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Novel Endoperoxide Antimalarials: Synthesis, Heme Binding, and Antimalarial Activity

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We report the synthesis of a series of novel epoxy endoperoxide compounds that can be prepared in high yields in one to three steps from simple starting materials. Some of these compounds inhibit the growth of *Plasmodium falciparum* in vitro. Structure−activity studies indicate that an endoperoxide ring bisubstituted with saturated cyclic moieties is the pharmacophore. To study the molecular basis of the action of these novel antimalarial compounds, we examined their ability to interact with oxidized and reduced forms of heme. Some of the compounds interact with oxidized heme in a fashion similar to chloroquine and other 4-aminoquinolines, while some of the compounds interact with reduced heme. However, the level of antimalarial potency is not well correlated with these activities, suggesting that some of the endoperoxides may exert their antimalarial activities by a novel mechanism of action.

Introduction

Malaria is a debilitating parasitic disease that is responsible for the deaths of about two million children each year. Artemisinin (Figure 1) has become increasingly important as a malaria treatment because of the fact that cheap alternatives such as chloroquine (CQ, Figure 1) and Fansidar (S/P, sulfadoxine/pyrimethamine) have become ineffective with the emergence of drug-resistant organisms. However, the use of artemisinin is somewhat limited because of its relatively high cost, limited production to GMP standards, and reports of toxicity.[1,2] The current routes for the total chemical synthesis of artemisinin (qinghaosu) remain too complex for commercial production.[3−6] It is currently prepared by large-scale extraction from *Artemisia annua* (sweet wormwood), and derivatives such as artemether, artesunate, arteether, and dihydroartemisinin are prepared semisynthetically from the purified extract. Difficulties have been encountered in the production of high-quality material.[1]

Artemisinin is a sesquiterpene lactone that contains a 1,2,4-trioxane ring system. The endoperoxide bond is an essential feature of artemisinin and its active derivatives. It has been proposed that active endoperoxides accumulate in the parasite cytosol and membranes and interact with the reduced form of heme (ferroprotoporphyrin IX, FP-Fe(II)[7−9]). In this reducing environment, the peroxide moiety is thought to react with FP-Fe(II) to form a cytotoxic carbon-centered radical intermediate that further reacts with susceptible groups within parasite enzymes and lipids.[2,9−11] Very recently, Eckstein-Ludwig et al.[12] showed that the *P. falciparum* homologue of the sarco/endoplasmic reticulum Ca^{2+}-ATPase (SERCA) is likely to be the major downstream target of activated artemisinin in the parasite cytosol.

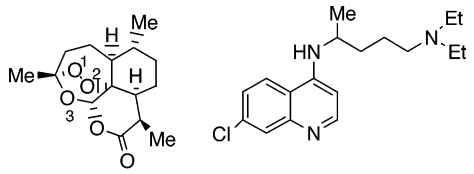

Figure 1. Structures of artemisinin and chloroquine.

By contrast, CQ and other quinoline antimalarial drugs accumulate in the acidic vacuole of the malaria parasite where they are thought to interact with oxidized heme (ferriprotoporphyrin IX, FP-Fe(III)) and to inhibit the formation of β-hematin (the form of FP present in the malaria pigment, hemozoin).[13−15] Thus, the quinoline antimalarial drugs are thought to exert their activities by inhibiting FP detoxification, causing a buildup of toxic FP molecules that eventually inhibit parasite enzymes and destroy the integrity of the membranes.

In this work, we extend our recently published procedure for the synthesis of the relatively unknown epoxy endoperoxides that can be readily generated from inexpensive starting materials.[16] We have examined the abilities of these compounds to inhibit the growth of malaria parasites in vitro and shown that a subset of compounds possess substantial antimalarial activity. We have shown that some of the compounds interact with oxidized or reduced FP, which may provide some information about their mechanism of action.

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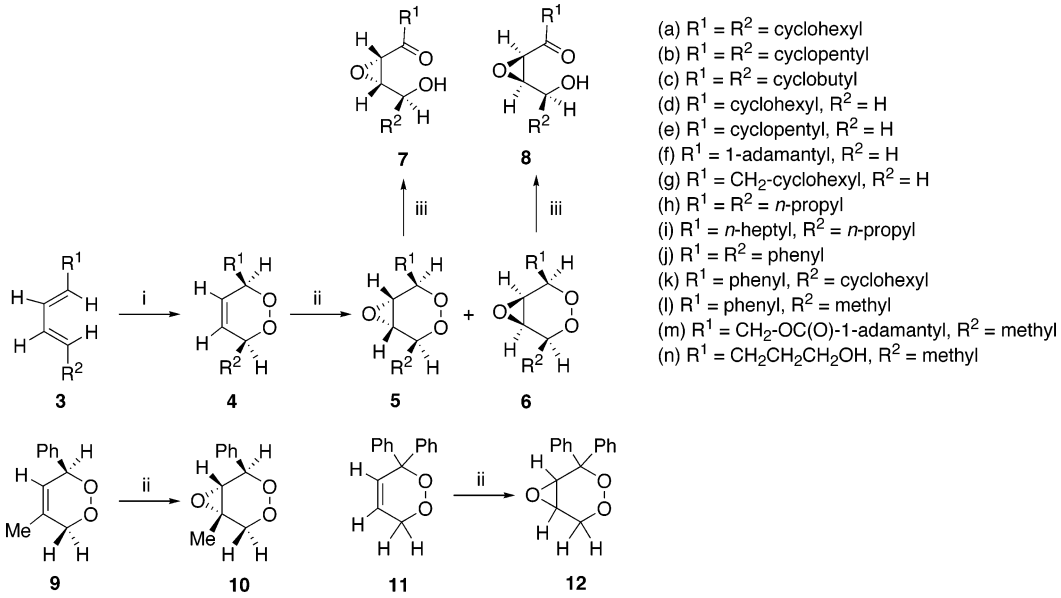
Scheme 1[a]



[a] (i) O_2, Rose Bengal bis(triethylammonium) salt, CH_2Cl_2, 7 h; (ii) *m*-CPBA, CH_2Cl_2, room temp; (iii) Co(II)salen or NEt_3.

Results

Synthesis. We have developed a robust synthetic procedure for the ready construction of endoperoxides of types **4a**−**n**, **9**, and **11** and epoxy endoperoxides (**5**, **6**, **10**, and **12**) according to generalized Scheme 1. Key features include a [4 + 2] cycloaddition of singlet oxygen with the appropriate 1,3-butadiene **3a**−**n** to generate the core of the endoperoxide ring and afford compounds of type **4a**−**n**. Further oxidation with *m*-CPBA at ambient temperature furnishes the epoxy endoperoxides **5** and **6**.[16] This latter conversion affords from a single precursor, both endoperoxides **5** and **6** which have the epoxide oxygen atom either on the opposite face of (compounds **5**) or on the same face (compounds **6**) as the alkyl or aryl substituents. Therefore, the epoxide moiety of these epoxy endoperoxides mimics the oxygen atom (3) of artemisinin (Figure 1). Single X-ray analysis of epoxy endoperoxide **5f** not only confirmed our structural assignments but also highlights the proximity of the epoxy oxygen to those of the endoperoxide moiety (Figure 2). Consequently, two unique versions of these new prodrugs are readily available for probing structure−activity relationships. Additionally, it is well established that exposure of artemisinin to Fe(II) results in the cleavage of the peroxide linkage and the generation of free radical intermediates, which ultimately lead to ring-opened products in which the peroxide linkage has been destroyed.[17,18] Furthermore, we have also reported that exposure of endoperoxides of type **4a**−**n** and their epoxy counterparts (**5** and **6**) to catalytic amounts of cobalt(II) salen complexes results in a clean free radical rearrangement resulting in the formation of the downstream products (**7** or **8**).[16,19] Consequently, given that exposure of the epoxy endoperoxides (**5** and **6**) to Fe(II) will result in significant formation of these downstream products (**7** or **8**), we also independently prepared pure samples of several of these ring-opened products (**7a**, **7j**, and **8a**) in order to evaluate their activities.

Antimalarial Activities. The abilities of the endoperoxides to inhibit the growth of the D10 strain of *P. falciparum* during in vitro culture were determined

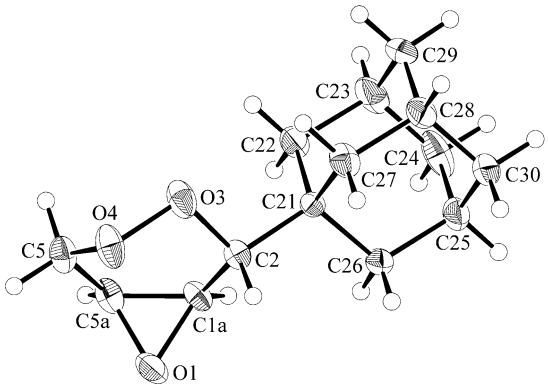


Figure 2. Molecular structure and crystallographic numbering scheme for $C_{14}H_{20}O_3$. Key geometric parameters are the following: O1−C1a 1.443(2) Å, O1−C5a 1.437(2) Å, C1a−C5a 1.461(2) Å, C2−O3 1.434(2) Å, O3−O4 1.462(2) Å, O4−C5 1.411(2) Å, C5−C5a 1.486(3) Å; C1a−O1−C5a 60.9(1)°, C2−O3−O4 106.9(1)°, O3−O4−C5 106.4(1)°.

(Table 1) and compared with data for CQ and artemisinin. A number of the compounds prepared in this study showed some antimalarial activity, with compounds **5a**, **5b**, **6a**, and **6b** showing the highest activity. These compounds comprise epoxy endoperoxide ring systems with saturated five- or six-member ring systems attached in the 3 and 6 positions. The dicyclobutyl derivatives (**5c**, **6c**) were less active, indicating that the larger ring system is favored. The presence of the epoxy group appears to be important for good antimalarial activity but is not essential because compounds **4a** and **4b** retained significant activity. Ring-opened derivatives of the cyclohexyl derivatives (**7a**, **8a**) showed no activity in the range tested, indicating that the endoperoxide ring system is critical for activity. The equivalent compounds with phenyl substituents (**4j**, **5j**) or alkyl chain substituents (**4h**) did not show activity, indicating that the nature of the substituent groups is critical. Similarly, compounds with a single cyclohexyl or cyclopentyl moiety (**4d**, **4e**, **5e**, **5d**, **5g**, **6g**) also had much lower activities, indicating that the double substitution is preferred. However, the monosubstituted *cis*-epoxy

Table 1. Biological Activities of Some of the Novel Endoperoxides

compd	hemolysis[a] (%)	antimalarial activity,[b] IC_{50} (μM)
ART	4	0.01
CQ	1	0.03
4a	100	1.2
4b	52	0.6
4c	32	>10
4d	100	3.2
4e	11	>10
4f	100	1.3
4g	100	9.5
4h	2	>10
4i	62	>10
4j[c]	4	6.0
4k	9	8.1
4l[c]	32	>10
4m	27	1.0
4n	0	2.5
5a	6	0.5
5b	8	0.5
5c	1	2.9
5d	2	>10
5e[d]	1	1.4
5f	41	4.0
5g	3	>10
5i	47	>10
5j	5	>10
5k	2	>10
5l	2	>10
5m	6	1.3
6a	5	0.32
6b	9	0.5
6c	1	1.6
6e[e]	1	1.4
6g	6	2.0
6i	51	>10
6m	4	5.0
7a	8	>10
7j[c]	2	>10
8a	14	>10
10	2	>10
12[c]	1	5.0

[a] Hemolysis of erythrocytes induced by 1 mM compound. [b] Antimalarial activity of compounds against the D10 parasite strain. [c] Compounds were not completely soluble in ethanol. Assays were performed using suspensions and activities expressed using the total concentration. [d] Used as a 75:25 ratio of trans/cis isomers. [e] Used as a 75:25 ratio of cis/trans isomers.

cycloalkyl derivatives (**6e**, **6g**) did show moderate activity. Indeed the *cis*-epoxy derivatives (**6a**, **6b**, **6c**, **6e**, **6g**) in general showed higher activity than their *trans*-epoxy counterparts. Similarly, compounds with mixed substituents (**5l**, **10**, **12**, **4l**) had variable activity. Some of the adamantane-linked compounds did show activity (**4f**), which was improved when the adamantane group was attached to the ring by an ester linkage (**4m**, **5m**).

Interaction with FP. In an effort to determine the likely mode of action of these novel endoperoxides, we have examined their interaction with oxidized and reduced FP. The binding of different quinoline antimalarial drugs to FP-Fe(III) has previously been shown to be important for the activity of these drugs. The binding of drugs such as CQ to FP modifies the spectral characteristics of FP, producing a decrease in the absorption and a broadening of the main Soret band at 400 nm even when it is present in stoichiometric quantities (Figure 3A). These effects are also observed in the presence of phenothiazines and may reflect the formation a $\pi-\pi$ donor−acceptor complex between the

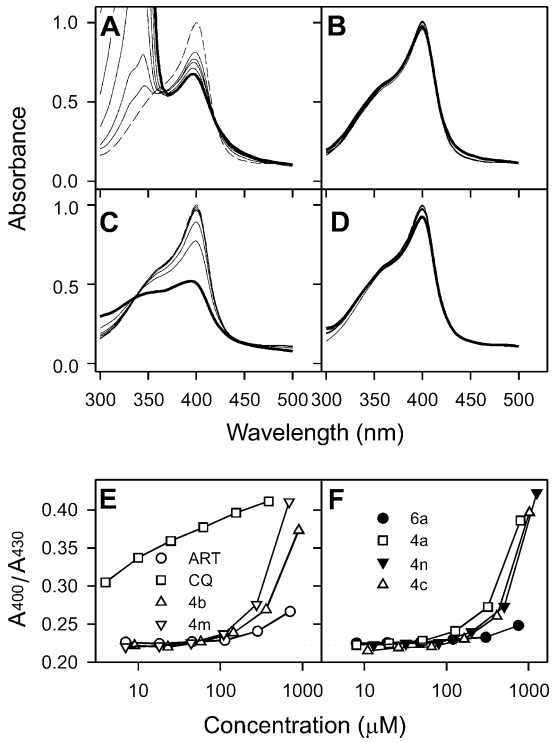


Figure 3. Spectral analysis of the interactions of CQ, artemisinin, and some novel endoperoxides with FP-Fe(III): absorption spectra of 50 μM FP-Fe(III) in the presence of (A) CQ, (B) artemisinin, (C) **4b**, and (D) **6a** under nonreducing conditions. The spectra in the presence of the highest concentration of compound (390, 710, 900, and 750 μM compound, respectively) are represented by the thick curves, whereas the spectra for FP-Fe(III) alone are the dashed curves. The dilution factor for intermediate concentrations is 2.5. CQ and **4b** possess some absorption below 400 nm and contribute to the absorption in this region. Spectra were normalized to that of FP in the absence of compound. The absorbance ratio at 430 and 400 nm (A_{430}/A_{400}) is a useful indicator of changes to the structure of the Soret band in the presence of compounds, and the dose dependence of this parameter for a number of compounds is shown in (E) and (F).

drug and the FP μ-oxo dimer.[20−22] In contrast, artemisinin produces a detectable change in the absorption spectrum only at a 100-fold greater concentration than FP-Fe(III) (Figure 3B,E), although a time-dependent decrease in the absorption of the Soret band has been reported in dimethyl sulfoxide−water mixtures over a 24 h period.[23] The addition of some of the novel endoperoxides (especially those lacking the epoxy moiety such as **4a**, **4b**, **4c**, **4m**) resulted in dose-dependent changes in the absorption spectrum of FP that resembled the changes observed with CQ (Figure 3C−F). This suggests that the compounds are binding to FP in a manner that produces a similar spectral change. Interestingly, some of the endoperoxides that do not contain π bonds such as **5d** and **6b** also exhibited this intermediate strength binding (Table SI, Supporting Information). It is important to note, however, that the binding of the endoperoxides to FP-Fe(III) did not seem to correlate with antimalarial activity because some of the most active antimalarials, such as **5a**, **5b**, **6a**, and **6b**, do not appear to interact with FP-Fe(III) or interact only very weakly under the conditions of the assay (Figure 3B and Table SI, Supporting Information).

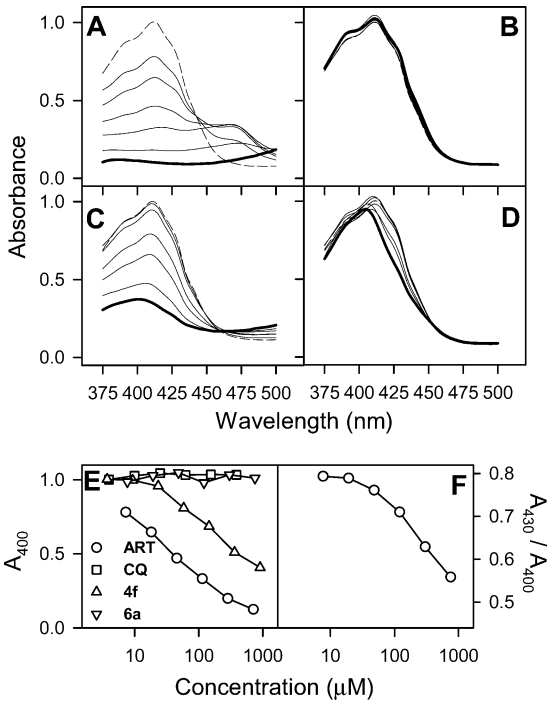


Figure 4. Spectral analysis of the interactions of CQ, artemisinin, and some novel endoperoxides with FP-Fe(II): absorption spectra of 30 μM FP-Fe(II) in the presence of (A) artemisinin, (B) CQ, (C) **4f**, and (D) **6a** under reducing conditions. The spectra in the presence of the highest concentration of compound (710, 390, 900, and 750 μM compound, respectively) are represented by the thick curves, whereas the spectra for FP-Fe(II) alone are the dashed curves. The dilution factor for intermediate concentrations is 2.5. The spectra are normalized to that of FP in the absence of compound and are shown at wavelengths above 375 nm because of the large absorbance of the dithionite ion at shorter wavelengths. The dose dependence of the loss of the Soret absorption at 400 nm is shown in (E). In some cases, compounds induce a change in the structure of the Soret band rather than a loss of absorbance. The absorbance ratio at 430 and 400 nm (A_{430}/A_{400}) indicates changes to the structure of the Soret band. The dose dependence of this parameter for **6a** is shown in (F).

The antimalarial activity of artemisinin is thought to arise from its interaction with FP-Fe(II), resulting in the formation a cytotoxic carbon-centered radical intermediate that further reacts with susceptible groups within parasite enzymes and lipids and can result in the alkylation of FP itself.[2,9−11] The addition of artemisinin to FP in the presence of the reducing agent dithionite produced dose-dependent decreases in the Soret absorption band (Figure 4A,E) and the appearance of a new absorption band evident as a shoulder at 475 nm (Figure 4A). A similar system has been previously employed using artemisinin and its derivatives, and the spectral changes were shown to correlate reasonably well with the antimalarial activity.[4] In this previous study, the reduction in absorbance was attributed to a binding interaction between the drug and FP. However, the complete loss of the Soret absorbance suggests that it in fact reflects the destruction of the FP chromophore with the concomitant formation of a new chromophore with different spectral characteristics. In contrast to artemisinin, CQ, which acts via a different mechanism, does not perturb the FP-Fe(II) spectrum under the conditions of this assay (Figure 4B,E), further demonstrating the usefulness of this assay for screening for artemisinin-like activity.

We found that a number of the novel endoperoxides (e.g., **4f**, Figure 4C) appeared to react with FP-Fe(II) in a manner similar to artemisinin, albeit at much higher concentrations. In general, compounds containing the epoxy group and two cyclic substituents showed the highest activity (Table SI, Supporting Information). Interestingly, some of the endoperoxides such as **6a** (Figure 4D) induced a blue shift in the Soret band rather than its ablation, indicating a specific interaction with FP-Fe(II). The endoperoxides exhibiting this binding mode tended to be those lacking the epoxy group (Table SI, Supporting Information). Other endoperoxides appeared to produce both effects, i.e., a reduction in the Soret band occurring in concert with a blue shift (Table SI, Supporting Information). Of the endoperoxides exhibiting the greatest antimalarial activity, **5a**, **5b**, and **6b** exhibited artemisinin-like activity whereas **6a** exhibited a shift in the Soret band (Figure 4 and Table SI, Supporting Information). As with the binding to oxidized FP, no clear correlation was observed with the antimalarial activity. For example, **6e** and **6g** exhibited moderate antimalarial activity but no detectable interaction with FP-Fe(II). In contrast, **4c** exhibited some interaction with FP-Fe(II) but no detectable antimalarial activity (Table SI, Supporting Information).

Membrane Activity. To determine whether any of the compounds showed membrane activity, we have examined their ability to lyse human erythrocytes. None of the compounds induced hemolysis at the highest concentration (10 μM) used in the antimalarial drug assays. However at a concentration of 1 mM and in the absence of protein in the bathing medium, some of the compounds did induce hemolysis (Table 1). This appeared to be directly related to the nature of the substituents. For example, alkyl-chain-substituted endoperoxides were significantly more active than ring-substituted compounds. This probably reflects the tendency of the alkyl chain to insert into the lipid bilayer. Similarly, the adamantyl group in **4f** appears to confer detergent-like properties. The epoxy endoperoxides were less membrane-active than their endoperoxide counterparts, presumably because of the additional polarity offered by the epoxy substituent. Again, there was no correlation between membrane activity and antimalarial activity, indicating that the compounds do not act by a nonspecific membrane perturbation mechanism.

Discussion

During intraerythrocytic growth, the malaria parasite feeds by degrading hemoglobin in an acidic food vacuole. It digests about 75% of the 20 mM hemoglobin in its host cell cytosol.[21,23,24] The free FP, which is produced as a byproduct of hemoglobin degradation, creates a waste disposal problem for the parasite (see ref 25 for review). At the pH of the food vacuole (estimated to be ~pH 5.2),[26−28] the FP in oxyhemoglobin is oxidized from the Fe(II) state to the Fe(III). Thus, the parasite needs to deal with FP-Fe(III) waste, which could reach a cellular concentration of about 15 mM. A major route for disposal of toxic FP is via a novel process known as biomineralization. The FP-Fe(III) is sequestered into a crystalline form, referred to as hemozoin.[13] This leads

to the formation of the characteristic malaria pigment that is visible in smears of infected blood.

While most of the FP-Fe(III) appears to be efficiently detoxified within the food vacuole, it is likely that a part of the FP population escapes the crystallization process and diffuses down the concentration gradient into the parasite cytosol where it will be reduced to FP-Fe(II). The redistribution of even a small fraction of the 15 mM cellular load of FP could potentially damage host proteins and membranes. Indeed, studies have shown that nonsequestered FP can be present at concentrations up to 100 μM in parasitized erythrocytes.[21,29] Although this represents a small proportion (0.5%) of the total FP in the parasitized erythrocyte, free FP at levels much lower than this have been shown to inhibit parasite enzymes,[30,31] to lyse erythrocytes,[32] and to cause substantial redox damage.[21,33] Thus, the parasite-specific processes of hemoglobin degradation and FP detoxification leave the parasite susceptible to quinoline and endoperoxide drugs that interfere with aspects of the detoxification process.

In this work, we have prepared a novel series of endoperoxide compounds. The route for the construction of the new targets is robust, can be performed on a large scale in a safe manner, and is of low cost. These new endoperoxides not only have the endoperoxide linkage but also have a third oxygen atom that is positioned in an environment similar to that of oxygen atom 3 within artemisinin. We have also prepared a series of endoperoxides that lack the epoxide group but have additional functional groups attached to the endoperoxide ring. In addition, we have utilized cobalt catalysts to induce ring opening of these endoperoxides, providing a synthetic route to their isomeric ring-opened derivatives. This free radical mechanism of ring opening is likely to parallel the reaction of the endoperoxides with FP-Fe(II). We were therefore able to test the possible downstream products for activity.

We examined the antimalarial activities of the members of this novel class of endoperoxides. We identified a number of compounds with IC_{50} values in the range 300−500 nM. The minimum pharmacophore appears to be an endoperoxide ring system with saturated cyclic substituents at the 1 and 4 positions. In an effort to determine the mechanism of action of the novel endoperoxides, we examined their interactions with FP. CQ and other quinoline antimalarials have previously been shown to form complexes with FP.[34−36] The observed spectral changes are thought to be derived from $\pi-\pi$ complexation of the porphyrin and quinoline ring systems.[20] A number of the novel endoperoxides induce similar changes to the spectral properties of FP-Fe(III) as CQ. Interestingly, some of these compounds lacked a π system, indicating they bind to FP in a manner different from that of CQ. It is possible that these compounds may cause the bridging of two FP monomers, resulting in $\pi-\pi$ stacking. We found no correlation between the FP-Fe(III) binding and antimalarial activity of the compounds, which may suggest that the compounds are not efficiently targeted to the food vacuole of the parasite. Quinoline antimalarials are weak bases and are accumulated in the food vacuole, at least in part, by a proton-trapping mechanism (see ref 25 for review). It would be interesting to determine whether addition of a basic side chain to compounds such as **4a**, **4m**, **4b**, and **4c** would improve their antimalarial activities. The different FP-Fe(II) binding modes of these compounds and their different structures compared to that of CQ may allow activity against CQ-resistant strains of parasites.

We also examined the abilities of the compounds to react with FP-Fe(II). Activation of artemisinin by reaction with reduced FP has long been thought to be critical for its activity. Recent studies suggest that activated artemisinin targets the SERCA calcium transporter in the endoplasmic reticulum of *P. falciparum*.[12] These authors showed that desferoxamine antagonized the action of artemisinin against SERCA and suggested that Fe^{2+} is the likely activator of artemisinin. However, it has previously been shown that desferoxamine also interacts with FP,[37] and given that the concentration of free Fe^{2+} in the parasite is likely to be limited, it seems more likely that reduced FP functions as the activator of artemisinin in vivo. In this work we found that the antimalarial activities of the novel endoperoxides is not correlated with their FP reactivity, a feature which may reflect the inability of some of the compounds to be located to the appropriate intracellular site. The spectroscopic analysis indicated that artemisinin and a subset of the endoperoxide compounds resulted in the destruction of the FP chromophore under reducing conditions. Intriguingly, some of the compounds did not destroy the FP but appeared to form a complex with the reduced FP that exhibits a spectral blue shift. Although the novel endoperoxides undergo rapid scission upon interaction with metals as indicated by analysis of the products formed during interaction with the copper catalysts, it is possible that the interaction of the bicyclic endoperoxides with FP-Fe(II) stabilizes the porphyrin ring and thereby prevents transfer of the radical intermediates to the porphyrin following scission of the peroxide moiety. Another subset of the compounds exhibit both FP destruction and FP binding, suggesting in these cases that the reaction between the compound and the FP is slowed sufficiently so that the complex can be observed. In this respect, the novel compounds may prove to be useful in elucidating the complex interaction of artemisinin with FP and in the design of new artemisinin-like compounds. In this work, we have not examined the effect of the novel endoperoxides on SERCA activity and it is possible that some of the compounds target this enzyme. However, given the range of interactions with FP, we suggest that the compounds may act via a number of different molecular mechanisms.

In conclusion, we have identified a facile route for the synthesis of a series of novel endoperoxides with antimalarial activities in the submicromolar range. The IC_{50} values are relatively high compared with values for artemisinin (~10 nM) and CQ (~30 nM for a CQ-sensitive strain); however, they approach the IC_{50} values for CQ against CQ-resistant strains (300−600 nM)[38] and for compounds such as sulfadoxine (~100 nM for sensitive strains and up to 10 μM for resistant strains). The efficacy of these compounds may be further improved by adding substituents onto the cyclic ring systems. For example, addition of an amino side chain has been shown to improve the efficacy of artemisinin,[39] presum-

ably by enhancing drug uptake. The fact that some of the compounds appear to inhibit parasite growth by what appears to be a novel mechanism of action indicates that they are worth pursuing in an effort to obtain novel compounds to replenish our rapidly dwindling armory of useful antimalarials.

Experimental Section

General Synthetic Methods. Solvents were dried by appropriate methods wherever needed. Thin-layer chromatography (TLC) used aluminum sheets coated with silica gel 60 F_{254} (40 mm × 80 mm) from Merck, visualized under 254 nm light or developed in vanillin or permanganate dip. Flash chromatography was conducted using Merck silica gel 60 of particle size 0.040−0.063 mm. Melting points were taken on a Reichert Thermovar Kofler apparatus and are uncorrected. Infrared spectra were recorded on an ATI Mattson Genesis series FTIR spectrophotometer. ^{1}H NMR and ^{13}C NMR spectra were recorded in $CDCl_3$ solution on a Varian Gemini 2000 (200 MHz), Varian Gemini 2000 (300 MHz), or Varian INOVA (600 MHz) instrument, using TMS (0 ppm) and $CDCl_3$ (77.0 ppm) as internal standards. Electron impact mass spectra (EI-MS) were recorded at 70 eV. Accurate mass measurements were performed at the Central Science Laboratory, University of Tasmania, Tasmania, Australia, or at the Department of Chemistry, Monash University, Victoria, Australia. Microanalyses were performed in the Department of Chemistry, University of Otago, Dunedin, New Zealand. All yields reported refer to isolated material judged to be homogeneous by TLC and NMR spectrometry.

General Synthesis of Endoperoxides. All endoperoxides (**4**, **9**, and **11**) were prepared via photolysis (3 × 500 W tungsten/halogen lamps) of an oxygen-saturated dichloromethane solution of the 1,3-butadiene in the presence of the photosensitizer Rose Bengal, bis(triethylammonium) salt, for 7 h.[40] In all cases, starting diene was reclaimed from the photolysis reactions as the other major product.

General Synthesis of Epoxy Endoperoxides (5, 6, and 12).[16] To a stirred solution of 1,2-dioxine (1 mmol) in CH_2Cl_2 (5 mL) was added 70% *m*-chloroperbenzoic acid (2 mmol). The reaction was stirred at ambient temperature until complete by TLC. Dichloromethane (10 mL) was then added, and the solution was extracted with saturated $Na_2S_2O_3$ (10 mL) followed by $NaHCO_3$ (10 mL). The organic layer was dried over $MgSO_4$ and filtered, and volatiles were removed in vacuo. The crude epoxides were purified by column chromatography. Epoxides **5a**, **6a**, **5j**, **5l**, and **10** have previously been reported.[16]

Crystal Structure Determination of $C_{16}H_{14}O_3$ (5f). Crystal data for $C_{14}H_{20}O_3$: M = 236.30, T = 223(2) K, monoclinic, $P2_1/c$, a = 15.2364(13) Å, b = 7.2642(7) Å, c = 11.1369(10) Å, β = 98.777(4)°, V = 1218.20(19) Å^3, Z = 4, D_x = 1.288, $F(000)$ = 512, μ = 0.089 mm^{-1}, no. of unique data (Bruker AXS SMART CCD using Mo Kα radiation so that θ_{max} = 30.1°) = 3553, no. of parameters = 154, R (2185 data with $I \geq 2\sigma(I)$) = 0.062, wR (all data) = 0.193, ρ = 0.39 e Å^{-3}. The structure was solved by direct methods (SIR92) and refined (anisotropic displacement parameters, H atoms in the riding model approximation, and a weighting scheme $w = 1/[\sigma^2(F_o^2) + 0.1018P^2 + 0.047P]$ where $P = (F_o^2 + 2F_c^2)/3$) with SHELXL-97 on F^2.

Biochemical Materials. Fresh human erythrocytes were obtained from the Red Cross Transfusion Service, Melbourne, Australia. Chloroquine, FP (bovine hematin), and artemisinin were obtained from Sigma Chemical Company (St. Louis, MO). Stock solutions of FP were prepared daily in 50 mM NaOH.

Assessment of Antimalarial Activity of the Endoperoxides. D10 is a CQ-sensitive strain of *P. falciparum*.[41] Malaria parasites were plated at about 1% parasitemia (2% hematocrit) in 96-well trays, and different concentrations of the compounds were added as serial dilutions in complete medium from concentrated stocks in ethanol. Parasites were incubated for 72 h, with daily replacement of the drug-supplemented medium. Growth curves based on the uptake of [^{3}H]-hypoxanthine were obtained in triplicate as described previously,[38] and the concentration of compound required to produce 50% inhibition of growth (IC_{50}) was determined.

FP Binding Assays. The interaction of the compounds with FP was examined spectrophotometrically under reducing and nonreducing conditions by monitoring the Soret absorption band of FP. Compound stocks were prepared in ethanol and serially diluted in a 96-well microtiter plate into nonreducing (50 μM FP, 100 mM sodium phosphate, pH 7.4, 1% SDS) or reducing (30 μM FP, 100 mM sodium phosphate, pH 7.4, 1% SDS, 35 mM sodium dithionite) buffer. The incorporation of the SDS detergent in the buffers was necessary to produce a stable absorption because FP has a tendency to aggregate in aqueous solution and its absorption can exhibit a temporal dependence. The concentration of ethanol in the assay was less than 2% (v/v). Absorption spectra were collected using a Spectromax 250 plate reader.

Erythrocyte Lysis Assay. Washed human erythrocytes (400 μL, 0.4 × 10^8 cells) in phosphate-buffered saline were incubated with the compounds to a final concentration of 10 μM or 1 mM and incubated 30 min at 37 °C. Cells were pelleted, and the absorbance of the supernatant was compared with that for a sample of freeze−thawed cells to determine the percentage of hemolysis.

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Appendix

Abbreviations. FP-Fe(III) = oxidized heme = ferriprotoporphyrin IX, FP-Fe(II) = reduced heme = ferroprotoporphyrin IX; CQ = chloroquine; BSA = bovine serum albumin; SDS = sodium dodecyl sulfate; THF = tetrahydrofuran; *m*-CPBA = *m*-chloroperoxybenzoic acid.

Supporting Information Available: Table SI containing data for the interaction of the compounds in Table 1 with oxidized and reduced FP; synthetic protocols and NMR data for each of the compounds, and relevant references. This material is available free of charge via the Internet at http://pubs.acs.org.

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