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Review

The key role of heme to trigger the antimalarial activity of trioxanes

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Contents

Abstract

Artemisinin is an efficient antimalarial drug containing a 1,2,4-trioxane, which is able to alkylate heme both in vitro and in vivo, giving rise to covalent heme–artemisinin coupling products. The low valent iron(II) protoporphyrin-IX, which is the prosthetic group of hemoglobin, induces the homolysis of the peroxide bond of artemisinin by an inner-sphere electron transfer. The generated alkoxy radical is quickly rearranged to a C-centered radical, which efficiently alkylates the heme macrocycle at *meso* positions. Heme is therefore both the activating agent and the target of artemisinin.

On the basis of this mechanism of action, a new family of peroxide-based antimalarials named trioxaquines® has been synthesized. Trioxaquines are made by the covalent attachment of a trioxane, having alkylating ability, to a quinoline, known to easily penetrate within infected erythrocytes. Several trioxaquines are active in vitro on chloroquine resistant malaria parasite at nanomolar concentration, and are efficient by oral route to cure infected mice at 20–50 mg/kg.

Keywords: Alkylation; Heme; Artemisinin; Trioxaquine; Malaria

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1. Introduction: iron in human body

Metal ions are ubiquitous in living organisms. Besides the major sodium, magnesium, potassium and calcium ions, several transition metals are essential. Iron is the most abundant within an adult human body containing ca. 4 g of iron. Apart from the major sodium, magnesium or potassium which freely circulate in the plasma, ferric ions are bound with high affinity and selectivity to transferrin and ferritin, the proteins responsible for its transport and storage, respectively. The free iron concentration in human plasma, limited at physiological pH by the low solubility of iron hydroxide, is as low as 10^{-16} M.

Iron is an important component of many cellular redox reactions and is required for essential enzymes. Heme represents one of the most ubiquitous and stable forms of redox active iron among living organisms. It is required as a prosthetic group for a vast number of proteins or enzymes (e.g. hemoglobin that accounts for ca. 55 wt.% of iron in humans, myoglobin, cytochromes, peroxidases, catalases, nitric oxide synthase, etc.) involved in various processes such as oxygen and electron transport and metabolism of small molecules. Iron–sulfur clusters Fe_nS_n are also involved in electron transfer proteins.

The ability of iron of heme (or Fe–S clusters) to accept and donate electrons is a key feature, which contributes mostly to its potential toxicity in vivo. In normal metabolism, during the sequential reduction of molecular oxygen, radical intermediates can be formed, including superoxide (Fig. 1). Hydroxyl radical can also be formed by the Fenton-catalyzed cleavage of hydrogen peroxide. For this reason, all aerobic organisms have developed sophisticated regulatory systems (superoxide dismutase, peroxidases, catalases) to maintain the lowest possible concentration of reactive oxygen species (ROS). In addition, iron does not dissociate from heme in physiological conditions without the oxidation of the macrocycle itself by heme oxygenase. The redox potentials of iron within heme-proteins are adjusted by close interactions with essential residues of the apoprotein.

2. Heme in malaria-infected red blood cells

Malaria is a parasitic disease due to the erythrocyte infection by a *Plasmodium* species transmitted to man by the bite of an infected anopheles mosquito. *Plasmodium falciparum* is the parasite species responsible for the lethal cases that cause 1–3 million deaths each year. The parasites have become resistant to the main classes of drugs, making the formerly efficient chloroquine useless in many tropical countries, and leading to the come-back of a high level of malaria in recent decades [1,2]. Even for the reference drug quinine, there are now assessments of progressive emerging resistance [3]. Besides the tropical regions, owing to the different aspects of malaria evolution, areas that used to be affected by malaria in the past, namely the southern parts of USA and south of Europe, might not be out of concern in the next decades.

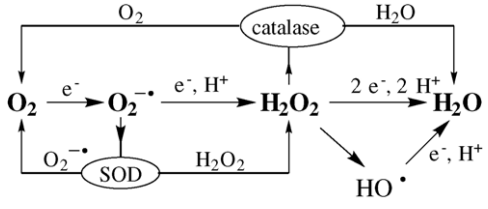


Fig. 1. Sequential reduction of molecular oxygen to water.

2.1. Digestion of hemoglobin

After inoculation, the parasite first invades and develops within hepatocytes, and then colonizes erythrocytes where its asexual multiplication is achieved. The symptomatology of malaria is due to this erythrocytic phase. The synchronous maturation of parasites leads to the erythrocyte burst together with an attack of fever and deep anemia. At each red blood cell burst (every 48 h for *P. falciparum*), parasites are released for further erythrocytic reinvasion.

Within infected erythocytes, up to 70% of the host hemoglobin is imported into a specialized acid compartment of the parasite (food vacuole), and broken down by specific proteases [4,5]. The amino acids released by this catabolic process are used by the parasite to build its own proteins. Of the four equivalents of heme by hemoglobin molecule, only a minor amount is degraded and used as source of iron for the parasite.

2.2. Aggregation of heme

In hemoglobin, iron is essentially in the ferrous state. Upon degradation of globin and release of heme, the iron(II) protoporphyrin-IX is able to reduce molecular oxygen which induces the cascade of reduced oxygen species. This results in a lethal oxidative stress within the parasite, which lacks the heme oxygenase that vertebrates use for heme catabolism. To avoid the heme-mediated oxidative damages, the released heme is converted to a redox inactive iron(III)–heme microcrystalline aggregate called hemozoin [6]. Hemozoin is primarily formed by the dimerization of heme, a carboxylate function of one heme molecule A being an axial ligand of the iron(III) of a second heme molecule B, and a carboxylate of B being an axial ligand of the iron of A. These dimers aggregate through hydrogen bonds of the remaining carboxylates, leading to a material, which is insoluble in biological conditions and accumulates in the parasite food vacuole [7]. Hemozoin is usually named a ‘polymer’, but it is not a covalent polymer, and the parasitic enzymes involved in its formation are not polymerases. In fact, the aggregation of heme dimers by non-covalent links is related to supramolecular chemistry.

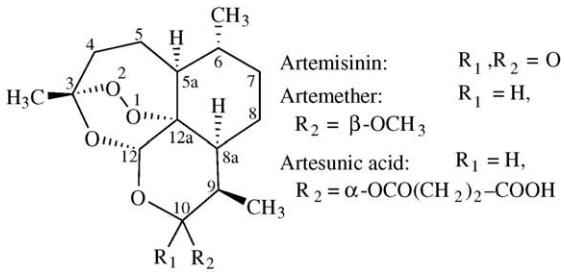


Fig. 2. Structure of artemisinin and some hemisynthetic derivatives.

Any perturbation of this heme detoxification process that is unique to *Plasmodium* is expected to have drastic consequences for the parasite survival, due to the accumulation of free redox active heme residues.

The control of malaria largely depends on drugs that disrupt this heme aggregation process, which is assisted by the parasitic histidine-rich protein (HRP). For instance, chloroquine has a high affinity for ferric heme (K_d = 3.5 nM) [8] as a result of a strong π-stacking between the quinoline ring of the drug and the porphyrin macrocycle [9–11]. Other quinoline-based antimalarials are supposed to interact with heme in a similar way.

3. Alkylating ability of artemisinin triggered by heme

3.1. The antimalarial peroxide artemisinin

Among the molecules that emerged over the past three decades as antimalarial drugs, artemisinin, extracted from the Chinese wormwood *Artemisia annua*, has a peculiar structure (Fig. 2), and thus a different mechanism of action is expected for this drug. Artemisinin is a sesquiterpene containing a 1,2,4-trioxane, and the dialkylperoxide function is the key of its biological activity, the deoxyartemisinin being completely devoid of biological activity [12,13]. However, artemisinin is poorly soluble in water and in oil, which are commonly used as vehicles for drug administration. For this reason, hemisynthetic derivatives obtained by reduction and functionalization of the lactone function of artemisinin are usually preferred (Fig. 2). These compounds are suitable for oral administration [14]. They have been intensively used for more than 20 years in south east Asia without any serious side effects or clinically relevant resistance up to now [15]. In addition, the induced development of resistance to artemisinin derivatives in mice was shown to be slow and reversible [16]. However, their use in monotherapy is associated with a high recrudescence rate (that should not be mistaken for inherent parasite resistance) due to their short plasma half-lives [17]. For this reason, they are now combined with more slowly eliminated drugs such as mefloquine or lumefantrine [18–20]. In addition, due to the erratic supply of the parent compound artemisinin, it is necessary to design new, cheap and accessible synthetic drugs based on the same mechanism of action. However, understanding the essential features of the mechanism of action of artemisinin is necessary to design synthetic antimalarial peroxides.

3.2. Mechanism of action of artemisinin

Artemisinin and related peroxide-containing drugs are active against malaria parasites at nanomolar concentrations in vitro, whereas the toxic concentration toward healthy erythrocytes is in the micromolar range. On the basis of in vitro experiments, it was initially proposed that the mechanism of action of this drug involved a heme-mediated oxidative stress leading to the destruction of the parasite. Heme was found to catalyze the reductive decomposition of artemisinin and dihydroartemisinin in vitro. When incubated with normal erythrocytes, artemisinin was shown to increase the methemoglobin concentration and to slightly reduce the intracellular glutathione and membrane fatty acid concentrations, resulting in a dose-dependent increase of cell lysis [21]. However, these experiments were performed at concentrations ranging from 50 to 1000 μM, that is 10^3 to 10^5 times higher than pharmacologically active drug concentrations. The parasite death in the presence of artemisinin at curative concentrations is therefore probably not due to non-specific or random cell damage caused by freely diffusing oxygen radical species, but might involve specific processes and targets, some of them now being under investigation.

When a peroxide is activated by a low-valent transition metal complex, the main reaction pathway is the homolytic cleavage of the weak peroxide bond [22,23]. The reductive activation of artemisinin or related peroxides by iron(II) heme or iron(II) salts have been reviewed [24–28].

3.3. Covalent heme–artemisinin adducts

Alkylating species generated by ferrous iron-mediated homolytic cleavage of the endoperoxide function of artemisinin, in particular the alkyl radical centered at position C4 of artemisinin (or related 1,2,4-trioxanes), were early proposed to be important [29]. However, the concentration of free iron ions in living cells is close to zero, but heme concentration is high in red blood cells. After preliminary experiments with synthetic metalloporphyrins [30], we have reported that iron(II) protoporphyrin-(IX) incubated with artemisinin, in the presence of a reducing agent able to generate iron(II) heme, was readily converted in high yield to heme–artemisinin covalent adducts. These adducts result from alkylation of the four *meso* positions of the macrocyclic ligand by the C4-alkyl radical derived from artemisinin (Fig. 3) [25,31,32]. The heme-mediated reductive activation of the peroxide bond generates an alkoxy radical, which quickly rearranges via a β-fragmentation process, to a primary alkyl radical centered at C4. Intramolecular addition of this akylating species occurs without regioselectivity on the four *meso* carbons of the protoporphyrin

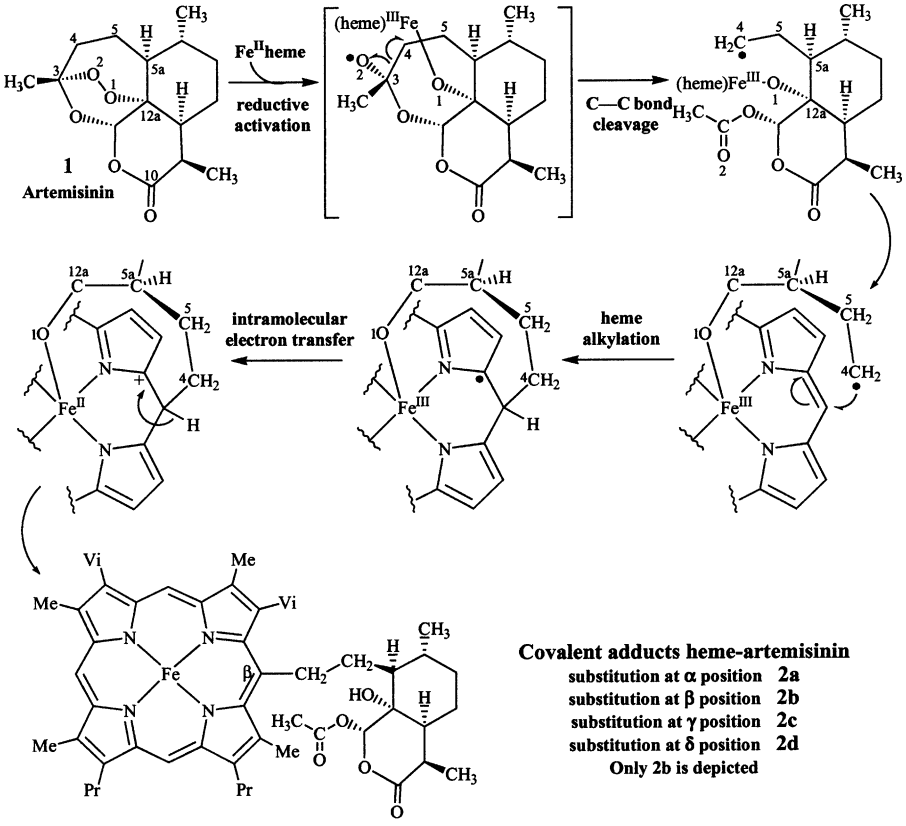


Fig. 3. Alkylation of heme by artemisinin. Alkylation occurred at four *meso* positions, only alkylation at β-position is depicted.

ligand. After demetallation of the heme moiety, complete NMR characterization of these adducts has been obtained [33].

These heme–artemisinin covalent adducts do not depend on the nature of the reducing agent used, provided that it is able to reduce iron(III) to iron(II)–heme (glutathione, hydroquinone derivatives, ascorbic acid or sodium dithionite were used).

In addition, artemisinin is able to alkylate heme in non-denaturated human iron(II) hemoglobin in the absence of any added protease, or even in the presence of high amounts of non-heme proteins when artemisinin was added to human, hemolyzed whole blood [34]. These results clearly indicate the high reactivity of this drug with hemoglobin under very mild conditions. It is also consistent with the fact that the inhibition of hemoglobin proteolysis does not antagonize the antimalarial activity of artemisinin derivatives, as it does for 4-aminoquinoline drugs [35]. In this regard, the selective toxicity of artemisinin to malarial parasites is probably due to the selective accumulation of the drug into the parasite within infected erythrocytes compared to non-infected erythrocytes [36,37]. It has been suggested that artemisinin might enter the parasite through the tubovesicular membrane network, a *Plasmodium*-specific transport process, which delivers essential extracellular nutriments to the parasite [38–40].

The alkylating ability of artemisinin toward heme or parasite proteins seems clear, but the correlation with its parasiticidal activity is still a matter of debate [41]. These heme–artemisinin covalent adducts **2** (Fig. 3) were recently detected in the spleen and urine of mice infected with *Plasmodium berghei* which have been treated with artemisinin by intraperitoneal or oral route. In the urine of these mice, the hydroxylated and glucuro-conjugated derivatives of the covalent adducts were also detected by LC–MS [42]. This result confirms that the alkylation of heme by artemisinin is not only a chemical laboratory production, but that these adducts are also generated in infected mice, but not in control animals.

3.4. Heme–drug adducts with other antimalarial peroxides?

By studying a series of trioxanes, a correlation was established between the alkylating ability of a drug-derived alkyl radical and the pharmacological activity. A heme model was used in these studies, namely manganese(II) tetraphenylporphyrin, having a fourth-order symmetry and only the β-pyrrolic positions as possible alkylation sites. When Mn^{II}TPP was reacted with artemisinin, artemether, or pharmacologically active trioxanes, a chlorin-type adduct was formed by reaction of the macrocycle with an alkyl radical generated

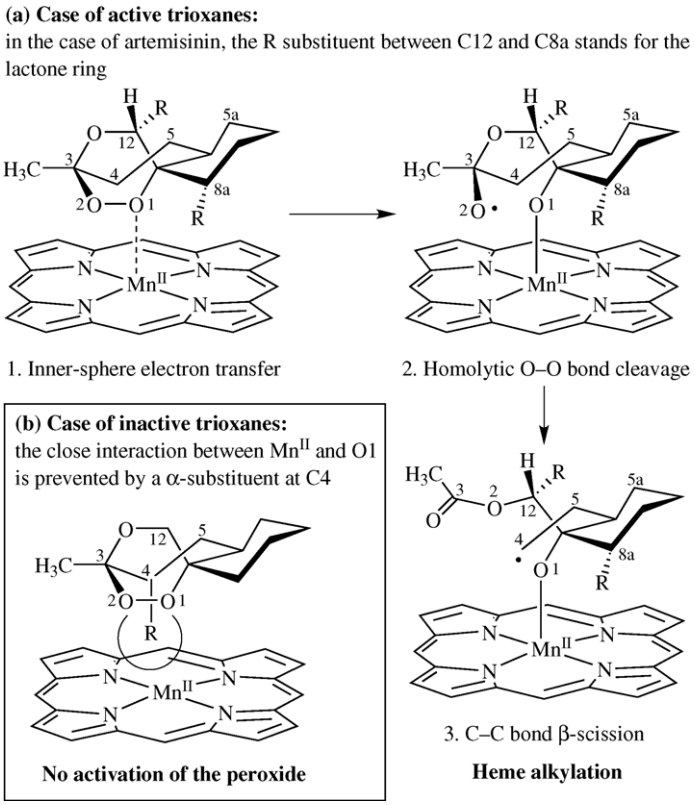


Fig. 4. Activation of trioxanes via an inner-sphere electron transfer. Possible correlation between pharmacological activity and alkylating ability.

by reductive activation of the drug endoperoxide [26,30,43]. On the contrary, most of the inactive drugs were unable to alkylate the porphyrin ring. Synthetic trioxanes bearing a bulky substituent on the α face (on the same side of the drug mean plane as the endoperoxide) were inactive toward malaria-infected red blood cells and also unable to alkylate the macrocyle (Fig. 4). α-Substituents at C5a position were also found detrimental to the docking of the trioxane derivative on heme [44]. These studies suggested that (i) a close interaction between the metal center and the peroxide bond is required, suggesting that this activation occurs through an inner-sphere electron transfer and (ii) alkylation ability is crucial for the antimalarial activity of artemisinin and is a general feature required for the biolog-

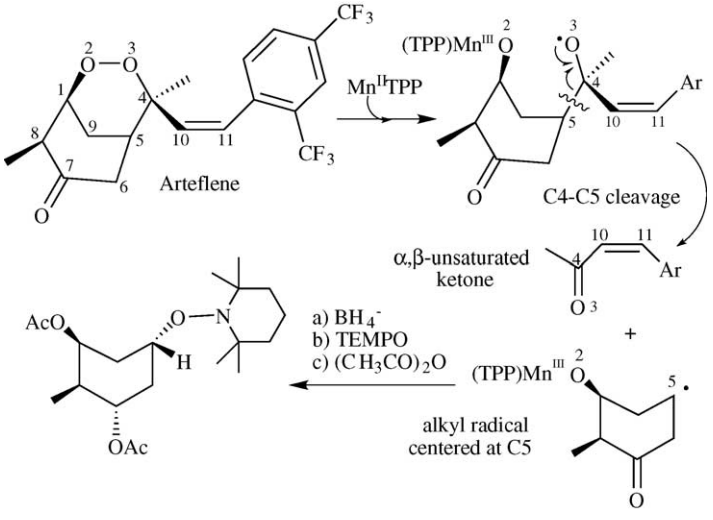
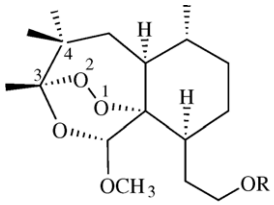


Fig. 5. Activation of arteflene by $Mn^{II}TPP$.



4,4'-dimethylsubstituted trioxane

Fig. 6. Structure of a 4,4′-disubstituted trioxane.

ical activity of endoperoxide-containing antimalarial drugs [26].

Another case is that of arteflene (Ro 42-1611, Fig. 5) which is efficient in vitro [45]. The reductive activation of the peroxide bond of arteflene by $Mn^{II}TPP$ induces the homolytic cleavage of the C4–C5 bond. The resulting secondary alkyl radical centered at C5 was not in suitable position to alkylate the porphyrin ligand via an intramolecular process, but it could be trapped by TEMPO, enabling the characterization of the two fragments of arteflene resulting from the peroxide homolysis [46,47]. It should be noted that, after incubation of [^{14}C11]arteflene with *P. falciparum*-infected red blood cells, the radioactive label was present in adducts between the drug and parasitic proteins [48]. Because C11 is not present in the alkyl radical at C5, this species cannot be involved in the arteflene-derived adducts with parasite proteins. In contrast, the C11 atom is incorporated in the α,β-unsaturated ketone which was generated in parallel with the alkyl radical at C5. In vivo, this enone may react with nucleophilic side chains of amino acid residues, leading to covalent adducts with parasitic proteins.

An exception to this feature is found in a 4,4′-*gem*-dimethylsubstituted trioxane (Fig. 6), which was activated by iron(II)–heme to a C4-centered radical, in spite of the presence of an α-methyl substituent, but was unable to alkylate the heme ring [41]. However, the 4,4′-dimethyl trioxanes and their 4α-methyl analogs are at least 100 times less active in vitro than the 4β-methyl derivatives [28].

Therefore, it appears that $Mn^{II}TPP$ alkylation may be a test for the screening of potentially antimalarial peroxides. However, the docking of active peroxides with this tetraarylporphyrin may be slightly different than with heme itself, due to the different substitution pattern.

3.5. Possible biological roles of covalent heme–artemisinin adducts

Although it is clear that artemisinin can efficiently alkylate heme, the role of this event in the in vivo antimalarial activity has been questioned. For example, interaction of heme–artemisinin adducts with *P. falciparum* histidine-rich protein (PfHRP-II), that promotes aggregation of heme to hemozoin, has been recently reported. This protein contains repeats of the sequence Ala-His-His, these two amino acids representing 76% of the mature protein [49]. HRP-II is able to bind approximately 50 molecules of heme at pH 7 [50], and 17 at pH 4.8, two histidine residues being axial ligands of each iron of heme. Thus, this histidine-rich protein acts as a scaffold for the initiation of hemozoin chains [51]. Recent studies suggest that heme–artemisinin adducts are able to bind PfHRP-II with a higher affinity than heme itself, to displace heme from PfHRP-II (either low pH or chloroquine can dissociate heme, but they cannot dissociate heme–artemisinin adducts from PfHRP-II [52]. However, the reason of such a high affinity and the nature of interactions between heme–artemisinin adducts and PfHRP-II are yet unclear. The binding of heme–artemisinin adducts to HRP-II with high affinity may hinder further sequestration of toxic heme as hemozoin, thus poisoning the parasite with its own waste [53,54].

In cell-free conditions, artemisinin inhibits hemozoin formation at micromolar concentrations [55]. The biological relevance of this fact remains controversial: artemisinin treatment of living parasites caused no measurable change in hemozoin content [56]. However, the concentration of the heme pool (hemoglobin + free heme) that accumulates within the parasite food vacuole during hemoglobin proteolysis may be as high as 400 mM [53], and free heme can damage cellular metabolism at micromolar concentration [54]. Consequently, a very small portion of heme that escapes the aggregation process (for example, 1 heme molecule over 10^4 or 10^5) should be sufficient to kill the parasite via a catalytic redox process without having a detectable effect on the hemozoin accumulation.

3.6. Other activators, other target(s) for artemisinin?

When malaria parasites are incubated in the presence of radiolabeled artemisinin, dihydroartemisinin or arteether, the radioactivity is associated with covalent adducts with hemozoin [57] and with a small number of specific parasite proteins, probably via alkylation [58]. Despite some difficulties to quantify the relative proportions of labeled heme and proteins, data indicate that drug-derived radioactivity is associated with heme on one side and with proteins on the other side. The reaction of artemisinin with proteins appears to be specific, since the alkylated proteins (detected by mass spectrometry) are not the most abundant in the parasites. This alkylation process is probably pharmacologically relevant, since all the related active peroxides alkylate the same proteins, while no proteins were alkylated by the inactive deoxyarteether (one oxygen atom is missing in the peroxide bridge) [48].

One of the most heavily labeled proteins was later isolated from parasite grown in the presence of [^{3}H]dihydroartemisinin and identified as a 25 kDa malaria translationally controlled tumor protein (TCTP) homolog that is able to bind heme with a modest affinity [59]. In vitro, the reaction of dihydroartemisinin with recombinant TCTP is clearly dependent on the presence of heme. The single cysteine residue

of this protein also appears to be necessary, probably serving as a source of electron for the heme-mediated activation of the drug. Although it is difficult to understand how alkylation of TCTP could kill the parasite because little is known about the physiological roles of this protein, the fact that the reaction occurs both in vitro and in vivo suggests that it is biologically relevant.

The selective inhibition of PfATP6, a sarco/endoplasmic reticulum Ca^{2+}-ATPase, by artemisinin has been recently reported [60]. The iron chelator desferrioxamine significantly abrogated the inhibition of PfATP6 by artemisinin, suggesting that an unidentified iron species was responsible for the activation of artemisinin leading to drug-derived radical species. The fact that the concentration inhibiting PfATP6 in oocyte membranes is two orders of magnitude higher than the in vitro IC_{50} values of this drug is a matter of question. However, the metabolism of calcium in *Plasmodium* may be a new drug target [61,62].

It was also reported that a high concentration of artemisinin (200 μM) inhibits the cysteine protease activity of purified food vacuoles of *Plasmodium*, which accounts for 30% of the parasite proteolytic activity. The remaining 70% of proteolysis, due to aspartic acid proteases that are responsible for the initial cleavage of the globin chains in large peptide fragments, is unchanged in the presence of artemisinin [55].

In addition, several hemoproteins, such as hemoglobin, catalase, and cytochrome *c* were found to easily react with artemisinin. After incubation with a labeled drug, a part of the hemoglobin- or catalase-bound radioactivity was associated with the protein moiety, rather than with heme. But heme-free globin did not react with artemisinin. These data suggest that, in hemoproteins, heme catalyzes the alkylation of the protein moiety [63]. The characterization of heme–artemisinin adducts indicates that heme can be both the trigger and one of the targets of artemisinin. But there is also evidence that heme is not the single target. Within a lifetime as short as 1–3 ns, a small organic molecule can move over a distance of 8–14 Å in a water solution at room temperature [64]. Such a displacement is sufficient for a C-centered radical generated by a heme residue to escape and alkylate a protein located in the close proximity of the heme, before being trapped by molecular oxygen. Finally, other biological reduced iron species may play the same activating role toward artemisinin than heme does.

4. Other antimalarial drugs related to artemisinin: trioxaquines®

A lot of effort has been made in recent years to develop new antimalarial drugs [65]. Among them, chloroquine has been modified by covalent link with a ferrocenyl entity. This drug named ferroquine is active against chloroquine-resistant strains of malaria parasite [66]. Bisquaternary ammonium salts were designed to target the parasite choline transporter [67]. Synthetic trioxanes, simplified analogs of artemisinin, supposed to act in the same way, have also been developed [68].

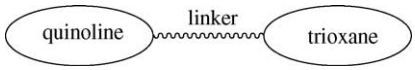


Fig. 7. Trioxaquines are dual antimalarial molecules.

The combination of artemisinin derivatives with a second drug having a different mode of action is a good way to increase the efficacy of the treatment, and to prevent the emergence and the spread of drug resistance [1,18]. As artemisinin and chloroquine both interact with heme, but by two different mechanisms, we designed new chimeric molecules named trioxaquines® (Fig. 7). They combine, in a single molecule, a potentially alkylating trioxane (as in artemisinin), and a 4-aminoquinoline (as in chloroquine) known to easily penetrate within infected red blood cells [69]. The first synthesized trioxaquines were found highly active in vitro on laboratory strains (chloroquine-sensitive and chloroquine-resistant ones) of *P. falciparum*. The drug concentrations necessary to reduce the parasitaemia by 50% (IC_{50} values) ranged from 8 to 40 nM, depending on the trioxane substituents, on both strains. Trioxaquine DU1102 has also been tested in vitro on human isolates in Yaoundé (Cameroon) and was found to be active on all isolates (IC_{50} mean value = 43 nM) [70].

4.1. Synthesis of trioxaquine DU1301

For obvious reasons, antimalarial drugs must be cheap and easily accessible. Trioxaquines were prepared through a convergent synthesis based on classical reactions. Many simple modulations are possible, leading to a large family of new potentially active molecules. As an example, the synthesis of a second-generation trioxaquine (DU1301) is depicted in Fig. 8. In trioxaquine DU1301, the amine and the peroxide substituent can be *trans* or *cis* with respect to the cyclohexane ring. The reductive amination reaction therefore provided two diastereomeric racemates *trans*-DU1301 and *cis*-DU1301 (50/50) (Fig. 9). For structure elucidation and biological evaluation, the two diastereomers of DU1301 have been separated and their structures were determined by X-ray diffraction. The structure of *trans*-DU1301 is depicted in Fig. 10 [71].

4.2. Alkylating ability of trioxaquine DU1301

The alkylating ability of trioxaquine DU1301 toward iron(II) heme was evaluated in similar conditions as reported for artemisinin derivatives. At 37 °C in 1 h, 60% of heme was alkylated by two alkyl radicals generated by the reductive activation of the peroxide bond (Fig. 11). With artemisinin, no alkylation product arising from a putative radical on O1 has been demonstrated (Fig. 3). On the other hand, in the case of DU1301, two different routes produced alkoxy radicals either on O1 or on O2, giving rise to two different heme–drug adducts **4** and **5** [72].

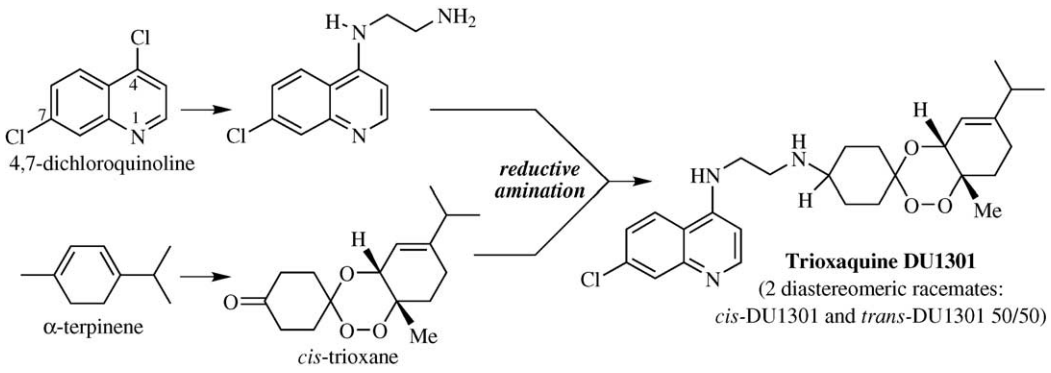


Fig. 8. Convergent synthesis of trioxaquine DU1301.

4.3. Biological activity of trioxaquine DU1301

The antimalarial activity of the dicitrate salt of trioxaquine DU1301 has been tested in vitro on a chloroquine-sensitive and two chloroquine-resistant strains. The *trans*-DU1301, *cis*-DU1301, and the 50/50 mixture of the two diastereomers exhibit similar activities, with IC_{50} values of 7–19, 5–11 and 6–17 nM, respectively. The dicitrate salt of trioxaquine DU1301 has also been tested in vivo, on mice infected by *Plasmodium vinckei*. The ED_{50} values were 5 and 18 mg/kg/d for a 4-day treatment by intraperitoneal or oral route, respectively. These values are in the range reported for artemisinin. Moreover, complete cure of parasitaemia without recrudescence has been obtained at 20 mg/kg/d (ip route) or 50 mg/kg/d (oral route). An absence of toxicity has been observed by oral route both on non-infected mice treated with 100 mg/kg/d for three consecutive days, and on infected mice treated with 120 mg/kg/d for 4 days [71].

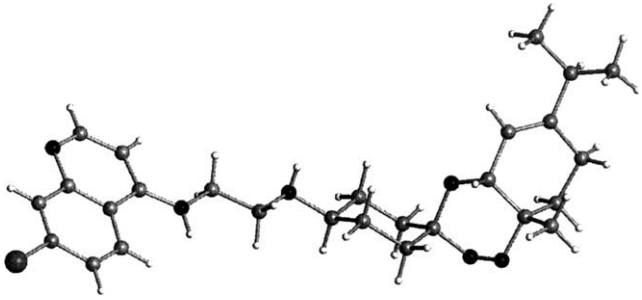


Fig. 10. X-ray structure of *trans*-DU1301 (conformer C).

Finally, the possible genotoxicity may be a matter of concern for compounds having alkylating ability. The genotoxicity of DU1301-$(citrate)_2$ has therefore been evaluated. Drugs that damage DNA induce systems of DNA repair such as the SOS-response, and the ability to induce this phenomenon in *Escherichia coli* has been shown to be correlated with

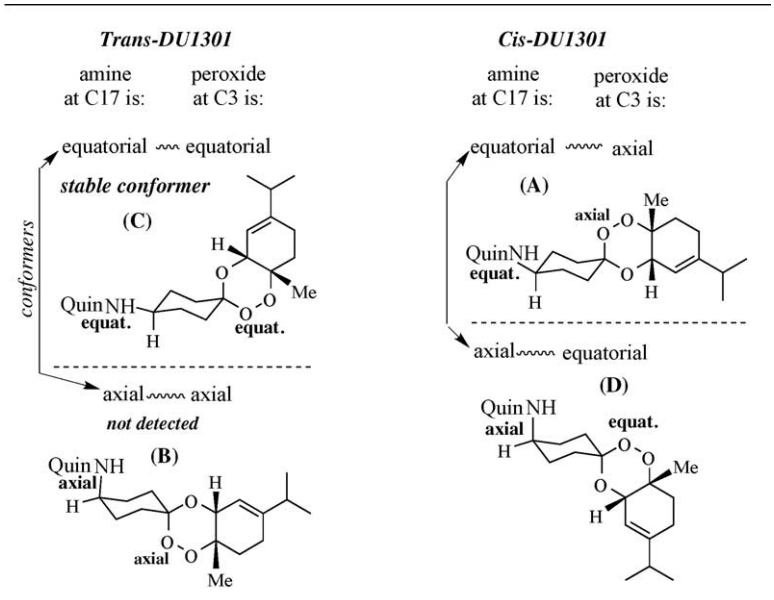


Fig. 9. The two diastereomeric racemates of DU1301, the amine and the peroxide function being either axial or equatorial with respect to the cyclohexane ring. Quin-NH refers to the aminoquinoline residue.

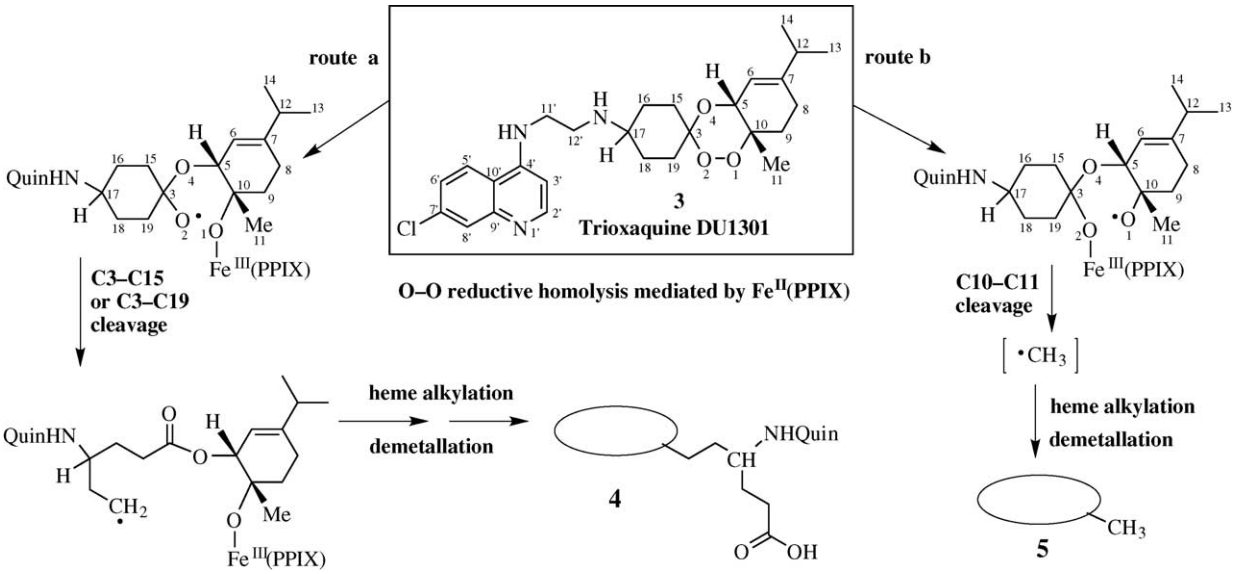


Fig. 11. Alkylation of heme by trioxaquine DU1301. Alkylation occurred at several *meso* positions, only alkylation at β position is depicted. The oval stands for the protoporphyrin-IX macrocycle.

the genotoxicity in humans [73]. The possible induction of the SOS-response by DU1301-(citrate)$_2$ in *E. coli* has been studied, using the anticancer drug mitomycin C as control. Whereas mitomycin C was genotoxic at 3 μM, the trioxaquine DU1301-(citrate)$_2$ was unable to induce the SOS-response up to 20 μM, a concentration 1000 times higher than its antimalarial IC_{50} value [71].

The high efficacy of DU1301 in vitro and in vivo, in particular on chloroquine-resistant strains, its easy synthesis and its chemical stability, its absence of toxicity are making this trioxaquine a promising drug candidate for antimalarial therapy.

5. Conclusion

At the interface of inorganic chemistry and biology, the few metal-based drugs known; have some spectacular large-scale medicinal applications (for example, *cis*-platin derivatives or MRI contrast agents). For these complexes, the metal plays a key role in the drug [74,75]. Beside this category, some organic compounds used as prodrugs must be activated in vivo by a metal center (for example, the heme of cytochrome P450) to generate the pharmacological reactive entity. The mechanism of action of artemisinin is different: this peroxide interacts with the metal center of heme, which acts at the same time as trigger and target. Artemisinin specifically enters infected red blood cells to reach its activator/target. Trioxaquines will probably do so.

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