Natural Products as potential antiparasitic drugs

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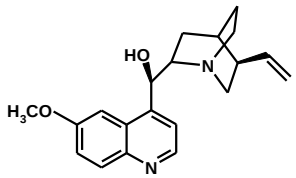
ABSTRACT: Pharmaceutical research in natural products represents a major strategy for discovering and developing new drugs. The use of medicinal plants for the treatment of parasitic diseases is well known and documented since ancient times e.g. by the use of *Cinchona succiruba* (Rubiaceae) as an antimalarial. This chapter provides a comprehensive review of the latest results in the field of antiparasitic drug development from biologic sources (plants, bacteria, fungi and marine organisms) focussing on the treatment of protozoal infections (*Plasmodium*, *Leishmania*, *Trypanosoma* spp.). The status of validated in vitro and in vivo assays is reviewed, discussing their different features, problems and limitations. Because of the high number of natural products tested against the aforesaid protozoa in the last years, we limit the discussion to lignans, phenolics, terpenoids, and alkaloids as defined natural product classes. The review also covers essential research topics of recent publications on specific natural products (e.g. licochalcone A, benzyl- and naphthylisoquinoline alkaloids, and artemisinin) and gives an outlook to semi-synthetic approaches of drugs already introduced in clinics or in clinical trial studies.

1. INTRODUCTION

The fascination of natural products, mostly as used as a preparation from a plant with known medicinal properties, goes back to ancient times. The discovery of pure compounds as active principles in plants was first described at the beginning of the 19th century, and the art of exploiting natural products has become part of the molecular sciences. The discovery of quinine (**1**) from *Cinchona succiruba* (Rubiaceae) and its subsequent development as an antimalarial drug represented a milestone in the history of antiparasitic drugs from nature for the treatment of all parasitic diseases – not just infections caused by only *Plasmodium, Leishmania* and *Trypanosoma* spp.. Early studies on plant products were followed by an era of organic chemistry that led to the development of arsenical and antimonial (melarsoprol, sodium stibogluconate), diamidine (pentamidine) and nitroheterocyclic (metronidazole) antiprotozoal drugs. In the past decades natural products have attracted renewed interest, especially with bacteria and fungi as important sources of biologically active compounds. Recently marine organisms have also been recognized as attractive source of antiparasitic compounds, and it can be expected that in the future other living organisms (for example, insects and amphibians) will provide additional sources. It is, therefore, not surprising that one of the most rewarding frontiers in modern science is the study of the chemistry and biology of natural products. Discovering untapped natural sources of novel antiprotozoal compounds from nature remains a major challenge and a source of novelty in the era of combinatorial chemistry and genomics. Since plants contain a high variety of constituents it is often claimed that the use of a whole plant rather than one single purified product may be more effective therapeutically. Because of the limited space in this contribution we restrict ourselves to defined natural products that have been tested in standard *in vitro* and *in vivo* assays.



(**1**) quinine

2. PRESENT SITUATION AND CHEMOTHERAPY OF MALARIA, LEISHMANIASIS AND TRYPANOSOMIASIS

2.1 MALARIA

It is estimated that there are 300-500 million cases of malaria annually with 1.75 to 2.5 million deaths. Malaria is a particularly important disease in sub-Saharan Africa, where about 90% of cases and deaths occur, but is also a serious public health problem in certain regions of southeast Asia and South America [1]. Human malaria is caused by four species of *Plasmodium, P. falciparum, P. vivax, P. ovale* and *P. malariae*, which are transmitted by female *Anopheles* mosquitoes. The majority of cases of malaria and deaths are caused by *P. falciparum* [1].

The life cycle, immunological defence mechanisms, and clinical development of malaria in humans is complex [2, 3]. The sporozoites that develop in the salivary glands of the female mosquito are inoculated into the human when then the insect bites to acquire a bloodmeal. The sporozoites travel in the bloodstream to the liver where they invade the hepatocytes, differentiate and undergo asexual division (the exoerythrocytic cycle) and form a schizont (a multiple division form). Mature tissue schizonts release thousands of merozoites after 5-15 days (depending on species). The merozoites invade erythrocytes where they appear initially as a ring stage, followed by a growing trophozoite stage, which develops into a dividing asexual schizont stage. During the erythrocytic stage, which lasts from 48-72 hours depending upon species, the parasite develops in a parasitophorous vacuole surrounded by host cell membrane. The *Plasmodium* parasite adapts to life within the erythrocyte, depending mainly on glycolysis for energy and altering the erythrocyte membrane with transporters that enable increased uptake of hexoses, amino acids and lipid precursors. During the growth cycle up to 80% of host cell haemoglobin is ingested by and digested in the food vacuole of the *Plasmodium* trophozoite. The trophozoite divides (schizogony) and the erythrocyte lyses releasing more merozoites to invade further erythrocytes. At some point during the infection the intraerythrocytic stage develops to form sexual stages, male or female gametocytes, rather than merozoites. These sexual stages are taken up

in a blood meal by another *Anopheles* mosquito where fertilisation occurs and the life cycle is completed.

Clinical malaria is characterised by periodic fever, which follows the lysis of infected erythrocytes, and caused mainly by the induction of cytokines interleukin-1 and tumour necrosis factor. *P. falciparum* infection can have serious effects, for example anaemia, cerebral complications (from coma to convulsions), hypoglycaemia and glomerulonephritis. The disease is most serious in the non-immune, including children, pregnant women and tourists. Humans in endemic areas, who have survived an attack of malaria, are semi-immune and disease can be characterised by headache and mild fever. Infection by the other species of *Plasmodium* is normally self-limiting although relapses may occur, particularly in *P. vivax* infections. The species of parasite and the age and immune status of the patient are important in considerations of treatment and interpretation of the effects of all medicines.

The chemotherapy and prophylaxis of malaria have been undermined by the development of worldwide resistance of *P. falciparum* to the 4-aminoquinoline chloroquine, first observed in the 1960s, as well as resistance to the antifolates pyrimethamine and cycloguanil. Resistance to quinine and other more recently developed drugs, for example mefloquine, have also been reported [4, 5]. The search for alternative antimalarials is one of the main themes of this chapter.

The re-emergence of malaria as a public health problem is due mainly to the development of resistance of *P. falciparum* to cheap highly effective drugs like chloroquine and pyrimethamine. As a consequence of this problem over 300,000 compounds were tested for antimalarial activity by the Walter Reed Army Institute of Research, USA between 1965 and 1986. The 4-quinolinemethanol mefloquine and the 9-phenanthrenemethanol halofantrine emerged from this programme. Mefloquine (see also section 6.2) has been registered for little over 10 years, but there is already resistance in South East Asia, concern over cross-resistance with quinine and controversy over toxic side effects. Chloroquine is still used in some low resistance areas in Africa and South America and quinine is used for the treatment of cerebral malaria (see section 6.2). The most important recent discovery for the therapy of *P. falciparum* malaria has been the identification of the sesquiterene lactone artemisinin (qinghaosu) from

Artemisia annua (Asteraceae) (see section 6.1). Artemisinin and its' derivatives, for example artemether and artesunate, are rapid acting antimalarials, effective against multidrug resistant *P. falciparum,* that have been used to treat over 3 million cases in South East Asia [6]. Another new drug, the hydroxynaphthoquinone atovaquone, identified as an antimalarial in the early 1980s [7], has proved to be highly effective in clinical trials but has to be used in combination with proguanil (as Malarone®) to prevent the development of resistance. The use of combinations to combat the development of resistance is a current strategy as demonstrated in the clinical use of other combinations for example mefloquine - artemisinin, co-artemether (lumefantrine/ benflumetol - artemether) and Lapdap® (chlorproguanil - dapsone) [8]. A new 8-aminoquinoline, tafenoquine, is on clinical trial as a potential replacement for primaquine to treat *P. vivax* malaria; it also holds promise as a prophylactic against *P. falciparum* [9].

2.2 LEISHMANIASIS

Protozoa of the genus *Leishmania* are obligate intracellular parasites of mononuclear phagocytes. Leishmaniasis is a spectral disease, depending on the *Leishmania* species involved and genetic potential and acute predisposition the hosts defense system. It ranges from self-healing ulcers (cutaneous leishmaniasis, CL) to progressive naso-pharyngal infections (mucocutaneous leishmaniasis, MCL) to disseminating visceral leishmaniasis (VL). While CL poses essentially cosmetic problems, and MCL leads to painful disfiguration, social stigmatization and often highly severe secondary infections, VL is generally lethal if left untreated. Leishmaniasis occurs from tropical to Mediterranean regions where the parasite is transmitted by female sandflies of the genus *Phlebotomus* in the Old World and *Lutzomyia* in the New World [10]. In the insect gut as well as in tissue culture media, the parasite exists as extracellular, elongated, flagellated promastigotes. Promastigotes are injected into the skin during a blood meal and rapidly taken up by mononuclear phagocytes where they reside in the parasitophorous vacuole. In contrast to other intracellular pathogens, for example *Toxoplasma*, *Leishmania* do not inhibit fusion

of lysosomes with the parasitophorous vacuole. Within the phagolysosome the parasites transform into and multiply as ovoid amastigotes, which are specially adapted to the elevated temperatures within the mammalian host and the hostile environment within these potent effector cells [11]. Massive amastigote multiplication leads to host cell disruption and release of parasites to infect freshly recruited host cells. While "resting" macrophages support parasite multiplication and thus spread of infection, these cells can also be activated by elements of the natural and the specific cellular immune response to kill intracellular parasites leading to cure. The prime signal for macrophage activation is the cytokine interferon IFN-γ, a glycopeptide released by Natural Killer (NK) cells and T lymphocytes. In the murine model for CL caused by *L. major* it was shown that distinct populations T cell populations exist with antagonizing effects. While T helper 1-type cells secrete IFN-γ and promote cure, Th2-type cells secrete interleukin IL-10, inhibiting macrophage activation and exacerbating disease. The exact factors promoting each type of immune response remain obscure but are in part genetically determined.

According to the World Health Organization (WHO) there are 2 million new cases of leishmaniasis per year. With the advent of AIDS, which currently affects over 30 million people in geographical regions largely overlapping with leishmaniasis, the nature of the disease has changed. In Mediterranean countries, where infant VL is endemic, adult VL is now considered a genuine AIDS-related opportunistic disease largely due to reactivation of latent infections by immunosuppression [12].

Although the three disease complexes leishmaniasis, human African trypanosomiasis (HAT, sleeping sickness) and South American trypanosomiasis (Chagas disease) are caused by closely related trypanosomatid parasites, the diseases are treated with different drugs and the parasites themselves have varying sensitivities to many compounds [13, 14]. The recommended drugs for both visceral and cutaneous leishmaniasis are the pentavalent antimonials sodium stibogluconate (Pentostam) and meglumine antimoniate (Glucantime). Both drugs have been used for over 50 years, require long courses of parenteral administration, and have toxic side effects. In addition there has been a dramatic increase in the number of cases

of VL in northeastern India that do not respond to antimonials. Alternative treatments for VL include the polyene antibiotic amphotericin B [15] that has highly effective less toxic lipid formulations [9]. A parenteral formulation of the aminoglycoside paromomycin (aminosidine), and the orally available alkyl-phospholipid miltefosine [16] are also potential treatments for VL. Interest in a new treatment for CL has focussed on different topical formulations of paromomycin. The increasingly observed partial resistance to antimonials has been overcome by higher dose regimens but in general therapy of MCL and VL is becoming increasingly problematic. Experimental studies and treatment of AIDS patients has revealed that successful treatment with some drugs requires the active participation of the immune system [12]. Antimonial drugs have been successfully combined with IFN-γ, although costs of such treatmenr render it useless for third world countries.

2.3 AFRICAN TRYPANOSOMIASIS

Flagellated protozoa of the genus *Trypanosoma* infect humans (sleeping sickness; *T. brucei rhodesiense*, *T. b. gambiense*) and domestic animals (nagana; *T. b. brucei*, *T. congolense*, *T. vivax*) causing major health and commercial problems in subSaharan Africa. In the 1960s African trypanosomiases was under control, mainly due to eradication of its insect vector, the tsetse fly. According to the WHO, sleeping sickness is again endemic in 36 African countries with over 250 thousand newly infected cases per year [17]. Following the bite of an infected tsetse (*Glossina* spp.) parasites multiply in the skin for one to three weeks before invading the haemolymphatic system. Early symptoms include high fever, swelling of lymph nodes (neck), hepatosplenomegaly, oedema and diarrhoea. Generalized inflam-mation of blood and lymph vessels may lead to myocarditis and encephalitis and often to death due to heart failure. Days or many weeks later, parasites invade the central nervous system causing the typical symptoms of sleeping disease: failures in speech and coordination of movement, epileptic episodes, somnolence, apathy, cachexy, and finally - if left untreated - coma and death [18].

Most *Trypanosoma* spp. are found only in wild and domestic animals. While for some species (e.g. antelopes, goats, pigs) infection with *T. brucei* produces no apparent symptoms, others (e.g. dogs, horses, cattle) often succumb to disease. Cattle show reduction in weight gain, milk yield, reproduction, and general performance. Laboratory strain rats and mice can also be infected, providing useful experimental models. In West African sleeping sickness, especially during epidemics, there is direct human-to-human transmission of *T. b. gambiense*, whereas the other species that infects humans, *T. b. rhodesiense*, is mainly transmitted between reservoir mammals.

T. brucei spp. are extracellular parasites living in blood and lymph as elongated trypomastigotes. Here, they obtain their energy by glycolysis, whereas in their insect vector they switch to oxidative metabolism using mainly proline. In contrast to intracellular parasites such as *Trypanosoma cruzi* and *Leishmania sp*., *T. brucei* are vulnerable for humoral defense mechanisms. Complement-activating antibodies may bind to their surface, facilitating recognition, phagocytosis and destruction by the monocytic phagocyte system. *Trypanosoma brucei* evade eradication by antigenic variation, sequentially producing new clones differing in their surface glycoproteins which are not susceptible to the prevailing antibody population. This strategy of antigen variation effectively neutralizes the hosts' immune defense mechanisms and has confounded all efforts of vaccine development. Furthermore, *T. brucei* organisms have been found to activate immunosuppressive macrophage functions.

Obviously, any successful strategy to combat the African trypanosomiases must be two-pronged: controlling the insect vector and combating the parasites by prophylactic and therapeutic measures. Chemotherapy of nagana has been reliant for over 40 years on diminazene (berenil), isometamidium and homidium. Due to the intensive use and structural similarities of these drugs, trypanosomes have developed multiple drug resistance in Ethiopia, Kenya, Somalia, and many other African countries.

Drugs for the treatment of human African trypanosomiasis are also inadequate. The diamidine pentamidine and the sulphonated naphthylamine suramin have been used for over 50 years, require parenteral administration and are only effective against the early haemolymphatic stage of the disease. The only drug currently available for the treatment of the late stage CNS infection is the

trivalent arsenical melarsoprol. Melarsoprol requires parenteral administration, has unacceptable side effects including 5 % mortality due to encephalopathy, and there are an increasing number of patients who are no longer responding to treatment. Eflornithine, an inhibitor of polyamine biosynthesis, proved to be an effective treatment in trials in the late 1980s and was registered in 1990 for the treatment of late stage infections caused by *T. b. gambiense* (but not *T. b. rhodesiense* which is refractory). Limited supplies of the eflornithine are available as the drug is no longer manufactured [21].

2.4 SOUTH AMERICAN TRYPANOSOMIASIS

South American trypanosomiasis (or Chagas disease) is caused by *Trypanosoma cruzi* and is widespread thoughout the subcontinent with an estimated 18 million people infected. The parasite is transmitted by triatomine insects, not directly by by contamination: the insect defaecates whilst taking a blood meal and parasites in the faeces can invade wounds, the eye or mucosal tissues. In mammals the parasite exists in two forms: the extracellular trypomastigote that is an invasive haemolymphatic form and is non-dividing, and the amastigote that divides in the cytoplasm of cells. *T. cruzi* trypomastigotes will invade many cell types, in particular macrophages, muscle cells and nerve cells. Chagas disease is characterised by three stages: (a) an early symptomatic acute phase during which the infection spreads throughout the body and up to 30% of deaths occur, (b) an asymptomatic indeterminate phase that may last for many years, and (c) a symptomatic chronic phase when immunopathological reactions to low numbers of parasites in heart and nerve cells cause illness and death. Some cases of transmission of Chagas disease in urban areas have resulted from blood transfusion.

Two drugs have been available for the treatment of Chagas disease since the early 1970s: the nitrofuran derivative nifurtimox (now no longer manufactured) and the 2-nitroimidiazole derivative benznidazole. Both drugs are only effective in the acute phase of the disease, have variable efficacy against different strains of *T. cruzi*, require long courses of (oral) treatment, and have high levels of side effects. Few drugs have proved effective against all stages of the

disease. The antifungal sterol biosynthesis inhibitors have shown the most promising activity against *T. cruzi* inexperimental models. Recently Urbina and colleagues [20] have identified several antifungal triazoles that are active against both acute and chronic *T. cruzi* infections in rodent models.

2.5 CHEMOTHERAPY OF OTHER PROTOZOAN DISEASES

For several other protozoan diseases there is adequate chemotherapy: the 5-nitroimidazoles (for example, metronidazole) for the treatment of amoebiasis, giardiasis and trichomoniasis, the hydroxy-naphthoquinone bupravaquone for theileriosis in cattle and other ungulates, and the polyene ionophores (for example monensin, lasalocid, narasin and salinomycin) for the prophylaxis of avian coccidiosis. However, improved therapies are required for some opportunistic parasites that cause disease in immunocompromised humans. Paromomycin and nitazoxanide have some effect in the treatment of cryptosporidiosis and albendazole appears to be effective for microsporidiosis caused by *Encephalitizoon intestinalis*.

3. IN VITRO AND IN VIVO ASSAYS FOR DETERMINATION OF DRUG ACTIVITY

3.1 ANTIPLASMODIAL ACTIVITY

Prior to 1976, when the asexual cycle of *P. falciparum* was successfully cultured in human erythrocytes, antimalarial drug screening was dependent upon *in vivo* avian and rodent models, using non-human *Plasmodium* species. Since 1976 *P. falciparum* has been used extensively in *in vitro* screens; techniques to culture the other three human *Plasmodium* species have been less successful. A semi-automated microdilution assay, in which parasite viability and drug activity is related to the uptake of a nucleic acid precursor [^{3}H]-hypoxanthine, was described by [22] and is still widely used in modified 96-well format versions [23]; 384 well format assays are also now being used. The sensitivity (and resistance) of a wide range of *P. falciparum* strains to the standard antimalarial drugs has been reported using this assay. The technique has some limitations but

modifications using synchronous cultures and altering the medium have enabled studies on differential drug effects on trophozoite stages and schizonts [24] or improved sensitivity to antifolates [25]. A colorimetric assay based on the reduction of nitroblue tetrazolium to formazan by lactate dehydrogenase (a glycolytic enzyme essential in *Plasmodium*) has been used to test drugs against *P. falciparum* in vitro [26], and is simpler and cheaper to use but less sensitive than the [^{3}H]-hypoxanthine assay.

Drug sensitivity testing for *P. vivax* and *P. ovale* remains a problem as continuous culure of these species is difficult due to nutritional and host cell requirements; only short term cultures have been used in drug tests [27]. *In vitro* culture of the exoerythrocytic liver stages of malaria infection have been achieved using hepatocytes and hepatomas to culture *P. berghei*, *P. vivax* and *P. falciparum* [28] but these models have not been used widely in drug evaluation studies.

None of the four *Plasmodium* species that cause disease in humans can infect rodents or other animals used in *in vivo* screens. For the past 40 years *in vivo* screens have based on rodent malaria models, in particular those using *P. berghei*, *P. yoelii* and *P. chabaudi* infections in mice. Rodent malaria has proved to be an essential part of the process of drug development for detecting blood schizonticidal, tissue schizonticidal and repository activity. The activity of standard antimalarials against a number of *P. berghei* and *P. yoelii* strains has been well characterised [29]. The development of drug-resistant strains of these parasites has added to their use in the identification of novel drugs and drug combinations. However, there are differences between the biology of these rodent species and that of *P. falciparum* and the pharmacokinetic properties of a drug in mouse and humans can be different. Candidate antimalarial drugs are therefore often tested in primate models of *P. falciparum* infection using *Aotus* and *Saimiri* monkeys.

3.2 ANTILEISHMANIAL ACTIVITY

In their mammalian hosts, *Leishmania* parasites exist primarily as amastigotes within phagolysosomes of macrophages. Extra- and intracellular promastigotes occur only during a few hours after infection and extracellular amastigotes appearing between disruption

of one host cells and uptake by the next. Antileishmanials must be also be accumulated by infected macrophages enter phagolysosomes and be active under the specific conditions within this compartment (e.g. low pH) and either kill amastigotes or reduce viability. The relative ease of mass cultivation of most *Leishmania* isolates in the laboratory and the availability of relevant animal models for human CL and VL facilitate adequate screening for novel antileishmanials at different levels of complexity.

Toxicity assays for extracellular promastigote are easy to perform and have been used in drug screening. However, they have limited as the intracellular amastigote has different biochemical and molecular properties. Promastigote *Leishmania* are cultured in a variety of liquid or two-phase semi-synthetic or fully synthetic media generally at 23-27 °C and direct cytotoxic effects can be assessed microscopically as reduced motility, altered morphology (rounded and bloated) or reduced numbers. Growth inhibition assays involving incorporation of radioactive nucleotides (e.g. [^{3}H]-thymidine) by proliferating cells or metabolization of chromophores (e.g. MTT) [30].

Assessments of effects on the intracellular survival of amastigotes is more complex. Primary macrophage cultures or monocytic cell lines are parasitized *in vitro* with promastigote *Leishmania* cultures or freshly isolated amastigotes. The former should be cultured at 37 °C at least over night to allow infection and intracellular transformation to amastigotes [31]. After exposure to test compounds, the cultures ells are stained and the average number of intracellular parasites/host cell determined in comparison to untreated controls. For mass screening, parasitized macrophages can be seeded into microtiter plates. After exposure to test substances, the host cells can be selectively lysed releasing intracellular parasites. The relative numbers of surviving transformed parasites can then be determined by the radiometric or colorimetric methods described above [31]. Indirect antileishmanial activity through activation of host macrophages needs to be assessed separately in adequate toxicological and immunological assays.

For many Leishmania spp. that cause visceral or cutaneous leishmaniasis, rodents are natural hosts and provide excellent laboratory models. Furthermore, genetically defined mouse strains vary according to their spontaneous healing/non-healing capacity and immune response patterns thus providing means for specific experimental design. For CL, highly susceptible, non-curing BALB/c

mice can be inoculated subcutaneously with *L. major* into a footpad or the shaven rump. Progression of disease is monitored by measuring the diameter of the developing lesion. Antileishmanial drugs can be given orally, as topical ointments, or injected into the lesion. As tissue response does not necessarily correlate with parasite numbers, impression smears of tissue samples taken from the periphery of the ulcer should be stained and evaluated microscopically. Alternatively, viable parasite numbers can be estimated from limiting dilution cultures of tissue homogenates. For VL, hamsters or mice should be inoculated intravenously or intracardically with *L. donovani*. Disease progression can be monitored by the extent of cachexia or development of ascites. Visceral leishmaniasis affects all internal organs, and parasite counts are performed at least on spleen, liver and bone marrow, again using impression smear or limiting dilution culture methods. Sensitive molecular techniques such RT-PCR - while providing little advantage when quantifying an acute infection - are useful for detection of latent infections after parasitological cure.

3.3 ANTITRYPANOSOMAL ACTIVITY (*TRYPANOSOMA BRUCEI*)

T . b. brucei, T.b. rhodesiense and *T.b. gambiense* extracellular blood-stream form trypomastigotes can be grown axenically in supplemented standard media. Trypanocidal activity may be tested by culturing a constant number of parasites (10^5/ ml) in serial dilutions of substances for 24-72 hours in microtitre plates at 37 °C and 5 % CO_2. For reference, standard drugs such as melarsoprol (Arsobal®), pentamidine (Pentacarinat®) or suramin (Germanin®) should be included. The % surviving trypomastigotes indrug treated cultures can be estimated colorimetrically using a *p*-nitrophenyl phosphate as substrate for acid phosphatase [32], the MTT assay (described in section 3.2) or a fluorochrome such as Alamar blue [33]. Plotting % growth inhibition {= [1 - δ_{sample} - $\delta_{max\ kill}$) / ($\delta_{max\ growth}$ - $\delta_{max\ kill}$)] x 100} against drug concentration, dose-response curves can be generated and EC_{50} and EC_{90} values calculated for better comparison. It should be noted, that for some drugs, the two major variants *T. b. gambiense* and *T. b.*

rhodesiense exhibit in vitro differences in drug sensitivity and some compounds, for example eflornithine are poorly active *in vitro* .

Most animal models are restricted to testing compounds against *T. b. brucei* or *T. b. rhodesiense*. In vivo tests against. *T. b. gambiense* are restricted to *Mastomys* rats or *scid* mice. However, some strains of *T. brucei* can establish chronic CNS infections in mice thus providing models for late-stage sleeping sickness. An effective new drug for sleeping sickness must also be capable to cross the blood/ brain barrier. Monkey models, available for testing of candidate drugs, can also provide data on the penetration of drugs into the CNS.

3.4 ANTITRYPANOSOMAL ACTIVITY (*TRYPANOSOMA CRUZI*)

The dividing *T. cruzi* amastigote can be cultured in vitro in a variety of host cells, most commonly macrophages, fibroblasts and myoblasts. Trypomastigotes used to infect these cells can also be cultured in vitro. Two points about the design and interpretation of the assay: (a) it is normally limited to 3-4 days, as intracellular amastigotes transform to trypomastigotes after this time, and (b) as drug activity is measured by determining the number of amastigotes/host cell in treated and untreated cultures, the effects of compounds on the division rate of host cells as well as amastigotes has to be considered. The standard drugs nifurtimox or benznidazole should be included for comparison, but as mentioned above their activity is variable depending upon the strain of *T. cruzi* used. This also underlines the importance of testing lead compounds against a number of strains of this parasite. Recently the in vitro amastigote assays have been automated through the use of beta-galactosidase transfected *T. cruzi* Tulahuen strain [19]. Active compounds can be tested further against the extracellular trypomastigotes over 24 hours at 4^{o}C as a new drug for sterilizing blood transfusions is also required.

Inbred mouse strains offer the most useful *in vivo* models, either for simple suppressive tests or more complex curative tests. Mice are infected by trypomastigotes and treatment starts when the parasitaemia is detectable in tail blood. To determine cure sensitive techniques that can detect low numbers of parasites (haemoculture, serology, immunohistopathology, PCR) have to be performed on

blood, muscle and other tissues [20]. Again it is important to include standard drugs and a number of strains of *T. cruzi* in the process of identifying a lead compound. Chronic infections can be established by infecting with a low number of parasites (10^3) and monitoring the infection by sensitive techniques during and after treatment [20].

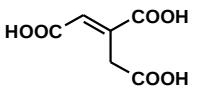
4. ANTIPROTOZOAL DRUGS FROM NATURE

4.1 LIPIDS AND RELATED ALIPHATICS

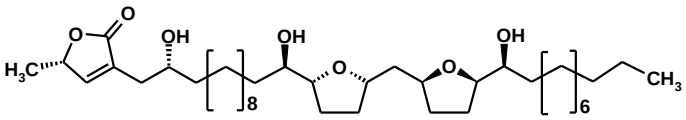
4.1.1 ORGANIC ACIDS, LIPIDS AND ACETOGENINS

Long chain hydrocarbons and fatty acids are best known as constitutents of waxes and lipophilic compounds. Some representatives of this natural product group show high antiprotozoal activity but mostly combined with a high levels of toxicity to mammalian cells. One example is *trans*-aconitic acid (**2**) that was used in combination with sodium stibogluconate, allopurinol, or pentamidine for experimental visceral leishmaniasis to determine synergistic effects [34]. When these three drugs (50, 15, 8 mM/kg/day, respectively) were used with *trans*-aconitic acid (5 mM/kg/day) the parasite load in BALB/c mice was inhibited by 100, 88, and 100%, respectively. At tested concentration *trans*-aconitic acid itself showed an inhibition of 59.2 %.

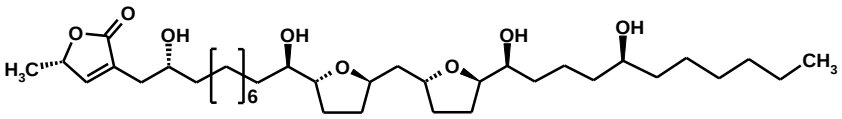
Four acetogenins from *Rollinia emarginta* (Annonaceae) were identified with antiprotozoal activity by bioassay-guided screening. Against different *Leishmania* and *T. cruzi* strains at 250 µg/mL inhibitory activity up to 89% and 67% was reported for rolliniastatin-1 (**3**) and squamocin (**4**) [35]. The leishmanicical activity was related to the number of hydroxy groups on these acetogenins. Maximum activity activity was observed in compounds that possessed three hydroxy groups, for example rolliniastatin-1 and squamocin, while activity was low in acetogenins having four or more hydroxy groups, e. g. sylvaticin and rollidecin.



(**2**) *trans*-aconitic acid



(**3**) rolliniastatin-1

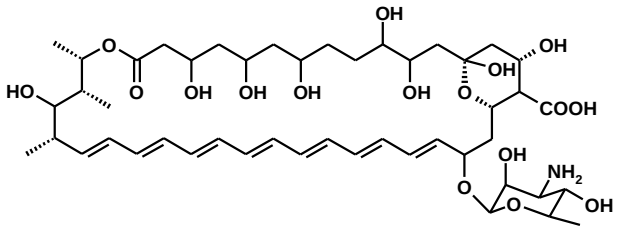


(**4**) squamocin

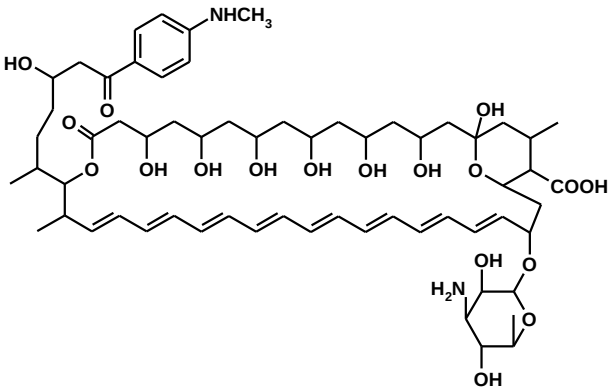
4.1.2 POLYENES

This class of antibiotics is well known because amphotericin B (**5**) is used as second line drug for the therapy of visceral and mucocutaneous leishmaniasis. Different polyene analogues, related to amphotericin B, also inhibit parasite growth. Polyene antibiotics can be divided into a non-aromatic group, which includes amphotericin B, and an aromatic group, which includes hamycins A and B another potent antiprotozoal agents. Hamycin (**6**), a polyene antibiotic, now in extensive use in the treatment of candidiasis and otomycosis, is found to be remarkably effective in killing *Leishmania donovani* promastigotes in a liquid medium at a concentration of 0.1 µg/mL. Glucose stimulated respiration and the uptake of 2-deoxy-D-[U-^{14}C]-glucose was inhibited in cells treated with the drug at a growth inhibitory concentration. The primary site of action of hamycin on *L. donovani* promastigote cells appears to be similar to amphotericin B, binding to membrane sterols, disrupting membrane stability with the loss of the permeability barrier to small metabolites. The lower minimum inhibitory concentration (MIC) of hamycin compared to other established drugs warrants further study in the

context of increasing reports of clinical resistance to pentavalent antimonials [36]. Despite the high activity of this class of compound there has been no discovery of new potent but less toxic polyene antibiotics in the past decade. Most interest has been focussed on the formulation of amphotericin B in colloidal drug carriers like liposomes, emulsions, micro-, and nanoparticles to improve bioavailability and reduce toxicity (AmBisome®, Ambicel®, Amphocil®).



(**5**) amphotericin B

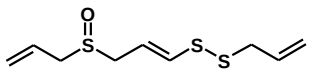


(**6**) Hamycin

4.1.3 AJOENES

Some simple fatty acid or aliphatics show antiprotozoal activity. Ajoenes metabolites (**7**) are a good example with the naturally

occuring allicin that has proved to be active against rodent malaria and *Trypanosoma cruzi*. These sulfur containing aliphatics, initially isolated from garlic (*Allium sativum*, Liliaceae), showed significant suppression of *Trypanosoma* parasitemia *in vivo* with daily doses of 50 mg/kg over 12 days [37]. Gallwitz et al. [38] identified ajoenes also as potential drugs effecting thiol metabolism by acting as a covalent inhibitor as well as a substrate of human glutathione reductase (GR) and secondly of the *Trypanosoma cruzi* trypanothione reductase (TR). The interactions between the flavoenzymes and ajoene lead to increased oxidative stress of the respective cell. The antiparasitic and cytostatic actions of ajoene may at least in part be due to the multiple effects on key enzymes of antioxidant thiol metabolism. Urbina et al. also demonstrated an effect on the phospholipoid biosynthesis of *Trypanosoma cruzi* with an alteration of the lipid composition of parasites from phosphatidylcholine to phosphatidylathanolamine [39]. Ajoenes also inhibited de novo synthesis of neutral lipids and sterols in *T. cruzi* epimastigotes, but these effects are not sufficient to explain antiproliferative effects of the drug.



(**7**) ajoene

4.2 PHENOLS

4.2.1 SIMPLE PHENOLS, PHENOLIC ACIDS AND COUMARINS

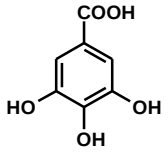
Simple phenols that are widely distributed in plants have been tested for their ability to inhibit parasite growth. For example, gallic acid (**8**) and its derivatives inhibit the proliferation of *Trypanosoma cruzi* trypomastigotes in vitro with an EC_{50} value of 15.6 µg/mL [40]. Higher activities were observed for the gallic acid esters ethyl-gallate and n-propyl-gallate which had EC_{50} values of 2.28 and 1.47 µg/mL, possibly due to increased lipophilicity. No *in vivo* data has been published. It seems unrealistic that such compounds, which form part of the daily diet, will have significant effects. The mechanism of

action remain obscure and the authors suggest that the formation of reactive oxygen species might be involved in the gallic acid induced apoptotic cell death [41]. Interestingly, Kayser et al. could not demonstrate any direct toxic effect of gallic acid and related gallotannins on *L. donovani* in infected macrophages [42]. Further studies should be conducted to clarify immunstimulatory activity of gallic acid [43].

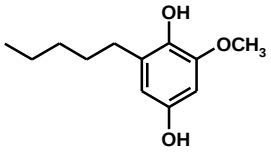
Ascofuranone (**9**), an isoprenoid prenylphenol antibiotic, derived from the fungus *Ascochyta visiae*, specifically inhibits mitochondrial glycerol-3-phosphate (G-3-P)-dependent electron transport in *T. b. brucei* [44]. Ascofuranone strongly inhibited both glucose-dependent cellular respiration and glycerol-3-phosphate-dependent mitochondrial oxygen consumption of *T. b. brucei* bloodstream form trypomastigotes. This inhibition was suggested to be due to inhibition of the mitochondrial electron-transport system, composed of glycerol-3-phosphate dehydrogenase and a plant-like alternative oxidase. Ascofuranone noncompetitively inhibited the reduced coenzyme Q1-dependent O_2 uptake of the mitochondrion with respect to ubiquinol (Ki = 2.38 nM). The site of action was deduced to be the ubiquinone redox machinery that links the two enzyme activities. Further, ascofuranone in combination with glycerol completely blocked energy production, and potently inhibited the in vitro growth of the parasite.

Other simple phenols include the hydroquinone derivatives miconidin (**10**) and espintanol (**11**), formed from its biosynthesis of a monoterpene to a phenolic, and pholoroglucinol derivatives from *Hypericum calycinum* (Hypericaceae). Quantitative data are not available for miconidin, but espintanol exhibited an IC_{90} in the 25-100 µg/mL range against twenty different *T. cruzi* strains [45], and a prenylated phloroglucinol derivative (**12**), inhibited *P. falciparum* growth *in vitro* with an EC_{50} of 0.88 µg/mL [46]. The mode of action of these semiquinones is unclear. It is attractive to speculate that the sensitivity of *Trypanosoma* and *Plasmodium* is due to oxidative stress resulting from the metabolic oxidation of semiquinone radicals or benzoquinones. The presence of antiplasmodial phloroglucinol derivatives has already been mentioned. Sarothalen B was found to be active *in vivo* in the 1950s [47] and the biogenetically related phloroglucinol derivative (**12**), which lacks the cyclohexadienone moiety of sarothalen B, is active in *in vitro* assays, indicating, that it conserves essential chemical features for this antiprotozoal activity.

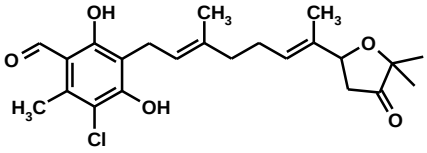
Oketch-Rabah et al. reported the antiprotozoal activity of 2´-epicycloisobrachycoumarinone epoxide (**13**) and its stereoisomer that has been isolated from *Vernonia brachycalyx* (Asteraceae) [48]. Both stereoisomers show similar *in vitro* activities against chloroquine-sensitive (CQ-S) and chloroquine-resistant (CQ-R) strains for *P. falciparum* as well as *L. major* promastigotes with EC_{50} values of 0.11µg/mL and 0.15 µg/mL for *Plasmodium* and 37.1 µg/mL and 39.2 µg/mL for *L. major*, respectively.



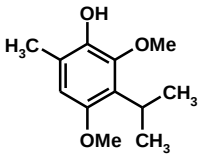
(**8**) gallic acid



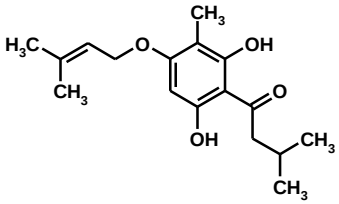
(**9**) ascofurane



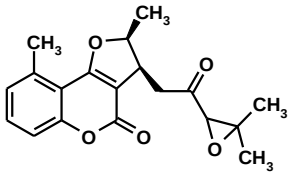
(**10**) miconidin



(**11**) espintanol



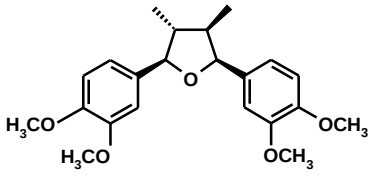
(**12**) phloroglucinol-derivate



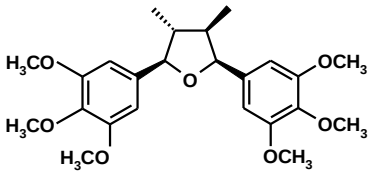
(**13**) 2´-epicycloisobrachy-coumarinone epoxide

4.2.2 LIGNANS

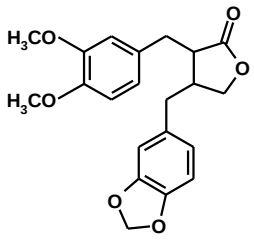
Lignans are a potent group of natural products with many toxic side effects, best represented by podophyllotoxin derivatives and the antineoplastic drug etoposide. Despite their known biological activities few lignans have been tested against parasitic protozoa. Lopes et al. demonstrated for the potential of the tetrahydrofuran lignans grandisin (**14**) and veraguensin (**15**) to prevent the transmission of Chagas disease by blood transfusion [49]. The activity of these terahydrofuran lignans (62 % and 87 % growth inhibition at 2.5 µg/mL, respectively) was forty times higher than that of the reference drug gentian violet. Lignans isolated from the hexane extract of the leaves of *Zanthoxyllum naranjillo* (Rutaceae) were tested in both *in vitro* and *in vivo* against two strains of *Trypanosoma cruzi* [50]. The compound (-)-methylpluviatolide (**16**) was highly effective for chemoprevention in the *in vitro* assay and healthy animals injected with the tested samples did not develop infection. Moreover, (-)-methylpluviatolide was also highly active against the bloodstream forms of two strains of *T. cruzi* in an *in vivo* assay [48].



(**14**) grandisin



(**15**) veraguensin



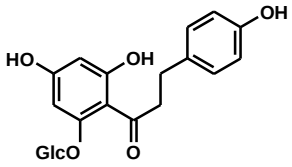
(**16**) (-)-methylpluviatolide

4.2.3 CHALCONES AND AURONES

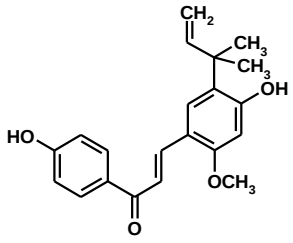
Phlorizidin (**17**), a naturally occurring dihydrochalcone glycoside from *Micromelum tephrocarpum* (Rutaceae), was one of first chalcones shown to possess antiparasitic activity. In ethnomedicine it was used for the treatment of malaria because of the bitter taste, a property shared with quinine and other antimalarials. Recent studies provide a rational basis for its antiplasmodial activity. Phlorizidin inhibits the induced permeability in *Plasmodium* infected erythrocytes to various substrates including glucose. The most promising compound out of this natural product class is licochalcone A (**18**). This compound was first isolated from *Glycyrrhiza glabra* (Fabaceae) and is the subject of intensive preclinical studies. The activity of licochalcone A is well documented in vitro and in vivo againsta panel of different parasites including *P.falciparum L. donovani* and *L. major*. The antileishmanial mechanism of action in through the inhibition of the electron transport in the mitochondrion [51]. Further biochemical studies were employed in *L. donovani* to demonstrated that growth inhibition is mitochondrial specific, and that the main targets are the fumarate dehydrogenase, succinate dehydrogenase and malate dehydrogenase, all essential for parasite viability [51]. Starting with licochalcone A as a lead structure, a large number of chalcones have been synthesized and structure-activity relationships determined with regard to their antiplasmodial, antileishmanial and trypanocidal activity [52].

Kayser et al. [53] demonstrated that structurally related aurones (**19**) share similar antiparasitic activites with chalcones. It is not kown, if this biogenetically related natural product group inhibits the same target sites as chalcones, but they have a similar size, integrated three-carbon linkers, and similar substituents on both aromatic rings. The main difference lies in the conjugation of the three-carbon linker that in aurones is linked to the B-ring, giving a two-member ring system. A planar structure is typical for all aurones and this conformation has a high similarity to compounds that Li et al. proposed as optimal lead structure of chalcones as protease inhibitors [54]. From molecular modeling studies it appears that chalcones are not only rigid but also adopt an extended structure due to the nature of the conjugated linker. The resulting linear, nearly planar structure, fits perfectly into the active site of *Trypanosoma* and *Plasmodium* cysteine proteases [54].

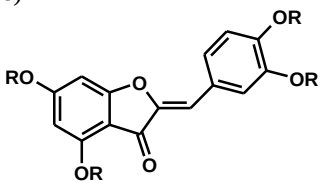
These findings suggest that both aurones and chalcones might interact with similar sites in essential parasite enzymes and thus have similar mechanisms of antiparasitic activity.



(**17**) phlorizidin
(Glc = Glucose)



(**18**) licochalcone A



(**19**) aurones (R = H or CH_3)

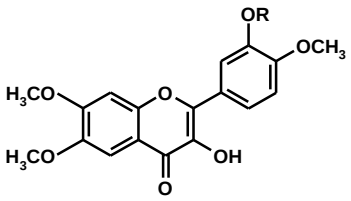
4.2.4 FLAVONOIDS

Flavonoids are widespread in the plant kingdom. In contrast to some ethnomedicinally reports up to 1986, there was no scientific evidence of their activity against *Leishmania* spp., *T.cruzi* and *P. falciparum* [55]. However, following the detection of antiplasmodial flavonoids from *Artemisia annua* (Asteraceae) this natural product group has attracted renewed interest. Elford et al. demonstrated that methoxylated flavonones artemetin (**20**) and casticin (**21**) act synergistically with artemisinin against *P. falciparum in vitro* [56]. The exact mechanism is unclear but tested flavonoids did inhibit the influx of L-glutamine and myoinositol into infected erythrocytes [57]. As a part of a multidisciplinary research programme in Thailand on antiplasmodial drugs, additional *Artemisia* species have been screened [58] and exiguaflavanone A (**22**) and B (**23**) isolated from *Artemisia indica* (Asteraceae) exhibited *in vitro* activity against *P. falciparum*

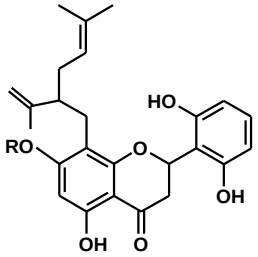
with EC_{50} values of 4.6 and 7.1 µg/mL, respectively. The flavonoids sakuranetin (**24**) and 7-methoxyaromadendrin (**25**) were also reported to be antiprotozoal natural products with inhibition rates of 100 % and 86 % at 500 µg/mL *in vitro* against *T. cruzi*, respectively. Despite the limited data of the antiprotozoal activity of flavonoids, it can be speculated that the mode of action is linked to the unusual antioxidant pathway. Ribeiro et al. discussed, that the lack of defense mechanisms against oxidative stress makes the parasites susceptible to drugs having an effect on the generation of reactive oxygen species [59]. Recently, Perez-Victoria et al. [60] suggested that specific flavonoids could effect transport mechanisms in *Leishmania*. The C-terminal nucleotide-binding domain of a P-glycoprotein-like transporter, encoded by the ltrmdr1 gene in *L. tropica* and involved in parasite multidrug resistance (MDR), was overexpressed in *Escherichia coli* as a hexahistidine tagged protein and purified. The *L. tropica* recombinant domain efficiently bound different classes of flavonoids with the following affinity: flavone > flavanone > isoflavone > glucorhamnosyl-flavone. The affinity was dependent on the presence of hydroxyl groups at positions C-5 and C-3 and was further increased by a hydrophobic 1,1-dimethylallyl substituent at position C-8. When flow cytometry was used to measure daunomycin accumulation in a *L. tropica* line, a reversing effect was observed with flavones such as dimethylallyl-kaempferide at low concentrations or apigenin at higher concentration, but not with the glucorhamnosyl derivative rutin nor with the isoflavone genistein (**26**). The *in vivo* reversing effect of dimethylallyl-kaempferide was correlated with a high inhibition of the *Leishmania* cell growth in the presence of daunomycin. The results suggest that flavone inhibition of both daunomycin efflux and parasite growth in the presence of the drug correlates to direct binding of the compound to cytosolic domain of the P-glycoprotein-like transporter [60]. Gale et al. also identified genistein (**26**) as potent natural compound as modulator of protein phosphorylation with effect on the SPK89 protein kinase in trypanosomes [61].

4.2.5 NAPHTHOQUINONES

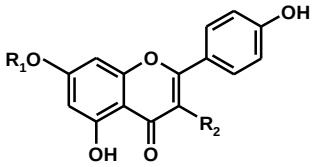
Naphthoquinones and other related quinoid compounds are one of the major natural product classes with significant activity against



(**20**) artemetin (R = CH_3)
(**21**) casticin (R = H)



(**22**) exiguaflavanone A (R = H)
(**23**) exiguaflavanone B (R = CH_3)



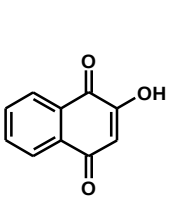
(**24**) sakuranetin (R_1 = CH_3, R_2 = H)
(**25**) 7-methoxyaromadendrin (R = OH)
(**26**) genistein

Leishmania, *Trypanosoma* and *Plasmodium*. Many naphthoquinones have been isolated but frequently their potential use has been limited by low bioavailability and high toxicity. Wright and Phillipson [62], Sepuvelda-Boza and Cassels [63], Fournet et al. [64] and Akendengue et al. [65] have reviewed much of the literature on naphthoquinones, we focus here on the latest developments and some new structures and their biological activities.

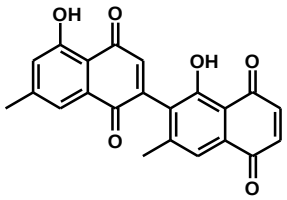
The plant derived product hydrolapachol (2-hydroxy-1,4-naphthoquinone) (**27**) [66] was shown to have activity against *Plasmodium lophurae* in ducks in the 1940s (see [67] for details). This observation provided the stimulus for the synthesis of hundreds of analogues including a series of 2-hydroxy-3-alkyl-1,4-naphthoquinones. One of this series, lapinone, synthesised in 1948, showed high activity in experimental models and was used to treat *P. vivax* infected patients in 1951. Due to high doses required and pharmacological problems interest in naphthoquinones faded. In the 1960s interest was revived and a new compound, menoctone, proved

to be highly active in the *P. berghei* mouse model but disappointing in clinical trials. In the late 1970s and 1980s a series of hydroxynaphthoquinones (HNQs) was synthesised at the Wellcome Laboratories, UK that overcame the problems of poor oral absorption, rapid metabolism and protein binding associated with previous series. These HNQs, with a cyclohexyl ring at the 2-position showed, showed activity against the apicomplexan parasites, *Eimeria*, *Toxoplasma*, *Theileria* and *Plasmodium* species [68]. This work resulted in the development of parvaquone and buparvaquone for the treatment of theileriosis in cattle and other ungulates and atovaquone for the treatment of malaria (in combination with proguanil) and *Pneumocystis carinii* pneumonia [69].

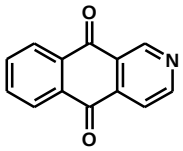
Besides this synthetic route, new and structurally interesting naphthoquinones have been isolated. A dimeric naphthoquinone diospyrin (**28**) from *Diospyros montana* (Ebenaceae) was found to be active against *L. donovani* [70]. The inhibition of Type I DNA-Topoisomerase in this parasite has been suggested as a mechanism of action [70]. Plumbagin and related HNQs have activity against Leishmania spp. in vitro and in vivo [64]. These compounds have been used as the basis for the synthetisis of naphthoquinones designed as subversive substrates of trypanothione reductase. Other rare naphthoquinones have been identified as potential antiparasitic drugs. From *Psychotria camponutans* (Rubiaceae) the benzisoquinoline-5-10-dione (**29**) has been isolated and tested against *P. falciparum* with an EC_{50} of 0.84 µg/ mL associated with significant cytotoxicity (EC_{50} = 1.62 µg/ mL, KB cells) [71].



(**27**) 2-hydroxy-1,4-naphthoquinone



(**28**) diospyrin



(**29**) benzisoquinoline-5,10-dione

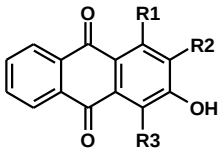
4.2.6 ANTHRAQUINONES AND XANTHONES

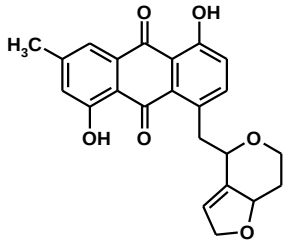
This natural product group is related to naphthoquinones in structure and biological activity. The main chemical difference between the groups is the tricyclic aromatic system with a para-quinoid substitution. Schnur et al. [71] and Fournet et al. [64] demonstrated that some derivatives have activity in vitro against *Leishmania* species, but few naturally occuring anthraquinones have been tested. Anthraquinones isolated from the tropical tree *Morinda lucida* (Rubiaceae) were tested for their antiplasmodial and antileishmanial activtity [72, 73]. In vitro, some (**30**-**32**) were more active against *L. major* promastigotes (EC_{50} ≅ 9.6 to 185 µM) than amastigotes and also acive against CQ-R strains of *P. falciparum* (EC_{50} ≅ 21.4 to 82.9 µM in vitro). The most active compounds have an aldehyde group at C-2, well known as a cytotoxic substructure in other natural products. The activity may also be explained by the cyclic planar structure that makes them potential DNA-intercalators. From the toxicological point, tested compounds showed moderate effects in the lymphocyte proliferation test with all EC_{50} values over 175 µM [75].

Antiprotozoal anthraquinones are known from microbial sources [76]. Mycotoxin MT81 (**33**) and some of its derivatives from *Penicillium nigricans* showed only moderate antileishmanial activity against *L. donovani* promastigotes (46% growth inhibition at 250 µg/mL in vitro). In parallel to the inhibitory effects of naphthoquinones on mitochondria, Majumdar et al. determined the effect of the drugs on the respiration of *L. donovani* cells [76]. The oxygen uptake was significantly inhibited (inhibition rate > 50 %) by all mycotoxins indicating a similar mode of actions as known for other naphthoquinones.

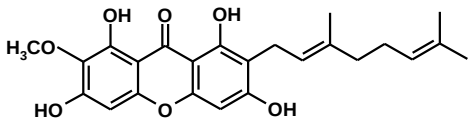
Antiplasmodial xanthones have been isolated from *Garcinia cowa* (Guttiferae) [77]. Preliminary screening of five prenylated xanthones dmonstrated significant activity against *P. falciparum* in vitro with EC_{50} concentrations between 1.5 and 3.0 µg/mL. Cowaxanthone (**34**) displayed an antiplasmodial potential (EC_{50} = 1.5 µg/mL) comparable to that of pyrimethamine (EC_{50} = 2.8 µg/mL). Although a number of biological properties are known about xanthones (antibacterial, antifungal and cytotoxicity) there have only been three reports on the

antiplasmodial activity of these and only one dealing with a pure natural product.





(**30**) digitolutein; R1 = H; R2 = CH_3 ; R3 = OCH_3 (**33**) mycotoxin MT81
(**31**) rubiadin-1-methyl ether; R1 = OCH_3; R2 = CH_3 ; R3 = H
(**32**) damnacanthal; R1 = OCH_3; R2 = CHO ; R3 = OCH_3



(**34**) cowaxanthone

4. 3 TERPENOIDS

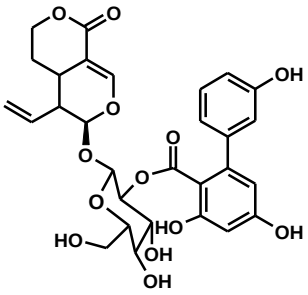
4.3.1 IRIDOIDS

The secoiridoid amarogentin (**35**) isolated from the upper parts of *Swertia chirata* (Loganiaceae) is a promising compound with leishmanicidal activity. This compound inhibited DNA-Topoisomerase I activity from *Leishmania donovani* at 30 μM [79]. This principle is quite interesting as most trypanocidal drugs target type II topoisomerases. There are a few compounds that specifically alter biological functions of toposisomerase I with the enzyme or DNA-enzyme complex, an exception being camptothecin an antineoplastic drug [80]. Other natural compounds with the iridoid parent structure also show significant activity against *Leishmania* parasites. A series of

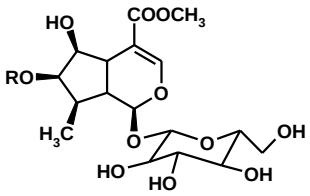
iridoids isolates from *Nyctanthes arbortristis* (Oleaceae), arbortristosides A, C (**36**, **37**), have antileishmanial activity. Unfortunately, although parasite growth was reduced in vitro at 100 µg/mL by 64.5% and 67.4 %, respectively, cytotoxicity was noted at 60 µg/mL [81].

4.3.2 MONOTERPENES

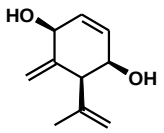
Monoterpenes are examples of simple antiprotozoal drugs. Espintanol (**11**) has already been discussed above and piquerol A (**38**) was active about 100 µg/mL [82]. Espintanol, isolated from the bark of *Oxandra espinata* (Annonaceae) and synthesised [83], is active against *Leishmania* promastigotes and *Trypanosoma* epimastigotes. In view of the sensitivity of pathogens from the genus *Trypanosoma* and *Leishmania* to oxidative stress, as discussed under section 4.2.1, piquerol A activity may also be due to interaction with the parasite redox cycling system leading to enzyme inhibition and parasite killing.



(**35**) amarogentin



(**36**) arbortristosides A;
R= p-methoxy cinnamoyl
(**37**) arbortristosides C;
R= coumaroyl



(**38**) piquerol A

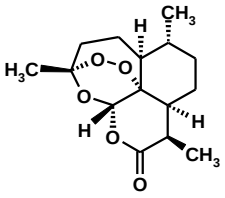
4.3.3 SESQUITERPENE LACTONES

The antiprotozoal potential of sesquiterpenes is well established since artemisinin (**39**) and a second endoperoxide sesquiterpene yinghaosu A, were identified as new drugs with high clinical relevance. In addition to artemisinin and other sequiterpene endoperoxides,

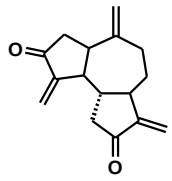
described in detail in Section 6.1, other series of sequiterpenes with antiprotozoal activity have been described. The sequiterpene lactone parthenin (**40**) has an EC_{50} value of 1.29 µg/mL against *P. falciparum* in vitro. Although parthenin is described as highly toxic, rats treated with 100 mg/kg/day did not show any signs of toxicity. From these results a series of parthenin derivatives have been synthesized and retested and those with an exocyclic methylene lactone have been identified as active. Exocyclic methylene lactones are well known as the allergic principle in medicinally used plants (mostly Asteraceae, e.g. *Arnica montana* (Asteraceae)). Picman et al. [84] demonstrated that parthenin (**40**) is capable of blocking parasite specific targets responsible for glutathinonylspermidine- and trypanothione synthesis from cysteine and glutathione precursors in *Leishmania* and *Trypanosoma* [38]. The sequiterpene lactones brevilin A (**41**) from *Centipeda minima* (Asteraceae) and dehydrozaluzanin C (**42**) from *Munnozia maronii* (Asteraceae) were discovered from ethnopharmacological screening. From *Neuroleaena lobata* (Asteraceae), a medicinal plant used in Guatemala for the treatment of *Plasmodium* infections, activity was documented for germacranolide sesquiterpene lactones as well for furanoheliangolides [85]. These compounds are also active against *Leishmania* promastigotes and *Trypanosoma epimastigotes in vitro. From preliminary structure-activity relationship analysis based on in* vitro EC_{50} data, germanocrenolide sesquiterpenes, like neurolenin A (EC_{50} = 0.92 µM) and B (**43**, **44**) (EC_{50} = 0.62 µM), were found to be more potent, than furanoheliangolides as represented by lobatin A and B (EC_{50} = 15.62 µM, 16.51 µM, respectively) (**45**, **46**) [85]. The main reason for the lower activity of the former could best be explained by the shift of the double bond from the 2,3 (neurolenin) into the 3,4 (lobatin) position, suggesting that one of the structural requirement in sesquiterpenes is an α/ß-unsaturated keto function.

Another approach for discovering antiprotozoal natural products is described by Koshimizu et al. [86]. Wild chimpanzees were observed to chew young stems of *Vernonia amygdalina* (Asteraceae) from which antiplasmodial sesquiterpenes (vernodalin (**47**), vernolide, hyroxyverniladin) have been isolated. Also unusual sesquiterpenes (**48**, **49**) with significant antiplasmodial activities (EC_{50} < 4 µg/mL) were isolated from marine red algae (*Laurencia implicata*,

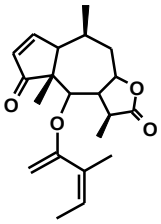
Rhodomelaceae) and brown algae (*Portiera hornemannii*, Rhizophylladaceae) [87].



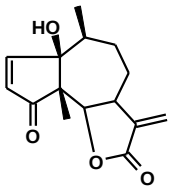
(**39**) artemisinin



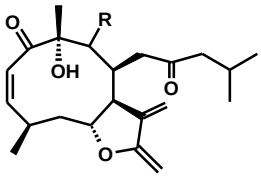
(**40**) parthenin



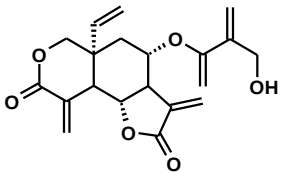
(**41**) brevilin A



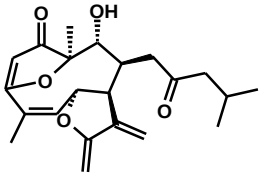
(**42**) dehydrozaluzanin C



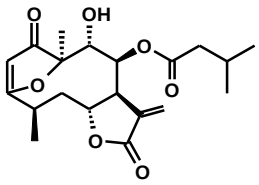
(**43**) neurolenin A; R = H
(**44**) neurolenin B; R = OAc



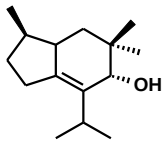
(**45**) lobatin A



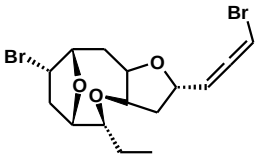
(**46**) lobatin B



(**47**) vernodalin



(**48**) 5-isopropyl-3,3,9-trimethylbicyclo-nona-5-en-4ol



(**49**) 9,10-trisepoxy-pentadec-12-1,2-diene

4.3.4 DITERPENES

Diterpenes from many species are well known for their biological activity and are amongst the most widely distributed terpenes in the plant kingdom. However, most of them combine both high antiparasitic activity as well as high cytotoxicity to mammalian cells. Both jatrogrossidione (**50**) and jatrophone, isolated from *Jatropha*

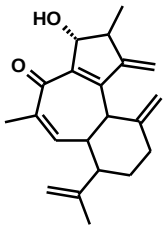
grossidentata and *J. isabelli* (Euphorbiaceae) respectively, showed significant activity against *Leishmania* promastigotes in vitro (EC_{50} values of 0.75 µg/mL (2.4 mM) and 5 µg/mL (16 mM)) and *L. amazonensis* amastigotes in vivo where jatrophone reduced parasite growth at a dose of 25 mg/kg/day. Unfortunately, both proved to have toxic effects of therapeutic doses [88].

A series of 80 labdane derivatives showed significant antileishmanial activity (*L. donovani, L. enriettii, L. major, L. infantum*), but cytotoxicity increased in parallel with the antiprotozoal effect [89]. From structure-activity studies overbridged tri- or tetracyclic ring labdanes had more significant activity in comparison to bicyclic labdanes. These results confirmed other data cited in literature, for example the subtype of overbridged labdane derivates (e.g. kauran-, trachyloban-type), as displayed by ent-kaur-16α-ol-19-oic acid (**51**) from *Mikania obtusata*, ent-kauran-16-en-19-oic acid from *Wedelia paludosa* (**52**), and (-)-trachyloban-19-oic (**53**) from *Viguirea aspillioides* (Asteraceae). Results of their extraordinary activity against *Leishmania* and *Trypanosoma* spp. have been published [90-92]. However, most antiprotozoal labdanes also show low EC_{50} values in vivo ($EC_{50} < 3.0$ µg/mL), and high toxicity ($EC_{50} <$ 10 µg/mL).

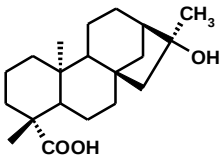
One compound isolated from the sponge *Acanthella klethra*, axisonitrile (**54**), a sequiterpene derivative, showed potent antiplasmodial activity with no detectable cytotoxic properties [93]. A series of different terpenes have been isolated from marine organisms and are of considerable interest for their unique structural features in antiparasitic drug research. In contrast to plant metabolites most of these 100 plus isolated natural products contain isonitrile, isothiocyanate, and thiocyanate functionalities. Major interest has been focussed on these marine drugs by [94], doubling the information in the literature and identifying novel compounds with EC_{50} values below 1 µg/mL with high selective indices (SI > 50) [95]. From the point of antiparasitic research four diterpene subclasses are of interest: kalihinane diterpenes, e.g. kalihinol A (**55**) [96], amphilectanes, for example 7-isocyano-11(20),14-epiamphilectadiene (**56**) [95], cycloamphilectanes, for example 7-isocyanocyclo-amphilect-10-ene (**57**) [95], and isocycloamphilectanes, for example 7,20-diisocanoisocycloamphilectane (**58**) [95]. The potent and

selective biological activities of these compounds represents an exciting advance in the search of novel antiplasmodial agents. *In vivo* studies are required now to validate the potential of these compounds.

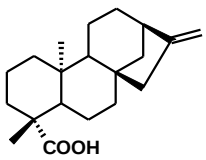
Not only novel compounds with unique structural features attract attention, but also well known compounds like macrocyclic terpenes used in other pharmacological fields, like taxol and epothilione (**59**) (potent antineoplastics) or highly toxic phorbol esters, have been tested in antiparasitic drug screens. Macrocyclic trichothecenes are known as mycotoxins, and a variety of biological activities have been reported. From the fungal culture of *Myrothecium verrucaria* BCC 112 (Hypomycetes) roridin E (**60**) show high antiplasmodial activity (EC_{50} = 0.15 ng/mL) but also significant cytotoxicity (EC_{50} = 0.5 ng/mL, KB cells (human epidermal nasopharyngeal cancer cells) [97]. It must also be noted that the selective index of roridin E (SI = 12) in comparison to artemisinin (SI > 7,100) was too low to make it useful for further *in vivo* investigation. The experience of research into antineoplastics with macrocyclic ring systems suggests that it maybe possible to find or synthesize new trichothecene derivatives with high antiplasmodial activity and low toxicity. Oketch-Rabah et al. showed that the macrocyclic germancrane dilactone 16,17-dihydrobrachycalyxolide (**61**), from *Vernonia brachycalyx* (Asteraceae), has both antileishmanial and antiplasmodial activity [98]. In *in vitro* tests the compound is strongly active against *L. major* (EC_{50} = 17 µg/mL) and *P. falciparum* (EC_{50} = 17 µg/mL), but also inhibits the proliferation of human lymphocytes at the same concentration indicating general toxicity [98].



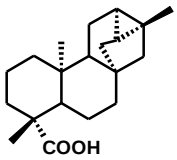
(**50**) jatrogrossidione



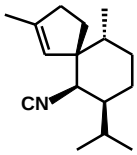
(**51**) ent-kaur-16α-ol-19-oic acid



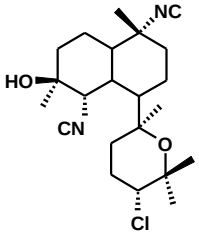
(**52**) ent-kauran-16-en-19-oic acid



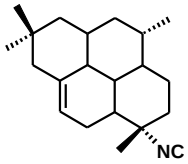
(**53**) (-)-trachyloban-19-oic



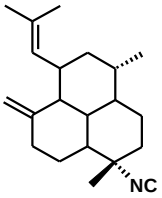
(**54**) axisonitrile-3



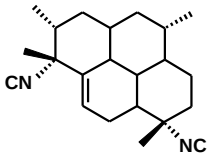
(**55**) kalihinol A



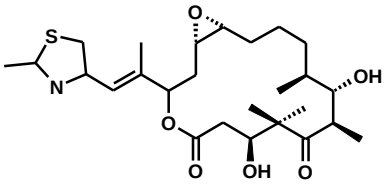
(**56**) 7-isocyano-11(20),14-epiamphilectadiene



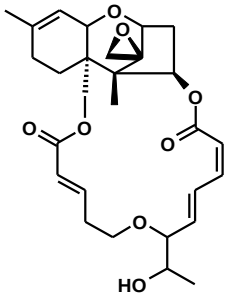
(**57**) 7-isocyanocyclo-amphilect-10-ene



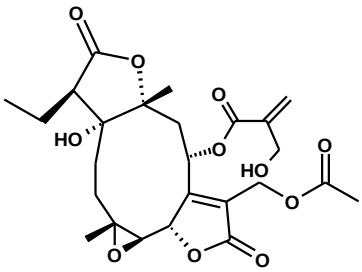
(**58**) 7,20-disiocanoiso-cycloamphilectane



(**59**) epothilone A



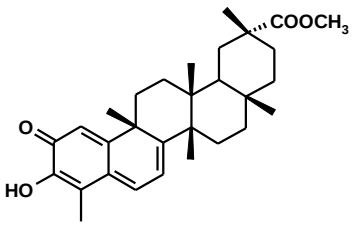
(**60**) roridin E



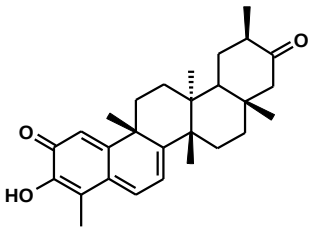
(**61**) 16,17-dihydrobrachylocalyxolide

4.3.5 TRITERPENES

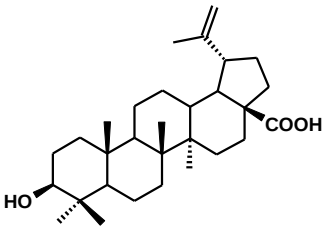
Triterpenes and saponins from plant sources are known for their biological activity (antineoplastic, anthelmintic and antiviral), but they exhibit some toxicity to humans and other mammals. Despite the fact that triterpene action in biological systems is well known, the first rational reports on their antiprotozoal activity were first described late 1970s. Tingenone (**62**) and pristimerin (**63**), from species of Celastraceae, have *in vitro* activity against *T. cruzi* amastigotes and *P. falciparum*. Tingenone could act through interaction with DNA or inhibition of DNA synthesis [45]. The lupane-type triterpene betulinic acid (**64**), also known for its antineoplastic effect, was identified by bioguided fractionation and identified as the antiplasmodial principle of *Triphyophyllum peltatum* and *Ancistrocladus heyneanus* (Dioncophyllaceae and Ancistrocladaceae, respectively) [99]. Against *P. falciparum in vitro* betulinic acid had an EC_{50} value of 10.46 µg/mL and with the exception of human melanoma cells (EC_{50} = 1-5 µg/mL), only moderate cytotoxicity (EC_{50} > 20 µg/mL). Based on saponins isolated from ivy, *Hedera helix* (Araliaceae), Majester-Sarvonin et al. gave a first insight in the structure-activity relationship of different antileishmanial saponin types [100]. Bidesmosides have no effects on the proliferation of either promastigote or amastigotes. In contrast, monodesmosides and hederagin (**65**) were highly active, especially the sodium salts of α- and ß-hederin (**66**, **67**) are highly active at concentration similar to that of pentamidine. Monodesmosides from *Hedera helix* damaged macrophages host cells at concentrations between 5 and 25 µg/mL, but this level of toxicity was in the same range as for Glucantime [100]. The use of saponins as drugs is limited due to poor bioavailability, reduced absorption in the gastrointestinal tract and their hemolytic toxicity when given by parenteral route. It is noteworthy that despite this fact medicinal plants that contain saponins are known. Oketch-Rabah et al. isolated a new steroidal saponin, muzanzagenin (**68**), from *Asparagus africanus* (Liliaceae) which had antileishmanial and antiplasmodial activity (EC_{50} = 70 µM, *L. major*; EC_{50} = 61 µM, *P. falciparum* K39) [101].



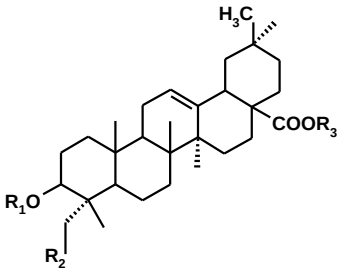
(**62**) tingenone



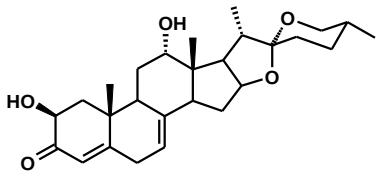
(**63**) pristimerin



(**64**) betulinic acid



(**65**) hederagin; R_1= H, R_2 = OH, R_3 = H
(**66**) α-hederin; R1= rham(1→2)ara
(1→, R2 = OH, R3 = H
(**67**) ß-hederin; R1= rham(1→2)ara
(1→, R2 = H, R3 = OH

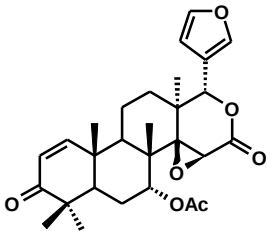


(**68**) muzanzagenin

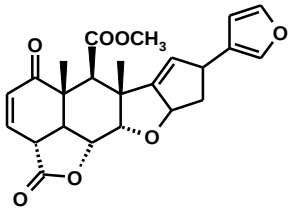
4.3.6 LIMONOIDS

Bitter terpenoids, known as limonoids, are biosynthetically related to the quassinoids that are produced by species of Meliaceae. One well known representative from this family is *Azadirachata indica*, the neem tree, widely used as an antiplasmodial plant in Asia. Rochanakij

et al. initially identified nimbolide (**69**) as the active antimalarial principle of the neem tree (EC_{50} = 0.95 ng/mL, *P. falciparum* K1) [102]. Nimbinin, geduin (**70**) (EC_{50} = 0.39 ng/mL, *P. falciparum* D6) and its dihydroderivative were also found to be active *in vitro* against *Plasmodium* parasites in the range of EC_{50} values of 0.72 - 1.74 µg/ml [103-105]. The mode of action of this natural product group is still unclear. The cytotoxic activity of gedunin was moderate (EC_{50} = 275 µg/mL). Geduin derivatives, D-seco-limonoids, do not show significant antiplasmodial activities (EC_{50} > 100 ng/mL, *P. falciparum* D6) comparable to the parent structure of geduin [105]. Insufficient geduin-related limonoids have been tested to allow a proper evaluation of this group.



(**69**) nimbolide

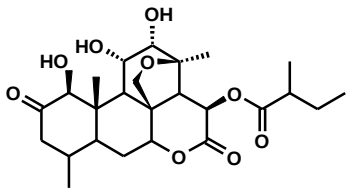


(**70**) geduin

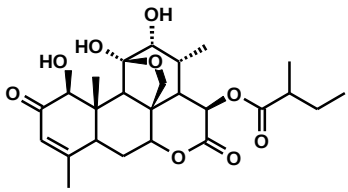
4.3.7 QUASSINOIDS

Quassinoids are biosynthetically related to triterpenes and share the same metabolic precursors. Most of the presently known quassinoids were found in the family Simbaroubaceae, and extracts and isolated natural compounds have been widely tested [106]. As deduced from structure-activity relationship analysis most potent quassionoids have a pentacyclic ring systems with a lactone function and a methylene-oxygen ring bridge linking C-8 and C-13 (e.g. brusatol (**71**)) or C-11 (e.g. ailanthinone (**72**)) [107]. Most of the quassinoids do not have a sufficient selective index to be considered as lead structures for clinical drugs. The antiplasmodial activity is high with EC_{50} values around 0.02 µg/mL, but the most active compounds like simalikalactone D (**73**) from *Simaba guianensis* (Simaroubaceae) [108], 15ß-heptylchaparrinone [109] and different sergolide

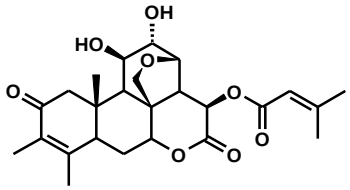
quassinoids [110] were too toxic in vivo. The mode of action of quassinoids seems to be the inhibition of protein synthesis [111]. Quassin (**74**) is inactive due to the missing methylene/oxygen bridge. A current research aim is to modify the parent compound synthetically, to find semisynthetic quassinoids with reduced toxicity and to improve the toxic/therapeutic ratio. As a potential lead structure for this approach Francois et al. identified chaparrinone (**75**) derivatives with an improved selective index [112]. The in vitro activities of chaparrinone and 15-desacaetylundulatone (**76**) were lower than reported activities of certain other quassinoids (EC_{50} = 0.037 µg/mL and 0.047 µg/mL, *P. falciparum* NF54, respectively). *In vivo*, however, when given at 50 mg/kg/day they produced significant reduction of parasitaemia with survival times similar to those of the control group with no signs of acute toxicity [112]. The contradictionary toxic/therapeutic ratio was explained by the occurence of the keto-function at C-2 and missing hydroxyl group at C-14 in a C8→C11 overbridged ring system improving its performance.



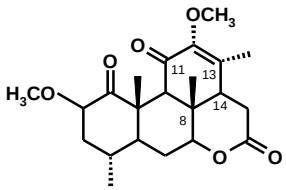
(**71**) brusatol



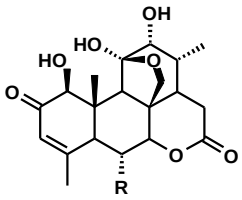
(**72**) ailanthinon



(**73**) simalikalactone D



(**74**) quassin



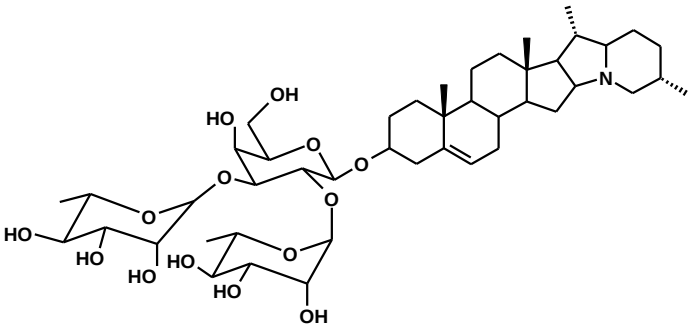
(**75**) chaparrinone, R = H
(**76**) 15-desacaetylundulatone; R = O-tiglate

4.4. N-CONTAINING NATURAL PRODUCTS (NON-ALKALOIDS)

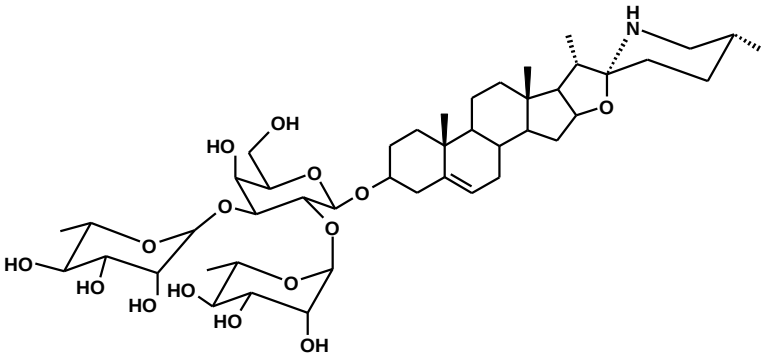
Besides the large group of alkaloids some nitrogen-containing natural products, do not fall under the definition of alkaloids, and are therefor discussed separately.

4.4.1 STEROIDAL ALKALOIDS

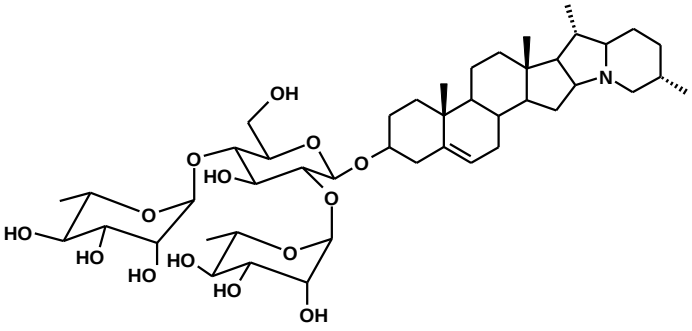
The literature contains numerous reports on biological activities of nitrogen-containing steroids of the *Solanum*-type. Most are quite common derivatives that occur in vegetables and thus in the daily diet; α-solanine (**77**), tomatine (**78**) which have been tested for their toxicological potential but their antiparasitic activity has not fully tested. Chataing et al. tested a series of *Solanum*-type steroid alkaloids against *Trypanosoma cruzi* in vitro in comparison to ketoconazole [113]. Glycoalkaloids containing a chacotriose sugar moiety showed trypanocidal activity against epimastigote and against metacyclic trypomastigote forms. The mechanism of action probably is the membrane, followed by structural changes of internal compartments, resulting in destruction of organelles such as mitochondria and glycosomes. The data indicate that steroid alkaloids containig ß-chacotriose trisacharide moiety, e.g. α-chaconine (**79**) and α-solarmargine (**80**), posses antitrypanosomal activity in the range of EC_{50} = 6.0 µM [113].



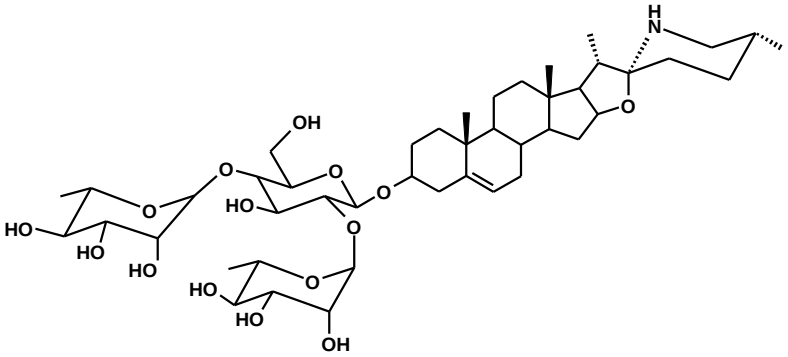
(**77**) α-solanine (solatriose)



(**78**) tomatine



(**79**) α-chaconine (chacotriose)

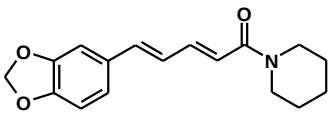


(**80**) α-solarmargine (chacotriose)

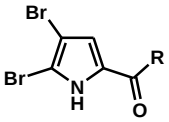
4.4.2 OTHER N-CONTAINING COMPOUNDS

Piperine (**81**), a major constitutent of pepper (*Piper nigrum*, Piperaceae), was tested against *L. donovani* promastigotes [114]. However, as this compound has been part of the daily diet over centuries, is cytotoxic potential, and only the moderate antileishmanial activity, it has not been considered as a potential antiparasitic agent.

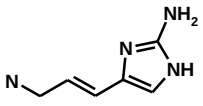
Recent studies found three halogenated pyrrole-2-carboxylic acids in a Maltese sample of the marine sponge *Agelas oroides* (Agelasidae) (**82**-**84**) [115]. Activity was evaluated against *P. falciparum* (strains D6 and W2) with EC_{50} values between 3.3 and 5.3 µg/mL. Parallel testing for against KB, Lu1, LNCaP and ZR-75-1 cells showed cytotoxic activity (EC_{50} = 2.0 - 14.5 µg/mL).



(**81**) piperine



(**82**) 4,5-Dibromopyrolle-2-carboxylic acid; R = OH
(**83**) 4,5-Dibromopyrolle-2-carboxylic methylester; R = OCH_3
(**84**) Oroidin; R =



4.5 ALKALOIDS

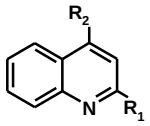
Alkaloids are one of the most important classes of natural product providing drugs for humans since ancient times. Most alkaloids are well known because of their toxicity or use as psychodelic drugs (e.g. cocaine, morphine or the semisynthetic LSD), but many alkaloids have had a deep impact on the treatment of parasitic infections. The outstanding example is quinine (**1**) from *Cinchona succirubra* (Rubiaceae) used for the treatment of malaria for more than three centuries.

4.5.1 QUINOLINES

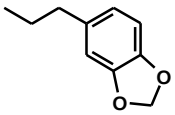
Up to the middle of this century quinine (**1**) was used for the treatment of malaria, and with the widespread development of chloroquine-resistant strains of *Plasmodium falciparum* it has become important again. Quinine has been used for the treatment of malaria for more than 350 years and has its origin in Peru in the early 17^{th} century. Quinine was the lead structure in the discovery of new synthetic derivatives like mefloquine that have higher antimalarial activity. This section will focus on other new quinoline alkaloids. The mechanism of antiplasmodial action and resistance of quinolines is well described elsewhere [116].

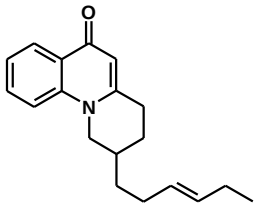
As the result of an ethnopharmacological search for new antileishmanial drugs aryl- and alkyl-quinolines were isolated from *Galipea longiflora* (Rutaceae) [117]. These simple natural quinoline derivatives 2-*n*-propylquinoline (**85**), chimanine B (**86**), chimanine D (**87**), 2-*n*-pentylquinoline (**88**), 4-methoxy-2-phenylquinoline (**89**), and 2-(3,4-methylenedioxyphenyl)-quinoline (**90**) were tested against strains of parasites causing cutaneous leishmaniasis and exhibited activities of EC_{50} = 25-50 µg/mL or 150 - 300 µM [117, 118]. Only chimanine B was active in vivo (50 mg/kg, BALB/c mice); twice daily oral treatment results in a decrease of parasite load by 95 %, a similar activity to that of the standard drug Glucantime [119]. No mechanism has been found yet to explain these effects. Two piperidino-4-quinolinone alkaloids dictyolomide A (**91**) and B were identified from *Dictyoloma incanescens* (syn. *D. vandellianum*) and *D. peruviana* (Rutaceae), collected in South America (Bolivia). They induced a

lysis of various strains of *Leishmania* promastigotes in vitro at a concentration of 100 µg/mL [120].



(**85**) 2-*n*-propylquinoline; $R_1 = C_3H_7$, $R_2 = H$
(**86**) chimanine B; R_1= CH=CH-CH_3, $R_2 = H$
(**87**) chimanine D; R_1  , $R_2 = H$

(**88**) 2-n-pentylquinoline; $R_1 = C_5H_{11}$, $R_2 = H$
(**89**) 4-methoxy-2-phenylquinoline; R_1 = phenyl, $R_2 = OCH_3$
(**90**) 2-(3,4-methylenedioxyphenyl)quinoline,
R_1=  $R_2 = H$



(**91**) Dictyolomide A

4.5.2 BENZYL- AND NAPHTHYLISOQUINOLINE ALKALOIDS

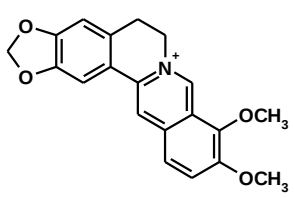
The chemical structure of this alkaloid group is well known through the widespread and abundant berberine (**92**). Many antiprotozoal isoquinolines have been isolated from the families Annonaceae, Berberidaceae, Menispaermaceae and Hernandiaceae [65]. Berberine is active at EC_{50} = 10 µg/mL against *Leishmania* amastigotes within murine peritoneal macrophages. Vennerstrom et al. tested berberine and several of its derivatives for antileishmanial activity against *L. donovani* and *L. panamensis* in golden hamsters [121]. Tetrahydro-

berberine is less toxic and more potent than berberine against *L. donovani* but was not as potent as meglumine antimonate (Glucantime). Only berberine, the natural product, showed significant activity (greater than 50% suppression of lesion size) against *L. panamensis*. Berberine was used for cutaneous leishmaniasis in India but was not effective when applied topically [122]. Recently catecholic berberines, (-)-pessione (**93**) and (-)-spinosine (**94**), have been isolated and tested for antileishmanial and trypanocidal (*T. cruzi*) activity in vitro. At a single concentration of 250 µg/mL 50 % inhibition for *T. cruzi* is found, indicating low trypanocidal activity [123].

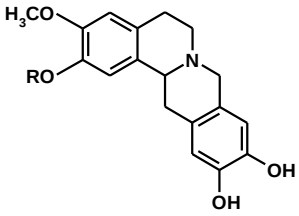
Naphthylisoquinoline alkaloids isolated from tropical llianas have been identified as new promising leads as antiprotozoals. They show remarkable activity against *P. falciparum in vitro* and *in vivo*, as well against *Leishmania* and *Trypanosoma* species [124]. Extracts from the single species of *Triphophyllum peltatum* (Dioncophyllaceae) dioncopeltine A (**95**) and, in particular, dioncophylline B (**96**) and dioncophylline C (**97**) exhibited high antiplasmodial activity [124]. Dioncopeltine A is able to suppress parasitaemia almost totally, while dioncophylline C cured infected mice completely after oral treatment with 50 mg/kg per day for 4 days without noticeable toxic effects. Analysis of the dose-response relationship of dioncophylline C revealed an ED_{50} dose of 10.71 mg/kg/day. Although four daily treatments with 50 mg/kg/day are needed to achieve parasitological cure, one oral dose is sufficient to kill 99.6% of the parasites. Intravenous application of dioncophylline C is even more effective, with an ED_{50} of 1.90 mg/kg/day and no significant toxic effects. The compound also suppressed more established *P. berghei* infections when applied orally at day three post infection. Both dioncopeltine A and dioncophylline C are active against the chloroquine-resistant *P. berghei* Anka CRS parasites.

Structure-activity relationships indicate that the presence of a secondary amine function, and the absence of an oxygen substituent at C-6 and R-configuration at C-3 are important. Recently, a novel dimeric antiplasmodial naphthylisoquinoline alkaloid heterodimer, korundamine A (**98**), has been isolated from another species, *Ancistrocladus korupensis* belonging to the family Ancistrocladaceae that is biogenetically related to Dioncophyllaceae. Korundamide A is one of the most potent naturally occuring naphthylisoquinoline dimers

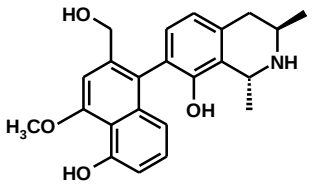
yet identified in antiplasmodial in vitro screening with an EC_{50} of 1.1 µg/mL against *P. falciparum* [125].



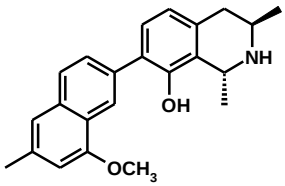
(**92**) berberine



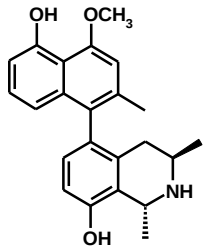
(**93**) (-)-pessione; R = H
(**94**) (-)-spinosine; R = CH_3



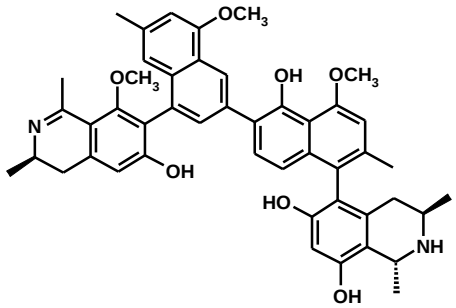
(**95**) dioncopeltine A



(**96**) dioncophylline B



(**97**) dioncophylline C



(**98**) korundamine A

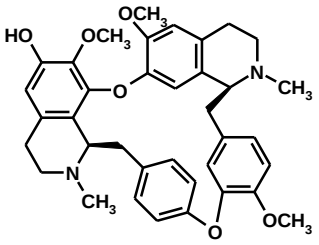
4.5.3 BISBENZYLISOQUINOLINES

A number of different bisbenzylisoquinolines with antiprotozoal activity have been identified. Although their antiparasitic activity has been recognised for years, particularly the antiplasmodial activity, the mechanism of action of these alkaloids is still unclear. So far in vivo activity has not been demonstrated. *In vitro* most bisbenzylisoquinolines exhibit activities in vitro far below 1 µg, close to the EC_{50} value of chloroquine ($EC_{50} \approx 0.2$ µM). Some bisbenzylisoquinolines like gyrocarpine (**99**), daphnandrine (**100**) and obaberine (**101**) are more potent than antimonials and nifurtimox and benznidazole, respectively, against *Leishmania* and *Trypanosoma* parasites ($EC_{50} < 50$ µg/mL) [123, 127]. Despite the fact that a large number of bisbenzylisoquinolines has been tested, a clear structure-activity relationship is not clear. Some structural features that appear to be important include the linkage of the heteromers and the number of ether bonds. Studies on *Triclisia* alkaloids showed that those compounds with two ether bridges (e.g. pycnamine) (**102**) are more potent than those with three ether bridges such as cosculine (**103**) (EC_{50} values of 0.15 µg and 15.56 ng/mL, respectively) [128]. Recently, Angerhofer et al. published an intensive study on structure-activity/toxicity-relationship of a series of 53 structurally different bis-isoquinolines [129]. More than half of the compounds tested against KB cells for cytotoxicity and *P. falciparum* strains W2 and D6, however, showed selective antiplasmodial activity, with >100-fold greater toxicity toward one or both of the *P. falciparum* clones, relative to cultured mammalian cells. The most selective alkaloids were (-)-cycleanine (**104**), (+)-cycleatjehine (**105**), (+)-cycleatjehenine (**106**), (+)-malekulatine (**107**), (-)-repandine (**108**), and (+)-temuconine (**109**). As a result of these studies, an understanding of the relationships between the structures, the stereochemistry, the substitution patterns of these alkaloids and their in vitro antiplasmodial and cytotoxic activities are beginning to emerge. The quaternarization of one or two nitrogen atoms, presence of an acetyl function at N-2', and N-oxide formation leads to a loss of toxicity and antiplasmodial activity. The decrease in lipophilicity (membrane permeability) of all of these alkaloids probably contributes to the lower toxicity observed. Within each subgroup of bisbenzyliso-

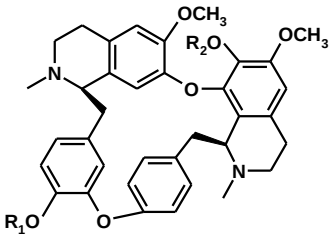
quinolines a change of configuration of the chiral center, as well as modification of substituents, may lead to independent changes in cytotoxicity and antiplasmodial activity. However, except for the three one-bridged compounds, (+)-neothalibrine (**110**), (+)-temuconine, and (+)-malekulatine, which show low toxicity and appreciable antiplasmodial activity, the current results do not reveal any clear structure-activity relationship between subgroups of bisbenzylisoquinoline alkaloids. With the exception of the one-bridged bisbenzylisoquinolines, all possess a large heterocycle of 18 to 20 atoms, which confers flexibility to the molecule. A study of the conformations assumed by compounds of the same subgroup (e.g., modification of conformation with the change of configuration at C-1 and C-1') should give more information on the structure-activity relationship. As the therapeutic index of the most antiplasmodial alkaloids is around 100 and those of chloroquinine, quinine and artemisinin, are 5460, >285 and >4680 respectively, the bisbenzyl-isoquinolines do not appear to be promising candidates as antimalarial agents.

Monomeric benzylisoquinolines do not appear to have potential. The activity of some aporhinoids, like isoguattouredigine (**111**) (from *Guatteria foliosa*, Annonaceae) argentinine (**112**), unonopsine (**113**) and hydroxynornuciferine (**114**) show only minor activity against *T. cruzi* in vitro ($EC_{50} \geq 250$ µM) [130, 131].

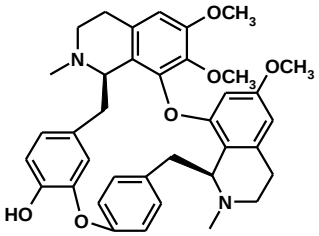
The isoquinoline derivate camptothecin (**115**), a well known antineoplastic drug and a topoisomerase I inhibitor, showed antiprotozoal activity when tested against *L. donovani*, *T. cruzi* and *T. b. brucei* with EC_{50} values of 1.5, 1.6 and 3.6 µM [132, 133]. For these parasites, camptothecin is an important lead for much-needed new chemotherapy, as well as being a valuable tool for further study of topoisomerase I activity.



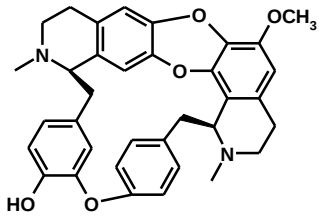
(**99**) (+)-gyrocarpine



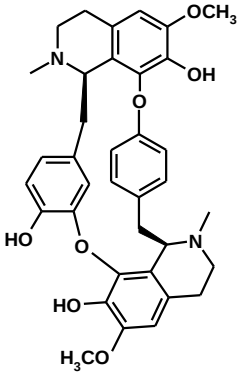
(**100**) (+)-daphnandrine; $R_1 = CH_3$, $R_2 = H$
(**101**) (+)-obaberine; $R_1 = R_2 = CH_3$
(**108**) (-)-repandine; $R_1 = H$, $R_2 = CH_3$



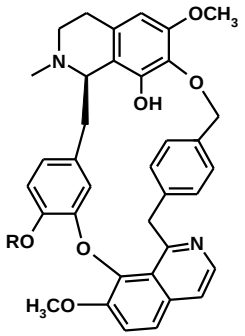
(**102**) (+)-pycnamine



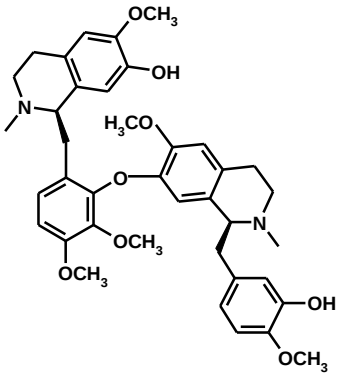
(**103**) (+)-cosculine



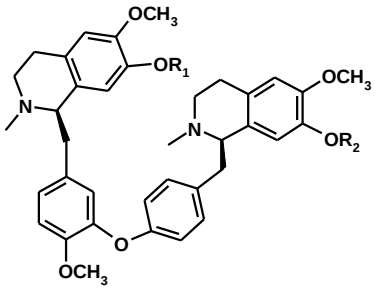
(**104**) (-)-cycleanine



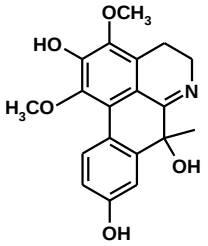
(**105**) (+)-cycleatjehine; R = H
(**106**) (+)-cycleatjehinine; $R = CH_3$



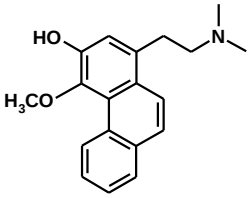
(**107**) (+)-malekulatine



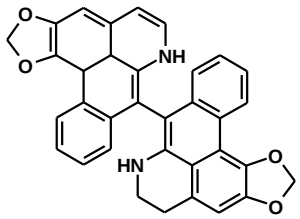
(**109**) (+)-temuconine; R_1 = H, R_2 = CH_3
(**110**) (+)-neothalibrine; R_1 = CH_3, R_2 = H



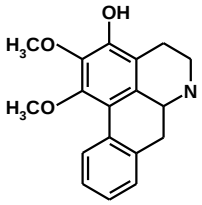
(**111**) isoguattouredigine



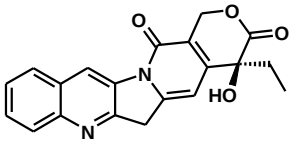
(**112**) argentinine



(**113**) unonpsine



(**114**) hydroxynornuciferine



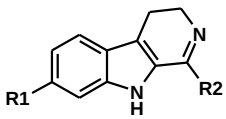
(**115**) camptothecin

4.5.4 INDOLES

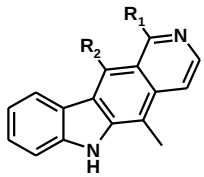
Indoles comprise another group of alkaloids with high biological activity. The indole sub-structure is widely distributed in the plant kingdom. Some indole derivatives have been reported to possess antiprotozoal activity. Indoles are biosynthetically derived from

tryptophan metabolism, which appears to be important in protozoa such as *Leishmania* and *Trypanosoma*. The end products of the tryptophan metabolism are thought to be involved in carbohydrate metabolism [134]. A simple derivative with antileishmanial activity (*L. amazonensis* amastigotes) is harmaline (**116**), often found in indole containing plants, e. g. *Peganum harmala* (Rutaceae). Harmaline, a harmane-type (**117**) indole alkaloid, is active at EC_{50} = 24 µg/mL, but too toxic for human use. The relevant pharmacological and antiprotozoal action of harmaline and related tryptamine derivatives is intensively discussed by [135]. Other monomeric indole derivatives are olivacine (**118**) and ellipticine (**119**). Both were identified as antiprotozoal compounds in the1970s [136], and both showed in vitro activity against *T. cruzi* epimastigotes with EC_{50} values of 2.5 and 5.0 µg/mL, respectively. In contrast, both were inactive in vivo, maybe because of inactivation through first pass metabolism. Cryptolepine (**120**) and related alkaloids, indole-quinolines, have been isolated from *Cryptolepis sanguinolenta* (Periplocaceae) and were active in vitro against *P. falciparum* in vitro (EC_{50} = 27-41 ng/mL, *P. falciparum*W2, D6, and K1) but failed in vivo (only 10.8 - 19.4 % suppression of *P. yoelii* at 100 mg/kg/day) [137]. Among the group of "dimeric" indole alkaloids the tubulin polymerisation inhibitor and antineoplastic agent vinblastine (**121**) is of experimental interest. In the therapy of protozoa infections its use is limited because of the poor therapeutic ratio against T*rypanosoma gambiense, L. donovani* and *P. falciparum* [138, 139]. Conodurine (**122**) and conoduramine from *Peschiera van heurkii* (Apocyanaceae) (**123**) showed antileishmanial activity with EC_{50} value of 50 µg/mL against *L. amazonensis* promastigotes in vitro [140]. Conodurine was less active than Glucantime (EC_{50} = 40 mg/kg/day, BALB/c mice) *in vivo* against *L. amazonensis*, and doses of conodurine at 200 mg/kg were toxic [140].

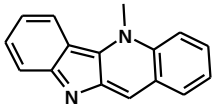
The mechanistically unusual antineoplastic product taxol (**124**), a diterpene-alkaloid, inhibits depolymerization of tubulin also in *Plasmodium, Trypanosoma* and *Leishmania* parasites, acting at concentrations as low as 0.1 µM. Because of toxicity reasons, this compound does not seem to be a particular attractive candidate for further development as antiparasitic agent.



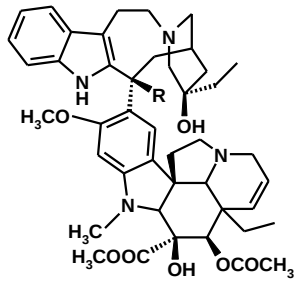
(**116**) harman; R_1 = H, R_2 = CH_3
(**117**) harmaline; R_1 = OCH_3, R_2 = CH_3



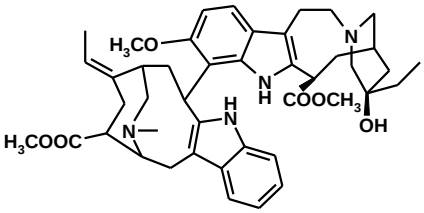
(**118**) olivacine; R_1 = CH_3, R_2 = H
(**119**) ellipticine; R_1 = H, R_2 = CH_3



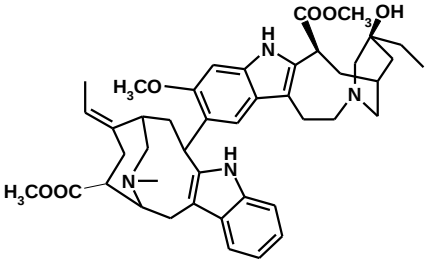
(**120**) cryptolepine



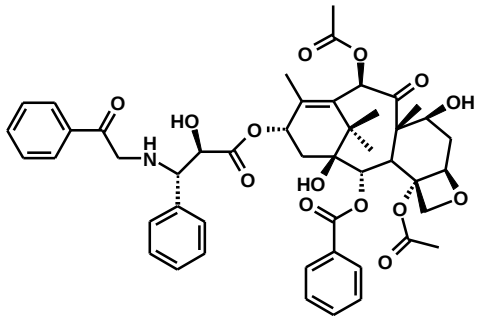
(**121**) vinblastine; R = $COOCH_3$



(**122**) conodurine



(**123**) conoduramine

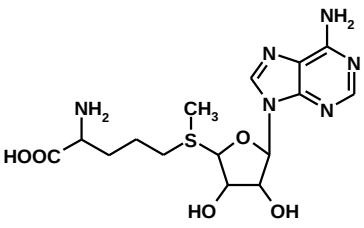


(**124**) taxol (paclitaxel)

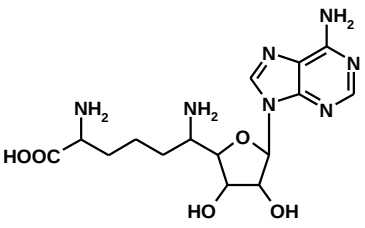
4.6 OTHER NATURAL PRODUCT CLASSES

4.6.1 NUCLEOSIDES

Sinefungin (**125**), a natural nucleoside isolated from cultures of *Streptomyces incarnatus* and *S. griseolus*, is structurally related to S-adenosylhomocysteine and S-adenosylmethionine (SAM) (**126**) [141]. Sinefungin has been shown to inhibit the growth of various fungi and viruses, but its major attraction resides in its potent antiparasitic activity. This natural product has attracted renewed interest since the synthetic S-adenosylmethionin-decarboxylase inhibitor 5'-([(Z)-4-amino-2-butenyl]-methylamino)-5'-deoxyadenosine (MDL 73811), a decarboxylated S-adenosyl-L-methionine analog, was introduced in experimental studies as a new drug for the treatment of Leishmania and *Trypanosoma* [142, 143]. Sinefungin does not inhibit S-adenosylmethionin-decarboxylase, but its action is focussed on SAM-synthases affecting methylation of macromolecules as nucleic acids and blocking of DNA polymerase by reduction of dATP.



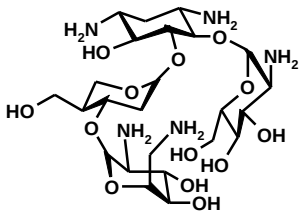
(**125**) sinefungin



(**126**) SAM

4.6.2 AMINOGLYCOSIDES

The aminoglycoside antibiotic, aminosidine (**127**), also known as paromomycin and monomycin, was first shown to be active against experimental cutaneous leishmaniasis in the early 1960s [144]. Later studies showed that is was the most potent among a series of tested compounds derived from microbiological sources [145]. Interest in the antileishmanial properties of this compound has been revived by the development of topical formulations for the treatment of cutaneous infections. It was found that topical application of either paromomycin or gentamicin, together with a transdermal enhancing agent, cured the parasite lesion, and that combined treatment with the two compounds had an additive effect [146]. The pharmacology and antiparasitic mechanism of these drugs formulations is discussed in detail by [147].



(**127**) aminosidine (paromomycin)

5. FROM MEDICINAL HERB TO THE DRUG MARKET

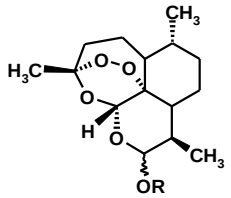
5.1 FROM ARTEMISININ TO ARTEMETHER (PALUTHER®), ARTEMETHER (ARTENAM®) AND ARTESUNATE (ARSUMAX®)

History. For thousands of years Chinese herbalists treated fever with a decoction of the plant called "qinghao", *Artemisia annua*, "sweet wormwood" or "annual wormwood" belonging to the family of Asteraccae. In the 1960s a program of the People Republic of China re-examined traditional herbal remedies on a rational scientific basis including the qinghao plant. Early efforts to isolate the active principle

were disappointing. In 1971 Chinese scientists followed an uncommon extraction route using diethyl ether at low temperatures obtaining an extract with a compound that was highly active in vivo against *P. berghei* in infected mice. The active ingredient was febrifuge, structurally elucidated in 1972, called mostly in China "qinghaosu", or "arteannuin" and in the west "artemisinin". Artemisinin, a sesquiterpene lactone, bears a peroxide group unlike most other antimalarials. It was also named artimisinine, but following IUPAC nomenclature a final "e" would suggest that it was a nitrogen-containing compound that is misleading and not favoured today.

Chemistry and Pharmacology. The chemistry and pharmacology of artemsinin has been reviewed in detail by Klayman [149], Luo and Shen [150], Woerdenbarg et al. [151], and van Agtmael et al. [152]. The limited stability as well as the poor solubility of artemisinin in water and oil, the two commonly used and approved media for parenteral administration, prompted scientist to prepare semisynthetic derivatives leading to improved solubility in water or higher chemical stability in oil formulations. Artemisinin is poorly soluble in water and decomposes in other protic solvents, probably by opening of the lactone ring. It is soluble in most aprotic solvents and is unaffected by them at temperatures up to 150°C and it shows a remarkable thermal stability. This section will focus on biological and pharmaceutical aspects; synthetic routes to improve antimalarial activity and to synthesize artemisinin derivatives with different substitutiuon patterns are reviewed elsewhere [151, 153]. Most of the chemical modifications were conducted to modify the lactone function of artemisinin to a lactol. In general alkylation, or a mixture of dihydroartemisinin epimers in the presence of an acidic catalyst, gave products with predominantly ß-orientation, whereas acylation in alkaline medium preferentially yields α-orientation (**128**-**132**). Artemether (**128**) as the active ingedient of Paluther® is prepared by treating a methanol solution of dihydroartemisinin with boron trifluoride-etherate yielding both epimers. The main goal was to obtain derivatives that show a higher stability when dissolved in oils to enable parenteral use. The α-epimer is slightly more active (EC_{50} = 1.02 mg/kg b.w.) than the ß epimer (EC_{50} = 1.02 mg/kg) and artemisinin itself (EC_{50} = 6.2 mg/kg) [154]. Synthesis of derivatives

with enhanced water solubility has been less successful. Sodium artesunate, Arsumax®; (**132**) has been introduced in clinics, is well tolerated and less toxic than artemisinin. The synthetic routes start with dihydroartemisinin treated with succinic anhydride in the presence of DMAP.



(**128**) dihydroartemisin; R = H (α + ß)
(**129**) artemether; CH_3 (ß)
(**130**) arteether; CH_2CH_3 (ß)
(**131**) artelinate; $CH_2C_6H_4COONa$ (ß)
(**132**) artesunate; $COCH_2CH_2COONa$ (α)

Pharmacokinetics and Pharmacodynamics. A characteristic of artemisinin and its related endoperoxide drugs is the rapid clearance of parasites in the blood in almost 48 hours. Titulaer obtained pharmacokinetic data for the oral, intramuscular and rectal administration of artemisinin to volunteers [155]. Rapid but incomplete absorption of artemisinin given orally occurs in humans with a mean absorption time of 0.78 h with a absolute bioavailability of 15 % and relative bioavailability of 82%. Peak plasma concentrations at a given dose are reached after 1-2 h and the drug is eliminated after 1 to 3 hours. The mean residence time after intramuscular administration was three times that when given orally. Other routes of adminstration, for example rectal or transdermal, are of limited success, but for the treatment of convulsive malaria in children artemeether in a rectal formulation is favoured. Artesunate acts as a prodrug that is converted to dihydroartemisinin. When given orally the first pass mechanism in the gut wall takes places metabolizing half of the administered dose. Oral artemether is rapidly absorbed reaching maximum blood levels (C_{max}) within 2-3 hours. Intramuscular artemether is rapidly absorbed reaching C_{max} within 4-9 hours. It is metabolized in the liver to the demethylated derivative dihydroartemisinin. The elimination is rapid, with a half-life time

($T_{1/2}$) of 4 hours. In comparison, dihydroartemisinin (128) has a $T_{1/2}$ of more than 10 hours. The degree of binding to plasma proteins varies markedly according to the species considered. The binding of artemether to plasma protein was 58% in mice, 61% in monkeys and 77% in humans. Radioactive labeled artemether was found to be equally distributed in plasma as well as in red blood cells indicating an equal distribution of free drug between cells and plasma.

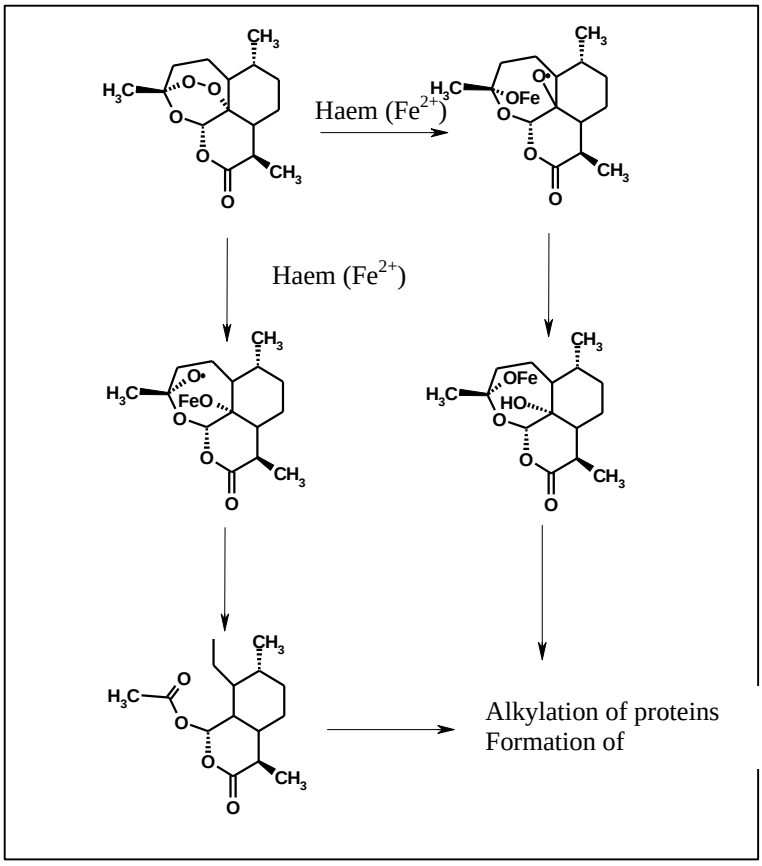


Fig. (1). Mechanism of action of artemisinin drugs, Active metabolites and formation of reactive epoxide intermediates (according to van Agtmael et al., 1999)

From the toxicological point of view artemisinin seems to be a safe drug for the use in humans. In animal tests neurotoxicity has been documented, but as yet this side effect has not been reported in

humans [156]. A major disadvantage of the artemisinin drugs is the occurrence of recrudescence when given in short monotherapy. So far no resistance has been observed clinically although it has been induced in rodent models in vivo. The mechanism of action is different from the other clinically used antimalarials. Artemisinin drugs act against the early trophozoite and ring stages, they are not active against gametocytes, and it affects blood- but not liver-stage parasites. The mode of action is explained by haem or Fe^{2+}, from parasite digested haemoglobin, catalysing the opening of the endoperoxide ring and forming free radicals. Malaria parasites are known to be sensitive to radicals because of the lack of enzymatic cleaving mechansims. The mechanism of action and the metabolism of reactive artemisinin metabolites is shown in **Fig. 1**.

Prospects. Other indications for malaria for the artemisinin drugs are currently under investigations. Without a final proof, other erythrocyte persisting parasites like *Babesia* are maybe another interesting target parasite. But also *Toxoplasma gondii*, *Pneumocystis carinii* infections in mice have been treated successfully with artemisinin drugs [157-159].

6.2 FROM QUININE TO MEFLOQUINE

History. Quinine has been listed as one of the six most important plant products that have influenced human history [160]. *Cinchona*, or "Quinine Bark" is one of the most famous plants from South America and most important discoveries. Legend says that the name "cinchona" comes from the Countess of Chinchon, the wife of a viceroy of Peru, who was cured in 1638 of a malarial type of fever by using the bark of the *Cinchona* tree. The legend starts with a misspelled name, continues with an extract named mistakenly by Linnaeus in 1742 as "quinquina", and maintains the reputed traditional use of plant extract for a disease probably introduced to that continent by Europeans and their African slaves [see 161, 162, 6]. Quinine bark was used by the Jesuits very early in its history, first advertized for sale in England in 1658 and was made official in the London Pharmacopoeia in 1677.

Several years after "Countess's powder" arrived in England, it arrived in Spain where the virtues of the bark were rapidly recognized, from this "tree of fever of the region of Loxa". Rapidly and due to the influence of the Company of Jesus, the "Jesuits' powder" became known all over Europe. Physicians gave credit to the drug, and because of the specificity of its action on malaria, it was recognized officially even when the identity of the producing species remained unknown.

Despite the confused history quinine still provides an important treatment for malaria, in particular cerebral malaria, with formulations of interest such as Quinimax, containing quinine and other active isomers, quinidine and cinchonine. Perhaps of greater significance to the history of malaria treatment, by a contorted route, is the work of William Perkins who in 1856 while trying to synthesise quinine discovered the first synthetic dye “mauve”. His work led to analine dyes that in turn led to methylene blue, the first compound rationally used for the treatment of malaria by Guttman and Ehrlich in 1891. Methylene blue provided the template for the design of the aminoquinoline drugs mepacrine, primaquine and chloroquine in the 1920s and 1930s [161]. Research on quinine also led to other drugs. In 1944 scientists were able to synthesise and structure elucidate (**Fig. 2**) the quinine alkaloid in the laboratory in Germany. This led to various synthesized quinine drugs to treat malaria and the use of the common bark and the natural quinine extracted from the bark and sold as antimalarial drugs fell out use. Indonesia and India still cultivates the *Cinchona*-tree, but Zaire has become the top supplier of a world market which is also supplied by other African countries (Burundi, Cameroon, Kenya), and much lower on the list of producers are the South American countries of Peru, Bolivia and Ecuador.

Plant products have also played a significant role in the treatment of another parasitic disease, amoebiasis. The root and corm extracts of *Cephaelis ipecuanhana* were traditionally and empirically for the treatment of dysentry and the active ingredient emetine was isolated in 1817. It was not until 1912 that the antiamoebic activity of the alkaloid emetine was placed on a rational basis. Another product cepahaeline proved to be less active than its methyl ester emetine. In the early 1960s the derivative dehydroemetine was shown to be more active against *E. histolytica* and less toxic than the parent compound.

This compound is still in use. Traditional uses of other plant products led to the identification of other compounds with activity against *Entamoeba* in the 1940s and 1950s, including the steroidal alkaloid conessine from *Holarrhena* sp. (Apocyanaceae), glaucarubin from *Simarouba amara* (Simaroubaceae), and henna from *Lawsonia alba* (Lythraceae) [163].

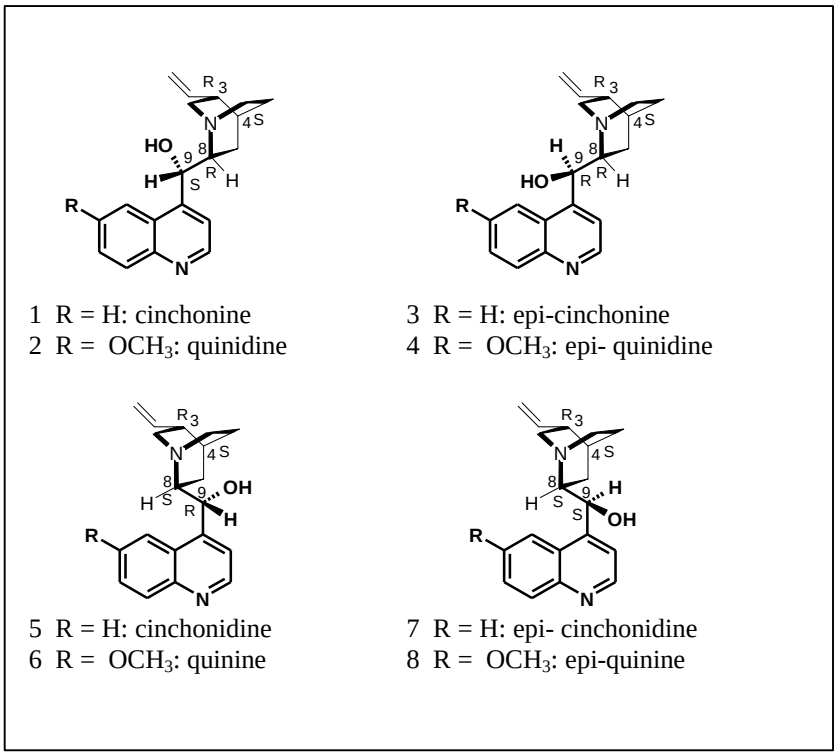


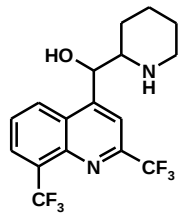
Fig. (2). The four principal *Cinchona*-alkaloids and their stereochemistry

Chemistry and Pharmacology. Quinine acts as a blood schizonticide although it also has gametocytocidal activity against *P. vivax* and *P. malariae*. Its effect is probably because of its properties as a weak base. As a schizonticidal drug, it is less effective and more toxic than chloroquine. However, it has a special place in the management of severe *P. falciparum* malaria in areas with known resistance to chloroquine [164]. Quinine is readily absorbed when given orally or intramuscularly. Peak plasma concentrations are achieved within 1 - 3

hours after oral dose and plasma half-life is about 11 hours. In acute malaria, the volume of distribution of quinine contracts and clearance is reduced, and the elimination half-life increases in proportion to the severity of the illness [165]. Therefore, the maintenance dose of the drug may have to be reduced if the treatment is continued for more than 48 hours. The drug is extensively metabolised in the liver and only 10% is excreted unchanged in the urine. Quinine is also a potentially toxic drug. The typical syndrome of quinine side effects is referred to as cinchonism and severity is related to size of dose. Mild cinchonism consists of ringing in the ears, headache, nausea and disturbed vision. Functional impairment of the eighth nerve results in tinnitus, decreased auditory acuity and vertigo. Visual symptoms consist of blurred vision, disturbed colour perception, photophobia, diplopia, night blindness, and rarely blindness. These changes are due to direct neurotoxicity, although vascular changes may contribute to the problem. Rashes, sweating, angioedema can occur. Excitement, confusion, delirium are also seen in some patients. Coma, respiratory arrest, hypotension, and death can occur with over dosage. Quinine can also cause renal failure. Massive hemolysis and hemoglobinuria can occur, especially in pregnancy or repeated use. Quinine has little effect on the heart in therapeutic doses and hence regular cardiac monitoring is not needed. However it can cause hypotension in the event of overdose. Quinine reduces the excitability of the motor end plate and thus antagonises the actions of physostigmine. It can cause respiratory distress and dysphagia in patients of myasthenia gravis. Quinine is administered orally at 10 mg/kg 8 hourly for 4 days and 5 mg/kg 8 hourly for 3 days, intravenous at 20 mg of salt/kg in 10 ml/kg isotonic saline or 5% dextrose over 4 hours, then 10 mg of salt/kg in saline or dextrose over 4 hours, every 8 hours until the patient is able complete oral administration or for 5-7 days, and finally intramuscularly at 20 mg/kg stat, followed by 10 mg/kg 8 hourly by deep intra muscular injections for 5-7 days [165].

Mefloquine (**133**) was developed during the Vietnam war, during a programme to find new antimalarials, to protect American soldiers from the multidrug resistant *P. falciparum* infection. The Walter Reed Army Institute for Research started preclinical development of mefloquine in 1972, filed and started human studies in the same year. After a successful evaluation of the drug potential further

development was done in a cooperation with Hoffman LaRoche leading to a final FDA-approval in 1988. Since then, it has been used worldwide for the treatment [166] and prophylaxis [167] of *P. falciparum* malaria, known under the trade name Lariam®. The antiplasmodial activity and mechanism of action is unknown. It probably affects the membranes of the parasites. It is effective against the blood forms of *P. falciparum* malaria, including the chloroquine resistant types. Mefloquine is available for oral administration only. It is absorbed rapidly and is extensively bound to plasma proteins [166]. The elimination half-life is about 2-3 weeks. It is mainly excreted in the faeces. It is generally well tolerated in therapeutic doses up to 1,500 mg. Nausea, vomiting, abdominal pain and dizziness can occur in doses exceeding 1 g [166]. Less frequently it can cause nightmares, sleeping disturbances, dizziness, ataxia, sinus bradycardia, sinus arrhythmia, postural hypotension, and an 'acute brain syndrome' consisting of fatigue, asthenia, seizures and psychosis. It is given at 25 mg/kg in a single dose.



(**133**) mefloquine (Lariam®)

6. FUTURE DIRECTIONS - DOES NATURE PROVIDE LEADS FOR NEW ANTIPARASITICS?

The widespread opinion that parasitic diseases no longer pose a problem thanks to antibiotics and vaccines is wishful thinking rather than reality. Today we are further away from controlling parasitic diseases than we were 20 years ago. Parasitic protozoa remain a major

threat to the health of human population throughout the world. Despite this fact, there are few effective drugs for the treatment of many protozoal diseases. However, the therapies for malaria, leishmaniasis and trypanosomiasis, diseases that threaten more than two billion people in mostly underdeveloped countries, are inadequate. This is now being recognised in multinational programmes such as "Roll back malaria". Traditionally, medicinal plants have already provided valuable leads for potential antiparasitic compounds, including naphthoquinones, terpenoids and alkaloids. The renewed interest in plant products has been stimulated in part by the identification of the antiplasmodial activity of the sesquiterpene lactone artemisinin (qinghaosu). This experience is not to be ignored as plants have frequently provided the template molecules on which to base further novel structures. For more than fifty years important antimicrobial and antiparasitic drugs have been identified from the products of fungi and bacteria and we should look to this source for future novel leads. In recent years marine organisms have been actively investigated and basic information has been made available to evaluate their potential. Although discovery of antiparasitic active compounds was not expected in the first pharmacological studies, promising leads have already been identified with new chemical types and active principles.

So what is the potential of natural products as a source of new antiparasitics? From the experiences with artemisinin (qinghaosu) it is wishful thinking to suppose that parasitic diseases can be treated with a single compound. Pure natural products are useful as lead structures, but in most cases high toxicity has restricted their use in humans. Many natural products with the desired activity and low toxicity have been identified, as reviewed here, but they did not progress through preclinical studies for evaluation as a potent drug because of low bioavailability and/or poor solubility. These pharmaceutical problems point towards the need for a rational, preferably mechanistically based, structural modification of chemical leads from nature. Natural products give new inputs to medicinal chemistry to develop new safe and effective drugs. By classical synthetic strategies organic chemists may create safer compounds close to lead to reduce toxicity, side effects or to improve bioavailability. This has been bone very successful for artemether from artemisinin and atovaquone from simple naphthoquinones.

Natural products have made an important contribution to antiparasitic drug research and despite all problems there is every indication that they will continue to make a contribution to the efforts to develop new and urgently needed drugs for the future.

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