 μM). For example, 9-chloro-10,11-methylenedioxy 47 was 40 times more active on T. b. brucei bloodstream forms only when combined with glycerol 4 mM (IC50 without glycerol = 250 μM, IC50 with glycerol = 0.03 μM). First in vivo studies have shown that 49 at 100 mg kg−1 combined with glycerol 3 g kg−1 given orally or at 25 mg kg−1 combined with glycerol 3 g kg−1 given intraperitoneally gave 100% cure of mice infected with T. b. brucei. In each experiment, 49 was given at a single dose and glycerol was given at 1 g kg−1 at 30 minute intervals. Living trypanosomes disappeared from the blood within 30 and 180 minutes after final intraperitoneal and oral treatments, respectively. Compound 49 was also shown to be highly non-toxic in vivo and able to cross the blood–brain barrier. A recent study by the same researchers has shown that treatment with 49 alone could also have therapeutic efficacy in mice if higher doses and a longer treatment were applied. 100 mg kg−1 of 49 given intraperitoneally for four consecutive days or 400 mg kg−1 given orally for eight consecutive days were necessary to cure mice.

2.2 Phenolic derivatives

Ascofuranone 49, a prenylated phenol antibiotic isolated from a phytopathogenic fungus, Ascochyta visiae, is a potent and specific inhibitor of the glycerol-3-phosphate-dependent mitochondrial oxygen consumption of T. b. brucei bloodstream forms. The specificity is due to the presence of a unique mitochondrial electron transport system in trypanosome bloodstream forms. It is composed of two enzymes, a glyceraldehyde-3-phosphate dehydrogenase and the trypanosome alternative oxidase (TAO), a ubiquinol-oxygen oxidoreductase. The two enzymes interact via ubiquinone, also known as coenzyme Q. The mechanism of action of 49, which has a structure analogous to that of coenzyme Q, is attributed to its binding at the coenzyme Q site of the ubiquinol oxidase, thus blocking the TAO. It has been demonstrated that in combination with glycerol, which suppresses a glycerol-producing anaerobic pathway by mass action, inhibitors of the TAO become trypanocidal due to a total block of the energy production of bloodstream forms. Compound 49 was potently trypanocidal in vitro for T. b. brucei bloodstream forms only when combined with glycerol 4 mM (IC50 without glycerol = 250 μM, IC50 with glycerol = 0.03 μM). First in vivo studies have shown that 49 at 100 mg kg−1 combined with glycerol 3 g kg−1 given orally or at 25 mg kg−1 combined with glycerol 3 g kg−1 given intraperitoneally gave 100% cure of mice infected with T. b. brucei. In each experiment, 49 was given at a single dose and glycerol was given at 1 g kg−1 at 30 minute intervals. Living trypanosomes disappeared from the blood within 30 and 180 minutes after final intraperitoneal and oral treatments, respectively. Compound 49 was also shown to be highly non-toxic in vivo and able to cross the blood–brain barrier. A recent study by the same researchers has shown that treatment with 49 alone could also have therapeutic efficacy in mice if higher doses and a longer treatment were applied. 100 mg kg−1 of 49 given intraperitoneally for four consecutive days or 400 mg kg−1 given orally for eight consecutive days were necessary to cure mice.

Other simple phenolic compounds which are widely distributed in plants have been tested for their antitrypanosomal activity as well. Gallic acid 50, a well-known component of hydrolysable tannins, is equally active on the bloodstream and procyclic forms of T. b. brucei (IC50 = 15.6 and 14.4 μM respectively). Gallic acid esters such as ethyl gallate 51 and n-propyl gallate 52 were more toxic to bloodstream forms than 50 (IC50 = 2.3 and 1.5 μM, respectively) but their activity on procyclic forms was weaker (32 and 29 μM, respectively). Syringic acid 53 and protocatechuic acid 54, which lack the pyrogallol moiety, were not toxic to bloodstream and procyclic forms (IC50 > 100 μM). The same researchers suggested that the formation of reactive oxygen species (such as the superoxide anion) might be involved in the gallic acid-induced trypanocidal activity, in other words, it would act as a pro-oxidant.

Bio-guided fractionation of a stem bark extract of Combretum molle (Combretaceae) led to the isolation of two classes of compounds: two inactive saponins and two hydrolysable tannins, punicagallin 55 and a structurally similar compound for which the full structure has not yet been elucidated (CM-A). Those two compounds exhibited activity on the mammalian
stage of T. b. rhodesiense (IC_{50} = 1.75 and 1.5 µM for 55 and CM-A) and were relatively less toxic to human epidermoid carcinoma KB cells (SI = 70 and 48, respectively, for 55 and CM-A). These two tannins showed a relative selectivity against trypanosomes, a result which contradicts the general belief that tannins are non-selective enzyme inhibitors due to their substantial number of phenolic groups. 

B. Ráez evaluated the in vitro antitrypanosomal activity of 132 flavonoids from the flavone, flavonol, flavanone, isoflavone and chalcone subclasses. Selectivity of the effect was determined by comparing the IC_{50} values of flavonoids against the parasite and adenocarcinoma cells (HT-29). 48 out of the 132 flavonoids displayed an IC_{50} value below 10 µM against T. b. rhodesiense bloodstream forms. Two compounds, 7,8-dihydroxyflavone 56 and quercetagetin 57, a flavonol, were selectively trypanocidal in the submicromolar range (IC_{50} = 0.16 and 0.8 µM, SI = 1019 and 571, respectively). Most of the compounds analyzed showed a selective trypanocidal activity with selectivity indices often greater than 100 when compared to the mammalian cell toxicity. In general, flavanones, which lack a double bond between C2 and C3, were less active than their flavone counterparts. Similarly, flavonols were more trypanocidal than their flavone counterparts, which lacks the hydroxyl substituent on C3. No clear structure–activity relationships could be drawn as a wider variety of substituents would need to be introduced more systematically. Camacho et al. confirmed the weak antitrypanosomal activity of quercetin 58 (IC_{50} = 13.2 µM on T. b. brucei bloodstream forms), a flavonol which only differs from quercetagetin 57 by the absence of an hydroxyl on C3. Compound 58 has been shown to be able to inhibit the F_{1}-ATPase of Trypanosoma cruzi, responsible for Chagas’ disease.

Four methoxylated flavones were isolated by bio-guided fractionation from the leaves of Ehretia amoena (Boraginaeae), a plant traditionally used in Uganda to treat sleeping sickness: chrysosolepinetin 59, chrysolepelon D 60, retusin 61 and artemetin 62. Compounds 59 and 60 were the most potent on T. b. rhodesiense bloodstream trypomastigotes (IC_{50} = 2.9 and 4.7 µM, respectively) while 61 was less active (IC_{50} = 14.2 µM). Although 62 was inactive (IC_{50} = 189 µM), the author showed that it increases synergistically in vitro the trypanocidal activity of the three other flavones. This synergy was already reported for 62 which enhanced the antilasmoidal activity of artemisinin in Artemisia annua (Asteraceae). On the other hand, an antagonistic effect was shown when 59 and 61 were combined, which clearly illustrates the complexity of crude extract activities.

From the roots of Tephrosia aquilata (Papilionaceae), four B-oxgenated chalcones were isolated. Demethylpraeclansone B 63 and praeclansone B 64 showed a low activity on the mammalian stage of T. b. rhodesiense (IC_{50} = 15–16 µM) and no cytotoxicity towards L6 cells, while praeclansone A 65 and its Z-isomer 66 were inactive.

Cissampelolavone 67 is a chalcone-flavone dimer isolated from the aerial parts of Cissampelos pareira (Menispermacaeae) with a good activity on T. b. rhodesiense bloodstream forms (IC_{50} = 1 µM) and a SI of 173 when compared to KB cells. However, other biflavonoids such as amonoflavone 68 and podocarpuflavones A 69 and B 70 isolated from the leaves of Celaenodonbrom mexicanum (Euphorbiaceae) were inactive on T. b. brucel.

Several diarylheptanoids isolated from species of the Zingiberaceae family have been tested for their antitrypanosomal activity. Curcumin 71, isolated from the rhizomes of Curcuma longa, displayed IC_{50} values of 0.83 µM and 4.35 µM for the bloodstream and procyclic forms of T. b. brucei, respectively. Four diarylheptanoids were isolated from the seeds of Aframomum letestuianum and showed different antitrypanosomal activity. Letestuianin C 72 and (4Z,6E)-5-hydroxy-1,7-bis(4-hydroxyphenyl)-hepta-4,6-dien-3-one 73 were effective with IC_{50} values in the range of 4–9 µM on the bloodstream forms of different T. brucei spp. isolates. In comparison,
letuestianins A 74 and B 75 were inactive. It should be noted that the additional methoxy group in 74 compared to 73 renders it inactive.\(^{39}\)

Two arylnaphthalide lignans were isolated from the aerial parts of \emph{Phyllanthus piscatorum} (Euphorbiaceae): justicidin B 76 and its C\(_{11}\)-hydroxylated derivative, piscatorin 77. Compound 76 exhibited a strong activity against the bloodstream trypomastigotes of \emph{T. b. rhodesiense} (IC\(_{50}\) = 0.55 \(\mu\)M) while 77 was 11 times less active (IC\(_{50}\) = 6.1 \(\mu\)M). Compound 76 was also more cytotoxic than 77, with IC\(_{50}\) values ranging from 0.6 to 13 \(\mu\)M depending on the mammalian cell line tested.\(^{39}\)

59 Two arylnaphthalide lignans were isolated from the aerial parts of \emph{Phyllanthus piscatorum} (Euphorbiaceae): justicidin B 76 and its C\(_{11}\)-hydroxylated derivative, piscatorin 77. Compound 76 exhibited a strong activity against the bloodstream trypomastigotes of \emph{T. b. rhodesiense} (IC\(_{50}\) = 0.55 \(\mu\)M) while 77 was 11 times less active (IC\(_{50}\) = 6.1 \(\mu\)M). Compound 76 was also more cytotoxic than 77, with IC\(_{50}\) values ranging from 0.6 to 13 \(\mu\)M depending on the mammalian cell line tested.\(^{39}\)

2.3 Quinones

It has been reported that quinones, especially 1,4-naphthoquinones such as plumbagin 78, can induce oxidative stress in trypanosomes (\emph{T. congolense})\(^{41}\) and \emph{T. cruzi}\(^{42}\). This may be explained by their reduction to semi-quinone radicals by enzymes such as those present in the mitochondrial electron transport chain and the trypanothione reductase, a key enzyme of the trypanosomal antioxidant thiol metabolism. Compound 78, which can be found in \emph{Drosera} species (Droseraceae), is active on the mammalian stage of \emph{T. b. brucei} (IC\(_{50}\) = 1.5–6.5 \(\mu\)M)\(^{24,62}\) while its reduced derivative, \emph{trans-}isoshinanolone 79 is inactive (IC\(_{50}\) > 65 \(\mu\)M).\(^{24}\)

Diospyrin 80, a bis-naphthoquinone isolated from the bark of \emph{Diospyros montana} (Ebenaceae), as well as semi-synthetic derivatives, have been investigated for their antitrypanosomal activity \emph{in vitro} on \emph{T. b. brucei} bloodstream forms. The IC\(_{50}\) of 80 was 50 \(\mu\)M while diospyrin dimethyl ether 81 and its hydroquinone form 82 were respectively 24 and 71 times more active than the parent compound (IC\(_{50}\) = 2.1 and 0.7 \(\mu\)M respectively).\(^{43}\) According to the authors, the enhanced activity of 82 as compared to the parent compound 80 could be due to the presence of the 4 hydroxyl groups in 82 which generate semi-quinone radicals more easily through one-electron shift leading thus to an increase of the oxidative stress in trypanosomes. On the contrary, in 80, the hydrogen bond between the carbonyl and phenolic groups of one naphthalene ring reduces the electron availability explaining the lower activity of 80.\(^{63}\)

Activity-guided fractionation of stem bark and root bark extracts of \emph{Kigelia pinnata} (Bignoniaceae) allowed the isolation of one furanonaphthoquinone, 2-(1-hydroxyethyl)-naphtho[2,3-b]furanyl-4,9-quinone 83, and three naphthoquinoids: isopinnatal 84, kigelinol 85, and isokigelinol 86. Compounds 83 and 84 possessed a pronounced activity against both \emph{T. b. brucei} and \emph{T. b. rhodesiense} bloodstream forms (IC\(_{50}\) = 0.12 \(\mu\)M and 0.045 \(\mu\)M respectively for 83 and 0.37 \(\mu\)M and 0.73 \(\mu\)M for 84) with a certain selectivity compared to KB cells (IC\(_{50}\) = 3.9 \(\mu\)M and 14.8 \(\mu\)M for 83 and 84 respectively). Compounds 85 and 86 had a less potent antitrypanosomal activity with IC\(_{50}\) values from 1.4 to 21.3 \(\mu\)M depending on the trypanosome tested.\(^{64}\)

Bioactivity-guided fractionation of an extract from the aerial parts of \emph{Stephania dinklagei} (Menispermaceae) led to the isolation of only one furanophthoquinone, 2-(1-hydroxyethyl)-naphtho[2,3-b]furanyl-4,9-quinone 83, and three naphthoquinoids: isopinnatal 84, kigelinol 85, and isokigelinol 86. Compounds 83 and 84 possessed a pronounced activity against both \emph{T. b. brucei} and \emph{T. b. rhodesiense} bloodstream forms (IC\(_{50}\) = 0.12 \(\mu\)M and 0.045 \(\mu\)M respectively for 83 and 0.37 \(\mu\)M and 0.73 \(\mu\)M for 84) with a certain selectivity compared to KB cells (IC\(_{50}\) = 3.9 \(\mu\)M and 14.8 \(\mu\)M for 83 and 84 respectively). Compounds 85 and 86 had a less potent antitrypanosomal activity with IC\(_{50}\) values from 1.4 to 21.3 \(\mu\)M depending on the trypanosome tested.\(^{64}\)

Phenylanthraquinones are constitutionally unsymmetric biaryls that have already shown potential as antiplasmodial
The sesquiterpene lactones tested, which possessed only one alkylation center, exhibited a less potent and less selective trypanocidal activity (IC50 = 1.4–23.6 µM). The authors postulate that the mechanism of action of these molecules towards trypanosomes might depend on interference with trypanothione metabolism (for example trypanothione reductase) leading to increased oxidative stress in the parasite.72

A diastereoisomer of the diterpene kolavenol 106, was isolated from a rootbark extract of *Entada abyssinica* (Leguminosae), a plant traditionally used in Uganda to treat sleeping sickness. It showed a trypanocidal activity with an IC50 value of 8.6 µM against *T. b. rhodesiense* bloodstream trypanomastigotes.73

**2.4 Terpenes**

Mikus et al. evaluated the effect of several mono- and sesquiterpenes, which are frequently present in essential oils, on the viability of *T. b. brucei* bloodstream forms and of human HL-60 cells. The only active monoterpene was terpinen-4-ol 95, a monoterpene alcohol, which exhibited a high antitrypanosomal activity (IC50 = 0.13 µM) with a remarkable SI of 1000 (IC50 on HL-60 cells = 133 µM). The sesquiterpene allomadendrene 96 had a moderate activity (IC50 = 9.3 µM) but aromadendrene 97, a diastereoisomer, was 10 times less active on trypanosomes.69 (−)-Boscalin 98, a volatile aroma component of various plants, as well as three of its stereoisomers obtained synthetically (99,100,101), were tested for their antitrypanosomal activity and cytotoxicity on human HT-29 cancer cells.
Several terpenoids, as well as a coumarin, were isolated from the leaves of *Guarea rhophalocarpa* (Malpighiaceae): only two lanostane triterpenoids showed some effect against the mammalian stage of *T. b. brucei*: 23-Hydroxy-5a-lanosta-7,9(11),24-triene-3-one 107 was active with an IC\(_{50}\) of 5 µM. 5a-Lanosta-7,9(11),24-triene-3a,23-diol 108, with a hydroxyl group on C3 instead of a ketone had an enhanced activity (IC\(_{50}\) = 1.75 µM) and was 12 times less cytotoxic to KB cells (IC\(_{50}\) = 21.2 µM).

Various terpenoids, as well as a coumarin, were isolated from the leaves of *Vernonia guineensis* (Asteraceae), exhibited significant inhibitory activity against four strains of *T. b. rhodesiense* mammalian trypomastigotes with IC\(_{50}\) values in the range of 5–10 µM. Other sterols isolated from *Guarea rhophalocarpa* (Malpighiaceae) and *Galphinia glauca* (Malpighiaceae) were inactive on trypomastigones.

### 2.5 Other metabolites

Bio-guided-fractionation of a methylene chloride extract of the stems of *Uvaria klaineana* (Annonaceae) led to the isolation of klahvanolide 112 (5-acetoxy-7-benzoyloxymethyl-7H-oxepin-2-one), a bisunsaturated seven-membered lactone which has a moderate activity on *T. b. brucei* bloodstream forms (IC\(_{50}\) = 33.2 µM). 109, a triruicalla-type triterpene, had IC\(_{50}\) values of 16.8 µM and 137.6 µM on trypanosomes and KB cells respectively.57 Two stigmastane-type steroids, vernoguinoside 110 and vernoguinoside 111, isolated from the stem bark of *Vernonia guineensis* (Asteraceae), exhibited significant inhibitory activity against four strains of *T. b. rhodesiense* mammalian trypomastigotes with IC\(_{50}\) values in the range of 5–10 µM.55 Other sterols isolated from *Guarea rhophalocarpa* (Malpighiaceae) and *Galpithia glauca* (Malpighiaceae) were inactive on trypomastigones.

Aculeatins, a class of compounds with a 1,7-dioxadispiro-[5.1.5.2]-pentadecan skeleton, have been isolated from the rhizome of *Annonom aculeatum* (Zingiberaceae) and exhibit a strong, but non selective, activity against the bloodstream trypanomastigotes of *T. b. rhodesiense*. For example, aculeatin D 113 had an IC\(_{50}\) of 0.5 µM and of 0.9 µM on *T. b. rhodesiense* and KB cells respectively.77,79

Acetogenins, such as senegalenic 114 and squamocine 115, isolated from the seeds of *Annona senegalensis* (Annonaceae) show activity against bloodstream forms of *T. b. brucei* (IC\(_{50}\) = 16 µM). However, these metabolites also show a cytotoxicity against KB and monkey kidney cells (VERO cells) greater than that of vincristine, an antitumor compound taken as reference.79

Different sulfur containing aliphatics, initially isolated from garlic bulb (*Allium sativum* Liliaceae), have been studied for their antitrypanosomal activity.50–52 Diallyl trisulfide 116 is a chemically stable final transformation product of allicin that can be synthesized and is used in China to treat bacterial, fungal and parasitic infections in man. This product exhibits IC\(_{50}\) values in the range of 10–15 µM when tested in vitro on bloodstream forms of *T. b. brucei*, *T. b. gambiens*, *T. b. rhodesiense* and of 30 µM when tested on *T. congolense* bloodstream forms. Up to 140 µM, no noticeable morphological changes could be observed on fibroblasts.20 A fraction of the oily extract from garlic bulb, which apparently contained mainly diallyl disulfide 117, cured mice infected with *T. b. brucei* in four days when given intraperitoneally at a dose of 120 mg kg^{-1} per day. Nok et al. showed that the extract interferes with the parasites’ synthesis of membrane lipids.83 The effects of garlic extract and various of its purified components on metabolic enzymes, especially thiol-containing enzymes, have already been reported (e.g. Gallwitz et al.).82 They showed that ajoenic 118, another garlic-derived natural product, affects the antioxidant thiol metabolism of *T. cruzi*, leading to increased oxidative stress by inhibition of the trypanothione reductase.82

Manumycin A 119, an antibiotic produced by *Streptomyces* microorganisms, is an inhibitor of Ras farnesylationtransferase, which is of interest for antitumour therapy. Compound 119 is potently active in vitro against the growth of both bloodstream and procyclic forms of *T. b. brucei* (IC\(_{50}\) = 1.5 and 0.4 µM respectively). Its mode of action seems complex: it does not affect trypanosomal protein or DNA synthesis nor cell cycle progression but inhibits farnesylation and causes significant mitochondrial damage possibly by interference with electron/proton transport systems. In vivo 119 is well tolerated but fails to cure experimental trypanosomiasis in mice when administered intraperitoneally.83

Sinefungin 120, a natural nucleoside produced by *Streptomyces griseolus* and *S. incarnatus*, is a structural analog of S-adenosylmethionine 121 (SAM), a molecule which plays an important role in the biosynthesis of polyamines and in the trans-methylation of proteins and lipids. It has been shown that 120 is a strong inhibitor of SAM-dependent transmethylation reactions.84 Compound 120 potently inhibits the in vitro growth of *T. b. rhodesiense* bloodstream trypanomastigotes (IC\(_{50}\) = 0.0004 kg per day. Nok et al. showed that the extract interferes with the parasites’ synthesis of membrane lipids.83 The effects of garlic extract and various of its purified components on metabolic enzymes, especially thiol-containing enzymes, have already been reported (e.g. Gallwitz et al.).82 They showed that ajoenic 118, another garlic-derived natural product, affects the antioxidant thiol metabolism of *T. cruzi*, leading to increased oxidative stress by inhibition of the trypanothione reductase.82

Manumycin A 119, an antibiotic produced by *Streptomyces* microorganisms, is an inhibitor of Ras farnesylationtransferase, which is of interest for antitumour therapy. Compound 119 is potently active in vitro against the growth of both bloodstream and procyclic forms of *T. b. brucei* (IC\(_{50}\) = 1.5 and 0.4 µM respectively). Its mode of action seems complex: it does not affect trypanosomal protein or DNA synthesis nor cell cycle progression but inhibits farnesylation and causes significant mitochondrial damage possibly by interference with electron/proton transport systems. In vivo 119 is well tolerated but fails to cure experimental trypanosomiasis in mice when administered intraperitoneally.83

Sinefungin 120, a natural nucleoside produced by *Streptomyces griseolus* and *S. incarnatus*, is a structural analog of S-adenosylmethionine 121 (SAM), a molecule which plays an important role in the biosynthesis of polyamines and in the trans-methylation of proteins and lipids. It has been shown that 120 is a strong inhibitor of SAM-dependent transmethylation reactions.84 Compound 120 potently inhibits the in vitro growth of *T. b. rhodesiense* bloodstream trypanomastigotes (IC\(_{50}\) = 0.0004
The support by the Belgian National Fund for Scientific Research is gratefully acknowledged (fellowship to S. H.).