From Mechanistic Studies on Artemisinin Derivatives to New Modular Antimalarial Drugs

ANNE ROBERT, ODILE DECHY-CABARET, JÉRÔME CAZELLES, AND BERNARD MEUNIER*

Laboratoire de Chimie de Coordination du CNRS, 205 route de Narbonne, 31077 Toulouse Cedex 4, France

Received March 2, 2001

ABSTRACT

In the first part of this account, the antimalarial drug artemisinin is presented, and the current hypotheses on the mechanism of action of this endoperoxide-based drug are reviewed. The alkylating ability of artemisinin and synthetic analogues toward heme related to their antimalarial efficacy are underlined. Some possible ways for discovery of new drugs, especially the design of trioxaquines, new active molecules recently patented that have been prepared by covalent attachment of a trioxane residue having alkylating ability to a quinoline moiety known to easily penetrate within infected erythrocytes, are presented.

Introduction

The world population is exposed to three major infectious diseases: tuberculosis, AIDS, and malaria. There is an urgent need to develop new efficient drugs to cure these diseases because of the increasing resistance of *Mycobacterium tuberculosis*, HIV, and the malaria parasite to classical drugs. Malaria was successfully reduced after World War II because of the easy access to a cheap insecticide, such as DDT, and readily available drugs, such as chloroquine and, later, mefloquine (two mimics of quinine, the natural antimalarial drug extracted from the bark of the cinchona tree, Figure 1). These drugs were highly efficient against the different species of the malaria parasite, including *Plasmodium falciparum*, responsible for severe malaria (four different species of the parasite are responsible for the contamination of 200 million people and the death of more than 1 million people each year: *Plasmodium vivax*, *Plasmodium malariae*, *Plasmodium ovale*, and *Plasmodium falciparum*).[1,2] Malaria is transmitted by inoculation of sporozoites during the bite of an infected female *Anopheles* mosquito. The parasite invades and develops within hepatocytes to release merozoites, which quickly invade erythrocytes to grow from "rings" to mature trophozoites, then to schizonts, and finally to release merozoites that invade more erythrocytes[3a] (Scheme 1). The multidrug-resistant falciparum malaria is now widespread in many tropical countries. In addition, the spread of malaria is also dependent on the increase in the total population, the development of transportation and migrations, and finally, climate modifications related to global warming. These different aspects of malarial evolution allow the prediction of a major health problem,[3b] not only in tropical countries but also in areas that used to be affected by malaria, namely the southern parts of the U.S. and of Europe. What are the possible weapons to "roll back malaria" as demanded by the World Health Organization (WHO) in 1998? Many different courses of action are currently being explored, such as

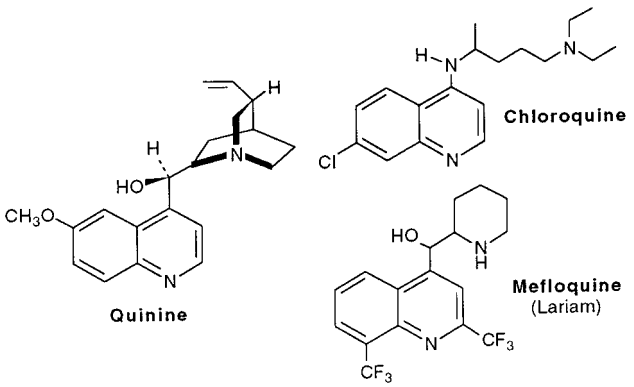
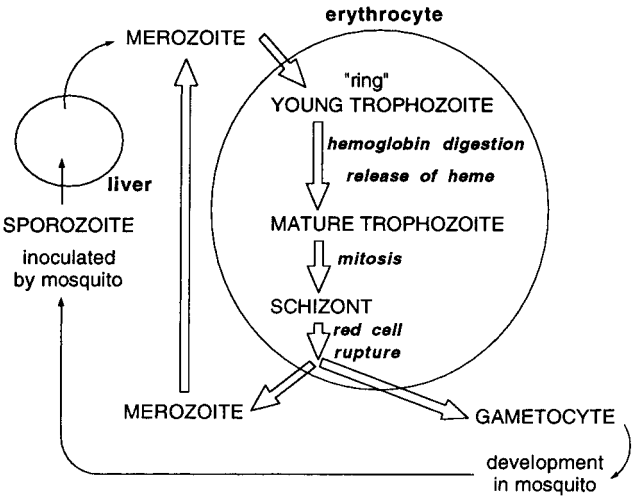


FIGURE 1. Quinoline-based antimalarial drugs: quinine, chloroquine, and mefloquine.

Scheme 1. *Plasmodium falciparum* Cycle within Human[a]



[a] Modified after Ref 3a.

Anne Robert was born in 1959. She is a researcher in the group of Bernard Meunier. Her current interests include oxidation reactions catalyzed by metalloporphyrins, the mechanism of action of artemisinin, and synthesis of new peroxide-based antimalarials.

Odile Dechy-Cabaret was born in 1975. She entered the École Normale Supérieure of Cachan in 1994 and is currently a Ph.D. student in the group of Bernard Meunier working on the synthesis of new modular antimalarials.

Jérôme Cazelles was born in 1974. After studying biochemistry at the University of Toulouse from 1993 to 1997, he obtained his Ph.D. in 2000 with Bernard Meunier on the mechanism of action of antimalarial endoperoxides.

Bernard Meunier was born in 1947 and is currently heading a research group at the Laboratoire de Chimie de Coordination du CNRS. He is a Director of Research at the CNRS and Associate Professor at the École Polytechnique (Palaiseau). His current research interests include catalytic oxidations, oxidative DNA cleavage, and the mechanism of action of antimalarial drugs. He is the coauthor of more than 260 articles. He has been a Member of the French Academy of Sciences since 1999.

* To whom correspondence should be addressed. E-mail: bmeunier@lcc-toulouse.fr

10.1021/ar990164o CCC: $22.00
Published on Web 02/12/2002

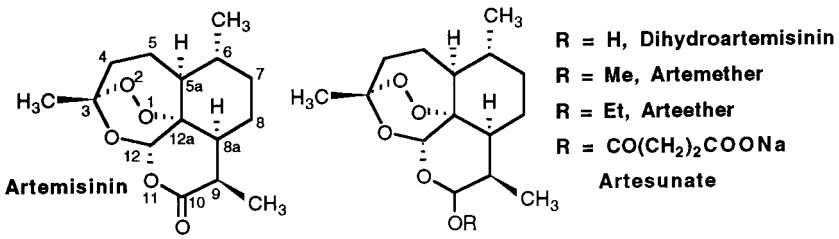


FIGURE 2. Structure of artemisinin and its derivatives.

(i) *The Development of Drug Combinations To Overcome the Antimalarial Resistance.* This resistance can be due to single mutations on parasite proteins (for example, pyrimethamine or sulfadoxine[4]) or multigenic factors, as for aminoquinoline derivatives.[5] The mechanism of the widespread chloroquine resistance is still not completely elucidated. Many genes have been invoked: *cg2*,[6] pfmdr, a *mdr*-like gene,[7] and more recently, *pfcrt*.[8]

To prevent drug resistance, a combination of drugs having different modes of action has been developed in antituberculosis therapies, cancer chemotherapies, and more recently, for antiretroviral treatments. In the case of malaria, several combinations of different drugs are used: quinine/tetracycline, pyrimethamine/sulfadoxine, chloroquine/proguanil, atovaquone/proguanil, and chlorproguanil/dapsone, artemisinin derivatives with mefloquine or benflumetol.[9]

(ii) *The Search for an Effective Vaccine against Malaria That Would Protect Nonimmune Individuals from the Disease*. Twenty years of antigen identification and gene cloning and expression have produced many vaccine candidates.[10] But there are many difficulties in the elaboration of malaria vaccines: none of the available in vitro assays is predictive of functional immunity in vivo, and the cycle of the malaria parasite is complex, the several stages in humans are morphologically and antigenically distinct, and immunity is stage-specific. Then multivalent antigen vaccines are required. Hybrid proteins that contains sequences from a number of asexual blood-stage antigens or sporozoite and merozoite antigens have been produced and are currently being evaluated.[10] The vaccine approach will benefit from the complete sequencing of the entire genome of *P. falciparum*. DNA vaccines are currently under investigation.[11]

(iii) *The Genomic Approach*. The complete sequencing of the genome of *P. falciparum* is now close to completion.[12] This work will undoubtly reveal many genes that code for yet unknown proteins that may be new candidates for vaccine development or targets for new enzyme inhibitors.

(iv) *The Search for New Antimalarial Drugs*. Soon, because of the troublesome evolution of the disease (WHO is predicting that the number of people suffering from malaria will double by the year 2010 in the absence of new antimalarial strategies), there is an urgent need for the discovery of cheap, well-tolerated, oral-efficient drugs to fight chloroquine-resistant strains of *P. falciparum*. *The search of new antimalarial drugs can be efficiently guided by considering that free heme within the food vacuole of the parasite is a key pharmacological target*. Many antimalarial drugs are active only on the erythrocytic forms of *Plasmodium* when the parasite is digesting between 25 and 80% of the 5 mM concentration of hemoglobin within erythrocytes to use the resulting amino acids.[13] The released free Fe(II)-heme, which is able to generate a strong oxidative stress, is potentially toxic for the parasite: Fe(II)-heme can be oxidized by molecular oxygen, leading to superoxide anion, to hydrogen peroxide, and then to hydroxyl radicals via heme-catalyzed cycles involving reductants, such as glutathion or NAD(P)H, both present in high concentrations within erythrocytes. The polymerization of the released heme to hemozoin assisted by the parasitic histidine-rich protein (HRP) is the means for *Plasmodium* to avoid the heme-mediated oxidative stress.[14,15] Chloroquine has a high affinity for ferric heme ($K_d = 3.5$ nM) as a result of a strong π-stacking between the quinoline ring and the porphyrin macrocycle.[16] We will see below that the released heme is also one of the main targets of endoperoxide-containing drugs, such as artemisinin derivatives. The specificity of heme-targeted antimalarial drugs is due to the absence of free (or easily accessible) heme in normal erythrocytes.

The present review is focused on the mechanism of action of artemisinin, a peroxide-containing drug. The preparation of new antimalarial agents named "trioxaquines" will be also briefly reported.

Artemisinin and its Derivatives. Among the molecules that emerged over the past three decades as antimalarial drugs, artemisinin has a peculiar structure (Figure 2). The potential value of this sesquiterpene (from decoctions of leaves of *Artemisia annua*) has been used in traditional Chinese medicine for the treatment of fevers for more than 2000 years.[17,18] Artemisinin is present in 0.01 to 0.8% of the leaves dry weight.[19] This trioxane is highly active against multidrug-resistant strains of human malaria and is recommended for the chemotherapy of cerebral malaria (severe malaria, the encephalitis responsible for fatal issues). Artemisinin is absorbed rapidly, the peak plasma concentration is reached within 1−2 h, and the elimination is fast, the half-life time of the drug being 2−3 h.[20] Semisynthetic analogues of artemisinin, artemether, and artesunate (Figure 2), are currently used in the curative treatment of life-threatening infections with *P. falciparum*. These compounds are well-tolerated, and no major side effects are observed in patients.[21,22] It is now the WHO's policy to develop the use of these artemisinin derivatives as first-intention drugs to treat severe malaria. Another key advantage of these endoperoxide-containing antima-

larial agents is the absence of drug resistance. When parasites are exposed to selection pressure within mice infected by *Plasmodium berghei*, a low level of resistance has been observed which disappears as soon as the drug-selection pressure is withdrawn.[23] Remarkably, the introduction of artemisinin derivatives in routine treatment in some areas of Southeast Asia was associated with a significant reduction of falciparum malaria. However, the poor pharmacokinetic parameters of this natural compound make necessary the development of new synthetic endoperoxide-based antimalarials based on the understanding of the mechanism of action of artemisinin at the molecular level.

Current Hypotheses on the Mechanism of Action of Artemisinin. Artemisinin and its derivatives are toxic to malaria parasites at nanomolar concentrations, but micromolar concentrations are required for toxicity to mammalian cells. One reason for this selectivity is the enhanced uptake of the drug by the parasite: *P. falciparum*-infected erythrocytes concentrate [^{3}H]-dihydroartemisinin and [^{14}C]-artemisinin to a >100-fold higher concentration than do uninfected erythrocytes.[17] An artemisinin derivative lacking the endoperoxide bridge (deoxyartemisinin) is devoid of antimalarial activity,[24] indicating that this peroxide function is the key factor of the pharmacological activity of these trioxanes.

Oxidative Stress. An artemisinin-mediated oxidative stress killing the parasite has been proposed on the basis of in vitro experiments with infected human red blood cells or with parasite membranes.[25] In fact, the intraerythrocytic activation of the drug peroxidic bond by iron(II)-heme produced during hemoglobin degradation should generate radical oxygen species. In vitro, hemin catalyzes the reductive decomposition of artemisinin and dihydroartemisinin.[26] When incubated with normal erythrocytes, artemisinin was shown to increase the concentration of methemoglobin and to slightly reduce the intracellular glutathione and membrane fatty acid concentrations, resulting in a dose-dependent increase of cell lysis.[27] However, these experiments were made at concentrations ranging from 50 to 1000 mM, that is, at doses 10^3 to 10^5 times higher than effective in vitro drug concentrations. Actually, the parasite death in the presence of artemisinin is probably not due to a nonspecific or random cell damage caused by freely diffusing radical oxygen species, but might involve *specific radicals and targets* which have yet to be identified at the molecular level.[28]

Acid-Mediated Opening of the Peroxide Bridge. Recent articles proposed an acid-mediated opening of the peroxidic function of artemisinin, generating a hydroperoxide, the source of an electrophilic oxygenating species. Such an endoperoxide opening has been produced in vitro by sulfuric acid-impregnated silica gel.[29] However, artemisinin was recovered unchanged after incubation with 10% aqueous acetic acid (pH 2.1) during 18 h at 37 °C.[30a] Artemisinin derivatives are also highly stable as a suspension in simulated stomach acid (hydrochloric acid pH 2 at 37 °C).[30b] In these conditions, the half-life of artemisinin is 24 h, far longer than its half-life in humans. Furthermore, an artemisinin derivative without lactone at C10 (i.e., deoxoartemisinin) was found to be 10 times more stable than artemisinin itself. This clearly indicates that (i) the lactone ring is much more acid-sensitive than the peroxidic bond, and (ii) the biological activity of artemisinin derivatives cannot be imputed to the acid decomposition of the peroxide.[30b]

The Iron−Oxo Route. The transfer of an oxygen atom from the peroxide function of artemisinin to a chelated iron ion to generate an Fe(IV)═O species has also been proposed.[17,18,31] The Raman resonance spectra proposed as "direct evidence for a heme ferryl intermediate" exhibit a signal/noise ratio below 2.[32] Metal−oxo RR signals have signal/noise ratios as high as 10, 20, or more when they undoubtedly exist.[33] Furthermore, most of the experiences supporting the iron−oxo hypothesis, namely, the radical-chain aromatization of hexamethyl Dewar benzene,[34] the sulfide oxidation to sulfoxide, and the allylic hydroxylation of tetrahydronaphthalene, can be performed by efficient one-electron oxidants. To check the possible generation of a high-valent metal−oxo species using artemisinin as the oxygen atom donor, we tried to epoxidize reactive olefins, such as cyclohexene[35] and 3,4-dihydro-2*H*-pyran[36] with artemisinin associated with manganese(III)-tetraphenylporphyrin. No conversion occurred after several hours. Alternatively, when artemisinin was replaced by NaOCl, a very efficient oxygen-atom donor in metalloporphyrin-catalyzed epoxidations,[37] we observed a complete conversion for both cyclohexene and dihydropyran in a few minutes.[36] The oxygen atom transfer from artemisinin peroxide to create an iron−oxo species with epoxidizing ability was also unsuccessfully investigated with $FeCl_2$.[38] These results confirmed that artemisinin, with its rather symmetrical O−O bond, is not an efficient oxygen atom donor. Only peroxides with a good leaving group, such as $KHSO_5$, are able to transfer an oxygen atom on a metalloporphyrin in order to generate a high-valent metal−oxo species. The differences between oxidations involving metal−oxo species and autoxidation reactions has been pertinently reviewed by Ingold.[39]

The Target(s) of Artemisinin. When artemisinin or other active trioxanes were incubated at pharmacologically relevant concentrations within human red blood cells infected by *P. falciparum*, a heme-catalyzed cleavage of the peroxide bond was reported to be responsible for the alkylation of heme[40a] and a small number of specific parasite proteins, one of which has a molecular size similar to those of a histidine-rich protein (42 kDa).[40b] Another possible target protein is the *P. falciparum* translationnally controlled tumor protein (TCTP).[41] Artemisinin may also be involved in the specific inhibition of malarial cysteine protease activity.[42] Furthermore, the incubation of purified hemozoin with artemisinin at a pH value close to that of the parasite vacuole (pH = 5), resulted in the loss of hemozoin, indicating that hemozoin may be dismantled by drug interactions, leading to the building up of the free heme pool.[42]

Alkylation Reactions. The importance of alkylating species generated by the homolytic cleavage of the arte-

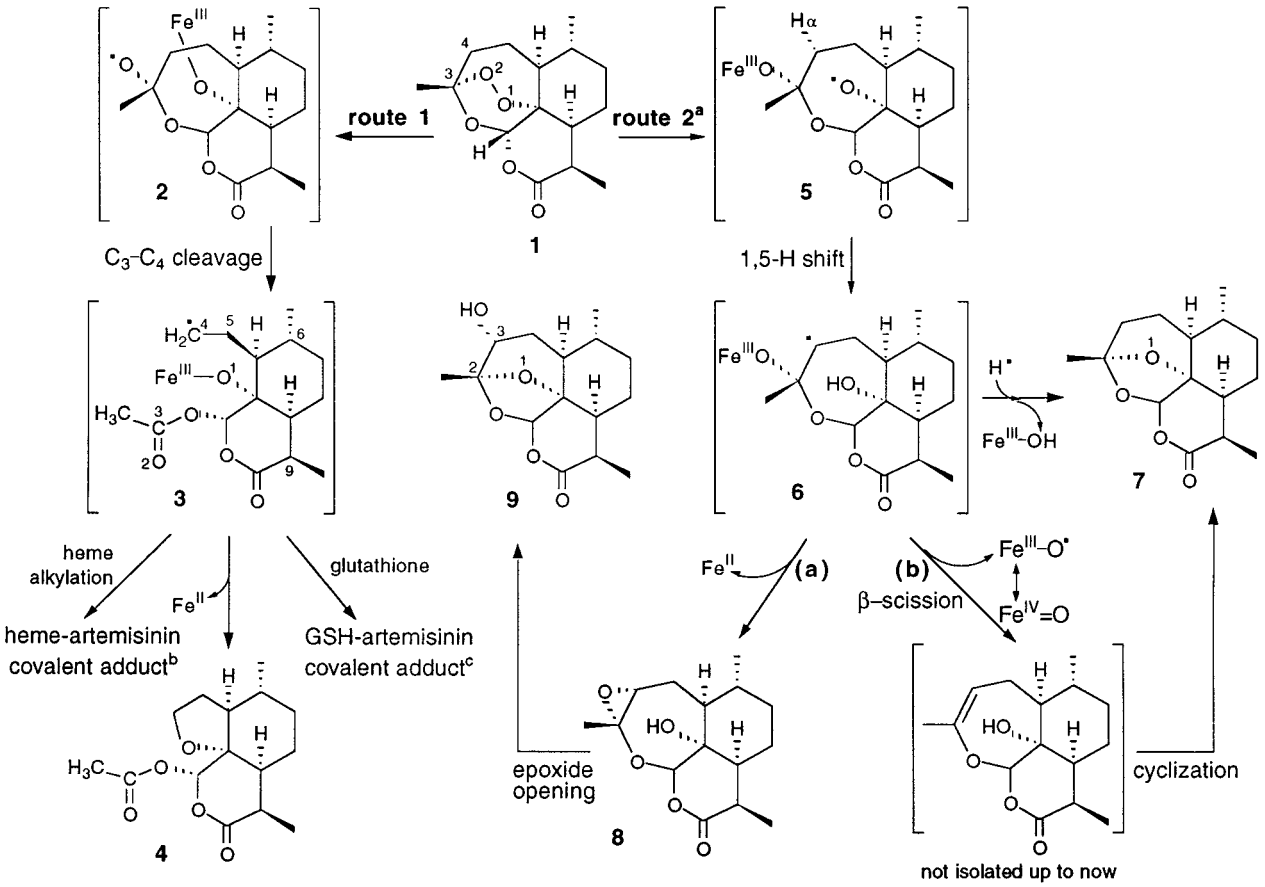


FIGURE 3. General reactivity of artemisinin after reductive activation of the endoperoxide function (reactions also observed with synthetic trioxanes). [a] Most of the route 2 is inspired from ref 19. [b] Ref 29. [c] Ref 50.

misinin endoperoxide function, in particular the alkyl radical centered at position C4 of artemisinin or related trioxanes, was earlier proposed by Meshnick and Posner.[43]

Product analyses in model studies using either iron(II) salts or iron− or manganese−porphyrin complexes support the formation of alkoxyl radicals (**2** and **5**, Figure 3) after reductive activation and homolysis of the peroxidic bond. These radicals quickly isomerize to produce the alkyl radicals **3** and **6** via a C−C β-scission or a 1,5 H-shift, respectively, although the distance for the H-atom transfer from C4 to O1 probably exceeds the critical limit distance of 2.1 Å for such migration.[44] Release of iron(II) from the C4-centered radical **6** produces the intermediate 3,4-epoxide **8** first postulated[31] and later isolated in low yield.[45] The intramolecular attack of the hydroxyl of **8** on C3 induces the epoxide ring opening, giving rise to the 3α-hydroxy-deoxyartemisinin **9**. The deoxyartemisinin **7** may be produced from **6** by H-atom abstraction and $Fe^{III}OH$ release. This compound, produced during the metabolism of artemisinin,[46] is probably the result of an adventitious enzymatic deoxygenation not related to the antimalarial activity.[38,47] Route 1, namely the evolution of the alkyl radical **3**, may be interpreted as the loss of M^{II} (M = Fe or Mn), which generates the tetrahydrofuran derivative **4**. However, the nonsterically hindered radical **3** also exhibits alkylating properties that have been evidenced by isolation of covalent adducts either with synthetic metalloporphyrins used as heme models,[48] with heme itself,[49] or with cysteine or glutathione[50] via a thioether linkage (epoxide **8**, route 2, may also be able to act as electrophilic agent with alkylating ability). When artemisinin is activated by hemin plus thiol in tetrahydrofuran or by ferrous chloride in acetonitrile, route 1 is the major one, as compared with route 2 (the amount of isolated compounds indicates a ratio route 1/route 2 of 90/10 and 86/14, respectively, in these cases)[31] and is the only one evidenced with Mn^{II}TPP in dichloromethane.[36] Route 2 becomes significant only in the presence of iron(II) bromide in tetrahydrofuran. Route 1 is also the major pathway for biologically active trioxane derivatives, whereas route 2 is more important with inactive trioxanes.[51]

Heme and Heme Model Alkylation. Alkylation of heme by artemisinin was first reported by Meshnick after identification of heme−drug adducts by mass spectrometry, but no structures were proposed for the resulting covalent adducts.[40a] We investigated this crucial feature of the reactivity of artemisinin with synthetic metalloporphyrins. Because of the variety of possible alkylation sites on iron protoporphyrin-IX, we first studied the alkylating activity of artemisinin with manganese(II) tetraphenylporphyrin, a synthetic metalloporphyrin having a fourth-order symmetry and only the eight equivalent β-pyrrolic positions as possible alkylation sites. By reacting Mn^{II}TPP with artemisinin, artemether, or several synthetic trioxanes in dichloromethane, chlorin-type adducts were formed by reaction of the macrocycle with an alkyl radical generated

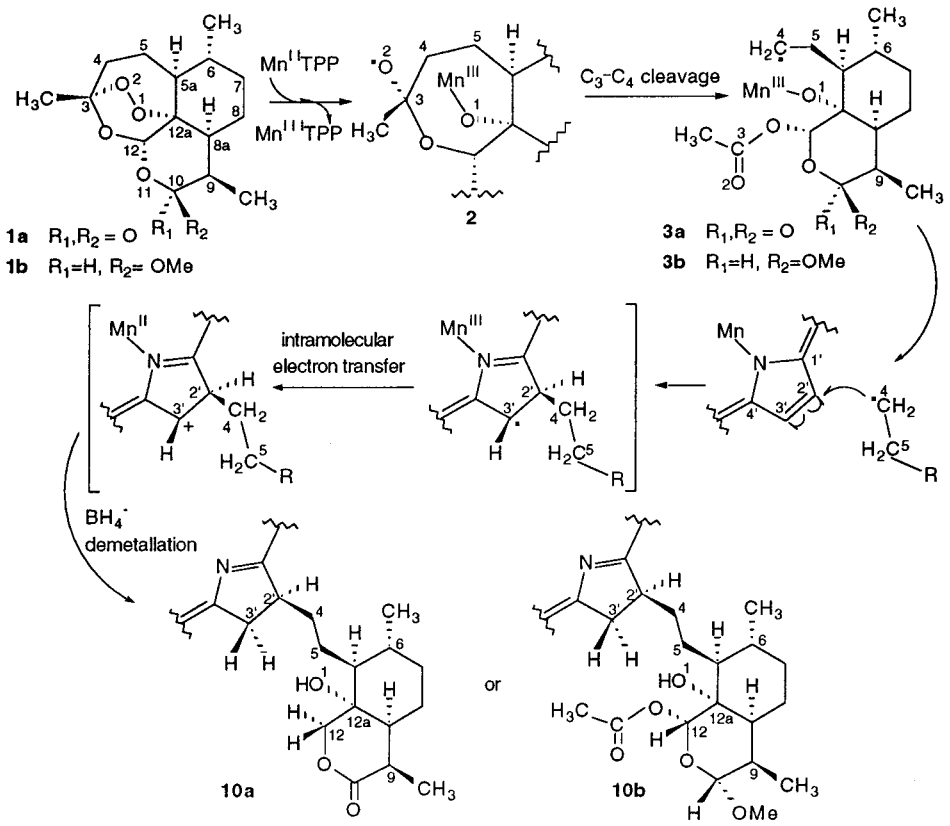


FIGURE 4. Alkylation of manganese(II) tetraphenylporphyrin by artemisinin or artemether.

by reductive activation of the drug endoperoxide.[48] The structure and mechanism of formation of the artemisinin- and artemether-TPP adducts are reported in Figure 4. [The reduction of the porphyrin macrocycle to a chlorin was mediated by the borohydride used to generate Mn^{II}TPP from Mn^{III}(TPP)Cl. In the absence of a hydride able to trap the cation generated at C3′, a proton release at C2′ regenerates the porphyrin ring. This rearomatization of the macrocycle is competitive with reduction by hydride, and a mixture chlorin/porphyrin adducts was obtained in the activation of the trioxane BO7 by Mn^{II}TPP[48b]]

By studying a large series of trioxanes,[36,48,52] it has been possible to correlate the alkylating ability toward Mn^{II}TPP and the pharmacological activity: efficient drugs behaving as good alkylating agents and most of the inactive drugs being unable to alkylate the porphyrin ring. Furthermore, it appeared that trioxanes bearing a bulky substituent on the α face close to the endoperoxide (i.e., on the same side of the endoperoxide with respect to the mean drug plane) were inactive on infected cells and unable to alkylate the macrocycle (Figure 5). These data confirmed that (i) a close interaction between the metal center and the peroxide bond is required, suggesting that this activation occurred through an inner-sphere electron transfer, (ii) the alkylation ability that is crucial for the antimalarial activity of artemisinin is a general feature required for the biological activity of endoperoxide-containing antimalarial drugs.

It was of particular interest to investigate the reactivity of artemisinin toward iron^{II}–heme itself, the biological activating system and one of the expected targets. For this purpose, iron(III) protoporphyrin-IX dimethylester was exposed to artemisinin in the presence of a hydroquinone derivative (or a thiol) used as a mild reducing agent to generate the iron(II) heme. Heme was readily converted in high yield to heme–artemisinin adducts.[49a] The demetalation of this mixture of three adducts to facilitate the NMR characterization indicated that the α, *β*, and *δ* meso carbons were alkylated (Figure 6).[49b] Heme–artemether adducts were also obtained.[49a]

No bis-alkylated adducts could be obtained, either with heme or with the heme model Mn^{II}TPP, not even in the presence of an excess of drug, although the metal center is expected to keep its redox properties after the first alkylation. That means that a bulky drug-derived substituent at the periphery of the macrocycle precludes the close interaction of a second drug molecule required for bis-alkylation.

The artemisinin chemistry generated by the monoelectronic reduction of the peroxide bridge in the presence of heme or models can probably be considered as a radical chemistry occurring within a cage and, therefore, with a relatively high selectivity. Such results suggest that the low and transient concentration of free heme generated by hemoglobin degradation in vivo is probably responsible for the reductive activation of the endoperoxide function of active trioxanes. This pathway generates alkylating species, such as radical **3**, and is likely to disrupt vital biochemical processes of the parasite via alkylation of biomolecules located in the close vicinity of the free heme.

The retention of configuration observed with drug-derived chiral C-centered radicals[36] suggests that alkyla-

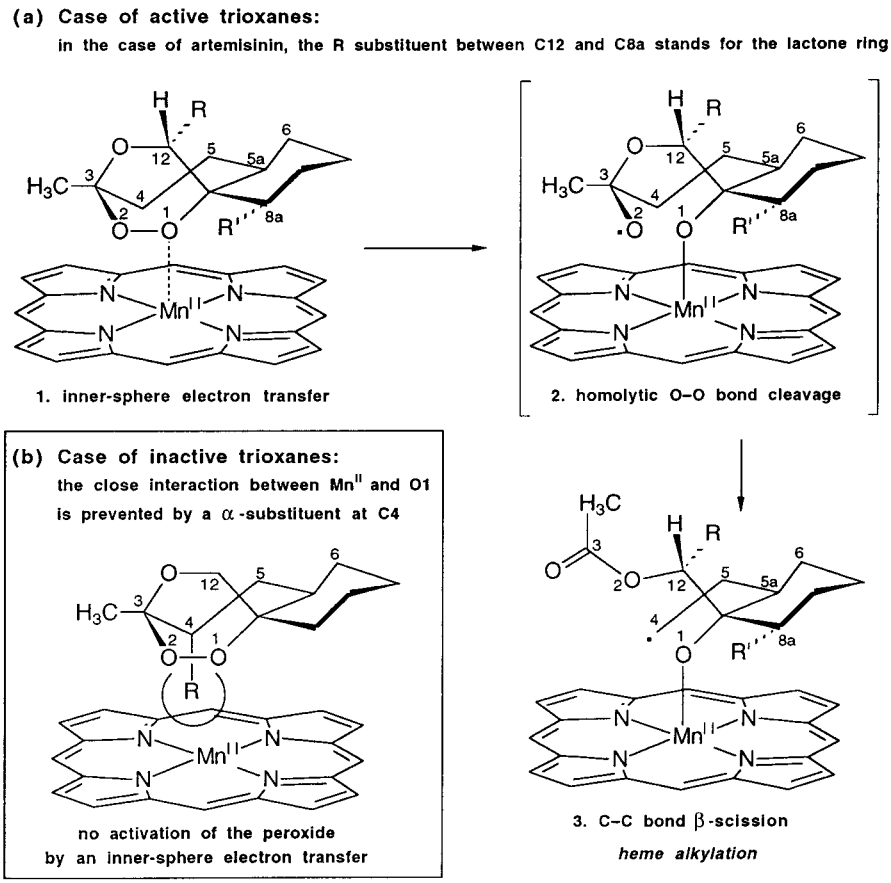


FIGURE 5. Activation of trioxanes by an inner-sphere electron transfer: possible correlation between pharmacological activity and alkylating properties.

tion reactions are very fast, occurring within few nanoseconds. Within 1−3 ns, a small organic molecule can move over a distance of 8 to 14 Å in a water solution at room temperature.[53] Such displacement is sufficient for a C-centered radical generated by a free-heme molecule to escape and alkylate an essential parasite protein located in the close proximity of heme before being trapped by molecular oxygen.

New Antimalarial Modular Molecules: "Trioxaquines". For reasons mentioned in the first part of this review, there is an urgent need for highly effective, well-tolerated and affordable antimalarial drugs. New targets have been proposed. Drugs have been designed to inhibit the phospholipid (PL) metabolism of infected erythrocytes, which is specific. The malaria parasite needs large amounts of phosphatidylcholine (PC) to grow and divide. The supply of choline, vital for the parasite, is achieved via a choline carrier. Quaternary ammonium and bis-ammonium salts, designed as choline analogues, are highly active in vitro, even against multiresistant isolates.[54]

The free heme liberated in the parasite food vacuole is an "old" but always attractive pharmacological target, the most specific one since it comes from the hemoglobin digestion by the parasite, that occurs only in infected erythrocytes. Chloroquine and artemisinin are directed toward this target. Many chemical modifications on the chloroquine skeleton have been performed to obtain molecules active on resistant strains: substitutions on the quinoline nucleus, variations on the side chain, synthesis of bisquinolines, and more recently, introduction of a ferrocenyl moiety.[55] Trioxane analogues of artemisinin containing the crucial endoperoxide bridge have been developped, but until now, none of them have been successfully used in clinical trials.[2] In our group, we decided to prepare new chimeric molecules by covalent attachment of a trioxane moiety to a 4-aminoquinoline entity.[56] These molecules, named trioxaquines (Figure 7a) were designed according to the current knowledge of the mechanism of action of artemisinin derivatives: they combine in a single molecule a peroxidic entity acting as a potential alkylating agent and an aminoquinoline known to easily penetrate within infected erythrocytes. Better than a simple combination of an aminoquinoline with a short-life trioxane, this "covalent bitherapy" is expected to considerably reduce the risk of drug resistance. For obvious reasons, these molecules must be cheap and easily accessible; thus, we used a convergent synthesis based on classical reactions. Many simple modulations (quinoline, diamine, diene, diketone) are possible, leading to a large family of new potential antimalarial compounds. The first synthesized trioxaquines (Figure 7b) have been tested in vitro on laboratory strains (chloroquine-sensitive and chloroquine-resistant ones) of *P. falciparum*: all IC_{50} values obtained ranged from 8 to 40 nM, depending on the trioxaquine substituents, for both strains. DU-1102 has also been tested in vitro on human isolates in Yaoundé

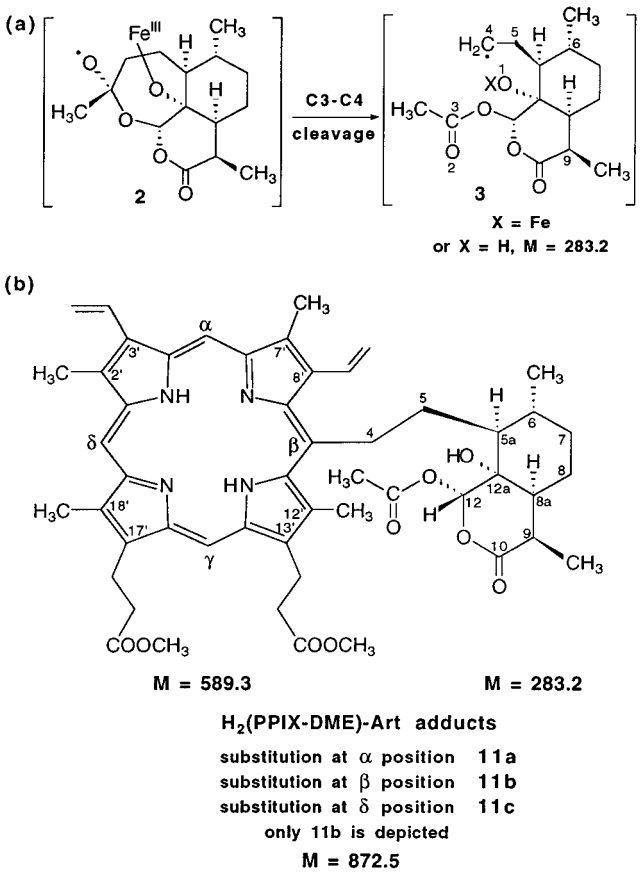


FIGURE 6. (a) Formation of a C4-centered radical after reductive activation of the endoperoxide function of artemisinin, leading to (b) the alkylation of heme−dimethylester.

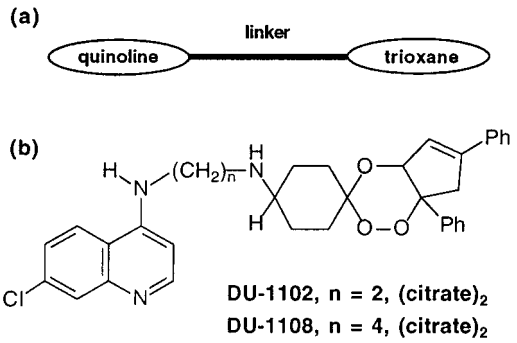


FIGURE 7. (a) General structure of trioxaquines. (b) Structures of trioxaquines DU-1102 and DU-1108.

(Cameroon) and was found to be highly active (IC_{50} mean value = 43 nM) on chloroquine- and pyrimethamine-sensitive or resistant isolates.[57] No cross-resistance was observed with chloroquine and pyrimethamine. Preliminary results indicated that some trioxaquines are active by oral administration on infected mice (unpublished data). We are currently working on the in vivo evaluation of these drug candidates that can be developed for clinical trials in a reasonable time frame in collaboration with a newly created company named Palumed.[58]

CNRS is acknowledged for financial support, and the French Ministry of Education for Ph.D. grants to J.C. and O.D.C.

References

(1) Balter, M.; Marshall, E.; Vogel, G.; Taubes, G.; Pennisi, E.; Enserink, M. A renewed assault on an old and deadly foe. *Science* **2000**, *290*, 428−441.
(2) Newton, P.; White, N. Malaria: new developments in treatment and prevention. *Annu. Rev. Med.* **1999**, *50*, 179−192.
(3) (a) O'Neill, P. M.; Bray, P. G.; Hawley, S. R.; Ward, S. A.; Park, B. K. 4-Aminoquinolines − past, present, and future: a chemical perspective. *Pharmacol. Ther.* **1998**, *77*, 29−58. (b) White, N. J.; Fosten, F.; Looareesuwan, S.; Watkins, W. M.; Marsh, K.; Snow, R. W.; Kokwaro, G.; Ouma, J.; Hien, T. T.; Molyneux, M. E.; Taylor, T. E.; Newbold, C. I.; Ruebush, T. K.; Danis, M.; Greenwood, B. M.; Anderson, R. M.; Olliaro, P. Averting a malaria disaster. *Lancet* **1999**, *353*, 1695−1967.
(4) Peterson, D. S.; Milhous, W. K.; Wellems, T. E. Molecular basis of differential resistance to cycloguanil and pyrimethamine in *Plasmodium falciparum* malaria. *Proc. Natl. Acad. Sci. U.S.A.* **1990**, *87*, 3018−3022.
(5) (c) McCutcheon, K. R. G.; Freese, J. A.; Frean, J. A.; Sharp, B. L.; Markus, M. B. Two mutations in the multidrug-resistance gene homologue of *Plasmodium falciparum*, *pfmdr1*, are not useful predictors of in-vivo or in-vitro resistance in southern Africa. *Trans. R. Soc. Trop. Med. Hyg.* **1999**, *93*, 300−302.
(6) Sanchez, C. P.; Horrocks, P.; Lanzer, M. Is the putative chloroquine resistance mediator CG2 the Na^+/H^+ exchanger of *Plasmodium falciparum*? *Cell* **1998**, *92*, 601−602.
(7) Price, R. N.; Cassar, C.; Brockman, A.; Duraisingh, M.; Van Vugt, M.; White, N. J.; Nosten, F.; Krishna, S. The *pfmdr1* gene is associated with a multidrug-resistance phenotype in *Plasmodium falciparum* from the western border of Thailand. *Antimicrob. Agents Chemother.* **1999**, *43*, 2943−2949.
(8) Djimdé, A.; Doumbo, O. K.; Cortese, J. F.; Kayentao, K.; Doumbo S..; Diourté, Y.; Dicko, A.; Su, X.-Z.; Nomura, T.; Fidock, D. A.; Wellems, T. E.; Plowe, C. V. A molecular marker for chloroquine-resistant falciparum malaria. *N. Engl. J. Med.* **2001**, *344*, 257−263.
(9) White, N. J. Preventing antimalarial drug resistance through combinations. *Drug Resistance Updates* **1998**, *1*, 3−9.
(10) (a) Holder, A. A. Malaria vaccines. *Proc. Natl. Acad. Sci. U.S.A.* **1999**, *96*, 1167−1169. (b) Gilbert, S. C.; Plebanski, M.; Harris, S. J.; Allsoop, C. E. M.; Thomas, R.; Layton, G. T.; Hill, A. V. S. A protein particle vaccine containing multiple malaria epitopes. *Nat. Biotechnol.* **1997**, *15*, 1280−1284. (c) Daubersies, P.; Thomas, A. W.; Millet, P.; Brahimi, K.; Langermans, J. A. M.; Ollomo, B.; Ben Mohamed, L.; Slierendregt, B.; Eling, W.; Van Belkum, A.; Dubreuil, G.; Meis, J. F. G. M.; Guérin-Marchand, C.; Cayphas, S.; Cohen, J.; Gras-Masse, H.; Druilhe, P. Protection against *Plasmodium falciparum* malaria in chimpanzees by immunization with the conserved pre-erythrocytic liver-stage antigen 3. *Nat. Med.* **2000**, *6*, 1258−1263.
(11) Sanchez, G. I.; Sedegah, M.; Rogers, W. O.; Jones T. R.; Sacci, J.; Witney, A.; Carucci, D. J.; Kumar, N.; Hoffman, S. L. Immunogenicity and protective efficacy of a *Plasmodium yoelli* Hsp60 DNA vaccine in BALB/c mice. *Infect. Immun.* **2001**, *69*, 3897−3905.
(12) Su, X. Z.; Ferdig, M. T.; Huang Y.; Huynh, C. Q.; Liu, A.; You, J.; Wootton, J. C.; Wellems, T. E. A genetic map and recombination parameters of the human malaria parasite *Plasmodium falciparum*. *Science* **1999**, *286*, 1351−1353.
(13) Francis, S. E.; Sullivan, D. J.; Goldberg, D. E. Hemoglobin metabolism in the malaria parasite *Plasmodium falciparum*. *Annu. Rev. Microbiol.* **1997**, *51*, 97−123.
(14) Choi, C. Y. H.; Cerda, J. F.; Chu, H. A.; Babcock, G. T.; Marletta, M. A. Spectroscopic characterization of the heme-binding sites in *Plasmodium falciparum* histidine-rich protein 2. *Biochemistry* **1999**, *38*, 16916−16924.
(15) Pagola, S.; Stephens, P. W.; Bohle, D. S.; Kosar, A. D.; Madsen, S. K. The structure of malaria pigment b-haematin. *Nature* **2000**, *404*, 307−310.
(16) (a) Moreau, S.; Perly, B.; Chachaty, C.; Deleuze, C. A nuclear magnetic resonance study of the interactions of antimalarial drugs with porphyrins. *Biochim. Biophys. Acta* **1985**, *840*, 107−116. (b) Egan, T. J.; Marques, H. M. The role of haem in the activity of chloroquine and related antimalarial drugs. *Coord. Chem. Rev.* **1999**, *190−192*, 493−517.
(17) Meshnick, S. R.; Taylor, T. E.; Kamchonwongpaisan, S. Artemisinin and the antimalarial endoperoxides: from herbal remedy to targeted chemotherapy. *Microbiol. Rev.* **1996**, *60*, 301−315.
(18) Cumming, J. N.; Ploypradith, P.; Posner, G. H. Antimalarial activity of artemisinin (qinghaosu) and related trioxanes: mechanism(s) of action. *Adv. Pharmacol.* **1997**, *37*, 2253−297.
(19) Van Geldre, E.; Vergauwe, A.; Van den Eeckhout, E. State of the art of the production of the antimalarial compound artemisinin in plants. *Plant Mol. Biol.* **1997**, *33*, 199−209.

(20) Duc, D. D.; de Vries, P. J.; Khanh, N. X.; Binh, L. N.; Kager, P. A.; van Boxtel, C. J. The pharmacokinetics of a single dose of artemisinin in healthy Vietnamese subjects. *Am. J. Trop. Med. Hyg.* **1994**, *51*, 785−790.

(21) Price, R.; Van Vugt, M.; Phaipun, L.; Luxemberger, C.; Simpson, J.; McGready, R.; ter Kuile, F.; Kham, A.; Chongsuphajaisiddhi, T.; White, N. J.; Nosten, F. Adverse effects in patients with acute falciparum malaria treated with artemisinin derivatives. *Am. J. Trop. Med. Hyg.* **1999**, *60*, 547−555.

(22) Bindschedler, M.; Lefevre, G.; Ezzet, F.; Schaeffer, N.; Meyer, I.; Tomsen, M. S. Cardiac effects of co-artemether (artemether/lumefantrine) and mefloquine given alone or in combination to healthy volunteers. *Eur. J. Clin. Pharmacol.* **2000**, *56*, 375−381.

(23) Peters, W.; Robinson, B. L. The chemotherapy of rodent malaria. LVI. Studies on the development of resistance to natural and synthetic endoperoxides. *Ann. Trop. Med. Parasitol.* **1999**, *93*, 325−339.

(24) Klayman, D. L. Qinghaosu (artemisinin): an antimalarial drug from China. *Science* **1985**, *228*, 1049−1055.

(25) Krungkrai, S. R.; Yuthavong, Y. The antimalarial action on *Plasmodium falciparum* of qinghaosu and artesunate in combination with agents which modulate oxidant stress. *Trans. R. Soc. Trop. Med. Hyg.* **1987**, *81*, 710−714.

(26) Zhang, F.; Gosser, D. K.; Meshnick, S. R. Hemin-catalyzed decomposition of artemisinin (qinghaosu), *Biochem. Pharmacol.* **1992**, *43*, 1805−1809.

(27) Scott, M. D.; Meshnick, S. R.; Williams, R. A.; Chiu, D. T.-Y.; Pan, H. C.; Lubin, B. H.; Kuypers, F. A. Qinghaosu-mediated oxidation in normal and abnormal erythrocytes. *J. Lab. Clin. Med.* **1989**, *114*, 401−406.

(28) Meshnick, S. R. Free radicals and antioxidants. *Lancet* **1994**, *344*, 1441−1442.

(29) Haynes, R. K.; Pai, H. H.-O.; Voerste, A. Ring opening of artemisinin (qinghaosu) and dihydroartemisinin and interception of the open hydroperoxides with formation of *N*-oxides-A chemical model for antimalarial mode of action. *Tetrahedron Lett.* **1999**, *40*, 4715−4718.

(30) (a) Robert, A.; Meunier, B. Unpublished results. (b) Jung, M.; Lee, S. Stability of acetal and nonacetal-type analogues of artemisinin in simulated stomach acid. *Bioorg. Med. Lett.* **1998**, *8*, 1003−1006.

(31) Posner, G. H.; Cumming, J. N.; Ployprodith, P.; Oh, C. H. Evidence for Fe(IV)═O in the molecular mechanism of action of the trioxane antimalarial artemisinin. *J. Am. Chem. Soc.* **1995**, *117*, 5885−5886.

(32) Kapetanaki, S.; Varotsis, C. Ferryl-oxo heme intermediate in the antimalarial mode of action of artemisinin. *FEBS Lett.* **2000**, *474*, 238−241.

(33) Czernuszewicz, R. S.; Su, Y. O.; Stern, M. K.; Makor, K. A.; Kim, D.; Groves, J. T.; Spiro, T. G. Oxomanganese(IV) porphyrins identified by resonance Raman and infrared spectroscopy: weak bonds and the stability of the half-filled t2g subshell. *J. Am. Chem. Soc.* **1988**, *110*, 4158−4165.

(34) Traylor, T. G.; Miksztal, A. R. Mechanism of hemin-catalyzed epoxidations: electron transfer from alkenes. *J. Am. Chem. Soc.* **1987**, *109*, 2770−2774.

(35) Robert, A.; Boularan, M.; Meunier, B. Interaction of artemisinin (qinghaosu) with the tetraphenylporphyrinato-manganese(II) complex. *C. R. Acad. Sci.* **1997**, *324-IIb*, 59−66.

(36) Cazelles, J.; Robert, A.; Meunier, B. Alkylating Capacity and Reaction Products of Antimalarial Trioxanes after Activation by a Heme Model. *J. Org. Chem.*, in press.

(37) Meunier, B.; Guilmet, E.; De Carvalho, M.-E.; Poilblanc, R. Sodium hypochlorite: a convenient oxygen source for olefin epoxidation catalyzed by (porphyrinato)manganese complexes. *J. Am. Chem. Soc.* **1984**, *106*, 6668−6676.

(38) Jefford, C. W.; Favarger, F.; Vicente, M. G. H.; Jacquier, Y. The decomposition of *cis*-fused cyclopenteno-1,2,4-trioxanes induced by ferrous salts and some oxophilic reagents. *Helv. Chim. Acta* **1995**, *78*, 452−458.

(39) Ingold, K. U.; MacFaul, P. A. Distinguishing biomimetic oxidations from oxidations mediated by freely diffusing radicals. In *Biomimetic Oxidations Catalyzed by Transition Metal Complexes*; Meunier, B., Ed.; Imperial College Press: London, 2000, pp 45−89.

(40) (a) Hong, Y.-L.; Yang, Y.-Z.; Meshnick, S. R. The interaction of artemisinin with malarial hemozoin. *Mol. Biochem. Parasitol.* **1994**, *63*, 121−128. (b) Asawamahasakda, W.; Ittarat, I.; Pu, Y.-M.; Ziffer, H.; Meshnick, S. R. Reaction of antimalarial endoperoxides with specific parasite proteins. *Antimicrob. Agents Chemother.* **1994**, *38*, 1854−1858.

(41) Bhisutthibhan, J.; Pan, X.-Q.; Hossler, P. A.; Walker, D. J.; Yowell, C. A.; Carlton, J.; Dame, J. B.; Meshnick, S. R. The *Plasmodium falciparum* translationally controlled tumor protein homolog and its reaction with the antimalarial drug artemisinin. *J. Biol. Chem.* **1998**, *273*, 16192−16198.

(42) Pandey, A. V.; Tekwani, B. L.; Singh, R. L.; Chauhan, V. S. Artemisinin, an endoperoxide antimalarial, disrupts the hemoglobin catabolism and heme detoxification systems in malarial parasite. *J. Biol. Chem.* **1999**, *274*, 19383−19388.

(43) Posner, G. H.; Oh, C. H.; Wang, D.; Gerena, L.; Milhous, W. K.; Meshnick, S. R.; Asawamahasakda, W. Mechanism-based design, synthesis, and in vitro antimalarial testing of new 4-methylated trioxanes structurally related to artemisinin: the importance of a carbon-centered radical for antimalarial activity. *J. Med. Chem.* **1994**, *37*, 1256−1258.

(44) Jefford, C. W.; Vicente, M. G. H.; Jacquier, Y.; Favarger, F.; Mareda, J.; Millasson-Schmidt, P.; Brunner, G.; Burger, U. The deoxygenation and isomerization of artemisinin and artemether and their relevance to antimalarial action. *Helv. Chim. Acta* **1996**, *79*, 1475−1487.

(45) Wu, W.-M.; Wu, Y.; Wu, Y.-L.; Yao, Z.-J.; Zhou, C.-M.; Li, Y.; Shan, F. Unified mechanistic framework for the Fe(II)-induced cleavage of qinghaosu and derivatives/analogues. The first spin-trapping evidence for the previously postulated secondary C-4 radical. *J. Am. Chem. Soc.* **1998**, *120*, 3316−3325.

(46) Lee, I.-S.; Hufford, C. D. Metabolism of antimalarial sesquiterpene lactones. *Pharmacol. Ther.* **1990**, *48*, 345−355.

(47) Robert, A.; Meunier, B. Is alkylation the main mechanism of action of the antimalarial drug artemisinin? *Chem. Soc. Rev.* **1998**, *27*, 273−279.

(48) (a) Robert, A.; Meunier, B. Characterization of the first covalent adduct between artemisinin and a heme model. *J. Am. Chem. Soc.* **1997**, *119*, 9, 5968−5969. (b) Robert, A.; Meunier, B. Alkylating properties of antimalarial artemisinin derivatives and synthetic trioxanes when activated by a reduced heme model. *Chem. Eur. J.* **1998**, *4*, 1287−1296.

(49) (a) Cazelles, J.; Robert, A.; Meunier, B. Alkylation of heme by artemisinin, an antimalarial drug. *C. R. Acad. Sci. Paris* **2001**, *Chem. 4*, 85−89. (b) Robert, A.; Cazelles, J.; Meunier, B. Characterization of the alkylation product of heme by the antimalarial drug artemisinin. *Angew. Chem., Int. Ed.* **2001**, *40*, 1954−1957.

(50) Wang, D.-Y.; Wu, Y.-L. A possible antimalarial action mode of qinghaosu (artemisinin) series compound. Alkylation of reduced glutathione by *C*-centered primary radicals produced from antimalarial compound qinghaosu and 12-(2,4-dimethoxyphenyl)-12-deoxoqinghaosu. *Chem. Commun.* **2000**, 2193-2194.

(51) Posner, G. H.; O'Dowd, H.; Caferro, T.; Cumming, J. N.; Ploypradith, P.; Xie, S.; Shapiro, T. A. Antimalarial sulfone trioxanes. *Tetrahedron Lett.* **1998**, *39*, 2273−2276.

(52) (a) Provot, O.; Camuzat-Dedenis, B.; O. Hamzaoui, M.; Moskowitz, H.; Mayrargue, J.; Robert, A.; Cazelles, J.; Meunier, B.; Zouhiri, F.; Desmaële, D.; d'Angelo, J.; Mahuteau, J.; Gay, F.; Cicéron, L. Structure−activity relationships of synthetic tricyclic trioxanes related to artemisinin: the unexpected alkylative property of a 3-(methoxymethyl) analog. *Eur. J. Org. Chem.* **1999**, 1935−1938. (b) Cazelles, J.; Camuzat-Dedenis, B.; Provot, O.; Robert, A.; Mayrargue, J.; Meunier, B. Alkylating properties of synthetic trioxanes related to artemisinin. *J. Chem. Soc., Perkin Trans. 1* **2000**, 1265−1270.

(53) The average distance travelled by a diffusing particle in a time t is $\langle x \rangle = 2(Dt/\pi)^{1/2}$. A typical diffusion coefficient for a small organic molecule is $D = 5 \times 10^{-10}$ m^2 s^{-1} (sucrose in water). See: Atkins, P. W. In *Physical Chemistry*, 5th ed.; Oxford University Press: Oxford, 1994, pp 846−856.

(54) Calas, M.; Ancelin, M. L.; Cordina, G.; Portefaix, P.; Piquet, G.; Vidal-Sailhan, V.; Vial, H. Antimalarial activity of compounds interfering with *Plasmodium falciparum* phospholipid metabolism: comparison between mono- and bisquaternary ammonium salts. *J. Med. Chem.* **2000**, *43*, 505−516.

(55) Biot, C.; Glorian, G.; Maciejewski, L. A.; Brocard, J. S. Synthesis and antimalarial activity in vitro and in vivo of a new ferrocene−chloroquine analogue. *J. Med. Chem.* **1997**, *40*, 3715−3718.

(56) Dechy-Cabaret, O.; Benoit-Vical, F.; Robert, A.; Meunier, B. Preparation and antimalarial activities of "trioxaquines", new modular molecules with a trioxane skeleton linked to a 4-aminoquinoline. *ChemBioChem.* **2000**, *1*, 281−283.

(57) Basco, L. K.; Dechy-Cabaret, O.; Ndounga, M.; Meche, F. S.; Robert, A.; Meunier, B. In vitro activity of DU-1102, a new trioxaquine derivative, against *Plasmodium falciparum* isolates. *Antimicrob. Agents Chemother.* **2001**, *45*, 1886−1888.

(58) For information on the development of antimalarial trioxaquines, see http://www.palumed.com.

AR990164O