Drug Resistance in *Plasmodium*: Natural Products in the Fight Against Malaria

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Abstract. Malaria, perhaps one of the most serious and widespread diseases encountered by mankind, continues to be a major threat to about 40 % of the world's population, especially in the developing world. As malaria vaccines remain problematic, chemotherapy still is the most important weapon in the fight against the disease. However, almost all available drugs have been compromised by the highly adaptable parasite, and the increasing drug resistance of *Plasmodium falciparum* continues to be the main problem. Therefore, the limited clinical repertoire of effective drugs and the emergence of multi-resistant strains substantiate the need for new anti-malarials. Plant-derived artemisinin is currently the only available drug that is globally effective, but alarmingly, recent studies suggest that resistance already may be developing. Nevertheless, the success story of artemisinin from the herb *Qing Hao* (*Artemisia annua* L.), used as a remedy in traditional Chinese medicine for more than two thousand years, shows once again that natural products serve as an invaluable reservoir of lead compounds for sophisticated small molecules.

This review outlines the major anti-malarials, summarizing recent knowledge about their mode of action and the development of drug resistance. Furthermore, the most promising and recently discovered natural products with anti-malarial potential will be introduced.

Key Words: Artemisinin, chemotherapy, drug resistance, malaria, natural products, pharmacognosy, *Plasmodium falciparum.*

1. INTRODUCTION

1.1. Disease and Clinical Manifestations

Malaria is still one of the most important diseases in the developing world accounting for 1.1 – 2.7 million deaths per annum as estimated by the World Health Organization (WHO) [1]. Over 40% of the world's population are at risk of malaria infection and between 350-500 million people become seriously ill every year [2]. Furthermore, the economic burden of malaria in the developing world is enormous. The gross national product per capita has been estimated to be reduced by more than 50% in countries suffering from malaria compared to countries without malaria [3]. The disease is spread in the tropics and subtropics with sub-Saharan Africa being most affected with about 90% of all cases [1], followed by India, Brazil, Afghanistan, Sri Lanka, Thailand, Indonesia, Vietnam, Cambodia and China [4, 5].

Malaria is transmitted by the female *Anopheles* mosquito, and upon its bite, the eukaryotic, unicellular parasites can enter the bloodstream. In the course of the infection, the protozoa with genus *Plasmodium* cause common first symptoms such as fever, headache, chills and vomiting. If untreated, however, malaria can cause severe illness that is often fatal. The clinical manifestations range from anemia, delirium and metabolic acidosis to cerebral malaria including neuronal failures and brain damage, until multi-organ system failure, coma and finally, death. Among the different types of malaria caused by four different species of *Plasmodium*, infections with *P. vivax* and *P. falciparum* are the most common. The latter causes also cerebral malaria and accounts for the majority of all malaria cases [2, 6]. It is by far the deadliest type, especially in young children, killing one in four Africans under the age of five [7]. Considering that *P. falciparum* is the most dangerous parasite, this review will mainly concentrate on this type.

1.2. Life Cycle of the Parasite

The complex life cycle of the parasite begins when the sporozoite forms enter the human host. Waiting in the salivary glands of the mosquito, they are injected into subcutaneous tissue or directly into the bloodstream upon the mosquito's bite. Subsequently, the sporozoites advance to the liver and invade hepatocytes where they undergo asexual replication. They remain in this stage for 9-16 days [8]. Inside the liver cells one sporozoite develops into tens of thousands of merozoites, each of them able to invade one red blood cell (RBC) [9]. When released by rupture of the hepatocytes, they invade the erythrocytes [10] and replicate through another asexual reproduction process. This process advances through different stages, illustrated in [11]. In the early trophozoite state, the parasite develops a characteristic 'ring-form' morphology from where it proceeds further into a metabolically highly active trophozoite stage with glycolysis of large amounts of imported glucose, ingestion of the host cell cytoplasm and proteolysis of hemoglobin into con-

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stituent amino acids. The potentially toxic heme by-product cannot be degraded by the malaria parasites and is stored in their food vacuole in a polymerised, crystalline form called hemozoin (malaria pigment) [8]. The trophozoites transform into schizonts, each containing about 20 new merozoites that are released with lysis of the RBCs to invade new uninfected erythrocytes [9]. The released cell contents of the lysed RBCs stimulate the production of tumor necrosis factor and other cytokines, which are responsible for the characteristic clinical manifestations. The cycle of invasion, replication and release is repeated periodically about every 48 (*P. falciparum*) to 72 hours, depending on the *Plasmodium* species [8]. *P. falciparum* has the additional feature of modifying the surface of RBCs so that it can adhere to endothelia and the placenta, which is probably responsible for the deadly complications in many organs and the brain (cerebral malaria) [9]. A small subset of the asexual parasites in the RBCs differentiates into male and female gametocytes that are essential for the transmission of the disease through the female *Anopheles* mosquito. When the mosquito takes a bloodmeal of an infected person, gametocytes may be ingested into the midgut where they perform sexual recombination and replication. The resulting sporozoites migrate into the salivary glands where the cycle starts again.

2. STANDARD CHEMOTHERAPEUTICS, THEIR MODE OF ACTION AND DEVELOPMENT OF RESISTANCE

Despite many years of extensive research, there is currently no effective malaria vaccine available, however, hope exists that the development may be realistic [12]. Until then, chemotherapy and chemoprophylaxis will remain the principal weapons in the fight against malaria. This section will outline the major anti-malarials including recent knowledge of their mode of action and the development of drug resistance.

2.1. Quinolines

Quinolines are historically the most important anti-malarial drugs with quinine from the bark of the Andean *Cinchona* tree being the first widely used anti-malarial. The history of this natural remedy goes back more than 350 years [13] and is used until today against severe forms of malaria. From the 1930s, its synthetic relative chloroquine (4-aminoquinoline) was the safe, cheap and effective first line drug, which was used with great success in the fight against malaria. However, resistance to chloroquine was observed in East Asia in 1957, followed by South America in 1959 and finally Africa in the late 1970s. By the mid-1980s, resistant parasites were reported from almost all countries of sub-Saharan Africa and today chloroquine has lost its efficacy in nearly all areas of the world.

The exact mechanism of action of chloroquine, despite years of research, is still not completely understood. Nevertheless, it is generally accepted that the weak base selectively accumulates in the acidic food vacuole of the parasite [14] and exerts its anti-malarial activity by interfering with the polymerisation of toxic heme moieties, the by-products of hemoglobin digestion in the blood stage of the parasite [15-17]. Upon the different models of chloroquine uptake and action [18], all have to include the '*Plasmodium falciparum* chloroquine resistance transporter' (PfCRT), which is a key determinant of decreased susceptibility to several principal anti-malarial drugs [19]. Mutations in the corresponding *pfcrt* gene are linked directly to the chloroquine resistance phenotype [20] with all strains having one consistent amino acid change at K76 [21, 22] in combination with several others [23].

As chloroquine-resistant parasites started appearing in East Asia, efforts for the development of new anti-malarials were initiated and other quinoline derivatives were found. These derivatives, such as amodiaquine, piperaquine, mefloquine and halofantrine, also target the food vacuole whereas the precise mechanism of action is unknown.

The membrane of the food vacuole carries more transporters involved in the development of resistance [19]. Another important transporter type was identified to be similar to P-glycoprotein of the human multidrug resistance gene *mdr*. The pendant in *P. falciparum*, Pgh1, is an ATP-binding cassette (ABC)-type transporter that seems to be involved in food vacuole import [24]. It has been implicated in decreased susceptibility to several anti-malarial drugs. Mutations in the Pgh1-encoding gene, *pfmdr1*, were shown to enhance chloroquine resistance *in vitro*, but were not able to confer resistance on their own [25]. An observation, which is supported by some clinical studies [26-28]. Additionally, point mutations showed an association between *in vitro* susceptibility to other anti-malarial drugs, including mefloquine, halofantrine, lumefantrine, quinine and artemisinin [25, 29, 30]. Further studies discovered a correlation between drug resistance and *pfmdr1* expression in *P. falciparum*, suggesting an equivalent multidrug resistance mechanism as observed in mammalian tumor cells [19]. In a study with drug-sensitive strains, transcript levels of *pfmdr1* increased after treatment with chloroquine, mefloquine and quinine [31]. Other studies observed an association between a higher *pfmdr1* copy number and *in vitro* mefloquine resistance [29, 32, 33].

Primaquine (8-aminoquinoline), another chloroquine relative, was used primarily to clear the liver of the latent hypnozoite stages of *P. vivax* to counteract a malaria relapse [34]. However, the observation that it can be used as a chloroquine resistance reversal agent in *P. falciparum*, leads to the suggestion of combining these drugs [35].

2.2. Antifolates

Antifolates, such as pyrimethamine and proguanil, were initially used alone after their introduction in the 1940s/50s. However, resistance evolved rapidly and the combination of pyrimethamine and sulfadoxine, another antifolate, became popular as the first-line alternative to chloroquine. Despite a rapid development of pyrimethamine-sulfadoxine resistance in South East Asia beginning in the mid-1960s, the relatively cheap drug combination has been used in Africa since the early 1990s, though, with reducing efficacy. The principal antifolate drugs include pyrimethamine, proguanil, a prodrug metabolized to the active form cycloguanil, and the so-called sulfa drugs involving sulfadoxine, sulfalene and sulfone as well as dapsone.

The mode of action of the antifolates is well known and the molecular targets have long been established. The drugs interact with the folate pathway of the parasite by inhibiting essential enzymes. Pyrimethamine and cycloguanil act on the dihydrofolate reductase (DHFR), while the sulfa drugs inhibit the dihydropteroate synthetase (DHPS). DHPS is unique to the parasite and is responsible for the biosynthesis of key folate coenzymes, whereas DHFR is also present in humans, basically responsible for the provision of nucleotides for DNA synthesis and the metabolism of certain amino acids. The drugs that target DHFR bind several hundred times more strongly to the parasite equivalent.

Development of resistance arises from point mutations of the target genes *dhfr* and *dhps*. The stable transfection of resistant-type *dhfr* and *dhps* sequences into the genome of sensitive parasites showed direct association of mutations and resistance in *P. falciparum* [36]. It is well established that accumulations of mutations in *dhfr*, mainly at codons 108, 59 and 51, cause high-level pyrimethamine resistance [37]. Likewise, certain mutations in *dhps* affect sulfadoxine efficacy, resulting in resistant genotypes with slightly different patterns found in Africa, South East Asia and South America, respectively [37, 38]. In Africa, the highly pyrimethamine-sulfadoxine resistant parasites widely share the genotypes that combine triple mutated *dhfr* with double mutated *dhps*, a reliable molecular marker for this phenotype [39]. An additional mutation in *dhfr*, often found in South America and South East Asia, confers almost full resistance to pyrimethamine-sulfadoxine in combination with the above genotype [40]. Considering the fact that still many countries in Africa are using pyrimethamine-sulfadoxine as their first-line treatment and that the most recent antifolate combination chlorproguanil-dapsone might be compromised, a wide spread of such resistance would be a tragedy, however, such resistant parasites were recently reported in East Africa [41].

2.3. Atovaquone

Atovaquone, a drug type acting on the parasite mitochondria, was already found in the 1940s, but only recently licensed as a clinical anti-malarial in combination with proguanil in 1997. It has been used successfully for some time in South East Asia where multi-resistant parasites are common.

The 2-hydroxynaphtoquinone derivative is a structural analogue of coenzyme Q (ubiquinone) in the electron transport chain and causes the mitochondrial membrane potential to collapse, thus leaving respiration and essential pyrimidine biosynthesis of the parasite arrested [42]. Atovaquone targets the cytochrome bc_1 complex (complex III) without affecting human mitochondria due to differences in the parasite (CoQ_8) and human (CoQ_{10}) complex [43]. Atovaquone is proposed to block a large-scale domain movement involving the iron-sulfur protein subunit that is required for electron transfer from cytochrome *b*-bound ubihydroquinone to cytochrome c_1 within the bc_1 complex [44].

Resistance emerges rapidly upon atovaquone monotherapy and is due to point mutations in the cytochrome *b* gene, which strongly reduces atovaquone susceptibility *in vitro* and *in vivo* [45, 46]. Therefore, atovaquone is used in combination with the antifolate precursor proguanil to counteract resistance development. The synergistic effect of the combined drugs, although not yet fully understood, seems to be associated with an additional function of proguanil, namely acting to sensitize the mitochondrion by blocking a secondary mechanism for maintaining its membrane potential [47]. Although the drug is still successful, cases of resistance have been reported recently [48, 49]. Interestingly, not all of them were associated with mutations in the cytochrome *b* gene [50], suggesting a possible alternative resistance mechanism.

2.4. Artemisinin

Artemisinins are sesquiterpene lactones derived from the Chinese herb *Qing Hao* (*Artemisia annua* L.) and have been used in traditional Chinese medicine for the treatment of fevers over two millennia [51, 52]. In 1967 in the course of the Vietnam War, the People's Republic of China initiated a program to identify anti-malarials in plants used traditionally in Chinese medicine [51]. In 1971 it was observed that extracts of *Qing Hao* were effective against malaria in mice [53] and one year later the active ingredient *qinghaosu* ("active principle of *Qing Hao*") could be identified, which is now referred to as artemisinin [52]. After the structure was resolved in 1977, modifications were introduced to improve solubility of the natural drug whose derivatives today comprise the most important class of anti-malarials [54]. The artemisinin derivates, such as artemether, arteether and sodium artesunate, are metabolized to dihydroartemisinin [54], which is highly effective and can rapidly reduce parasite burden. The only drawback is the relatively short plasma half-life in the body and thus the danger of recrudescent parasites when used in monotherapy [55]. In order to circumvent this problem and to minimize the development of resistance, artemisinins are used principally in combination with other anti-malarials operating on longer time scales, such as mefloquine, lumefantrine, piperaquine, amodiaquine and sulfadoxine-pyrimethamine, generally referred to as artemisinin combination therapies (ACTs) [56]. However, there is some skepticism about certain combinations where the partner drug already has been compromised [57]. Ominously, there is also recent evidence that resistance to one of the major ACTs, artemether-lumefantrine, may already be developing in parts of East Africa [58, 59].

The artemisinins mode of action is based on the highly reactive epoxide bridge within the molecule. The peroxide within the 1,2,4-trioxane system is essential for antiparasitic activity [54], although the exact mechanism remains elusive. However, some studies suggest that upon activation by Fe^{2+}, artemisinins inhibit the Ca^{2+}-dependent SERCA-like ATPase PfATP6 [60]. Furthermore, artemisinins might exert their antiplasmodial potential through the formation of free radicals that can modify proteins and other molecules, as observed *in vitro* [60, 61]. Another mechanism might be the disruption of the mitochondrion membrane potential, which has been proposed by studies in a yeast model [62]. On the other hand, localization of artemisinins to mitochondria in *P. falciparum* has not been observed, which leaves the relevance of this mechanism questionable [61]. However, the PfATP6 hypothesis has recently been supported by alarming field isolates that associated reduced susceptibility to arteme-

ther with a S769N mutation in PfATP6 found in French Guiana, where artemisinins were used without control [63]. These results imply that PfATP6 plays a prominent role in the artemisinin mode of action, although it may possibly not be the only target. Interestingly, resistance to artemisinin and artesunate has been induced in the rodent parasite *Plasmodium chabaudi*, though, without mutations or copy number changes in potential resistance gene homologues including *pfatp6* [64]. This might be due to differences between the *Plasmodium* species and their host system but on the other hand these results could also be a forewarning that there are other resistance mechanisms yet undiscovered.

3. ANTI-MALARIAL POTENTIAL OF NATURAL PRODUCTS

As reported in the previous section, emerging resistance against essentially every major drug type and their combinations is a serious problem and the demand for effective compounds to fight malaria is increasing rapidly. The search for synthetic lead compounds may be promising [65], however, it has long been recognized that structures of natural products are highly diverse and offer interesting biochemical specificity in combination with other molecular properties that make them favorable as compounds for drug development [66]. Natural products were the basis of sophisticated traditional medicine for more than 2,000 years and more recently served as an important source of lead compounds, especially against infectious diseases [67]. They dominate the recent malaria patent literature [68] and always have been essential in the fight against malaria, beginning with the most important lead compound quinine, the template for chloroquine and other quinolines, until the discovery of the artemisinins, inspiring the synthesis of new promising derivatives [69, 70]. Furthermore, there are interesting effects also against cancer [71], other parasites [72, 73] or viruses [74, 75], to take only the example of artemisinin.

Several screenings of different natural sources were performed in the last decades resulting in some interesting candidates. In this review the most promising and recently discovered compounds with anti-malarial potential will be introduced ordered by their chemical class.

3.1. Alkaloids

The Indoloquinoline cryptolepine (**1**) from the West African shrub *Cryptolepsis sanguinolenta*, used traditionally against malaria and other infectious diseases, has been identified as a lead towards new anti-malarial agents [76]. With IC_{50} values against chloroquine-sensitive and -resistant strains *in vitro* of 0.27 μM and 0.44 μM respectively, it is probably inhibiting hemozoin formation in the parasite [76]. However, it was also found to be cytotoxic mainly by intercalating into DNA and interacting with topoisomerase II [77]. Therefore, several cryptolepine analogues have been synthesized with 2,7-dibromocryptolepine (**2**) showing the most promising *in vitro* and *in vivo* anti-malarial activity about 10-fold higher than the lead drug (IC_{50}= 0.049 μM) with low cytotoxicity [78]. Further development of these analogues would be worthwhile, particularly as there appears to be no cross-resistance with chloroquine [78]. Neocryptolepine (**3**), also isolated from the roots of *Cryptolepsis sp.*, was evaluated in a screen of some derivatives where 2-bromoneocryptolepine (**4**) was identified as favorable lead compound with good antiplasmodial activity (IC_{50}=4.0 μM) and no obvious cytotoxicity [79].

The indoloquinazolin tryptanthrin (**5**), well known for its anti-microbial properties, was firstly discovered in *Candida lipolytica* cultures [80] and has been found also in higher plants [81]. Tests of tryptanthrin and several analogues showed low IC_{50} values for tryptanthrin with the most potent analogues in the range of 0.43 to 10 ng/mL, particularly active against strains resistant to chloroquine, mefloquine and atovaquone [82]. Promising compounds with increased solubility have been recently synthesized waiting for *in vivo* studies [82]. Additionally, tryptanthrin has immunostimulating properties, an interesting detail that might be useful in the fight against malaria [83].

Naphtylisoquinoline alkaloids have been extracted from various species of the tropical lianas *Dioncophyllacea* and *Ancistrocladacea*, widely used in traditional medicine. In a study, the isolated alkaloids dioncophylline B (**6**) and C (**7**) showed high antiplasmodial activity where dioncophylline C cured malaria-infected mice completely after a 4-day oral treatment with 50 mg kg^{-1} day^{-1} without noticeable toxic effects [84]. Recent structural studies indicate complex formation of dioncophylline C with heme in solution and provide important structural information to further optimize naphtylisoquinoline alkaloids as anti-malarials [85].

β-Carboline alkaloids isolated from marine sources showed high *in vitro* and *in vivo* anti-malarial potential with manzamine A (**8**) being the most active compound, inhibiting the growth of *P. falciparum* after a single intraperitoneal injection (50 μg/mL, 95nM) into infected mice [86]. Initially discovered in sponges from Manzamo in Japan, they appear to be produced by symbiotic actinomycetes of *Micromonospora* [87]. However, support by the nonprofit foundation Medicines for Malaria Venture to deliver affordable anti-malarial drugs was stopped in 2005 because of the unfavorable toxicology profile.

Quinazoline type alkaloids like febrifugine (**9**), isolated from the Chinese herb *Dichroa febrifugar*, which has been used for treatment against fevers caused by malaria, show powerful antiplasmodial activity but along with undesirable side effects [88]. Synthetic febrifugines, however, demonstrated anti-malarial activity of similar potency as the natural product with high selectivity where one compound (**10**) exhibited extremely high *in vivo* activity in infected mice (ED_{50}= 0.6 mg/kg), thus, promising to be a lead compound for the development of new anti-malarials [88].

A novel morphinan alkaloid called tazopsine (**11**), isolated from *Strychnopsis thouarsii*, used in Madagaskar as a traditional remedy against malaria, is specifically active against the liver stages of the parasite [89]. Chemical modifications led to N-cyclopentyl (NCP)-tazopsine (**12**) with a better toxicity profile and full malaria protection in a mouse model with IC_{50} values and therapeutic indices in the area of primaquine [89], the only licensed drug against hepatic stages of malaria, however, with some unfavorable side effects [34]. Thus, NCP-tazopsine serves as an excellent lead

compound. Moreover, while acting on the early hepatic stages, it exerts only mild selective pressure on the parasites in contrast to inhibitors of the erythrocytic stage, where the high parasite load puts high pressure on the selection of drug resistant parasites. Therefore, it could lead to the development of a true causal prophylactic drug.

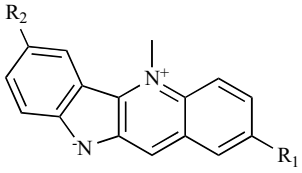
The monoindole alkaloid malagashanine (**13**) from *Strychnos myrtoides*, used in traditional medicine in Madagaskar, shows only weak antiplasmodial activity (IC_{50}~100 μM) [90]. However, associated with chloroquine it is able to reverse *in vitro* resistance in *P. falciparum* [90] and acts as a resistance reversal agent also for quinine, mefloquine, halofantrine and other anti-malarials [91]. It is active also *in vivo* [90] and was recently found to stimulate chloroquine influx and reduce its efflux in resistant *P. falciparum* [92]. This rather unconventional approach looks quite interesting but further clinical assays have to confirm its usefulness.

3.2. Terpenes

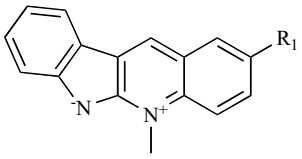
Sesquiterpene peroxides like artemisinin and its synthesized derivatives are highly effective against malaria. They served as leads for second generation endoperoxides with promising new compounds like OZ 277 (**14**), a trioxolane that is now in phase II clinical trials [70], or several trioxane dimers (**15**), which cure malaria-infected mice after only one single dose [69]. *Yingzhaosu* (**16**), another sesquiterpene peroxide isolated from *Artabotrys uncinatus*, showed activity in infected mice comparable to quinine or mefloquine but 10-fold less than that of artemisinin [93]. Its highly active derivative arteflene (**17**) has reached clinical trials in humans, but was discontinued because its synthesis was not suitable for industrial production.

Terpene isonitriles isolated from marine sponges were reported to possess anti-malarial activity [94]. Axisonitrile-3 (**18**), a sesquiterpene derivative from the sponge *Acanthella klethra*, shows potent antiplasmodial activity with no detectable cytotoxicity [95]. It may exert its anti-malarial potential through the inhibition of heme detoxification processes [96] and merits closer examination for further development.

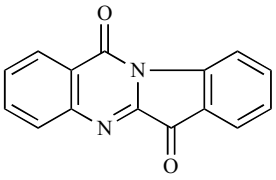
Quassinoids from S*imaroubacceaes* showed high *in vitro* anti-malarial activity with the most active compounds bruceantin (**19**) and simalikalactone D (**20**) (IC_{50}=0.8 and 0.9 ng/mL) [97], though, their high cytotoxicity prevented them from further development [98]. Nevertheless, the new quassinoid orinocinolide (**21**) was recently isolated from *Simaba orinocensis* and found to be equally potent to si-



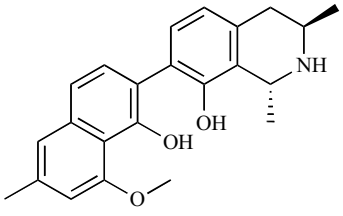
(**1**) R1 = R2 = H Cryptolepine
(**2**) R1 = R2 = Br 2,7-Dibromocryptolepine



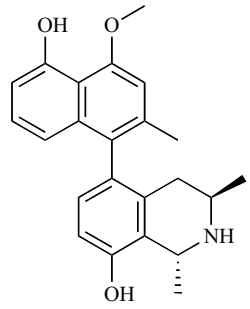
(**3**) R1 = H Neocryptolepine
(**4**) R1 = Br 2-Bromoneocryptolepine



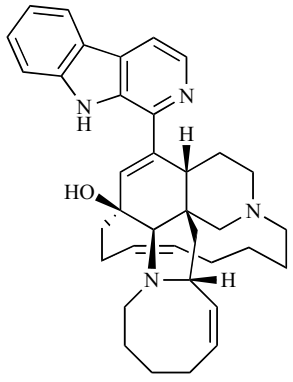
(**5**) Tryptanthrin



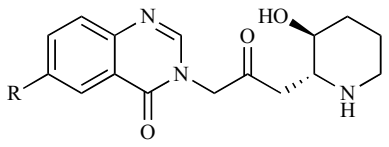
(**6**) Dioncophylline B



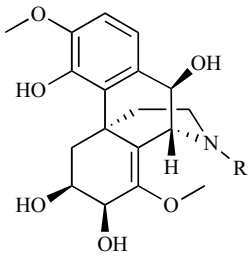
(**7**) Dioncophylline C



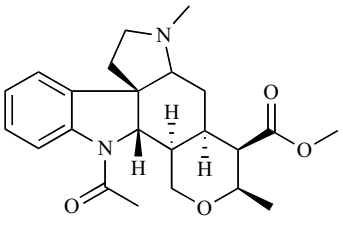
(**8**) Manzamine A



(**9**) R = H Febrifugine
(**10**) R = OH



(**11**) R = H Tazopsine
(**12**) R = cyclopentyl NCP-Tazopsine



(**13**) Malagashanine

Fig. (1). Chemical structures of natural products with anti-malarial potential (**1-13**).

malikalactone against *P. falciparum* strains D6 and W2 with IC_{50} of 3.27 and 8.53 ng/mL versus 3.0 and 3.67 ng/mL, respectively, with up to 28-fold less toxicity [99]. Furthermore, derivatives of bruceolide (**22**) exhibited potent *in vitro* and *in vivo* antiplasmodial activity with an ED_{50} value of 3,15-*O*-diacetylbruceolide (**23**) of 0.46 mg/kg compared to chloroquine with 2.0 mg/kg in infected mice [100].

3.3. Phenolics

Chalcone anti-malarial activity has been of interest since the identification of Licochalcone A (**24**), isolated from Chinese licorice *Glycyrrhiza inflata* that is used in traditional Chinese medicine against different diseases [101]. It was tested successfully against *P. falciparum in vitro* as well as *in vivo* and strategies for synthesis to optimize drug action have been developed [101]. More recently, synthetic new phenylurenyl chalcones were reported to have high anti-malarial activity with the most active compound (**25**) displaying an IC_{50} of 1.76 μM against *P. falciparum* [102]. Further analysis of different chalcones [103, 104] may guide the design of novel and more potent anti-malarial agents.

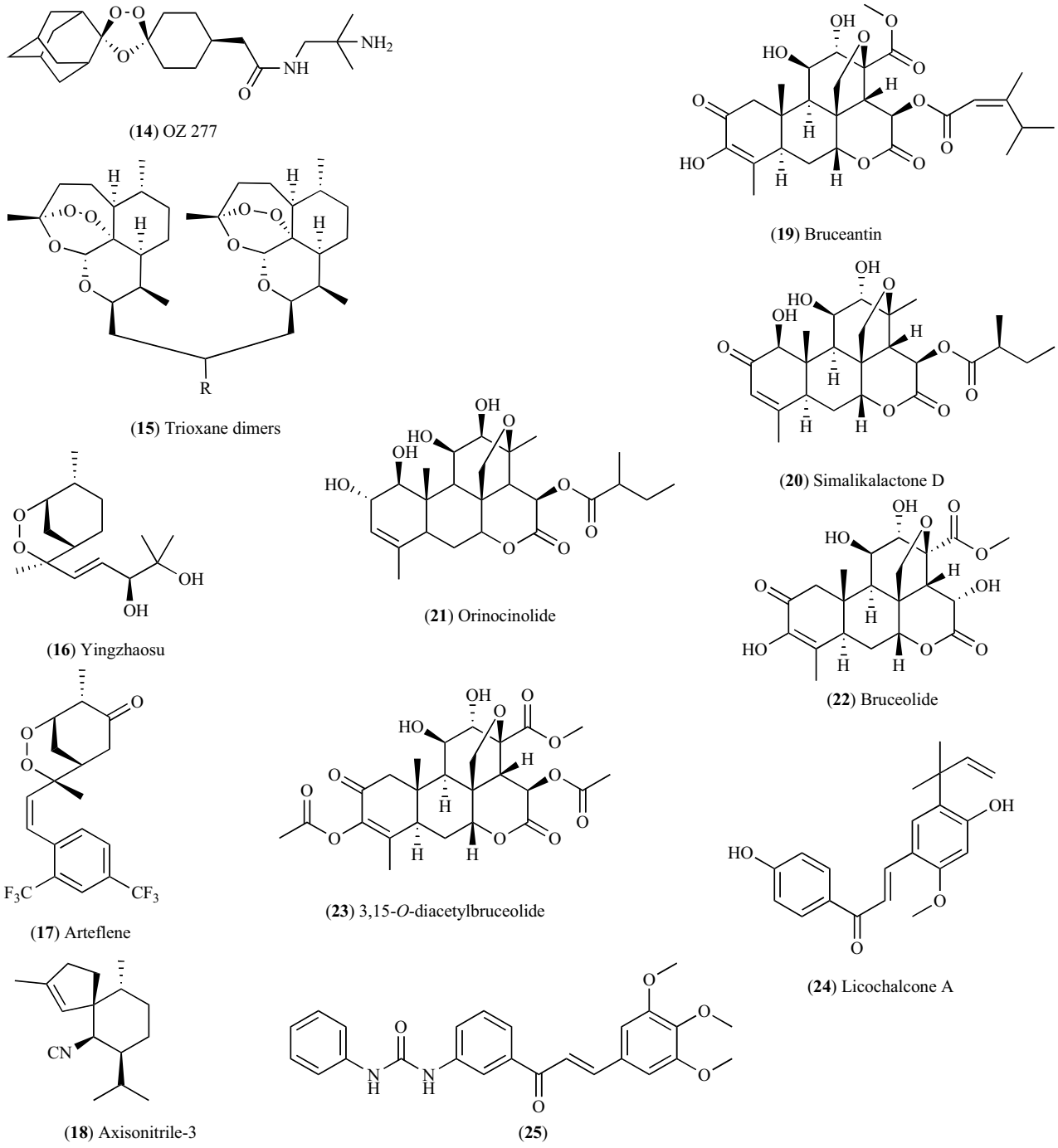


Fig. (2). Chemical structures of natural products with anti-malarial potential (**14**-**25**).

CONCLUSION

The major threat to effective malaria control is the emergence of drug-resistant malaria parasites. The WHO states that there are currently no effective alternatives to artemisinins for the treatment of *P. falciparum* malaria either on the market or towards the end of the development pipeline. Furthermore, the increased malaria control and the support for commodities and health systems in endemic countries, regarding the artemisinin-based combination therapies in particular, leads to a high drug pressure on the parasite that increases the probability of selecting for resistant strains. Ominously, the first genotypes have been found to be associated with the resistance to artemisinins, which underscores the urgent need of new anti-malarials. To this end, several interesting lead compounds have been discovered in the last decades and among these, nature's small molecules continue to play the dominant role. From the rainforest to the deep sea, from exotic microorganisms to plants, used over centuries in traditional medicine, the remarkable chemical diversity of natural products is still the most important weapon in the fight against malaria. However, the mode of action of these compounds is mostly unknown and research focused on this area would be worthwhile, providing a better understanding of the resistance mechanisms as well as inspiring drug development for the future to keep pace with the highly adaptive malaria parasite.

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