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Trioxaquines: hybrid molecules for the treatment of malaria.

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ABSTRACT

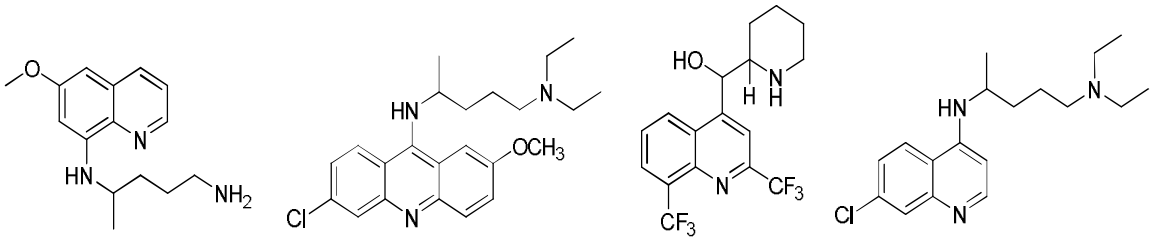
Artemisinin, with its 1,2,4-trioxane as active motif, is now the first-line treatment for multidrug-resistant malaria. The endoperoxide ring is essential for the antimalarial activity of artemisinin. Based on its mechanism of action new hybrid molecules, named trioxaquines with dual mode of action has been designed. Trioxaquines are made by the covalent attachment of a trioxane, having alkylating ability, to a quinoline, known to easily penetrate within infected erythrocytes. This review discusses the importance of various hybrid molecules of artemisinin and 4-aminoquinoline in the treatment of malaria and the evolution of trioxaquine hybrid, as a promising antimalarial drug candidate.

Key words: Antimalarial, Hybrid, 4-aminoquinoline, Artemisinin, Trioxaquine.

Introduction

Malaria is one of the most widespread infectious diseases of our time, particularly in tropic and sub-tropic regions of the world. The disease is caused by protozoan parasites of the genus *Plasmodium*. In humans, it is the four species *P. falciparum, P. vivax, P. malariae* and *P. ovale* that are responsible for the spread of the disease. Among these species, *P. falciparum* is the most fatal. It is estimated that 40% of the worldôs population is exposed to the threat of malaria. According to the WHO, between 300 million and 500 million clinical cases of malaria occur every year. Malaria kills between 1 and 3 million people annually, most of whom are children under the age of 5 and pregnant women. It is estimated that every 40 seconds a child dies from malaria [1-5]. The two antimalarial natural products quinine and artemisinin (quinhaosu) have been proved to be the lead structures for the development of antimalarial agents

Quinine, isolated from cinchona bark, was the first drug used to fight against malaria [6-8]. It served as a template for the development of structurally simpler analogues such as primaquine **(1)**, mepacrine **(2)**, mefloquine **(3)** and chloroquine **(4)** as effective antimalarials. But the emergence of resistance to these most commonly employed 4-aminoquinoline based drugs has limited their use in treatment [9].



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

Artemisinin, isolated from the herb *Artemisia annua*, and its derivatives are currently the drugs of choice for the treatment of malaria caused by multi-drug resistant *Plasmodium falciparum* [10-14]. Artemisinin-based combination therapies are the first choice of combination therapy for the treatment of malaria [15, 16]. However lower abundance and high cost of artemisinin and related products motivate the medicinal chemists to search for new chemical pharmacophores which may prove effective as antimalarials. The peroxide group, present in the form of a 1,2,4-trioxane, is essential for the antimalarial activity of these compounds. Since then, several synthetic 1,2,4-trioxanes have been prepared that have shown good antimalarial activity [17-22].

Designing hybrid drugs, by covalently linking two distinct chemical moieties with multiple effects, is a common strategy in todayôs search for new treatment of malaria. In recent years, various structurally diverse hybrid molecules were reported for the antimalarial activity. Based on the concept of hybrid molecules with a dual mode of action, new antimalarial agents named trioxaquines have been designed [23, 24]. Trioxaquine moiety contain two covalently linked pharmacophores, a 1,2,4-trioxane as in artemisinin (**5**) and a 4-aminoquinoline as in chloroquine (**4**). These molecules have shown promising activity against multi-drug resistant malarial parasites which has been discussed later in this review.

Hybrid molecules: Discovery and Development

The progression of drug development applies rigorous selection criteria and consequently compounds that have reached to market have attained favorable physiochemical properties. Medicinal chemists have made considerable efforts to gear up the sensitivity and selectivity of known targets via more elaborated and rational drug designing [25]. In this concern, nature plays a promising role, a glycopeptide-bleomycin, an efficient anticancer drug, originally isolated as an antibiotic from *Streptomyces verticilus,* is a paradigm for hybrid molecule based chemotherapy [26, 27]. Bleomycin consists structurally and functionally of three different core unit (i) DNA binding unit (ii) Metal binding unit and (iii) Carbohydrate core [28]. This anticancer agent is an outstanding prototype of the hybrid molecules designed by nature (microorganism). Such a hybrid molecule based approach or a bitherapy strategy in malaria was first introduced by Peter et al (1987) [29]. However, earlier attempts for such bitherapy in malaria failed to prevent the emergence of resistance [30]. These failures were attributed either to the drugs used having similar mode of action or resistance already been developed to them. Therefore one of the greatest challenges of future malarial chemotherapy is the development of those compounds which have promising activity against multi-drug resistance (MDR) strain of the pathogen along with high selectivity. Many approaches to antimalarial drug discovery are currently being developed which includes combination therapy [31], modifications of existing drugs [32], assessment of effective agents from natural products especially plant derived [33], ñpiggyback approachò [34] and evaluation of drug-resistance reversers (chemosensitizers) [35]. Recently through rational drug design approach, hybrid molecules emerge as a beneficial tool against infectious diseases, specifically against those which are resistant or possess MDR [36-39].

Considering the different approaches regarding to the development of hybrid molecules [40-44], hybrid molecules are defined as a chemical entity with two (or more than two) structural domains which acts on different/same biological functions or targets via different mode of action.ò Dual activity of hybrid molecule indicates that it acts as two distinct pharmacophores inside the biological system.

Classification of hybrids:

On the basis of types of linkers connecting the two pharmacophores, hybrid molecules can be classified as follows: (Fig.1)

1. Conjugate hybrids, molecules in which both the pharmacophores are joined through a metabolically stable linker which is not a part of either of the individual pharmacophores. The best example of this type is trioxaquine that have ñdual warheadsò [40, 45]. This case has also been recently illustrated by our group, where dihydrofolate reductase inhibitor (triazine or pyrimidine) has been covalently attached to the haloquinoline core of the chloroquine, to develop new hybrid antimalarial agents [46-48].

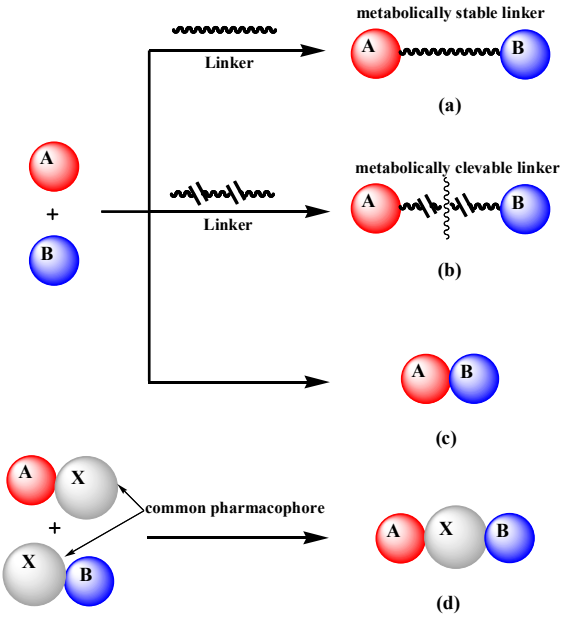


Fig. 1 Different hybridization approaches (a) Conjugate hybrid, (b) Cleavage conjugate hybrid, (c) Fused hybrid, (d) Merged hybrid

2. Cleavage conjugate hybrids, molecules joined through a linker designed to be metabolized inside the biological system to release the two pharmacophoric units that interact independently with different targets [49]. The CF_3-artemisinin derivative, bound to the mefloquine via a diester linker may be a prototype for this kind of hybrid. [30].

3. Fused hybrids, molecules, in which the size of the linker is decreased / removed such that the framework of the pharmacophores is in contact [50].

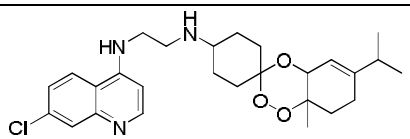
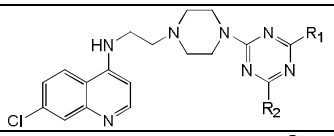
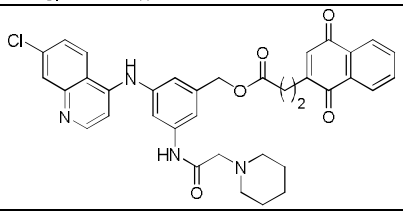
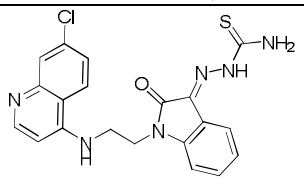
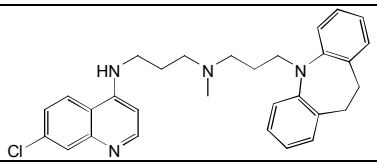
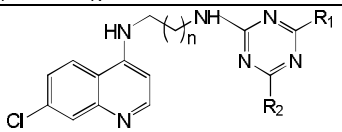
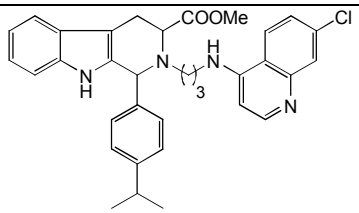
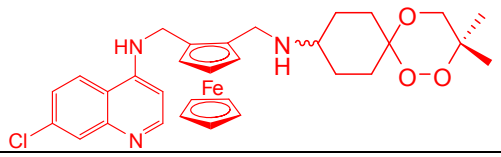
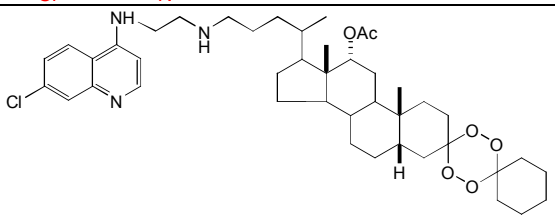
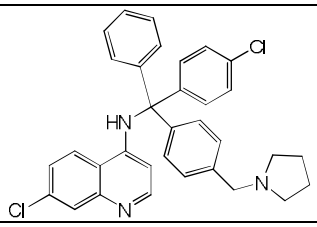
4. Merged hybrids, have their frameworks merged by taking advantage of the common pharmacophore in the structures of the starting compounds, which give rise to smaller and simpler molecules [50]. Dual-function acridones refer to molecule designed by taking the commonilities of the 4-aminoquinoline and chemosensitizers 9-acridone [51].

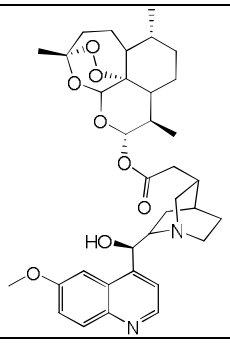
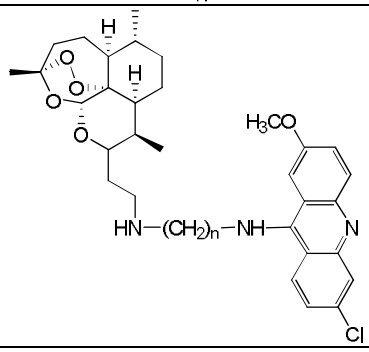
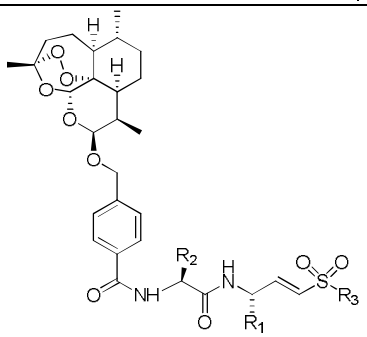
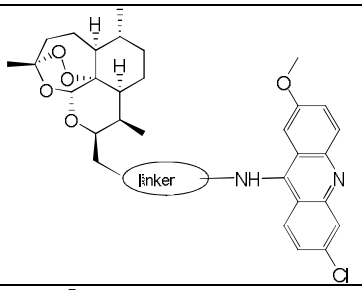
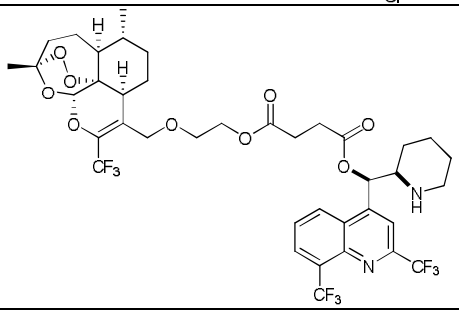
Hybrid Antimalarials:

Due to its advanced mode of action and high selectivity, the hybrid molecule based chemotherapy emerges as a beneficial tool in contemporary trend of antimalarial drug discovery. Several developments have given a new impetus to antimalarial chemotherapy and have demonstrated that approach to synthesize hybrid molecule is feasible. Here we limit our discussion to 4-aminoquinoline and artemisinin hybrids i.e. Trioxaquines. Few of the hybrid molecules and their pattern of linkage are briefly discussed in tabulated form (Table-1).

Table-1: Hybrid molecules in malaria based on 4-Aminoquinoline and Artemisinin

S. No.	Classification	Prototype Structures	Target of distinct Pharmacophores	Ref. No.

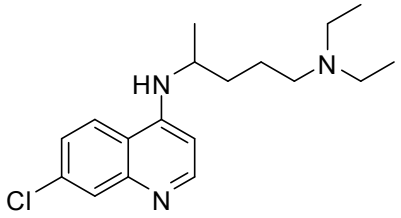
1.	Aminoquinoline-trioxane hybrid		Targeting Heme polymerization	40, 52
2.	Aminoquinoline-triazine hybrid		Targeting Heme polymerization and DHFR	49
3.	Aminoquinoline-naphthoquinone hybrid		Targeting Heme polymerization and glutathione reductase	53
4.	Aminoquinoline-thiosemicarbazone hybrid		Targeting Heme polymerization and cysteine proteases	54
5.	Aminoquinoline-imipramine hybrid		Targeting Heme polymerization and PfCRT protein	55
6.	Aminoquinoline-triazine hybrid		Targeting Heme polymerization and DHFR	48
7.	Aminoquinoline-beta- carboline hybrid		Targeting Heme polymerization and DNA synthesis	56
8.	Aminoquinoline-ferrocene trioxane hybrid		Targeting Heme polymerization	57
9	Aminoquinoline-tetraoxane hybrid		Targeting heme polymerisation	58
10.	Quinoline-clotrimazole hybrid		Targeting Heme polymerization	59

11.	Artemisinin-quinine hybrid		Targeting Heme polymerization	60
12	Artemisinin-acridine hybrid		Targeting Heme polymerization	61
13	Artemisinin-vinyl sulfone hybrid		Targeting Heme polymerization and cysteine proteases	62
14	Artemisinin-acridine hybrid		Targeting Heme polymerization	63
15	Artemisinin-mefloquine hybrid		Targeting Heme polymerization	30

Development of Trioxaquines: Designing and Rational Selection

4-Aminoquinolines as antimalarials:

In ancient time malaria was cured by the powdered bark of the cinchona tree, whose constituents were later characterized as quinine and quinidine (diastereomer), the first medicine used against malaria [64]. The characterization of the quinine gives a platform for the development of synthetic antimalarials. Consequently, in 1934 Hans Andersag from Bayer Company discovered Chloroquine (CQ), related to quinine, as the first synthetic antimalarial drug, produced on industrial scale [65].



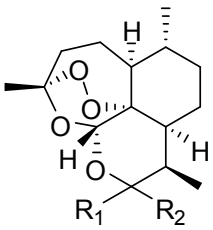
4

Chloroquine is a safe, affordable and a relatively well-tolerated drug [66]. Chloroquine acts by binding to heme molecule, released from hemoglobin that is digested by malaria parasites via successive action of various proteolytic enzymes, the aspartate proteases plasmepsins Iï IV, followed by falcipains (cysteine proteases) and the metallo proteases falcilysine [67]. The resulting small peptides and possibly free amino acids are transported across the vacuole membrane into the cytoplasm, leaving the heme behind, as residue. Chloroquine binds with this residual heme and oxidizes the central Fe (II) atom of heme to ferriprotoporphyrin IX (FPIX or FPPIX). Quinoline-based antimalarial drugs kill the malarial parasites by inhibiting the polymerization of ferriprotoporphyrin IX (FP) into hemozoin (HZ). However the PfCRT (plasmodium falciparum chloroquine resistance transporter) associated efflux of the chloroquine is the major problem in present day chemotherapy [68-70]. The PfCRT protein localizes into the digestive vacuole membrane and contains 10 putative transmembrane domains. Evidences show, that the amino acid substitutions in PfCRT (including a key Lys^{76} Ÿ Thr change) determine the chloroquine resistant (CQR) phenotype of *P. falciparum* in all malarious regions where it occurs [71]. Despite the worldwide resistance to chloroquine, there is still a significant potential to discover new quinoline antimalarials based on chloroquine.

Artemisinin and artemisinin derivatives as antimalarials:

Among the molecules that emerged over the past three decades as antimalarial drugs, artemisinin **(5)** has a unique skeletal arrangement. This sesquiterpene (from decoctions of leaves of *Artemisia annua*) has been used in traditional Chinese medicine for the treatment of fever for more than 2000 years [72, 73]. Artemether **(6)** and arteether **(7)**, the semisynthetic first-generation analogues of artemisinin, are more potent than artemisinin but have certain side effects. This naturally occurring endoperoxide, has been shown to possess good antimalarial activity against drug-resistant strains of *P. falciparum* [74, 72, 75]. Chemically, artemisinin is a sesquiterpene lactone having an endoperoxide bridge, which appears to be essential for its antimalarial activity [76]. An artemisinin derivative lacking the endoperoxide bridge (deoxyartemisinin) is devoid of antimalarial

activity, which shows that the peroxide functionality is essential for the pharmacological activity of these trioxanes [77].



Artemisinin (5) : R_1=R_2=OH
Artemether (6) :R_1=H, R_2 =β-OCH_3
Artemether (7) :R_1=H, R_2 =β-OC_2H_5
Artesunic acid (8) :R_1=H, R_2=α-$OCO(CH_2)_2$-COOH

Iron-mediated cleavage of artemisinin endoperoxide bridge was first proposed by the Meshnick group, who isolated heme-artemisinin adducts from artemisinin-treated *P. falciparum* [78]. The endoperoxide ring of artemisinin is activated on interaction with heme, resulting into the homolysis of the Oï O bond of the trioxane generating more stable carbon-centered radical (fig. 2) which is able to alkylate the meso positions of heme [79-82]. This alkylating capacity is the key step of all artemisinin derivatives and other trioxane containing antimalarial drugs [83, 84]. However, molecular target of artemisinin in *Plasmodia* is still the topic of debate though there are strong evidences that suggest that iron is a primary activator in mechanistic pathway of artemisinin. Athough protein alkylation in *Plasmodia* is well established, a single molecular target that has a direct role in cell death is still not implicated [85].

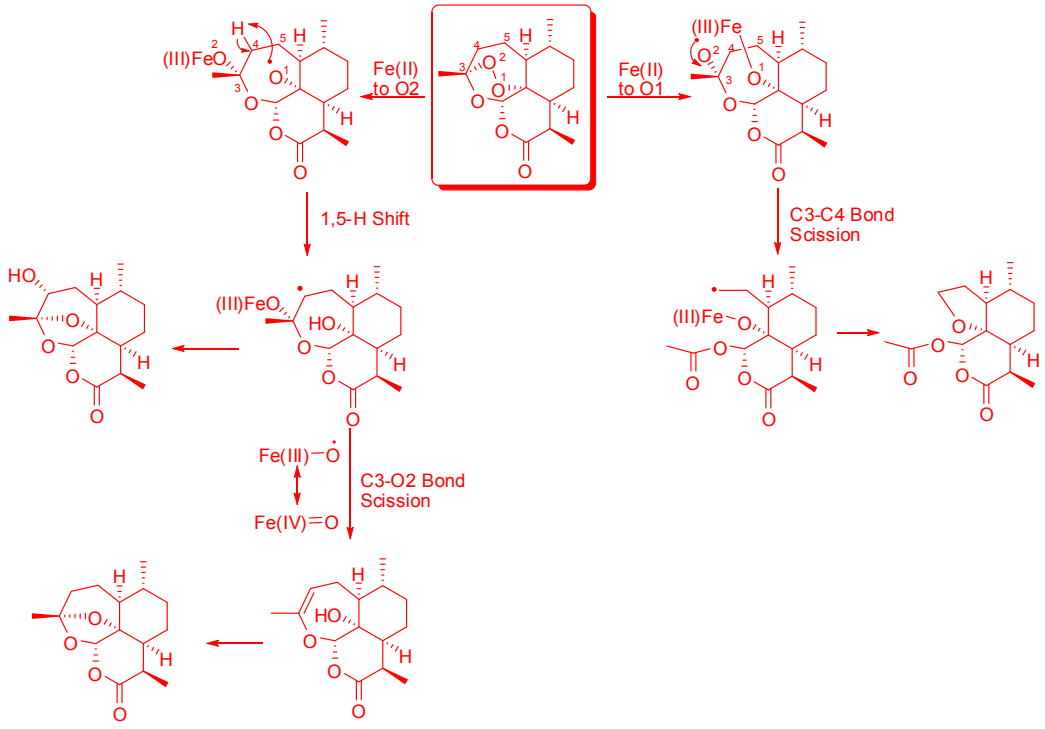


Fig 2: Proposed activation pathway for artemisinin by ferrous haem (Fe(II)

Furthermore, due to poor solubility of artemisinin in both water and oil, hemisynthetic derivatives obtained by reduction and functionalization of the lactone function of artemisinin are usually preferred. Artesunic acid **(8)** (or its sodium salt, artesunate) is the most widely used drug. Sodium artesunate, due to its high recrudescence rate is normally administered in combination therapy and therefore, among all the first generation derivatives, it is currently considered the drug of choice [86, 87]. Due to a short half life of elimination and appearance of recrudescence, artemisinin derivatives are combined with more slowly eliminated drugs such as mefloquine or lumefantrine, inorder to increase the efficiency of treatment and overcome the development of resistance [88, 99, 92]. But, unfortunately, these artemisinin-based combination therapies are very expensive. In addition to this the availability of artemisinin is also unreliable [90, 91]. So, it is necessary to design economically affordable synthetic endoperoxide-based antimalarials [89, 92].

Trioxaquine hybrid as antimalarials

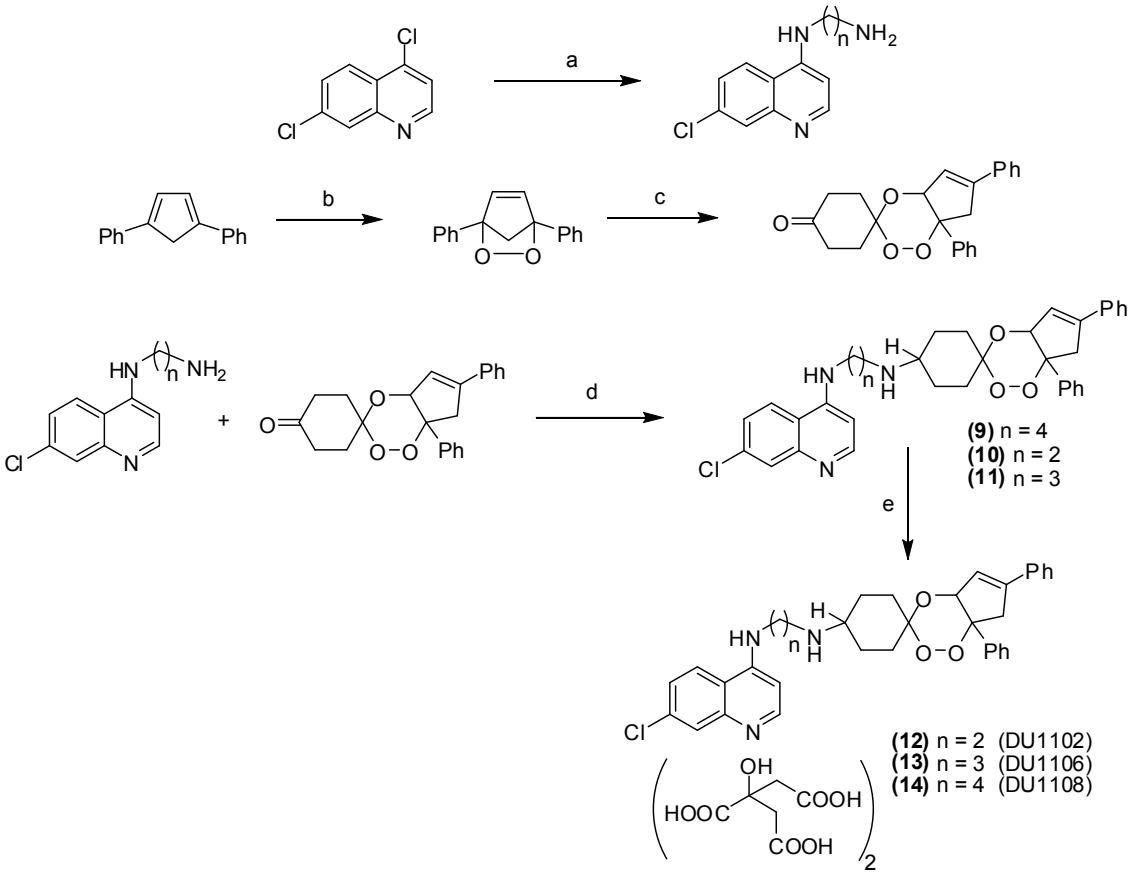
During their erythrocytic life stage, the malarial parasite degrades hemoglobin into heme, within the food vacuole, as waste residue, which is a common target for both, 4-aminoquinolines and endoperoxide derivatives [93-97].

Meunier and co-workers prepared new chimeric molecules by covalent attachment of a trioxane moiety to a 4-aminoquinoline entity named as trioxaquines (Fig. 3). As the trioxane moiety is a potential alkylating agent after reductive activation by heme [81, 98-99] and the 4-aminoquinoline entity is known to easily penetrate within infected erythrocytes [100] and then interact with heme. These trioxaquine molecules will penetrate the infected red blood cells and then interact with the free heme liberated during the hemoglobin digestion by the schizonts within these cells. Such modular molecules are expected to combine the properties of both fragments.

Fig 3: General structure of trioxaquines

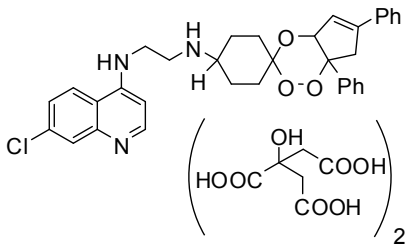
The first trioxaquine, DU1102 **(12)**, synthesized by Meunier and co-workers, showed high in vitro activity against chloroquine-resistant as well as pyrimethamine-resistant human isolates of *P. falciparum.* They synthesized four trioxaquine derivatives **(9), (10), (11), (12)** (Scheme 1) and tested them against three different strains of *P. falciparum*: Nigerian CQ-sensitive (CQS) strain, FcB1 CQ-resistant and FcM29 highly CQ-resistant strains. The IC_{50} values of different trioxaquines ranged from 2 to 86 nM. It was observed that the IC_{50} values on Nigerian CQ strain was dependent on the length of the linker between the trioxane and aminoquinoline entity. The ones with shorter chain (n=2) showed better IC_{50} values than the ones with longer chain (n=3, 4). Among the four synthesized compounds the dicitrate-trioxaquine **(12)** and its base analogue **(10)** were more active against *P. falciparum* than chloroquine. These trioxaquine derivatives are highly active on chloroquine resistant strains (IC_{50} values at 72 h being 9 and 21 nM for

(10) and **(12)** respectively, compared to 116 nM for chloroquine) [101]. This molecule DU1102 **(12)** further showed high activity against Cameroonian isolates (mean 50% inhibitory concentration of 43 nmol/liter) of *Plasmodium falciparum* [102].

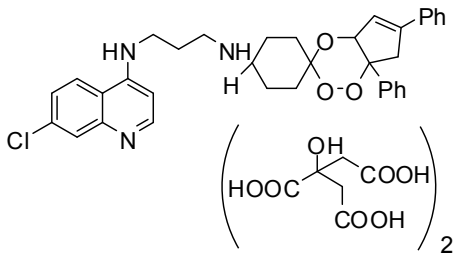


Scheme 1: (a) $H_2N(CH)_nNH_2$, 85°C (b) hɜ, O_2, TPP, CH_2Cl_2, 5°C ; (c) 1,4-cyclohexadione, Me_3SiOTf, CH_2Cl_2, -78°C; (d) $NaBH(OAc)_3$, CH_2Cl_2; (e) citric acid, acetone.

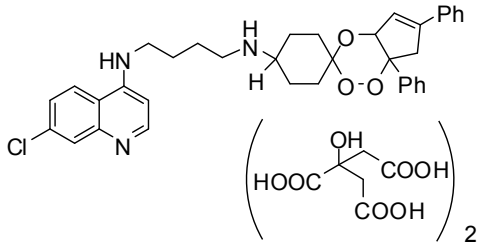
Inspired by the high in vitro antimalarial activity of DU-1102 **(12)** and in an effort to increase the antimalarial activity, a series of new trioxaquines were synthesized [52]. (Fig.4)



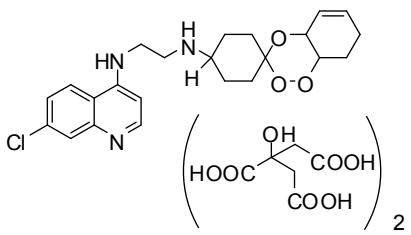
(12) DU1102



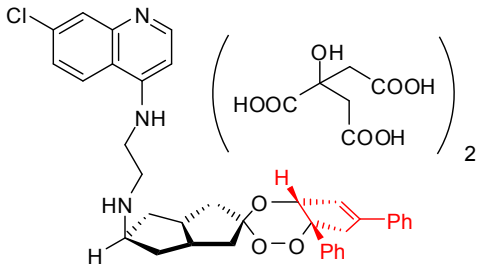
(13) DU1106



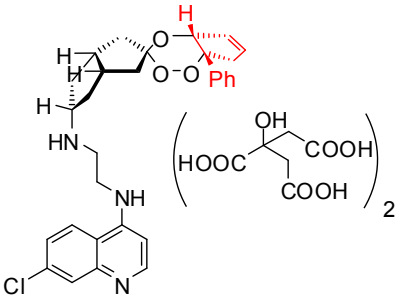
(14) DU1108



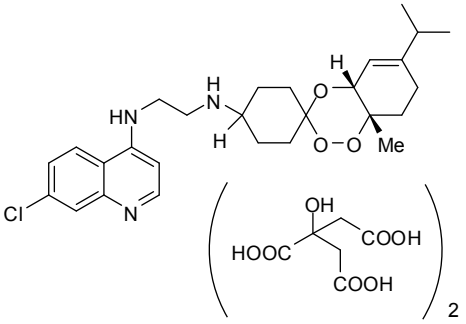
(15) DU1402



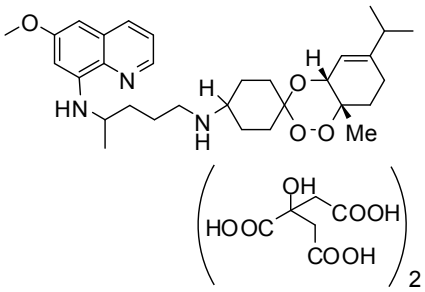
(16) DU1112



(17) DU1114



cis-18 DU1302



(19) DU2302

Fig 4: Structure of trioxaquines citrates

The aim was to study the influence of structural parameters on the antimalarial activity. Various molecules synthesized were: (1) DU1106 **(13)** and DU1108 **(14)** by varying the length of the linker between 4-aminoquinoline and trioxane fragment; (2) DU1402 **(15)** and DU1302 **(*cis*-18)** obtained from 1,3-cyclohexadiene and alpha-terpinene respectively i.e., using different dienes; (3) DU1112 **(16)** and DU1114 **(17)** obtained from *cis*-bicyclo[3.3.0]octane-3,8-dione, rather than cyclohexane-1,4-dione i.e., by changing the nature of diketone used; (4) DU2302 **(19)** obtained from primaquine, a 8-aminoquinoline based antimalarial drug instead of chloroquine, a 4-aminoquinoline motif [52].

In vitro activity of the synthesized compounds were tested on different strains of *P. falciparum*: a Nigerian strain which is chloroquine sensitive (CQS), FcB1-Columbia which is chloroquine resistant (CQR) and FcM29 which is highly chloroqine resistant (CQR+) [Table 2], inorder to determine the influence of different structural modification on the biological activity of these modular molecules. These molecules showed high antimalarial activity both on chloroquine sensitive as well as chloroquine resistant strain of *P.falciparum.* Amongst all the drugs, the best activities were shown by DU1102 (IC_{50}

22-27 nM) and DU1302 (IC_{50} 5-19 nM) [52]. Change in the length of lateral chloroquine chain has influence on the antimalarial activity and, in some cases, is able to overcome chloroquine- resistance of *P.falciparum* [103, 104]. Here, no such significant change in the activity was observed by varying the length of linker between the trioxane and 4-aminoquinoline moieties, though activity was lowered when diaminoalkyl chain was longer than two carbon units. The trioxaquine molecule with two carbon atom tether showed maximum activity (IC_{50} 22 and 27 nM) [52].

Similarly, there was also no significant influence of stereochemical modulations on the antimalarial activity of these trioxaquines. The two trioxaquines **(16)** and **(17)** had no marked difference in their IC_{50} values, so we can conclude that the C3 stereochemistry of these trioxaquines showed no remarkable influence on their antimalarial activity. There was also no effect of change in the starting dienes in **(12)** (1,4-diphenyl-1,3-cyclopentadinene to **(15)** or alpha-terpinene in *cis*-**18** (1, 3-cyclohexadiene) though they too were quite active. Out of the two, *cis*-**18** (DU1302) was most active against both CQS and CQR strains. The two diastereomers of *cis*-**18** (*trans, cis*-**18** and *cis, cis*-**18**) were independently tested and found to have activities in the same range. Furthermore, molecules containing 8-aminoquinoline as quinoline moiety (i.e., **19**), showed lower antimalarial activity, confirming that the presence of 4-aminoquinoline as an essential entity for antimalarial activity in trioxaquines moiety. However, since it showed better activity in comparison to its base moiety primaquine, this showed that covalent coupling of trioxane moiety with primaquine results into an increase in intraerythrocytic antimalarial activity. Thus, this trioxaquine can be potentially used against both sexual and asexual erythrocytic stages [52].

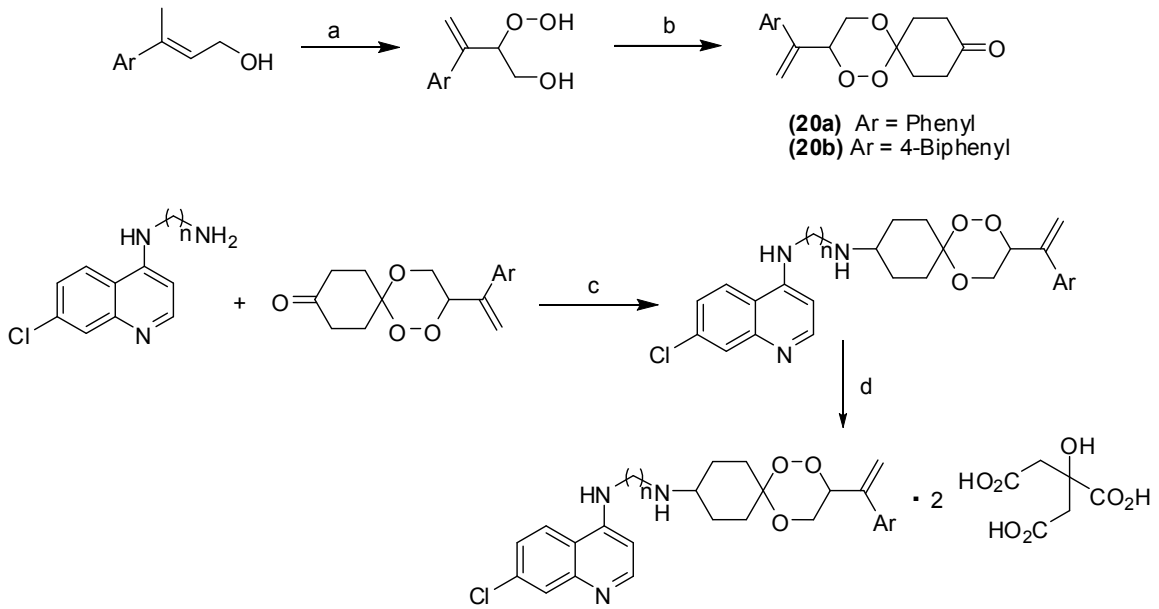
Table 2: IC_{50} values [nM] for the trioxaquine citrates against *Plasmodium falciparum*

Trioxaquine citrates	IC_{50} [nM] Nigerian (CQS)	FcB1-Columbia (CQR)	FcM29-Cameroon (CQR+)
(12) (DU1102)	22	27	27
(13) (DU1106)	181	41	133
(14) (DU1108)	87	41	41
(16) (DU1112)	112	31	39
(17) (DU1114)	108	63	85
(15) (DU1402)	36	71	49
cis-**18** (DU1302)	17	15	6
trans,cis-**18**	9	19	7
cis,cis-**18**	5	11	5
(19) (DU2302)	176	112	108
Chloroquine	62	116	174
Artemisinin	6	5	8
Primaquine	875	1075	1400

The results indicated that, ***cis*-18** is efficient on a wide range of strains. This molecule showed IC_{50} values between 5-19 nM which is lower than the range expected (10-20 nM) for efficient antimalarial based on trioxaquine moiety [105, 106]. Due to its high efficacy and solubility, ***cis*-18** (DU1302) is an attractive trioxaquine.

The in vivo results revealed that this drug showed promising activity compared to those of trioxane containing antimalarial drugs. Based on the level of curative doses, trioxaquines, in particular, DU1302, are more active than artemisinin and artemether and exhibit activities similar to that of artesunate [107]. Previously, it has been confirmed in clinical trials that artesunate is potent against gametocytes [108, 109]. Similarly trioxaquines DU1302 and DU2302 were also most potent against gametocytes of *P. falciparum* which showed activities (IC_{50}, 46-69 nM) slightly greater than that of artesunate (IC_{50}, 72-108 nM) showing that difference in the quinoline moieties of the two drugs DU1302 (with 4-aminoquinoline) and DU2302 (with 8-aminoquinoline) did not show any effect in their activities against asexual and sexual stages of *P. falciparum.* Further studies confirmed that the trioxaquines DU1302 showed the absence of toxicity on the cell lines and in mice model. [107].

A novel photooxygenation route was used for the preparation of 1, 2, 4trioxanes [110], a number of which have already shown promising antimalarial activity [111-114]. The extension of this methodology was further used in the preparation of a new series of trioxaquines (scheme 2) [115].



Scheme 2: Reaction conditions: (a) hɜ, O_2, methylene blue, MeCN, -10 to 0°C, 4 h; (b) 1,4-cyclohexadione. conc. HCl, 5°C, 18 h; (c) $NaBH(OAc)_3$, CH_2Cl_2, r.t., 18 h; (d) citric acid, acetone, r.t.

These compounds were tested against *P. yoelii* in Swiss mice at 96 mg/kg by intramuscular as well as oral routes. Though the trioxaquine molecules **21a-23a** showed no improvement in activity over their parent compound **20a,** when given through intramuscular route, they were active over their parent compound when given via oral

route. Compounds **21b-23b** showed comparable activities to their parent compound **20b** when administered both by intramuscular or oral route. The biphenyl derivatives were found to be orally more active than the phenyl derivatives basically due to high lipophilicity of biphenyl group as compared to phenyl group. However, these hybrid molecules suffer from serious limitations such as poor stability and poor solubility both in oil and water. They form an inseparable mixture of diastereomers which is another serious limitation [115].

Trioxaquines with a 1, 2, 4-trioxane cycle have been designed in order to mimic the alkylating ability of artemisinin, and is responsible for its biological activity. Trioxaquines efficiently alkylates the heme macrocycle, after the activation of its peroxide function by Fe (II) and thereby forms a heme-drug adducts [116] (Fig.5).

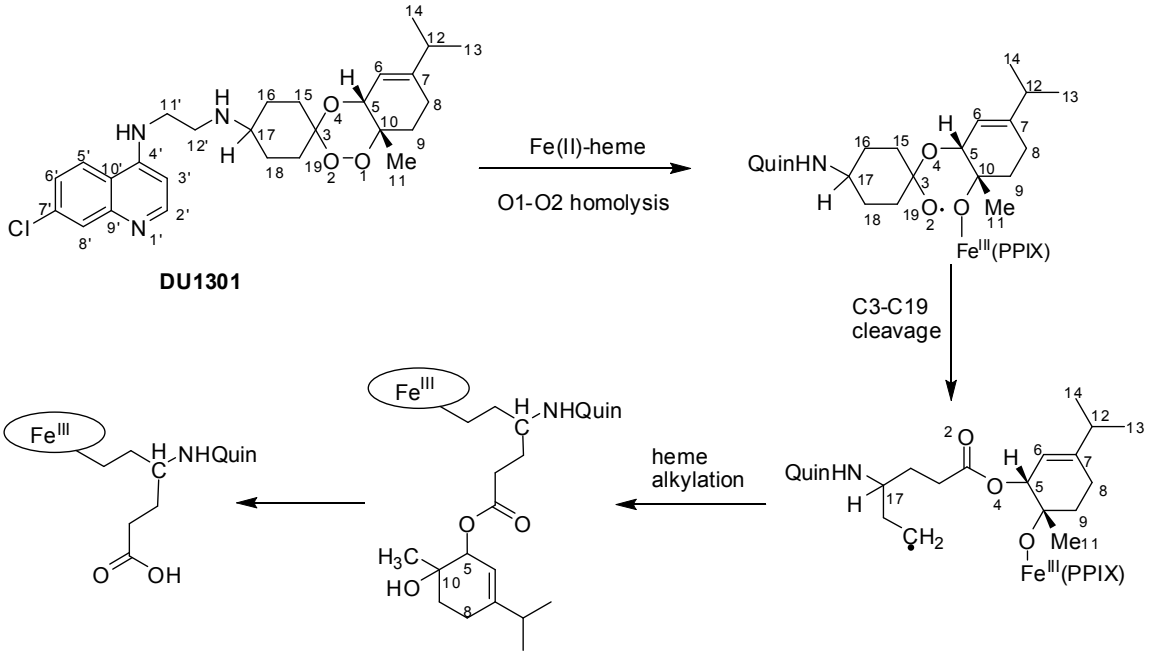


Fig 5: Alkylation of heme by trioxaquines DU1301.

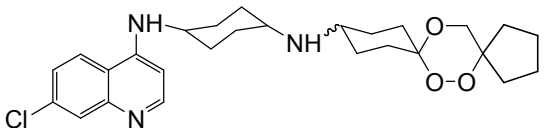
Further, the in vivo alkylating ability of trioxaquine DU1301 was examined in parasite infected mice. The detection of heme-DU1301 adducts within a *Plasmodium*-infected mammal indicates that the heme alkylation by DU1301 is an efficient reaction that occurs in vivo and plays the basic role in the mechanism of trioxaquines in antimalarial activity. These results further confirm that artemisinin and trioxaquines share the same heme-alkylating properties and the heme-trioxaquine adduct which has been characterized is also similar to that obtained with artemisinin derivatives [117].

Trioxaquines and heme-artemisinin adducts, resulting from alkylation of heme by artemisinin, are able to inhibit beta-hematin formation [86]. The inhibition of beta-hematin formation by chloroquine or *N*-(7-chloro-4-quinolinyl)-1,2-ethanediamine has previously been reported [118]. Thus it is a reasonable assumption that 4-aminoquinoline entity of trioxaquine inhibits the formation of beta-hematin via a noncovalent interaction

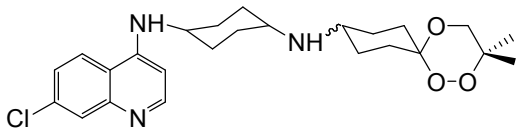
with heme [119]. It was tested that trioxane-containing molecules like artemisinin, artesunate and artemether did not interfere with beta-hematin formation as the mechanism of these trioxane compounds is different from that of aminoquinolines [120, 121]. Thus these trioxaquines have dual mode of action i.e. inhibition of beta-hematin formation which is chloroquine-like mechanism and heme alkylation which is artemisinin like mechanism [107].

Trioxaquines show better antimalarial activity than simple trioxane and the linked aminoquinoline entity largely improves their antiplasmodial activities. This concept was proved experimently, by testing the activities of its precursors, quinoline and trioxane, against different strains of *P.falciparum.* The trioxane motif exhibited IC_{50} values ranging from 200 nM to 600 nM and the IC_{50} values of quinoline alone ranged from 120 nM to 2 nM. When both the entities were tested together, in combination, in the same well, showed IC_{50} values in the range of 40 nM to 180 nM, whereas the trioxaquine show IC_{50} values between 4 nM to 32 nM. Thus the link between both the active pharmacophores of trioxaquines is essential for their activity and trioxaquines are more active than simple trioxane or quinoline moiety [52].

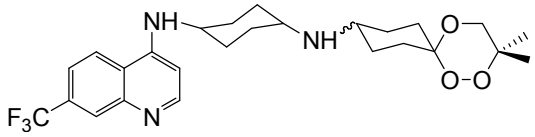
Recently, a new series of trioxaquines **(24-29)** have been synthesized that showed good in vitro activity against *Schistosoma mansoni* [122]. (Fig.6)



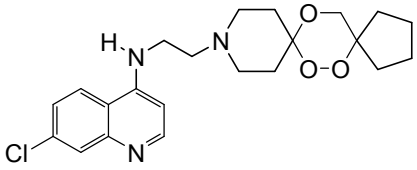
(24) PA1259



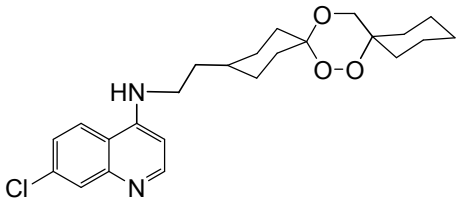
(25) PA1103



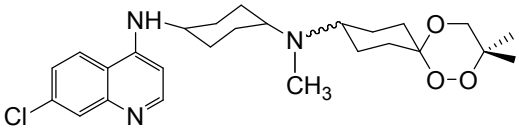
(26) PA1252



(27) PA1120



(28) PA1110



(29) PA1302

Fig 6: Structutres of trioxaquines active against *Schistosoma mansoni*

Hemozoin is a disposal product shared by *Plasmodium* and *Schistosoma* [123] and all these trioxaquines were also active against *P. falciparum*. It is for this reason that the activities against plasmodia were compared with antischistosomal activities. These trioxaquines showed in vitro activities, among which PA1259 **(24)** was found to be the most active. It was found to be as active as praziquantel, the well known drug currently used for schistosomiasis [122]. This recent study shows that trioxaquine is not only active against malaria but can also be used as potential antischistosomal drugs in future.

Conclusion

In conclusion, development of molecules with dual mode of action is a milestone in the field of drug discovery. It is one possible approach to create drugs via a rational drug design to prepare drugs at affordable price and overcome the emergence of drug resistant strains. Trioxaquine is the best example of one such molecule. Trioxaquines with heme-alkylation and hemozoin-inhibiting capacities can help in avoiding the development of resistant strains of parasites. Moreover, the trioxaquines also showed good activity against gametocytes which would limit the transmission of the malaria parasite. Thus, trioxaquines with such biological profile can be considered as a promising antimalarial candidate that is able to fight malaria by treating individual symptoms and limiting parasite transmission.

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