Mefloquine derivatives: synthesis, mechanisms of action, antimicrobial activities.

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Malaria is a serious public health problem. More than 100 tropical and sub-tropical countries are endemic for malaria. Pregnant women and children are the most sensitive to this disease and more than 750 000 people die of malaria each year. Among the five species of Plasmodium, P. falciparum is the parasite responsible for the most serious form of the disease and it has developed varying degrees of resistance to many classes of antimalarial drugs; consequently the development of new drugs to treat malaria is in full rise. Mefloquine was proposed, for a long time, as the drug of choice for chloroquine-resistant malaria. Emergence of resistance to mefloquine and its associated severe side effects have limited the use of this drug. Nowadays, there is no effective alternative, especially, in the zone of multi-drug resistance. Since a few years, the mechanisms of resistance to antimalarial drugs, the mechanism of action of the mefloquine and the origin of the side effects begin to be understood. On these bases, several approaches are considered to enhance the design of the mefloquine including its synthesis as a pure enantiomer or even the modulation of the mefloquine core as a pure enantiomer.

Keywords: malaria, mefloquine, mechanisms of action, resistance, quinolinemethanol.

1. Introduction

According to the World Health Organization (WHO), more than 100 tropical and sub-tropical countries are endemic for malaria and as many as 3.3 billion people are exposed to malarial infection [1]. Each year, more than 250 million people suffer from acute malaria, and over 750,000 people die of malaria. Most of the deaths are among young children under the age of five. Malaria also represents a significant threat to travelers and military personnel deployed for long periods of time in countries where malaria is endemic [2]. Human malaria is caused by protozoa belonging to five species of the genus Plasmodium. The parasites are transmitted to humans through bites of female Anopheles mosquitoes. Plasmodium falciparum, which is dominant in Africa, is the most pathogenic one and responsible for most of the fatalities. P. falciparum has developed varying degrees of resistance to many classes of antimalarial drugs; consequently the development of new highly efficacious drugs to treat malaria remains a public health priority. Surprisingly whereas the development of chloroquine analogues has been extensively reported without much success, fewer research is carried out on mefloquine analogues [3]. Since mefloquine was proposed as the drug of choice for chloroquine-resistant malaria, the synthesis of new analogues keeps all its interest to overcome the emergence of resistance toward mefloquine. Throughout this review, we will give an overview of the development of mefloquine derivatives. The mechanism of action, resistance, synthesis and structure-activity relationships as well as other activities of antimalarial amino-4-quinolinemethanol will be described.

2. Mefloquine

The first treatment of malaria dates back to the early 18th century and made use of the bark of Cinchona trees. In 1820, Pelletier and Caventou isolated quinine (Fig. 1), a 4-quinolinemethanol, as the active compound of this crude bark. Thus, quinine was the first pure chemical compound used to treat malaria, it represents the first generation of quinolinemethanol [4]. During World War I, quinine was used for prophylaxis and routine treatment by Allied and German armies. Unfortunately, natural production became insufficient and its total synthesis was too complex for commercial production. In addition, important side effects such as fever, confusion, respiratory arrests and arrhythmias were frequently associated with quinine treatment [4]. These data led British and German scientists to develop synthetic alternatives. Shortly after World War II, chloroquine (CQ), a 4-aminoquinoline, and pyrimethamine largely replaced quinine. CQ is the most used because it is effective, inexpensive and involves little side effects when used at the prescribed doses. In the early 1960s, in an effort to eradicate malaria, CQ was used extensively. Afterwards, the first case of CQ resistance appeared and today, CQ -resistance strains of P. falciparum are common in all endemic areas throughout the world. CQ resistance was responsible of heavy losses during the Vietnam War. Consequently, a massive screening program, undertaken by the Walter Reed Army Institute of Research (WRAIR), started in USA and a score of years later some of 300,000 compounds had been screened [5]. Among these compounds, more than 300 4-quinolinemethanols were included among which approximately one hundred 2-phenyl-4-quinolinemethanol-type compounds [6]. The most promising drug to emerge was SN 10275 (Fig. 1), a 6,8-dichloro-2-phenyl-α-(2-piperidyl)-4-quinolinemethanol. It was tested in man but provided a long-lasting phototoxic reaction [7]. After pharmacomodulation
of the 2-phenylquinolinemethanol core to reduce phototoxicity, WR030090 (Fig.1) was discovered. Its high activity with less phototoxic effects led to its use by the U.S. Army for the treatment of recrudescent malaria infections during the 1970s [8]. Unfortunately, this compound was only partially effective as a prophylactic agent and required a dosing regimen similar to that of quinine. These problems were due to its unfavorable pharmacokinetic characteristics and subsequently it was abandoned. SN 10275 and WR030090, in which a 2-phenyl substituent is introduced, represent the second generation of quinolinemethanol. During this same period, a third generation of quinolinemethanol was discovered and is represented by WR 142490 (Fig. 1), subsequently named mefloquine (MQ). In this structure, the 2-phenyl moiety of the previous generation is replaced by a trifluoromethyl group. Due to its more potent activity with no appreciable phototoxicity, this quinine derivative was thus developed and marketed, under the name Lariam® by Hoffman La Roche and the U.S. Army [9]. For a long time, MQ was the drug of choice for U.S. military deployments because its longer half-life allows weekly administration and thereby makes compliance less problematic. But, MQ use was rapidly associated with neuropsychiatric side effects and, for some years now, the spread of resistance to MQ has limited its use. In addition, MQ is relatively expensive compared to other antimalarial drugs, which limits its accessibility to developing countries. Yet, MQ is particularly efficient against chloroquine-resistant strains of malaria parasites. In addition to its longer half-life as compared to others antimalarial drugs, MQ is safe in the second half of pregnancy [10].

![Fig. 1 Structures of some quinoline-based antimalarial drugs.](image1)

2.1 Mefloquine structure and synthesis of different stereoisomers.

MQ is a α-2-piperidinyl-2,8-bis(trifluoromethyl)-4-quinolinemethanol and possesses two asymmetric carbon atoms. It exists under two racemic forms, *erythro* and *threo*, each one composed of a pair of enantiomers (Fig. 2). In 1974, Caroll and Blackwell prepared all four optical isomers and assigned the both relative and absolute configuration of the *erythro* and *threo* racemates by NMR and circular dichroism studies [11]. One the one hand, the (+)- and (-)-*erythro* have the (11R, 12S) and (11S, 12R) configurations, respectively. On the other hand, the (+)- and (-)-*threo* have the (11R,12R) and (11S,12S) configurations, respectively.

![Fig. 2 Optical isomers of mefloquine](image2)

In clinical practice, MQ is commonly used as a racemic mixture of *erythro* enantiomers; (+)-(11R,12S)-α-2-piperidinyl-2,8-bis(trifluoromethyl)-4-quinolinemethanol and (-)-(11S,12R)-α-2-piperidinyl-2,8-bis(trifluoromethyl)-4-quinolinemethanol. Two studies have shown an equal activity for the four optical isomers of mefloquine against *P. berghei* in mice [11] and against *P. yoelii* [12]. Basco et al. [13] reported a best activity for the *erythro* racemate against *P. falciparum in vitro* as compared with pure enantiomer, (+)-*erythro* and (-)-*erythro*. But, the use of MQ as an enantiopur isomer could be more beneficial. Indeed, in another study, Karle et al. showed that (+)-enantiomers of MQ were more potent than (-)-enantiomers by a factor of 1.69 for *erythro* mefloquine and 1.95 for *threo* mefloquine (IC50 values against Sierra Leone and Indochina *P. falciparum* strains) [14]. Moreover, neurological side effects associated with MQ use seems to be due to the (+)-enantiomer of the mefloquine [15]. Consequently, due to the different properties and activities of the different MQ enantiomers, several syntheses have been realized in order to obtain enantiopur MQ.

The first synthesis of racemic MQ was published in 1971 by Ohnmacht et al. [16]. As shown in Fig. 3, this synthesis begins with the condensation of *p*-trifluoromethylaniline with ethyl 4,4,4-trifluoroacetate in the presence of polyphosphoric acid to provide a 4-quinolone which was converted to 4-bromoquinoline 3 using POBr3. A carboxylation reaction led to cinchonic acid 4 with 86% yield. Addition of 2-pyridyl lithium salt to this acid provided a pyridylketone 5 which, is reduced with H2/Pt, gave MQ. This stereospecific reduction led only to the *erythro* enantiomers.
Since this first synthesis, new synthetic routes have been explored, in particular to improve the process of preparation of the pyridylketone 5 previously reported by Ohnmacht in 1971 [17]. Figure 4 shows the first asymmetric synthesis of (11R,12S)-mefloquine which was reported in 1993 by Roche’s group using an enantioselective Rhodium-catalyzed hydrogenation of 2-pyridyl-2,8-bis(trifluoromethyl)-quinolineketone 5 [18]. The corresponding (R)-alcohol (R)-6 was obtained using an unsymmetrical ligand containing one dicyclohexylphosphino and one diphenylphosphino moiety, which afforded 91% ee. Later, Schmid’s group tried to improve this enantioselective hydrogenation using several others Rh catalyst but the best result was obtained with the ligand previously reported by Roche [19]. In the two cases, the second hydrogenation leads to a mixture of (11R,R)-α-2-piperidinylquinolinemethanol and (11R,S)-α-2-piperidinylquinolinemethanol with quantitative yield in a ratio of 5.7:1, respectively.

More recently, Xie et al. [20] reported a new and enantioselective synthesis of the (11R,12S)-MQ hydrochloride using a proline-catalyzed asymmetric direct aldol reaction and a Beckmann rearrangement as key steps as shown in Fig. 5. Formylation of 4-bromoquinoline 3 was conducted in presence of n-BuLi/DMF to give aldehyde 7 in 71% yield. The key aldol reaction is carried out by reaction of this aldehyde with cyclopentanone in presence of 30% of proline as catalyst. After chromatography purification, the sym-aldol 8 and the anti-aldol 9 were obtained with 69% yield with a ratio of 6.8:1. Then sym-aldol 8 reacted with NH₂OH.HCl to give oxime 10 which was put in presence of TsCl in a Beckmann rearrangement. Finally, reduction with BH₃.DMS followed by addition of condensed HCl affords to give (11R,12S)-MQ hydrochloride in a 70% yield. The experimental analysis confirmed the absolute configuration of (+)-MQ as (11R,12S) as previously reported by Caroll and Blackwell [11].

2.2 Mechanisms of action of Mefloquine and derivatives

The life cycle of malaria parasites is complex and consists in several distinct phases. Briefly, there are two basic cycles; an asexual cycle in humans and a sexual cycle in the female Anopheles mosquito. The asexual cycle can be further divided into a pre-erythrocytic stage (in liver) and an erythrocytic stage. The clinical symptoms of malaria, such as intense fever, exclusively occur during this erythrocytic phase. Indeed, just after the bite of an infected mosquito, the parasite establishes an asymptomatic infection of liver cells (lasting 7 to 15 days for P. falciparum). Incubation thus begins and will at least last one week. Plasmodia parasites are then released in the blood stream develop and multiply.
inside host erythrocytes. During this intra-erythrocytic stage, the parasite ingests the cytosol of the infected cell by a mechanism of endocytosis into its food vacuole. This cytosol contains more than 95% of hemoglobin which is an essential source of amino acids for the parasite. Hemoglobin digestion within the parasite food vacuole by aspartic proteases leads to the release of a proteic moiety (globin), potential source of aminoacids, and a free heme moiety, ferrirrprotoporphyrin IX. The latter moiety is toxic for the parasite. Indeed, it can generate reactive oxygen species which may induce membrane and DNA damages [21]. To overcome the free heme toxicity, the malaria parasite possesses efficient heme detoxification systems. The most important mechanism of detoxification takes place in the parasite food vacuole. The acid conditions (pH = 5.2) of this digestive vacuole promote the conversion of heme into insoluble and non-toxic crystals termed hemozoin or malaria pigment [22]. The remaining free heme passes through the food vacuole membrane and reaches the cytosol of the parasite. Within the cytosol three additional systems of detoxification exist. Thus, free heme can either be neutralized; (i) by interaction with glutathione (GSH), (ii) by binding with others proteins such as glutathione S-transferase or \textit{P. falciparum} glutathione reductase [23] or by (iii) reaction with H$_2$O$_2$, which is generated by spontaneous oxidation of the released heme in the cytosol [24].

Like 4-aminoquinolines, ary laminoalcohols as MQ primarily acts on the intra-erythrocytic asexual stages but its mechanism of action is not completely understood. It is known that 4-aminoquinolines inhibit both the hemozoin formation and the oxidative and glutathione-dependent degradation of heme [25]. Heme interaction is the major mode of action of 4-aminoquinolines. The resulting quinoline-heme complexes formed are toxic for the parasite, just like free heme is. Studies showed that MQ and quinine inhibit the accumulation of hemozoin in infected cells [26]. However, MQ interacts relatively weakly with free heme ($K_a = 3 \times 10^{-7}$ M to $1.6 \times 10^{-5}$ M) as compared to CQ ($K_a \approx 3.5 \times 10^{-9}$ M) [27]. Indeed, due to its lower basicity (pKa$_1 = 2$/pKa$_2 = 8.6$ vs pKa$_1 = 8.1$/pKa$_2 = 10.2$ for CQ), MQ accumulates less inside the acidic food vacuole and the concentration required to inhibit heme polymerization is not achieved. Other mechanisms of action could explain the antimalarial activity of MQ. In 1982, Chevli \textit{et al.} [28] showed that MQ binds with phospholipids with a good affinity. Since the malaria parasites are enriched in phosphatidylinositol, these authors suggested that the possible interaction between MQ and phosphatidylinositol could be the second mode of action of MQ. In 1996, Desneves \textit{et al.} [29] identified two high-affinity MQ-binding proteins with apparent molecular masses of 22–23 kDa and 36 kDa in \textit{P. falciparum}-infected erythrocytes. The identities of these polypeptides have not been established yet, but they may be involved in MQ uptake or action. In 1999, Famin \textit{et al.} [30] and more recently our group [31] showed \textit{in vitro} activity of MQ on GSH-mediated hemin degradation. However, when a red blood cell leaky white ghost was used as model membrane (membrane being a target for the toxic activity of free heme): the amount of heme dissolved in ghost membranes as well as partition coefficients were lower for MQ and quinine as compared to CQ [30]. These authors are also showed that both MQ and quinine no affect GSH-mediated degradation of membrane-associated hemin. Maertins \textit{et al.} [32] described that MQ affects volume-regulated anion channels (VRAC). This transmembrane pathway would be used by the parasite to bring substrates necessary to its growth and to allow the efflux of toxic compounds. This suggests that VRAC may also be a target of action for MQ. In 2002, Famin and Ginsburg reported a reduction of hemoglobin levels in MQ–treated \textit{P. falciparum} FCR3 parasites [33]. They suggest that MQ could act by blockage of endocytosis. Indeed, two years later, Hoppe \textit{et al.} [34] showed that MQ inhibits endocytosis in the \textit{P. falciparum} D10 strain.

### 2.3 Mefloquine resistance

The first case of resistance to arylaminoalcohols was observed back in 1910 with quinine. Resistance to MQ appeared in 1982 in Thailand where MQ was used intensively [35]. Then, pockets of clinical resistance to MQ were reported in Southeastern Asia [36]. In order to overcome the spread of the MQ resistance, drug combinations such as MQ/artesunate [37] or MQ/artemether [38] were introduced. Thus, most of the parasites are removed by the most active

### 3.5 Mefloquine resistance

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a transport of the drugs out of the cell. Consequently, as previously suggested by Foley and Tilley [25], an overexpression of Pgh-1 could result in an enhancing transport of MQ. However clinical resistance to MQ may also occur in the absence of pfmdr1 amplification in some cases [38]. Indeed, some strains such as D6 strain, derived from Sierra Leone P. falciparum isolate, exhibit a natural resistance to MQ. Some molecules, such as reversal agents or chemosensitizers, are able to interact with efflux pumps in order to restore the activity of antimalarial drugs. In 1987, verapamil, a calcium channel-blocker, was the first antimalarial reversal agent discovered [47]. Verapamil reverses CQ-resistance but possesses no effect on MQ-resistance. Reversal of MQ-resistance was observed with penfluridol, an antipsychotic agent [48]. Recently, others reversal agents of MQ-resistance have been reported such as cyproheptadine, NP-30 and icajine [49].

2.4 Side effects of Mefloquine

MQ use is associated with undesirable side effects on the central nervous system (CNS) such as nausea, vertigo, and more serious ones such as disturbed sleep, heightened anxiety, hallucinations, depression and acute psychosis [50]. Indeed, several experiments showed that MQ accumulates in the CNS and interacts with cellular targets within a pharmacological concentration range. The incidence of severe neuropsychiatric symptoms were reported to be 1 out of 10,000 with prophylactic use and 1 out of 200-1200 patients with therapeutic use. Efforts were made to explain the neuropsychiatric disturbances associated with MQ use. Several mechanisms are proposed including interactions with the cholinergic system, interactions with the human P-glycoprotein, blockage of the human adenosine A2A receptor, inhibition of ATP-sensitive potassium(K)-channels or even perturbations of the endoplasmic reticulum (ER).

The effects of MQ on the cholinergic system were demonstrated in 1994 by Speich and Haller [51]. They reported that physostigmine, an anticholinesterase agent, could reverse the neuropsychiatric side effects due to MQ. In 1997, Baudry et al. [52] demonstrated that MQ crosses the blood-brain barrier in the rat but not its quinoline carboxylic acid derivative metabolite. A stereoselective cerebral transport of MQ has been established, in rat [53], in mice [54] and in human [55], in each specie the cerebral concentration of (-)-MQ being higher than that of (+)-MQ. These data suggest that the brain penetration of (-)-MQ is greater than its corresponding enantiomer. In contrast, there is a species-dependent stereoselective pharmacokinetic in the plasma. In rat, plasma concentrations of (+)-MQ were higher than those of its enantiomer, opposite to those observed in mice and in human [53-55]. Pharmacokinetics differences between the two enantiomers were already reported by these authors [56]. Baudry et al. [52] proposed four mechanisms which could explain the stereoselective (-)-MQ brain distribution; (i) active passage through the blood-brain barrier; (ii) passive passage through the blood-brain barrier with higher availability of (-)-MQ in the plasma compartment; (ii) possible metabolism in the brain; and (iv) stereospecific efflux. In 2004, Barraud et al. [54] demonstrated, for the first time, in vivo (in mice), that an enantioselective cerebral efflux transport is implied. MQ and most particularly (+)-MQ would be able to interact with human P-glycoprotein (P-gp) pumps. P-gp is a membrane-bound ATP-dependent drug efflux pump which plays important detoxification roles, particularly at the blood-brain barrier. Its activity results in the decrease of the intracellular concentration of toxic agents and xenobiotics such as MQ. Experiments showed that (+)-MQ was excreted back to the central nervous system more rapidly than its enantiomer. Indeed, Gillespie et al. [57] reported a stereoselective antagonism effect of MQ on central nervous adenosine receptors. The (-)-enantiomer of MQ binds to the adenosine receptor, particularly subtypes A2, while (+)-enantiomer is without significant activity at this binding site. Adenosine receptor antagonists such as theophylline, a bronchodilator drug which is a mixed antagonist of adenosine A1 and A2A receptor, or caffeine may be involved in seizure disorders [58]. The inhibition of ATP-sensitive K-channels, which are present in neurons of the CNS may also be an explanation for the neurotoxicity of MQ as mentioned by Gribble et al. [59]. In 2003, Dow et al. [60] indicated that neurotoxicity of MQ could be mediated through a perturbation of ER function via a disruption of calcium homeostasis. Indeed, prolonged disruptions in Ca$$^{2+}$$ homeostasis can result in damage of neuronal function and cell death [61]. Neuronal death in specific brain regions could explain symptoms as vertigo, anxiety and disturbances in emotion. As previously mentioned by Dow in 2004 [62], several approaches could be considered to decrease the MQ neurotoxicity; (i) administration of neuroprotective drugs (physostigmine); (ii) reformulation of MQ as a pure enantiomer ((+)-erythro-MQ); (iii) modulation of MQ core and; (iv) modulation of MQ core as a pure enantiomer.

3. Mefloquine derivatives

The structure-activity relationships of chloroquine and related quinoline antimalarial compounds were reviewed extensively [4,5,63]. Structure-activity studies of MQ indicated that the presence and the orientation of the hydroxyl and amine groups are essential to antimalarial activity [64]. Consequently, the synthesis of MQ analogues has been developed.
3.1 Quinolinemethanol derivatives

In 1971, Cheng [65] then Chien and Cheng [66] showed, using molecular models and mass spectral data, that the active conformation of antimalarial compounds (analogues of MQ) is such that it allows hydrogen bonding between the amine of the piperidine and the hydroxyl function. These data are in accord with other results found in a series of phenanthrenemethanols [67]. The presence of a substituent such as phenyl group into the 2-position of the quinoline ring (in place of trifluoromethyl group) can induce, or greatly augmente, the antimalarial activity but increases the phototoxic effects of the quinoline [68]. Sweeney et al. [69] reported that the aromatic ring system could be interchangeable with a phenanthrene or a pyridine ring. Since some years, the interest to synthesis of MQ derivatives have increased and complementary structure-activity relationships have been established.

In order to reduce MQ neurotoxicity, Dow et al. [62] synthesized C2, C6 and C8 quinoline substituted MQ analogues. Some of them showed curative antimalarial activities in vivo (P. falciparum strains W2, D6 and multi-drug resistant TM91C235) with a higher therapeutic index than MQ against TM91C235 (Fig. 6). Among the most active compounds, the compound WR007930 is neutrutoxic in the same concentration range as MQ, as shown in Table 1. Shortly after, the same authors reported the antimalarial activity and pharmacological properties of a library of 21 2-phenyl substituted dialkylaminoquinolinemethanols related to WR030090 cited above (Fig. 1) [70].

![Fig. 6 Two mefloquine-derivatives (Dow et al. 2004-2006)](image)

The most promising compound in terms of activity and neurotoxicity was the compound WR069878 (Fig. 6). This non-piperidine analogue is even less neurotoxic than piperidine analogues previously described by Dow due to the opening of piperidine ring. Moreover, the cost of treatment with WR069878 is estimated much lower than that with MQ. Nevertheless, complementary studies concerning the phototoxicity, which is the main side effect of the second generation of quinolinemethanols, remain to be carried out. This compound also showed a weak metabolic stability.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Antimalarial activity (IC50 against P. falciparum strain in nM)</th>
<th>Neurotoxicity (IC50 in µM)</th>
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<tbody>
<tr>
<td>MQ</td>
<td>9.9, 33</td>
<td></td>
</tr>
</tbody>
</table>

a. chloroquine resistant and mefloquine sensitive.
b. chloroquine sensitive but naturally less susceptible to mefloquine.
c. resistant to mefloquine, chloroquine and pyrimethamine.

Recently, Dow et al. [71] described several libraries of new racemic 2,8-trifluoromethylquinolinemethanol compounds with their antimalarial activities and physicochemical properties. The main modification of the quinoline scaffold concerns the 4-position which is substituted by a non-piperidine moiety in order to reduce neurotoxicity by a limited absorption through the blood-brain barrier. These analogues were prepared from a racemic epoxide 14 which can be synthesized by two routes shown in Fig. 7. The first one begins by the bromination of the 2,8-trifluoromethyl-4-quinolinol with phosphorus oxybromide to afford a 4-bromo-2,8-trifluoromethylquinoline. This compound was put in reaction with n-BuLi/DMF to give a quinoline-4-carboxaldehyde 7. Racemic epoxide 14 was then obtained using Corey’s dimethylsulfonium methylide. The first step of the second way consists equally to the formation of the 4-bromo-2,8-trifluoromethylquinoline. The second step is a Stille reaction catalyzed by Pd2(dba)3 which leads to a vinyl compound intermediate 13 with 62% yield. An oxidation with meta-chloro peroxybenzoic acid (m-CPBA) affords the racemic epoxide. The ring epoxide opening by different commercially available amines provided a library of 200 4-position MQ analogues.
The structure-activity relationships principally concern the nature of the amino side chain. The authors have established that: (i) a reduction of electron density around the nitrogen atom diminishes the antimalarial activity (for example a phenylamino group has a much lower activity than a benzylamino group); (ii) the presence of a second nitrogen atom on the side chain reduces the ability to cross the blood-brain barrier and therefore the corresponding neurotoxicity; (iii) access of the second amine must be restricted for good potency (for example methylamine was less active than propylamine); (iv) the presence of two carbon atoms between the two nitrogen atoms, like in WR308396, and/or a second amine cyclic promoted antimalarial activity (Fig. 8).

Among the most promising compounds, the activities and physicochemical properties of WR 177000 and WR 308336 were compared between. Both compounds were active in vivo after oral administration in the blood schizonticidal P. berghei-ICR mouse model and were more potent than MQ against different strains of P. falciparum as shown in table 2. WR 177000 is the most active compound against MQ-resistant strains of P. falciparum (C235 and C2A strains) and is metabolically stable. But, due to its lipophilicity, it has a greater propensity for accumulation into the CNS than MQ, as showed by the measure of permeability across MDR1-transfected MDCK cell monolayers in table 2. WR308336 which contains an additional H-donor (vs. WR177000) exhibited a lower permeability as compared to WR177000 but has an equivalent permeability compared to MQ. Thus, authors are currently investigating new libraries of compounds based on the rational lead optimization of diaminoquinolinemethanols such as WR308336. This second generation of diaminequinolinemethanols should have an equivalent potency to MQ with a reduced permeability across the blood-brain barrier. Since these compounds have been evaluated as a racemate mixture, the biological evaluation of the corresponding pure enantiomers or diastereoisomers is envisaged.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Physicochemical properties</th>
<th>Antimalarial activity (IC₉₀ against P. falciparum strain in ng/ml)</th>
<th>Permeability</th>
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<tr>
<td></td>
<td>logD</td>
<td>HBDs&lt;sup&gt;a&lt;/sup&gt;</td>
<td>W²&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>MQ</td>
<td>3.1</td>
<td>2</td>
<td>6.2 ± 2.8</td>
</tr>
<tr>
<td>WR177000</td>
<td>2.8</td>
<td>2</td>
<td>1.7</td>
</tr>
<tr>
<td>WR308396</td>
<td>3.3</td>
<td>3</td>
<td>6.2</td>
</tr>
</tbody>
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<sup>a</sup> HBDs = H-bond donors
<sup>b</sup> chloroquine resistant and mefloquine sensitive.
<sup>c</sup> chloroquine sensitive but naturally less susceptible to mefloquine.
<sup>d</sup> resistant to mefloquine, chloroquine and pyrimethamine.
<sup>e</sup> resistant to mefloquine, chloroquine and pyrimethamine (two pfmdr1 copy strain).
<sup>f</sup> apparent permeability in the typical direction across MDR1-transfected MDCK cell monolayers (units in 10<sup>-6</sup> cm/s).
Several compounds showed a promising antimalarial activity, in the range of nanomolar, against *P. falciparum* W2 and 3D7 strains. These compounds exhibited equally good antimicrobial activities. One of the enantiomers is two to fifty times more active than the others [75]. These results are supplementary proofs concerning the fact that the antimalarial activity is dependent on the stereoselectivity of the drugs. A more detailed structure-activity relationships is currently being developed through the synthesis of additional analogues. The elucidation of the mechanism of action of the lead compounds is still under investigation.

### 3.2 Quinolinemethanol dual drugs

Since April 2001, [76] to overcome the resistance of malaria to conventional drugs, the use of artemisinin-based combination therapy (ACT) is the treatment of choice in the corresponding countries. Artemisinin derivatives are particularly effective in combination because of their very high killing rates, lack of adverse effects, and absence of significant resistance. In malaria drug combination therapy, the current trend is to co-formulate two or more agents into a single tablet, termed as multi-component drug (e.g., Coartem®, a lumefantrine-artemether association) as opposed to the traditional cocktail therapy, so as to improve patient compliance. However, based on the wide interest in hybrid molecules and the numerous encouraging efficacy and toxicity reports, the next generation of antimalarial drugs will undoubtedly be hybrid drugs. As aforementioned, MQ combined to artemisinin derivatives (artesunate, artemether...) constitutes one of the most effective combinations for the treatment of MQ- and multidrug-resistant *P. falciparum*. It combines the immediate effect of artemisinin derivatives with the prolonged effect of MQ. In 2005, Grellepois *et al.* [77] described two new dual molecules, shown in Fig. 10, in which a fluorinated artemisinin derivative is covalently linked to a MQ moiety; (i) by an easily cleavable ester bond (19), or (ii) by a non cleavable bond (20). The two chimeras were highly active against both CQ- and MQ-sensitive and resistant strains in the low nanomolar range. The divisible molecule 19 was slightly more efficient (IC50 = 2.4-6.6 nM) than the indivisible chimera (IC50 = 10.6-17.2 nM) in vitro. This difference was confirmed by preliminary *in vivo* experiments in mice. Moreover, the divisible chimera was as active the reference drug, artemether, in inhibiting the parasite growth. These results showed that the diester linker must be hydrolyzed *in vivo* to liberate the two pharmacophores and achieve maximum efficiency.

Recently, Varotti *et al.* have studied a salt 21 derived from MQ·HCl and artesunate (Fig. 10) [78]. This salt exhibited better activity than artesunate and MQ against *P. falciparum* strains (W2 and 3D7). Its mechanism of action showed that two intracellular targets are involved: endoplasmic reticulum via disruption of Ca2+ homeostasis in the parasite and food vacuole by alteration of pH gradient. Other drugs could be combined covalently to MQ as for example chemosensitizers. This concept was previously studied with success with the association of CQ and imipramine [79].
4. Other antimicrobial properties of mefloquine and its derivatives

Racemic mixtures of MQ analogues were synthesized and evaluated for other antimicrobial properties such as the treatment of bacterial infections. Like for antimalarial drugs, there is a great urge to propose new drugs due to the multidrug resistance of bacteria. During a screening carried out at the Walter Reed Army institute of research, MQ and its derivatives were tested as antibacterial agents [80]. MQ exhibited bactericidal activity against several Gram-positive bacteria such as *Staphylococcus aureus* (Minimal Inhibitory Concentration (MIC) = 16 µg/ml) or *Streptococcus pneumoniae* (MIC = 0.4-4.8 µg/ml). However, MQ was not active against gram-negative strains. Some MQ derivatives that possess a piperidine attached to methanol in its 2-position were more active than MQ against staphylococci, enterococci and other Gram-positive bacteria except *S. pneumoniae*. Other substitutions led to compounds without activity [81]. MQ was tested against different strains of *Streptococcus spp.*, *Escherichia spp.* and *Neisseria gonorrhoeae* and demonstrated even a high activity against *S. pneumoniae* (MIC = 0.06 µg/ml vs 4 µg/ml for sulphadoxine-pyrimethamine as reference). Previous studies showed that its target would be a bifunctional ATP synthase/ATPase [82]. In 2009, Vidal-Aroca et al. [83] reported MQ as an efflux-pump inhibitor of the Resistance-Nodulation-Division (RND) family in *Pseudomonas aeruginosa*. This efflux pump contributes to multi-drug resistance and, thus, MQ could be used in combination with drug whose activities are compromised by efflux to restore its activity.

MQ was found to be relatively active against non-replicating persistent (NRP) tuberculosis strain (NRP-TB) [84]. Stereoselective activity was reported, indeed, in this study, the (+)-erythro-MQ (MIC = 7 µM) were more active than the (±)-threo-MQ (MIC = 17 µM) against NRP *Mycobacterium tuberculosis* and the (+)-erythro isomer was less cytotoxic than the (-)- erythro isomer as in the case of antimalarial activity. Several libraries of MQ-derivatives were synthesized and tested against replicating (R-TB) and NRP tuberculosis strains and two hit compounds (Fig. 11) emerged of these structural modifications; (i) hydrazone 22 which has an improved anti-TB activity with both a lesser cytotoxicity and a lesser predicted CNS side effects than MQ [85] and (ii) isoxazole derivative 23, the isoxazole scaffold emerged following a screening against *M. tuberculosis* [86]. As expected by the authors, this last compound is the most active against R-TB (MIC = 0.9 µM vs 4 µM for 22 and 13 µM for MQ) with a less cytotoxicity (IC50 > 128 µM) than both MQ (IC50 = 11 µM) and 22 (IC50 = 37 µM).

5. Conclusion

Since a few years, many efforts have been made in order to find alternatives compounds to current antimalarial drugs. MQ has several advantages; a good activity against chloroquine-resistant stains, a long half-life and a relatively safety in pregnancy. The newest data concerning its mechanism of action, the origin of its side effects and the structure-activity relationships already established should permit a better design of the MQ core to propose new antimicrobial agents. For example, MQ derivatives as an enantiomeric pure compounds could reduce the side effects of MQ and an hybrid molecule with a MQ derivative moiety could limit the resistance spread.
References


