Tropical Journal of Pharmaceutical Research December 2021; 20 (12): 2663-2674
ISSN: 1596-5996 (print); 1596-9827 (electronic)

Available online at http://www.tjpr.org
http://dx.doi.org/10.4314/tjpr.v20i12.29

Review Article

Chloroquine bioconjugates and hybrid compounds: past and recent developments in combatting chloroquine resistant malaria

Abdul Wahab Aliyu[1,2] Khairul Mohd Fadzli Mustaffa[1]*, Lee Chong Yew[3] Low Jen Hou[4]

[1]Institute for Research in Molecular Medicine, Health Campus, Universiti Sains Malaysia, 16150 Kubang Kerian, Kota Bharu, Kelantan, [2]Pharmaceutical Microbiology and Biotechnology, Faculty of Pharmaceutical Sciences, Gombe State University, P.M.B 127, Tudun Wada Gombe, Gombe State, Nigeria, [3]Department of Pharmaceutical Chemistry, School of Pharmaceutical Sciences, Main Campus, Universiti Sains Malaysia, 11800 Penang, Malaysia, [4]Department of Pharmacology, School of Medical Sciences , Universiti Sains Malaysia, 16150 Kubang Kerian, Kota Bharu, Kelantan, Malaysia

For correspondence:* ***Email:* *khairulmf@usm.my*

Sent for review: 28 July 2021 *Revised accepted: 22 December 2021*

Abstract

Drug structural modification through conjugation with bioactive chemical compound or biological agent provides the opportunity to combine their intrinsic desirable properties as a single agent to combat drug resistance. By focusing on chloroquine (CQ) as therapeutic agent to reverse CQ resistance in Plasmodium falciparum, this review initially discusses the development of chloroquine hybrid compound through covalent biotherapy and bioconjugation. Chloroquine-bioconjugate containing amino acids, peptide, polysaccharides and bio-organometals were highlighted. Then, the use of chloroquine hybrid and conjugates in patients and in clinical trial were comprehensively enumerated and lastly, the review summarizes the challenges and current trend in reversing CQ resistance in malaria.

Keywords: *Chloroquine, Hybrid compounds, Bioconjugates, Resistance and P. falciparum*

Tropical Journal of Pharmaceutical Research is indexed by Science Citation Index (SciSearch), Scopus, International Pharmaceutical Abstract, Chemical Abstracts, Embase, Index Copernicus, EBSCO, African Index Medicus, JournalSeek, Journal Citation Reports/Science Edition, Directory of Open Access Journals (DOAJ), African Journal Online, Bioline International, Open-J-Gate and Pharmacy Abstracts

INTRODUCTION

Malaria is a parasitic infection caused by at least one of the five protozoans of the genus *Plasmodium* (*P. falcirarum, P. vivax, P. oval, Malaria and P. knowlesi.*) [1] . The 2020 World Health Organization (WHO) report indicates that malaria claimed the life of almost 409,000 individuals in the year 2019 [2]. The mortality rate was found to be higher in the tropical region of Africa such as Nigeria (23%), Congo (11%), Tanzania (5%), Niger (4%) Mozambique (4%) and Burkinafaso (4%), than the death rate which occurred in South East Asia (3%) and South America (0.4%) [2-3]. The higher burden of malaria especially around the endemic areas was found to be associated with the evolution of resistance to pharmacological agents used for

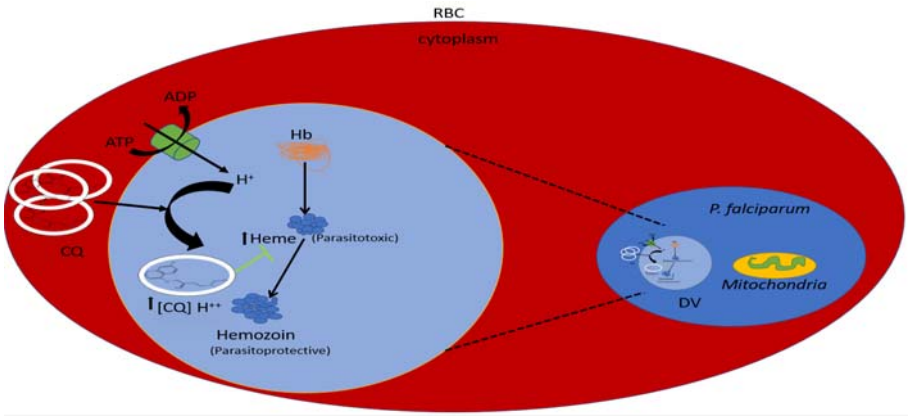


Figure 1: The Mode of action of Chloroquine. *After internalization of the P. falciparum into the host red blood cell (RBC), the parasite engulfs the host hemoglobin (Hb) and subject it to proteolytic degradation. The resultant heme from proteolytic process accumulates and exerts parasitotoxic effect on malaria parasite. To evade the parasiticidal actions, the parasite polymerizes heme to a Parasitoprotective hemozoin. CQ block conversion of heme to hemozoin and thus kill the parasite through the action of heme*

malaria therapy [4]. This has rendered many antimalarials including CQ practically not useful in treating the disease.

Chloroquine belongs to the 4-aminoquinolines family of antimalarial agents and was the first-line drug for treatment of malaria infection because of its safety and effectiveness in eradicating blood schizont of all strains of malaria parasites [5]. Due to its relative safety and efficacy especially to pregnant women and children, it was considered as a relevant choice of treatment for malaria until the emergence of CQ resistance plasmodium strain in the year 1961 [5-6]. Thus, the use of chloroquine became limited to those CQ sensitive parasites. It is well established that drug structural modification is particularly vital when the resistance is transport mediated in nature [7] , as with CQ resistance [6] .

Drug structural modification is mainly aim at "hibernating" the active domain of the drug in a way that their pharmacodynamics is either maintain or enhance while toxicokinetic are decrease and resistance mechanisms evade. Amongst other prominent approach adapted by several researchers in achieving that is "Covalent biotherapy", an approach that combines at least two bioactive compounds in a single molecule to achieve multiple potency. In this review, CQ resistance reversal strategies involving CQ-bioconjugation and CQ-hybrid compounds generation were discussed. The review also highlights CQ-bioconjugate/ hybrid compounds in clinical use and those under pre-clinical and clinical testing. Lastly future directions on the CQ bioconjugates development was highlighted

CHLOROQUINE MECHANISM OF ACTION

Chloroquine acts by inhibiting the conversion of heme to hemozoin by plasmodium parasites. Heme is toxic to the plasmodium and its accumulation kills the parasite. This process takes place during the erythrocytic stage of malaria infection. At this stage, the parasite engulfs hemoglobin into its digestive vacuole (DV) where it undergoes degradation [8]. The by-product produced from the degradation process is heme which is highly toxic to the parasite. Polymerization of heme to hemozoin is a parasite strategy to detoxify the effect of heme as malaria parasite do not possess enzymes to deactivate the heme [9]. Inhibition of hemozoin formation thus, is the mechanism by which CQ mediates its therapeutic effect (Figure 1). At the cellular level, CQ is easily transported into the DV, as it enters the digestive vacuole, the CQ gets doubly protonated. This doubly protonated CQ has high affinity for heme and thus forms complex with it and in turn prevents the formation of hemozoin [10]

CHLOROQUINE RESISTANCE MECHANISM

The emergence of resistant strains of malaria parasite have retarded the effort toward control of malaria infection. The parasite was reported to have developed means of decreasing the concentration of CQ at its site of action which is the parasite DV via a mutated *Plasmodium falciparum* CQ resistant transporter (PfCRT) [6, 11]. The efflux mechanism through which CQ is removed from DV is not fully understood.

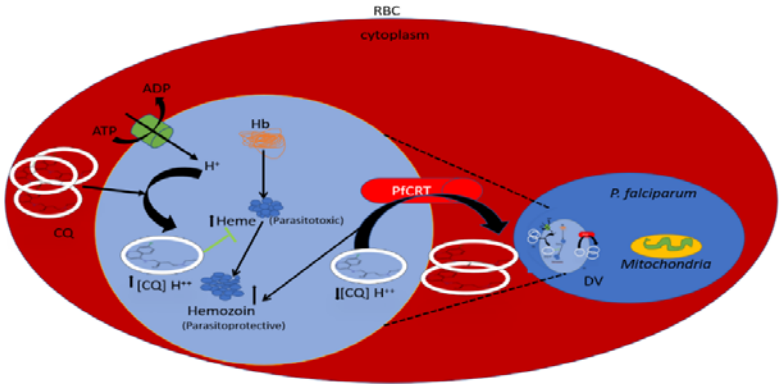


Figure 2: The Chloroquine resistance mechanism**.** *The P. falciparum developed a survival tactics in the presence of CQ through formation of P. falciparum CQ resistance transporter (PfCRT) which mediates efflux of CQ out of the parasite digestive vacuole. As the CQ concentration drops, polymerization of heme to hemozoin continues. Depletion of parasitotoxic heme and accumulation of Parasitoprotective hemozoin allows the parasite to survive the therapeutic concentration of CQ and is said to have developed CQ resistance*

However, it was hypothesized to be via alteration of hydrogen ion concentration potentials within the parasite DV which affects accumulation of antimalarial agents including CQ (Figure 2) [12] PfCRT-mediated CQ resistance has led to withdrawal of this agent from the drug market and clinical use. However, limited studies demonstrated that CQ is still effective in control of malaria in some region of African continent such as Kenya, Ethiopia, and Malawi [13]. Other than PfCRT there are few more postulated receptor/pumps associated to the CQ resistant typify by P-glycoprotein homologue 1 (Pgh-1) and ATPase pump.

CHLOROQUINE RESISTANCE REVERSAL STRATEGIES

Modifying the compound, CQ, has been adopted as a strategy in reversing CQ resistance. These modified CQ based-drugs are subclassified based on the nature of the modifying agent coupled to the CQ molecule. A vast majority of recently documented modified CQ compounds can be categorized as CQ bioconjugates and CQ hybrid compounds.

Chloroquine bioconjugates compounds

Bioconjugation in chemistry involves covalent linkages of at least two pharmacophore molecules, one of which is of biological origin. In therapeutics it implies the coupling of drug with biologics such as peptides, oligonucleotides, carbohydrates or lipid molecules in order to enhance the drug-dynamics/kinetic, to reduce serum toxicity or to stabilize it against resistance machineries of the cellular microenvironment [14]. Over the decades, CQ bioconjugates have been developed to either reverse the transport mediated CQ resistance or to prevent invasiveness in pancreatic cancer chemotherapy [15]. CQ -TP10 peptide conjugate, CQ -ornithine conjugate, CQ-Chitosan conjugate and CQ-cinnamic acid conjugate are typical examples of CQ containing bioconjugate.

Chloroquine -ornithine conjugate

Noopur and Aditya [14], utilized the concept of "covalent biotherapy" to fuse CQ bioactive moiety with ornithine (Figure 3) The resultant bioconjugate was then tested for in vivo anti-plasmodial activity using male Balb/c mice infected with *P. berghei*. Prior to treatment with the conjugate, the mice were infected with 0.2 ml of inoculum containing infected red blood cells. Increasing doses of CQ ornithine bioconjugate (10, 25, 50, 100 mg/kg) and standard drug CQ diphosphate (10 mg/kg/day × 40) were administered into the mice via oral route, 6 hourly for three days. The findings of Noopur and Aditya [14] showed that, curative activity was achieved with 10 mg/kg dose of the bioconjugate which is lower than that achieved by the standard drug. Although the work of this group claimed that, the conjugate demonstrates anti-plasmodial effectivity at lower doses, initial invitro anti-plasmodial potential of the conjugate should have been established prior to in vivo studies. Similarly, other strains of *Plasmodium* particularly, *P. falciparum* (both CQ sensitive and resistant strains) need to be tested to establish the effectiveness of the conjugate against these strains and its subsequent potential to reverse CQ resistance in the respective plasmodium strains.

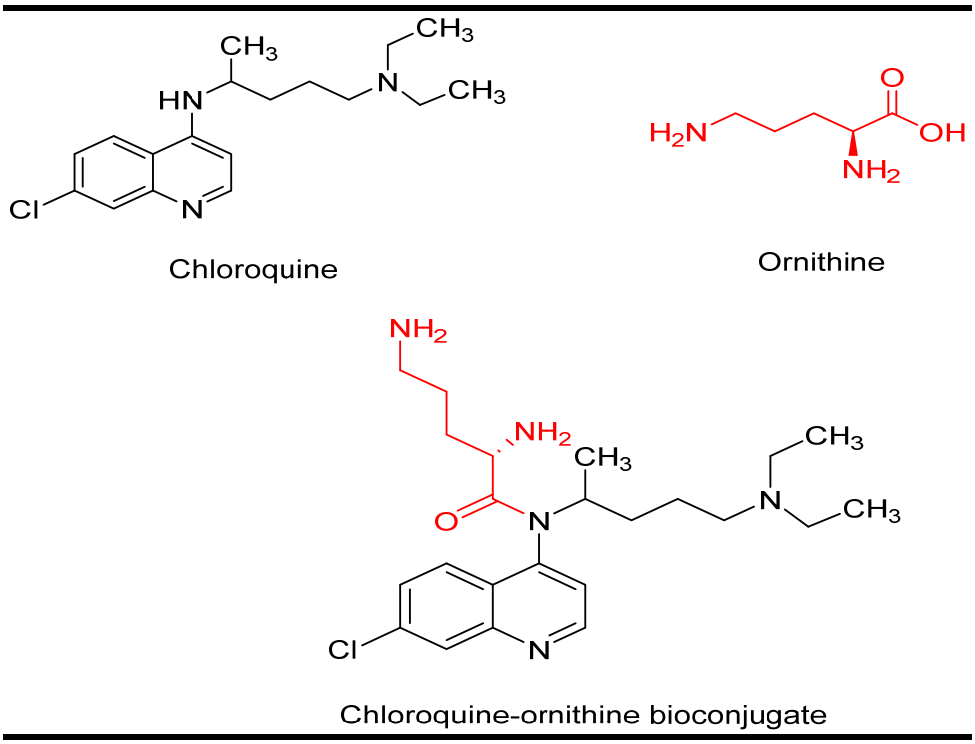


Figure 3: Structures of CQ, ornithine, and CQ-ornithine bioconjugate

Chloroquine -TP10 peptide conjugate

Some peptides possess intrinsic cell internalization properties which gave them the ability to deliver "load" into cell cytoplasm through interaction with specific membrane-receptor proteins. Most of these peptide, if not all, contains few sequences of not more than 30 residues [16]. Cell penetrating peptides differ in their sources, while some are obtained naturally from proteins others are synthetic in nature. Semi natural cell penetrating peptide also occur and are obtained often by linking two natural occurring sequences. Different cell penetrating peptides show different penetration kinetics and dynamics [17], and their application in improving drug kinetic is increasing, for instance Aguiar, *et al* [18], successfully conjugated nine different cell penetrating peptide with CQ analogue to generate CQ-peptide bioconjugates (Figure 4) by combination of solution and solid-phase synthesis methods. The bioconjugate were pharmacologically screened in vitro for anti-plasmodial activity against CQ resistant P. *falciparum* (W2) strain. Although the findings of Aguiar, *et al* [18] demonstrated that, the bioconjugates have shown anti-plasmodial activity, but none of the compounds have shown potency equal to or higher than the standard CQ (IC50 = 699 nM) [19] probably because of the different in the parasite strain and developmental stage used for testing (*P. berghei vs. P. falciparum*) and (liver vs. blood) respectively. Among the bioconjugates, 5a (IC50= 1.5 /µM) and 5b (IC50 = 5.2 /µM) are the most potent. There is need to establish sound correlation between CQ-peptide bioconjugate and CQ through in vitro and in vivo anti-plasmodial screening using the same conditions and parameters in terms of *plasmodium* strain and developmental stage of the parasitic growth.

Chloroquine-chitosan conjugate

Chitosan, a derivative of chitin is naturally occurring polymer made up of monomers of sugar moieties with no serum and tissue toxicity Due to its cell penetration power, chitosan has found wide application in pharmaceutical industries as drug career [20]. To explore such benefit, Tripathy, *et al* [21], prepared CQ-chitosan conjugate by ionotropic gelation. The nano-CQ bioconjugate was tested to examine its anti-plasmodial, antioxidant and pro/anti-inflammatory effects using CQ resistant *P. berghei* strain, NK65-*infected Swiss mice as a model. Plasmodium infection was achieved ten days after intraperitoneally injecting the mice with* 200 µl of infected red blood cells harboring 1 x 10^5 parasites. The intervening nano-CQ biocon-

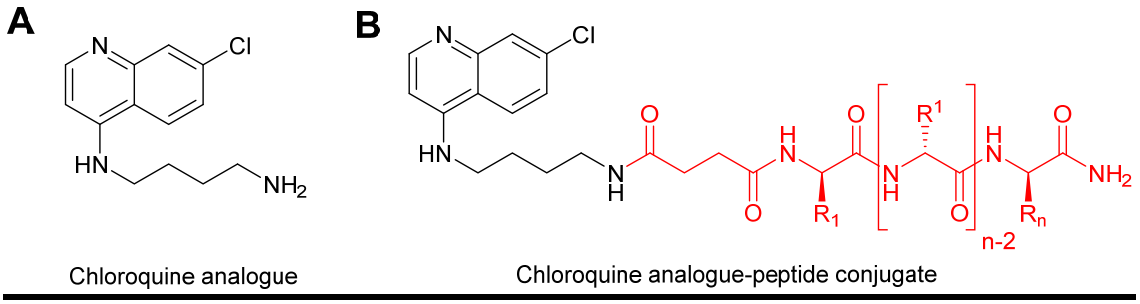
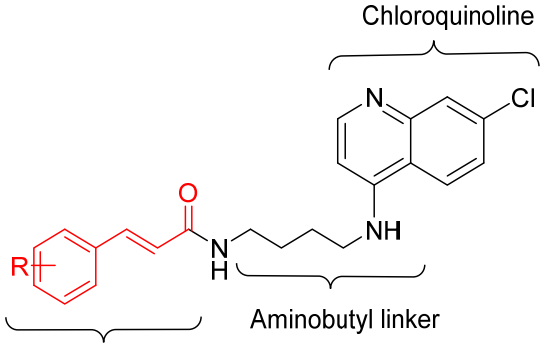


Figure 4: structure of CQ analogue (A) and CQ analogue-peptide (B: a = TP10, b= Transportan, c=DPT-sh1, d=DPT-sh2, e=IDR-1018, f=TAT, g=PasTAT, h=R9 and I= Penetratin) bioconjugate.

jugate and the standard CQ were administered at the doses of 250 mg/kg body weight and 68.4 mg/kg respectively via intraperitoneal route for the therapeutic duration of 15 days. The findings of this work demonstrated that, CQ-chitosan bioconjugate significantly reduced parasitemia by 97.59% compared to the unconjugated CQ that only produced 67.02% reduction after 15-day treatment. The high potency exhibited by the nano-CQ bioconjugate in *P. berghei need to be investigated in a CQ resistant/sensitive P. falciparum to establish the broad-spectrum activity of the bio-conjugate and its resistance reversing potentials.*

Chloroquine-cinnamic acid conjugate

Cinnamic acids are secondary phytoconstituents distributed in various plants that exert many physiological function such as guarding against microbial intruders and other forms of xenobiotics they also serve as chemo-attractants for insect pollinators [22]. Their therapeutic value has been extensively documented through the work of several researchers and are found to possess various medicinal properties such as anti-infective activity including anti-plasmodial effects . Cinnamic acid forms conjugate with other moieties easily. As such, Pérez, *et al.* **[**23**],** applied the concept of "covalent biotherapy" that combines the individual efficacy of two different drug in a single molecule by linking the active part of one to the other. Through this approach, the group prepared a dozen of new CQ bioconjugates (Figure 5) containing 1, 4-amino-7-chloroquinoline in aminobutyl mediated covalent linkage with various substituted cinnamoyl functional groups. The dual anti-*P falciparum* bio-activity incorporated in the CQ-cinnamic acid conjugate is inhibition of both hemozoin formation and new permeability pathway, a pathway believed to developed following parasite invasion of the red blood cell [25]. The bioconjugates were tested for anti-plasmodial activity against CQ-resistant *P. falciparum* (W2) in vitro. All the conjugates produced IC_{50} (< 60 nM) which is less than that produced by the standard CQ (183nM). The most potent was found to be the compound, 7c (IC50 = 11.0 nM). Presence of linker between the two pharmacophore is necessary for anti-plasmodial effect in a similar way lipophilicity of bioconjugates determines their therapeutic potential.



Hybrid compounds	R substituent
Compound 7a	H
Compound 7b	*Para*-methyl
Compound 7c	*Para*-isopropyl
Compound 7d	*Para*-methoxy
Compound 7e	*Para*-amino
Compound 7f	*Meta*-fluoro
Compound 7g	*Para*-fluoro
Compound 7h	*Para*-chloro
Compound 7i	*Para*-bromo
Compound 7j	*Ortho*-nitro
Compound 7k	*Meta*-nitro
Compound 7l	*Para*-nitro

Figure 5: structure of CQ-cinnamic acid bioconjugates 7a-l

Chloroquine hybrid compounds

A hybrid compound is often generated by chemical synthesis where two or more distinct

pharmacologically active agents are coupled together through single or multiple chemical bonds thus combining their individual potency into single agent [26]. To reverse resistance in malaria, several researchers [28-34] have hybridized CQ with other potent anti-malarial agent or other compounds that augment the effects of such agents.

Chloroquine chemosensitizers hybrids

Chloroquine chemosensitizers otherwise called CQ resistance reversal agents are pharmacological agents capable of augmenting the therapeutic effect of CQ in resistance strain of *P falciparum* [27]. A clear example of chemosensitizer is verapamil which increases the parasite DV concentration of CQ by inhibiting the H^+-pumping V-type ATPase [11]. Substantial effort has been made using such agent to develop relatively new molecules with potent activity against CQ resistant *Plasmodium*. For instance, Boudhar, *et al.* [28] synthesized a sequence of CQ-based hybrid molecules by linking a CQ sensitizing agents derived from different chemical compounds including loperamide and octoclothepin to CQ active moiety, (Figure 6) to restore the therapeutic effect of CQ in a single agent against various resistant strains of malaria parasite. The hybrid compounds were tested in vitro for antimalarial activity against both CQ sensitive (3D7) and resistance (K1) cell lines. Among the series of these compounds, the most promising were compound 27a, 27b, 31, and 35 (Figure 6) possessing IC50s equal to or lower than 200 nM for both strains. Compound 35 with IC_{50} of 190nM for K1 was found to be more potent than CQ and similarly retained profound effect against 3D7 with IC_{50} of 32.4 nM. Further anti-plasmodial screening was conducted for the most potent compounds (27, 31 and 35) against other multiple lab and field isolates ranging from CQ sensitive (Hb3)/resistance (Dd2 and NHP-04559) to CQ-artemisinin resistant (ARS-233, ARS-272 and NHP-04773) strains. The group found that, unlike compound 31 which showed lower IC50 than that of standard (CQ), compound 27 and 35 retained their potentials indicating that the activity observed was not limited by the presence of the aromatic ring in the structure of hybrid compounds (absent in 27 but present in 31). Due to the high potency demonstrated by compound 27 and 35, the team conducted

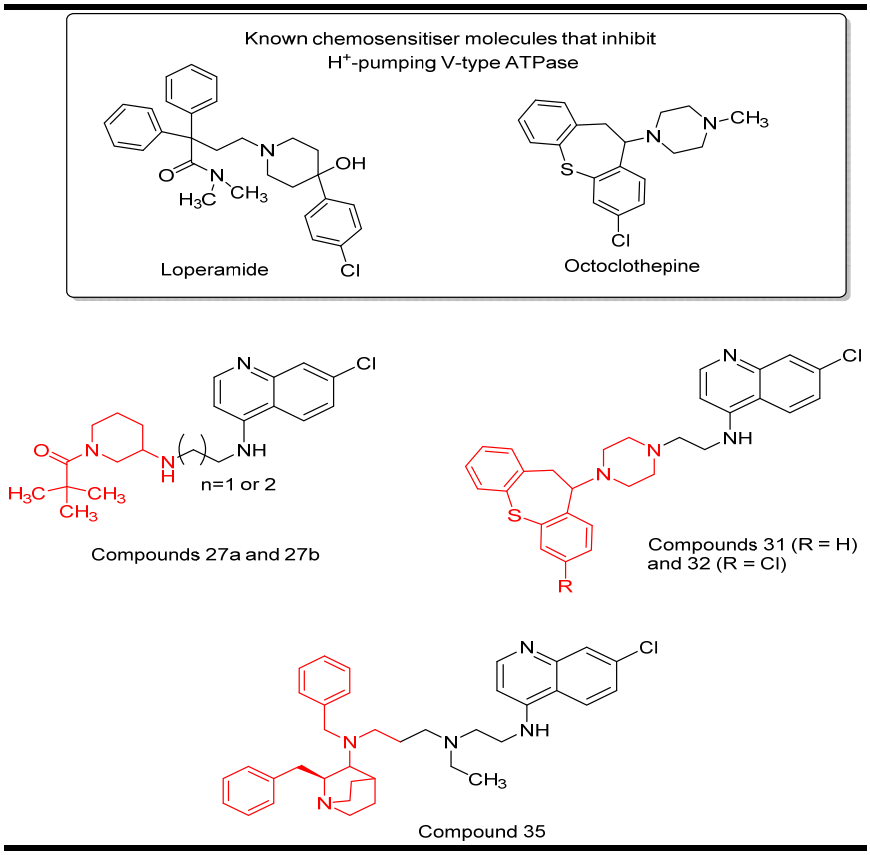


Figure 6. Structures of CQ hybridized with loperamide (compounds 27a, 27b, and 35) and octoclothepine (compound 35)

further investigations such as aqueous solubility, membrane permeability and toxicity studies to establish the pharmacokinetic and dynamic of these hybrids. Their findings suggested that compound 35 possessed good water solubility, permeation through lipid bilayer and had broad spectrum of bioactivity with profound potency against resistance malaria parasite. Compound 35 similar demonstrated good safety profile when tested using hepatocyte and a cardiomyocyte cell lines. The group did not carry out in vivo pharmacological studies for this compound which is required to further establish it pharmacokinetic and dynamic potentials.

Chloroquine simple reversal agent hybrids

Gunsaru, *et al.* [29] studied the relationship between the bioactivity of CQ hybrid compounds and the structural aromaticity of the reversal agent coupled to the quinoline pharmacophore. The group synthesized simple forms of the re-sensitizing agent. Several hybrid compounds with promising CQ resistance reversing properties were generated (Figure 7) and their findings base on heme binding affinity and hematin inhibition assays conducted, indicated that, reducing two aromatic rings to a single ring in the chemosensitizer do not have profound alteration in the anti-plasmodial activity of the CQ hybrid compound. Among these compounds, compound 9 was found to be more promising with IC_{50} of 0.4 nM, 0.7nM and 0.6 nM against D6, Dd2 and 7G8 strains of *P. falciparum* respectively which is more potent than the standard drug (CQ) with higher IC_{50} values against the respective strains (6.9nM, 102 nM and 108nM).

The group further modified the structure of compound 9 to investigate the effect of introducing different substituents on the single phenyl ring of the reversal agent and the effect of introducing of a linker between the phenyl and piperazine rings. They synthesized compound 10 to 15 through various substitution on the phenyl ring and these compounds retained higher potency than CQ with varying IC50s values but all values lower than that of the standard drug (less than 5 nM) against D6, Dd2 and 7G8 strains of *P. falciparum*. On the other hand, the researchers generated compounds 17, 18 and 20 through insertion of linker between the phenyl and piperazine rings. Surprisingly, all these changes did not decrease the potency of the newly synthesized CQ hybrid as compared to that the parent compound.

To further ascertained the potency of these new hybrid compounds in reversing CQ resistance in plasmodium parasite the group performed in vitro heme binding test where they unveiled that hybrid compounds 9, 11 – 13 bind to heme with dissociation constant of almost 5 nM to the same extent as CQ (kd = 5.4nM) does. This implies that these hybrids have the potential to prevent formation of hemozoin and thus allow heme to aggregate and mediate it toxic effects against the parasite. Another test to confirm the efficacy of these compound which is hematin inhibition test was similarly conducted where the findings indicated that the hybrid inhibit hematin production with IC_{50} of between 5 nM – 43 nM in similar way CQ does (IC_{50} = 35nM). This further confirmed the potentials of the new hybrid to reverse resistance to CQ in plasmodium species. The promising heme binding and hematin inhibition potential demonstrated by these hybrid compounds motivated the group to extend the work to in vivo anti-plasmodial study.

For appropriate extrapolation to human, in vivo pharmacological screening of a test drug is often design to assess the safety profile and efficacy of the lead compound in test model with similar characteristic to the intended target population and via the intended route of administration. Here, the group selected the best performing compounds (hybrid 9, 10, 12, 21,23 and 24) and *P. yoelii* murine malaria model as test items and system, respectively. To obtained aqueous form of the test hybrid, the compound was formulated in either phosphate or hydrochloride salt forms. The in vivo preclinical testing was conducted using 4 varying doses with five mice for each dose using oral route of drug administration. The outcome of the in vivo assays demonstrated that compounds 9 and 21 tend to be more potent with lower 50% effective dose (ED50) of 2 mg/kg/day and 6 mg/kg/day respectively. Similarly, these two compounds demonstrated good therapeutic margin as higher dose of 300 mg/kg and 453 mg/kg of respective compounds were well tolerated with no evident toxicity.

CLINICAL TRIAL REPORT OF SOME CHLOROQUINOLINE HYBRID/ BIOCONJUGATE

The effort toward combatting CQ resistance in plasmodium parasite, using CQ-hybrid/bioconjugate was not limited to in vitro and in vivo animal studies only, Clinical testing in human subject have been carried out on such agents. Drug such as Ferroquine, Napthoquine and aminoquinine are examples of CQ hybrid that underwent clinical trials [34,37,39]. Similarly, CQ-hybrid/conjugate in combination with other

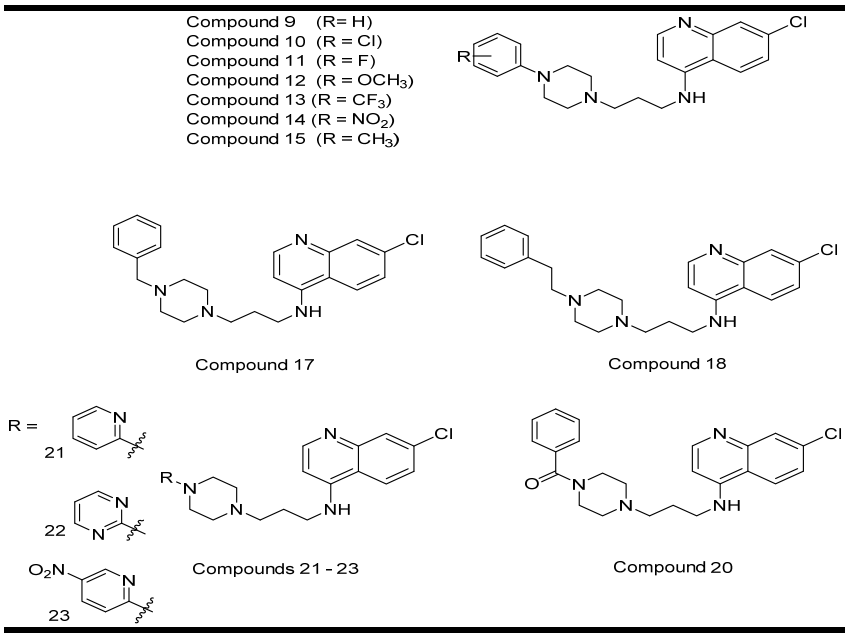


Figure 7: Structures of CQ hybrids 9-15, 17, 18, 20, 21, 23 and 24

antimalarial drugs have also been tested clinically. Artesunate-amodiaquine, dihydro-artemisinic-piperaquine and Ferroquine-Antigenome are classical examples of such combination [31,34,37].

In a clinical trial, Chotsiri, *et al.* [30], reported that, there was a profound effort to replace the well-known prophylactic anti-malarial regimen, sulfadoxine-pyrimethamine and amodiaquine with dihydroartemisinic- piperaquine in children for seasonal malaria prophylaxes. In this study, a population-based simulations and a covariate-free sigmoidal E_{MAX}-model indicated that dosage increment and duration of prophylactic-therapy extension up to four monthly predicts decrease malaria incidence by 58% despite the high transfection season.

In another study, Olliaro *et al*. [31] highlighted that, co-administration of artesunate with amodiaquine enhanced therapeutic efficacy across the multicenter where the trial took placed (Senegal, Gobon and Kenya).

Similarly, efficacy of the range of 97 – 99% cure has been documented in an 74hrs phase II trial when ferroquine was concomitantly administered with Artesunate (4mg/kg/24hr) with minimal dose of 2, 4 and 6 mg/kg/24hr ferroquine according to Mombo *et al.* [34]. Another Phase II clinical testing conducted by Sanofi, for combination of Ferroquine and Antigenome has recently been terminated (NCT02497612). The trial was stopped to reserved resources as all the treatment wings have met futility criteria for efficacy as revealed by pre-planned provisional assessment [35].

A phase IV clinical trial investigating the possibility of replacing Dihydroartemisinin-piperaquine (DHP), 3-day regimen with Artemisinin-Napthoquine (AN) single dose for *P. vivax* therapy indicated both arms possessed similar efficacy and safety profile. About 158 *P. vivax* infected individuals were recruited. 80 patients were treated with AN and 78 with DHP. Both treatment wings cleared parasite within 64 hours [37].

Clinical trial of a member of aminoquinoline group of CQ analogue, AQ-13, has been clinically tested in comparison with artesunate-lumefantrine for the therapeutic cure of uncomplicated *P. falciparum* infection. The finding of this trial indicated that, both treatment groups achieved similar end point, since erythrocytic parasite were eliminated by day seven in both arms and no serious untoward effects reported in both groups [39].

CHLOROQUINOLINE HYBRID/ BIOCONJUGATE IN CLINICAL USE

The effort toward combatting CQ resistance in plasmodium parasite, using the concept of conjugation biology and chemistry have seen

progress from bench tops (preclinical pharmacological screening) to bedside (clinical testing) in healthy volunteers and in intended patients' settings. With regards to combatting CQ resistance through "covalent biotherapy" involving hybrid compound generation or bioconjugate formations, some drug like piperaquine, amodiaquine, Ferroquine and Napthoquine are currently in clinical use in either single or combination forms.

Piperaquine

Piperaquine a hybrid obtained by coupling of CQ active portion with that of piperazine through replacement of nitrogen (at position 4) of piperazine with a 7-chloroquinolin-4-yl group is a clear example of CQ hybrid in clinical use for malaria treatment. It was developed through the independent work of Chinese and France pharmaceuticals in the year 1960s and was patronized for the treatment of *Plasmodium falciparum* until 1980 when its popularity declined due to development of resistance. However piperaquine returns to bedside but in combination with artemisinin derivatives for treatment of *P. falciparum* [30].

Amodiaquine

Amodiaquine is another member of chloroquinoline hybrids with related structure to that of CQ but more pharmaco-economical compared to the later. It is readily obtainable in many countries. Amodiaquine is more children-friendly due to its palatable taste unlike CQ [31]. In accordance with the WHO guidelines of using combination of drugs in combating malaria infection, Amodiaquine is used concurrently with Artesunate. This combination has produced excellent result in cure for malaria infections in most African countries [32].

Ferroquine

Ferroquine, a CQ bio-organometallic conjugate generated by coupling active ferrocenyl moiety with that of CQ [33] has received a lot of attention from various researchers due its promising CQ resistance reversing property. The mechanism via which ferroquine mediates it anti-plasmodial activity was suggested to be via hemozoin inhibition and generation of free radicals (ROS) which are both detrimental to the parasite survival [33]. The safety profile of ferroquine has been established and was found to possess a tolerable single dose of about 1.6g and 0.8g divided dose [34].

Napthoquine

Napthoquine another CQ hybrid and a product of "covalent biotherapy" was first developed in China around 1986 and has entered clinical trials about a decade later. It was formulated both as single and in combination form with artemisinin-derived anti-malarial agents in a dose ration of 2:5 mg/kg of artemisinin derivative to Napthoquine for the therapy of uncomplicated *Plasmodium falciparum and vivax* for the duration of 3 days in accordance the WHO guidelines [36].

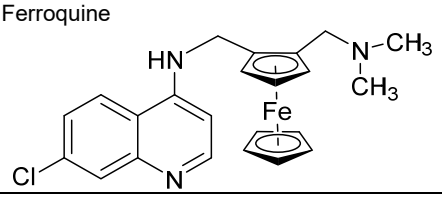
Aminoquinine

Aminoquinoline are CQ equivalent compound with reduced side chains. The work of Gunsaru *el al.* [38] demonstrated to the scientific community that, compounds with very close structure to CQ possessed CQ resistance reversing properties confirming that, the resistance to CQ is structure related. Investigation into the efficacy of these agents gained tremendous attention not only at preclinical level but also clinical trials as discussed above [39].

DISCUSSION

Bioconjugation involves covalent linkage of two or more pharmacophores one of which is of biological origin. On the other hand, a hybrid compound contains two or more chemical moieties obtained by chemical synthesis. In both

Table 1: Chloroquinoline hybrid/bioconjugate under clinical trials

Chloroquine hybrid/bioconjugate	Clinical trial phase	Status	Clinical trial.org ID	References
Ferroquine 	Phase II	Terminated	NCT02497612	[34]

cases, the intrinsic bioactive property of individual pharmacophore was coupled to one another and combined in a single agent as bioconjugate or hybrid compound [14, 26]. To tackle the problem of CQ-resistance in malaria, several researchers have hybridized CQ with other potent anti-malarial agent or other compounds that augment the effects of such agents. Similarly, CQ bioconjugates have been developed over the years to solve the problem of resistance in malaria and to stop pancreatic cancer metastasis in another instances [15].

Based on the findings of this review, various biologics and chemical compound can be used for bioconjugation or hybridization with CQ active moiety toward reversing resistance in malaria parasite such as amino acids, polysaccharides, cell penetrating peptide and organometallic compounds [14, 18, 21].

Findings from invitro anti-plasmodial assay against various strain of malaria parasites showed increasing trend in effectiveness with CQ structural modification via hybrid compound generation. The potency observed does not depend on aromaticity of the reversal agent according to Boudhar, *et al* [28]. While Gunsaru, *et al* [29] described the mode of their action as like that of CQ.

Bioconjugation of CQ with biologic is often receiving attention because of its relative cost effectiveness as alternative approach to restore CQ potency in malaria resistance parasite strain [14]. Pérez, *et al*.[25] reported that, conjugating cinnamic accid with QC resensitized resistant plasmodiun to CQ ,however the group emphasiszed the need to have a linker between the two pharmacophores for anti-plasmodial effect. Likewise, in an in vivo study, conducted by Tripathy, *et al* [21], CQ-Chitosan conjugate was found to significantly reduced *P. berghei* parasitemia by 97.59% as against 67.02% reduction produced by CQ. This need to be investigated in *P falciparum* resistance parasite.

The efforts toward reversal of CQ resistance through structural modification via hybrid compound generation or bioconjugate formation has reached clinical settings. Many antimalarials obtained through the above-mentioned approaches are either in phases II - IV trial (Table 1) or in clinical use such Amodiaquine [31], Ferroquine [34], Napthoquine [37] and Aminoquinoline [39].

This review has shown that, tremendous effort has been invested into the development of new chloroquinoline hybrids [28-34], with full determination to combat CQ resistance, but little has been done with regards to chloroquinoline bioconjugates development [14,18,21,25]. Conjugating biologic with CQ active moiety is an aspect that need to be given due attention. A CQ-antibody conjugate, CQ-aptamer Biocon-jugate are clear examples of possible future CQ-bioconjugates. For instance, an antibody or an aptamer with specific affinity for the PfCRT could be developed and conjugated with CQ. While the biologics will target PfCRT, CQ can concentrate in the parasite digestive vacuole and exert its therapeutic activity. It is good to notice that heme binding aptamer was successfully selected, and our group is currently working toward developing CQ-Aptamer bioconjugates.

Since development of aptamer is more economical and less complicated compared to generation of antibodies, an aptamer that has the potential to mimic the mechanism of action of other forms of antimalarial can be selected. For instance, aptamer with binding specific affinity to the enzyme mediating the parasite DNA synthesis, *P. falciparum* dihydrofolate reductase (PfDHFR) can be selected to mimic the mode of action of cycloguanil and pyrimethamine. Another aptamer capable of mimicking the mechanism of action of atovaquone that interferes with the pathway for the synthesis of pyrimidine through downregulating the expression of mitochondrial gene encoding the *P. falciparum* cytochrome b can also be developed. These aptamers when available can be used to form CQ-aptamer bioconjugates with dual mechanism of actions.

CONCLUSION

Most of the published article reviewed in this paper have demonstrated the need to put more efforts toward combatting CQ resistance in malaria parasite. Modification of CQ structure either by hybrid compound generation or bioconjugate formation have shown significant impact in improving the potency of modified CQ in comparison with the standard CQ. Due to high cost and time required for development of new antimalarial drug, molecular modification of CQ provides more pharmaco-economic approach toward combatting chloroquine resistance as indicated by most of the articled included in this review. CQ structural modification through CQ-hybrid generation and CQ-bioconjugate formation have received profound attention over the years. To ensure optimum resistance reversal potency with good safety profile, the new bioconjugate should consist of a near ideal biologic like an aptamer instead of antibody. This

is because, aptamers can be easily selected, are not antigenic and have high affinity for their target. Their three-dimensional structure gives them more advantage as a biological agent for modifying drug molecular structure.

DECLARATIONS

Acknowledgement

The authors would like to thank Individual Research University Grant by Universiti Sains Malaysia (RUI_1001/CIPPM/8012336), Higher Institution Centre of Excellent Grant by the Ministry of Education Malaysia (311/CIPPM/ 4401005) and Tetfund Nigeria for providing the scholarship.

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

We declare that this work was performed by the authors named in this article, and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. AWA and KMFM came up with the idea and drafted the initial manuscript, LCY drew the chemical structures using Chemdraw software and LJH helped in manuscript revision. All Authors read and approved the final manuscript.

REFERENCES

1. *Yu T, Fu Y, Kong X, Liu X, Yan G, Wang Y. Epidemiological characteristics of imported malaria in Shandong Province, China, from 2012 to 2017. Scientific Reports 2020; 10 (1):1-8.*
2. *WHO, World malaria report 2020: 20 years of global progress and challenges; 2020 [cited 2020 August 10] Available from http:// www.who.int/teams/global-malaria-programme/reports/world-malaria-report-2020.*
3. *Blasco B, Leroy D, Fidock DA. Antimalarial drug resistance: linking Plasmodium falciparum parasite biology to the clinic. Nature medicine 2017; 23 (8):917.*
4. *Roldan, EQ, Biasiotto G, Magro P, Zanella I. The possible mechanisms of action of 4-aminoquinolines (chloroquine/hydroxychloroquine) against Sars-Cov-2 infection (COVID-19): A role for iron homeostasis Pharmacological research 2020:104904.*
5. *Kim J, Tan YZ, Wicht KJ, Erramilli SK, Dhingra SK, Okombo J, Vendome J, Hagenah LM, Giacometti, SI, Warren AL. Structure and drug resistance of the Plasmodium falciparum transporter PfCRT. Nature 2019;576 (7786): 315-320.*
6. *Ch'ng JH, Mok S, Bozdech Z, Lear MJ, Boudhar A, Russell B, Nosten F, Tan KS. A whole cell pathway screen reveals seven novel chemosensitizers to combat chloroquine resistant malaria. Scientific reports 2013; (3) :1734.*
7. *Agrawal P, Manjithaya R, Surolia N. Autophagy-related protein PfATG18 participates in food vacuole dynamics and autophagy-like pathway in Plasmodium falciparum. Molecular microbiology 2020; 113 (4) :766-782.*
8. *Coronado LM., Nadovich CT, Spadafora C. Malarial hemozoin: from target to tool. Biochimica et Biophysica Acta (BBA)-General Subjects 2014;1840 (6): 2032-2041.*
9. *Rifaie-Graham O, Pollard J, Raccio S, Balog S, Rusch S, Hernández-Castañeda MA, Mantel PY, Beck HP, Bruns N. Hemozoin-catalyzed precipitation polymerization as an assay for malaria diagnosis. Nature communications 2019; 10 (1): 1-8.*
10. *Ecker A, Lehane AM, Clain J Fidock DA. PfCRT and its role in antimalarial drug resistance. Trends in parasitology 2012; 28 (11): 504-514.*
11. *Henry M, Alibert S, Rogier C, Barbe J, Pradines B Inhibition of efflux of quinolines as new therapeutic strategy in malaria. Current topics in medicinal chemistry 2008; 8 (7) : 563-578.*
12. *Kiarie WC, Wangai L, Agola E, Kimani FT, Hungu, C. Chloroquine sensitivity: diminished prevalence of chloroquine-resistant gene marker pfcrt-76 13 years after cessation of chloroquine use in Msambweni, Kenya. Malaria journal 2015; 14 (1): 328.*
13. *Noopur, D.; Aditya, T., Synthesis of safer chloroquine by ornithine bioconjugation. International Journal of Chemical and Natural Sciences 2014; 2 (5):145-148.*
14. *Sleightholm R Yang B, Yu F, Xie Y, Oupicky D. Chloroquine-modified hydroxyethyl starch as a polymeric drug for cancer therapy. Biomacromolecules 2017; 18 (8): 2247-2257.*
15. *Rádis-Baptista G, Campelo IS, Morlighem JE, Melo LM, Freitas VJ. Cell-penetrating peptides (CPPs): From delivery of nucleic acids and antigens to transduction of engineered nucleases for application in transgenesis. Journal of biotechnology 2017; 252 :15-26.*
16. *Benizri S, Gissot A, Martin A, Vialet B, Grinstaff MW, Barthelemy P. Bioconjugated oligonucleotides: recent developments and therapeutic applications. Bioconjugate chemistry 2019; 30 (2) :366-383.*
17. *Aguiar L, Biosca A, Lantero E, Gut J, Vale N, Rosenthal PJ, Nogueira F, Andreu D, Fernàndez-Busquets X, Gomes P. Coupling the antimalarial cell penetrating peptide TP10 to classical antimalarial drugs primaquine and chloroquine produces strongly hemolytic conjugates. Molecules 2019; 24 (24): 4559.*
18. *Pradines B, Alibert-Franco S, Houdoin C, Mosnier J, Santelli-Rouvier C, Papa V, Rogier C, Fusai T, Barbe J, Parzy D. In vitro reversal of chloroquine resistance in Plasmodium falciparum with dihydroethanoanthracene derivatives. The American journal of tropical medicine and hygiene 2002; 66 (6):661-666.*
19. *Li D, Fu D, Kang H, Rong G, Jin Z , Wang X, Zhao K. Advances and potential applications of chitosan nanoparticles as a delivery carrier for the mucosal immunity of vaccine. Current drug delivery 2017; 14 (1): 27-35.*
20. *Tripathy S, Chattopadhyay S, Dash SK, Chowdhuri AR,*

Das S, Sahu SK, Majumdar S, Roy S. Chitosan conjugated chloroquine: Proficient to protect the induction of liver apoptosis during malaria. International journal of biological macromolecules 2015; 74: 585-600.

21. *Silva AT, Bento CM, Pena AC, Figueiredo LM, Prudêncio C, Aguiar L, Silva T, Ferraz R, Gomes MS, Teixeira C. Cinnamic acid conjugates in the rescuing and repurposing of classical antimalarial drugs. Molecules 2020; 25 (1): 66.*
22. *Zhou, K.; Chen, D.; Li, B.; Zhang, B.; Miao, F.; Zhou, L., Bioactivity and structure-activity relationship of cinnamic acid esters and their derivatives as potential antifungal agents for plant protection. PLoS One 2017, 12 (4), e0176189.*
23. *Perez B, Teixeira C, Gut J, Rosenthal PJ, Gomes JR, Gomes P. Cinnamic acid/chloroquinoline conjugates as potent agents against chloroquine-resistant Plasmodium falciparum. ChemMedChem 2012; 7 (9):1537.*
24. *Pawełczyk A,. Olender D, Sowa-Kasprzak K, Zaprutko L. Hybrid compounds strategy in the synthesis of oleanolic acid skeleton-NSAID derivatives. Molecules 2016; 21 (4): 420.*
25. *Kanaani J,Ginsburg H. Effects of cinnamic acid derivatives on in vitro growth of Plasmodium falciparum and on the permeability of the membrane of malaria-infected erythrocytes. Antimicrobial agents and chemotherapy 1992; 36 (5): 1102-1108.*
26. *Egan TJ, Kaschula CH. Strategies to reverse drug resistance in malaria. Current opinion in infectious diseases 2007; 20 (6):598-604.*
27. *Boudhar A, Ng XW, Loh CY, Chia WN, Tan ZM, Nosten F, Dymock BW, Tan KS. Overcoming chloroquine resistance in malaria: design, synthesis, and structure-activity relationships of novel hybrid compounds. Antimicrobial agents and Chemotherapy 2016; 60 (5): 3076-3089.*
28. *Gunsaru B, Burgess SJ, Morrill W, Kelly JX, Shomloo S, Smilkstein MJ, Liebman K, Peyton DH. Simplified reversed chloroquines to overcome malaria resistance to quinoline-based drugs. Antimicrobial agents and chemotherapy 2017; 61 (5).*
29. *Desai C. Meyler's side effects of drugs: The international encyclopedia of adverse drug reactions and interactions. Indian Journal of Pharmacology 2016; 48 (2): 224.*
30. *Chotsiri P, Zongo I, Milligan P, Compaore YD, Some AF, Chandramohan D, Hanpithakpong W, Nosten F, Greenwood B, Rosenthal PJ. Optimal dosing of dihydroartemisinin-piperaquine for seasonal malaria chemoprevention in young children. Nature communications 2019; 10 (1): 1-12.*
31. *Olliaro PL, Mussano P. Amodiaquine for treating malaria. Cochrane Database of Systematic Reviews 2003; (2).*
32. *Adjuik M, Agnamey P, Babiker A, Borrmann S, Brasseur P, Cisse M, Cobelens F, Diallo S, Faucher J, Garner P. Amodiaquine-artesunate versus amodiaquine for uncomplicated Plasmodium falciparum malaria in African children: a randomised, multicentre trial. The Lancet 2002;359 (9315):1365-1372.*
33. *Biot C, Glorian G, Maciejewski LA, Brocard JS, Domarle O, Blampain G, Millet P, Georges AJ, Abessolo H, Dive D. Synthesis and antimalarial activity in vitro and in vivo of a new ferrocene− chloroquine analogue. Journal of medicinal chemistry 1997; 40 (23): 3715-3718.*
34. *Mombo-Ngoma, G, Supan C, Dal-Bianco MP, Missinou MA, Matsiegui PB, Salazar CLO, Issifou S, Ter-Minassian D, Ramharter M, Kombila M. Phase I randomized dose-ascending placebo-controlled trials of ferroquine-a candidate anti-malarial drug-in adults with asymptomatic Plasmodium falciparum infection. Malaria journal 2011;10 (1) 53.*
35. *Held J, Supan C, Salazar CL, Tinto H, Bonkian LN, Nahum A, Moulero B, Sié A, Coulibaly B, Sirima SB. Ferroquine and artesunate in African adults and children with Plasmodium falciparum malaria: a phase 2, multicentre, randomised, double-blind, dose-ranging, non-inferiority study. The Lancet Infectious Diseases 2015;15 (12):1409-1419.*
36. *Dubar F, Slomianny C, Khalife J, Dive D, Kalamou H, Guerardel Y, Grellier P, Biot C. The ferroquine antimalarial conundrum: redox activation and reinvasion inhibition. Angewandte Chemie 2013;125 (30):7844-7847.*
37. *Moore BR, Laman M, Salman S, Batty KT, Page-Sharp M, Hombhanje F, Manning L, Davis TM. Naphthoquine: an emerging candidate for artemisinin combination therapy. Drugs 2016; 76 (7):789-804.*
38. *Hasugian AR, Siswantoro H, Fay MP, Tjitra E. Artemisinin-napthoquine versus dihydroartemisinin-piperaquine in adult subjects with Plasmodium vivax infection. Health Science Journal of Indonesia 2015;6 (2): 92-98.*
39. *Koita OA, Sangaré L, Miller HD, Sissako A, Coulibaly M, Thompson TA, Fongoro S, Diarra Y, Ba M, Maiga A. AQ-13, an investigational antimalarial, versus artemether plus lumefantrine for the treatment of uncomplicated Plasmodium falciparum malaria: a randomised, phase 2, non-inferiority clinical trial. The Lancet Infectious Diseases 2017;17 (12) :1266-1275.*

.