Non-nitrogenous Plant-derived Constituents with Antiplasmodial Activity

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Dedicated to the memory of Professor Ivano Morelli.

The paper is a compilation of the studies reported in the literature concerning non-nitrogenous natural constituents that have shown antiplasmodial activity and aims to provide a basis for further *in vivo* studies as well as for clinical trials to develop new antimalarial agents. Due to the increasingly unsatisfactory outcomes for *N*-heterocyclic drugs, coupled with the rising incidence of the deadly *falciparum* malaria, the advent of non-nitrogenous lead compounds is timely, signaling a new era of antimalarial chemotherapy. Currently a few non-nitrogenous molecules are used in therapy, but many promising molecules of plant origin are under study, such as peroxide sesquiterpenes, quinoid triterpenes, quassinoids, gallic acid derivatives, lignans, flavonoids and biflavonoids, xanthones, naphthoquinones and phenylanthraquinones. Many of these constituents are isolated from plants used traditionally to treat malaria and fever. Ethnopharmacology can still be considered as a rich source of lead molecules.

Keywords: Plant-derived non-nitrogenous, malaria, *in vitro* and *in vivo* studies, terpenoids, polyphenols.

Malaria is one of the oldest life-threatening parasitic diseases diffused in the tropical regions of the world. It causes more than 300 million acute illnesses and at least 1-2.7 million deaths annually (mainly children under the age of five in sub-Saharan Africa). The majority of malaria deaths are due to cerebral malaria and other complications as a result of malaria-related anemia, and the cost in human life, incapacity for work, programs of control and medical treatment are enormous [1,2]. There are four types of human malaria: *Plasmodium vivax, P. falciparum*, *P. malariae,* and *P. ovale,* the first two of which are the most common, and *P. falciparum* is the most deadly type of malaria infection. The malaria situation is aggravated by the appearance of strains of *P. falciparum* resistant to antimalarial drugs as well as by the resistance of vector *Anopheles* mosquitoes to DDT and other insecticides. These are the principal factors that contribute to the difficulty of malaria control and it is unrealistic to think about eradication of this disease by means of destruction of the vector or use of vaccination. Studies in a number of African countries have shown that the emergence of chloroquine-resistant malaria parasites is associated with a two-fold increase in malaria deaths, but in one study in Mlomp, Senegal it was shown that malaria mortality in children under the age of four increased 11-fold within six years of the emergence of chloroquine-resistance [3]. Thus, chloroquine (**1**) represents one of the most effective anti-malarial drugs, but if used as monotherapy its effectiveness is rapidly lost.
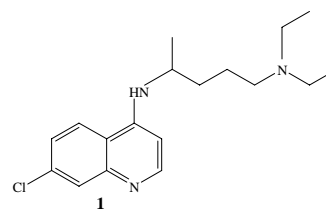
Chloroquine is an analog of quinine (**2**), a natural constituent, which is not only considered as the most important lead molecule for the synthesis of the majority of the existing antimalarial drugs but is also currently used in therapy, especially in severe and

complicated cases of malaria caused by chloroquine-resistant strains of *P. falciparum.*

Quinine was isolated in 1820 from *Cinchona* sp. bark, because the antimalarial properties of these plants had been known for several centuries. Jesuit missionaries in Peru around 1630 discovered that the bark of the cinchona tree allayed fever and a few years later exported the bark to Europe, where it was included in pharmacopoeias to treat fever.
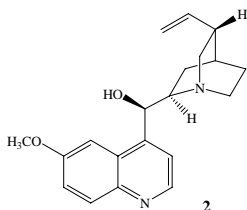

From the 1930s, chemically related molecules such as chloroquine (**1**), mefloquine, amodiaquine, mepaquine and pamaquine were developed [1,2]. These molecules are all characterized by the presence of nitrogen heterocycle moieties, and apart from the diminished effectiveness due to resistance by *P. falciparum*, they generally present some disadvantages and risks for the patient or user. Chloroquine is limited in its geographical use, only working in the Middle East, Mexico and Central America. Mefloquine is expensive, 100 times more so than chloroquine, and has resulted in seizures and psychiatric disorders. Halofantrine is equally expensive, unsuitable for prophylaxis, and has led to cases of cardiotoxicity. Even quinine is never totally effective, and its toxic side effects deter its usage [1,2]. Against this disheartening backdrop of the increasingly unsatisfactory performance of the *N*-heterocyclic drugs coupled with the rising incidence of the deadly *falciparum* malaria, the advent of non-nitrogenous lead compounds was not only timely, but also fortuitous and signalled a new era of antimalarial chemotherapy.

Plant-derived non-nitrogenous antimalarials have made, and continue to make, an immense contribution to malaria chemotherapy. In particular, artemisinin (**3**), isolated from the Chinese plant *Artemisia annua* L., has recently been used successfully against malaria resistant to chloroquine and the aim of this review is to consider the potential of plants to provide new antimalarial treatments.

In the last decades many plant extracts, especially those from species with a reputation for use in

traditional medicines, have been evaluated in the laboratory for their *in vitro* antiplasmodial activities and some have also been tested *in vivo*, usually in mice infected with *P. berghei* or *P. yoelii* [4,5]. In some cases, the constituent(s) responsible for the observed activities have been isolated by bioassay-guided fractionation and their structures elucidated, many of them being non-nitrogenous derivatives.
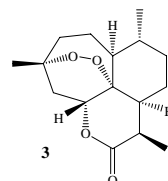
This paper reports on the antiplasmodial evaluation of these constituents according to their structures: terpenoids, polyphenols and other constituents.

Terpenoids

In the last three decades all the classes of terpenoids have been investigated to evaluate their antimalarial potency.
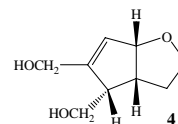

Among monoterpenoids, a simple molecule, an iridoid related aglycone, compound **4**, isolated from the roots of *Scrophularia lepidota* Boiss. (Scrophulariaceae), showed a low anti-plasmodial activity (the 50% inhibitory concentration (IC_{50}) was 240 μM). The result was nevertheless interesting because **4** showed a weak FabI enzyme inhibitory activity (IC_{50}=590 μM): FabI is a key enzyme of *Plasmodium falciparum* fatty acid biosynthesis and it can be used as a novel biological target to be used in the search for novel antiplasmodial constituents [6].

Bioassay-guided fractionation of the dichloromethane extract of the fruits of *Renealmia cincinnata* (Zingiberaceae), whose fruits are widely used in Cameroon to treat fevers, led to the isolation of six sesquiterpenoids of which two known ones, **5** and **6**, were the most active; their IC_{50} values were 6.8 and 7.4 μM, respectively, using 3D7 chloroquine-sensitive *P. falciparum* strains [7].

Among sesquiterpenes some lactone derivatives deserve to be mentioned because of the interesting activity [8-11]. A bioassay-guided fractionation of *Neurolaena lobata* (L.) R. Br. (Asteraceae), an

important medicinal plant in Central America and the Caribbean region, where it is used for a variety of diseases including malaria, resulted in the isolation of seven sesquiterpene lactones that showed IC_{50} values ranging from 0.62 to 19.27 μM against the NF54 strain (chloroquine-sensitive) and the clone A1A9 (chloroquine-resistant) of *P. falciparum in vitro* [8]. The most active components were neurolenin A (**7**) with IC_{50} of 0.92 μM and neurolin B (**8**) with IC_{50} of 0.62 μM. It was found that the structural requirements for high antiplasmodial activity *in vitro* is an *α,β*-unsaturated keto function. Additionally, a free hydroxy function at C-8 increased the antiplasmodial activity [8, 12].

7 R= H
8 R=OAc
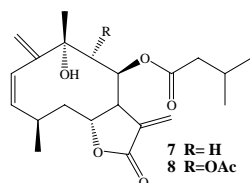
The sesquiterpene dilactone 16,17-dihydro-brachycalyxolide (**9**) was isolated from *Vernonia brachycalyx* (Asteraceae), a herb growing in East Africa and used by the Maasai, the Kipsigis and other East African tribes as a treatment for parasitic diseases [9]. This compound showed an IC_{50} of 26.9 μM using the 3D7 chloroquine-sensitive *P. falciparum* strain. The IC_{50} values for other tested strains, K39, V1/S and Dd2, were in a similar range, 8.3, 5.9 and 32 μM. This compound also strongly inhibited the proliferation of human lymphocytes at the same concentrations [13].


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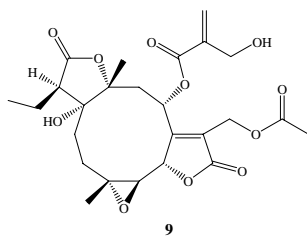
Another known sesqiterpene lactone, brevilin A was isolated from *Centipeda minima,* a plant used by the Chinese people to treat colds, nasal allergies, asthma, malaria and amoebiasis [10]. Brevilin A showed an IC_{50} of 9.42 μM against the W2 chloroquine-resistant strain [10].

Recently, the antimalarial activity of lactucin and lactupicrin isolated from *Cichorium intybus* L. (Asteraceae) was also determined against the HB3 clone of strain *Honduras-1* of *Plasmodium* which is

chloroquine sensitive and pyrimethamine resistant. The complete inhibitory activity (IC_{100}) for lactucin was 38.5 μM and the value for lactucopicrin was 126 μM [11].

Four sesquiterpene lactones of the pseudo-guaianolide type, the typical constituents of *Arnica montana* L. (Asteraceae), i.e. helenalin (**10**), dihydrohelenalin and their acetates, have shown activities against asexual blood forms of *Plasmodium falciparum in vitro* cultures (NF54, clone A1A9) [14]. The IC_{50} values of the four compounds were in the range from 0.23 to 7.41 μM and the most active constituent was helenalin (**10**), whose potency was comparable to that found for artemisinin (IC_{50} 0.14 μM).

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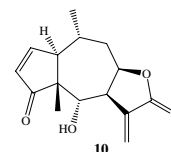
Because of the cytotoxic effects of sesquiterpene lactones on various types of cells, the cytotoxic/antiplasmodial ratio was also evaluated as a measure of therapeutic efficiency. Using the cytotoxicity data obtained for helenalin and artemisinin against the human carcinoma cell lines GLC4 and COLO 320, ten times higher toxicity was found for helenanin, which makes its therapeutic usefulness questionable [14].

Several sesquiterpene lactones isolated from *Eupatorium semialatum* Benth. (Asteraceae), a plant used in the traditional medicine of Central America for malaria and dysentery, were assayed *in vitro* for their activities against *Plasmodium falciparum* (K1 strain) using the pLDH-assay [15]. All the compounds were tested and exhibited a moderate activity (IC_{50} 8.9-31.7 μM) if compared to chloroquine (IC_{50} 0.18 μM). Nevertheless these results concerning their *in vitro* activity could justify the traditional use of the plant against malaria [15].

Several diterpenoids with different structures were also reported for their antiplasmodial activity. Among the abietane-type derivatives, 3-*O*-benzoyl-hosloppone (**11**) was isolated from the roots of *Hoslundia opposita* (Lamiaceae) used in East and West Africa to treat malaria [16]. The IC_{50} against the multidrug resistant strain K_1 of *Plasmodium falciparum* was 0.95 μM and the activity of this molecule was attributed to the presence of an *α,β*-unsaturated carbonyl moiety [16,17].
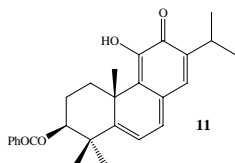
Several studies have recently been carried out on labdane and isopimarane diterpenoids, but most of them have shown a modest *in vitro* activity against chloroquine-sensitive *P. falciparum* strains [18-20]. Among the tested constituents only 8(9),15-isopimaradien-3β-ol, isolated from *Platycladus orientalis* (L.) Franco (Cupressaceae), gave interesting IC$_{50}$ values (7.1 μg/mL, 24.6 μM) in the inhibition of the growth of 3D7 *P. falciparum* strain [18].

Clerodane diterpenoids with a mild antiplasmodial activity have recently been isolated from two species of Flacourtiaceae, *Laetia procera* (Poepp.) Eichler a typical species of French Guiana [21] and *Casearia grewiifolia* Vent., growing widely in the northern and northeastern parts of Thailand and used traditionally as a tonic and a febrifuge [22].
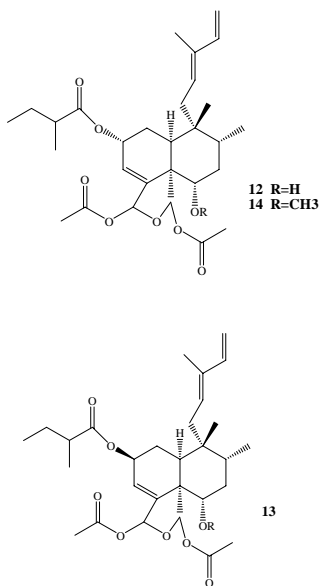
The compounds isolated from *C. grewiifolia* were tested against K1 multidrug resistant strains using artemisinin as positive control, while the derivatives isolated from *L. procera* were tested against F32 Tanzania (a chloroquine-sensitive strain) and FcB1-Columbia (a chloroquine-resistant strain), using chloroquine as a positive control. The most active clerodane diterpenoids were compounds **12**-**14** isolated from *L. procera,* showing activities against *P. falciparum* with an IC$_{50}$ as low as 0.5 μM on both FCb1 and F32 strains. The IC$_{50}$ values were 0.62 and

0.54 μM, respectively, in the two strains for compound **12**, 0.57 and 0.59 μM for **13,** and 0.58 and 0.66 μM for **14** [21]. It was also observed in this study that the hydrolysis of the diacetal moiety lowered their biological activity [21].

In 1997 Bringmann and coworkers [23] first reported on the moderate activity of betulinic acid against *P. falciparum in vitro*, with an IC$_{50}$ of 23.0 μM. This widespread constituent was isolated after a bioassay-guided fractionation from *Triphyophyllum peltatum* (Dioncophyllaceae) and *Ancistrocladus heyneanus* (Anciostrocladaceae). In 1999 Steele et al. [24] confirmed the *in vitro* activity of betulinic acid, but found that it was ineffective in *in vivo* experiments. Betulinic acid was also isolated from an ethanol extract of the root bark of the Tanzanian tree *Uapaca nitida* Mull-Arg. (Euphorbiaceae) used in Tanzania to treat malaria. It showed *in vitro* antiplasmodial IC$_{50}$ values similar to those obtained in the study of Bringmann et al. in 1997 [24]; the IC$_{50}$ values against chloroquine resistant (K1) and sensitive (T9-96) *P. falciparum* strains were 43.0 μM and 63.6 μM, respectively. The *in vitro* activities of several related triterpenes were also evaluated. Betulin was found to be inactive at 1164 μM for both K1 and T9-96. Ursolic acid exhibited IC$_{50}$ values similar to betulinic acid, 80.0 μM and 61.4 μM, respectively. Oleanolic acid exhibited higher IC$_{50}$ values, 194.7 μM and 154.8 μM against K1 and T9-96, respectively. Thus, among the triterpenes, betulinic acid showed the highest activity and for this reason was further tested for *in vivo* activity in a murine malaria model (*P. berghei*). However, the top dosage of 250 mg/kg/day was ineffective in reducing parasitaemia and exhibited some toxicity, and thus not advisable for clinical use [24].

An investigation of *Gardenia saxatilis* Geddes (family Rubiaceae), a plant with folkloric use against malaria and distributed in the northeastern part of Thailand, led to the isolation of several triterpenoids which were assayed for antiplasmodial activity using the K1 multidrug resistant strain [25].

Four compounds, namely messagenic acid A (**15**) and messagenic acid B (**16**), the 27-*O*-*p*-(*Z*)- and 27-*O*-*p*-(*E*)-coumarate esters of betulinic acid, and a mixture of uncarinic acid E (27-*O*-*p*-(*E*)-coumaroyloxy-oleanolic acid) (**17**) and 27-*O*-*p*-(*E*)-coumaroyloxy-ursolic acid (**18**) showed moderate activity with IC$_{50}$ values of 2.43, 6.14 and 4.69 μM, respectively. The results indicated that *p*-coumarate moieties at the

27-position contributed to antiplasmodial activity. As both the *p*-(*Z*)-coumarate ester **15** and the isomeric *p*-(*E*)-coumarate ester **16** were active in the assay, it was noteworthy that the difference in geometry of the double bond in the ester moieties did not significantly effect antiplasmodial activity of the triterpenes, while the introduction of a methoxyl group to the 3-position of *p*-(*E*)-coumarate moiety gave a ferulate moiety which resulted in a loss of activity [25].

15 R=*p*-(*Z*)-coumaroyl
16 R=*p*-(*E*)-coumaroyl

17 R$_1$=H   R$_2$=CH$_3$
18 R$_1$=CH$_3$ R$_2$=H
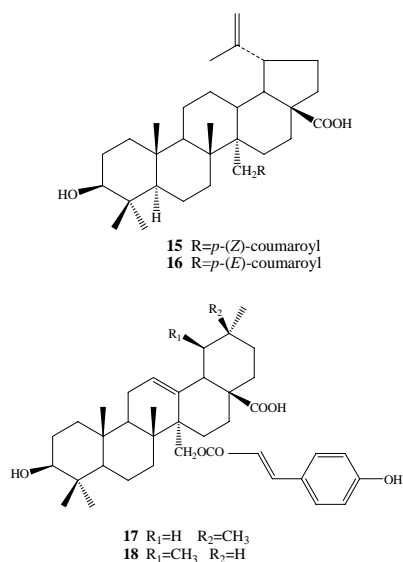
A very recent investigation [26] tested several ceanothane- and lupane-type triterpenes isolated from the root bark of *Ziziphus cambodiana* Pierre (Rhamnaceae) were antiplasmodial activity. 3-*O*-Vanillylceanothic acid (**19**), 2-*O*-*E*-*p*-coumaroyl alphitolic acid (**20**) and zizyberenalic acid (**21**) exhibited significant *in vitro* antiplasmodial activity against the parasite *Plasmodium falciparum* (K1 multidrug resistant strain), with IC$_{50}$ values of 5.81, 1.45 and 6.61 μM, respectively.


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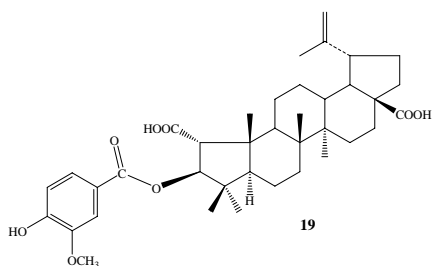
A comparison of the structures of the tested compounds indicated that the *p*-coumaroyl moiety in **20** and the vanillyl group of compound **19** were crucial for high antiplasmodial potential. Introduction of a double bond in ring A of the ceanothane-type triterpene **21** greatly increased the inhibitory activity in the antiplasmodial assay [26].


20 R=*p*-(*E*)-coumaroyl

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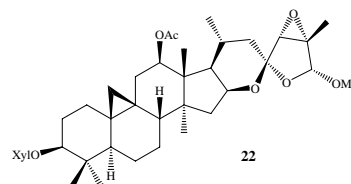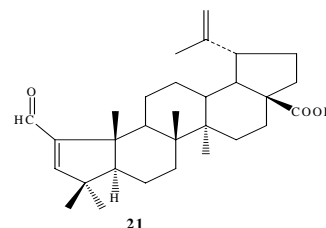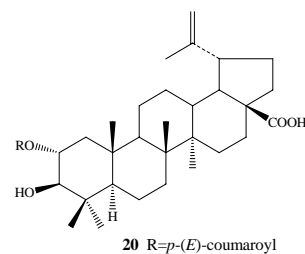
Another extensive investigation on the antimalarial effects of triterpenoids isolated from several species of the genus *Cimicifuga* was carried out by Takahara and coworkers [27]. Fifty-nine compounds belonging to five different structural groups were investigated. Almost all the compounds tested showed activity in the 1–56 μM concentration range against *Plasmodium falciparum* FCR-3 strain.

Twenty-five compounds had an IC$_{50}$ 1-3 μM and nineteen of them had a common 16, 23:23, 26:24, 25-triepoxy group in the side-chain moieties. The most active compound was (26*S*)-*O*-methylactein (**22**) [27].

Studies on a species of the Celastraceae family, *Celastrus paniculatus* Willd. from Thailand, known locally as Kra-Thong-Lai and sold in the form of pressed pills for the treatment of malaria, led to the isolation of a moderately active antiplasmodial constituent, a quinonoid triterpene, pristimerin (**23**). The IC$_{50}$ value against K1 strain was 0.42 μM [28].

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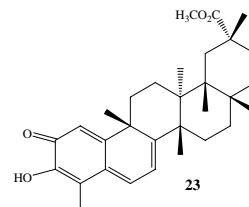
These findings were also confirmed by an investigation on another species of the Celastraceae family, *Salacia krauss,* a small shrub growing in Mozambique and KwaZulu-Natal Province, South Africa and traditionally used to treat bilharzia and dysentery. Thus, a bioassay-guided fractionation of the roots resulted in the isolation of six quinone methides including pristimerin [29]. Each of these compounds was tested *in vitro* against two strains of *P. falciparum*, a chloroquine-resistant strain (K1) and a chloroquine-sensitive reference strain (NF54). The highest activities were found for isoiguesterol (**24**) with an IC_{50} of 22.9 ng/mL (51.1 nM) against K1 and IC_{50} of 54.1 ng/mL (127 nM) against NF54.
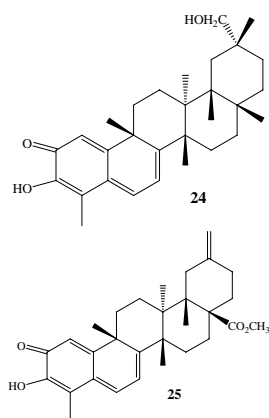
Another constituent, 17-(methoxycarbonyl)-28-*nor*isoiguesterin (**25**), displayed an IC_{50} of 27.6 ng/mL (60.9 nM) against K1 and an IC_{50} of 37.1 ng/mL (81.9 nM) against NF54. In addition, all the six isolated quinone methides were found to be cytotoxic against the human adenocarcinoma cell line HT-29 in the range of 1300 ng/mL up to 6060 ng/mL. They displayed, however, a 10-100-fold higher activity against plasmodia than against HT-29 cells, thus indicating some selectivity. Furthermore, compound **25** was also tested *in vivo* against *P. berghei* in mice. However, parenteral administration at 10 mg/kg body weight lead to the death of mice after 1 day, whereas 5 mg/kg and 1mg/kg parenteral as well as 30 mg/kg per oral neither cured mice nor reduced parasitaemia of *Plasmodium berghei* significantly [29].

Recently four tanshinones, i.e. 20-norditerpenes with an abietane-type skeleton containing a quinone moiety in the C-ring, were isolated from *Perovskia abrotanoides* Kar. (Lamiaceae) and moderately inhibit growth of cultured malaria parasites (3D7 strain of *Plasmodium falciparum*); the IC_{50} values ranged from 12.5 to 26.9 μM [30].

Artemisinin and other peroxides

Among the terpenoid derivatives, artemisinin (quinghaosu) (**3**) is one of the most well-known antiplasmodial drugs, it has few adverse side effects, making this by far the most useful natural product discovered to date to treat chloroquine-resistant malaria.

Artemisinin is an unusual sesquiterpene trioxane lactone containing an endoperoxide bridge which is essential for its activity. It (**3**) was isolated in 1972 by Chinese scientists from *Artemisia annua* (Asteraceae), a Chinese herb that has been used for over 2,000 years as a remedy for chills and fever. It was quickly observed that this molecule is a rapidly acting antimalarial drug effective against chloroquine and other drug-resistant parasites, and was as good as quinine (but less toxic) for the treatment of cerebral malaria. It is very active *in vitro*, with IC_{50} values between 1-100 nM depending on the *Plasmodium* strain [31,32]. As artemisinin is a non-polar compound, derivatives including ethers (artemether, arteether) and esters (sodium artesunate, sodium artelinate) were prepared to improve its formulation characteristics. These derivatives are now increasingly used as an alternative to quinine [33].

A number of other naturally occurring peroxides, not only from *Artemisia* sp. but also from other members of the Asteraceae (*Achillea millefolium*, *Anthemis nobilis*, *Heterothalamus psiadioides*), have also been tested [34]. It was found that although all of them showed some activity, none was as active as artemisinin (**3**). A weakly active peroxide (1*S*)-1-hydroxy-α-bisabolol oxide A acetate was isolated from *Artemisia abrotanum,* a plant widely cultivated in Europe for its aromatic properties. This compound showed interesting antiplasmodial *in vitro* activity, the IC_{50} being 17.9 μM [35].
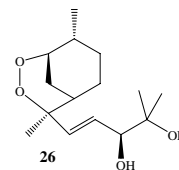
The functional group associated with the activity, namely the endoperoxide, is also present in the structure of another natural antimalarial, yingzhaosu A (**26**) first isolated in 1979 from another Chinese plant, *Artabotrys uncinatus* (Lam.) Merr. (Annonaceae). This constituent is a typical 1,2-dioxane and it occurs as a decomposition product from the stored roots of a sparsely growing vine [36].

Although the evidence of its antimalarial activity is largely anecdotal, **26** is reported to be active against *P. berghei.* However, owing to the limitations imposed by a poor supply of yingzhaosu A, the total synthesis of **26** was proposed in 1991 starting from R-(-)-carvone [37]. Total synthesis proved to be long and tedious, but in 1994 efforts led to semisynthetic first generation derivatives as potential drug candidates. Structurally related but simplified analogues containing the 2,3-dioxabicyclo [3.3.1] nonanes were synthesized. The analogue arteflene (Ro 42-1611, **27**) is a highly active, synthetic antimalarial endoperoxide [38] which can be considered a new lead molecule because of its lower rate of recrudescence, longer lasting therapeutic effects, and a longer half-life than that of artemisinin (**3**) and its commercial derivatives [39]. Later on, a series of endoperoxides containing a sulfide or a sulfone group were synthesized and some members of this class of sulfone endoperoxides have a good *in vivo* therapeutic index (efficacy/toxicity) [40].


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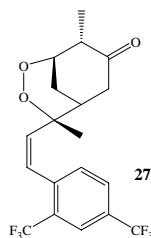
Several other endoperoxides have been isolated from plant sources, but most of them did not show high potency against *P. falciparum* strains. The exception to this is ascaridole (**28**), isolated from *Chenopodium ambrosioides* (Chenopodiaceae) and reported to be a potent inhibitor of plasmodial growth; at a concentration of 0.05 μM, development of plasmodium was arrested after 3 days [41]. Zingiberene 3,6-β-endoperoxide and zingiberene 3,6-α-endoperoxide isolated from two Brazilian species, *Eupatorium rufescens* and *Senecio selloi*, were reported to be active with an IC$_{50}$ value of 49 μM against FCH-5 *Plasmodium* strains [42]. 10,12-Peroxycalamenene (**29**), a sesquiterpene with an endoperoxide group similar in structure to artemisinin, was isolated from *Cyperus rotundus*, a Tanzanian plant used traditionally to treat malaria; it showed an IC$_{50}$ value of 2.33 μM against the K1 strain [43].

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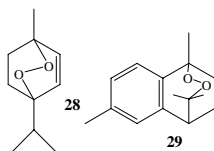
Two epimers, nardoperoxide (**30**) and isonardoperoxide (**31**) were isolated from *Nardostachys chinensis* (Valerianaceae) and tested for antimalarial activity [44-46]. Their EC$_{50}$ values against *P. falciparum* were 1.5 μM and 0.6 μM, respectively, values comparable with that of quinine (0.11 μM). In addition, studies of cytotoxicity against FM3A and KB cells showed that the selectivity (cytotoxicity/antimalarial activity) of these compounds was comparable to that of quinine. Therefore, these compounds could be considered as promising leads for a new class of antimalarial drugs.

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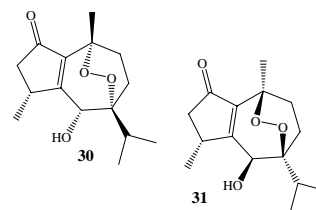
Finally, a diterpene peroxide (**32**) isolated from the spice cardamom, *Amomum krevanh* Pierre (Zingiberaceae) showed an antiplasmodial activity about one-tenth that of artemisinin, having an IC$_{50}$ of 0.17 μM [47].

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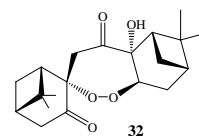
Quassinoids

Quassinoids are terpenoid bitter principles of the *Simaroubaceae* family, including the genera *Ailanthus*, *Brucea*, *Eurycoma* and *Simarouba*. Originally, these bitter substances were termed *quassin*, after a man by the name of "Quassi" who treated fever with the bark of these plants [48, 49]. Chemically they are degraded triterpenes and are categorized into five groups according to their basic skeleton.

Many quassinoids display a wide range of biological activities *in vitro* and/or *in vivo,* and their activity is related to both the position and nature of the ester group and, on the other hand, to the substitution of the A nucleus [48]. Constituents with antiplasmodial activity are mainly represented by the C-20 skeleton. An α,β-unsaturated ketone in ring A and an oxymethylene bridge in ring C are generally considered necessary for antimalarial activity [49].

At the end of the 1940s it was demonstrated that the majority of *Simaroubaceae* have activity on malaria in birds [50] and at the beginning of the 1980s a strong antimalarial activity *in vitro* of many derivatives was demonstrated [51]. Although several quassinoids are cytotoxic, results do indicate that cytotoxicity and antimalarial activity are not correlated, suggesting that the antimalarial activity is not merely cytotoxicity, but that selectivity is present [52]. Therefore, more investigations should be carried out in order to obtain specific information regarding the mechanism of action of these compounds.

The first molecules tested *in vivo* using *P. berghei*-infected mice were bruceine B (**33**) and brusatol (**34**). They showed some activity, but they were found to be toxic at higher levels than were necessary for antimalarial activity [53].

33 R=CH₃
34 R= CH=C(CH₃)₂
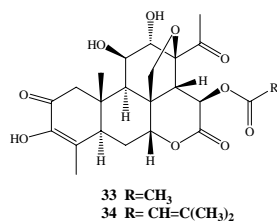
Active quassinoids have also been isolated from the fruits of *Simarouba amara* of the Republic of Panama [54], *Ailanthus altissima* [55], *Simana cedron* [56], the Brazilian plant *Simaba guianensis* [57]*, Eurycoma longifolia* [58], the Central African *Hannoa chlorantha* and *Hannoa klaineana* [59], the Guinanan *Picrolemma pseudocoffea* [60], and stems of the Indonesian plant *Quassia indica* [61]. All the tested quassinoids showed good activity against chloroquine-resistant and chloroquine-sensitive strains of *P. falciparum* and against *P. vinckei petteri* or *P. berghei* in mice. Studies on the structure–activity relationships of the quassinoids [48,62] indicated that the type and presence of an ester group at C-15 was vital for the antiplasmodial activity. Ring A substitution also affected the activity, with a diosphenol moiety in ring A giving the highest activity. The glycosides were found to be generally less active than the corresponding aglycones [48,62].

The most active quassinoids reported in the literature are gutolactone (**35**) and simalikalactone D (**36**) isolated from the bark of *Simaba guianensis* collected near Manaus, Brazil. They were tested against two *Plasmodium falciparum* strains: the W-2 Indochina, a chloroquine-resistant strain, and the D-6 Sierra

Leone, a mefloquine-resistant strain. Most notably, the activity was the same for the two different strains, since compound **35** showed IC_{50} values of about 9 nM and compound **36** displayed an IC_{50} of about 3.4 nM. Both compounds presented *in vitro* antimalarial activity similar to or better than that of known antimalarials used as standards (chloroquine, mefloquine, artemisinin, quinine) [57].
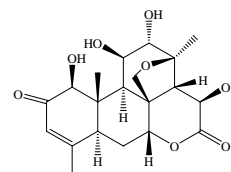

**35 R=COCHC(CH₃)₂**
36 R=COCH(CH₃)CH₂CH₃

Limonoids

Limonoids are bitter constituents which have a polyoxygenated triterpenoid skeleton biosynthetically connected to the quassinoids. Limonoids are most often found in the family Meliaceae and less frequently in the families Rutaceae and Cneoraceae. Of the over 300 limonoids known today, about one-third are accounted for by neem (*Azadirachta indica*) and Chinaberry (*Melia azedarach*) [63, 64]. The first limonoid found active against *Plasmodium* was gedunin (**37**) with an IC_{50} of 0.040 μM (0.02μg/mL) isolated from *Melia azedarach* [65]. However, this compound was not active *in vivo* against *Plasmodium berghei* in mice. [66].

On the other hand, recently it has been found that the combination of gedunin with chloroquine has an additive effect [67]. In addition, a recent *in vivo* reinvestigation [68] of the antimalarial activity of gedunin (**37**) in CD-1 mice infected with *Plasmodium berghei* led to some interesting results. When orally administered at 50 mg kg^{-1} day^{-1} for four days, gedunin (**37**) was able to suppress the parasitaemia level by 44%. However, no clear dose-response effects were observed in the 0-100 mg kg^{-1} day^{-1} dose range. Preliminary pharmacokinetics in Sprague-Dawley rats showed poor absorption, but a binary treatment of 50 mg kg^{-1} day^{-1} gedunin with 25 mg kg^{-1} day^{-1} dillapiol, a cytochrome P450 inhibitor, increased parasitaemia clearance in mice to 75%. A clear dose-response curve was observed in the 0-50 mg kg^{-1} day^{-1} gedunin dose range when administration was combined with 25 mg kg^{-1} day^{-1} dillapiol. In addition, 7-methoxygedunin, a semi-synthetic derivative which is more stable to degradation than gedunin, suppressed the level in

mice by 67% at 50 mg kg^{-1} day^{-1}. When administered at this dose in combination with 25 mg kg^{-1} day^{-1} dillapiol, clearance increased to 80%. These results demonstrate the potential efficacy of gedunin and the value of combination therapy [68].

37
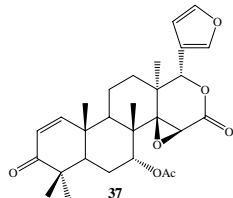
Studies on the leaves of *Azadirachta indica* collected in India resulted in the isolation of four limonoids active against the chloroquine-resistant K1 strain of *P. falciparum* [69]. Further investigations on *A. indica* have been carried out by Jones and coworkers [70] and Dhar and coworkers [71]. Jones and his co-workers looked at azadirachtin (**38**) and a series of 17 semisynthetic derivatives and their effects *in vitro* on male gamete production from malarial microgametocytes.


38
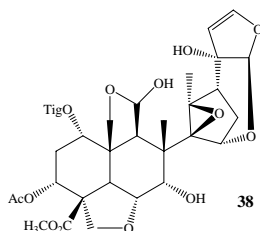
Azadirachtin (**38**) and three of the semisynthetic derivatives were found to inhibit the formation of mobile male gametes *in vitro*. This study indicated that the presence of a hemiacetal group at C-11 was vital to the activity. Dhar and coworkers [71] investigated the seeds of *A. indica* and found that the extract was active against all the erythrocytic stages of *P. falciparum*. In addition, the neem extracts also revealed a gametocytocidal effect with inhibition of the asexual stages of the parasite. All stages of maturation of the gametocytes were affected, unlike artemisinin and primaquine which only affect the immature stages [71].

Polyphenols

Over the last three decades studies on polyphenol plant constituents have shown antiplasmodial activity by almost all the classes of polyphenols. Simple galloyl derivatives isolated from *Swintonia forworthyi* Elmer (Anacardiaceae), a large tree of the Philippines, showed activity against two strains of *P. falciparum* (W-2, a chloroquine-resistant one and

D-6, a chloroquine-sensitive one). Methyl gallate showed an IC$_{50}$ of 19 μM for the D6 and an IC$_{50}$ of 10.9 μM for the W2 strain. Methyl 3-*O*-galloylgallate showed an IC$_{50}$ of 28.8 μM and for W2 a value of 13.7 μM against D6. Methyl gallate demonstrated a selectivity index of >5 towards the D6 strain and >8 towards the W2 strain when compared with cytotoxicity towards BC1, Lu1, CoI2, KB-V1, and LNCaP cancer cells, while methyldigallate demonstrated a selectivity index of >4 against both strains [72].

β-Glucogallin, the ester glucoside of gallic acid, and 1-*O*-galloyl-6-*O*-luteoyl-α-D-glucose are constituents of *Phyllanthus niruri* L. (Euphorbiaceae), a medicinal plant widely distributed in Indonesia that is often used in folk medicine to treat malaria and other diseases. They were active against the chloroquine-susceptible *P. falciparum* strain FCR-3 (IC$_{50}$ 14.6 and 2.21 μM, respectively) [73].

39
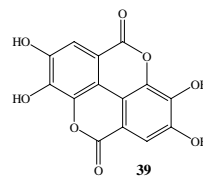
Ellagic acid (**39**) and 3,4,5-trimethoxyphenyl-(6´-*O*-galloyl)-*O*-β-D-glucopyranoside, isolated from *Tristaniopsis calobuxus* Brongiart & Gris, *T. yatensis* J.W. Dawson and *T. glauca* Brongiart & Gris (Myrtaceae) inhibited the growth of chloroquine-sensitive and resistant clones. Their IC$_{50}$ values were 0.5 and 3.2 μM, respectively [74].

Gossypol (**40**), the most abundant component of cottonseed (cotton=*Gossypium* sp., Malvaceae), is known for a variety of biological activities, including antispermatogenic, anticancer, antiparasitic and antiviral activity. It also demonstrated a weak antimalarial activity against both chloroquine-sensitive and chloroquine-resistant strains of *P. falciparum*, with IC$_{50}$ values in the order of 10 μM. [75].

40
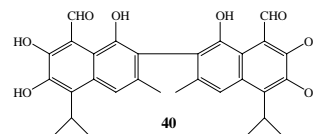
Three prenylated stilbenes, isolated from the edible fruits of *Artocarpus integer* (Moraceae), popular among the people in Thailand, exhibited moderate activity. Their EC$_{50}$ values against the K1 multidrug

resistant strain were 5.66 μM, 26.3 μM and 32.0 μM, respectively, with the novel compound **41**, being the most active [76]. Two other stilbenes, longistylin A and C, isolated from the roots and leaves of *Cajanus cajan* (L.) Millsp. (Fabaceae) showed a moderately high activity *in vitro* against the chloroquine-sensitive *Plasmodium falciparum* strain 3D7 [77].

41
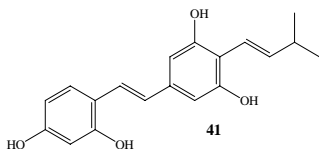
Bioassay-guided fractionation of the leaves from *Andira inermis* led to the isolation of numerous polyphenol constituents including isoflavones, dihydroflavonols and three novel 2-arylbenzofuran-3-carbaldehydes, andinermal A–C. Andinermal A (**42**) exhibited the strongest antiplasmodial activity *in vitro* with IC$_{50}$ values of 6.69 μM against the poW strain (chloroquine-sensitive) and 11.3 μM against the Dd2 strain (chloroquine-resistant). Andirnermal C (**43**) was slightly less active and the values were 17.8 μM (poW) and 19.0 μM (Dd2), respectively [78].


42 R=CH$_3$
43 R=H
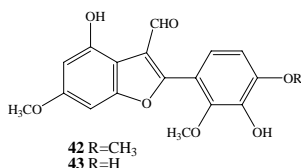
Two 5-methylcoumarin epoxides and several 4-phenylcoumarins have been found to be active against *P. falciparum* strains *in vitro*. The first compounds were isolated from the roots of *Vernonia brachycalyx* Hoffm. (Asteraceae), an herb used by the Maasai, the Kipsigis and other East African tribes as a treatment for parasitic diseases [79]. Their structures were 2′-epicycloisobrachycoumarinone epoxide (**44**) and cycloisobrachycoumarinone (**45**) epoxide, both of which showed antiplasmodial activity against chloroquinesensitive (3D7) and chloroquine-resistant (Dd2) strains of *P. falciparum in vitro*. IC$_{50}$ values for the strain 3D7 were 160 and 111 μM, respectively, while the IC$_{50}$ values for the strain Dd2 were 54 μM for both compounds [79].

A second group of coumarins active against *Plasmodium* strains was isolated from the stem bark of *Exostema mexicanum* (Rubiaceae), used in Latin American folk medicine as a quinine substitute for malaria treatment. The most lipophilic compound, 4′,5,7,8-tetramethoxy-4-phenylcoumarin (*O*-methyl-

exostemin), revealed the strongest antiplasmodial activity with IC$_{50}$ values of 10.5 μM (poW strain *P. falciparum*) and 4.68 μM (Dd2 strain) [80].

Four coumarins, theraphins A-D, isolated from *Kayea assamica* King & Prain (Clusiaceae), an evergreen tree used as a remedy for treating fevers in India, were tested against a panel of human cancer cell lines to assay their cytotoxicity, and tested for antimalarial activity against the D6 (chloroquine-sensitive) and W2 (chloroquine-resistant) clones of *Plasmodium falciparum*. The constituents were characterized by a 1-hydroxypropyl moiety linked to C-4, a 1-oxobutyl moiety linked to C-8 and an isoprenyl chain linked to C-6. They showed modest antiplasmodial activities, with IC$_{50}$ values in the range 9.7–11.1 μM against the D6 clone, and IC$_{50}$ values in the range 5.1–10.4 μM against the W2 clone. However, their Selectivity Indices (SI=KB IC$_{50}$/*P. falciparum* IC$_{50}$) were less than 1.0, although the values for theraphin D (i.e. 11(S)-(-)-8,8-dimethyl-5-hydroxy-4-(1-hydroxypropyl)-10-(1-oxobutyl)-2*H*,8*H*-benzo(1,2-b:3,4-b′)di-pyran-2-one) were 4.70 and 5.02 for the D6 and W2 clones, respectively. These observations indicated that the coumarin derivatives possess little potential as antimalarial drugs, although appropriate structure modifications of some of them might improve the SI level leading to derivatives of greater antimalarial potential [81].

Among neolignan derivatives, polysyphorin (**46**), isolated from *Rhaphidophora decursiva* (Araceae), a vine growing in Vietnam, showed antiplasmodial activity. From the same plant was also isolated a new active benzoperoxide, rhaphidecurperoxin (**47**) [82]. Compounds **46** and **47** were tested against the oral epidermoid cancer line KB and cultures of the chloroquine-sensitive clone D6 and chloroquine-resistance clone W2 of *P. falciparum*. The IC$_{50}$ of the neolignan was 0.92 μM (D6 strain) and 0.84 μM (W2 strain) with selectivity indices of 5 and 6, respectively, compared to the KB cell line.

46   **47**
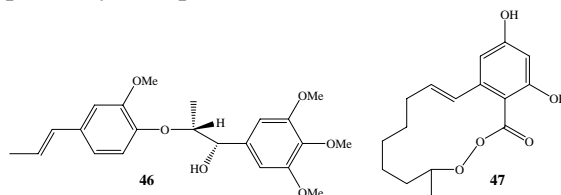
Therefore, compound **46** appears promising and further evaluation in *in vivo* antimalarial models should be pursued. It was also interesting to note that

47, which contains a peroxide ester, a moiety similar to the endoperoxide bridge of artemisinin, showed a moderate antimalarial activity; its IC_{50} against the D6 strain was 1.76 μM and against W2 was 1.37 μM. However, due to its poor SI values (0.7 and 1, respectively), this compound is not considered of great interest as an antimalarial agent [82].

Recently the neolignan nitidanin (**48**) has been isolated from *Grewia bilamellata* Gagnep. (Tiliaceae). It displayed weak antimalarial activity in cultures of *P. falciparum* clones D6 and W2 (IC_{50} 21.2 and 18.4 *μ*M, respectively). The same derivative tested against the human oral epidermoid carcinoma cell line (KB) showed a minimal cytotoxicity (ED_{50} >99.0 *μ*M) and thus its selectivity index (SI) expressed as ED_{50} (KB)/IC_{50} (*P. falciparum*) was high against both *Plasmodium* clones (>4.6 and 5.4, respectively). Thus, this molecule could represent a model structure because several neolignans with antimalarial activity were previously reported, but were highly cytotoxic [83].


48
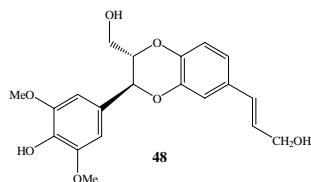
Several other lignans are reported to have antiplasmodial activity. Two of them, termilignan and anolignan B were isolated from *Terminalia bellerica* (Combretaceae), a species extensively used in the Indian system of traditional medicine for the treatment of fever, cough, diarrhea, dysentery and skin conditions [84]. These compounds were tested against the chloroquine-susceptible strain 3D7 of *Plasmodium falciparum* and showed IC_{50} values of 9.6 and 20.5 μM, respectively [84].

**44** R=CH_3 R_1=H
45 R=H R_1=CH_3
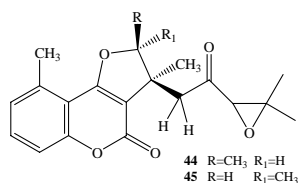
Another antiplasmodial lignan was isolated from a palm, *Euterpe precatoria* Mart. (Aracaceae): it was the 8-5´linked lignan dehydrodiconiferyl dibenzoate showing a similar antiplasmodial activity. The IC_{50} value was 12 μM when the compound was tested against the chloroquine-sensitive 3D7 *Plasmodium falciparum*.[85]

Phytochemical investigation of the aerial parts of *Bonamia spectabilis* (Choisy) Hall. (Convolvulaceae) led to the isolation of some active tetrahydrofuran-type sesquilignans (**49-52**). The derivatives were tested for their antiplasmodial activity against a chloroquine-sensitive strain (PoW) and a chloroquine-resistant clone (Dd2) of *Plasmodium falciparum*. Bonaspectin C 4´´-O-glucoside (**49**), its aglycone (**49a**), and bonaspectin D 4´´-O-glucoside (**50**) revealed the highest antiplasmodial activities (IC_{50} values: 1.3, 2.0, 6.5 μM [PoW]; 1.7, 4.6, 3.7 μM [Dd2], respectively.

49 R=β-glucose
49a R=H

50
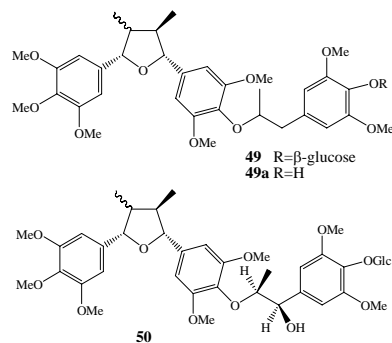
The sesquineolignans **51** and **52** revealed antiplasmodial activity with IC_{50} values of 9.9, 3.0 μM (PoW) and 10.9, 8.5 μM (Dd2), respectively.

51

52
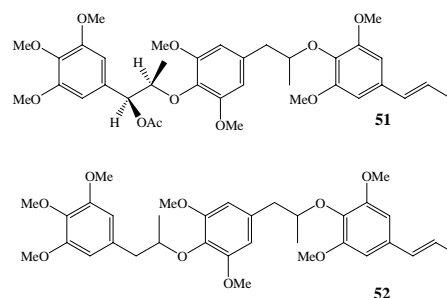
There was no significant difference of activity between the chloroquine-sensitive strain PoW and the chloroquine-resistant clone Dd2, however the phenylpropanoid dimers showed lower antiparasitic activities than the related trimers [86].

Several flavonoids, including biflavonoids, have been recognised for their antiplasmodial activity. Among them two flavanones, exiguaflavanone A (**53**) and exiguaflavanone B (**54**), were isolated from Artemisia indica from Thailand [87]. The assay was carried out with P. falciparum (K1, multidrug-resistant strain) and the constituents exhibited an IC_{50} of 10.8 μM and 16.0 μM, respectively [87].

53 R=CH₃
54 R=H
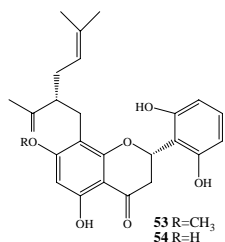
Two flavones, 5,7,4'-trimethoxyflavone and 5,7,3',4'-tetramethoxyflavone isolated from *Kaempferia parviflora* (Zingiberaceae), another plant from Thailand, [88] showed a weak antiplasmodial activity (IC₅₀ values were 11.9 and 12.5 μM, respectively).

Three flavonol glycosides (all kaempferol derivatives) isolated from *Hydrangea macrophylla* Seringe var. *thunbergii* Makino (Hydrangeaceae), a Japanese plant, were tested for the antimalarial properties and the cytotoxic activity against KB3-1 cells. The compounds exhibited characteristic antimalarial activity: in particular, approximately 60% of proliferation of the parasite was inhibited even at the concentration of 0.5 ng/mL. On the other hand, these flavonol glycosides have little influence on the growth of KB 3-1 representing the host cell [89].

Other flavonoids, namely (R)-4"-methoxydalbergione, obtusafuran, 7,4'-dihydroxy-3'-methoxyisoflavone, and isoliquiritigenin, isolated from the heartwood of *Dalbergia louvelii*, inhibit the growth of *P. falciparum in vitro*. Their IC₅₀ values ranged from 5.8 to 8.7 μM [90].

Five rotenoids, a chalcone and an isoflavone isolated from the stem bark of *Milletia usaramensis* subsp. *usaramensis* (Fabaceae), a plant of Kenya were tested against chloroquine-resistant (W2) and chloroquine-sensitive (D6) strains of *P. falciparum*. The chalcone 4'-O-geranylisoliquiritigenin was the most potent compound (IC₅₀ values were 8.7 and 10.6 μM, respectively). Among the rotenoids, those containing a prenyl or a 2,2-dimethylpyrano substituent were most potent (IC₅₀ values were between 19.4 and 70.1 μM) [91].


55
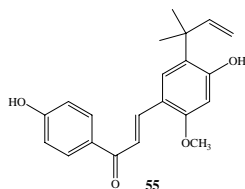
Another simple chalcone, licochalcone A (**55**) isolated from *Glycyrrhiza* species in different amounts, is reported as having *in vitro* and *in vivo* antimalarial activity [92, 93].

In *in vivo* tests against *P. yoelii* in mice, oral doses of 1000 mg/kg resulted in the complete eradication of the malaria parasite and no toxicity was noted [92]. *In vitro* the IC₅₀ was 1.78 μM (0.6 μg/mL) on the chloroquine-sensitive (3D7) and chloroquine-resistant (Dd2) strain of *P. falciparum*. An intraperitoneal injection of 15 mg/kg/ of licochalcone A twice daily for three days led to the survival of mice infected with *P. yoelli* and clearance of parasites. Oral administration (50 mg/kg) of a suspension of this flavonoid to mice infected with *P. yoelli* revealed that after four days, that the animals were no longer infected, thus it is efficient in controlling the infection [93]. A further investigation on licochalcone A showed that it is a potent membrane-active agent that transforms normal erythrocytes into echinocytes in parallel with the inhibition of growth of *Plasmodium falciparum* cultures. Thus, the *in vitro* antiplasmodial effect apparently is an indirect effect on the host cell. This effect could also be transiently observed *in vivo* after intravenous administration of the compound, but the cells returned quickly to the normal shape, presumably as the result of redistribution of licochalcone A in lipophilic compartments of the body or removal of damaged erythrocytes [94]. Recently it has also been demonstrated that licochalcone A can inhibit the bc(1) complex (ubiquinol-cytochrome c reductase) as well as complex II (succinate ubiquinone reductase, SQR) of *Plasmodium falciparum* mitochondria at very low concentrations. Because the property of the *P. falciparum* bc(1) complex is different from that of the mammalian host, chalcones could be promising candidates for a new antimalarial drug [95].

Xanthohumol (**56**), an isomer of licochalcone A, and seven derivatives isolated from *Humulus lupulus* L (Cannabinaceae) were tested for their *in vitro* antiplasmodial activity against the chloroquine-sensitive strain poW and the multiresistant clone Dd2. Of the eight compounds tested, four possessed activity with IC₅₀ values <25 μM against at least one of the two strains of *Plasmodium falciparum*. The main hop constituent, the chalcone xanthohumol, was the most active with IC₅₀ values of 8.2 μM (poW) and 24.0 μM (Dd2). Three of these compounds were additionally active in the haemin-degradation assay [96].

56
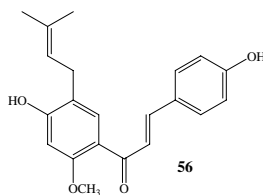
Other prenylated chalcones isolated from *Crotalaria orixensis* L. (Fabaceae) have been tested for *in vitro* antiplasmodial activity against NF-54 chloroquine sensitive strains. The most active compound was 3',5'-diprenyl-4,2',4'-trihydroxy chalcone, which inhibited the parasites 100% at 5.09 μM [97]. Within the same study it was shown that substitution at the 4' and 4-hydroxyl groups decreases the activity. The presence of prenyl moieties can affect the activity positively especially with free 4,4'-dihydroxy systems [97].

Structure-activity relationship studies of antimalarial chalcones were carried out using a series of forty oxygenated derivatives obtained by synthesis [98]. Good antimalarial activity was found among alkoxylated chalcones with polar A rings, in particular those substituted with electron-withdrawing groups or replaced by quinoline rings. The size characteristics of ring B (large, alkoxylated) and the electronic properties of ring A (electron deficient) are considered as important for antimalarial activity [98].

Two studies reported the antiplasmodial activity of prenylated flavonoids isolated from *Erythrina abyssinica* L. (Leguminose), a species widely used in Africa to treat infectious diseases. Flavonoids were tested against two *Plasmodium* strains: the chloroquine-sensitive D6 and the chloroquine-resistant W2 clones. Chalcones, flavanones and isoflavones with prenyl moieties showed weak activity against both strains with IC_{50} values ranging from 4.9 to 27.7 μM [99,100]. From another *Erythrina* species, *Erythrina sacleuxii*, several flavanones, isoflavones, and isoflavanones with isoprenyl moieties were isolated. These compounds displayed a similar antiplasmodial activity against the chloroquine-sensitive D6 and the chloroquine-resistant W2 *Plasmodium* strains. Their IC_{50} values ranged from 4.9 to 28.0 μM [101].

Three new prenylated flavonoids, namely the two flavanones 5,7,3'-trihydroxy-4',5'-(2''',2'''-dimethyl-pyran)-8,2'-di(3-methyl-2-butenyl)-(2S)-flavanone and 5,7,3'-trihydroxy-4'-methoxy-8,2'-di(3-methyl-2-

butenyl)-(2S)-flavanone and the flavan 7,3',4'-trihydroxy-6-methoxy-8,2'-di(3-methyl-2-butenyl)-(2S)-flavan, were isolated from the roots of *Dendrolobium lanceolatum* (Dunn) Schindl. (Fabaceae) and assayed against the parasite *Plasmodium falciparum* (K1, multidrug-resistant strain) and several cancer cell lines. They exhibited antimalarial activity with IC_{50} values of 5.3, 7.1, and 6.9 μM, respectively. However the flavanones were also cytotoxic, in particular the first flavanones showed strong cytotoxicity against the cancer cell lines KB, BC, and NCI-H187 with IC50 values of 2.4, 3.3, and 1.2 μM, respectively, while the latter showed moderate cytotoxicity against the NCI-H187 cell line with an IC_{50} value of 17.5 μM [102].

The dihydrochalcone, 2',4,6'-trihydroxy-4'-methoxydihydrochalcone (asebogenin) isolated from *Piper hispidum* Sw (Piperaceae), a species used by the indigenous population of Central America to treat malaria or fever, exhibited an IC_{50} of 56 μM for poW strains and 35 μM for Dd2 strains [103].

Two common flavone glycosides, luteolin 7-*O*-β-D-glucopyranoside (**57**) and chrysoeriol 7-*O*-β-D-glucopyranoside (**58**), isolated from *Phlomis brunneogaleata* Hub.-Mor. (Lamiaceae), were determined to be the major anti-malarial principles of this plant. Their IC_{50} values were 5 and 13 μM, respectively, using a K1 strain (chloroquine- and pyrimethamine-resistant). The same compounds, tested with skeletal myoblast L6 cells in order to evaluate their cytotoxicity, did not show any activity at the maximum tested dose of 90 μg/mL (about 200 μM). In addition, compound **57** showed a promising FabI-inhibiting effect (the IC_{50} was about 22.2 μM) [104].

57 R=H
58 R=CH₃
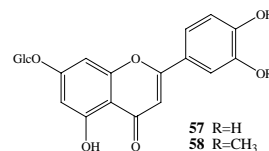
Bioassay-guided fractionation of a *Satureja parvifolia* (Philippi) Epling. (Lamiaceae) MeOH extract led to the isolation, among others, of eriodictyol and luteolin as its active components against *Plasmodium falciparum* K1 strain. The IC_{50} value of luteolin was 22.3 μM while that of eriodictyol was 59.7 μM. Besides their moderate antiplasmodial activity, flavonoids showed a very low toxicity on the mammalian KB cell line and

eriodictyol was the most selective compound as a result of its rather low cytotoxicity (IC$_{50}$ 604.2 µM) [105].

A very recent investigation reported on the inhibition by several flavonoids of different enzymes of *Plasmodium falciparum* fatty acid biosynthesis: α-ketoacyl-ACP-reductase (FabG), α-hydroxacyl-ACP-dehydratase (FabZ), and enoyl-ACP-reductase (FabI). About forty related structures were investigated and several compounds were found to have very good activity against all three enzymes. The flavones and flavonols exhibiting a simple substitution pattern (that is, no hydroxy groups on ring B and one or two hydroxy groups on rings A/C) show moderate inhibition effects toward FabG (10-100 µM), FabZ (20-30 µM), and FabI (10 µM) while flavonoids having more than one hydroxyl substitution on ring B exhibited strong activity toward all three enzymes (IC$_{50}$ 0.5-8 µM). The methylation of any of the hydroxy groups in flavonols generally abolishes almost all activity against all three enzymes. Among the flavanones tested, only 5,7-dimethoxy-8-methylflavanone showed some inhibitory activity against FabZ (40 µM). The isoflavonoids tested showed moderate and selective activity only against FabZ with IC$_{50}$ values in the range of 7-30 µM. The most active compounds were C-3 galloyl acid esters of catechins, which are strong inhibitors of all three enzymes (IC$_{50}$ 0.2-1.1 µM). Catechins and epicatechins, carrying a free hydroxy group at C-3, neither inhibit the enzymes nor have antiplasmodial activity. This study suggests that flavonoids and analogues are promising antimalarial agents, thus adding new targets to the broad spectrum of biological activities demonstrated by these compounds [106].

59
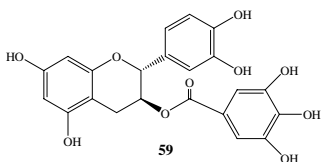
Within the same investigation it was shown for the first time that C-3 galloyl acid esters of catechins had *in vitro* activity against chloroquine-sensitive (NF54) and -resistant (K1) *P. falciparum* strains in the low to submicromolar range. The most active compound was (-)catechin gallate (**59**) with EC$_{50}$ values of 3.2 and 0.4 µM, respectively [106].

The same finding was also confirmed by another recent investigation [107]. Within this study two *P. falciparum* strains were investigated, namely 3D7, a chloroquine-sensitive one, and F9CR-1/FVO, a chloroquine-resistant one. Remarkably, pronounced plasmodicidal effects on both tested parasite strains were measured for (-)epigallocatechin gallate (**60**, IC$_{50}$ 30 µM for 3D7 and 20 µM for F9CR-1/FVO), and (-)epicatechin gallate (**61**, IC$_{50}$ 7 µM for 3D7 and 5 µM for F9CR-1/FVO).

60 R=OH
61 R=H
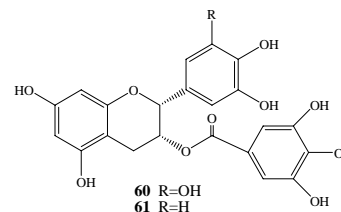
Furthermore a synergism was observed between artemisinin and these two derivatives on the 3D7 drug-sensitive parasite strain using sublethal doses of artemisinin, ranging from 1 to 10 nM, both of them in the presence (and in the absence) of 15 µM (-) epigallocatechin gallate (**60**) or of 5 µM (-) epicatechin gallate (**61**) [107].

Several biflavonoids have shown antiplasmodial activity. Among biflavanones, 7,7'-di-*O*-methyltetrahydromentoflavone (**62**) isolated from *Rhus retinorrhoea* (Anacardiaceae), a tree growing in the southern parts of Saudi Arabia, showed weak antiplasmodial activity but no cytotoxicity [108].

62
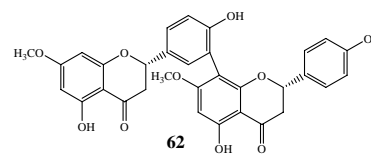
The compound exhibited weak antimalarial activity against *Plasmodium falciparum* (W2 clone) with an IC$_{50}$ of 1.6 µM, and activity against *P. falciparum* (D6 clone) with an IC$_{50}$ of 4.6 µM. [108].

63

64
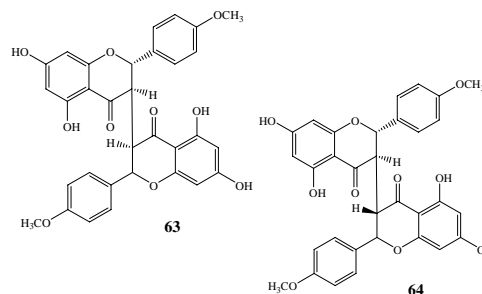
Two biflavanone isomers of **62,** namely sikokianin B (**63**) and sikokianin C (**64**) with moderate activity (IC$_{50}$ values of about 1 μM) against a chloroquine-resistant strain (K1) and a drug-sensitive strain (FCR3) of *Plasmodium falciparum* were also isolated from *Wikstroemia indica* (Linne) C.A. Meyer (Thymelaeceae). Their activity for the K1 strain was nearly the same as chloroquine but they were less than 2% as active as artemisinin [109].

65
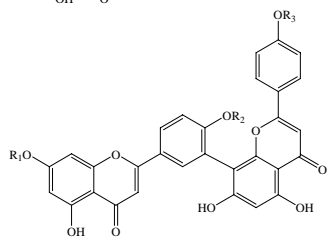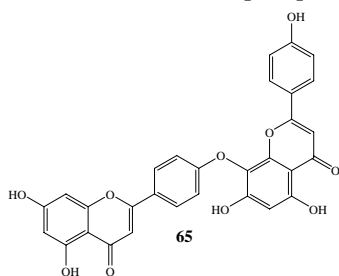
66 R$_1$= H, R$_2$=CH$_3$, R$_3$=H
67 R$_1$= CH$_3$, R$_2$=CH$_3$, R$_3$=H
68 R$_1$= H, R$_2$=CH$_3$, R$_3$=CH$_3$
69 R$_1$= CH$_3$, R$_2$=CH$_3$, R$_3$=CH$_3$

Recently, the antiplasmodial activity of eight other natural biflavones was evaluated [110]. Lanaroflavone (**65**) showed the highest antiplasmodial activity (IC$_{50}$ of 0.48 μM) when studied *in vitro* on a K1 chloroquine-resistant strain of *Plasmodium falciparum*. Other biflavones of the amentoflavone type, namely bilobetin (**66**), ginkgetin (**67**), isoginkgogetin (**68**) and sciadopitysin (**69**) showed medium activity (IC$_{50}$ values were 6.7, 2.0, 3.5, and 1.4 μM, respectively). Lanaroflavone also exhibited a high selectivity index value (SI=159), indicating selective antiplasmodial activity and no significant cytotoxicity [110].

A new biflavanoid, ent-naringeninyl-(I-3α,II-8)-4'-*O*-methylnaringenin, isolated from the root bark of *Garcinia livingstonei* collected in Tanzania, showed moderate activity against *P. falciparum* (chloroquine-sensitive Ghana strain); the IC$_{50}$ was 6.7 μM. Within the same assay the biflavonoids (**+**)-volkensiflavone and (**+**)-morelloflavone were also tested and displayed IC$_{50}$ values of 6.0 and 48.0 μM, respectively [111].

Several phenylanthraquinones showed considerable activity with only a little cytotoxicity as well, whereas the individual anthraquinone and phenyl moieties were completely inactive. Knipholone (**70**) and three of its natural derivatives, along with seven structurally-related but simplified compounds, have been examined for their antiplasmodial activity against asexual erythrocytic stages of two strains of *Plasmodium falciparum in vitro* (K1/chloroquine-resistant and NF54/chloroquine-sensitive). All the phenylanthraquinones showed considerable activity with IC$_{50}$ values 0.38-2.37 μM for the K1 strain and 0.42-2.64 for the NF 54 strain. Knipholone (**70**) and its natural derivatives can therefore be considered a new group of potential antimalarials [112].

From another *Bulbine* species, *B. frutescens* (L.) Wild (Asphodelaceae), three novel phenylanthra-quinones were isolated, namely 4'-O-demethyl-knipholone-4'-O-beta-D-glucopyranoside (**70a** a glycoside derivative of knipholone), and gaboroquinones A and B. These were tested against the chloroquine- and pyrimethamine-resistant K1 strain and against the strain NF54 of *P. falciparum* which is sensitive to all known drugs.

The glycoside **70a** displayed the best activity (IC$_{50}$ 0.7 μM for both strains) and did not exhibit any cytotoxic effects on mammalian cells, at least at concentration below 0.15 mM, the highest concentrations tested [113].

70 R= OCH$_3$
70a R= OGlc
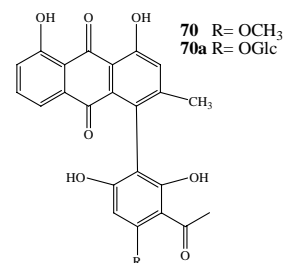
Morinda lucida is widely used in West Africa to treat malaria and other tropical diseases. Anthraquinones isolated from this plant have been tested against chloroquine–susceptible (3D7) and chloroquine-resistant (Dd2) strains. Their activity was moderate in both strains, with IC$_{50}$ values between 21.4 and 87.8 μM. Structure-activity relationships studies showed that an aldehyde group at C-2 and a phenolic hydroxy group at C-3 enhance activity of these anthraquinones against *Plasmodium* strains [114,115]

Xanthones from *Garcinia dulcis* and *G. cowa* (Clusiaceae) have been investigated for antiplasmodial activity [116,117]. *G. cowa* is widely distributed in Thailand where it is used as an antipyretic, while *G. dulcis* is mostly known for its disinfective activity [116,117]. Among the five xanthones isolated from *G. dulcis*, the most active against chloroquine-sensitive strains of *P. falciparum* ((T9/94 line) is garciniaxanthone (**71**) with an IC_{50} of 2.06 μM. The presence of isoprenyl moieties at C-2, C-7 or C-8 enhanced the antiplasmodial activity [116,117].

71
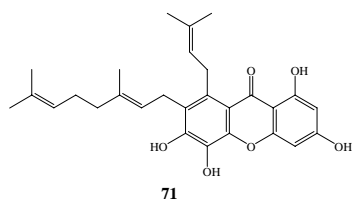
Recently, twenty-two xanthones isolated from *Calophyllum caledonicum* and *Garcinia vieillardii*, (Clusiaceae) were tested against chloroquine-resistant strains of *Plasmodium falciparum* (FcB1/colombia) [118]. The most potent xanthones were found to be **72**, **73** and **74** (IC_{50} of c.a. 1.0 μg/mL) which are 1,3,7 trioxygenated and prenylated at the positions 2 and 8. The relationship between antimalarial activity and molecular structure of xanthones has been explored. Firstly, the position of the hydroxyl groups appears to be important, as indicated by the observed differences in activity. Indeed, oxygenation at the positions 1, 3 and 7, seems to improve antimalarial activity. Secondly, substitution with a 1,1-dimethylallyl chain or the presence of an additional pyran ring appear to be activity-enhancing factors, as well as substitution with two isopentenyl chains or combination of one isopentenyl chain and a pyranic ring. Moreover, hydroxylation of the prenyl side chain is not required for activity [118].

72 R=isoprenyl
73 R=H

74
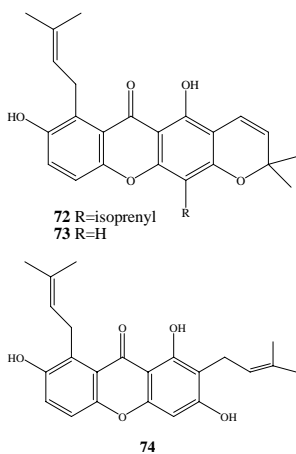
In addition, the *in vivo* antimalarial activity of some hydroxyxanthones was recently demonstrated for the first time [119].

Another study reported on a series of oxygenated xanthones which were synthesized and evaluated *in vivo*, using four-day suppressive assays against *Plasmodium berghei* ANKA in BALB/c mice. When given at a dose of 20 mg/kg/day for four days, most of the compounds produced significant chemosuppression of parasitaemia. The most active compound was 1,3,6,8-tetrahydroxyxanthone, which reduced the percentage of erythrocytes infected by 70.5%, followed by norlichexanthone (44.3%) and its isomer, 1,3,8-trihydroxy-6-methylxanthone (37.0%). While di-*C*-allyl-dihydroxyxanthone showed lower but still notable activity (33.4%), 1,3-dihydroxyxanthone was much less active (15.1%). This is the first demonstration of the antimalarial activity of some hydroxyxanthones *in vivo* [119]. In a different investigation, four xanthones isolated from the roots of *Andrographis paniculata* Nees (Acanthaceae), namely 1,8-di-hydroxy-3,7-dimethoxy-xanthone, 4,8-dihydroxy-2,7-dimethoxy-xanthone,1,2-dihydroxy-6,8-dimethoxyxanthone and 3,7,8-trimethoxy-1-hydroxy xanthone, were assayed *in vitro* using a chloroquine-sensitive strain FSG. 1,2-Dihydroxy-6,8-dimethoxy-xanthone was the most active (IC_{50} of 4 μg/mL), and it was tested *in vivo* in mice with a *Plasmodium berghei* infection using the Peters' 4-day test. A substantial reduction (62%) of parasitaemia was observed in mice with a 30 mg/kg dose. *In vitro* cytotoxicity against mammalian cells revealed that 1,2-dihydroxy-6,8-dimethoxy-xanthone is non-cytotoxic with an $IC_{50}>32$μg/mL [120].

75
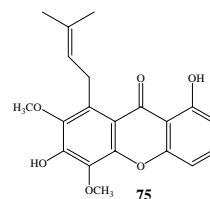
Finally, in 2006 several papers reported on the antimalarial activity of some natural xanthones. A new prenylated xanthone, 5-O-methylcelebixanthone (**75**), together with six related constituents from the roots of Cratoxylum cochinchinense (Lour.) Blume (Clusiaceae) have been tested for antiplasmodial and cytotoxic activity. Four derivatives including the new one showed cytotoxic activity against the human lung cancer cell line (NCI-H187) with IC_{50} values ranging from 1.4 μM to 0.011 mM. In the same concentration

ranges they showed antimalarial activity against Plasmodium falciparum with IC$_{50}$ values from 5.62 μM and 0.015 mM [121].

Five other previously known prenylated xanthones isolated from the root bark of *Garcinia livingstonei* collected in Tanzania, were tested against a chloroquine-sensitive Ghana strain of *P. falciparum.* The dimeric xanthone garcilivin A (**76**) showed the highest antiparasitic activity (IC$_{50}$ 6.7 μM) but it was cytotoxic in the same range of concentration (IC$_{50}$ 2.0 μM against MRC-5 cells). Its diastereoisomer garcilivin C and the monomeric xanthones showed IC$_{50}$ values ranging from 10 to 68 μM against *Plasmodium* with remarkable selectivity against MRC-5 cells (IC$_{50}$>32 μM) [111].

Three polyprenylated structurally related xanthones (gaboxanthone, symphonin and globuliferin) isolated from *Symphonia globulifera* L (Guttiferae), a tree whose bark is used in the Northwestern province of Cameroon to treat malaria, were tested for their anti-plasmodial activity against the W2 strain of *P. falciparum*, which is resistant to chloroquine and other antimalarials.
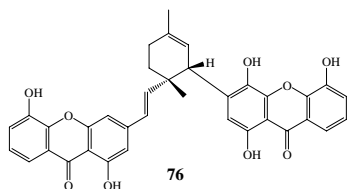
They all exhibited good to moderate activity relative to chloroquine, and symphonin (**77**) had the best potency (IC$_{50}$ was 1.29 μM). From the structure–activity relationship, it appeared that the cyclization of one of the isopentenyl groups (positions 2 and 4) to give a pyran ring increases the potency of xanthones. The best result was obtained when the dimethylpyran ring is attached to positions 3 and 4 of the xanthone nucleus as in symphonin (**77**) [122]. A benzophenone, guttiferone A was also isolated and found to be moderately active (IC$_{50}$ 3.17 μM) [122].

A further study on prenylated xanthones was carried out on a new prenylated xanthenedione, 1,2-dihydro-3,6,8-trihydroxy-1,1,7-tri(3-methylbut-2-enyl) xanthen-2,9-dione and five known xanthones isolated from
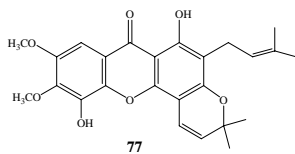
the stem bark of *Allanblackia monticola* Staner L.C. The compounds were tested on two strains of *Plasmodium falciparum*, F32 (chloroquine sensitive) and FcM29 (chloroquine resistant). The IC$_{50}$ values obtained ranged from 1.4 to 21 μM. Their cytotoxicity was estimated on human melanoma cells (A375) and the cytotoxicity/antiplasmodial ratio was found to be between 40 and 70 [123].

Other Constituents

Lapachol (**78**), a simple hydroxynaphthoquinone, is known for many pharmacological properties including antimalarial activity. It is present in many members of the Bignoniaceae family and it has been used as a template for the synthesis of the antimalarial drug atovaquone (**79**) [124].
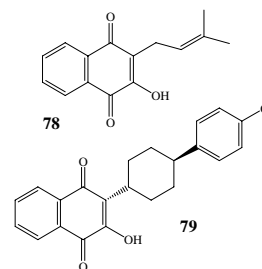
In a very recent paper a naphthoquinone–anthraquinone coupled pigment named newbouldiaquinone A (**80**) together with other naphthoquinones isolated from *Newbouldia laevis* Seem. (Bignoniaceae), a tropical African species widely used for the treatment of several diseases including malaria, were tested *in vitro* against *P. falciparum* NF54 and R strains [125].
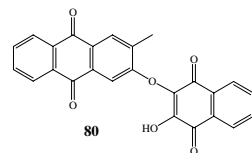
The most active compounds were newbouldiaquinone A, lapachol, α-lapachone and β-lapachone which showed a moderate suppression of parasitic growth [125].

Several papers report the isolation of active naphthoquinones from Bignoniaceae. Five furanonaphthoquinones isolated from *Tabebuia ochracea* ssp. *neochrysantha* (Bignoniaceae), a plant used traditionally in the Amazon to treat malaria, were tested against *P. falciparum* and *P. berghei in vitro.* The most active constituent was represented by a mixture of two compounds that could not be

separated: 5- and 8-hydroxy-2-(1'-hydroxy ethyl)naphtho[2,3-*b*]furan-4,9-dione. The IC$_{50}$ values obtained with this mixture were 0.17 μM (against *P. berghei*) and 0.67 μM (against FcB2 chloroquine-resistant strain of *P. falciparum*). For the former parasite, the IC$_{50}$ value for chloroquine was 0.05 μM, while for *P. falciparum* the IC$_{50}$ value was 0.11 μM. These results indicate that the furanonaphthoquinones isolated from *T. ochracea* are potential antimalarial compounds [126]. Four naphthoquinoids isolated from *Kigelia pinnata* (Bignoniaceae) root bark were assessed *in vitro* against chloroquine-sensitive (T9-96) and chloroquine-resistant (K1) *Plasmodium falciparum* strains and for cytotoxicity using KB cells. The most active one, 2-(1-hydroxyethyl)naphtho[2,3-*b*]furan-4,9-dione (**81**), has good activity against both strains; IC$_{50}$ values were 627 nM for the K1 strain and 718 nM for the T9-96 strain [127].

81
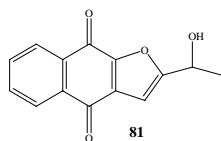
Several novel structurally related, prenylated naphthoquinones (sterekunthals A and B, pyranokunthones A and B) and one novel prenylated anthraquinone (anthrakunthone) isolated from the root bark of *Stereospermum kunthianum* Cham (Bignoniaceae), a plant used in Uganda to treat fever, have been tested against the chloroquine-sensitive strain poW and the chloroquine-resistant clone Dd2. The quinones showed different degrees of activity against the two strains of *P. falciparum* and sterekunthal A (**82**) was the most effective one [IC$_{50}$ values: 3.85 μM (PoW); 1.18 μM (Dd2)].

82
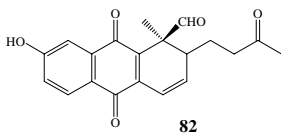
It was also shown that the 4-hydroxy group is an important structural feature for the antiplasmodial activity of these compounds, as sterekunthal B is distinctly less active than pinnatal [128]. The IC$_{50}$ values were comparable to those of related naphthoquinones isolated from *Kigelia pinnata* DC [127]. On the other hand, these compounds also exhibited marked toxicity against endothelial ECV-304 cells and hence their antiplasmodial effect seems to be due to general cytotoxicity [129].

A number of isofuranonaphthoquinones isolated from *Bulbine capitata* Poelln. (Asphodelaceae) showed only weak antiplasmodial activity both against the 3D7 (chloroquine-sensitive) and the K1 (chloroquine-resistant) strains. The plant is used in Botswana for its claimed antibiotic and antipyretic properties. The IC$_{50}$ values for both strains were between 23 and 92 μM , suggesting that these compounds are unlikely to have a significant *in vivo* activity when used alone [129].

Plumbagin (5-hydroxy-2-methyl-1,4-naphthoquin-one), isolated from *Nephenthes thorelii*, a species related to *N. ampullaria* and used to treat malaria in Malaysia, was active against *P. falciparum*, with an IC$_{50}$ value of 0.27 μM. The quinone structure is believed to be essential for the activity whereas the presence of a heteroatom such as oxygen or chlorine in synthetic derivatives at position 3 of the naphthoquinone nucleus causes weakening or loss of activity [130].

Another interesting group of constituents tested for antimalarial activity are the anthranoids. A highly active derivative of this class is vismione H (**83**), isolated from *Vismia guineensis* (Clusiaceae). The IC$_{50}$ against the sexual erythrocytic stages of *P. falciparum* (NF 54, clone A1A9) was 0.23 μM [131].

83

84
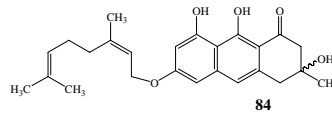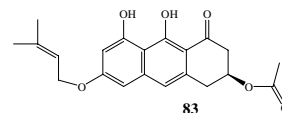
From another species of Vismia, *V. orientalis* Engl., a plant used in traditional medicine in Tanzania, vismione D (84) was isolated and exhibited antiprotozoal activity against *Plasmodium falciparum* strain K1 (IC$_{50}$ 2.4 μM). However, it was also found slightly cytotoxic against human L6 cells (IC$_{50}$ 10 μM) [132]

Concluding remarks

The prevalence of malaria in tropical zones worldwide, together with the lack of a vaccine and the appearance of strains of malaria parasite resistant to commercially available anti-malarial drugs based

on quinoline derivatives, makes the search for new effective anti-malarial drugs a global demand.

From the examination of the literature of the last decades it appears that a large number of plants used as antimalarial in the traditional medicine or related species have been investigated. Bioassay-guided fractionation of the extracts was generally used to find the active constituents and a large number of non-nitrogenous molecules have been found to possess a moderate to high *in vitro* antiplasmodial activity. However, only a few compounds have also been tested for *in vivo* antimalarial activities. Based on the literature compilation reported here the following three main conclusions can be drawn.

Firstly, only a few molecules result possessing a moderate to high activity and therefore should be considered for further investigations. They including peroxide sesquiterpenes, quinoid triterpenes, quassinoids, gallic acid derivatives, lignans, flavonoids and biflavonoids, xanthones, naphthoquinones and phenylanthraquinones.

Secondly, cytotoxicity of many of these derivatives has been evaluated in order to obtain the selectivity index, and results indicate that cytotoxicity and antimalarial activity are generally not correlated. It would be highly advantageous to consider these molecules as potential new antimalarial drugs.

Thirdly, although some of the investigated compounds are not particularly active, they are nevertheless interesting because they might strengthen chloroquine activity or restore chloroquine sensitivity in resistant strains of *P. falciparum.* Partially effective treatments might be beneficial in that the course of the disease is shortened, perhaps reducing anaemia and lowering the risk of death or serious illness from other anaemia-related diseases. Other possible benefits could be the alleviation of symptoms such as pain and fever and immunomodulation leading to increased immunity.

Another important aspect, not yet developed, is the search for molecules with little or no antiplasmodial activity which can synergistically act with known antimalarial drugs against *Plasmodium*. Thus, it is known that several flavonoids of *A. annua* can promote and enhance the antiplasmodic activity of artemisinin [133, 134], and recently it has been demonstrated that epigallocatechin gallate, epicatechin gallate and green tea extract not only have moderate antiplasmodial activity but also produce synergism in the presence of sublethal doses of artemisinin [107]. Also these molecules could have an important role in fighting malaria.

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