Journal of Medicinal Chemistry

Volume 47, Number 12 June 3, 2004

Perspective

A Medicinal Chemistry Perspective on Artemisinin and Related Endoperoxides

Paul M. O'Neill*,† and Gary H. Posner‡

Department of Chemistry, The Robert Robinson Laboratories, University of Liverpool, Liverpool L69 7ZD, U.K., and Department of Chemistry, Remsen Hall, The Johns Hopkins University, Baltimore, Maryland 21218

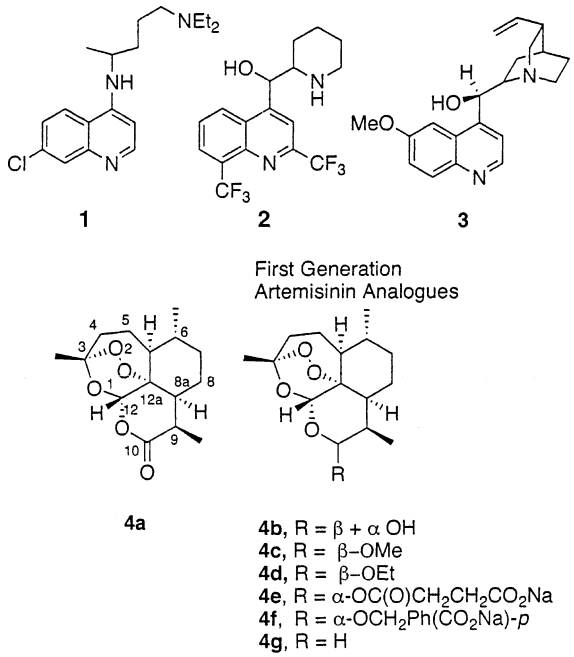
Received November 11, 2003

Introduction

Currently, nearly two billion people are at risk of contracting malaria and the incidence of this disease is dramatically increasing because many *Plasmodium falciparum* strains, the parasite responsible for the majority of fatal malaria infections, have become resistant to chloroquine (**1**).[1,2] Some strains have also developed resistance to mefloquine (**2**)[3] and even to the naturally occurring and highly efficient antimalarial quinine (**3**).[4]

Resistance to chloroquine first appeared in Thailand and South America in the early 1960s, and many strains of *P. falciparum* resistant to nearly all quinoline drugs are now present in large parts of the world.[5] Resistance is now so widespread that chloroquine is virtually useless in some parts of the world. The alarming spread of drug resistance has led the World Health Organization (WHO) to predict that in the absence of new antimalarial strategies the number of people suffering from malaria will double by the year 2010. Thus, to circumvent this phenomenon of drug resistance, it is imperative that novel drugs should be developed to treat the disease.[6] In the absence of an effective malaria vaccine, the design and discovery of new antimalarial drugs that are active against chloroquine-resistant parasites is the best way to achieve the goal of "rolling back malaria".[7]

In 1967, the Chinese government launched a program to discover new antimalarial drugs, and indigeneous plants used in traditional medicine were systematically examined.[8,9] The first written record of the antipyretic activity of tea-brewed leaves of *Artemisia annua* was described in "The Handbook of Prescriptions for Emergency Treatments" written by Ge Hong (281−340 A.D.). Li Shizen's "Compendium of Materia Medica" (published in 1596) cited the prescription from Ge Hong's book. In 1972, Chinese researchers isolated, by extraction at low temperature from this plant, a crystalline compound that they named *qinghaosu* [the name artemisinin (**4a**) is preferred by Chemical Abstracts, Registry No. 63968-64-9]. The source of artemisinin is the plant *A. annua* (Sweet wormwood), and the fact that artemisinin is a



* To whom correspondence should be addressed.′ Phone: 0151-794-3553. Fax: 0151-794-3588. E-mail: p.m.oneill01@liv.ac.uk.
† University of Liverpool.
‡ The Johns Hopkins University.

10.1021/jm030571c CCC: $27.50
Published on Web 05/12/2004

stable, easily crystallizable compound renders the extraction and purification processes reasonably straightforward.[10] In Vietnam, the total amount of artemisinin produced in 1999 was approximately 3 tonnes. It is difficult to estimate production in China, but figures close to 5 tonnes per year (from wild *A. annua*) have been reported.[11] Thus, as cultivation of *A. annua* increases, artemisinin should prove to be a cheap and readily available building block for improved analogue synthesis.[12−14] In this Perspective, we will review aspects of the chemistry, mechanism of action, metabolism, and toxicity of the endoperoxide class of drugs and will focus on lead compounds (semisynthetic and synthetic) that have emerged as potential next-generation analogues. We will also briefly cover artemisinin combination therapy (ACT) and will discuss the potential of drug hybrids and prodrugs as new approaches to delivering combination chemotherapy through a single chemical entity.

Artemisinin and First-Generation Derivatives

Although artemisinin (**4a**, qinghaosu) has been used clinically in China for the treatment of multidrug-resistant *Plasmodium falciparum* malaria, the therapeutic value of **4a** is limited to a great extent by its low solubility in both oil and water. Consequently, in the search for more effective and soluble drugs, Chinese researchers prepared a number of derivatives of the parent drug.[15] Reduction of artemisinin produces dihydroartemisinin (**4b**), which has in turn led to the preparation of a series of semisynthetic first-generation analogues that include artemether (**4c**, R = −Me) and arteether (**4d**, R = −Et). Both of these compounds are more potent than artemisinin but have short plasma half-lives and produce fatal central nervous system (CNS) toxicity in chronically dosed rats and dogs.[16−18] Although neurotoxicity is an issue in animal models, recent studies by White and co-workers have shown a lack of neuronal death in patients who had received high doses of artemether by intramuscular injection.[19] Despite these observations, there is no comparative data on oral dosing with first-generation alkyl ether prodrugs (i.e., artemether, arteether) of the neurotoxic dihydroartemisinin.

For treatment of advanced cases of *P. falciparum* malaria, a water-soluble derivative of artemisinin is desired. A water-soluble derivative can be injected intravenously (iv), and thus, the drug can be delivered more quickly than by intramuscular (im) injection (Lin et al., 1989).[20] The sodium salt of artesunic acid (**4e**) is such a water-soluble derivative, capable of rapidly diminishing parasitaemia and restoring consciousness of comatose cerebral malaria patients.[20] Because of the high recrudescence rate, however, sodium artesunate (**4e**) is normally administered in combination therapy, most often with mefloquine.[21] Out of all the first-generation derivatives, sodium artesunate (**4e**) is currently the drug of choice.[22]

Sodium artelinate (**4f**), the sodium salt of artelinic acid, was designed to overcome the hydrolytic instability experienced with artesunate.[23,24] In comparison to artemether and arteether, **4f** not only is more stable in aqueous solution but also has a much longer half-life (1.5−3 h) than oil-soluble analogues. In addition, recent findings suggest that **4f** is substantially less CNS-toxic in rats and dogs than either of the first-generation ethers **4c** and **4d**.[25]

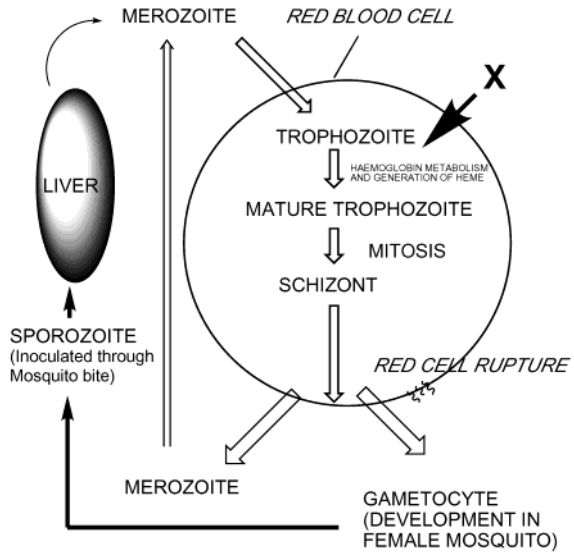


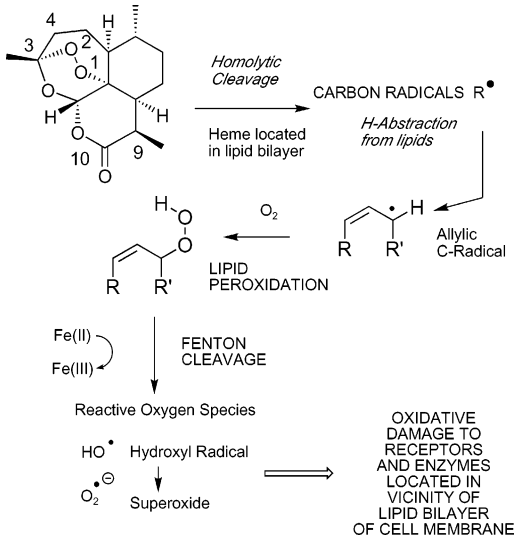
Figure 1. Life cycle of the malaria parasite. Site X is the principal point of attack for peroxide-based antimalarials.

Current Theories on the Mechanism of Action

Figure 1 illustrates the life cycle of *Plasmodium falciparum*, one of the four species of parasite that are pathogenic in man (and also the most dangerous). As can be seen, a portion of the life cycle occurs within the erythrocyte (red blood cell) of the human host. During this stage of the cycle, the parasite utilizes host hemoglobin as a food source. Hemoglobin is imported into a specialized acidic compartment in the parasite, known as a food vacuole (FV), and broken down by proteolytic enzymes called plasmepsins[26] to peptides that are subsequently degraded to amino acids by a number of food vacuole cysteine proteases, known as falcipains.[27−32] In the process an amount of 4 equiv of heme [ferroprotoporphyrin IX, or Fe(II)PPIX] is released and oxidized to hematin Fe(III)PPIX. Hematin in its reduced ferrous state [Fe(II)PPIX will be referred to as heme in this review] is toxic to microorganisms in its free form and, in the parasite, is detoxified by conversion to an insoluble compound known as malaria pigment or hemozoin. Recent evidence suggests that disruption of this detoxification process is the chemical mechanism of action of chloroquine and related drugs.[33−36] As we will see below, it has been widely proposed that hematin, upon reduction to heme, is the source of ferrous iron that is responsible for bioactivation of the endoperoxide bridge of artemisinin to cytotoxic radical species (vide infra).

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 trioxane 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.[37] An artemisinin derivative lacking the endoperoxide bridge (deoxyartemisinin) is devoid of antimalarial activity, indicating that this peroxide functionality is the key factor of the pharmacological activity of these trioxanes.[8]

Scheme 1. Proposed Chemical Mechanism for the Observed Artemisinin Mediated Lipid Peroxidation of Cell Membranes[a]



[a] Carbon radicals, generated from artemisinin, abstract allylic hydrogen atoms from unsaturated lipid bilayers to set in motion the downstream generation of a variety of reactive oxygen species by a classic lipid peroxidation mechanism. Heme or hematin/thiols associated with the lipid bilayer may be the catalysts responsible for initiation of these processes.

Oxidative Stress. Artemisinin-mediated oxidative stress has been proposed as a mechanism of action on the basis of in vitro experiments with infected human red blood cells or with parasite membranes.[38] In fact, the intraerythrocytic activation of the trioxane peroxidic bond by iron(II)-heme produced during hemoglobin degradation should generate oxygen radical species. In vitro, heme catalyzes the reductive decomposition of artemisinin and dihydroartemisinin.[39] 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. However, these experiments were made at concentrations ranging from 50 to 1000 mM, that is, at doses 10^3-10^5 times higher than effective in vitro drug concentrations.[40]

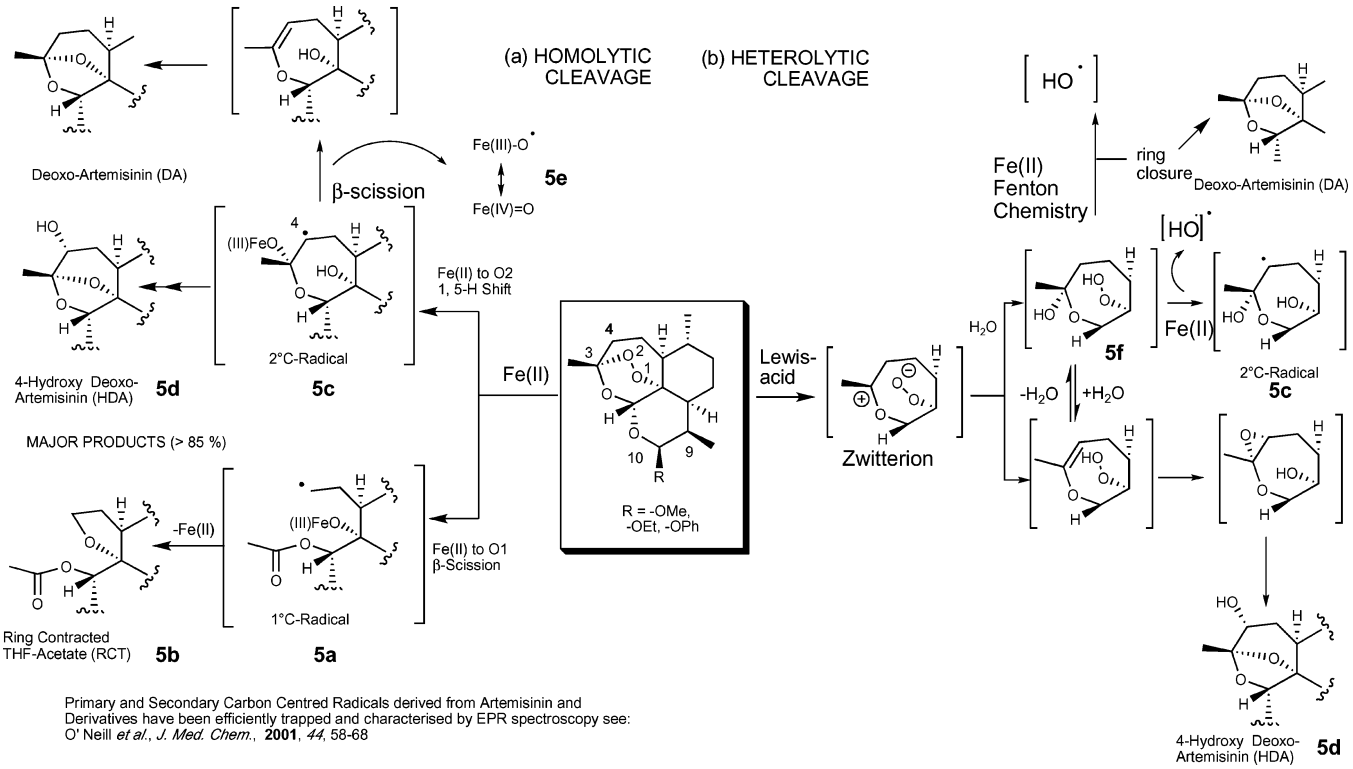
Biomimetic studies by Berman and Adams have clearly demonstrated that artemisinin can effect a 6-fold increase in heme-mediated lipid membrane damage.[41] Of importance to this observation are the findings of Fitch and colleagues who have recently demonstrated that *unsaturated* lipids coprecipitate with hematin in the parasite's acidic food vacuole and also dissolve sufficient monomeric hematin to allow efficient polymerization.[42,43] A possible mechanism of artemisinin-induced lipid peroxidation is depicted in Scheme 1, and this mechanism provides access downstream to typical reactive oxygen species such as the hydroxyl radicals and the superoxide anion. Interaction of lipid solubilized heme with artemisinin followed by ferrous-mediated generation of oxyl and carbon radicals places these reactive intermediates in the vicinity of target allylic hydrogens of unsaturated lipid bilayers. Hydrogen abstraction and allylic carbon radical formation with subsequent triplet ground-state oxygen capture result ultimately in the formation of lipid hydroperoxides. The explicit mechanism depicted in Scheme 1 is supported by the work of Berman and Adams and others, and it was proposed that the damage caused to the parasite's FV membrane leads to vacuolar rupture and parasite autodigestion.[41] This mechanism is consistent with the observed oxygen dependence of antimalarial action of artemisinin and the morphological changes seen following artemisinin administration to parasites in vitro.[44] The biological significance of hydroperoxides in relation to biological hydroxylation and autoxidation of, for example, lipids and membrane bilayers is well established. The generation of unsaturated lipid hydroperoxides provides a means of initiation of such processes. In contrast to these proposals, other workers in the field have suggested that membrane-bound heme may have a role to play in reducing the effectiveness of endoperoxides such as dihydroartemisinin. Further work is required to clarify the role of *vacuolar membrane bound heme* in the mechanism of action of endoperoxide antimalarials.[45] An alternative nonlipid "artemisinin derived source of hydroperoxide" is discussed below.

Although Scheme 1 would appear chemically plausible, several workers have proposed that the parasite death in the presence of artemisinin is probably not due to nonspecific or random cell damage caused by freely diffusing oxygen radical species but might involve *specific radicals and targets*, some of which are described later in this review. The following section details the specific "transitory" species that may be responsible for the antimalarial mechanism of action of artemisinin. Targets of these species are discussed and will conclude with the most recent studies by Eckstein-Ludwig who suggest that artemisinin derivatives target the PfATP6, the sarco/endoplasmic reticulum Ca^{2+}-ATPase (SERCA) of the parasite.[46]

Transitory Species Mediating the Antimalarial Activity of Artemisinin: Carbon Radicals, Carbocations, Hydroperoxides, and High-Valent Iron−Oxo Species. On the basis of the seminal work of Posner and co-workers in the early 1990s, the free radical chemistry of artemisinin is now very well-defined and has been shown to involve an initial chemical decomposition induced by heme Fe(II) (reduced hemin) or other sources of ferrous iron within the malaria parasite to produce initially an *oxy radical* that subsequently rearranges into one or both of two distinctive *carbon-centered radical species*.[47] Scheme 2a summarizes the main radical pathways available for artemisinin following endoperoxide-mediated bioactivation. Since artemisinin is an unsymmetrical endoperoxide, the oxygen atoms of the peroxide linkage can associate with reducing ferrous ions in two ways. Association of Fe(II) with oxygen 1 provides an oxy radical that goes on to produce a primary carbon-centered radical (**5a**). A surrogate marker for the intermediacy of this radical species is the ring-contracted tetrahydrofuran (RCT) product **5b**. Alternatively, association with oxygen 2 provides an oxy radical species that, via a 1,5-H shift, can produce a secondary carbon-centered radical (**5c**). Again, like the previous route, a stable end-product, hydroxydeoxoartemisinin (HDA) (**5d**), functions as a surrogate marker for this secondary carbon-centered radical species.

It has been proposed that final alkylation by these reactive intermediates of biomacromolecules such as

Scheme 2. Homolytic and Heterolytic Mechanisms of Bioactivation of the Endoperoxide Bridge of Artemisinin and Derivatives



Primary and Secondary Carbon Centred Radicals derived from Artemisinin and Derivatives have been efficiently trapped and characterised by EPR spectroscopy see: O' Neill *et al.*, *J. Med. Chem.*, **2001**, *44*, 58-68

heme, specific proteins, and other targets result in the death of malaria parasites.[48,49] The secondary C-radical intermediate (**5c**) has also been implicated as the precursor to a *high-valent iron−oxo species* (**5e**), and several experimental results support the intermediacy of such a potentially toxic species.[50,51] Although Varotsis has provided Raman spectroscopic support for the generation of a high-valent iron−oxo species during ferrous-mediated endoperoxide decay,[52,53] the groups of Meunier[54] and Jefford have contested this chemical mechanism.[55,56]

For artemisinin, Wu has argued that since the formation of the surrogate markers **5b** and **5d** proceeds in an intramolecular fashion, only a small proportion of the carbon radicals generated during iron-mediated cleavage have the ability to react with biological targets in a less favorable intermolecular sense.[57] This theory may not be unreasonable because, although there are now over 100 publications on biomimetic iron-mediated degradations on endoperoxide-based systems, to date there are only a handful of model studies where C radicals have been shown to be capable of alkylating protein models. (A literature review using ISI Web of Knowledge reveals that carbon radicals are poor protein alkylators). Models presented by Wu, where carbon radical species can react in a favored intramolecular sense with thiol residues of amino acid derivatives, include the reaction of the primary radical intermediate **5a** with iron sulfur chelates.[58,59] It follows that C−S bond formation at the enzyme active site remains a plausible but unproven chemical mechanism of action for the artemisinin class of antimalarial. At this point in the discussion, it is important to note that many endoperoxides and spirotrioxanes have been identified that can generate *carbon radicals* but *are not antimalarially* active. In our view, this does not rule out the role of carbon radicals in the mechanism of action of endoperoxide antimalarials, since careful examination of many endoperoxides that generate carbon radical species but are poor antimalarials reveal that structurally their endoperoxide function is chemically exposed and not protected (sterically shielded) compared with the trioxane bridge in artemisinin. It is likely that such systems can be detoxified by direct reaction with the parasites' antioxidant defense system or by glutathione before they have the opportunity to gain entry to the parasite cytosol and ferrous-rich food vacuole.[60,61]

Further objections to the C-4 carbon centered radical hypothesis have been put forward on the basis of careful considerations of the SAR of a ring-opened/rearranged derivative of dihydroartemisinin **5g** and Avery's *seco*-4,5-artemisinin analogues **5h**−**k** (Figure 2).[62] First, it is claimed that rearranged **5g**, which retains substantial antimalarial activity, cannot generate C-4 radicals in a manner analogous to that of artemisinin.[49] The main problem with this proposal is that in acid, **5g** can be rearranged back to the parent drug dihydroartemisinin (DHA) by an acid-catalyzed unzipping recyclization process. Thus, the activity of this analogue may stem from its reconversion to DHA (**4b**). Two of the seco analogues, **5h** and **5k**, have poor antimalarial activity, but remarkably **5i** has activity equivalent to that of artemisinin. Clearly **5i** cannot generate either a secondary or primary C-4 radical in the same manner as the parent drug artemisinin. However, this analogue still has the capacity to extrude a primary methyl radical by C−C scission after homolytic cleavage by association of reducing Fe(II) with oxygen O1. For association of ferrous iron with O2, it is not unreasonable in this case to suggest that the oxyl radical generated, without the option to generate a carbon radical in an intramolecular sense, may well be involved in intermolecular H-

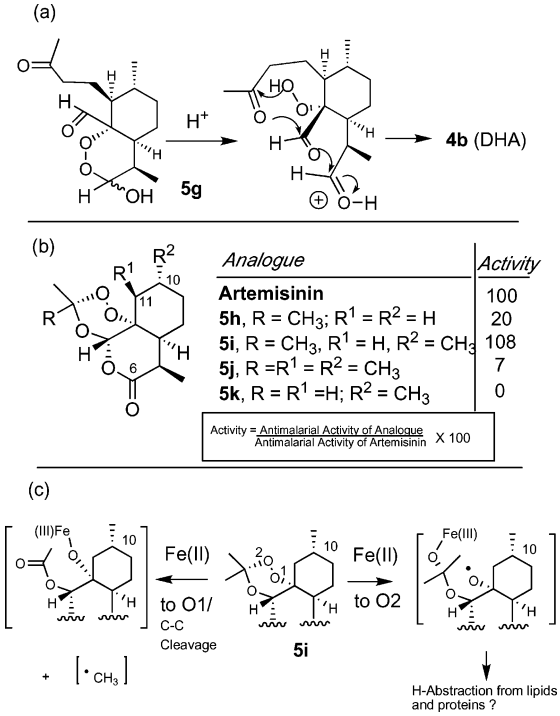
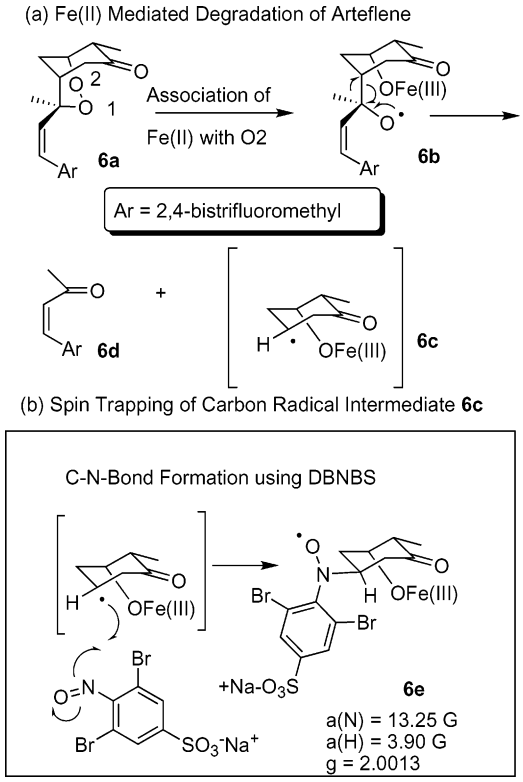


Figure 2. (a) Interconversion of a ring-opened form of DHA to DHA by an acid-catalyzed unzipping, recyclization pathway and (b) variations in the antimalarial activities for *seco*-4,5-artemisinin derivatives. (c) Possible routes of ferrous mediated degradations of a *seco* analogue **5i**.

abstractions with target proteins or lipids. Further work is required here, but what these diverse antimalarial SAR results certainly indicate is that very minor chemical changes have a profound effect on activity, suggesting that *a specific biological target* may indeed be involved and that no simple explanation can be used to fully explain the results for these seco analogues, particularly the extraordinary beneficial effect of C-10 α methyl substitution in **5i** that leads to a 5-fold (15-fold versus W-2 strain) increase in antimalarial activity versus the D2 strain of *Plasmodium falciparum* in vitro.

An alternative view to the iron-induced homolytic endoperoxide cleavage hypothesis is that artemisinin acts as a masked source of hydroperoxide (Scheme 2, b).[63−65] Heterolytic cleavage of the endoperoxide bridge and formation of an unsaturated hydroperoxide is followed by capture by water (or other nucleophile). Subsequent Fenton like degradation of the hydroperoxide produces the hydroxyl radical, a species that can go on to oxidize target amino acid residues; this alternative pathway provides a mechanism of producing a whole host of reactive oxygen species (ROS) that may have an equally important role to play in the antimalarial activity of these compounds. It has been proposed that the heterolytic step is aided by the nonendoperoxide bridging oxygen of the trioxane ring, where the carbocation can be stabilized by resonance.[63] Presumably, this species as shown can readily react with water to provide a neutral source of hydroperoxide following loss of a proton (direct loss of a proton could also generate a neutral species).[49] Haynes and co-workers have demonstrated that artemisinin can mediate N-oxidation of tertiary alkylamine derivatives via the intermediacy of such a ring-opened peroxide form of artemisinin.[63] If at this point we return to the difficulties in explaining the

Scheme 3. (a) Ferrous Mediated Bioactivation of Arteflene and (b) Spin-Trapping of Secondary Carbon-Centered Radical **6c** with the Water-Soluble Nitroso Spin-Trap DBNBS

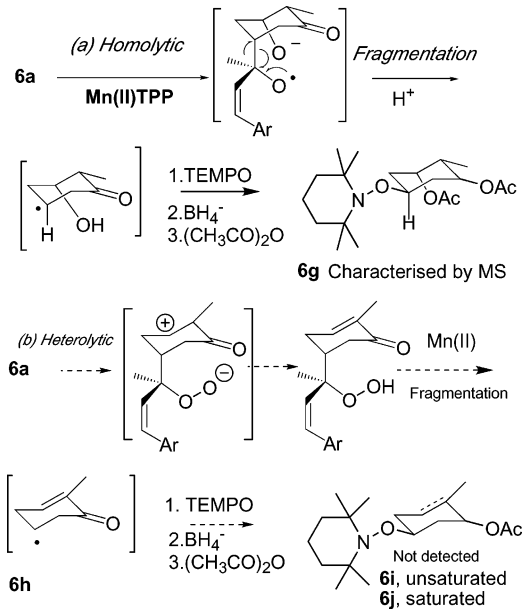


activity of *seco*-4,5-artemisinin analogues by the carbon-centered radical hypothesis, it is clear that the heterolytic cleavage mechanism is supported by the excellent activity of **5i** but not by analogue **5h**. The two seco analogues **5h** and **5i** should have no difficulty in undergoing a heterolytic cleavage step to form virtually the same carbocation, yet they have very different antimalarial activities, thereby providing some evidence against an initial heterolytic cleavage mechanism.

Definitive evidence for the generation of carbon radical intermediates during ferrous-mediated endoperoxide degradation of both artemisinin and arteflene has been provided by EPR spin-trapping techniques.[66] For artemisinin, both the primary and secondary carbon-centered radicals have been efficiently spin-trapped after iron-mediated activation.[67,68] In contrast, the endoperoxide arteflene (**6a**) cleanly generates only a secondary carbon-centered radical (**6c**), again via the intermediacy of an oxy radical (**6b**), that has been trapped by the nitroso compounds DBNBS and DMPO (Scheme 3).[68] For **6a**, carbon radical formation is accompanied by formation of enone (**6b**), a compound capable of acting as a potential Michael acceptor of thiols.[69]

While heterolytic cleavage may be possible in the trioxane class of antimalarial, it does not appear to be operative, in biomimetic studies at least, in the endoperoxide class of antimalarial (e.g., arteflene) on the basis of TEMPO spin-trapping experiments that clearly show that homolysis is the only route of degradation with *no evidence* of heterolytic retro-Michael addition of the endoperoxide bridge. Heterolytic cleavage of arteflene would necessitate the formation of a carbon−carbon

Scheme 4. Mn(II) Mediated Degradation of Arteflene[a]



[a] (a) Manganese(II) TPP mediated bioactivation of arteflene and subsequent TEMPO spin-trapping and derivatization of the adduct by acetylation. Only adduct **6g** derived from a homolytic cleavage pathway was detected, ruling out (b) the heterolytic pathway that would result in adducts **6i** and/or **6j**.

double bond containing a cyclohexyl secondary carbon-centered radical fragment (**6h**). This is not observed in spin-trapping studies conducted with TEMPO using MnTPP (or $FeCl_2 \cdot 4H_2O$) as reductants (Scheme 4), and the only TEMPO adduct characterized was **6g** with no evidence for the formation of **6i** or the double bond reduced **6j**.[70]

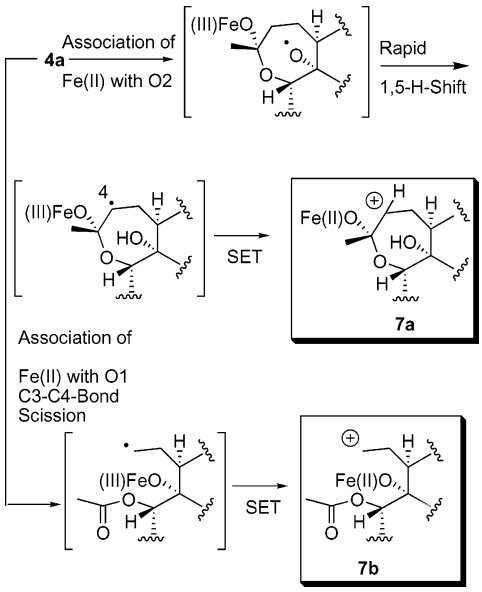
There is no question that carbon-centered radicals are generated during ferrous-mediated cleavage of trioxanes, but are these species simply intermediates en route to the ultimate electrophilic species responsible for the observed alkylation of proteins? An alternative mechanism that also renders degradation of the trioxane bridge catalytic in ferrous iron involves oxidation of the carbon radical species to an electrophilic carbocation by a single electron transfer (SET); for artemisinin this process can formally regenerate ferrous iron (Fe(II)) and carbocation species **7a** and **7b** that would appear to be better candidates as potential protein alkylating agents (Scheme 5). Bachi has recently demonstrated that oxidation of carbon radicals readily occurs during biomimetic Fe(II) degradation reactions of bicyclic endoperoxides.[71]

We have seen that artemisinin and endoperoxide-based drugs have the ability to generate a range of different reactive intermediates, and many of these have been proposed as the mediators of the phenomenal antimalarial activity of this class of drug, and we will now briefly summarize the main suggested biological targets.

Target(s) of Artemisinin Derivatives

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[39]

Scheme 5. Oxidation of Carbon Radicals to Potentially Toxic Carbocations via Fe(III) Reduction to Fe(II)



and a small number of specific parasite proteins,[72,73] one of which has a molecular size similar to those of a histidine-rich protein (42 kDa).[74] Another possible target protein is the *P. falciparum* translationally controlled tumor protein (TCTP).[75,76] Artemisinin may also be involved in the specific inhibition of malarial cysteine protease activity[77] (vide infra). Furthermore, the incubation of purified hemozoin with artemisinin at a pH value close to that of the parasite vacuole (pH 5.5) resulted in the loss of hemozoin, indicating that hemozoin may be dismantled by drug interactions, leading to the building up of the free hematin pool.[77,78]

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.[39,78] Because of the variety of possible alkylation sites on iron protoporphyrin IX, Meunier 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. When $Mn^{II}TPP$ was reacted with artemisinin (or artemether and several related synthetic trioxanes) in dichloromethane, a chlorin-type adduct (e.g., **7c**) was formed by reaction of the macrocycle with an alkyl radical generated by reductive activation of the drug endoperoxide (Scheme 6).[79]

From studies with synthetic trioxanes it was also shown that analogues 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 toward malaria infected cells and also unable to alkylate the macrocycle (Figure 3). This work suggested 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, and (ii) alkylation ability is crucial for the antimalarial activity of artemisinin and is a general feature required for the biological activity of endoperoxide-containing antimalarial drugs.[54,80]

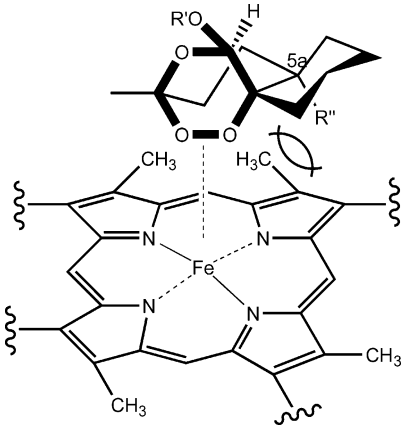
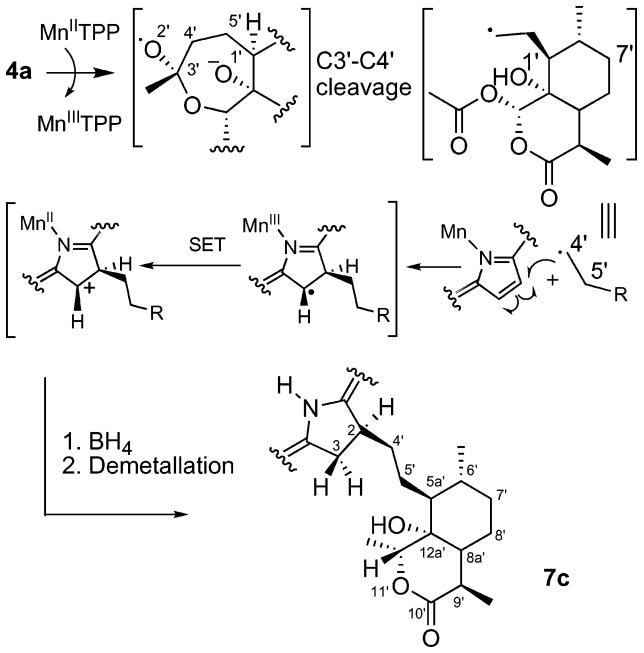


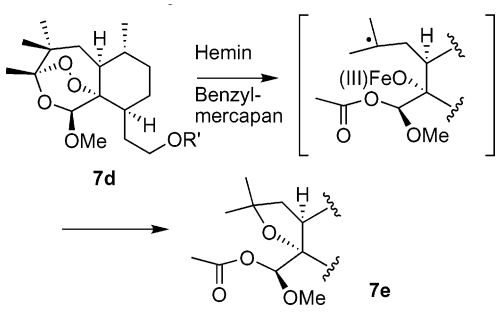
Figure 3. Detrimental effect of **5a** substitution on the docking of trioxane derivatives on heme.

Scheme 6. Reaction of an Artemisinin-Derived Primary Carbon Centered Radical with $Mn^{II}TPP$ To Give Chlorin Adduct **7c**



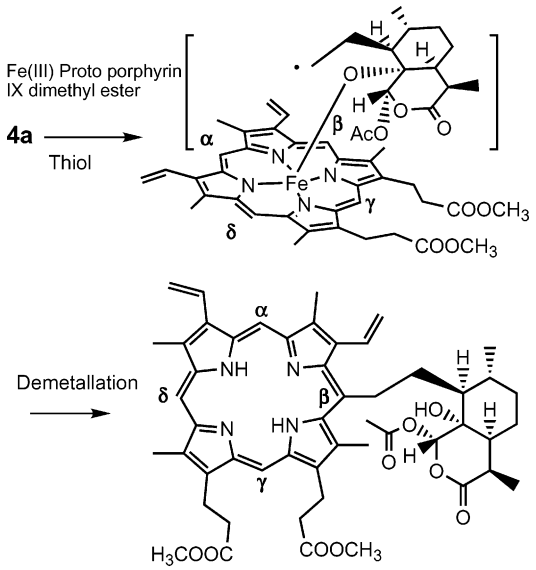
A vitally important exception to the generalizations discussed above is the observation that the 4,4′-*gem*-dimethyl-substituted trioxane **7d** is smoothly isomerized by hemin in the presence of benzyl mercaptan as reducing agent. Neither hemin alone or benzyl mercaptan alone converts trioxane **7d** into RCT product **7e**.[81] That mercaptan-reduced hemin can mediate isomerization of **7d** suggests that *4α substitution does not block trioxane* docking on heme and that other factors must account for the antimalarial inactivity of such compounds. Clearly **7d** has generated a carbon radical en route to the observed RCT product **7e** but cannot form a secondary radical in a manner analogous to that of artemisinin. Additionally, this analogue should have no problem undergoing heterolytic cleavage to an open hydroperoxide. It would appear difficult to provide a convincing explanation for the inability of the radical species from **7d** to alkylate heme or to explain this compound's antimalarial inactivity (Scheme 7). Indeed, Scheme 7 and the lack of antimalarial activity of the 4,4-dimethyltrioxane **7d** have been used to support the

Scheme 7. Hemin/Benzyl Mercaptan Mediated Isomerization of a 4,4′-*gem*-Dimethyltrioxane Analogue of Artemisinin[a]



[a] The formation of **7e** clearly demonstrates that 4-α-methyl substitution *does not prevent* close docking on heme.

Scheme 8. Reaction of an Artemisinin-Derived Primary Carbon Centered Radical with Fe(III) Protoporphyrin IX Dimethyl Ester[a]



[a] Adducts are also obtained from reaction at the α and δ carbon atoms.

importance of C-4 secondary carbon centered radical intermediates for high antimalarial activity of any trioxane. Likewise, the antimalarial inactivity of the corresponding 4α-methyltrioxane and the very potent antimalarial activity of the corresponding 4*β*-methyltrioxane both support the critical role of the secondary C-4 radical intermediate.[81]

Further studies involved the investigation of the reactivity of artemisinin toward the pharmacologically more relevant iron(II)-containing model of ferriprotoporphyrin IX.[82] For this purpose, iron(III) protoporphyrin IX dimethyl ester was exposed to artemisinin in the presence of a hydroquinone derivative (or a thiol) as reducing agent to generate the requisite iron(II) heme species (Scheme 8). Heme was readily converted in high yield to heme−artemisinin adducts.[83] The demetalation of this mixture of three adducts to facilitate the NMR characterization indicated that the α, *β*, and *δ* meso carbons were alkylated. Such results prompted Meunier to suggest that the low and transient concentration of free heme generated by hemoglobin degradation in vivo may be responsible for the reductive activation of the

endoperoxide function of active trioxanes. This pathway generates alkylating species, such as the primary carbon radical (**5a)**, which are likely to disrupt vital biochemical processes of the parasite via alkylation of biomolecules located in the vicinity of the free heme. This proposal is based on the assumption that the primary C radical has sufficient lifetime to migrate from the face of the porphyrin metallocycle and subsequently to interact with its biological target.[49]

Although it is clear in model systems that artemisinin can efficiently alkylate heme-based models, the role of this event in the mechanism of action of artemisinin has been questioned. For example, it has been proposed that the formation of heme−artemisinin adducts of the type described could result in the prevention of heme polymerization to nontoxic hemozoin. The resultant buildup of redox active alkylated porphyrins could in theory lead to parasite death by a mechanism similar to that proposed for the quinoline-based antimalarials. However, Haynes and co-workers have ruled out this potential mechanism by demonstrating clearly that although artemisinin (**4a**) and dihydroartemisinin (**4b**) have the ability to inhibit β-hematin formation in vitro, the closely related and antimalarially potent C-10 deoxoartemisinin (**4g**) has no effect on polymerization. Thus, it was proposed that the observed inhibitory activities in the heme polymerization inhibitory assay (HPIA) for **4a** and **4b** are a reactivity or property not related to the inherent antimalarial mode of action of this class of drugs.[84]

Other workers have questioned the role of heme as the catalyst for artemisinin bioactivation on the basis of isobologram analysis with a variety of different protease inhibitors and artemisinin derivatives. By use of a number of peptide-based inhibitors, it has clearly been shown that inhibition of hemoglobin proteolysis does not antagonize the antimalarial mechanism of dihydroartemisinin. This is in sharp contrast to the situation with chloroquine, where protease inhibition results in marked antimalarial antagonism (this result provides evidence to support the widely accepted theory that hematin is the target for the 4-aminoquinoline class of drugs).[85,86] This result implies that heme may not be the iron source responsible for endoperoxide bioactivation. If there are catalytic amounts of heme present after protease inhibition, then the efficient alkylation processes described above[54,83,87] would be expected to rapidly consume any residual quantities of "free heme", thus rendering further endoperoxide cleavage impossible. Apparently, this is not observed.

Other evidence against the "alkylation of heme theory" includes the fact that arteflene and other endoperoxide analogues do not form covalent adducts with iron-based[69] and manganese-based heme models.[70]

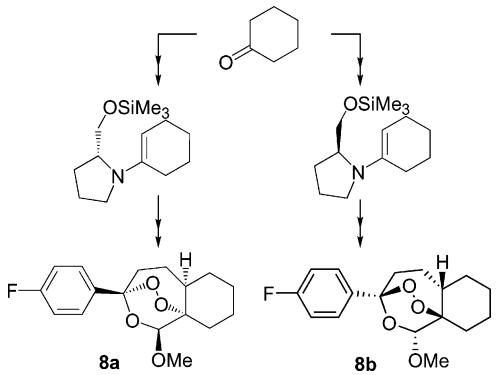
It is emphasized that virtually all of the above discussion is based on *biomimetic chemistry* where the Fe(II) source varies from salts such $FeSO_4$ to the more reactive $FeCl_2 \cdot 4H_2O$ as well as heme mimetics (TPP) and ester hematin variants. When heme models are used, since porphyrin alkylation is a favored process, end-product distributions of products can be very different from when a free ferrous ion source is employed. Furthermore, solvent has been shown to have a profound effect on the products obtained in iron-mediated endoperoxide degradation. Thus, all of these studies are truly only approximate models of the actual events within the malaria parasites.[48,57] Future work is needed to correlate the results of biomimetic chemistry with the actual situation within the parasite. In our view, the endoperoxide class of drugs should be viewed as an iron-triggered "cluster bomb" with many of the reactive intermediate species that we have described having some role to play in the expression of the observed potent antimalarial activity. It also seems reasonable that specific noncovalent interactions with the biological targets are involved prior to the iron-triggered release of the toxic entities we have described. It may be necessary to postulate such drug−target interactions to explain the observed and very diverse SAR in this field.

Enzymes as Targets. As described earlier, erythrocytic malaria parasites degrade hemoglobin to acquire amino acids for protein synthesis. Falcipain 2 (FP-2)[29] is a papain family cysteine protease that appears to act in concert with other enzymes including two aspartic proteases[88,89] to degrade hemoglobin. Incubation of erythrocytic parasites with inhibitors of FP-2 blocks hemoglobin degradation and parasite development. Pandey has demonstrated that, in purified digestive vacuoles from *P. yoelli*, cysteine protease activity can be inhibited by artemisinin in a manner similar to that of the potent cysteine protease inhibitor E-64.[77] Inhibition of falcipain-mediated cleavage of the fluorogenic peptide substrate Z-Phe-Arg-AMC was also demonstrated in a continuous fluorometric assay, and surprisingly protease inhibition was increased in the presence of heme (suprising in the sense that strong arguments have been made that the heme-generated radical species cannot escape the porphyrin macrocycle and hit biological targets after reductive endoperoxide cleavage).

To fully validate falcipain 2 and falcipain 3 as targets for endoperoxide drugs, it is essential that these studies be expanded to human forms of the parasite. In addition, it would be of great interest to compare the efficiency of falcipain inhibition with the known antimalarial activities of a series of artemisinin analogues of varying potency.

Krishna and co-workers[46] have very recently provided compelling evidence that artemisinins act by inhibiting PfATP6, the sarco/endoplasmic reticulum Ca^{2+}-ATPase (SERCA) orthologue of *Plasmodium falciparum*. When expressed in *Xenopus* oocytes, Ca^{2+}-ATPase activity of PfATP6 is inhibited by artemisinin with potency similar to that of thapsigargin (another sesquiterpene lactone and highly specific SERCA inhibitor) but not by quinine or chloroquine. As predicted from this observation, thapsigargin antagonizes the parasiticidal activity of artemisinin. Desoxyartemisinin is ineffective as an antimalarial and was not shown to inhibit PfATP6 activity. Chelation of iron by desferrioxamine abrogates the antiparasitic activity of artemisinins and correspondingly attenuates inhibition of PfATP6. Single-cell imaging of living parasites with BODIPY-thapsigargin demonstrates cytosolic labeling that is competed for by an excess of artemisinin. Furthermore, similar labeling is observed with a novel fluorescent artemisinin derivative. These studies support PfATP6 as a target of artemisinins operating via an Fe^{2+}-dependent activation mechanism. This information may allow, for the first

Scheme 9. Asymmetric Synthesis of Chiral 1,2,4-Trioxanes[92]



time, rational biological target-guided drug design efforts to be carried out.[90]

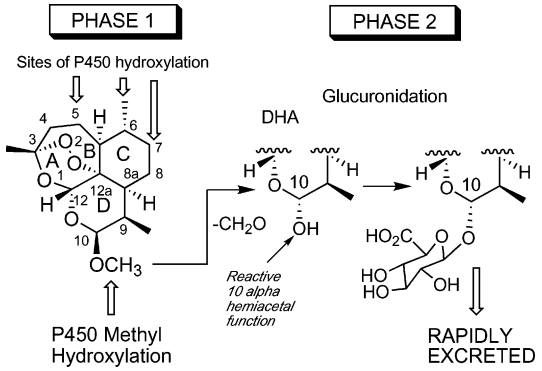
If trioxanes and endoperoxides mediate their activity through hitting a single enzyme target such as falcipain or the SERCA, then why has resistance to these derivatives not been rapidly developed?[91] The most probable answer to this question is that artemisinin derivatives do not exert their antimalarial effects by hitting a *single biological target* but rather by simultaneously hitting several targets with very high precision and efficiency.[90] These obviously involve enzyme inhibition, lipid peroxidation, membrane damage in the vicinity of vital enzymes, and some effect on heme detoxification (this is clearly analogue-dependent). That artemisinin derivatives' activities are mediated solely by interaction with a single enzyme seems unlikely, and this can be critically addressed by the use of enantiomerically enriched 1,2,4-trioxane analogues.[92] These should have very different K_d values for the enzyme and potentially should have differential in vitro activities. Conveniently, the authors have developed routes to chiral nonracemic 1,2,4 trioxane alcohols and C-3 aryltrioxanes **8a** and **8b** (Scheme 9),[92] and methods are also in place for the synthesis of nonracemic endoperoxide analogues to test some of these newly suggested enzyme targets.

Chemical Stability and Drug Metabolism

Human plasma concentrations of oral, intravenous, or intrarectal artemisinin and its derivatives artemether, arteether, and artesunate reach a maximum within 1−3 h when the concentration of the metabolite DHA is included.[93] Intramuscular administration of the ethers in sesame oil or peanut oil results in a different plasma profile because of the slow release from the injection depot. Time-dependent first-pass metabolism in the gut and liver is observed for artemisinin derivatives.[94] In addition to metabolic degradation, artesunate has also been shown to hydrolyze to dihydroartemisinin in vitro.[95] Thus, its half-life is several minutes, whereas that of DHA, at 40−60 min, is similar to that of artemether. As a hemiester, artesunate is intrinsically unstable, and it seems likely that its hydrolysis in vivo may not be enzyme-mediated. Artemisinin undergoes phase I metabolism by CYP2B6, CYP2C19, CYP3A4, and at least one other member of the P-450 enzyme superfamily.[96]

CYP3A4 is primarily involved in artemether metabolism, and apart from oxidative dealkylation to DHA,[97,98]

Scheme 10. Sites of P450 Monohydroxylation on the Artemisinin Tetracycle[a]



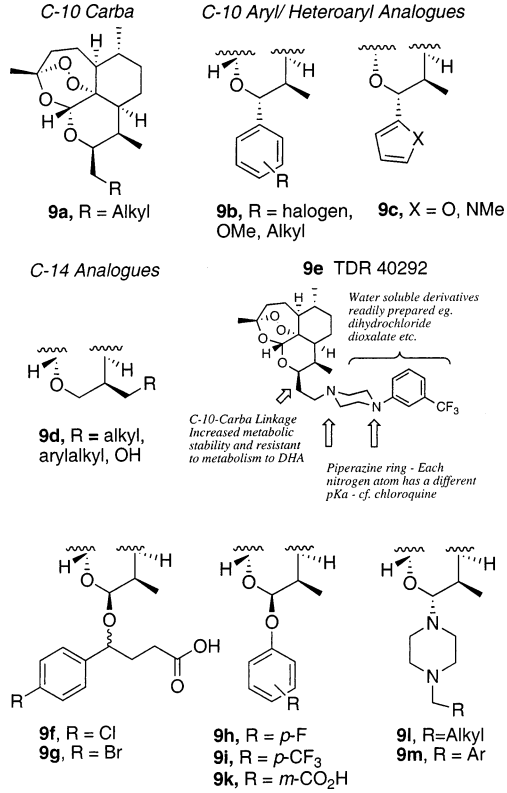
[a] For artemether, P450-catalyzed oxidation on the methyl group of the C-10 methoxy function liberates a hemiacetal, which collapses to DHA. Subsequently, DHA is rapidly glucuronidated and excreted.

the prime pathway is hydroxylation in the liver and intestine. Some but not all of the B- and C-ring hydroxylated metabolites derived from artemisinin derivatives are active antimalarials, although these may be eliminated through phase II metabolism via generation of water-soluble conjugates such as glucuronides. For example, Maggs and co-workers have demonstrated that the major biliary metabolite of artemether in rats is the 7α-hydroxyglucuronide.[99] Hydroxylation by P-450 involves substrate, reducing cofactor, and heme iron that proceeds through the Fe(II) and Fe(III) oxidation states. Oxygen transfer occurs from a perferryl Fe(V)═O species generated by a reaction between dioxygen (O_2) and the Fe(II) complex. The sites of hydroxylation are derived from presentation of the B- or C-ring end of the artemisinin tetracycle to the reactive perferryl center of the P450 enzyme active site (Scheme 10). It is remarkable to note that in all mammalian drug metabolism studies to date, there is no evidence for artemisinin analogues acting as irreversible inhibitors of the cytochrome P450 subfamily. In other words, heme-based P450 iron does not reductively activate artemisinins to radical species that subsequently alkylate the protein cavity surrounding the active site of the enzyme. The restrained metabolism of endoperoxides contrasts with their reactivity to inorganic(II), which exceeds that of simple dialkyl peroxides.[100]

The basis for the very minor reductive isomerization that has been observed in drug metabolism studies on artemisinin or artemether (ie formation of RCT, **5b** and HAD, **5d**, Scheme 2) may be as a result of prostacyclin and thromboxane synthase heme thiolate enzymes (compare with the reductive isomerizations of 1,3-endoperoxide PGH_2 in the prostacyclin (PGI_2) and thromboxane (TXA_2) biosynthetic pathways)[101] or may be the result of trace amounts of non-transferrin-bound iron. Overall, it is clear that cytochrome P450 catalyzes extensive hydroxylation and O-dealkylation without obtaining effective access to the shielded endoperoxide bridge. In addition, these observations emphasize the fact that heme, in its redox active form in the food vacuole, is unlikely to be associated with protein and is likely to be in an environment very different from that of the CYP P450 active site.

From a medicinal chemistry perspective, the coupled phase 1 and phase 2 conjugation reactions (Scheme 10,

Chart 1. Second-Generation Artemisinin Derivatives



shown for artemether) of **4c**−**e** are the principal means through which these derivatives have a short half-life.[102] As a result, a huge amount of work has been carried out to improve the potency and stability of the first-generation analogues. The following section summarizes the main lead compounds that have emerged as potential next-generation peroxide analogues.

Semisynthetic and Synthetic Endoperoxide Analogues

The synthesis of metabolically more robust C-10 carba analogues **9a** and C-10 aryl analogues **9b** of DHA has been the focus of medicinal chemists for the past 10 years.[103] Notable analogues that emerged include the C-10 alkyldeoxo analogues prepared by Haynes,[104,105] Posner,[103,106−108] O'Neill,[109−111] Jung,[112−114] and Ziffer[115−117] and the C-10 aryl or heteroaromatic derivatives **9b** and **9c** prepared by the groups of Haynes[118] and Posner[103] (Chart 1). Equally impressive in terms of antimalarial activity profiles are the C-14 modified analogues **9d** prepared by elegant total synthesis and semisynthesis by the Avery[119−121] and Jung[122] groups, respectively. With an ever-expanding data set for artemisinin analogues, Avery has recently developed a predictive 3-D QSAR for artemisinin analogues, and this is being used to design even more potent peroxide derivatives.[123,124] Future work in this area will no doubt combine the computer-guided C-10 and C-14 functional group optimization of antimalarial activity coupled with careful incorporation of functional groups that provide both water solubility and resistance to first-pass metabolism.

It is important to stress that a factor not taken into account by many of the C-10 and C-14 analogues described above is the concern about potential neurotoxicity. Any analogue with a log *P* higher than that of artemether (3.3−3.5) can cross the blood−brain barrier. Recently, with this in mind, Haynes and co-workers have succeeded in preparing new analogues that have considerably reduced neurotoxicity. By use of the ADME (absorption, distribution, metabolism, and excretion) paradigm for enhancing efficacy through increased absorption, the application of "Lipinski's Rule of Five" to the design of new semisynthetic analogues has been employed with the exception that the new semisynthetic derivatives do not have a log *P* greater than 2.5.[11,125] As discussed in a recent review by Haynes, there are many ways in which a medicinal chemist can achieve such criteria by the incorporation of suitable polar residues and their isosteres. Artemisone is undergoing development by Baeyer as an analogue with much improved properties over the first-generation analogues and represents the success of the ADME approach to drug design.

Recently, in research in collaboration with WHO, a C-10 carba analogue (TDR 40292) **9e** has been compared with artemether. This compound cannot form DHA as a metabolite and contains a side chain that can be formulated as a water-soluble salt. In addition, antimalarial assessment both in vitro and in vivo demonstrates that this compound is superior to artemether and artesunate.[110] Furthermore, from initial pharmacokinetic data, **4d** has a higher volume of distribution than artemether and is considerably more orally bioavailable (16% versus 1.5% for artemether) (unpublished results).

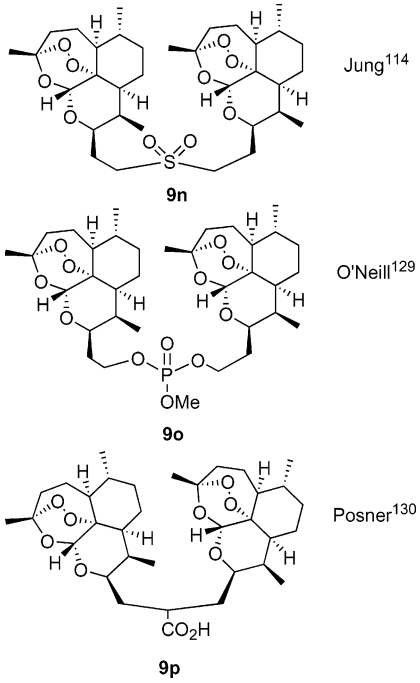
On the basis of the chemical instability of artesunate and the improved stability of artelinate, Lin prepared new water-soluble analogues **9f** and **9g**.[126] Analogue **9f** was shown to be superior by oral administration to artelinate in in vivo studies against *Plasmodium berghei* in the mouse model of malaria.

An alternative approach to preventing the formation of dihydroartemisinin by simple P450 metabolism is to replace the methyl function in artemether with an aryl function. Phenoxy analogues of DHA can easily be prepared in a one-step synthesis from **4b**.[127] In addition to having in vivo activity superior to that of artesunate and artemether, analogues substituted with a *p*-fluoro (**9h**, R = *p*-F) or trifluoromethyl group (**9i**, R = *p*-CF_3) resist metabolism to DHA. To improve water solubility, a novel meta carboxylic acid phenoxy derivative (**9k**, R = *m*-CO_2H) has recently been prepared as a metabolically more robust alternative to artesunate and artelinic acid. Preliminary in vivo assessment of this analogue is encouraging. Finally and closely related to **9e**, Haynes has recently patented several C-10 α-amino analogues (**9l**, **9m**) of dihydroartemisinin.[11] Several of these analogues are reported to have exceptional antimalarial activity both in vivo and in vitro.

Attempts to increase the metabolic and chemical stability of arteether and DHA by fluorine substitution have been reported. For example, the stability of DHA can be markedly increased by the incorporation of a C-10 CF_3 group. Bonnet-Delphon and co-workers have revealed that the CF_3 analogue of arteether is 45 times more stable than arteether itself under "simulated stomach acid conditions".[128]

Finally, apart from monomeric semisynthetic derivatives, C-10 non-acetal carba dimers have attracted attention,[107] and representative lead compounds (**9n**,[114]

Chart 2. Lead Antimalarial and Antitumor C-10 Non-Acetal Dimers

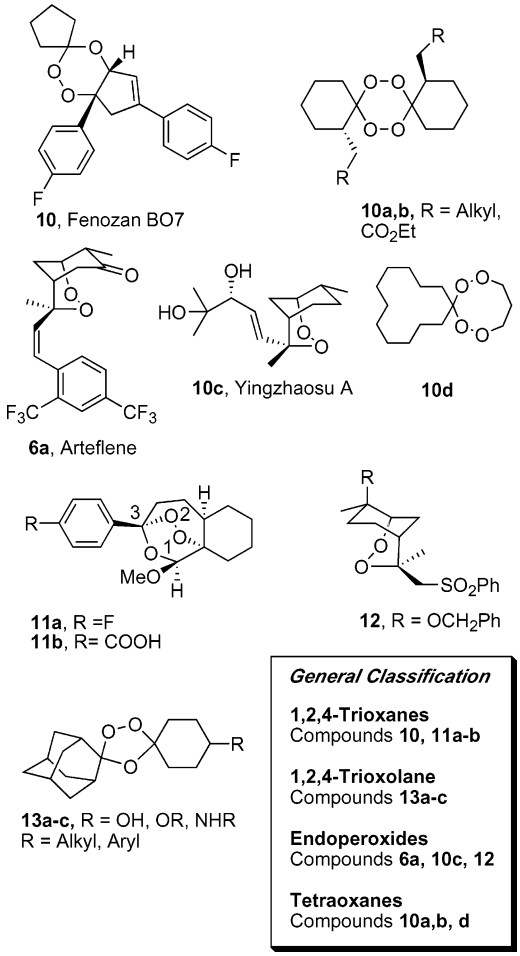


9o,[129] and **9p**[130]) are depicted in Chart 2. Not only are these analogues potent as antimalarials, both in vitro and in vivo, but several of these dimers also have potent in vitro anticancer properties against a variety of different cell lines. Furthermore, very recent studies have demonstrated that water-soluble dimer **9p** has a therapeutic index (maximum tolerated dose/EC_{50}) of 167. This value compares favorably with that for sodium artesunate, which has an MTD of only 23. Further work is ongoing with these lead dimeric trioxanes.

The disadvantage of all of the semisynthetic compounds described is that their production requires **1** as starting material.[131,132] As noted in the Introduction, artemisinin is extracted from the plant *Artemisinia annua* in low yield, a fact that necessitates significant crop production. To circumvent this problem, a number of groups have attempted to produce totally synthetic peroxide analogues, some of which demonstrate remarkable antimalarial activity.[103,119,133] These include the synthetic 1,2,4-trioxane, fenozan B0-7 (**10**),[56,134] the dispiro tetraoxanes [**10a**, **10b**],[135−137] and the endoperoxide analogues such as arteflene (**6a**),[138] a synthetic analogue of the naturally occurring yingzhaozu A (**10c**).[139] More recently, tetraoxane (**10d**) with an IC_{50} as low as 3 nM has been discovered (artemisinin = 10 nM) and analogues in this class have been shown to be effective when given orally in mice infected with *Plasmodium berghei* with no observable toxic side effects (Chart 3).[140]

Following a considerable amount of SAR work in the trioxane area, a number of potent, water-insoluble 3-substituted trioxanes (e.g., **11a**, R = F**)** were disclosed by the Posner group in a U.S. patent and subsequently in a full paper.[141] These compounds retain the A, B, and C rings of the tetracyclic natural product artemisinin. Carboxylic acid **11b** was shown to be efficacious and have lower toxicity than artelinic acid.[141] Although in most cases synthetic trioxanes are superior to endoperoxide derivatives, Bachi and co-workers have very recently discovered bicyclic endoperoxide analogues **12** with oral activity (ED_{50}) as low as 0.5 mg/kg.[142] Further work is ongoing with these exciting derivatives.

Chart 3. Structure of the Natural Product Yingzhaosu A and Lead Synthetic Endoperoxides



Out of all the synthetic endoperoxide projects to date, the most significant discovery in the past 5 years has been the revelation by Vennerstrom and co-workers that ozonides (1,2,4-trioxolanes) substituted with an adamantane ring (**13a**−**c**) are not only chemically stable but active against *P. falciparum* in the low nanomolar range.[143] Furthermore, these compounds are orally active in mice and have a prolonged duration of action when compared with the currently available synthetic and semisynthetic derivatives. Currently this research is supported by the Medicines for Malaria Venture (MMV, Geneva, Switzerland, http: http://www.mmv.org/pages/page_main.htm) and a 2-year objective is underway to advance this project from preclinical development to phase 1 clinical trials. Ranbaxy (an Indian pharmaceutical company) has been identified as a partner to help MMV move the project forward. This project was the MMV project of the year in 2001, and excellent progress has been made since then. Water-soluble compounds that retain good oral activity in the *P. berghei* mouse model have been identified. These compounds generate longer-lasting activity than current artemisinin derivatives, suggesting that treatment courses of 3 days or less are feasible (see http://www.mmv.org/pages/page_main.htm).

Table 1. Lead Synthetic Endoperoxide Antimalarials[a]

endoperoxide analogue	no. of synthetic steps (NR, A, or R)[b]	method of O_2 incorporation	in vitro IC_{50} (nM)[c] and in vivo ED_{50} (oral) (mg/kg)[d]	metabolism and toxicity studies	refs
10	4 (R)	dye-sensitized photooxygenation	4nM (K1) 2.7 mg/kg	Not reported.	134
10d	3 (A)	hydrogen peroxide/ozone	25 nM (FCR-3) 20 mg/kg	Not reported.	140
11a,**b**	5 (R)	dye-sensitized photooxygenation	30 nM (NF54) 10 mg/kg	Toxicity studies reveal that **11b** has a therapeutic index similar to that of artelinic acid.	141, 177
12	4 (NR)	molecular oxygen (TOCO) reaction	9.4 nM (NF54) 0.43 mg/kg	Initial toxicity studies reveal no observable toxicity in mice at doses up to 200 mg/kg.	142
13a−**c**	2 (A)	ozone	3−10 nM (K1) 1−3 mg/kg	Ongoing in a project sponsored by MMV.	143

[a] The table compares the antimalarial activities, the method of synthesis, and the number of synthetic steps required for drug synthesis. [b] NR, nonracemic; A, achiral; R, racemic. [c] In vitro assays were performed by the method of Desjardins as reported in refs 134, 140, 141, 177. [d] In vivo assays were performed using the Peters 4-day test. The activity refers to oral antimalarial activity and reflects the concentration of drug required to reduce parasitaemia in mice infected with *Plasmodium berghei* by 50% of the control.

In contrast to the notion that the preparation of synthetic endoperoxides may not be cost-effective, several new methodologies have now been developed that incorporate the peroxide function by use of efficient radical[144,145] and catalytic aerobic oxidation processes[146] and controlled use of ozonolysis to incorporate the key endoperoxide function.[143] Table 1 summarizes features of the leading synthetic trioxanes, endoperoxides, tetraoxanes, and trioxolanes that have been prepared in the past 10 years. Clearly, compounds of the type **10**, **10d**, **11b**, **12**, and **13a**−**c** are analogues with profiles that warrant further investigation.

Strategies To Combat Resistance Development

Artemisinin Combination Therapy (ACT). The idea of combining antimalarials in order to prevent the emergence of resistant organisms is not new. Work by Peters et al. has elegantly shown how the judicious combination of drugs can delay the selection of resistant mutants in vitro.[147] During treatment with two (or more) drugs, the chance of a mutant emerging resistant to both drugs can be calculated from the product of the individual per parasite mutation rates (assuming that the resistance mutations are not "linked"). The principles of drug combinations have also been applied to the treatment of bacterial infections such as tuberculosis, for HIV/AIDS,[148] and for the chemotherapy of certain cancers.[149] Earlier examples of combination therapy in malaria include pyrimethamine/sulfadoxine, pyrimethamine/dapsone, and quinine plus tetracyclines. More recently, mefloquine has been combined with pyrimethamine/sulfadoxine and atovaquone with proguanil. However, resistance to all these combinations has emerged. This is either because the compounds used had linked modes of action (i.e., were susceptible to the same parasite mutations) or because resistance had developed already to one of the compounds.[150,151]

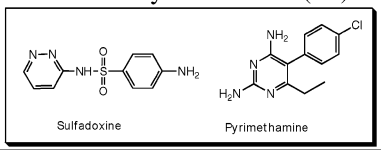
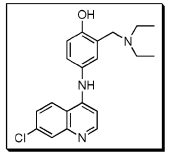
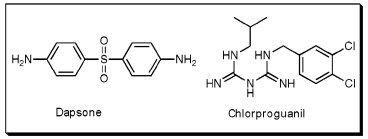
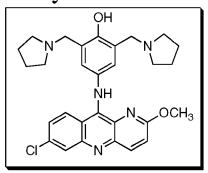
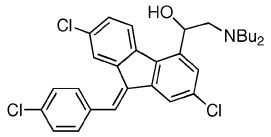
The artemisinin derivatives reduce the parasite biomass by around 4 log units for each asexual cycle. This makes them the most rapidly efficacious antimalarial drugs in use.[152,153] This rapid reduction of the parasite biomass has a major theoretical role when artemisinin derivatives are combined with another antimalarial drug (artemisinin combination therapy (ACT));[4,151,154−160] the parasite population available to develop mutations to the second drug is reduced by several log orders. As an example of the success of the ACT approach, when mefloquine was used in combination with ARTs in Thailand, the rate of development of mefloquine resistance was reduced.[161] WHO recommends ACT as part of the "ideal" strategy for malaria control in Africa, but there are practical concerns over the cost of such combinations, and although ACT approaches have been shown to be successful in Thailand, it is still not fully established that this will be the case in Africa. A further concern is the fact that artemisinins are embryotoxic in rats and rabbits, and this will mean that such drug combinations will have to undergo careful scrutiny before they can be widely advocated.[162] Table 2 lists the drugs that are currently undergoing investigation for use in ACT.[151]

Drug Hybrid and Prodrug Approaches. Combination Chemotherapy

Trioxaquines and Trioxolaquines. The hemoglobin degradation pathway in *P. falciparum* is a specialized parasite process with a proven history as an exploitable therapeutic target as exemplified by the 4-aminoquinolines and the endoperoxide derivatives.[36,77,86,163]

Meunier and co-workers recently prepared new chimeric molecules by covalent attachment of a trioxane moiety to a 4-aminoquinoline entity. These molecules, named trioxaquines (**14a**, **14b**, Figure 4), were designed according to the current knowledge of the mechanism of action of artemisinin derivatives: they combine in a single molecule a peroxidic entity that can function as a potential alkylating agent and an aminoquinoline, known to easily penetrate infected erythrocytes.[40,164−167] It was proposed that this "covalent bitherapy" would be expected to considerably reduce the risk of drug-resistant antimalarial compounds. The first synthesized trioxaquines demonstrated potent activity in vitro against a variety of chloroquine-resistant parasites. Preliminary results indicated that some trioxaquines are active by oral administration to infected mice. The main drawbacks with these first-generation chimeras are the facts that diastereomers are formed in the synthetic approaches used and that antimalarial testing has been done thus far using such diastereomeric mixtures of the trioxaquines.

Table 2. Candidates for Use in Artemisinin-Based Combination Therapy (ACT)[151,152,178]

Drug	Drug Uses	Limitations	Examples to date/ references
Chloroquine (**1**)	It has been proposed that in areas where *P. falciparum* is still sensitive to chloroquine combination with artesunate should permit continued use of this safe and effective	Spread of chloroquine resistance probably means that implementation of chloroquine drug combinations will be limited	A recent study by Sutherland *et al* demonstrated that addition of artesunate to chloroquine delays, but does not prevent treatment failure. [154]
Sulfadoxine/Pyrimethamine (SP) 	Like chloroquine, the combination of SP should prevent resistance development in areas where SP is still effective.	Likely to have greater use than chloroquine in an ACT.	Has recently been shown to be effective in a recent study in Africa. SP plus artesunate should reduce transmission of resistance. [151, 155]
Amodiaquine 	Since amodiaquine retains substantial activity versus highly chloroquine resistant parasites, combination with an artemisinin derivative has been considered in areas of chloroquine resistance	Amodiaquine has a range of toxic side-effects. This may limit its use in a drug combination.	Studies *in vitro* demonstrate synergism with artemisinin. Still under consideration as a viable ACT component.[156]
Chlorproguanil/Dapsone (LAPDAP™) 	This combination has been developed as an alternative to chloroquine. It has a different mechanism of action to chloroquine, faster absorption and faster rate of elimination than SP. It is proposed that addition of artesunate to LAPDAP™ (Lapdap™+) will delay resistance development.	Addition of a third component will increase cost.	In 2002 an MMV/GlaxoSmithkline (GSK) sponsored phase 1 study was completed to assess pharmacokinetic interactions in man. Phase 2 dose-ranging studies are ongoing to determine the optimum ratio of artesunate to the fixed combination of chlorproguanil/dapsone.[4] (see http://www.mmv.org/pages/page_main.htm)
Mefloquine (**2**)	The most widely studied drug combination with artesunate. Use has halted progression of mefloquine resistance	Mefloquine is associated with adverse effects and has a long half-life (3 weeks) which carries the risk of selecting for resistant strains should reinfection occur	Recommended in parts of Thailand, Cambodia and Vietnam.[157-159]
Pyronaridine 	Pyronaridine is an azaacridine that was discovered in China in 1970. It is highly effective against chloroquine resistant parasites. Artesunate is the trioxane partner undergoing evaluation.	More studies are required to determine the pharmacokinetic and pharmaco-dynamic properties as well as toxicity prior to recommendation in ACT.	Undergoing MMV sponsored investigation for safety and efficacy in the treatment of P. falciparum malaria in South East Asia and Afria.[153] (see http://www.mmv.org/pages/page_main.htm)
Lumefantrine 	This compound is a relative of mefloquine With a half-life of 3-6 days this drug is an excellent candidate for ACT. It has been developed as a highly efficacious combination with artemether (Coartem®).	In spite of the excellent efficacy, cost is still an issue with this drug combination.	The optimum regimen consists of 2 doses per day for 3 days. Novartis is developing a fixed-dose combination of Coartem® at cost for a paediatric form of this drug which will be distributed by the WHO as part of the Roll Back Malaria initiative. (http://www.who.rbm/).[160]

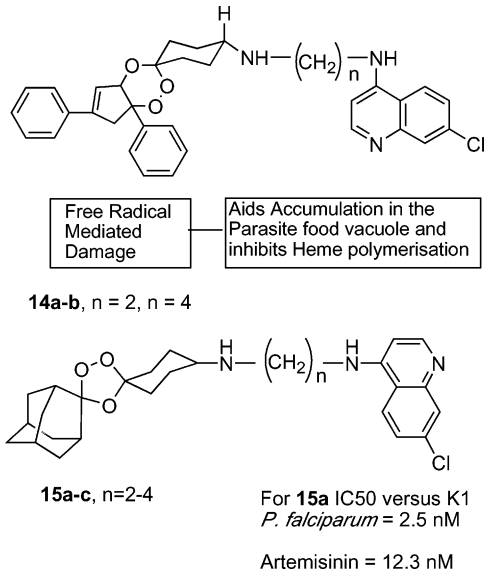


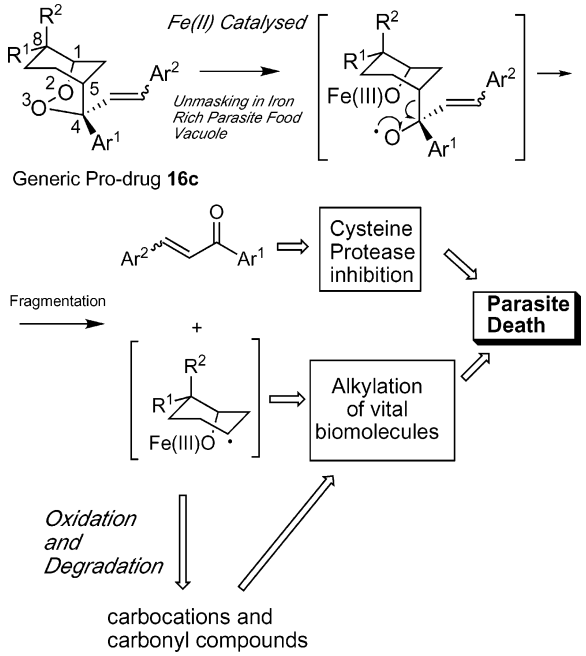
Figure 4. Trioxaquines (**14a**,**b**) and trioxalaquines (**15a**−**c**).

More recently and on the basis of the same concept, O'Neill et al. have prepared a series of related systems (**15a**−**c**, Figure 4) based on Vennerstrom's adamantyltrioxolane unit.[168] These derivatives, which are synthesized in only three steps from adamantanone, have activity in the low nanomolar region (<3 nM versus K1 *P. falciparum*). Like the prototype synthesized by Meunier, these compounds can be formulated as water-soluble salts, and it is anticipated that these agents may have the capacity to hit the parasite by two distinctive mechanisms. Indeed, any chemical or metabolic degradation of the endoperoxide bridge in these compounds will result in metabolites that may still have the ability to function as inhibitors of heme polymerization provided that they do not become covalently attached to proteins in the bioactivation process.

Endoperoxide Cysteine Protease Inhibitor (ECPI) Prodrugs. Among potential new targets for antimalarial drugs is the FV *Plasmodium falciparum* hemoglobinase falcipains 2 and 3.[29,169−171] Molecular modeling studies using the ligand identification algorithm DOCK and homology-based models of the trophozoite malaria cysteine protease have identified several novel antimalarial cysteine protease inhibitors that include the bisaryl chalcones **16a**.[172] At about the same time as this work, a paper was published on the potent antimalarial activity of the natural product licochalcone A **16b**.[173] These derivatives have good antimalarial potency both in vitro against chloroquine-resistant *P. falciparum* and in vivo versus *Plasmodium berghei* in mice.[174] Despite the promising antimalarial activities of these derivatives, a potential concern with the chalcone class of drug is that they might be expected to react with host proteins, thereby causing toxicity. In line with this, researchers have shown that, in vitro, licochacone A **16b** readily reacts with sulfur-based biological nucleophiles.[175] Therefore, a masked prodrug based approach to delivering the chalcone selectively to the parasite would be desirable.[51]

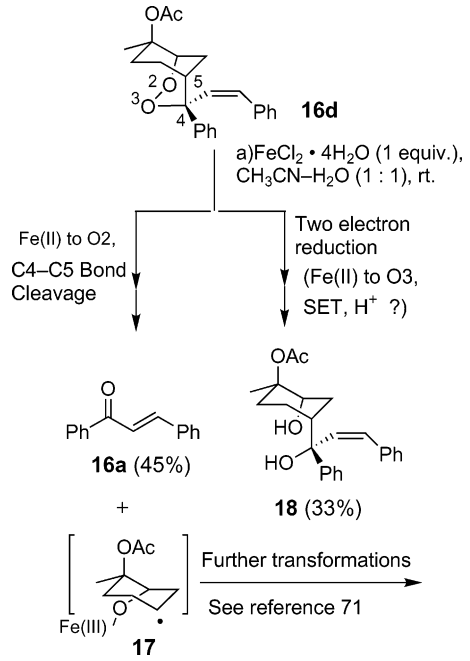
As described earlier, O'Neill et al. have recently defined the mechanism of action of the second-generation peroxide arteflene (**6a**, Ar = 2,4-bistrifluoromethylphenyl), which involves ferrous-mediated fragmentation to a stable enone system and a cyclohexyl carbon centered radical.[68,69] On the basis of this knowledge, we have designed a novel class of endoperoxide−chalcone based prodrugs, **16c**, and have recently completed the synthesis of several prototypes, **16d**−**f**. It was proposed that these prodrugs will selectively deliver the chalcone-based cysteine protease inhibitor to its site of action by means of ferrous-catalyzed unmasking in the parasite food vacuole. This represents an exciting and totally new approach to antiparasitic drug design in that besides the selective generation of C-centered radicals in the parasite, a second drug molecule with a different

Scheme 11. Ferrous-Mediated Degradation of an Endoperoxide Chalcone Prodrug **16c**[a]

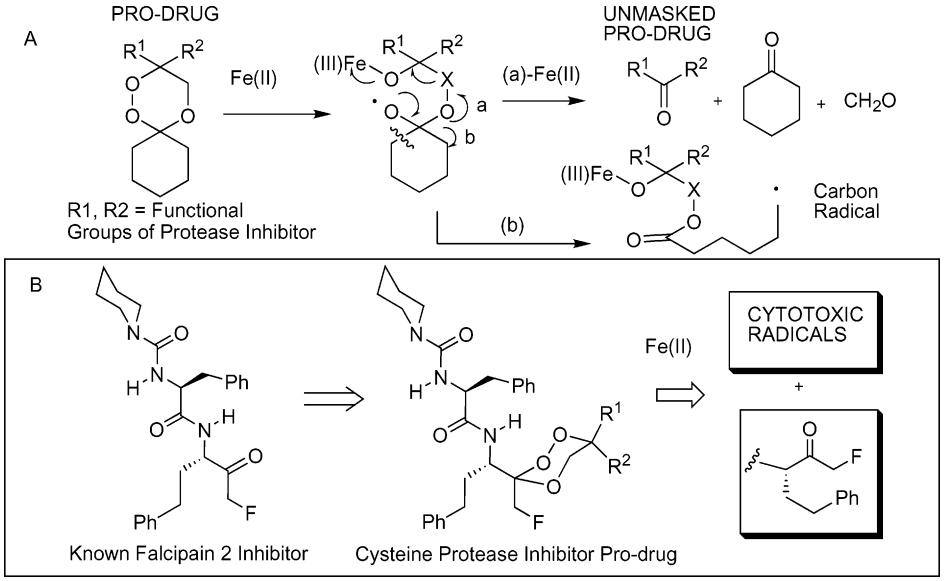


[a] Ferrous-mediated activation of the prodrug releases not only carbon radicals and other noxious reactive species but also a chalcone, a species reported to inhibit parasite cysteine proteases.

Scheme 12. Preliminary Results of Ferrous-Mediated ($FeCl_2 \cdot 4H_2O$) Degradation of Prodrug **16d**



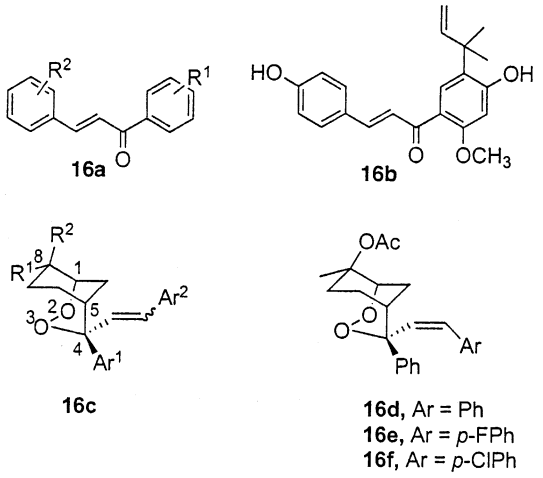
Scheme 13. Fe(II)/Heme Fe(II) Mediated Degradation Reactions of 1,2,4-Trioxanes and Endoperoxide Prodrug Design[a]



[a] (a) Known degradation of endoperoxides to carbonyls and carbon radicals. Pathway a is the major route for these systems, resulting in good yields of ketone/aldehyde products. Route b is also possible, and this type of C−C cleavage has been observed for artemisinin and other endoperoxides. The whole process can be achieved by using catalytic ferrous ions (<1 mol %) in the presence of glutathione. (b) In order to mask the reactive carbonyl function of the known fluoro ketone falcipain 2 inhibitor, incorporation into a 1,2,4-trioxane or 1,2,4-trioxalane provides a prodrug that should be activated by iron in the ferrous-rich parasite food vacuole to release the inhibitor and free radicals at the cellular location of the malarial cysteine proteases.

mechanism of drug action will be produced. Scheme 11 depicts the Fe(II)-mediated fragmentation of a generic prototype **16c**, a compound that is a structural analogue of the Roche antimalarial arteflene **6a**.

In effect, antimalarial "Trojan horse" drugs of this type should deliver a double blow to the parasite by exploiting the presence of high concentrations of ferrous ion present in the parasite food vacuole as the trigger for protease inhibitor release. In model studies with prototype **16d**, in the presence of ferrous ions, these systems readily degrade to produce the desired chalcone (**16a**, R = H, 45% yield from **16d**) in tandem with secondary carbon centered radical **17** (Scheme 12). Furthermore, analogues **16d**−**f** have in vitro antimalarial activity superior to that of arteflene.



Currently, O'Neill and Bachi groups are synthesising new versions of endoperoxide−chalcone prodrugs incorporating chalcone units that have already been shown to display potent activity versus *Plasmodium falciparum* both in vitro and in vivo to fully validate this novel approach to combination chemotherapy.[176]

This "prodrug" approach provides a paradigm for future antimalarial drug discovery efforts in the sense that this approach can be extended to any protease inhibitor that contains a carbonyl group as the reactive protease inhibitor "warhead". Simply masking the carbonyl group within a trioxane or endoperoxide provides a unique and selective mechanism for targeting the malaria parasite by two different mechanisms (Scheme 13A). One such example undergoing investigation is the peptide-based falcipain 2 inhibitor shown in Scheme 13B.

Conclusion

As described in this review, significant progress has been made in the elucidation of the chemical mechanisms of action and identification of potential biological targets for the endoperoxide class of drug. Equally, medicinal chemists have had success in the preparation of both semisynthetic artemisinin analogues and simplified cyclic peroxides; new design strategies encompassing hybrid drugs and identification of chemically and metabolically stable artemisinin derivatives have been explored. These efforts are now producing candidates worthy of further preclinical evaluation. Future efforts on establishing lead compounds should focus on in vivo efficacy, pharmacokinetics, neurotoxicity, in vivo toxicity, and direct head to head comparisons of the most promising drug candidates. Successful development of many of the lead synthetic analogues described will require a large investment of money and time, and the overriding issue in this area is that for use in the Third World the compounds should be cheap to prepare. Critical appraisal of the candidates prepared to date reveals that only a few synthetic and semisynthetic derivatives can be prepared cheaply enough to be

worthy of entering full-scale drug development. However, with the establishment of the Medicines for Malaria Venture (MMV) and with the increasing participation of pharmaceutical companies in antimalarial drug research, the resources may now be available for development of effective, affordable, and improved peroxide-based antimalarial drugs.

Acknowledgment. P.M.O. thanks Pfizer, Sandwich, Kent, U.K., Ultrafine Chemicals, Manchester, U.K., the EPSRC (Grant GR/N65875/01), and the BMS Committee of the BBSRC (Grant 26/B13581) for financial support of some of work described in this Perspective. P.M.O. also thanks the Drug Discovery Committee of the UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases (TDR) (Grants 980176 and A10043). G.H.P. thanks the NIH (Grant AI34885) for financial support and the Department of Chemistry, University of Liverpool, for kind hospitality in August and September 2003.

Biographies

Paul M. O'Neill graduated with a first class Honors degree in Chemistry and Pharmacology at the University of Liverpool in 1990 and subsequently received a Ph.D degree under the guidance of Dr. Richard C. Storr and Professor B. Kevin Park. Following graduation, he was appointed Roche Lecturer in Medicinal Chemistry in the Department of Pharmacology at Liverpool from 1995 to 1996. In 1997 Dr. O'Neill carried out postdoctoral with Professor Gary H. Posner at the Johns Hopkins University before returning to Liverpool in 1997 where he is currently a Senior Lecturer in Organic and Medicinal Chemistry. His main research interests include catalytic oxidation processes, new reagents for singlet oxygenation, fluorine substitution in bioorganic chemistry, drug metabolism, and malaria chemotherapy.

Gary H. Posner received a Ph.D. degree from Harvard University in 1968 under the guidance of Professor E. J. Corey. He has been at The Johns Hopkins University since 1969, holding the Scowe Professorship in Chemistry since 1989. He is a 2004 recipient of the Cope Senior Scholar Award from the American Chemical Society. His research interests include organocopper chemistry, asymmetric synthesis, multicomponent organic reactions, organic reactions on solid surfaces, pyrone [4 + 2]-cycloadditions, design and synthesis of new vitamin D analogues, and design and synthesis of new antimalarial peroxides.

References

(1) Warhurst, D. C. Drug Resistance in Plasmodium Falciparum Malaria. *Infection* **1999**, *27*, S55−S58.
(2) Trape, J. F. The Public Health Impact of Chloroquine Resistance in Africa. *Am. J. Trop. Med. Hyg.* **2001**, *64*, 12−17.
(3) Marquino, W.; Huilca, M.; Calampa, C.; Falconi, E.; Cabezas, C.; et al. Efficacy of Mefloquine and a Mefloquine−Artesunate Combination Therapy for the Treatment of Uncomplicated Plasmodium Falciparum Malaria in the Amazon Basin of Peru. *Am. J. Trop. Med. Hyg.* **2003** , *68*, 608−612.
(4) Winstanley, P. A.; Ward, S. A.; Snow, R. W. Clinical Status and Implications of Antimalarial Drug Resistance. *Microbes Infect.* **2002**, *4*, 157−164.
(5) Wongsrichanalai, C.; Pickard, A. L.; Wernsdorfer, W. H.; Meshnick, S. R. Epidemiology of Drug-Resistant Malaria. *Lancet Infect. Dis.* **2002**, *2*, 209−218.
(6) Kumar, A.; Katiyar, S. B.; Agarwal, A.; Chauhan, P. M. S. Perspective in Antimalarial Chemotherapy. *Curr. Med. Chem.* **2003**, *10*, 1137−1150.
(7) Narasimhan, V.; Attaran, A. Roll Back Malaria? The Scarcity of International Aid for Malaria Control. *Malar. J.* **2003**, *2* (article 8).
(8) Klayman, D. L. Qinghaosu (Artemisinin)−An Antimalarial Drug from China. *Science* **1985**, *228*, 1049−1055.
(9) Butler, A. R.; Wu, Y. L. Artemisinin (Qinghaosu)−A New Type of Antimalarial Drug. *Chem. Soc. Rev.* **1992**, *21*, 85−90.
(10) Klayman, D. L. Artemisia-Annua−From Weed to Respectable Antimalarial Plant. *ACS Symp. Ser.* **1993**, *534*, 242−255.
(11) Haynes, R. K. Artemisinin and Derivatives: The Future for Malaria Treatment? *Curr. Opin. Infect. Dis.* **2001**, *14*, 719−726.
(12) Brown, G. D. Cadinanes from Artemisia-Annua That May Be Intermediates in the Biosynthesis of Artemisinin. *Phytochemistry* **1994**, *36*, 637−641.
(13) Fulzele, D. P.; Heble, M. R.; Rao, P. S. Production of Terpenoid from Artemisia-Annua L Plantlet Cultures in Bioreactor. *J. Biotechnol.* **1995**, *40*, 139−143.
(14) Tandon, S.; Kahol, A. P.; Jain, D. C.; Bhakuni, R. S.; Kumar, S. Pilot Plant Processing Data for the Isolation of Artemisinin from the New Variety of Artemisia Annua "Jeevanraksha". *J. Sci. Ind. Res.* **2003**, *62*, 457−461.
(15) Ravindranathan, T. Artemisinin (Qinghaosu). *Curr. Sci.* **1994**, *66*, 35−41.
(16) Brewer, T. G.; Grate, S. J.; Peggins, J. O.; Weina, P. J.; Petras, J. M.; et al. Fatal Neurotoxicity of Arteether and Artemether. *Am. J. Trop. Med. Hyg.* **1994**, *51*, 251−259.
(17) Brewer, T. G.; Peggins, J. O.; Grate, S. J.; Petras, J. M.; Levine, B. S.; et al. Neurotoxicity in Animals Due to Arteether and Artemether. *Trans. R. Soc. Trop. Med. Hyg.* **1994**, *88*, 33−36.
(18) Kamchonwongpaisan, S.; McKeever, P.; Hossler, P.; Ziffer, H.; Meshnick, S. R. Artemisinin Neurotoxicity: Neuropathology in Rats and Mechanistic Studies in Vitro. *Am. J. Trop. Med. Hyg.* **1997**, *56*, 7−12.
(19) Hien, T. T.; Turner, G. D. H.; Mai, N. T. H.; Phu, N. H.; Bethell, D.; et al. Neuropathological Assessment of Artemether-Treated Severe Malaria. *Lancet* **2003**, *362*, 295−296.
(20) Lin, A. J.; Lee, M.; Klayman, D. L. Antimalarial Activity of New Water-Soluble Dihydroartemisinin Derivatives. 2. Stereospecificity of the Ether Side-Chain. *J. Med. Chem.* **1989**, *32*, 1249−1252.
(21) Barradell, L. B.; Fitton, A. Artesunate−A Review of Its Pharmacology and Therapeutic Efficacy in the Treatment of Malaria. *Drugs* **1995**, *50*, 714−741.
(22) Awad, M. I.; Alkadru, A. M. Y.; Behrens, R. H.; Baraka, O. Z.; Eltayeb, I. B. Descriptive Study on the Efficacy and Safety of Artesunate Suppository in Combination with Other Antimalarials in the Treatment of Severe Malaria in Sudan. *Am. J. Trop. Med. Hyg.* **2003**, *68*, 153−158.
(23) Vanvianen, P. H.; Klayman, D. L.; Lin, A. J.; Lugt, C. B.; Vanengen, A. L.; et al. Plasmodium-Berghei−The Antimalarial Action of Artemisinin and Sodium Artelinate Invivo and Invitro, Studied by Flow-Cytometry. *Exp. Parasitol.* **1990**, *70*, 115−123.
(24) Shrimali, M.; Bhattacharya, A. K.; Jain, D. C.; Bhakuni, R. S.; Sharma, R. P. Sodium Artelinate: A Potential Antimalarial. *Indian J. Chem., Sect. B: Org. Chem. Incl. Med. Chem.* **1998**, *37*, 1161−1163.
(25) Li, Q. G.; Peggins, J. O.; Lin, A. J.; Masonic, K. J.; Trotman, K. M.; et al. Pharmacology and Toxicology of Artelinic Acid: Preclinical Investigations on Pharmacokinetics, Metabolism, Protein and Red Blood Cell Binding, and Acute and Anorectic Toxicities. *Trans. R. Soc. Trop. Med. Hyg.* **1998**, *92*, 332−340.
(26) Semenov, A.; Olson, J. E.; Rosenthal, P. J. Antimalarial Synergy of Cysteine and Aspartic Protease Inhibitors. *Antimicrob. Agents Chemother.* **1998**, *42*, 2254−2258.
(27) Li, R. S.; Chen, X. W.; Gong, B. Q.; Selzer, P. M.; Li, Z.; et al. Structure-Based Design of Parasitic Protease Inhibitors. *Bioorg. Med. Chem.* **1996**, *4*, 1421−1427.
(28) Olson, J. E.; Lee, G. K.; Semenov, A.; Rosenthal, P. J. Antimalarial Effects in Mice of Orally Administered Peptidyl Cysteine Protease Inhibitors. *Bioorg. Med. Chem.* **1999**, *7*, 633−638.
(29) Dua, M.; Raphael, P.; Sijwali, P. S.; Rosenthal, P. J.; Chishti, A. H.; et al. Falcipain-2: Molecular Cloning and Characterization of Plasmodium Falciparum Cysteine Protease That Cleaves Erythrocyte Ankyrin and Protein 4.1. *Blood* **2000**, *96*, 946.
(30) Shenai, B. R.; Sijwali, P. S.; Singh, A.; Rosenthal, P. J. Characterization of Native and Recombinant Falcipain-2, a Principal Trophozoite Cysteine Protease and Essential Hemoglobinase of Plasmodium Falciparum. *J. Biol. Chem.* **2000**, *275*, 29000−29010.
(31) Singh, A.; Rosenthal, P. J. Comparison of Efficacies of Cysteine Protease Inhibitors against Five Strains of Plasmodium Falciparum. *Antimicrob. Agents Chemother.* **2001**, *45*, 949−951.
(32) Shenai, B. R.; Lee, B. J.; Alvarez-Hernandez, A.; Chong, P. Y.; Emal, C. D.; et al. Structure−Activity Relationships for Inhibition of Cysteine Protease Activity and Development of Plasmodium Falciparum by Peptidyl Vinyl Sulfones. *Antimicrob. Agents Chemother.* **2003**, *47*, 154−160.
(33) 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.
(34) Egan, T. J. Quinoline Antimalarials. *Expert Opin. Ther. Pat.* **2001**, *11*, 185−209.
(35) Foley, M.; Tilley, L. Quinoline Antimalarials: Mechanisms of Action and Resistance and Prospects for New Agents. *Pharmacol. Ther.* **1998**, *79*, 55−87.

(36) Francis, S. E.; Sullivan, D. J.; Goldberg, D. E. Hemoglobin Metabolism in the Malaria Parasite Plasmodium Falciparum. *Annu. Rev. Microbiol.* **1997**, *51*, 97−123.
(37) 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.
(38) 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.
(39) Zhang, F.; Gosser, D. K.; Meshnick, S. R. Hemin-Catalyzed Decomposition of Artemisinin (Qinghaosu). *Biochem. Pharmacol.* **1992**, *43*, 1805−1809.
(40) Meunier, B. From Studies on Artemisinin Derivatives to Trioxaquines. *J. Porphyrins Phthalocyanines* **2002**, *6*, 271−273.
(41) Berman, P. A.; Adams, P. A. Artemisinin Enhances Heme-Catalysed Oxidation of Lipid Membranes. *Free Radical Biol. Med.* **1997**, *22*, 1283−1288.
(42) Fitch, C. D.; Cai, G. Z.; Chen, Y. F.; Shoemaker, J. D. Involvement of Lipids in Ferriprotoporphyrin IX Polymerization in Malaria. *Biochim. Biophys. Acta* **1999**, *1454*, 31−37.
(43) Fitch, C. D.; Cai, G. Z.; Shoemaker, J. D. A Role for Linoleic Acid in Erythrocytes Infected with Plasmodium Berghei. *Biochim. Biophys. Acta* **2000**, *1535*, 45−49.
(44) Maeno, Y.; Toyoshima, T.; Fujioka, H.; Ito, Y.; Meshnick, S. R.; et al. Morphologic Effects of Artemisinin in Plasmodium−Falciparum. *Am. J. Trop. Med. Hyg.* **1993**, *49*, 485−491.
(45) Vattanaviboon, P.; Siritanaratkul, N.; Ketpirune, J.; Wilairat, P.; Yuthavong, Y. Membrane Heme as a Host Factor in Reducing Effectiveness of Dihydroartemisinin. *Biochem. Pharmacol.* **2002**, *64*, 91−98.
(46) Eckstein-Ludwig, U.; Webb, R. J.; van Goethem, I. D. A.; East, J. M.; Lee, A. G.; et al. Artemisinins Target the Serca of Plasmodium Falciparum. *Nature* **2003**, *424*, 957−961.
(47) Posner, G. H.; Oh, C. H. A Regiospecifically O-18 Labeled 1,2,4-Trioxane, a Simple Chemical-Model System To Probe the Mechanism(s) for the Antimalarial Activity of Artemisinin (Qinghaosu). *J. Am. Chem. Soc.* **1992**, *114*, 8328−8329.
(48) Posner, G. H.; Meshnick, S. R. Radical Mechanism of Action of the Artemisinin-Type Compounds. *Trends Parasitol.* **2001**, *17*, 266−267.
(49) Olliaro, P. L.; Haynes, R. K.; Meunier, B.; Yuthavong, Y. Possible Modes of Action of the Artemisinin-Type Compounds. *Trends Parasitol.* **2001**, *17*, 122−126.
(50) Posner, G. H.; Cumming, J. N.; Ploypradith, P.; Chang, H. O. Evidence for Fe(IV)═O in the Molecular Mechanism of Action of the Trioxane Antimalarial Artemisinin. *J. Am. Chem. Soc.* **1995**, *117*, 5885−5886.
(51) Posner, G. H.; Park, S. B.; Gonzalez, L.; Wang, D. S.; Cumming, J. N.; et al. Evidence for the Importance of High-Valent Fe═O and of a Diketone in the Molecular Mechanism of Action of Antimalarial Trioxane Analogs of Artemisinin. *J. Am. Chem. Soc.* **1996**, *118*, 3537−3538.
(52) Kapetanaki, S.; Varotsis, C. Ferryl−Oxo Heme Intermediate in the Antimalarial Mode of Action of Artemisinin. *FEBS Lett.* **2000**, *474*, 238−241.
(53) Kapetanaki, S.; Varotsis, C. Fourier Transform Infrared Investigation of Non-Heme Fe(III) and Fe(II) Decomposition of Artemisinin and of a Simplified Trioxane Alcohol. *J. Med. Chem.* **2001**, *44*, 3150−3156.
(54) Robert, A.; Meunier, B. Is Alkylation the Main Mechanism of Action of the Antimalarial Drug Artemisinin? *Chem. Soc. Rev.* **1998**, *27*, 273−279.
(55) Jefford, C. W.; Vicente, M. G. H.; Jacquier, Y.; Favarger, F.; Mareda, J.; et al. The Deoxygenation and Isomerization of Artemisinin and Artemether and Their Relevance to Antimalarial Action. *Helv. Chim. Acta* **1996**, *79*, 1475−1487.
(56) Jefford, C. W. Why Artemisinin and Certain Synthetic Peroxides Are Potent Antimalarials. Implications for the Mode of Action. *Curr. Med. Chem.* **2001**, *8*, 1803−1826.
(57) Wu, Y. K. How Might Qinghaosu (Artemisinin) and Related Compounds Kill the Intraerythrocytic Malaria Parasite? A Chemists View. *Acc. Chem. Res.* **2002**, *35*, 255−259.
(58) Wu, Y. K.; Yue, Z. Y.; Liu, H. H. New Insights into the Degradation or Qinghaosu (Artemisinin) Mediated by Non-Heme-Iron Chelates, and Their Relevance to the Antimalarial Mechanism. *Helv. Chim. Acta* **2001**, *84*, 928−932.
(59) Liu, H. H.; Wu, Y. K.; Shen, X. Alkylation of Sulfur Ligand in Cysteinate−Iron Chelates by a 1,2,4,5-Tetraoxane. *Chin. J. Chem.* **2003**, *21*, 875−877.
(60) Meshnick, S. R. Free-Radicals and Antioxidants. *Lancet* **1994**, *344*, 1441−1442.
(61) Becuwe, P.; Gratepanche, S.; Fourmaux, M. N.; VanBeeumen, J.; Samyn, B.; et al. Characterization of Iron-Dependent Endogenous Superoxide Dismutase of Plasmodium Falciparum? *Mol. Biochem. Parasitol.* **1996**, *76*, 125−134.
(62) Avery, M. A.; Gao, F. L.; Chong, W. K. M.; Hendrickson, T. F.; Inman, W. D.; et al. Synthesis, Conformational-Analysis, and Antimalarial Activity of Tricyclic Analogs of Artemisinin. *Tetrahedron* **1994**, *50*, 957−972.
(63) 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.
(64) Haynes, R. K.; Vonwiller, S. C. The Behaviour of Qinghaosu (Artemisinin) in the Presence of Heme Iron(II) and (III). *Tetrahedron Lett.* **1996**, *37*, 253−256.
(65) Haynes, R. K.; Vonwiller, S. C. The Behaviour of Qinghaosu (Artemisinin) in the Presence of Non-Heme Iron(II) and (III). *Tetrahedron Lett.* **1996**, *37*, 257−260.
(66) Butler, A. R.; Gilbert, B. C.; Hulme, P.; Irvine, L. R.; Renton, L.; et al. EPR Evidence for the Involvement of Free Radicals in the Iron-Catalysed Decomposition of Qinghaosu (Artemisinin) and Some Derivatives; Antimalarial Action of Some Polycyclic Endoperoxides. *Free Radical Res.* **1998**, *28*, 471−476.
(67) Wu, W. M.; Wu, Y. K.; Wu, Y. L.; Yao, Z. J.; Zhou, C. M.; et al. 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.
(68) O'Neill, P. M.; Bishop, L. P. D.; Searle, N. L.; Maggs, J. L.; Storr, R. C.; et al. Biomimetic Fe(II)-Mediated Degradation of Arteflene (Ro-42-1611). The First EPR Spin-Trapping Evidence for the Previously Postulated Secondary Carbon-Centered Cyclohexyl Radical. *J. Org. Chem.* **2000**, *65*, 1578−1582.
(69) O'Neill, P. M.; Bishop, L. P.; Searle, N. L.; Maggs, J. L.; Ward, S. A.; et al. The Biomimetic Iron-Mediated Degradation of Arteflene (Ro-42-1611), an Endoperoxide Antimalarial: Implications for the Mechanism of Antimalarial Activity. *Tetrahedron Lett.* **1997**, *38*, 4263−4266.
(70) Cazelles, J.; Robert, A.; Meunier, B. Characterization of the Main Radical and Products Resulting from a Reductive Activation of the Antimalarial Arteflene (Ro 42-1611). *J. Org. Chem.* **1999**, *64*, 6776−6781.
(71) Szpilman, A. M.; Korshin, E. E.; Hoos, R.; Posner, G. H.; Bachi, M. D. Iron(II)-Induced Degradation of Antimalarial β-Sulfonyl Endoperoxides. Evidence for the Generation of Potentially Cytotoxic Carbocations. *J. Org. Chem.* **2001**, *66*, 6531−6540.
(72) Yang, Y. Z.; Little, B.; Meshnick, S. R. Alkylation of Proteins by Artemisinin−Effects of Heme, pH, and Drug Structure. *Biochem. Pharmacol.* **1994**, *48*, 569−573.
(73) Yang, Y. Z.; Asawamahasakda, W.; Meshnick, S. R. Alkylation of Human Albumin by the Antimalarial Artemisinin. *Biochem. Pharmacol.* **1993**, *46*, 336−339.
(74) 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.
(75) Bhisutthibhan, J.; Philbert, M. A.; Fujioka, H.; Aikawa, M.; Meshnick, S. R. The Plasmodium Falciparum Translationally Controlled Tumor Protein: Subcellular Localisation and Calcium Binding. *Eur. J. Cell Biol.* **1999**, *78*, 665−670.
(76) Bhisutthibhan, J.; Meshnick, S. R. Immunoprecipitation of H-3 Dihydroartemisinin Translationally Controlled Tumor Protein (Tctp) Adducts from Plasmodium Falciparum-Infected Erythrocytes by Using Anti-Tctp Antibodies. *Antimicrob. Agents Chemother.* **2001**, *45*, 2397−2399.
(77) 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.
(78) Hong, Y. L.; Yang, Y. Z.; Meshnick, S. R. The Interaction of Artemisinin with Malarial Hemozoin. *Mol. Biochem. Parasitol.* **1994**, *63*, 121−128.
(79) Robert, A.; Boularan, M.; Meunier, B. Interaction of Artemisinin (Qinghaosu) with the Tetraphenylporphyrinatomanganese(II) Complex. *C. R. Acad. Sci., Ser. IIb* **1997**, *324*, 59−66.
(80) Cazelles, J.; Robert, A.; Meunier, B. Alkylating Capacity and Reaction Products of Antimalarial Trioxanes after Activation by a Heme Model. *J. Org. Chem.* **2002**, *67*, 609−619.
(81) Posner, G. H.; Oh, C. H.; Wang, D. S.; Gerena, L.; Milhous, W. K.; et al. 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.
(82) 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.

(83) Cazelles, J.; Robert, A.; Meunier, B. Alkylation of Heme by Artemisinin, an Antimalarial Drug. *C. R. Acad. Sci., Ser. IIc* **2001**, *4*, 85−89.
(84) Haynes, R. K.; Monti, D.; Taramelli, D.; Basilico, N.; Parapini, S.; et al. Artemisinin Antimalarials Do Not Inhibit Hemozoin Formation. *Antimicrob. Agents Chemother.* **2003**. *47*, 1175.
(85) Bray, P. G.; Janneh, O.; Raynes, K. J.; Mungthin, M.; Ginsburg, H.; et al. Cellular Uptake of Chloroquine Is Dependent on Binding to Ferriprotoporphyrin IX and Is Independent of the Activity in Plasmodium Falciparum. *J. Cell Biol.* **1999**, *145*, 363−376.
(86) Mungthin, M.; Bray, P. G.; Ridley, R. G.; Ward, S. A. Central Role of Hemoglobin Degradation in Mechanisms of Action of 4-Aminoquinolines, Quinoline Methanols, and Phenanthrene Methanols. *Antimicrob. Agents Chemother.* **1998**, *42*, 2973−2977.
(87) Robert, A.; Coppel, Y.; Meunier, B. NMR Characterization of Covalent Adducts Obtained by Alkylation of Heme with the Antimalarial Drug Artemisinin. *Inorg. Chim. Acta* **2002**, *339*, 488−496.
(88) Banerjee, R.; Francis, S. E.; Goldberg, D. E. Food Vacuole Plasmepsins Are Processed at a Conserved Site by an Acidic Convertase Activity in Plasmodium Falciparum. *Mol. Biochem. Parasitol.* **2003**, *129*, 157−165.
(89) Boss, C.; Richard-Bildstein, S.; Weller, T.; Fischli, W.; Meyer, S.; et al. Inhibitors of the Plasmodium Falciparum Parasite Aspartic Protease Plasmepsin II as Potential Antimalarial Agents. *Curr. Med. Chem.* **2003**, *10*, 883−907.
(90) Ridley, R. G. Malaria—To Kill a Parasite. *Nature* **2003**, *424*, 887.
(91) Meshnick, S. R. Artemisinin: Mechanisms of Action, Resistance and Toxicity. *Int. J. Parasitol.* **2002**, *32*, 1655−1660.
(92) O'Neill, P. M.; Miller, A.; Bickley, J. F.; Scheinmann, F.; Chang, H. O.; et al. Asymmetric Syntheses of Enantiomeric 3-P-Fluorophenyl 1,2,4-Trioxane Analogues of the Antimalarial Artemisinin. *Tetrahedron Lett.* **1999**, *40*, 9133−9136.
(93) Grace, J. M.; Aguilar, A. J.; Trotman, K. M.; Peggins, J. O.; Brewer, T. G. Metabolism of Beta-Arteether to Dihydroqinghaosu by Human Liver Microsomes and Recombinant Cytochrome P450. *Drug Metab. Dispos.* **1998**, *26*, 704−704.
(94) van Agtmael, M. A.; Eggelte, T. A.; van Boxtel, C. J. Artemisinin Drugs in the Treatment of Malaria: From Medicinal Herb to Registered Medication. *Trends Pharmacol. Sci.* **1999**, *20*, 199−205.
(95) Batty, K. T.; Ilett, K. F.; Powell, S. M.; Martin, J.; Davis, T. M. E. Relative Bioavailability of Artesunate and Dihydroartemisinin: Investigations in the Isolated Perfused Rat Liver and in Healthy Caucasian Volunteers. *Am. J. Trop. Med. Hyg.* **2002**, *66*, 130−136.
(96) Svensson, U. S. H.; Ashton, M. Identification of the Human Cytochrome P450 Enzymes Involved in the in Vitro Metabolism of Artemisinin. *Br. J. Clin. Pharmacol.* **1999**, *48*, 528−535.
(97) Souppart, C.; Gauducheau, N.; Sandrenan, N.; Richard, F. Development and Validation of a High-Performance Liquid Chromatography−Mass Spectrometry Assay for the Determination of Artemether and Its Metabolite Dihydroartemisinin in Human Plasma. *J. Chromatogr. B* **2002**, *774*, 195−203.
(98) Terkuile, F.; White, N. J.; Holloway, P.; Pasvol, G.; Krishna, S. Plasmodium-Falciparum—Invitro Studies of the Pharmacodynamic Properties of Drugs Used for the Treatment of Severe Malaria. *Exp. Parasitol.* **1993**, *76*, 85−95.
(99) Maggs, J. L.; Bishop, L. P. D.; Edwards, G.; O'Neill, P. M.; Ward, S. A.; et al. Biliary Metabolites of Beta-Artemether in Rats: Biotransformations of an Antimalarial Endoperoxide. *Drug Metab. Dispos.* **2000**, *28*, 209−217.
(100) Maggs, J. L.; Madden, S.; Bishop, L. P.; Oneill, P. M.; Park, B. K. The Rat Biliary Metabolites of Dihydroartemisinin, an Antimalarial Endoperoxide. *Drug Metab. Dispos.* **1997**, *25*, 1200−1204.
(101) Ullrich, V.; Brugger, R. Prostacyclin and Thromboxane Synthase—New Aspects of Hemethiolate Catalysis. *Angew. Chem., Int. Ed. Engl.* **1994**, *33*, 1911−1919.
(102) Ilett, K. F.; Ethell, B. T.; Maggs, J. L.; Davis, T. M. E.; Batty, K. T.; et al. Glucuronidation of Dihydroartemisinin in Vivo and by Human Liver Microsomes and Expressed Udp-Glucuronosyltransferases. *Drug Metab. Dispos.* **2002**, *30*, 1005−1012.
(103) Borstnik, K.; Paik, I. H.; Shapiro, T. A.; Posner, G. H. Antimalarial Chemotherapeutic Peroxides: Artemisinin, Yingzhaosu a and Related Compounds. *Int. J. Parasitol.* **2002**, *32*, 1661−1667.
(104) Haynes, R. K.; Vonwiller, S. C. From Qinghao, Marvelous Herb of Antiquity, to the Antimalarial Trioxane Qinghaosu and Some Remarkable New Chemistry. *Acc. Chem. Res.* **1997**, *30*, 73−79.
(105) Haynes, R. K.; Vonwiller, S. C. Efficient Preparation of Novel Qinghaosu (Artemisinin) Derivatives—Conversion of Qinghao (Artemisinic) Acid into Deoxoqinghaosu Derivatives and 5-Carba-4-Deoxoartesunic Acid. *Synlett* **1992**, 481−483.
(106) O'Dowd, H.; Ploypradith, P.; Xie, S. J.; Shapiro, T. A.; Posner, G. H. Antimalarial Artemisinin Analogs. Synthesis via Chemoselective C−C Bond Formation and Preliminary Biological Evaluation. *Tetrahedron* **1999**, *55*, 3625−3636.
(107) Posner, G. H.; Paik, I. H.; Sur, S.; McRiner, A. J.; Borstnik, K.; et al. Orally Active, Antimalarial, Anticancer, Artemisinin-Derived Trioxane Dimers with High Stability and Efficacy. *J. Med. Chem.* **2003**, *46*, 1060−1065.
(108) Posner, G. H.; Parker, M. H.; Northrop, J.; Elias, J. S.; Ploypradith, P.; et al. Orally Active, Hydrolytically Stable, Semisynthetic, Antimalarial Trioxanes in the Artemisinin Family. *J. Med. Chem.* **1999**, *42*, 300−304.
(109) O'Neill, P. M.; Pugh, M.; Stachulski, A. V.; Ward, S. A.; Davies, J.; et al. Optimisation of the Allylsilane Approach to C-10 Deoxo Carba Analogues of Dihydroartemisinin: Synthesis and in Vitro Antimalarial Activity of New, Metabolically Stable C-10 Analogues. *J. Chem. Soc., Perkin Trans. 1* **2001**, 2682−2689.
(110) Hindley, S.; Ward, S. A.; Storr, R. C.; Searle, N. L.; Bray, P. G.; et al. Mechanism-Based Design of Parasite-Targeted Artemisinin Derivatives: Synthesis and Antimalarial Activity of New Diamine Containing Analogues. *J. Med. Chem.* **2002**, *45*, 1052−1063.
(111) O'Neill, P. M.; Searle, N. L.; Kan, K. W.; Storr, R. C.; Maggs, J. L.; et al. Novel, Potent, Semisynthetic Antimalarial Carba Analogues of the First-Generation 1,2,4-Trioxane Artemether. *J. Med. Chem.* **1999**, *42*, 5487−5493.
(112) Jung, M.; Bae, J. An Efficient Synthesis of New Analogs of Water-Soluble and Hydrolytically Stable Deoxoartemisinin. *Heterocycles* **2000**, *53*, 261.
(113) Jung, M.; Yu, D.; Bustos, D.; Elsohly, H. N.; McChesney, J. D. A Concise Synthesis of 12-(3′-Hydroxy-*N*-propyl)-deoxoartemisinin. *Bioorg. Med. Chem. Lett.* **1991**, *1*, 741−744.
(114) Jung, M.; Lee, S.; Ham, J.; Lee, K.; Kim, H.; et al. Antitumor Activity of Novel Deoxoartemisinin Monomers, Dimers, and Trimer. *J. Med. Chem.* **2003**, *46*, 987−994.
(115) Ma, J. Y.; Katz, E.; Kyle, D. E.; Ziffer, H. Syntheses and Antimalarial Activities of 10-Substituted Deoxoartemisinins. *J. Med. Chem.* **2000**, *43*, 4228−4232.
(116) Ma, J. Y.; Katz, E.; Ziffer, H. A New Synthetic Route to 10 Beta-Alkyldeoxoartemisinins. *Tetrahedron Lett.* **1999**, *40*, 8543−8545.
(117) Pu, Y. M.; Ziffer, H. Synthesis and Antimalarial Activities of 12-β-Allyldeoxoartemisinin and Its Derivatives. *J. Med. Chem.* **1995**, *38*, 613−616.
(118) Haynes, R. K.; Monti, D.; Taramelli, D.; Basilico, N.; Parapini, S.; et al. Artemisinin and Heme—Authors' Reply. *Antimicrob. Agents Chemother.* **2003**, *47*, 2712−2713.
(119) Vroman, J. A.; Alvim-Gaston, M.; Avery, M. A. Current Progress in the Chemistry, Medicinal Chemistry and Drug Design of Artemisinin Based Antimalarials. *Curr. Pharm. Des.* **1999**, *5*, 101−138.
(120) Avery, M. A.; Bonk, J. D.; Bupp, J. Radiolabeled Antimalarials: Synthesis of C-14-Artemisinin. *J. Labelled Compd. Radiopharm.* **1996**, *38*, 263−267.
(121) Avery, M. A.; Alvim-Gaston, M.; Vroman, J. A.; Wu, B.; Ager, A.; et al. Structure−Activity Relationships of the Antimalarial Agent Artemisinin. 7. Direct Modification of (+)-Artemisinin and in Vivo Antimalarial Screening of New, Potential Preclinical Antimalarial Candidates. *J. Med. Chem.* **2002**, *45*, 4321−4335.
(122) Jung, M.; Lee, K.; Jung, H. First Synthesis of (+)-Deoxoartemisitene and Its Novel C-11 Derivatives. *Tetrahedron Lett.* **2001**, *42*, 3997−4000.
(123) Avery, M. A.; Alvim-Gaston, M.; Rodrigues, C. R.; Barreiro, E. J.; Cohen, F. E.; et al. Structure−Activity Relationships of the Antimalarial Agent Artemisinin. 6. The Development of Predictive in Vitro Potency Models Using COMFA and HQSAR Methodologies. *J. Med. Chem.* **2002**, *45*, 292−303.
(124) Alvim-Gaston, M.; Woolfrey, J. R.; Avery, M. A. 3D-QSAR of Peroxidic Antimalarials Based on Artemisinin. *Abstr. Pap.—Am. Chem. Soc.* **1999**, *217*, 138-COMP.
(125) Andrews, C. W.; Bennett, L.; Yu, L. X. Predicting Human Oral Bioavailability of a Compound: Development of a Novel Quantitative Structure−Bioavailability Relationship. *Pharm. Res.* **2000**, *17*, 639−644.
(126) Lin, A. J.; Zikry, A. B.; Kyle, D. E. Antimalarial Activity of New Dihydroartemisinin Derivatives. 7. 4-(P-substituted phenyl)-4(*R* or *S*)-10(α or β)-Dihydroartemisininoxy Butyric Acids. *J. Med. Chem.* **1997**, *40*, 1396−1400.
(127) O'Neill, P. M.; Miller, A.; Bishop, L. P. D.; Hindley, S.; Maggs, J. L.; et al. Synthesis, Antimalarial Activity, Biomimetic Iron(II) Chemistry, and in Vivo Metabolism of Novel, Potent C-10-Phenoxy Derivatives of Dihydroartemisinin. *J. Med. Chem.* **2001**, *44*, 58−68.
(128) Magueur, G.; Crousse, B.; Charneau, S.; Grellier, P.; Bégué, J-P.; Bonnet-Delpon, D. *J. Med. Chem.* **2004**, in press.
(129) Jeyadevan, J. P.; Bray, P. G.; Chadwick, J.; Mercer, A. E. et al. Antimalarial and Antitumour Evaluation of Novel C-10 Non-acetal Dimers of 10β-(2-hydroxyethyl)-deoxoartemisinin. *J. Med. Chem.* **2004**, *47*, 1290−1298.
(130) Posner, G. H.; McRiner, A. J.; Paik, I. H.; Sur, S.; Borstnik, K. et al. Anticancer and antimalarial Efficacy of Artemisinin-Derived trioxane Dimers in Rodents. *J. Med. Chem.* **2004**, *47*, 1299−1301.

(131) Klayman, D. L.; Lin, A. J.; Acton, N.; Scovill, J. P.; Hoch, J. M.; et al. Isolation of Artemisinin Qinghaosu from Artemisia-Annua Growing in the United-States. *J. Nat. Prod.* **1984**, *47*, 715−717.
(132) Woerdenbag, H. J.; Luers, J. F. J.; Vanuden, W.; Pras, N.; Malingre, T. M.; et al. Production of the New Antimalarial Drug Artemisinin in Shoot Cultures of Artemisia-Annua L. *Plant Cell, Tissue Organ Cult.* **1993**, *32*, 247−257.
(133) Cumming, J. N.; Wang, D. S.; Park, S. B.; Shapiro, T. A.; Posner, G. H. Design, Synthesis, Derivatization, and Structure−Activity Relationships of Simplified, Tricyclic, 1,2,4-Trioxane Alcohol Analogues of the Antimalarial Artemisinin. *J. Med. Chem.* **1998**, *41*, 952−964.
(134) Peters, W.; Robinson, B. L.; Rossier, J. C.; Jefford, C. W. The Chemotherapy of Rodent Malaria. 48. The Activities of Some Synthetic 1,2,4-Trioxanes against Chloroquine-Sensitive and Chloroquine-Resistant Parasites. 1. Studies Leading to the Development of Novel Cis-Fused Cyclopenteno Derivatives. *Ann. Trop. Med. Parasitol.* **1993**, *87*, 1−7.
(135) Vennerstrom, J. L.; Ager, A. L.; Andersen, S. L.; Grace, J. M.; Wongpanich, V.; et al. Assessment of the Antimalarial Potential of Tetraoxane Wr 148999. *Am. J. Trop. Med. Hyg.* **2000**, *62*, 573−578.
(136) Vennerstrom, J. L.; Dong, Y. X.; Andersen, S. L.; Ager, A. L.; Fu, H. N.; et al. Synthesis and Antimalarial Activity of Sixteen Dispiro-1,2,4,5-tetraoxanes: Alkyl-Substituted 7,8,15,16-Tetraoxadispiro 5.2.5.2 Hexadecanes. *J. Med. Chem.* **2000**, *43*, 2753−2758.
(137) Kim, H. S.; Shibata, Y.; Wataya, Y.; Tsuchiya, K.; Masuyama, A.; et al. Synthesis and Antimalarial Activity of Cyclic Peroxides, 1,2,4,5,7-Pentoxocanes and 1,2,4,5-Tetroxanes. *J. Med. Chem.* **1999**, *42*, 2604−2609.
(138) Hofheinz, W.; Burgin, H.; Gocke, E.; Jaquet, C.; Masciadri, R.; et al. Ro-42-1611 (Arteflene), a New Effective Antimalarial−Chemical-Structure and Biological-Activity. *Trop. Med. Parasitol.* **1994**, *45*, 261−265.
(139) Zhou, W. S.; Xu, X. X. Total Synthesis of the Antimalarial Sesquiterpene Peroxide Qinghaosu and Yingzhaosu-A. *Acc. Chem. Res.* **1994**, *27*, 211−216.
(140) Kim, H. S.; Nagai, Y.; Ono, K.; Begum, K.; Wataya, Y.; et al. Synthesis and Antimalarial Activity of Novel Medium-Sized 1,2,4,5-Tetraoxacycloalkanes. *J. Med. Chem.* **2001**, *44*, 2357−2361.
(141) Posner, G. H.; Jeon, H. B.; Ploypradith, P.; Paik, I. H.; Borstnik, K.; et al. Orally Active, Water-Soluble Antimalarial 3-Aryltrioxanes: Short Synthesis and Preclinical Efficacy Testing in Rodents. *J. Med. Chem.* **2002**, *45*, 3824−3828.
(142) Bachi, M. D.; Korshin, E. E.; Hoos, R.; Szpilman, A. M.; Ploypradith, P.; et al. A Short Synthesis and Biological Evaluation of Potent and Nontoxic Antimalarial Bridged Bicyclic *β*-Sulfonyl-endoperoxides. *J. Med. Chem.* **2003**, *46*, 2516−2533.
(143) Vennerstrom, J. L.; Dong, Y. X.; Chollet, J.; Matile, H. Spiro and Dispiro 1,2,4-Trioxolane Antimalarials. (Medicines for Malaria Venture (MMV)). U.S. Patent 6,486,199 B1, 2002.
(144) Bachi, M. D.; Korshin, E. E.; Ploypradith, P.; Cumming, J. N.; Xie, S. J.; et al. Synthesis and in Vitro Antimalarial Activity of Sulfone Endoperoxides. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 903−908.
(145) Korshin, E. E.; Hoos, R.; Szpilman, A. M.; Konstantinovski, L.; Posner, G. H.; et al. An Efficient Synthesis of Bridged-Bicyclic Peroxides Structurally Related to Antimalarial Yingzhaosu Based on Radical Co-Oxygenation of Thiols and Monoterpenes. *Tetrahedron* **2002**, *58*, 2449−2469.
(146) O'Neill, P. M.; Pugh, M.; Davies, J.; Ward, S. A.; Park, B. K. Regioselective Mukaiyama Hydroperoxysilylation of 2-Alkyl- or 2-Aryl-prop-2-en-1-ols: Application to a New Synthesis of 1,2,4-Trioxanes. *Tetrahedron Lett.* **2001**, *42*, 4569−4571.
(147) Peters, W. The Problem of Drug-Resistance in Malaria. *Parasitology* **1985**, *90*, 705−715.
(148) Grosset, J. Basis for Short-Course Chemotherapy. *Chest* **1981**, *80*, 719−720.
(149) Montaner, J. S. G.; Montessori, V.; Harrigan, R.; O'Shaughnessy, M.; Hogg, R. Antiretroviral Therapy: "The State of the Art". *Biomed. Pharmacother.* **1999**, *53*, 63−72.
(150) Bloland, P. B.; Ettling, M.; Meek, S. Combination Therapy for Malaria in Africa: Hype or Hope? *Bull. W. H. O.* **2000**, *78*, 1378−1388.
(151) Nosten, F.; Brasseur, P. Combination Therapy for Malaria−The Way Forward? *Drugs* **2002**, *62*, 1315−1329.
(152) White, N. Antimalarial Drug Resistance and Combination Chemotherapy. *Philos. Trans. R. Soc. London, Ser. B* **1999**, *354*, 739−749.
(153) White, N. J. Preventing Antimalarial Drug Resistance through Combinations. *Drug Resist. Updates* **1998**, *1*, 3−9.
(154) Sutherland, C. J.; Drakeley, C. J.; Obisike, U.; Coleman, R.; Jawara, M.; et al. The Addition of Artesunate to Chloroquine for Treatment of Plasmodium Falciparum Malaria in Gambian Children Delays, but Does Not Prevent Treatment Failure. *Am. J. Trop. Med. Hyg.* **2003**, *69*, 19−25.
(155) Tjitra, E.; Baker, J.; Suprianto, S.; Cheng, Q.; Anstey, N. M. Therapeutic Efficacies of Artesunate−Sulfadoxine−Pyrimethamine and Chloroquine−Sulfadoxine−Pyrimethamine in Vivax Malaria Pilot Studies: Relationship to Plasmodium Vivax Dhfr Mutations. *Antimicrob. Agents Chemother.* **2002**, *46*, 3947−3953.
(156) Gupta, S.; Thapar, M. M.; Mariga, S. T.; Wernsdorfer, W. H.; Bjorkman, A. Plasmodium Falciparum: In Vitro Interactions of Artemisinin with Amodiaquine, Pyronaridine, and Chloroquine. *Exp. Parasitol.* **2002**, *100*, 28−35.
(157) Krudsood, S.; Looareesuwan, S.; Silachamroon, U.; Chalermrut, K.; Pittrow, D.; et al. Artesunate and Mefloquine Given Simultaneously for Three Days via a Prepacked Blister Is Equally Effective and Tolerated as a Standard Sequential Treatment of Uncomplicated Acute Plasmodium Falciparum Malaria: Randomized, Double-Blind Study in Thailand. *Am. J. Trop. Med. Hyg.* **2002**, *67*, 465−472.
(158) Lefevre, G.; Bindschedler, M.; Ezzet, F.; Schaeffer, N.; Meyer, I.; et al. Pharmacokinetic Interaction Trial between Co-Artemether and Mefloquine. *Eur. J. Pharm. Sci.* **2000**, *10*, 141−151.
(159) Svensson, U. S. H.; Alin, M. H.; Karlsson, M. O.; Bergqvist, Y.; Ashton, M. Population Pharmacokinetic and Pharmacodynamic Modelling of Artemisinin and Mefloquine Enantiomers in Patients with Falciparum Malaria. *Eur. J. Clin. Pharmacol.* **2002**, *58*, 339−351.
(160) van Vugt, M.; Looareesuwan, S.; Wilairatana, P.; McGready, R.; Villegas, L.; et al. Artemether−Lumefantrine for the Treatment of Multidrug-Resistant Falciparum Malaria. *Trans. R. Soc. Trop. Med. Hyg.* **2000**, *94*, 545−548.
(161) Nosten, F.; Luxemburger, C.; Terkuile, F. O.; Woodrow, C.; Eh, J. P.; et al. Treatment of Multidrug-Resistant Plasmodium−Falciparum Malaria with 3-Day Artesunate Mefloquine Combination. *J. Infect. Dis.* **1994**, *170*, 971−977.
(162) Lebrun-Vignes, B. Expert Review−Antimalarial Drugs Pharmacology, Pharmacokinetics and Toxicity. *Med. Mal. Infect.* **1999**, *29*, 229S−248S.
(163) Rosenthal, P. J.; Meshnick, S. R. Hemoglobin Catabolism and Iron Utilization by Malaria Parasites. *Mol. Biochem. Parasitol.* **1996**, *83*, 131−139.
(164) 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.
(165) Dechy-Cabaret, O.; Benoit-Vical, F.; Robert, A.; Meunier, B. Preparation and Antimalarial Activities of "4 Trioxaquines", New Modular Molecules with a Trioxane Skeleton Linked to a 4-Aminoquinoline. *Actual. Chim.* **2001**, 9−11.
(166) Basco, L. K.; Dechy-Cabaret, O.; Ndounga, M.; Meche, F. S.; Robert, A.; et al. In Vitro Activities of Du-1102, a New Trioxaquine Derivative, against Plasmodium Falciparum Isolates. *Antimicrob. Agents Chemother.* **2001**, *45*, 1886−1888.
(167) Dechy-Cabaret, O.; Robert, A.; Meunier, B. Synthesis and Stereochemical Study of a Trioxaquine Prepared from *cis*-Bicyclo 3.3.0 Octane-3,7-dione. *C. R. Chim.* **2002**, *5*, 297−302.
(168) Araujo, N. C.; Ward, S. A.; Davies, J.; Bray, P. G.; O'Neill, P. M. Semisynthetic and Synthetic 1,2,4-Trioxaquines and 1,2,4-Trioxoquines: Synthesis and Preliminary SAR. *J. Med. Chem.*, in press.
(169) Sabnis, Y. A.; Desai, P. V.; Rosenthal, P. J.; Avery, M. A. Probing the Structure of Falcipain-3, a Cysteine Protease from Plasmodium Falciparum: Comparative Protein Modeling and Docking Studies. *Protein Sci.* **2003**, *12*, 501−509.
(170) Sabnis, Y.; Rosenthal, P. J.; Desai, P.; Avery, M. A. Homology Modeling of Falcipain-2: Validation, de Novo Ligand Design and Synthesis of Novel Inhibitors. *J. Biomol. Struct. Dyn.* **2002**, *19*, 765−774.
(171) Rosenthal, P. J.; Olson, J. E.; Lee, G. K.; Palmer, J. T.; Klaus, J. L.; et al. Antimalarial Effects of Vinyl Sulfone Cysteine Proteinase Inhibitors. *Antimicrob. Agents Chemother.* **1996**, *40*, 1600−1603.
(172) Li, R. S.; Kenyon, G. L.; Cohen, F. E.; Chen, X. W.; Gong, B. Q.; et al. In Vitro Antimalarial Activity of Chalcones and Their Derivatives. *J. Med. Chem.* **1995**, *38*, 5031−5037.
(173) Chen, M.; Theander, T. G.; Christensen, S. B.; Hviid, L.; Zhai, L.; et al. Licochalcone-a, a New Antimalarial Agent, Inhibits in-Vitro Growth of the Human Malaria Parasite Plasmodium−Falciparum and Protects Mice from P-Yoelii Infection. *Antimicrob. Agents Chemother.* **1994**, *38*, 1470−1475.
(174) Dimmock, J. R.; Elias, D. W.; Beazely, M. A.; Kandepu, N. M. Bioactivities of Chalcones. *Curr. Med. Chem.* **1999**, *6*, 1125−1149.

(175) Nadelmann, L.; Tjornelund, J.; Hansen, S. H.; Cornett, C.; Sidelmann, U. G.; et al. Synthesis, Isolation and Identification of Glucuronides and Mercapturic Acids of a Novel Antiparasitic Agent, Licochalcone A. *Xenobiotica* **1997**, *27*, 667−680.

(176) O'Neill, P. M.; Stocks, P. A.; Pugh, M. D.; Araujo, N. C.; Korshin, E. E.; Bickley, J. F.; Ward, S. A.; Bray, P. G.; Pasinin, E.; Davies, J.; Verissimo, E.; Bachi, M. D. Design and Synthesis of Endoperoxide Antimalarial Pro-Drug Models: Prototypes for Selective Intraparasitic Generation of Cysteine Protease Inhibitors and Other Parasitocidal Species. *Angew. Chem., Int. Ed.* **2004**, in press.

(177) Posner, G. H.; Jeon, H. B.; Parker, M. H.; Krasavin, M.; Paik, I. H.; et al. Antimalarial Simplified 3-Aryltrioxanes: Synthesis and Preclinical Efficacy/Toxicity Testing in Rodents. *J. Med. Chem.* **2001**, *44*, 3054−3058.

(178) White, N. J.; Olliaro, P. L. Strategies for the Prevention of Antimalarial Drug Resistance: Rationale for Combination Chemotherapy for Malaria. *Parasitol. Today* **1996**, *12*, 399−401.

JM030571C