Current Status and Progresses Made in Malaria Chemotherapy

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Abstract: Malaria is the most important parasitic disease worldwide, affecting more than 500 million people and causing close to 1 million deaths per annum. This serious fact is mainly attributable to the emergence of drug resistant strains of *Plasmodium falciparum*. The advances made in malaria chemotherapy based on unique aspects of the biochemistry and physiology of the responsible agents for this disease, parasites of *Plasmodium* genus, are covered in this review. Increasing resistance to conventional antimalarial drugs constitutes the main drawback for the persistence of this disease. In the present article, a comprehensive analysis of selected molecular targets is depicted in terms of their potential utility as chemotherapeutic agents. Our review focuses on different and important molecular targets for drug design that include proteases that hydrolyze hemoglobin, protein farnesyltransferase, heme detoxification pathway, polyamine pathways, dihydrofolate reductase, artemisinin-based combination therapies (ACTs), etc. Therefore, rational approaches to control malaria targeting metabolic pathways of malaria parasites which are essential for parasites survival are presented.

INTRODUCTION

Malaria can be considered as one of the deadliest disease around the world, which affects 500 million people causing 2.5 million deaths annually. In addition, it has been estimated that close to two billion individuals are at risk of developing malaria.

The existing chemotherapy is not satisfactory in terms of its lack of effectiveness and also due to the toxicity associated to long-term treatments with empirically discovered drugs. Drug resistance and different strain sensitivity to the available drugs are other drawbacks for the clinically accessible chemotherapy [1]. In fact, the drug discovery costs for the pharmaceutical industry to introduce new compounds into the market have risen dramatically from the last decades. Therefore, in the absence of vaccines, new chemotherapies are needed urgently to help in the prevention and control of this parasitic disease. The main focus of this article is the use of unique features of the biochemistry and physiology of the etiological agent of malaria.

Plasmodium falciparum

Malaria is a hematoprotozoan parasitic disease transmitted to humans by particular species of *Anopheline* mosquitoes. These insects inoculate *Plasmodium* sporozoites *via* a blood feeding process [2]. The genus *Plasmodium* is classified into nine subgenera. The species that infect humans are *P. falciparum*, *P. vivax*, *P. ovale* and *P. malariae*. Malaria caused by *P. falciparum* is the most critical form. The life cycle of *P. falciparum* is divided into three largely stages, mosquito bite, liver and blood stages. Sporozoites leave the salivary ducts to enter the bloodstream, where they circulate for a short period of time before invading the liver. At this stage, parasites form schizonts, which, after hepatocytes rupture, release thousands of merozoites into the bloodstream. Merozoites invade erythrocytes, initiating the asexual stage. Merozoites mature inside the erythrocyte and develop mature trophozoites followed by asexual division to form schizonts, which contain 24–32 merozoites. After schizonts rupture and erythrocytes lysis, merozoites are released into the bloodstream. Symptoms normally arise when schizont rupture takes place because parasite toxins (cytokines) act on the host cells [2–6]. A subpopulation of parasites differentiates into gametocytes, the sexual stages, which infect feeding mosquitoes closing the cycle. The particular mechanisms that trigger the change from asexual to sexual stage development are still unknown [2,7]. There are several features that promote gametocytogenesis, mostly those that impair asexual parasite proliferation [8]. After ingestion by an *Anopheles* mosquito, gametocytes convert into male and female forms. Fusion and meiosis takes place in the stomach to produce a zygote, which goes through the midgut wall and converts into an oocyst. Parasite growth continues by asexual division until the oocyst contains thousands of sporozoites. Once the oocyst rupture takes place, sporozoites move to the salivary glands, where they are able to invade the mammalian host [6–8].

A major problem in the treatment of malaria is the increasing resistance of some strains of *P. falciparum* to chloroquine (**1**), mefloquine (**2**), and the naturally occurring quinine (**3**), which are the commonly used drugs for the treatment of malaria [9,10]. In addition, the naturally occurring sesquiterpene artemisinin (**4**) proved to be a very effective agent to impair parasite levels in severe cases of cerebral malaria. Artemisinin has also been clinically employed in China for the treatment of malaria produced by drugs resistant strains of *P. falciparum* [11]. The source of artemisinin, known in China as qinghaosu, is the plant *Artemisia annua*. This compound, which possesses such an

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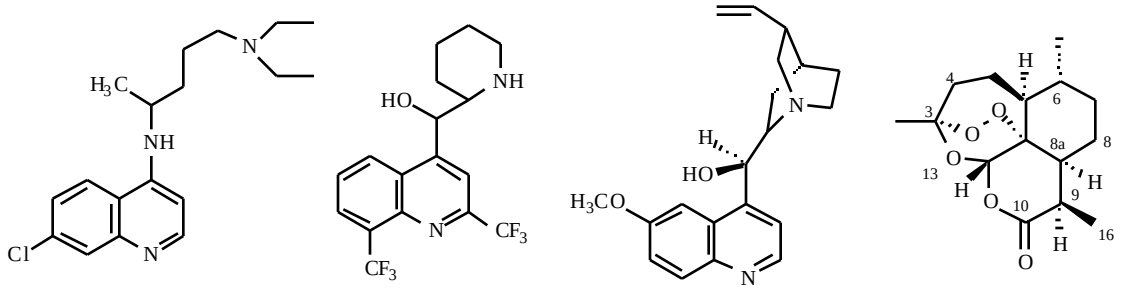


1 **2** **3** **4**

Fig. (1). Existing drugs currently employed for malaria chemotherapy.

interesting chemical structure, suffers from some disadvantages regarding its poor solubility in oil and water, short plasma half-life and a restricted yield from nature. On the basis of artemisinin chemical structure, many derivatives have been prepared, including semisynthetic approaches starting from artemisinin [12]. The recent advances made on chemotherapy based on artemisinin chemical structure will be discussed below (Fig. (**1**)).

MOLECULAR TARGETS

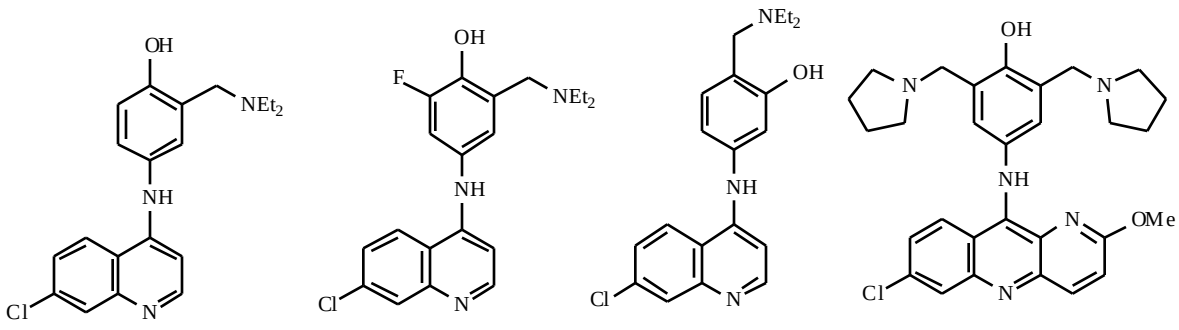
As mentioned previously, malaria is an endemic disease in many parts of the world. Approximately 40% of the world population is at risk of contracting malaria. Close to 500 million individuals suffer from malaria worldwide, of which more than one million die of severe and complicated malaria [13]. A major concern for malaria control is parasite resistance to antimalarial drugs. In addition, mosquitoes resistance to insecticides and the lack of an effective vaccine are other significant obstacles. These reasons justify the continued and considerable efforts in the development of novel structural classes of antimalarial agents.

Basically, three different strategies are under research: (a) the innovative exploitation of old drugs; (b) optimizing existing drugs such as quinolines and artemisinin derivatives; and (c) validation of novel molecular targets based on the knowledge of parasite physiology and biology. It is hope that these approaches might present good prospective in the search for safe and efficient new drugs for the treatment of malaria.

HEME DETOXIFICATION PATHWAY

The distinctive clinical symptoms produced by this disease arise during the intraerythrocytic phase of the parasite life cycle within its host. In the course of this phase, hemoglobin is employed as a major source of amino acids to stimulate the intense parasite growth. When hemoglobin is degraded, the potentially toxic iron-containing prosthetic group heme is released. However, *Plasmodium* parasites develop a particular heme detoxification mechanism where heme is transformed into a dimeric form, which finally gives rise to polymeric non-toxic hemozoin (malaria pigment) through hydrogen bonds between dimeric heme units [14].

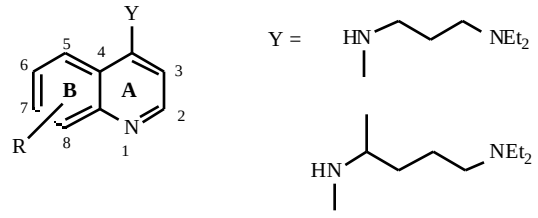
Quinolines derivatives such as chloroquine, amodiaquine (**5**), quinine and mefloquine, are the most commonly used drugs against *P. falciparum*. There is strong evidence to believe that these drugs would act by blocking this detoxification pathway; nevertheless, their efficacy has decreased due to the rapid development of parasite resistance. The mode of action of 4-aminoquinoline derivatives depends on hemoglobin degradation in the parasite food vacuole through binding to hematin, the aqua complex of ferriprotorphyrin IX (Fe(III)PPIX) [5]. These compounds block β-hematin formation, indicating that they would target hemozoin production [5]. Hemozoin formation and hematin degradation are the most likely detoxification mechanisms for these drugs. Amodiaquine is an effective drug against many cloroquine-resistant strains of *P. falciparum*, but it is associated with toxic side effects [15], probably due to the presence of a side chain containing an aminophenol moiety. Therefore, the design and synthesis of fluorine-containing



5 **6** **7** **8**

Fig. (2). Chemical structure of 4-aminoquinoline derivatives.

amodiaquine derivatives resulted in potent antimalarial agents [16], particularly, 5'-fluoroamodiaquine (**6**), which has proven to be a very potent drug in *in vitro* and *in vivo* studies. The IC_{50} values against two resistant *P. falciparum* strains such as K1 and T9-96 were estimated as 42 nM and 37 nM, respectively [16]. A more detailed discussion on fluorine-containing amodiaquine derivatives is described below. However, the synthesis of compound **6** turned out to be extremely expensive. In addition, the influence of different substituents on biological activity for 4-aminoquinolines has been investigated. It was reported that short chain-containing chloroquine analogs, especially those possessing a two-carbons *N*-side-chain like compound **6**, exhibited an extremely potent action against parasite resistant strains at the low nanomolar range in *in vitro* assays [17]. Unfortunately, this compound was not effective in *in vivo* assays. Moreover, these compounds underwent degradation leading to inactive metabolites [18]. For that reason, a new set of structurally related compounds bearing different side chains have been designed and prepared [19]. Some of these compounds were potent antimalarial agents *in vitro* and *in vivo*. It was observed that the presence of the 4-arylamino moiety and the aromatic hydroxyl group were very important for antiparasitic activity. Moreover, isoquine (**7**), which is an isomer of amodiaquine, showed a very potent activity as an antimalarial agent. It is currently under pre-clinical evaluation. Actually, two approaches are being evaluated in humans, which include short chain chloroquine derivatives and pyronaridine (**8**) [20] (Fig. (**2**)).



9, R = 7-chloro, 6-methyl; Y = *N*,*N*-(diethyl)-1,3-diaminopropane

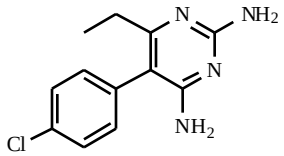
Fig. (3). SAR analysis varying the substituent at the B ring and the basic side chain at C-4 position.

In a recent study, the effect of diverse substitutions at the C-5, C-6, C-7 and C-8 positions of the quinoline ring has been analyzed [21]. Several synthetic chloroquine analogs showed interesting antimalarial activities against both a *P. falciparum* drug-sensitive strain and a highly drug-resistant strain. Chloroquine-containing side chain derivatives (Y = (*N*,*N*-diethyl)-1,4-diaminopentane) did not prove beneficial for biological activity because they exhibited weak potency especially against the drug resistant W2 strain of *P. falciparum.* However, a shorter side chain at C-4 such as Y = (*N*,*N*-diethyl)-1,3-diaminopropane restored some of the parasite resistance observed for chloroquine side chain derivatives [21]. For example, compound **9** (Y = (*N*,*N*-diethyl)-1,3-diaminopropane; R = 7-chloro, 6-methyl) exhibited IC_{50} values of 9.0 nM and 50 nM against 3D7 and W2 strains, respectively [21]. The above data reinforces the hypothesis that the length and nature of the basic side chain has a marked effect on antimalarial activity [21]. (Fig. (**3**)).

In an attempt to develop novel potent antimalarial agents minimizing parasite resistance, Viswanathan *et al*. *via* dynamic simulations, have found a three-point pharmacophore based on the chemical structures of known antimalarial drugs such as chloroquine (**1**), pyronaridine (**8**), pyrimethamine (**10**), quinacrine (**11**), and sulfadoxine (**12**) [22]. This pharmacophore led to 5-substituted amino-2,4-diamino-8-chloropyrimido-[4,5-*b*]quinoline derivatives such as compound **13**, which proved to be an effective drug against *P. berghei*-infected mice at 20 mg/kg (Fig. (**4**)).

Aminobenzenesulfonamides and aminophenol derivatives also showed an important level of protection against infected mice. Among them, compounds **14**–**16** are representative members being curative against *P. berghei* infected mice at a dose of 20 mg/kg [22] (Fig. (**5**)).

Schyf *et al*. designed and synthesized a series of thioacridone derivatives based on acronycine chemical structure (**17**), which possesses anticancer activity acting as a DNA intercalating unit [23]. These thioacridone derivatives have previously shown to have DNA binding interaction [24]. These compounds, which were straightforwardly prepared from *o*-chloro- or *o*-bromobenzoic acid, exhibited



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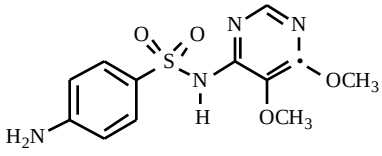

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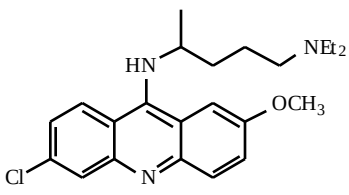

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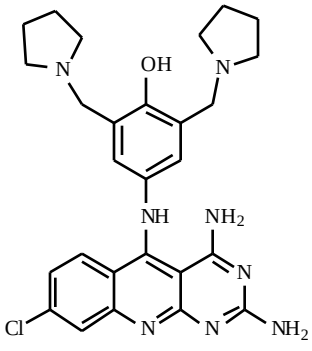

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Fig. (4). Chemical structure of antimalarials structurally related to chloroquine.

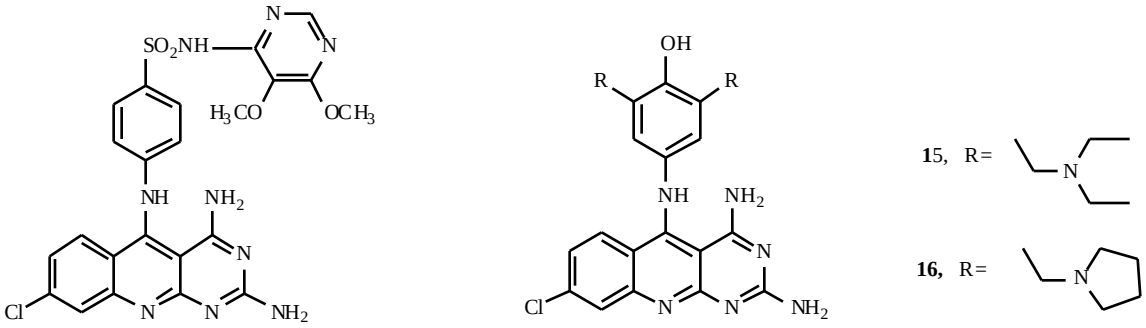


Fig. (5). Aminobenzenesulfonamides and aminophenol derivatives as antimalarial agents.

potent *in vitro* antimalarial activity against a chloroquine sensitive strain (D10) and a chloroquine resistant strain (RSA11) of *P. falciparum*. Compound **18** exhibited an IC_{50} value of 0.4 μg/mL and 1.0 μg/mL against the D10 and RSA11 strains, respectively [25]. These thioacridone compounds were less potent than chloroquine (**1**), which was used as a positive control (IC_{50} = 0.01 μg/mL and 0.16 μg/mL, respectively), but proved to be significantly more potent than doxycycline and proguanil, also used as positive controls. Doxycycline is an effective drug in the treatment of chloroquine-resistant strains [26]. Doxycycline and proguanil have been used for malaria prophylaxis [27] (Fig. (**6**)).

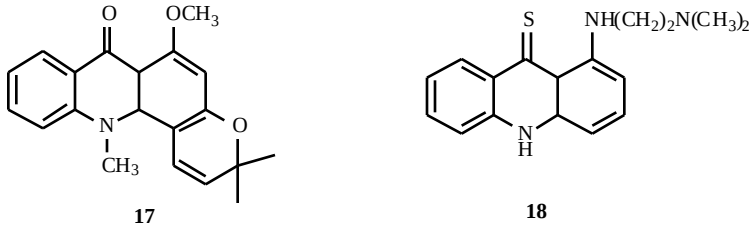
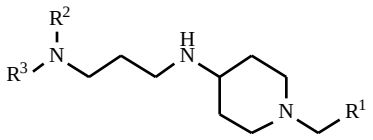


Fig. (6). Thioacridone derivatives as antimalarials.

Aminopiperidine-containing drugs are other class of antimalarial drugs [28]. This type of compounds has a similar basic side chain pattern as found in chloroquine. Compound **19** was a very potent drug against a chloroquine sensitive strain (3D7) of *P. falciparum* at the low nanomolar range (IC_{50} = 30 nM), but was not so efficient against the multi-drug resistant Dd2 strain of the parasite. On the other hand, some structurally related analogs showed promising antimalarial activity [29]. Compounds **20** and **21** were 15-fold (IC_{50} < 60 nM) and 10-fold (IC_{50} = 75 nM) more potent than **19** against *P. falciparum* Dd2 strain, respectively. These compounds exhibited good therapeutic indexes: 78 for **20**, and 100 for **21,** while **18** had a therapeutic index of 11 (Fig. (**7**)).

Simple structural variation on the chloroquine side chain had a profound effect on biological activity leading to significantly more potent drugs than chloroquine itself against *P. falciparum* resistant strains [19,30,31]. N^1-(7-Chloro-4-quinolyl)-1,4-bis(3-aminopropyl)piperazine derivatives are interesting lead compounds for malaria chemotherapy [30]. For example, although compound **22** was able to cure *P. berghei* infected mice, its high level of toxicity against treated mice exceeding 20 mg/kg restricted its further evaluation as a drug candidate but not as a lead drug [30] (Fig. (**8**)).

Therefore, considering **22** as a lead drug, diverse structural variations were considered. The replacement of one of the methylenecyclopropyl moieties by an amide group or a tertiary amine unit provided compounds with a broad range of antimalarial activities. The amine series proved to be more effective than the amide series, in most cases. Compounds **23**, **24** and **25** were the more representative members of this new series of N^1-(7-chloro-4-quinolyl)-1,4-bis(3-aminopropyl)piperazine derivatives exhibiting IC_{50} = 8.5 nM, 9.9 nM and 12.6 nM against FcB1 strain, respectively. Further, these drugs exhibited close to 10-fold and 20-fold more potency than chloroquine. In addition, compounds **23** and **24** presented low cytotoxicity. The introduction of cyclic tertiary amines as can be seen in drug **26** also gave rise to a potent antimalarial agent. In fact, compound **26** was slightly less potent than **25**, but was remarkably less cytotoxic than **24** or **25** [31] (Fig. (**9**)).



19, $R^1 = CHPh_2$; $R^2 = R^3 = Et$

20, $R^1 = CHPh_2$, $R^2 = R^3 = n$-Pr

21, $R^1 = CHPh_2$, $R^2 = Et$, $R^3 = CH_2C_6H_4(4\text{-}C_6H_5)$

Fig. (7). Chemical structure of representative aminopiperidine-containing antimalarials.

As mentioned before, amodiaquine (**5**) has been used for malaria prophylaxis. This drug has the disadvantage of presenting toxic side effects such as hepatotoxicity and

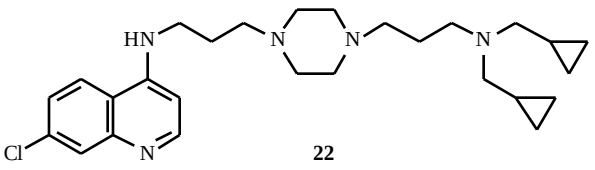


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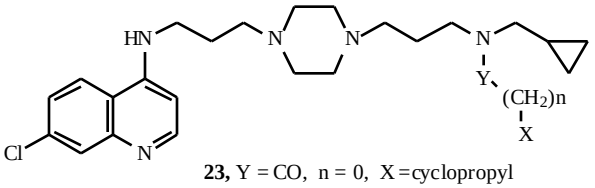
Fig. (8). Chemical structure of N^1-(7-chloro-4-quinolyl)-1,4-bis(3-aminopropyl)piperazine.



23, Y = CO, n = 0, X = cyclopropyl
24, Y = CH_2, n = 3, X = CN
25, Y = CH_2, n = 2, X = NHBoc

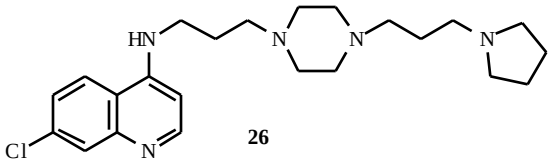


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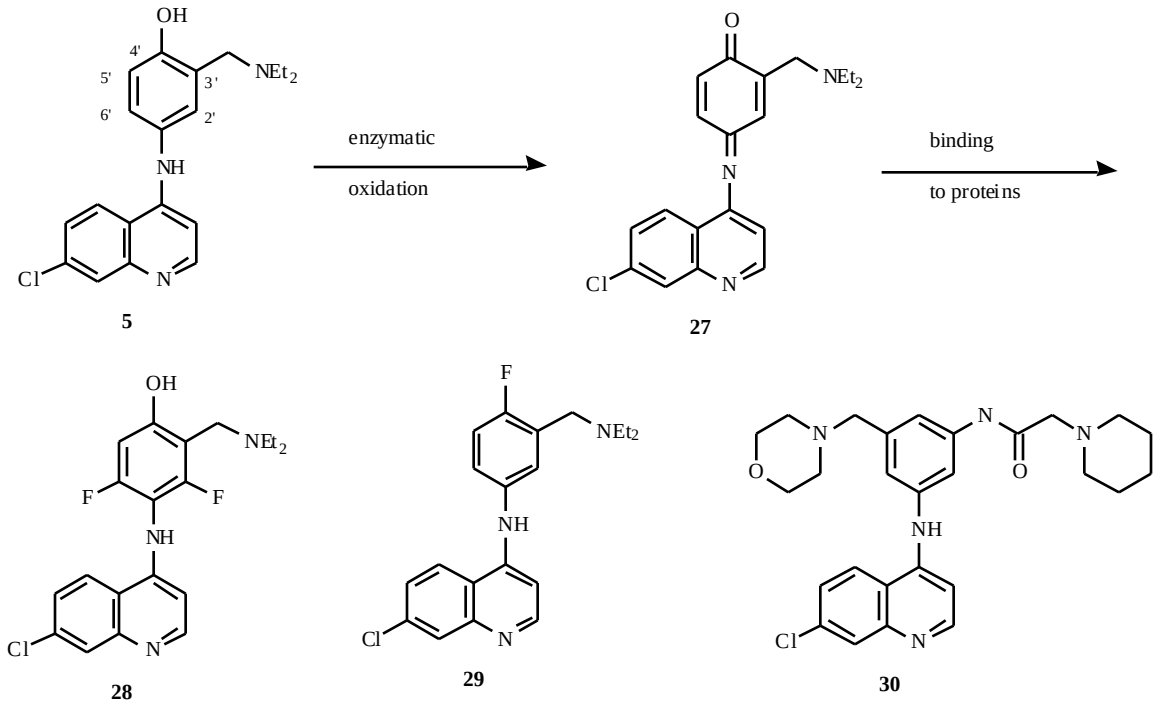
Fig. (9). N^1-(7-chloro-4-quinolyl)-1,4-bis(3-aminopropyl) piperazine derivatives as antimalarials.

agranulocytosis. Toxicity of amodiaquine has been attributed to an enzymatic oxidation of the hydroxyaniline unit to form amodiaquine quinone imine **27**, which would further undergo nucleophilic addition to cellular proteins [34] eliciting hypersensitive reactions [35,36]. This problem was overcome with the substitution of the hydrogen atoms at C-2' and C-6' positions by fluorine in the amodiaquine structure (compound **28**) or by the replacement of the hydroxyl group at C-4' by fluorine (compound **29**). In addition, the introduction of a fluorine atom into the aromatic side chain of amodiaquine has a strong influence on the critical balance between drug activation and bioactivation [16]. Compounds **28** and **29** exhibited similar efficacy to amodiaquine without forming a reactive metabolite of type **27**, responsible for the toxic effect. Both of these drugs acted at the low nanomolar level against chloroquine resistant K1 strain and chloroquine sensitive strain [16] (Fig. (**10**)).

Sergheraert *et al*. have developed a new series of structurally related drugs bearing two proton accepting side chains present at the phenyl substituent of amodiaquine [37]. The main feature of these compounds was the lack of the hydroxyl group at C-4' to avoid oxidative bioactivation. Taking compound **30** as a typical example, this drug exhibited a potent antimalarial action presenting IC_{50} values of 12.8 nM, 9.6 nM, and 12.4 nM against chloroquine-resistance THAI strain, FcB1 strain, and K1 strain, respectively [37]. Moreover, **30** completely cured *P. berghei*-infected mice at a dose of 20 mg/kg [37].

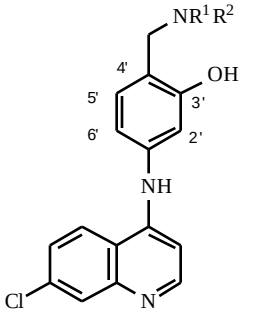
The substitution pattern at the aromatic side chain of isoquine (**7**) rendered this compound unable to undergo oxidation to the respective quinone imine derivative because of isoquine being a regioisomer of amodiaquine where the hydroxyl group and the Mannich side-chain of the amodiaquine chemical structure are interchanged [38]. Isoquine presents excellent prospective as a drug candidate, as its potency is similar to amodiaquine without exhibiting the toxic effects associated to the latter drug, its synthesis is cheap (the title compound is straightforwardly prepared from 3-hydroxyacetanilide and 4,7-dichloroquinoline using a classical synthetic approach) [39], and may be effective for both the treatment and prophylaxis of malaria [38]. X-ray analysis of **7** indicated that there is an intramolecular hydrogen bond between the hydroxyl group and the nitrogen



5 **27** **28** **29** **30**

Fig. (10). Chemical structure of some fluorine-containing amodiaquine derivatives.

atom of the Mannich base. It is worthy to mention the efficacy exhibited by closely related analogs of isoquine as antimalarial agents. For example, compounds **7**, **31**–**34** showed IC_{50} values below 20 nM against chloroquine sensitive strain HB3. In fact, these compounds exhibited IC_{50} values of 12.7 nM, 9.0 nM, 14.8 nM, 19.8 nM, 9.1 nM, and 16.2 nM for drugs **7**, isoquine diphosphate (**7b**), **31**, **32**, **33**, and **34**, respectively, while amodiaquine had a comparable potency (IC_{50} = 9.6 nM, positive control). Moreover, all of these drugs were also potent antimalarial agents against chloroquine resistant K1 strain of *P. falciparum*. In this case, isoquine (**7**) and its corresponding diphosphate (**7b**) showed IC_{50} values of 17.6 nM and 6.0 nM, respectively [38]. Moreover, isoquine diphosphate was also very potent in *in vivo* assays being almost three times more potent than amodiaquine against the murine *P. yoelii* NS strain (EC_{50} = 2.65 mg/kg). In summary, the phosphate salt of isoquine was shown to be superior to amodiaquine as antimalarial agent, it was not metabolized to toxic compounds, more did not it accumulate in animal models. [38] (Fig. (**11**)).



7, $R^1 = R^2 = Et$
31, $R^1 = R^2 = Me$
32, $R^1 = R^2 = n\text{-}Pr$
33, $R^1 = R^2 = -(CH_2)_5-$
34, $R^1 = H, R^2 = Et$

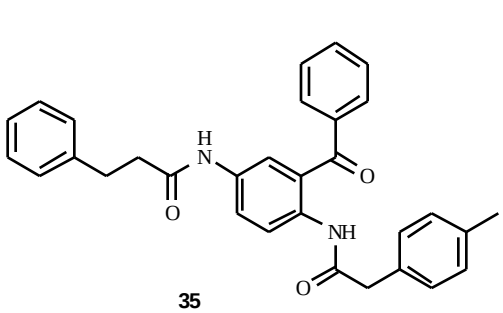
Fig. (11). Chemical structure of isoquine analogs.

PROTEIN FARNESYLTRANSFERASE

Different pathogenic parasites require protein prenylation for survival [40], a process that is responsible for the attachment of farnesyl and geranylgeranyl groups to the *C*-terminal cysteine residues of a number GTPase signaling proteins giving rise to farnesylated and geranylgeranylated proteins. These transfer reactions are catalyzed by at least three different cytoplasmic prenyl protein transferases [41]. These proteins are important signaling molecules involved in crucial cell processes concerning cell transformation and survival [42]. The attached farnesyl and geranylgeranyl groups seem to be crucial for anchoring proteins to membranes and consequently their biological action [41]. Selective inhibition of the enzymatic activity of prenyl protein transferases slows down the proliferation of human tumors due to farnesylation inhibition of oncogenic Ras [43]. On the basis of this result, many prenyl protein transferase inhibitors have been developed, which were not only potential antitumor agents [43,44], but also potent growth inhibitors of *T. cruzi* and *T. brucei* [40,45]. The signal recognition sequence for farnesyltransferase is called a CAAX box, which is constituted by four amino acids at the *C*-terminus where C is cysteine, A is an amino acid with aliphatic side chains and X is often methionine, glutamine or serine [46]. This farnesyltransferase activity has been identified in a number of different pathogenic parasites such as *T. brucei*, *T. cruzi* and *Leishmania mexicana amazonensis* [47]. In addition, an inhibitor of the PFTase activity was also able to inhibit *P. falciparum* growth in human red blood cells exhibiting an IC_{50} = 43 μM [48]. For the above reasons, farnesyltransferase constitutes a valid target for the development of new antimalarial drugs.

Ras is a protein that hydrolyzes GTP and regulates intracellular signal transduction acting as molecular switch between active GTP-bound and inactive GDP-bound state. In over 30% of human cancers, the Ras is mutated and this activity is blocked, leading to uncontrolled cell growth [44]. Once Ras is synthesized, the cysteine residue of the *C*-terminal sequence is farnesylated as a first step in a modification that conducted to the active form. Then, the AAX residues are cleaved and the free cysteine is converted into the corresponding methyl ester. As the farnesylation is fundamental to Ras activity, several peptidomimetics of the CAAX sequence farnesyltransferase inhibitors have been developed, which proved to be tumor growth inhibitors targeting FTase [49,50].

Benzophenone-type non-thiol farnesyltransferase inhibitors have been developed as potential antimalarial drugs. Schlitzer *et al*. discovered by random screening that compound **35** (IC_{50} = 2.7 μM and 3.4 μM against 3D7 and



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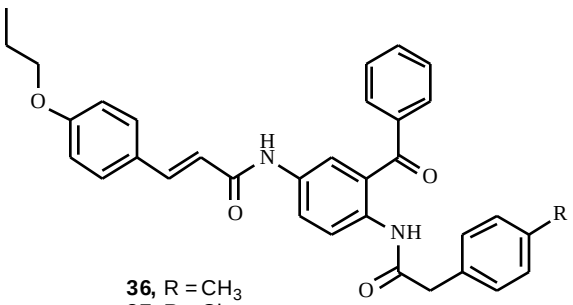


36, $R = CH_3$
37, $R = Cl$
38, $R = Br$
39, $R = CF_3$

Fig. (12). Chemical structure of benzophenone-type non-thiol farnesyltransferase inhibitors.

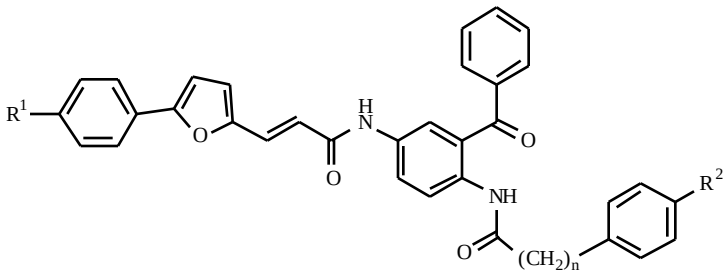
Dd2 strains of *P. falciparum*, respectively) can be considered as a new lead structure for the development of novel antimalarial agents against resistant strains of *P. falciparum* [51]. Structural variations on **35** led to **36**, which was significantly superior to **35** (IC_{50} = 0.2 μM against Dd2 strain of *P. falciparum*) [52]. (Fig. (**12**)).

Structural variations on chemical structure of **35** and **36** led to more potent antimalarial agents. Replacement of the methyl group at the tolylacetyl residue of **36** by chlorine or bromine atoms led to compounds **37** and **38**, which exhibited similar potency to **36** with IC_{50} values of 0.35 μM and 0.31 μM against Dd2 strain of *P. falciparum*, respectively. A comparable efficiency was observed against the resistant strain 3D7 (IC_{50} = 270 μM and 140 μM for **37** and **38**, respectively). Replacement of this methyl group by a trifluoromethyl group to form **39,** resulted in a more efficient drug exhibiting IC_{50} values of 0.12 nM and 0.10 nM against Dd2 and 3D7, respectively [53]. Structural variations on compound **36**, specifically the replacement of the 4-propoxyphenyl unit of the cinnamoyl fragment by a biaryl moiety like a terminal aryl residue and a central 2-furyl ring, gave **40**–**43** as representative drugs [54]. These compounds presented enhanced antimalarial activity compared with reference compound **36** exhibiting IC_{50} values of 88 nM, 85 nM, 84 nM, and 75 nM, respectively, against the intraerythrocytic forms of the Dd2 strain of *P. falciparum* (Fig. (**13**)).

Structural modifications on **43**, in which the isosteric replacement of the methyl group at the tolylacetyl residue by chlorine, bromine or a trifluoromethyl group was envisioned, led to slightly more potent drugs such as **44**–**46** [55] (Fig. (**13**)). These compounds were effective antimalarial agents exhibiting IC_{50} values against chloroquine-resistant *P. falciparum* Dd2 strain of 64 nM, 70 nM, and 47 nM, respectively [55]. Homologation in one carbon at the 2-arylacetylamino residue of **46** afforded the corresponding 2-arylpropionylamino derivatives, from which compound **47** was the main member of this new set of structurally related drugs. Compound **47** showed an IC_{50} = 61 nM against chloroquine-resistant Dd2 strain of *P. falciparum* [56].

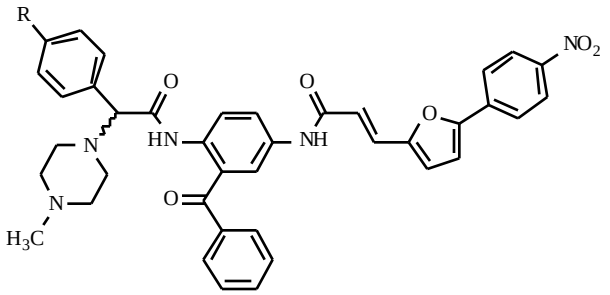
Replacement of the nitro group present in compound **43** by different polar groups that were able to accept hydrogen bonds produced slightly more potent or at least equipotent drugs compared to **43**. Further, representative members of this type of antimalarial agents (compounds **48**–**51**) are illustrated in (Fig. (**13**)). The trifluoromethyl analog **48** and the acetyl **49** presented a similar efficacy as **43** (IC_{50} = 77 nM and 67 nM, respectively), while the methyl and ethylsulfoxide analogs **50** and **51** were observed to be somewhat more potent than **43** against the Dd2 strain of *P. falciparum* (IC_{50} = 37 nM and 60 nM, respectively) [57].

Although many of these compounds were active against a multidrug resistant *P. falciparum* strain in *in vitro* assays presenting IC_{50} values at the low nanomolar range, their behavior was not satisfactory in a murine malaria model, probably due to insufficient solubility in water [58]. The introduction of a methyl piperazinyl moiety at the α-position of the phenyl acetyl fragment bonded to the 2-amino group of the benzophenone template led to compounds, which exhibited improved solubility in water [59]. Although the methylpiperazinyl derivatives **52** and **53** were almost 4-fold less potent than **44** in *in vitro* assays (*P. falciparum* Dd2 strain), they exhibited potent *in vivo* activity against *P. vinckei*-infected mice (EC_{50} = 30 mg/kg and 21 mg/kg, respectively), being the first inhibitors of the enzymatic activity of farnesyl transferase that were active in *in vivo* testing [59] (Fig. (**14**)).



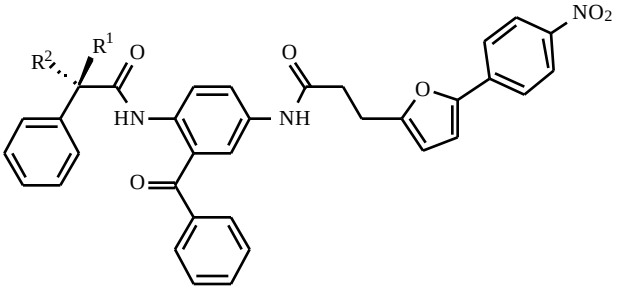
40, $R^1 = CH_2CH_3$, $R^2 = CH_3$, n = 1
41, $R^1 = CHCH_2$, $R^2 = CH_3$, n = 1
42, $R^1 = SCH_3$, $R^2 = CH_3$, n = 1
43, $R^1 = NO_2$, $R^2 = CH_3$, n = 1
44, $R^1 = NO_2$, $R^2 = Cl$, n = 1
45, $R^1 = NO_2$, $R^2 = Br$, n = 1
46, $R^1 = NO_2$, $R^2 = CF_3$, n = 1
47, $R^1 = NO_2$, $R^2 = CF_3$, n = 2
48, $R^1 = CF_3$, $R^2 = CH_3$, n = 1
49, $R^1 = C(O)CH_3$, $R^2 = CH_3$, n = 1
50, $R^1 = S(O)(O)CH_3$, $R^2 = CH_3$, n = 1
51, $R^1 = S(O)(O)CH_2CH_3$, $R^2 = CH_3$, n = 1

Fig. (13). Representative *N*-(3-benzoyl-4-tolylacetylaminophenyl)-3-(5-aryl-2-furyl)acryl amides as antimalarial agents.



52, R = H
53, R = Cl

Fig. (14). Chemical structure of methylpiperazinyl derivatives **52** and **53**.

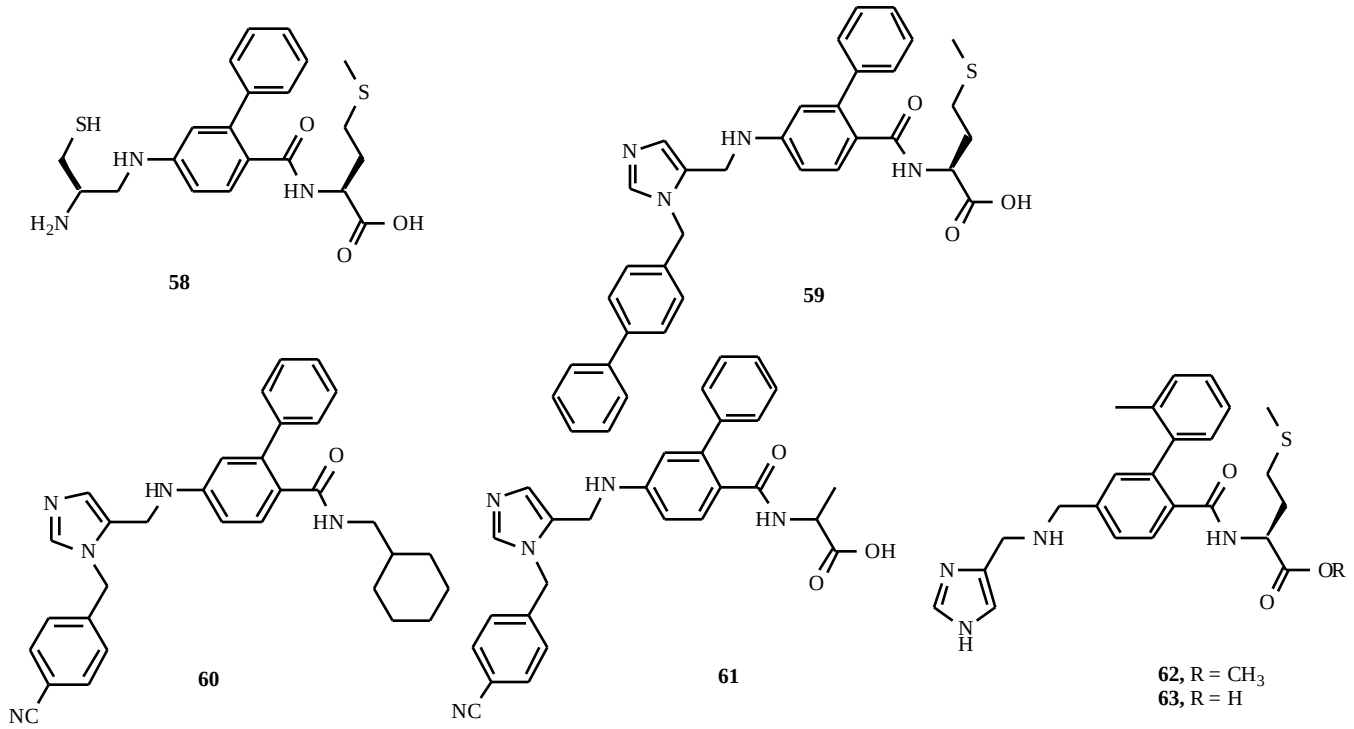


54, R^1 = NHC(O)O^{t}Bu, R^2 = H
55, R^1 = NH2, R^2 = H
56, R^1 = H, R^2 = NHC(O)O^{t}Bu
57, R^1 = H, R^2 = NH_2

Fig. (15). Chemical structure of α-amino phenylacetyl derivatives **54**–**57**.

Replacement of this piperazinyl moiety by an amino group at the α-position of the phenylacetic fragment gave rise to highly soluble compounds in water [60]. However, although some of these drugs were potent inhibitors of the enzymatic activity of farnesyl transferase, they exhibited inadequate action against *P. falciparum* cells due to poor pharmacokinetic properties [60]. Taking drugs **54**–**57** as representative examples, all of these compounds were potent inhibitors towards FTase activity exhibiting IC_{50} values of 8 nM, 46 nM, 10 nM, and 8 nM, respectively. In contrast, drugs **54**–**57** did not prove to be much effective against *P. falciparum* showing IC_{50} values of 0.15 μM, 3.2 μM, 0.23 μM, and 0.56 μM, respectively [60]. The chemical structures of these derivatives are illustrated in (Fig. (**15**)).

Another strategy for the design of non-thiol FTase inhibitors involved analogs structurally related to CAAX tetrapeptidic moiety. Therefore, the central dipeptide AA was changed to a substituted 4-aminobenzoyl group as a hydrophobic and rigid spacer considering compound **58** as a lead structure. Compound **58** had proven to be an effective inhibitor of *Trypanosoma brucei* growth at the low nanomolar range targeting *Tb*FTase (IC_{50} = 1.7 nM) [47]. The replacement of the cysteine residue of **58** by substituted imidazole moieties gave rise to different drugs (compounds **59**–**62** as main members), which presented antimalarial activity at the low micromolar range against the 3D7 strain of *P. falciparum* in red blood cells (ED_{50} =5 μg/mL, 3 μg/mL, 3 μg/mL, and 2 μg/mL, respectively). At a concentration of 20 μM, compound **62** was able to inhibit 99% growth of *P. falciparum*. It is worthy to point out that the free acid **63**, which is a potent inhibitor of mammalian FTase (IC_{50} = 1.0 nM), was devoid of activity against *P. falciparum* [61]. This lack of biological activity may be attributed to weak membrane permeability and not to poor enzymatic activity (Fig. (**16**)).



58 **59** **60** **61**

62, R = CH_3
63, R = H

Fig. (16). Peptidomimetic inhibitors of the enzymatic activity of protein farnesyltransferase.

A series of tetrahydroquinoline-containing protein farnesyltransferase inhibitors proved to be potent antimalarial agents [62]. Taking compounds **64**–**68** as representative examples, these drugs were able to inhibit *P. falciparum* 3D7 strain proliferation at the very low nanomolar level (ED_{50} = 5 nM, 7 nM, 6 nM, 5 nM, and 5 nM, respectively) [62]. These drugs were also effective against other *P. falciparum* strains such as HB3, W2, K1 and Dd2 [62]. This cellular activity correlated quite well with the inhibition of the enzymatic activity towards *P. falciparum* protein farnesyltransferase. Certainly, these drugs showed IC_{50} values of 0.9 nM, 0.6 nM, 0.4 nM, 1.2 nM, and 0.6 nM, respectively, against this protein [62] (Fig. (**17**)).

Compound **64** was further evaluated against *P. berghei*-infected mice. Protein farnesyltransferase from *P. berghei* was very sensitive to drug **64** (IC_{50} = 0.4 nM) [62]. *In vivo* studies indicated that **64** could be able to eradicate *P. berghei* infections. Although the selectivity for *Pf*PFT versus mammalian PFT was poor, no toxicity was observed in clinical trials where patients tolerated different classes of protein farnesyltransferases inhibitors employed as antitumor agents. These results together with the observations of Schlitzer *et al*. [59] validated the use of protein farnesyltransferases as a molecular target for malaria chemotherapy.

CYSTEINE PROTEASES

Cysteine proteases are proteolytic enzymes producing protein hydrolysis through a nucleophilic attack on the carbonyl group of a labile peptidic bond. Plasmodial proteases can be considered as potential molecular targets for an alternate malaria chemotherapy [63]. Proteases play important roles in *P. falciparum* life cycle such as parasite infection and development [63–65]. For example, two homologous serine proteases, subtilisin-like proteases 1 and 2 are involved in schizont rupture and merozoite invasion [66,67]; in addition, cysteine proteases are also associated with rupture/invasion process [68]; moreover, other cysteine proteases such as falcipain-1, -2 and -3 are involved in hemoglobin degradation [65]. The recent achievement of the genome sequence of *P. falciparum* [69] indicated that there exist more than ninety proteases. Cysteine proteases are one of the five clans according to their catalytic mechanism [63,64]. Clans are divided into families according to sequence identities and similarities. Clan CA, family C1 consists of four falcipains, three dipeptidyl peptidases, nine proteins related to the serine-rich antigen and a calpain homolog. Sequence analysis of Clan CD suggested that the members of the C13 and C14 families occurred in *P. falciparum* [63,64]. Clan CE proteases also correspond to *P. falciparum* genome [63,64]. Erythrocytic *P. falciparum* degrades hemoglobin in an acidic food vacuole to supply amino acids for parasite protein biosynthesis. It has been demonstrated that malarial cysteine proteinase not only prevented hydrolysis of globin, but was also involved in hemoglobin denaturation and heme release [70,71]. Further, cysteine, but not aspartic proteases, prevented hemoglobin degradation at an early step. In order to demonstrate this fact, parasites treated with (2*S*,3*S*)-3-(*N*-{(*S*)-1-[*N*-(4-guanidinobutyl)carbamoyl]3-methylbutyl}carbamoyl)oxirane-2-carboxylic acid (E-64; compound **69**) or Z-Phe-Arg-CH_2F, both well-known cysteine protease inhibitors, produced an inhibition of denaturation of hemoglobin tetramer and heme release from globin [70]. E-64 is a natural product, which was isolated from *Aspergillus japonicus* in 1978, being one of the first examples of cysteine protease inhibitors. These results indicated that falcipain had an important role in hemoglobin degradation together with globin hydrolysis [70]. As mentioned before, during the erythrocytic phase of *P. falciparum*, merozoites are released from infected hepatocytes. On host cells invasion, the parasites become enclosed in a parasitophorous vacuole in which they grow, followed by asexual division as trophozoites to produce daughter merozoites, which escape from this vacuole and the erythrocyte [72]. It has been determined that the lysis of the parasitophorous vacuole membrane occurred before the rupture of erythrocyte plasma membrane [72]. Both of these steps could be selectively controlled by the use of protease inhibitors. The former one is inhibited by the known irreversible inhibitor of cysteine proteases E-64; therefore, it was likely that the enzyme involved in parasitophorous vacuole rupture was a cysteine protease. On the other hand, there existed evidence that either a cysteine protease or an aspartic protease would be implicated in erythrocyte plasma

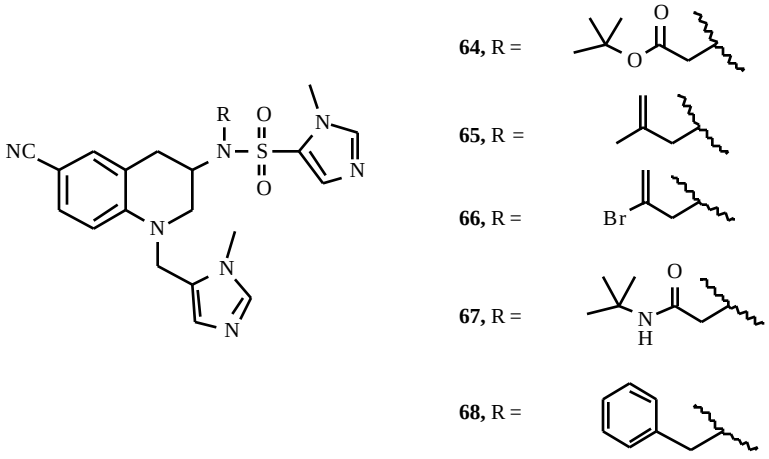


Fig. (17). Chemical structure of tetrahydroquinoline-containing derivatives as inhibitors of the enzymatic activity of farnesyltransferase.

membrane rupture [72]. The above data motivated the search of inhibitors of the cysteine proteases activities as potential antimalarial agents (Fig. (**18**)).

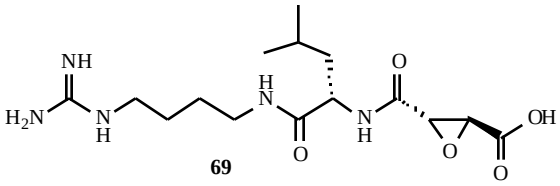


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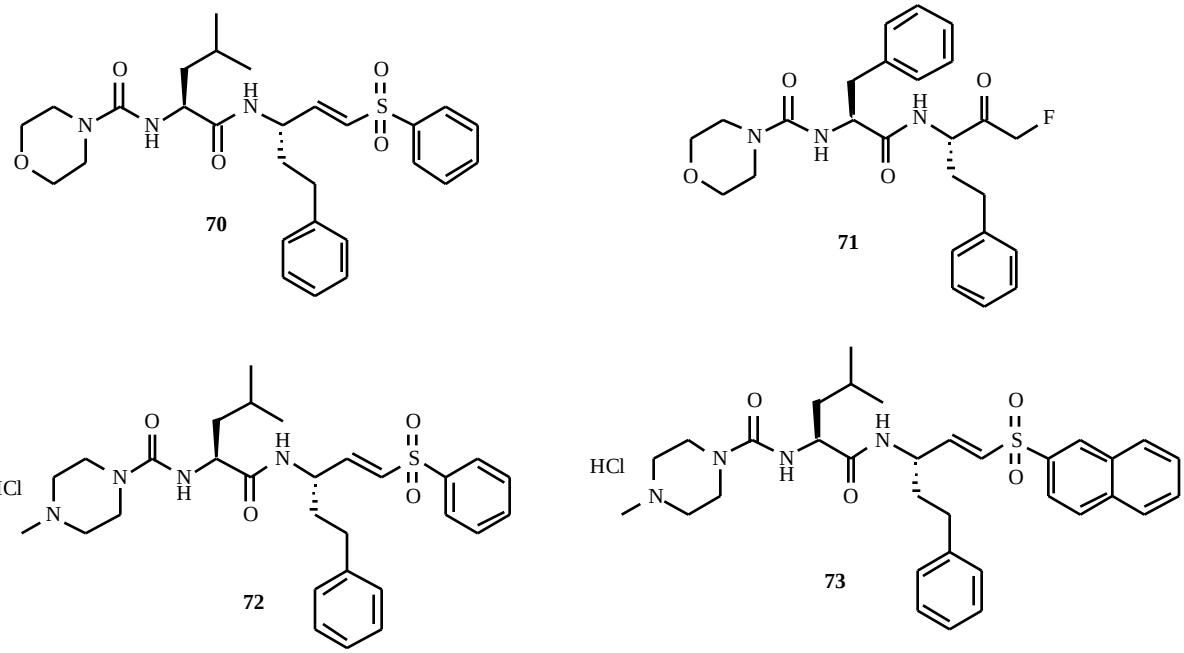
Fig. (18). Chemical structure of the cysteine protease inhibitor E-64.

Falcipains are the best identified and characterized cysteine proteases of *Plasmodium*. These enzymes constitute a family of four papain enzymes. Falcipain-1 was identified in erythrocytic *P. falciparum* as a potent hemoglobinase [73]. Falcipain-2 is a major trophozoite cysteine protease that starts cleavage of native hemoglobin playing an important function in hydrolyzing globin fragments [74]. In addition, falcipain-3 is a second *P. falciparum* hemoglobinase; this cysteine protease is also expressed by trophozoites and is found in the food vacuole [75]. Falcipain-2 and falcipain-3 are the more relevant *P. falciparum* cysteine proteases, which possess a key role in hemoglobin hydrolysis, while falcipain-1 does not seem to be crucial for hemoglobin hydrolysis by erythrocytic parasites [76,77]. Moreover, falcipain-2' has been recently described; this enzyme was found to be very similar in sequence to falcipain-2 [78]. It could be anticipated that inhibitors of falcipain-2 would behave similarly against falcipain-2'. These enzymes require a reducing environment and acidic p*H* values for optimal activity [79].

As a consequence of the importance of cysteine proteases, it would seem to be reasonable that inhibitors of these enzymes constituted new lead structures for drug design. For example, different peptide-based falcipain inhibitors proved to be effective agents against *P. falciparum*. Vinyl sulfone cysteine protease inhibitors proved to be potent inhibitors of falcipain and this inhibitory action was associated with an efficient antimalarial effect [80]. For example, compound **70** (*N*-methyl piperazine urea-L-leucine-L-homophenylalanine-phenyl vinyl sulfone), which had shown to be a potent cysteine protease inhibitor [81], was a very efficient *P. falciparum* and *P. vinckei* falcipain inhibitor (IC_{50} = 3 nM and 200 nM, respectively) [80]. This drug and other closely related compounds inhibited falcipain activity and blocked globin hydrolysis and the development of parasites at the nanomolar range [80]. In a further study, compounds **70**–**73** were evaluated as antimalarial agents targeting parasite cysteine protease [82]. Of special interest were piperazine derivatives where the morpholine urea group present in **70** was replaced by an *N*-methyl piperazine urea moiety to form **72** and **73**. These structural variations were motivated to improve aqueous solubility and bioavailability. Compounds **72** and **73** proved to be potent inhibitors of the enzymatic activity of *P. falciparum* cysteine proteases (IC_{50} = 5 nM and 2 nM, respectively). This enzymatic activity was also observed against the *P. vinckei* enzyme (IC_{50} = 200 nM and 20 nM, respectively). Compound **71** had shown to be an inhibitor of falcipain enzymatic activity and when administered parenterally, had been able to cure malaria in murine models but required repeated dosages [83]. In addition, compound **71**, when administered orally to *P. vinckei*–infected BALB/c mice, showed antimalarial effect, but not complete protection from the disease [82]. All of these compounds were also effective in inhibiting *P. falciparum* development at the very low nanomolar level [82]. Compound **73** significantly slowed down murine malaria development resulted in an almost cure and 40% of *P. vinckei*–infected Swiss Webster mice at a dose of 50 mg/kg twice a day [82] (Fig. (**19**)).

Bearing in mind the antimalarial activity exhibited by peptidyl vinyl sulfone derivatives [82], further studies were



70 **71** **72** **73**

Fig. (19). Chemical structure of representative peptide-based falcipain inhibitors.

conducted to optimize their biological action [84]. In this context, a set of closely related compounds, which included phenyl vinyl sulfones, vinyl *N*-benzyloxy sulfonamides and vinyl phenyl sulfonates, were designed, prepared and biologically evaluated [84]. The potent inhibition exhibited by some of the designed compounds towards falcipain-2 was associated with a strong control of *P. falciparum* growth (W2 strain) [84]. For example, sulfones **74** and **75** were potent inhibitors towards falcipain-2 with IC_{50} values of 6.9 nM and 6.7 nM, respectively, while they also showed IC_{50} values of 3.9 nM and 1.6 nM against parasite development, respectively [84]. Peptidyl sulfonamide derivatives such as **76** and **77** were slightly more potent than peptidyl sulfones **74** and **75**. Certainly, drugs **76** and **77** exhibited IC_{50} = 2.3 nM and 2.2 nM against falcipain-2 activity, respectively. Both of these drugs were very effective against *P. falciparum* showing IC_{50} = 4.4 nM and 1.6 nM, respectively [84]. Finally, the sulfonate esters **78** and **79** were very potent inhibitors against falcipain-2 (IC_{50} = 0.9 nM and 0.7 nM, respectively). This enzymatic activity was also associated with a potent inhibitory action against cultured parasites (IC_{50} = 9.7 nM and 42 nM, respectively) [84] (Fig. (**20**)).

It was also found that other closely related peptides such as peptidyl aldehyde and α-ketoamide derivatives were effective drugs to inhibit the enzymatic activity of falcipain-2 at the very low nanomolar range [85]. For example, **80** showed an IC_{50} value of 2 nM against native falcipain-2, while **81** exhibited an IC_{50} value of 1 nM. Other representative drugs like **82** and **83** were potent inhibitors of falcipain-2 activity exhibiting IC_{50} values of 3 nM and 1 nM, respectively. In addition, inhibition of parasite development by compounds **80**, **81** and **83** correlated quite well with the observed enzymatic activity. In fact, these compounds were potent inhibitors against five different resistant and sensitive strains of *P. falciparum* (W2, ItG, Dd2, D6 and HB3). Taking compound **83** as an example, this drug exhibited IC_{50} values of 1.1 nM, 0.96 nM, and 1.4 nM against *P. falciparum* resistant W2, ItG and Dd2 strains, respectively; while it showed IC_{50} values of 1.9 nM and 2.6 nM against *P. falciparum* sensitive D6 and HB3 strains, respectively [85]. However, compound **83** was not effective in *in vivo* assays against *P. vinckei*-infected mice [85]. Peptidyl aldehydes were prepared according to Nolan *et al*. [86], while peptidyl α-ketoamides were prepared as depicted by Semple *et al*. [87] (Fig. (**21**)).

The availability of homology models of falcipain-2 [88] and falcipain-3 [89] allowed the design of a new class of non-peptide falcipain inhibitors. Based on these studies, substituted isoquinoline derivatives have shown to be inhibitors of the enzymatic activity of falcipain-2 at the low micromolar level [88] as was the case of compounds **84** and

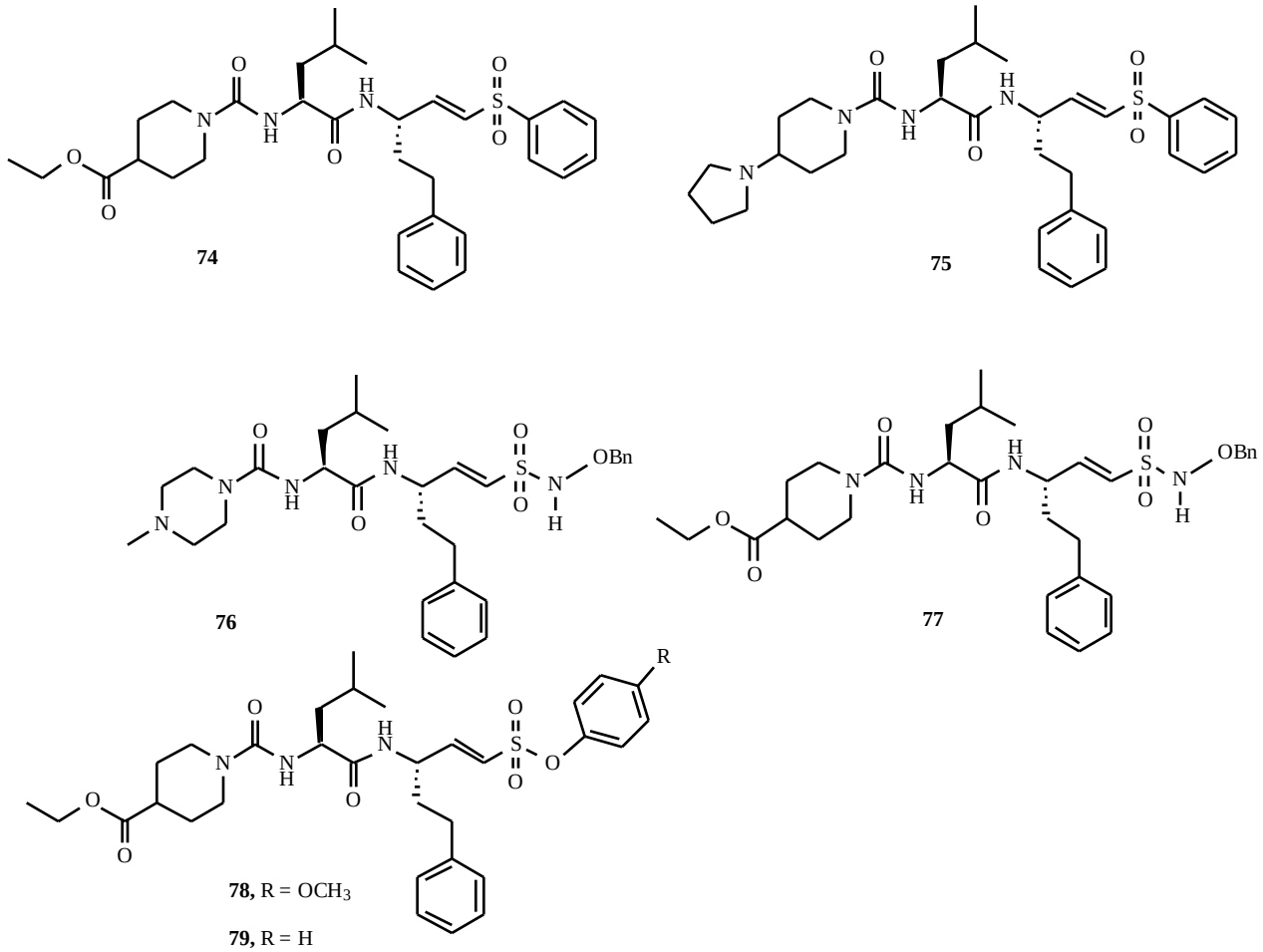


Fig. (20). Chemical structures of representative peptidyl sulfones, sulfonamides and sulfonates as inhibitors of the enzymatic activity of falcipain-2.

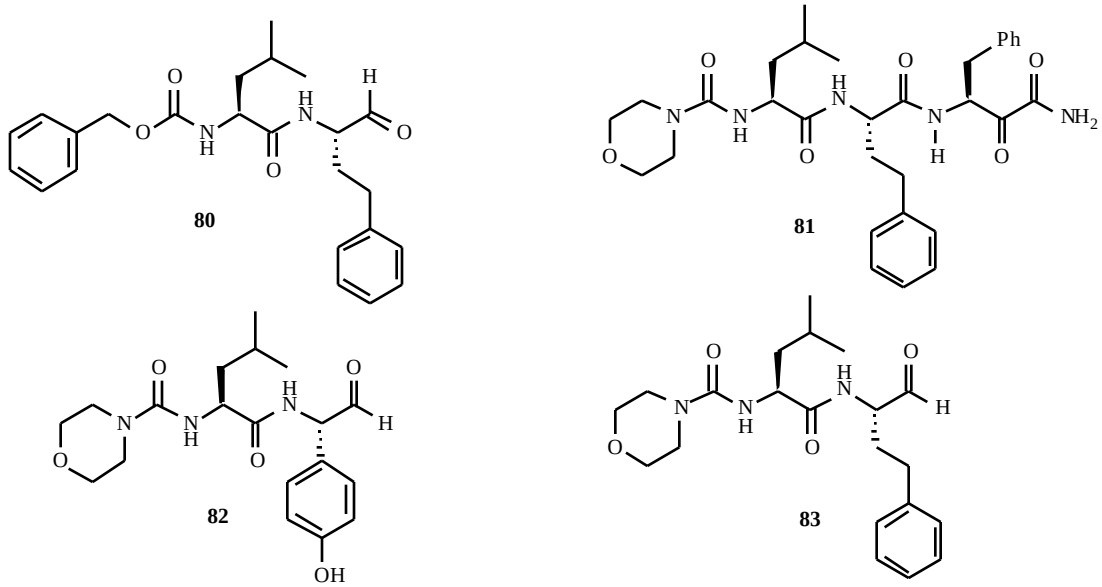


Fig. (21). Chemical structures of peptidyl aldehyde and α-ketoamide derivatives as inhibitors of antimalarials targeting falcipain-2.

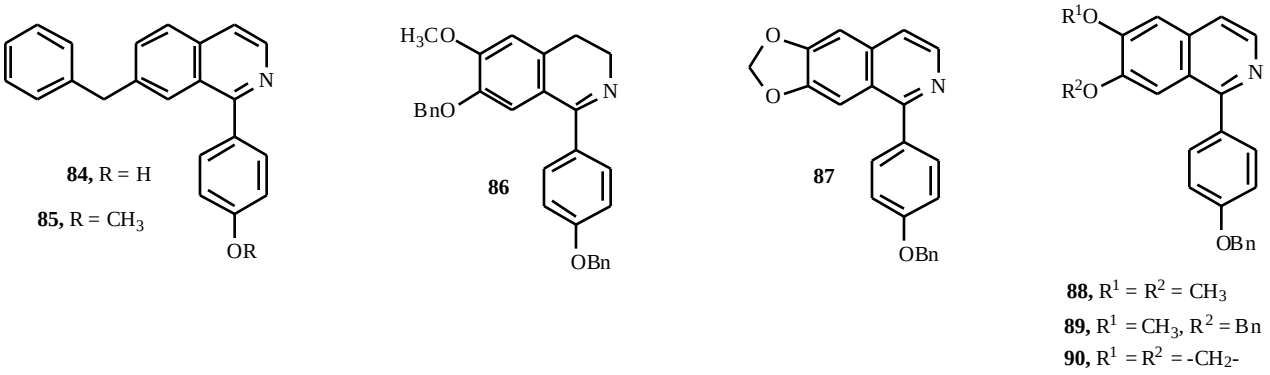


Fig. (22). Chemical structures of isoquinoline derivatives as inhibitors of falcipain-2.

85, which presented an isoquinoline ring as a basic skeleton rather than the peptidic nature of the known peptidyl vinyl sulfone inhibitors. Both compounds inhibited the enzymatic activity of falcipain-2 exhibiting IC_{50} values of 8 μM and 10 μM against this enzyme, respectively [88]. Further studies led to 1,6,7-trisubstituted isoquinoline and dihydroisoquinoline derivatives, which also proved to be inhibitors against *P. falciparum* falcipain-2 [90]. Representative drugs such as **86**–**90** were effective inhibitors at the low micromolar range and even slightly more potent than **84** and **85** (IC_{50} = 4 μM, 3 μM, 3 μM, 3 μM, and 3 μM, respectively) [90] (Fig. (**22**)).

Since the discovery of E-64, a significant number of compounds containing small rings as electrophilic centers such as epoxides, β-lactones, β-lactams, aziridines, thiiranes and cyclopropenones have been reported [91]. Among epoxides, several peptidyl epoxysuccinyl derivatives have been developed as antimalarial agents. Alkylation of cysteine residues should be the mode of action of these compounds. It has been reported that aziridine-2,3-dicarboxylic acid derivatives attached to biotin by a suitable spacer were active against plasmodial falcipain-2. The dibenzylester derivative, **91**, showed a relative potent inhibitory action with an IC_{50} = 11.5 ± 3.3 μM [92] (Fig. (**23**)).

Another example of non-peptidic inhibitors of cysteine proteases is compound **92**, which had shown an IC_{50} value of 6 μM against falcipain [93]. The structure of this drug was further optimized affording new antimalarial agents, from which compound **93** was obtained as a main member exhibiting an IC_{50} value of 0.19 μM [94] (Fig. (**24**)).

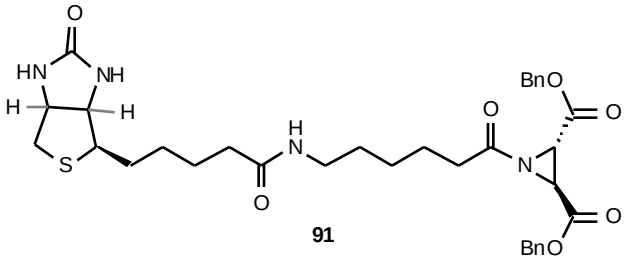
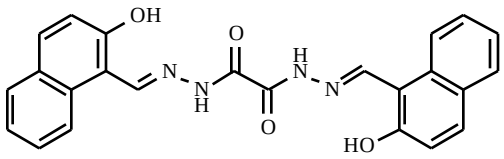


Fig. (23). Chemical structure of 1-{-6-(2-oxohexahydrothieno [3,4-d]imidazol-6-yl)-pentanoylamino]-hexanoyl}-aziridine-2,3-dicarboxylic acid dibenzyl ester.

Peptidomimetic cysteine protease inhibitors have recently been developed based on a 1,4-benzodiazepine scaffold. In this case, a series of arylcarbamic acid 1-[(2-hydroxy-5-oxo-tetrahydrofuran-3(*S*)-ylcarbamoyl)-methyl]-2-oxo-5-phenyl-2,3-dihydro-1*H* benzo[*e*][1,4]diazepin-3(*R*)-yl methyl esters



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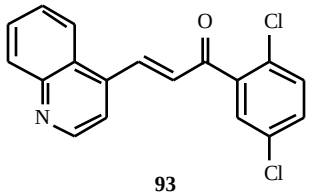

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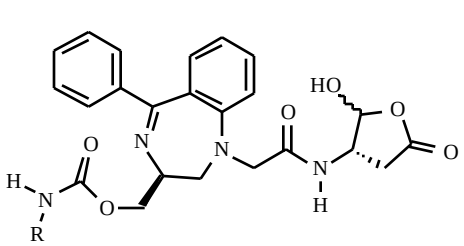
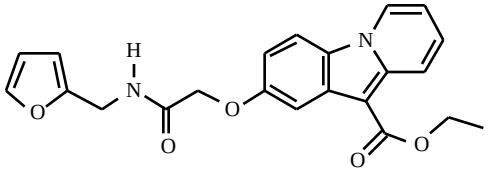
Fig. (24). Chemical structure of non-peptidic inhibitors of cysteine proteases.



94, R = 4-methoxyphenyl

95, R = 4-chloro-2-methylphenyl

96, R = 4-chloro-2-trifluoromethylphenyl



97

Fig. (25). Peptidomimetic inhibitors of falicipain-2 enzymatic activity.

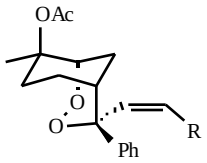
have been designed, synthesized and biologically evaluated against falcipain-2 [95]. Representative members of this new family of drugs were moderately effective against this enzyme. For example, compounds **94**–**96** exhibited IC_{50} values of 8.8 μM, 8.3 μM and 8.7 μM, respectively [95] (Fig. (**25**)).

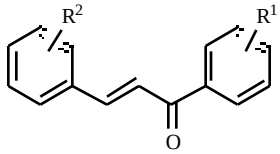
Very recently, the use of virtual screening [96], a technique that examines a significant number of compounds leading to a few number of drugs for biological evaluation, provided 22 non peptidic inhibitors of cysteine proteases from which 18 proved to be moderate inhibitors of falcipain-2 and falcipain-3 [97]. Only one of these compounds **97** exhibited activity against the W2 strain of *P. falciparum* (IC_{50} = 9.5 μM), which was associated with an inhibitory action towards falcipain-2 and falcipain-3 (IC_{50} = 13.8 μM and IC_{50} = 31.2 μM, respectively). Synthetic endoperoxides of type **98**–**100** proved to be potent antimalarial agents [98]. Further, these drugs were found to be potent *in vitro* antimalarial agents showing IC_{50} values of 34 nM, 29 nM and 23 nM, respectively. These compounds would act as prodrugs targeting cysteine proteases through *in situ* formation of the respective chalcone of general formula **101** by rupture of the C-4/C-5 bond. The motivation for the design and synthesis of compounds **98**–**100** was due to the fact that chalcones are considered as multi-target agents. In particular, different chalcones proved to be potent cysteine protease inhibitors [99]; however, the antimalarial activity of some chalcones was attributed to the inhibition of sorbitol transport in *Plasmodium*-infected erythrocytes [100] (Fig. (**26**)).

In summary, cysteine proteases constitute a promising and a valid target for malaria chemotherapy.

ARTEMISININ-BASED CHEMOTHERAPIES

As indicated previously, artemisinin (**4**) has a particular 1,2,4-trioxane moiety in its chemical structure, which exhibits a potent antimalarial action against drug resistant strains of *P. falciparum* and has the ability to quickly lower parasite levels, even in severe cases of cerebral malaria. However, several problems are associated with **4** such as (a) low solubility in oil and water, (b) a short plasma half-life and (c) limited bioavailability. In consequence, the development of new artemisinin derivatives was essential [101]. In this sense, dihydroartemisinin (**102**), obtained by the reduction of artemisinin, led to a group of first-generation analogs such as **103**–**106** (Fig. (**27**)). For example, artemether (**103**) and sodium artesunate (**104**), either alone or in combination with other drugs, are clinically employed in Southeast Asia. However, there are strong evidences that some artemisinin derivatives might be neurotoxic agents in chronically infected rats and dogs [102].





101

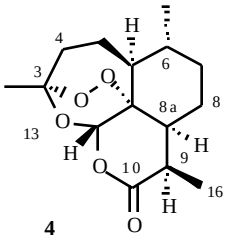
98, R = Ph

99, R = *p*-FC_6H_4

100, R = *p*-ClC_6H_4

Fig. (26). Synthetic endoperoxides as cysteine protease inhibitors.

The mechanism of action of artemisinin and analogs is still not completely understood. It is believed that free radical intermediates are involved. These species are formed by the interaction between the endoperoxide moiety and the heme group. The alkylation of macromolecules would result in parasite death [103]. Another hypothesis is that artemisinin would constitute as a source of hydroperoxide; the heterolytic cleavage of the endoperoxide bridge would produce reactive oxygen species, which would be the actual responsible agents for the antimalarial activity [104]. In fact,



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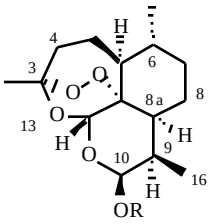
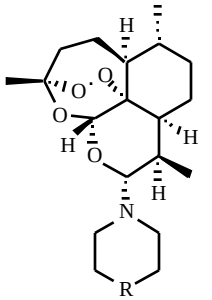


102, R = H,
103, R = CH_3
104, R = $COCH_2CH_2CO_2Na$
105, R = CH_2CH_3
106, R = CH_2-$C_6H_4CO_2H$

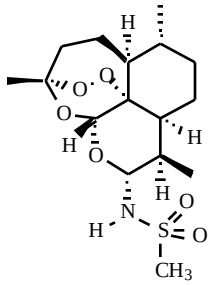
Fig. (27). Chemical structure of dihydroartemisinin analogues.

trioxanes would act as prodrugs, triggered by Fe^{2+}, which would yield a wide range of highly reactive paramagnetic species as well as different electrophiles such as epoxides, aldehydes, etc. This fact justifies why malaria parasites do not develop resistance to artemisinin and other closely related drugs [105−107]. Recently, it has been reported that the molecular target of artemisinin is the sarco/endoplasmic reticulum Ca^{2+}-dependent *P. falciparum* ATPase (*Pf*ATP6), which is a transmembrane enzyme associated with the *P. falciparum* endoplasmic reticulum [108,109]. However, some discrepancies arose about the real targets of artemisinins [110]. A more detailed account on drug metabolism and the mode of action of artemisinin and other closely related compounds has been recently reviewed [111]. Recently, the 3D structure of *Pf*ATP6 has been built by homology modeling and the binding modes of artemisinin were studied by docking simulations [112]. This study indicated that the main binding source of artemisinin to *Pf*ATP6 would be a hydrophobic interaction, which exposed the peroxide bonds to the outside of the binding pocket suggesting that activation of the peroxide bond by Fe^{2+} takes place after binding of artemisinin to *Pf*ATP6 [112].

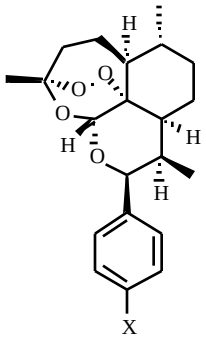
A great effort has been carried out by medicinal chemists to improve the efficacy and stability of artemisinin derivatives. Several C-10 alkyl deoxo and C-10 aryl or heteroaromatic analogs have been designed, prepared and evaluated against *P. falciparum* [20]. It is worth to point out that some artemisinin derivatives, especially dihydroartemisinin, exhibit neurotoxicity according to *in vitro* [113] and *in vivo* studies [114,115]. This fact should be taken into account in the design of closely related compounds. In this context, however, the hemiacetal-type structure should be definitely avoided. Any compound with a higher log P value than artemether, that is lipophilic artemisinins, will probably cause toxic effects. However, neurotoxicity may be attributed to a molecular target [116] rather than just to lypophilicity of the molecule. Haynes *et al.* have prepared new interesting derivatives, in which artemisone (compound **112**) emerged as an excellent drug candidate [117–121]. Compounds **107**–**116** were very potent drugs against either chloroquine-sensitive (D6 or 3D7) or chloroquine resistant (W2 or K1) strains of *P. falciparum* [118]. **107** and **108** were 20-fold more potent than artesunate; however, compounds **109**–**110** proved to be neurotoxic drugs. Artemisone was significantly more potent than artemisinin, chloroquine and pyrimethamine against both drug-resistance and drug-sensitive strains of *P. falciparum* exhibiting IC_{50} values at the very low nanomolar range in all cases [118]. Later, recrudescence was observed on *P. falciparum* FVO isolate infected-aotus monkeys treated with **112** compared to those treated with **104** [118]. Moreover, an important synergistic effect was observed when compound **112** was used together with mefloquine or amodiaquine. Complete cure was accomplished in infected monkeys when treated orally with **112** (10 mg/kg) plus mefloquine (5 mg/kg) or **112** (10 mg/kg) plus amodiaquine (20 mg/kg). In conclusion, the C-10 aminoalkyl derivative **112** has been depicted to be a promising antimalarial agent possessing an enhanced potency over the current artemisinins with minor neurotoxicity [118] (Fig. (**28**)).



107, R = NCH_2Ph
108, R = N-(2-pyrimidine)
109, R = O
110, R = S
111, R = S=O
112, R = SO_2
113, R = SO_2CH_3

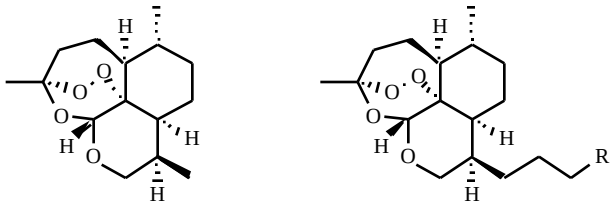


114



115, R = F
116, R = Cl

Fig. (28). Chemical structure of artemisone and closely related drugs.



117

118, R =CH_3
119, R = CF_3
120, R = C_6H_5
121, R = *p*-ClC_6H_4
122, R = *p*-FC_6H_4
123, R = *p*-$MeOC_6H_4$
124, R = 3,4-$Cl_2C_6H_3$
125, R = *m*-ClC_6H_4
126, R = *m*-FC_6H_4
127, R = *m*-$CF_3C_6H_4$
128, R = *p*-$CF_3C_6H_4$
129, R = 3,5-di$CF_3C_6H_3$
130, R = 3,5-diFC_6H_3

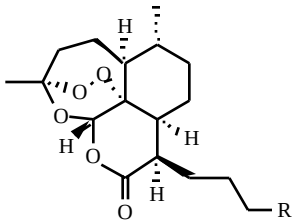
Fig. (29). Chemical structures of 10-deoxoartemisinin and closely related analogues.

There are many interesting chemotherapy strategies based on artemisinin chemical structure. It is worth pointing out the work of Avery *et al*., who used previous quantitative structure-activity relationship (QSAR) studies to design more efficient artemisinin derivatives [11]. Taking 10-deoxoartemisinin (**117**) as the lead drug, several structurally related compounds were designed and synthesized aimed at employing comparative molecular field analysis (CoMFA) techniques to predict the efficacy of new compounds [11]. Representative deoxoartemisinin derivatives are illustrated in (Fig. (**29**)). These compounds were evaluated *in vitro* against four *P. falciparum* strains. Most of the designed drugs were effective at the low nanomolar level. For example, **120** had IC_{50} values of 0.90 ng/mL (D6) and 1.35 ng/mL (W2) against parasite development, while **129** showed IC_{50} values of 0.78 ng/mL (D6), 7.11 ng/mL (W2), and 4.06 ng/mL (K1) and **130** had 0.82 ng/mL (D6), 6.50 ng/mL (W2) and 3.2 ng/mL (K1), respectively. Of the tested compounds, **118** (16-propyl-10-deoxoartemisinin) exhibited an interesting *in vivo* activity against *P. berghei*-infected mice when subcutaneously inoculated (IC_{100} = 8 mg/kg/day), but this compound did not work when it was orally administered. Compounds **120–124** were very effective drugs in *in vivo* assays when administered in peanut oil exhibiting complete protection when they were subcutaneously administered at a dose of 32 mg/kg/day [11].

Substituted phenyl groups at the side chain at C-16 produced potent antimalarial drugs according to *in vitro* and *in vivo* assays. Even their potency was higher than that observed for artemisinin, artemether, arteether and dihydroartemisinin. Compounds **118** and **120–124** did not show neurotoxicity. *In vivo* Peters' 4 day antimalarial testing [122] indicated that drugs **120**, **121** and **125–130** exhibited acceptable oral activities, with **127** and **124** being the most potent and less expensive drug, respectively [11].

Some 16-substituted artemisinin analogs exhibited a dual action as antimalarial drugs and also against leishmaniasis (Fig. (**30**)) [123]. Most of them were more potent than artemisinin against chloroquine-resistant *P. falciparum* strain (W2) and mefloquine-resistant *P. falciparum* strain (D6). For example, **131** exhibited IC_{50} values of 4.7 nM and 1.3 nM against D6 and W2 strains, respectively, while **133** showed IC_{50} values of 7.9 nM and 4.7 nM against D6 and W2 strains, respectively [123].

1,2,4-Trioxane dimers have shown *in vitro* antimalarial and antitumor activities. Recently, a new non-acetal trioxane dimer **134** was designed and synthesized [124]. In addition, the linker between the two 1,2,4-trioxane units was structurally modified to produce different analogues such as **135–137** as indicated in (Fig. (**31**)). These compounds were evaluated against chloroquine-sensitive *P. falciparum* NF. Alcohols **135** and **136** and ketone **137** showed an enhanced potency compared to compound **134**. These compounds were 10-fold more potent than artemisinin possessing IC_{50} values of 0.87 nM, 0.59 nM and 0.91 nM, respectively [124].



131, R = C_6H_5
132, R = CN
133, R = *p*-HOC_6H_4

Fig. (30). Chemical structure of 16-substituted artemisinin analogues.

In addition, several carboxylic acids derivatives of dimeric artemisinin analogs were prepared, motivated by the low water solubility of the corresponding dimeric precursors. The incorporation of polar groups into the molecule facilitated *in vivo* administration. Of special interest were compounds **138** and **139**, which can be considered as derivatives of **135** and **136**. These drugs were also more potent than artemisinin (**4**) having IC_{50} = 2.0 nM and 3.0 nM, respectively (artemisinin IC_{50} = 9.0 nM) [124] (Fig. (**32**)). Moreover, these compounds were more efficient than artenilic acid, when administered intravenously, and sodium artesunate, when administered orally. Compounds **138** and **139** showed ED_{50} values of 2.2 (9.0) mg/kg/day intravenously (orally) and 2.4 (4.8) mg/kg/day intravenously (orally), respectively. Moreover, both of these drugs, at a dose of 10 mg/kg, were able to suppress parasite growth >80% against *P. berghei* NY-infected mice [124]. In addition, **140** showed potent antimalarial activity in *in vitro* and *in vivo* assays [125]. This *N*-oxide dimer exhibited an ED_{50} = 0.56 nM against cultured chloroquine sensitive *P. falciparum* NF54 strain and ED_{50} = 0.8 (mg/kg)/day × 4 (intravenously administered) and 2.0 (mg/kg)/day × 4 (orally administered) [125].

Other interesting examples of orally active, artemisinin-derived trioxane derivatives are the methyl phthalate **141** and its diol derivative **142**, which were shown to be very potent

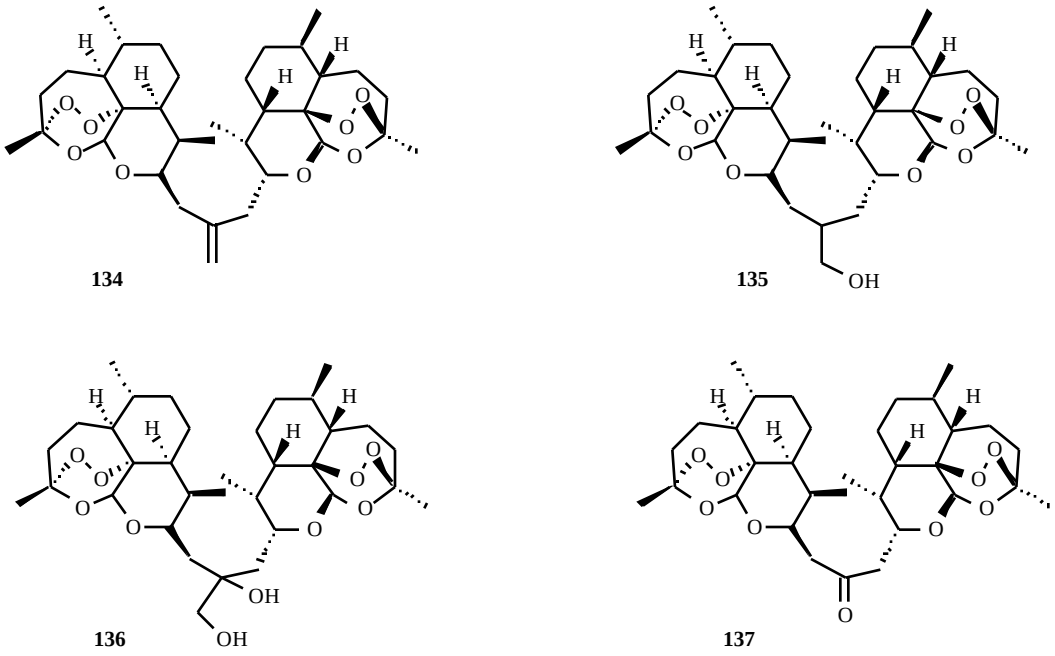


Fig. (31). Chemical structure of dimeric artemisinin analogues.

compounds against chloroquine-sensitive *P. falciparum* (NF 54) parasites with IC_{50} = 1.6 nM and 0.77 nM, respectively [126]. Compound **142** was ten-times more potent than artemisinin. Both of these drugs were also very potent antimalarial agents in *in vivo* assays according to a single administration protocol [127]. Compound **141** exhibited sc ED_{50} = 0.71 mg/kg and **142** sc ED_{50} = 0.06 mg/kg and po ED_{50} = 2.6 mg/kg [126]. The latter compound was almost 40 times more potent than artesunate administered sc under these assays conditions [126] (Fig. (**33**)).

Phosphates **143** and **144** are interesting dimeric antimalarial drugs structurally related to artemisinin. In this case, the C-10 position was also replaced by a methylene group giving rise to *carba* analogs, which were metabolically more stable than acetal models. Therefore, this isosteric replacement afforded more hydrolytic stable compounds, longer half life and less toxicity [128,129]. Phosphate **143** showed an IC_{50} value of 0.2 nM against chloroquine-resistant K1 strain of *P. falciparum*, while **144** presented an IC_{50} value of 0.5 nM, that is, almost 50-fold more potent than artemisinin (IC_{50} = 12.3 nM) and 15-fold more effective than the acetal artemether (Fig. (**34**)). Moreover, these drugs were 400 / 900-fold more potent than chloroquine (IC_{50} = 190 nM). In addition, **143** and **144** were much more potent against chloroquine-sensitive HB3 strain as well (IC_{50} = 0.09 nM and 0.18 nM, respectively) [129].

FATTY ACID BIOSYNTHESIS

Fatty acid biosynthetic pathway of *P. falciparum* also constitutes a valid target for malaria chemotherapy. *Plasmodium* fatty acid synthase (FAS) catalyzes the synthesis of fatty acids in the apicoplast. It is a type II enzyme complex, which is significantly different from human type I FAS. The antibiotic thiolactomycin (**145**), a well-known inhibitor of type II FAS (FAS-II) enzymes, has represented a new lead for the design of new antimalarial agents. The drug inhibits proliferation of cultured *P.*

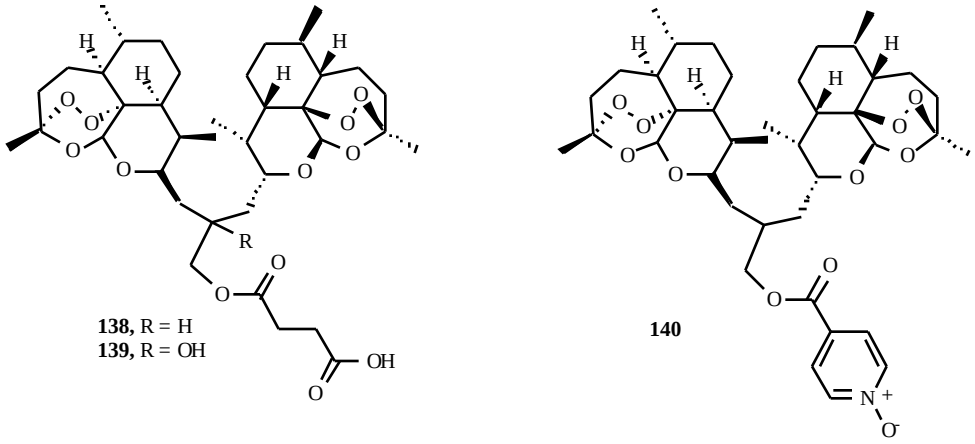
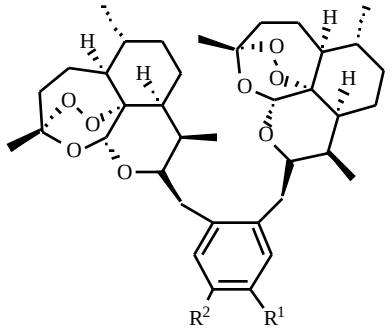


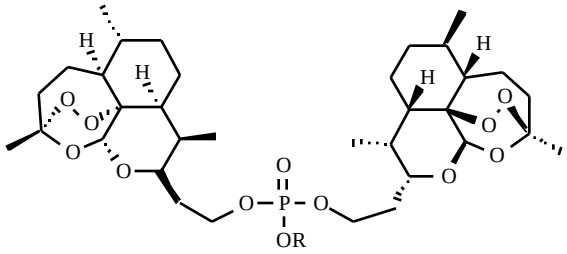
Fig. (32). Chemical structure of carboxylic acids derivatives of dimeric artemisinin analogues.



141, $R^1 = R^1 = CO_2CH_3$
142, $R^1 = R^1 = CH_2OH$

Fig. (33). Chemical structures of dimers **141** and **142** linked by an aromatic moiety.

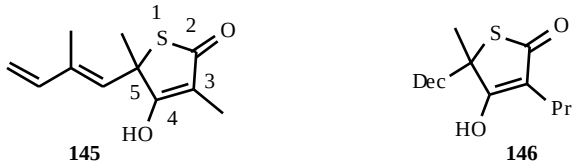
falciparum at the micromolar range (IC_{50} = 50 μM) [130]. In *P. falciparum*, three proteins initiate fatty acid biosynthesis: acyl carrier protein, malonyl-CoA/ACP acyl transferase and type III β-ketoacyl-carrier-protein synthase [131]. Taking **145** as the lead drug, several analogs were designed, synthesized and biologically evaluated against *P. falciparum*. Compound **146** was shown to be the most potent derivative possessing an IC_{50} value of 10 μM against *P. falciparum* cultured in red blood cells, which was14-fold more potent than **145** (IC_{50} = 143 μM) [132] (Fig. (**35**)).



143, R = CH_3
144, R = C_6H_5

Fig. (34). Chemical structure of dimeric phosphate analogues of artemisinin.

In order to improve the biological action against *P. falciparum*, several structural variations on **145** were carried out considering this compound as a lead drug [133]. The isosteric replacement of the heterocyclic sulfur atom by an oxygen atom led to less effective compounds. However, the isosteric replacement of a nitrogen atom instead of an oxygen atom bearing an acetyl group at the C-3 position afforded **147**, which exhibited to be 2-fold more potent than **145** against *P. falciparum* K1 strain (IC_{50} = 63 μM). Substitution at the C-5 position with lypophilic side-chains yielded potent compounds against cultured *P. falciparum*.



145 **146**

Fig. (35). *P. falciparum* fatty acid synthase inhibitors.

For example, compounds **148** and **149** exhibited IC_{50} values of 6 μM and 7 μM, respectively. Alkylation of the hydroxy group at C-4 led to the most potent drug belonging to this type of compounds. For example, compound **150** proved to be 143-fold more potent than **145** against *P. falciparum* K1 strain (IC_{50} = 1.0 μM). However, in this case, a weak correlation was observed between antiparasitic activity and the inhibition of the enzymatic activity of *pf*KASIII; these results suggested that this enzyme would not be the primary target of this family of antimalarial agents [133] (Fig. (**36**)).

Flavonoids display a wide range of biological properties as antiviral, antibacterial, anti-inflammatory, antineoplastic and antiprotozoal agents targeting specific enzymes. It has been reported that the flavonoid glucoside of **151**, luteolin-7-*O*-glucoside, was an antimalarial natural product targeting *P. falciparum* enoyl acyl carrier protein reductase (*Pf*FabI), a crucial enzyme for fatty acid biosynthesis [134]. As the mentioned luteolin derivative showed potent inhibitory action against the enzymatic activity of this enzyme, several flavonoids were investigated and further biologically evaluated against FabG (β-ketoacyl-ACP reductase), FabZ ((β-hydroxyacyl-ACP dehydratase), and FabI, all enzymes of the type-II fatty acid synthase (FAS-II) of *P. falciparum*. Compounds **151**–**155** exhibited inhibitory action against all of these enzymes with IC_{50} values at the low micromolar range [135]. For example, luteolin (**151**) exhibited IC_{50} values of 4 μM, 5 μM and 2 μM towards FabG, FabZ and FabI, respectively; while quercetin (**152**) showed IC_{50} values of 5.4 μM, 1.5 μM and 1.5 μM against the same enzymes respectively [135]. Fisetin, morin and myricetin (compounds **153**–**155**) presented similar enzymatic action to **151** and **152** against these enzymes involved in the FAS-II biosynthetic pathway [135]. Moreover, these drugs were also effective against cultured *P. falciparum*; for example, **153** exhibited IC_{50} values of 8.2 μM, and 6.5 μM against NF54 and K1 strains of *P. falciparum*, respectively [135] (Fig. (**37**)).

Catechin-type flavonoids esterified with gallic acid proved to be potent inhibitors against both the enzymatic activity of FabG, FabZ and FabI and *P. falciparum* development. Cathequines and epicathequines bearing a free hydroxyl group at C-3 were devoid of antiplasmodial activity. The gallates derivatives (**156**-**159**) showed potent inhibitory action towards all of these three enzymes as well as against cultured parasites at the micromolar level [135]. Taking **158** as a representative member, this drug showed IC_{50} values of 1.0 μM, 0.4 μM, 0.3 μM against FabG, FabZ, and FabI, respectively. This enzymatic activity correlated with the cellular activity presented by these compounds against *P. falciparum* with IC_{50} = 3.2 μM (NF54) and 0.4 μM (K1) [135].

In order to determine the corresponding mechanism of inhibition, kinetic studies were carried out considering **151** and **158** as representative compounds. The inhibition of FabG was noncompetitive with respect to the substrate acetoacetyl-CoA and the cofactor NADPH. However, these compounds behaved as competitive inhibitors towards FabZ [135] (Fig. (**38**)).

POLYAMINE PATHWAY

Polyamines, such as putrescine, spermidine and spermine, are involved in numerous cellular processes and

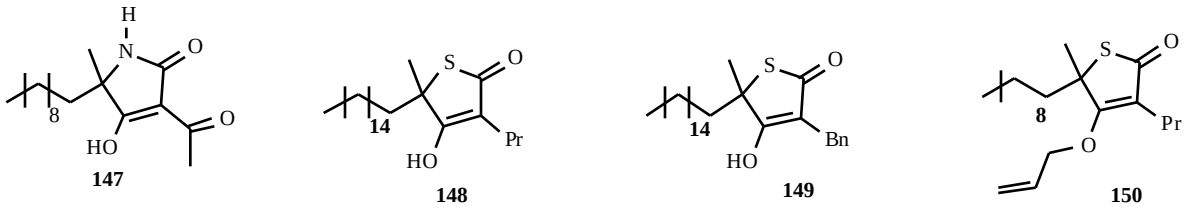
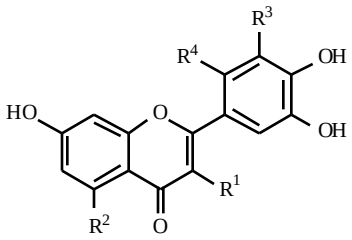


Fig. (36). Further chemical structures of *P. falciparum* fatty acid synthase inhibitors.

are essential metabolites for cell proliferation and differentiation; therefore, the blockade of parasitic biosynthesis of polyamines constitutes a valid chemotherapeutic approach. Human erythrocytes can not biosynthesize polyamines, because they lack the corresponding enzymes to produce them. For that reason, just vestige amounts of polyamines are present within them, but erythrocytes have a polyamine mechanism of transport [136]. During the asexual stage of *P. falciparum*, this parasite induces numerous biochemical, structural and functional variations in the erythrocytes. Especially, the parasite induces a stage-dependent increment in polyamines concentration. Two crucial enzymes, ornithine decarboxylase (ODC) and *S*-adenosylmethionine decarboxylase (AdoMetDC), are encoded by a common gene leading to a unique bifunctional protein with an *N*-terminal ODC and a *C*-terminal AdoMetDC combined by a hinge region [137]. Little attention has been focused on spermidine biosynthesis. The biochemical characterization of *P. falciparum* spermidine synthase has been reported. Unlike the plasmodial enzyme, which also has spermidine as a substrate and is able to form spermine, the mammalian counterpart, has a high specificity for putrescine. It has been shown that *trans*-4-methyl-cyclohexylamine (**160**) was an effective inhibitor of *P. falciparum* spermidine synthase activity (IC_{50} = 1.4 ± 0.1 μM). This enzymatic activity correlated with the antiproliferative action exhibited by **160** against parasites (IC_{50} = 34.2 ± 4.0 μM) [138] (Fig. (**39**)).



151, $R^1 = R^3 = R^4 = H$, $R^2 = OH$
152, $R^1 = R^2 = OH$, $R^3 = R^4 = H$
153, $R^2 = R^3 = R^4 = H$, $R^1 = OH$
154, $R^1 = R^2 = R^4 = OH$, $R^3 = H$
155, $R^1 = R^2 = R^3 = R^4 = OH$

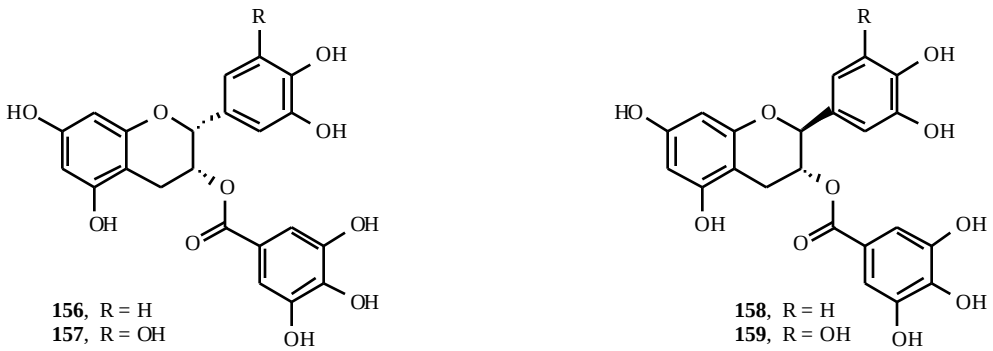
Fig. (37). Chemical structures of antimalarial flavonoids targeting fatty acid biosynthesis.

Taking into account that diverse polyamines derivatives present a broad range of pharmacological actions against several etiological agents of parasitic diseases, a synthesis of diamines derivatives, in this case on solid support, was carried out as possible antimalarials [139]. These drugs were evaluated against *P. falciparum* (D6 and W2 strains) and *L. donovani* (promastigotes). Polyamines **161**–**167** were representative drugs on the basis of the inhibitory action against *P. falciparum* (D6 and W2 strains) and *L. donovani*. For example, **161** showed ED_{50} values of 2.97 μM (D6) and 2.81 μM (W2), **162** exhibited ED_{50} values of 1.07 μM (D6) and 1.68 μM (W2), **163** showed ED_{50} values of 4.43 μM (D6) and 3.79 μM (W2) and **164** showed ED_{50} values of 5.73 μM (D6) and 7.78 μM (W2). Drugs **165**–**167** were also effective antimalarial agents; certainly, **167** was able to inhibit parasite development showing ED_{50} values of 0.68 μM and 2.16 μM against the D6 and W2 strains, respectively [139]. Compounds **161**–**167** were also found to be effective against *L. donovani* (**164**, ED_{50} = 0.37 μM); therefore, as these drugs presented this dual action against both *P. falciparum* and *L. donovani*, it can be anticipated that this family of drugs might be potentially useful as broad-spectrum antiparasitic agents [139] Fig. (**40**)).

THIOREDOXIN SYSTEM

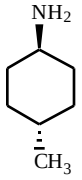
Living cells are exposed to oxidative stress through oxygenated species such as $O_2^{\cdot -}$, H_2O_2, $HO_2^{\cdot}$ and HO . These reactive oxygen-containing species (ROS) constitute harasser agents, which can damage macromolecules present in the cells [140]. Apicomplexan parasites like *Plasmodium* are particularly susceptible to oxidative stress; consequently, the glutathione system and the thioredoxin system are putative targets for drug design. The mechanism of defense against radical species is to trap them by sulfur-containing compounds of low molecular weight.

Thioredoxin reductase catalyzes the reduction of oxidized thioredoxin by NADPH according to the following equation:



156, R = H
157, R = OH
158, R = H
159, R = OH

Fig. (38). Chemical structures of catechins as antimalarial agents targeting FabG, FabZ, and FabI.



160

Fig. (39). Chemical structure of *trans*-4-methyl-cyclohexylamine.

$NADPH + H^+ + TrxS_2 \rightarrow NADP^+ + Trx(SH)_2$. Trx is a M_r 12000 protein containing a redox-active pair of cysteine residues. This enzyme is crucial in ribonucleotide reduction and transcription factor modulation. TrxR belongs to the disulfide reductase family of enzymes, which occurs in the low and high *M*r forms [141]. The *P. falciparum* TrxR is a homodimeric, FAD-dependent oxidoreductase of 55 kDa subunit molecular mass. Cys-88, Cys-93 and His-509 have been characterized as the functional amino acids present at the active site. Unlike the mammalian enzyme, *P. falciparum* TrxR, is a non-selenium dependent enzyme, which can be considered as a valid target for the development of antiparasitic drugs. TrxR presents several essential cellular functions. Besides its important role as a protecting protein against oxidative stress, it supplies reducing equivalents to enzymes such as ribonucleotide reductase and thioredoxin peroxidase (TPx) and also catalyzes the reduction of cysteine residues of other proteins. In contrast to glutathione reductase (GR), which specifically reduces glutathione disulfide, high molecular weight TrxRs has broad substrate specificity. TrxR from *P. falciparum* has been cloned and expressed [142,143] and the TrxR/Trx system has been well characterized. TrxR is a high M_r form [144] similar to the human enzyme. In addition, the sequence around the active cysteine pair is very similar. The distinct aspect is the protein sequence at the *C*-terminal redox center. *h*TrxR contains a cysteine-selenocysteine pair, while *Pf*TrxR has two cysteine residues separated by four amino acids. The *C*-terminal redox-active residues Cys-535' and Cys-540' are in redox communication with the active Cys-88 and Cys-93 pair of the other subunit [145,146].

Unsaturated Mannich bases such compounds **168** and **169** were shown to be inhibitors of the enzymatic activity of *Pf*TrxR [141]. There is strong evidence to believe that unsaturated Mannich bases inactivate TrxR by interacting with the *C*-terminal redox-active cysteine residues of the protein. A probable mechanism for inactivation by **168** would involve an alkylation reaction of the *C*-terminal thiol of Cys-540' as the first step. Further, the residue Hys-509' would catalyze the corresponding deamination followed by alkylation of Cys-535', leading to the formation of a macrocyclic ring between the Mannich base and the thiol groups. The *N*-terminal thiols are not modified. In the case of compound **169**, the first step is similar to that postulated for **168**, but the second step of deamination is not possible due to the absence of α-hydrogen atoms. Based on spectroscopic data, it has been postulated that inhibition would occur through a retro-Michael reaction. The inhibition exerted by **169** can be reversed by the addition of dithiothreitol (Fig. (**41**)).

Nitrophenyl-containing drugs have also been identified as inhibitors of the enzymatic activity of *P. falciparum* TrxR. Benzo[1,2,5]thiadiazole, nitroquinoxaline and nitrophenyl derivatives were found to be efficient inhibitors of the enzymatic activity of *Pf*TrxR. The more representative compounds in this study (**170**–**172**) are illustrated in (Fig. (**42**)). These drugs exhibited IC_{50} values of 2.0 μM, 0.5 μM and 0.5 μM, respectively and also showed good therapeutic indexes compared to human TrxR [147]. In addition, these drugs also acted as inhibitors towards the enzymatic activity of human and *Pf*GR. These results indicated that these inhibitors did not significantly interact neither with the *C*-terminal redox center of TrxR nor with the NADPH binding site [147].

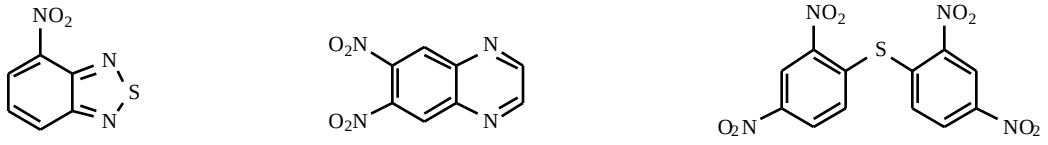
161, n = 1, $R^1 = R^2$ = 3-MeO-4-BnO-benzyl
162, n = 2, $R^1 = R^2$ = 3-MeO-4-BnO-benzyl
163, n = 1, R^1 = 4-benzyloxy-benzyl, R^2 = Bn
164, n = 2, R^1 = 4-benzyloxy-benzyl, R^2 = Bn

165, n = 1, R^1 = H, R^2 = 3-phenyl-propyl
166, n = 1, R^1 = H, R^2 = 4-bromo-benzyl
167, n = 2, R^1 = H, R^2 = 2,3,4,5,6-pentafluoro-benzyl

Fig. (40). Chemical structure of polyamines derivatives.

168 **169**

Fig. (41). Unsaturated Mannich bases that are inhibitors of the enzymatic activity of *Pf*TrxR.



170 **171** **172**

Fig. (42). Benzo[1,2,5]thiadiazole, nitroquinoxaline and nitrophenyl derivatives as inhibitors of the enzymatic activity of *Pf*TrxR.

GLUTATHIONE SYSTEM

As mentioned previously, malaria parasites are particularly susceptibile to oxidative stress due to the absence of antioxidant enzymes catalase and glutathione peroxidase [148,149]. Glutathione system is vital for parasite survival due to be the main defense mechanism against reactive oxygen species. The glutathione system of *P. falciparum* includes NADPH, glutathione reductase and glutathione [150,151]. Glutathione reductase (GR) catalyzes the NADPH-dependent reduction of glutathione disulfide to glutathione according to: GSSG + NADPH + H^+ → 2 GSH + $NADP^+$, and the decrease in GSH activity is proposed as a chemotherapeutic strategy. Glutathione, the thiolate anion GS^-, glutathione disulfide GSSG and *S*-glutathionylated proteins PSSG are the more important glutathione species occurring in trophozoite cytosol [152].

In *P. falciparum* infected red blood cells, the observed oxidative stress, characterized by the detection of O_2^- species [153] and increased levels of lipid peroxidation [149], is initiated from hemoglobin degradation in the acid food vacuole [154]. Hemin is not degraded but taken as hemozoin, which is bound to parasitic glutathione *S*-transferase [155]; on the other hand, the residual heme is the source of reactive oxygen species.

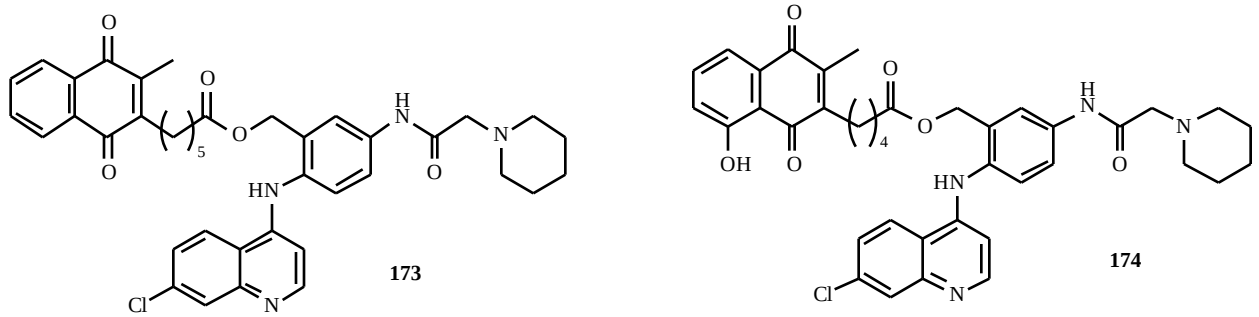
Glutathione system is essential for parasite survival because of its crucial role in regulating the cellular redox equilibrium. In normal erythrocytes, the GSH/GSSG ratio is close to 320. In exposed parasites to oxidative stress, GSSG concentration reached a GSH/GSSG ratio of 28 resulting in toxicity to the cells. In any case, concentration of total glutathione is comparable [149]. Therefore, decreasing GSH level has been proposed as an alternate chemotherapeutic strategy. Together with heme polymerization, glutathione system is another detoxification mechanism by degrading heme [156,157].

In infected cells, the rate of GSSG efflux is significantly increased, maintaining the ratio GSH/GSSG as high as in normal cells [158]. This increment in the efflux of GSSG (40-60 times with respect to normal erythrocytes) is allowed by the changes in the red blood cell plasma membrane permeability [159].

The GSSG/2GSH redox cycle is essential in glutathione metabolism. There are several processes for GSH oxidation, leading to GSSG, which is mainly reduced by the flavoenzyme glutathione reductase, which is rich in trophozoites. Protein thiol glutathionylation is another process that contributes to GSSG reduction [160].

P. falciparum glutathione reductase (*Pf*GR) has been characterized biochemically and kinetically both in its native and in its recombinant forms [161,162]. *Pf*GR shares 45% sequence identity with human GR presenting a comparable general shape [163]. However, both of these enzymes exhibit specific characteristics such as: (a) three major insertions in *Pf*GR; (b) the intersubunit cavity of *Pf*GR is different in size and chemical features from its mammalian counterpart and (c) different pairs of interface helices involved in protein folding and dimerization [162].

Different analogs of quinolines [37], nitrosoureas [164], flavins [165], quinones [166] and methylene blue [167] have been developed as inhibitors of the enzymatic activity of *Pf*GR. Their antimalarial effect was attributed to a decrease in glutathione concentration in *P. falciparum* trophozoite-infected red blood cells. 1,4-Naphthoquinones have also been depicted as GR inhibitors [168]. Synthetic esters derivatives of 1,4-naphthoquinones alcanoic acids giving rise to double-headed drugs presented interesting antimalarial properties. This family of drugs bears two fragments, which individually possess antimalarial activity; one of these fragments is the 1,4-naphthoquinone alcanoic acid unit and the another one is the alcoholic moiety. Compounds **173** and **174** were obtained as the main members of this class of inhibitors of the enzymatic activity of GR. At a concentration of 10 μM, compounds **173** and **174** blocked GSH-dependent heme degradation by 40% and 95%, respectively. In addition, both of these drugs possessed



173 **174**

Fig. (43). 1,4-Naphthoquinone alcanoic acid derivatives as antimalarials.

efficient antimalarial effect acting at the low nanomolar range. For example, **173** presented IC_{50} = 23.1 nM against the chloroquine-resistant FcB1R strain, while chloroquine exhibited IC_{50} = 126 nM (positive control). Compound **173** evaluated against several *Plasmodium* strains with different chloroquine resistance degrees showed similar IC_{50} values against all sensitive and resistant strains (closed to 28 nM). In addition, it was observed a significant level of protection in *P. berghei* infected mice [168] (Fig. (**43**)).

GLUTATHIONE TRANSFERASES

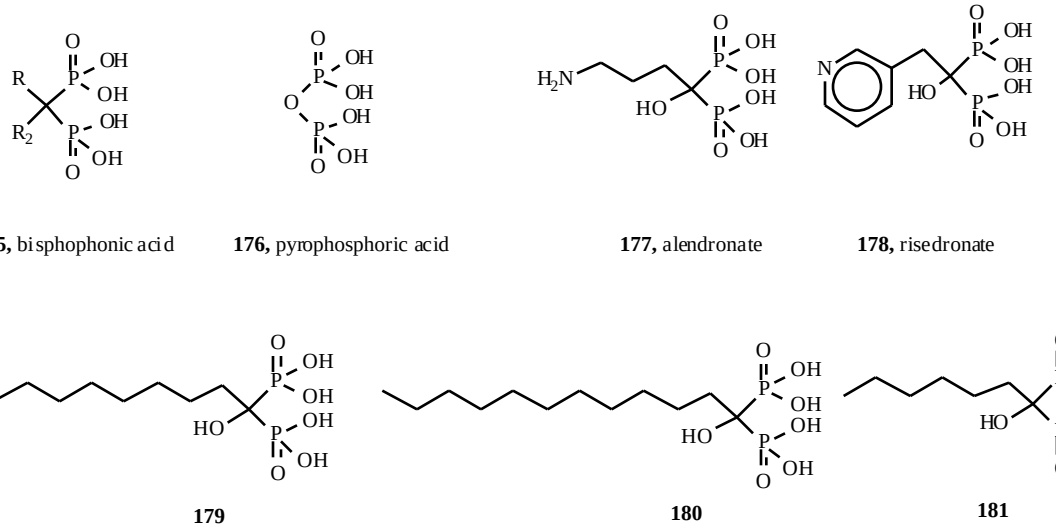
Glutathione transferases (GSTs) catalyze the nucleophilic attack of the sulfur atom present in glutathione on different electrophilic centers. These enzymes act in the intracellular detoxification of several toxic compounds. It has been suggested that GSTs may serve as carrier proteins of certain metabolites [169]. In addition, some GSTs have been shown to detoxify lipid peroxidation products [170]. GST activity has been detected in *P. falciparum*. Interestingly, significant increase in GST activity has been observed in chloroquine-resistant parasites compared to sensitive strains [171].

Glutathione *S*-transferases constitute a potential target for drug design of antitumoral and antiparasitic agents as these enzymes participate in cell protection as a consequence of several electrophilic attacks. As mentioned previously, GSH-dependent heme degradation represents an important intraparasitic pathway for detoxifying heme [9,10]. Recently, a glutathione *S*-transferase from *P. falciparum* (*Pf*GST) has been cloned and expressed [172], which consists of 2 exons. Each subunit has 211 amino acids and a molecular mass of 24.8 kDa. Molecular modeling studies indicated that *Pf*GST presented the typical features of glutathione *S*-transferases [173]. *Pf*GST is a homodimeric that catalyzes glutathione-dependent conjugation of different substrates such as 1-chloro-2,4-dinitrobenzene, ethacrynic acid and *o*-nitrophenyl acetate. Chloroquine is an inhibitor of the enzymatic activity of *Pf*GST at the micromolar range observing accumulation of chloroquine metabolites in the parasite [174]. Cibacron blue, ethacrynic acid and protoporphyrin IX inhibited the enzymatic activity of both *Pf*GST and human placenta GST, but to a lesser extent than chloroquine and even the observed IC_{50} values against *Pf*GST were shown to be higher than those against *h*GST. However, protoporphyrin IX was an exception presenting IC_{50} values of 11.0 μM and > 40 μM against *Pf*GST and *h*GST, respectively [172]. Glutathione *S*-transferase activity did not fluctuate among different chloroquine resistant and chloroquine sensitive strains of *P. falciparum* as observed in extracts from isolated trophozoites. There exists evidence to believe that the inhibitors of the enzymatic activity of GST would have synergistic effect when used together with chloroquine against *P. falciparum* [173,174].

MISCELLANEA

Very recently, the effect of several bisphosphonates against *P. falciparum* has been investigated [175,176]. Bisphosphonic acid derivatives of general formula **175** are compounds structurally related to inorganic pyrophosphate (**176**), in which a methylene group has replaced the oxygen bridge between the phosphorous atoms. Unlike pyrophosphate, geminal phosphonates present greater metabolic stability because they are not recognized by pyrophosphatases and are also stable to hydrolysis under acidic conditions. Some bisphosphonate derivatives are effective inhibitors of bone resorption and are currently being used for the treatment of several bone disorders [177–182]. Bisphosphonates were formerly designed to mimic the chemical structure of pyrophosphate. In addition, different bisphosphonates proved to be antitumor agents [43,44] as well as herbicides [184]. Moreover, the fact that some representative FDA-approved bisphosphonates such as pamidronate (**177**) and alendronate (**178**) inhibited *T. cruzi* proliferation *in vitro* and *in vivo* without toxicity to the host cells [185] encouraged the search for novel bisphosphonates to be used as antiparasitic agents. In this context, a number of bisphosphonates were found to be potent growth inhibitors of several pathogenic trypanosomatids (*T. cruzi*, *T. brucei rhodesiense*, and *L. donovani*) [185–188] and apicomplexan parasites (*Toxoplasma gondii* and *P. falciparum*) [176,189,190]. The molecular target of nitrogen-containing bisphosphonates in osteoclasts [191], plants [192] and *Dictyostelium discoideum* [193] is farnesyl pyrophosphate synthase (FPPS). This enzyme catalyzes the formation of the substrate for protein prenylation [194]. Inhibition of the FPPS enzymatic activity resulted in decreased content of sterols, dolichos and ubiquinones together with the inhibition of protein prenylation. It had been postulated that nitrogen-containing bisphosphonates, particularly those where the nitrogen atom is at the C-3 position, would act as carbocation transition state analogs of isoprenoid diphosphates for isoprenoid biosynthesis [195]. However, some questions have been raised about this assumption because several nitrogen-free bisphosphonates, namely 1-alkyl-1,1-bisphosphonates also target FPPS [186–188]. Of special interest are bisphosphonates derived from fatty acids such as **179** and **180**, which exhibited IC_{50} values of 0.83 μM and 1.07 μM, respectively, against intraerythrocytic growth of *P. falciparum* (3D7 strain) [175]. It is worthy to mention that bisphosphonates derived from fatty acids like **181**, which is a non-nitrogen containing drug, is a potent inhibitor of *T. cruzi* (amastigotes) proliferation (IC_{50} = 18.0 μM) [186,187] (Fig. (**44**)). Taking into account that bisphosphonates derivatives are FDA-approved drugs for long-term treatment of bone disorders low toxicity might be anticipated for new compounds bearing the bisphosphonate moiety. Bearing in mind that the pharmacophore corresponded to the *gem*-phosphonate unit, and on the basis of the potent inhibitory action exhibited by many bisphosphonates, it can be anticipated that bisphosphonates are excellent drug candidates to be used as chemotherapeutic agents against a number of parasitic diseases including malaria.

Phospholipid metabolism is essential for parasite survival and constitutes an interesting target for malaria chemotherapy [196]. This metabolism of infected erythrocytes is an important target due to its specificity and relevance for membrane biogenesis and parasite development [196]. In addition, phopholipid metabolism is not present in normal mature human erythrocytes, while the erythrocyte phopholipid content increases significantly after infection. Phosphatidylcholine constitutes almost 50% of full amount



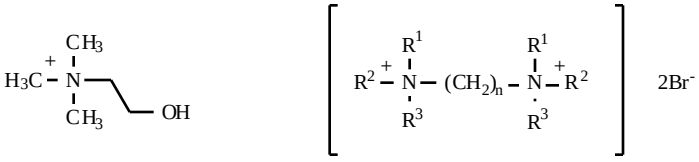
175, bisphophonic acid **176,** pyrophosphoric acid **177,** alendronate **178,** risedronate

179 **180** **181**

Fig. (44). Chemical structure of representative FDA-approved bisphosphonates and bisphosphonates derived from fatty acids.

of phospholipids of infected erythrocytes. Phosphatidylcholine is mostly synthesized from plasma-derived choline by the corresponding parasitic enzymes [196]. In order to design inhibitors of phosphatidylcholine biosynthesis, a classical approach was followed based on the chemical structure of the substrate, that is, choline ((β-hydroxyethyl) trimethylammonium, compound **182**) [197,198]. Some choline derivatives as bisquaternary ammonium salts linked by a long aliphatic chain, used as a hydrophobic spacer, proved to be exceptionally potent against *P. falciparum in vitro*. Taking compounds **183**–**186** as representative drugs, they exhibited IC_{50} values of 4 nM, 0.6 nM, 1.6 nM, and 0.003 nM, respectively (Fig. (**45**)). This parasitic activity was associated with the inhibition of choline uptake and consequently, with phosphatidylcholine biosynthesis by the parasite. This class of compounds does not interfere with other phospholipids biosynthesis [196]. Compound **184** was effective in *in vivo* assays against *P. chabaudi*-infected mice with an EC_{50} value of 0.08 mg/kg [198]. The efficacy of this drug was also observed against *P. falciparum* (FVO strain)-infected *Aotus* monkeys even at high parasitemia without recrudescence employing a very low dose of **184** [198]. For example, at 0.1% parasitemia, a dose of 0.03 mg/kg of **184** parasite clearance was observed on day 4, without recrudescence over 60 days [198].

1-Deoxy-D-xylulose 5-phosphate reductoisomerase (DXR) is a crucial enzyme of the nonmevalonate pathway of isoprenoid biosynthesis, which is not present in humans and is the second enzyme in this mevalonate-independent pathway [199]. This enzyme mediates the conversion of 1-deoxy-D-xylulose 5-phosphate into 2*C*-methyl-D-erithrytol 4-phosphate [199]. It has been found that the antibiotic fosmidomycin (**187**) exhibited a potent inhibitory action against *P. falciparum* targeting 1-deoxy-D-xylulose 5-phosphate reductoisomerase [200]. Conformationally restricted analogs of fosmidomycin also showed potent inhibitory action against the Dd2 and 3D7 strains of *P. falciparum in vitro* [201]. This cellular activity was associated with the inhibition of the enzymatic activity of DXR [201]. For example, conformationally constrained analogs **188** and **189** exhibited IC_{50} values of 0.48 μM (0.32 μM) and 2.0 μM (2.1 μM) against Dd2 (3D7) strain of *P. falciparum* [201]. The efficacy of compounds **188** and **189** was similar to fosmidomycin (positive control), which presented IC_{50} values of 0.48 μM and 0.40 μM against Dd2 and 3D7 strains, respectively [201]. In addition, compounds **188** and **189** inhibited the enzymatic activity of *E. coli* DXR presenting IC_{50} values of 50 nM and 0.313 μM, respectively, while fosmidomycin exhibited an IC_{50} = 48 nM towards recombinant *E. coli* DXR (Fig. (**46**)).

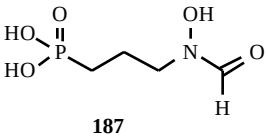


182

183, $R^1 = R^2 = R^3 = CH_3$, n = 16
184, $R^1 = CH_3$, $R^2 = R^3 = -(CH_2)_4-$, n = 16
185, $R^1 = R^2 = R^3 = C_2H_5$, n = 16
186, $R^1 = R^2 = R^3 = C_2H_5$, n = 21

Fig. (45). Chemical structure of bisquaternary ammonium salts structurally related to choline.

There are several different review articles, which describe additional targets and lead structures [202–204].



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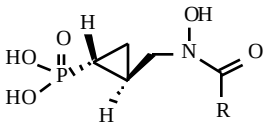

188, R = CH_3
189, R = H

Fig. (46). Chemical structures of fosmidomycin and conformationally constrained analogues.

CONCLUSION

The aim of this review was focused at presenting a broad scope of several important targets for rational design of drugs to control malaria, the deadliest parasitic disease worldwide. In the present study, the progresses made in the design, synthesis and biological evaluation of significant antimalarials were discussed. This disease constitutes at the present time, an important cause of morbidity and mortality mostly in the countries where this disease is endemic. Although there are other potentially valuable targets, only the more representative ones were treated in this article. However, the eradication of this parasitic disease will be possible only with the appropriate knowledge of the biochemistry and physiology of the involved etiological agents of malaria.

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