Mechanism-Based Design of Parasite-Targeted Artemisinin Derivatives: Synthesis and Antimalarial Activity of New Diamine Containing Analogues

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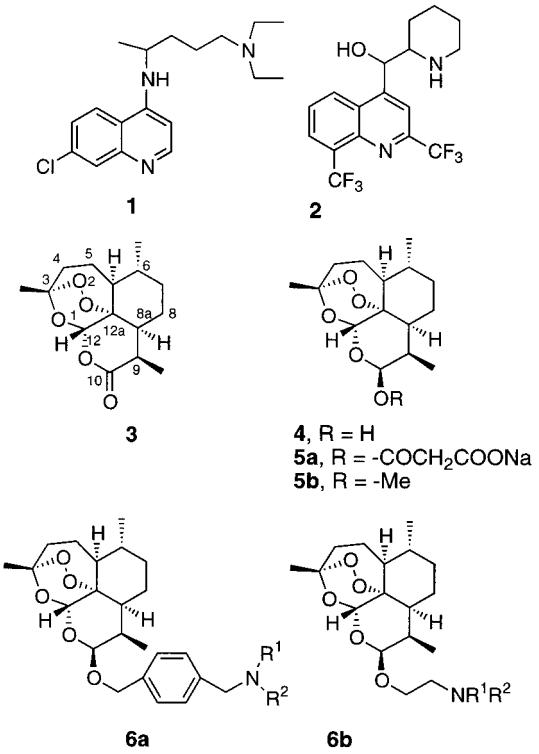
The potent antimalarial activity of chloroquine against chloroquine-sensitive strains can be attributed, in part, to its high accumulation in the acidic environment of the heme-rich parasite food vacuole. A key component of this intraparasitic chloroquine accumulation mechanism is a weak base "ion-trapping" effect whereupon the basic drug is concentrated in the acidic food vacuole in its membrane-impermeable diprotonated form. By the incorporation of amino functionality into target artemisinin analogues, we hoped to prepare a new series of analogues that, by virtue of increased accumulation into the ferrous-rich vacuole, would display enhanced antimalarial potency. The initial part of the project focused on the preparation of piperazine-linked analogues (series 1 (**7**−**16**)). Antimalarial evaluation of these derivatives demonstrated potent activity versus both chloroquine-sensitive and chloroquine-resistant parasites. On the basis of these observations, we then set about preparing a series of C-10 carba-linked amino derivatives. Optimization of the key synthetic step using a newly developed coupling protocol provided a key intermediate, allyldeoxoartemisinin (**17**) in 90% yield. Further elaboration, in three steps, provided nine target C-10 carba analogues (series 2 (**21**−**29**)) in good overall yields. Antimalarial assessment demonstrated that these compounds were 4-fold more potent than artemisinin and about twice as active as artemether in vitro versus chloroquine-resistant parasites. On the basis of the products obtained from biomimetic Fe(II) degradation of the C-10 carba analogue (**23**), we propose that these analogues may have a mode of action subtly different from that of the parent drug artemisinin (series 1 (**7**−**16**)) and other C-10 ether derivatives such as artemether. Preliminary in vivo testing by the WHO demonstrated that four of these compounds are active orally at doses of less than 10 mg/kg. Since these analogues are available as water-soluble salts and cannot form dihydroartemisinin by P450-catalyzed oxidation, they represent useful leads that might prove to be superior to the currently used derivatives, artemether and artesunate.

Introduction

Malaria is one of the world's deadliest diseases and is becoming an increasingly serious problem as malaria parasites develop resistance to drugs such as chloroquine (**1**) and mefloquine (**2**). There is, therefore, considerable urgency to develop new classes of antimalarials. Artemisinin (**3**) (qinghaosu) is an unusual 1,2,4-trioxane, which has been used clinically in China for the treatment of multidrug-resistant *Plasmodium falciparum* malaria.[1] However, the clinical application of artemisinin has been limited by the drug's pharmacokinetic properties. This has provided the impetus for the investigation of derivatives of this compound, some of which include esters (**5a**, R= −$COCH_2COONa$, artesunate) and ethers (**5b**, R= −Me, artemether) of the corresponding lactol, dihydroartemisinin (DHA) (**4**). First-generation analogues of this type are currently being developed as potent and rapidly acting antimalarials.[2]



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The mechanism of action of artemisinin is believed to involve an interaction with ferriprotoporphyrin IX ("heme"), or ferrous ions, in the acidic parasite food vacuole, which results in the generation of cytotoxic radical species. On the basis of this knowledge, several "mechanism-based approaches" have been investigated for improving the antimalarial activity of artemisinin derivatives. These include the incorporation of groups to enhance the stability of proposed "parasiticidal intermediates"[3] and the covalent attachment of "iron chelator functionality"[4] to enhance the interaction of the peroxide bridge with available "free iron" in the food vacuole of the parasite. More recently, we have prepared a number of benzylamino (**6a**) and alkylamino derivatives (**6b**) of dihydroartemisinin.[4] It was proposed that this chemical modification would enhance drug activity by increasing the cellular accumulation within the acidic parasite food vacuole by "ion trapping". The higher concentration of drug available for interaction with heme and hence generation of the required alkylating species may have been responsible for the increased antimalarial activity observed in this study.

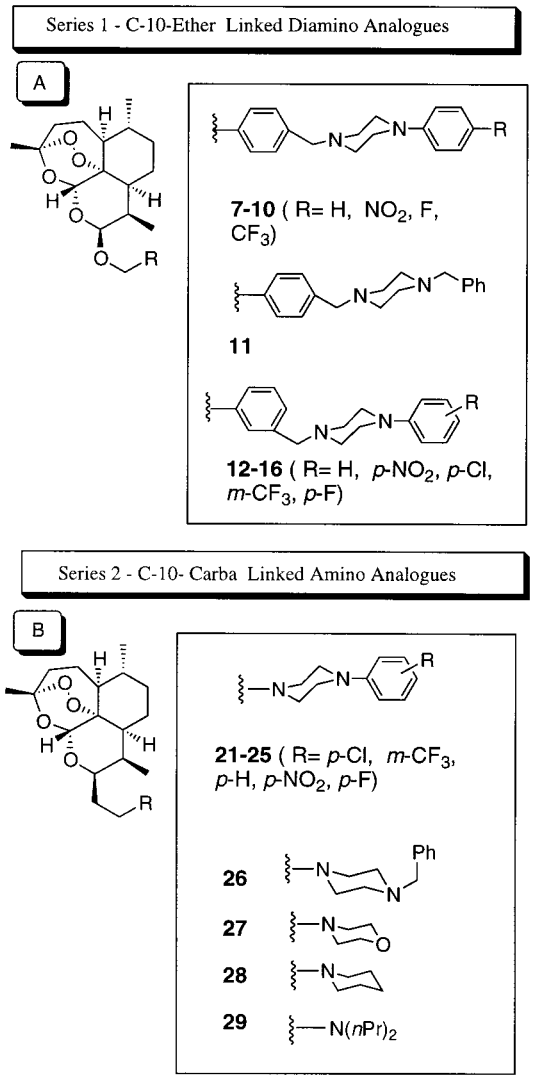
Chloroquine is dibasic with pK_a values of 8.1 (quinoline ring nitrogen) and 10.2 (diethylamino side chain) and accumulates in acidic vesicles to the square of the monobasic antimalarials such as mefloquine.[6] Experiments on the pH gradient between the external medium and the parasite food vacuole have shown that the value is around 2.2. On this basis, Ginsburg and co-workers suggested that chloroquine would be expected to accumulate (2.5×10^4)-fold compared with 160-fold for the monobasic antimalarials such as mefloquine.[6] On the basis of similar reasoning, the introduction of two basic amino groups into an artemisinin derivative would be expected to significantly increase the cellular accumulation of drug in the ferrous-rich parasite food vacuole. In theory, this approach should provide analogues with increased antimalarial potency, since more drug will be available for reductive endoperoxide bioactivation to radical species. In this work we have prepared two new series of artemisinin derivatives that incorporate two basic nitrogen atoms. Compounds in the first series (Chart 1A) are structurally related to our previously synthesized monoamine derivatives (**6a,** R = alkyl) and (**6b,** R = alkyl) and contain the metabolically susceptible C-10 ether linkage.

It was considered important, at an early stage, to demonstrate that the incorporation of amino functionality was not detrimental to antimalarial potency. On the basis of promising results, a second series of compounds (Chart 1B) were designed to both increase the intraparasitic cellular accumulation and to resist P450 metabolism to DHA, a metabolite associated with a short half-life and neurotoxicity. Both series of analogues have the added advantage of being available as water-soluble salt preparations.

Chemistry

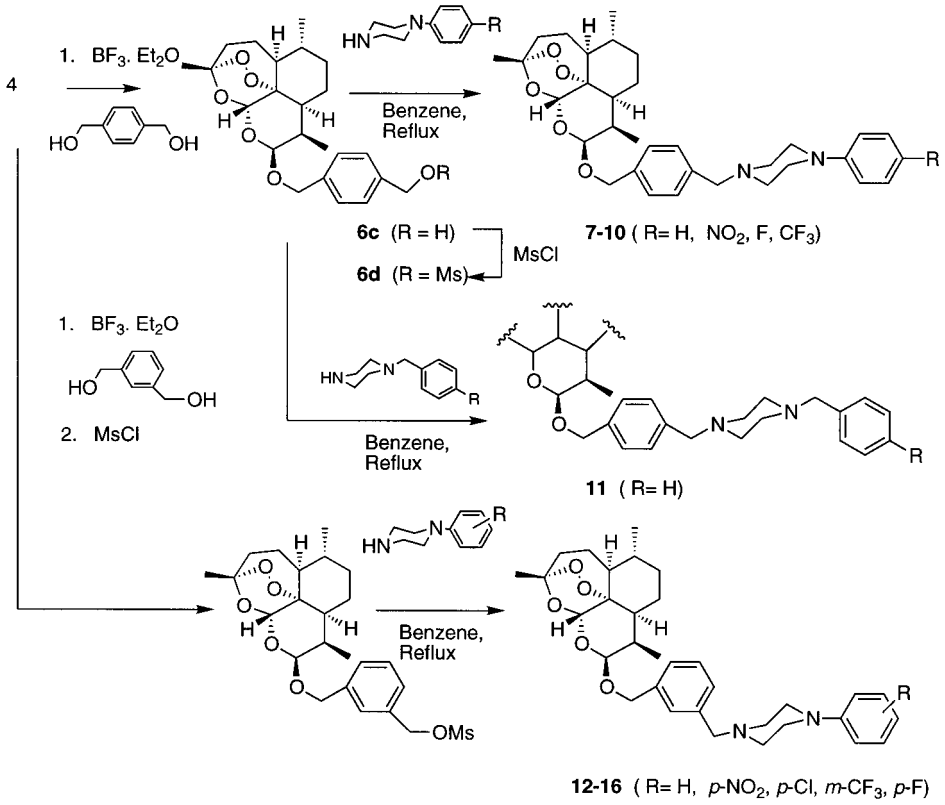
The first series of compounds were prepared as shown in Scheme 1. Dihydroartemisinin **(4)** was coupled with 1,4-dibenzene methanol to give the corresponding alcohol **(6c)** in high yield with excellent diastereoselectivity (β/α, 5:1). The alcohol was then converted into the mesylate **(6d)** in high yield by treatment with mesyl chloride and triethylamine. The key mesylate was then allowed to react with a range of diamino nucleophiles to provide compounds (**7**−**11**). As shown in Scheme 1, DHA was also coupled with 1,3-dibenzene methanol to provide the alcohol which could then be transformed into target analogues **(12**−**16)** using the same chemistry described for the para-substituted analogues.

Chart 1. New Parasite-Targeted Analogues of Artemisinin

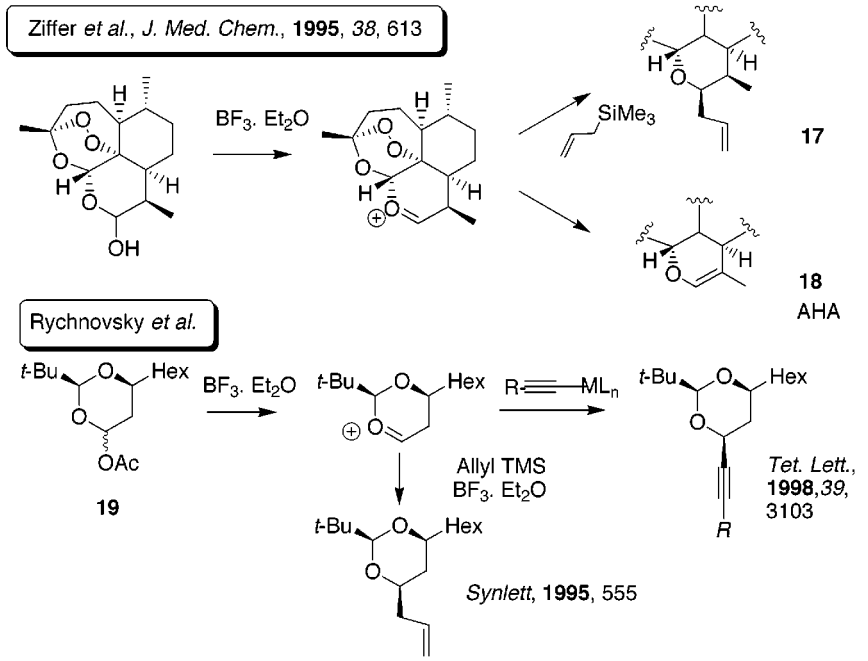


The literature synthesis of key intermediate (**17**), required for the preparation of target carba analogues, is complicated by the formation of the byproduct anhydroartemisinin (AHA) (**18**) (Scheme 2). Higher yields, ca. 55%, can be achieved by conversion of the anomeric hydroxyl group of DHA to an OTMS function. However, although the yield is improved using this approach, anhydroartemisinin is still formed as a byproduct and purification is complicated. Recent studies by Rychnovsky on 4-acetoxy-1,3-dioxanes (**19**) has shown that the use of the acetate group as an anomeric leaving group enables smooth formation of an oxonium intermediate that can be intercepted with a range of carbon-based nucleophiles (Scheme 2).[7] On the basis of this and recent work by Ziffer et al.,[8] we prepared the C-10 acetate and C-10 benzoate of dihydroartemisinin and investigated the reaction with allyltrimethylsilane using $BF_3{\cdot}Et_2O$ as catalyst. Once again, a reasonable yield of

Scheme 1. Synthesis of Ether-Linked Analogues (Series 1)



Scheme 2. Lewis Acid Mediated Oxonium Generation and Interception with Nucleophiles



product was obtained (52%), but the dehydration product AHA was also formed in the reaction. To improve the yield of C-10 deoxoartemisinin (**17**) and to minimize the formation of AHA, a variety of Lewis acids, solvents, and reaction temperatures were investigated.[9]

From this study, the optimized method for the synthesis of (**17**) involves reaction of the C-10 benzoate (**20**) with allyltrimethylsilane using anhydrous $ZnCl_2$ as Lewis acid in the presence of 4 Å molecular sieves and dichloroethane (DCE) as solvent. This provides the target molecule in 90% yield with no AHA byproduct (Scheme 3). The vinyl function of key intermediate (**17**) was ozonized and the ozonide reduced in situ to provide the key alcohol. (Mesylation and reaction with an appropriate amine gave the target molecules (**21**−**29**) in moderate yield (Scheme 3).)

Biology

In Vitro and in Vivo Antimalarial Activity. Analogues were tested in vitro against the K-1 chloroquine-resistant strain of *P. falciparum* and against the chloroquine-sensitive HB3 strain. The IC_{50} values of the

Scheme 3. Synthesis of C-10 Carba Amino Derivatives **21**−**29**

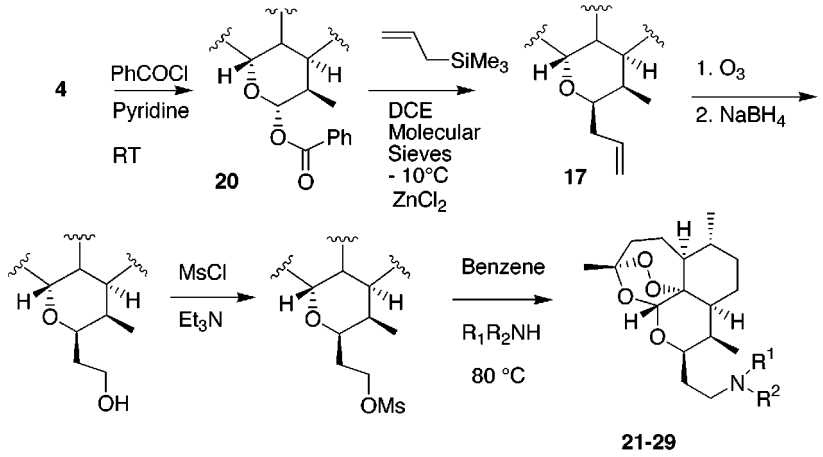


Table 1. IC_{50} of Amine Analogues versus the K1 Strain of *Plasmodium falciparum* in Vitro

compd	IC_{50} (nM)	SD ±
7	8.2	2.7
8	138.1	11.1
9	6.3	0.7
10	12.5	1.6
11	3.8	0.1
12	6.6	0.7
13	4.3	0.30
14	13.3	0.6
15	22.5	1.9
16	12.6	17
artemether	6.5	2
chloroquine	255	10

Table 2. IC_{50} of Amine Analogues versus the HB3 Strain of *Plasmodium Falciparum* in Vitro

compd	IC_{50} (nM)	SD ±
9	2.3	0.7
10	8.1	1.2
11	1.8	0.2
12	3.4	0.7
13	4.3	0.30
16	12.6	17
artemether	9.2	0.2

new ether-linked diamines are shown in Tables 1 and 2. From Table 1, it can be seen that several of these new derivatives are as potent or more potent than the first-generation derivative artemether. From this initial SAR work, it was clear that the incorporation of the *N*-benzyl- and *N*-phenylpiperazine ring systems provides analogues with excellent in vitro antimalarial potency. It is also interesting that the analogue that has lost substantial activity (**8**) contains a *p*-nitro function, which might be expected to decrease the basicity of the attached piperazine nitrogen atom. However, this argument does not explain why compound (**10**), an analogue containing the electron-withdrawing *p*-trifluoromethyl substituent, has potent antimalarial activity. Thus, the reduction in in vitro antimalarial activity observed with (**8**) cannot simply be a function of reduced pK_a alone. From Table 2, several compounds were more potent than artemether, compound (**11**) being 5 times more active.

As noted earlier, a drawback with these *parasite-targeted analogues* is the nature of the ether linkage in each of the candidate molecules. Metabolism studies have demonstrated that ether derivatives are rapidly

Table 3. IC_{50} of Carba Analogues versus the K1 Strain of *Plasmodium falciparum* in Vitro

compd	IC_{50} (nM)	SD ±
21	8.16	1.06
22	6.19	2.68
23	3.15	0.90
24	4.29	0.58
25	5.96	1.98
26	4.22	0.36
27	4.22	1.36
28	4.83	1.36
29	7.51	3.21
artemether	7.94	1.03
artemisinin	12.5	2.89

Table 4. In Vivo Testing (ED_50, ED_{90}) of C-10 Carba Analogues versus *P. yoeli* in Mice

compd	ED_{50} (mg/kg)	ED_{90} (mg/kg)
22	<10	<10
23	<10	>10
24	<10	>10
25	<10	~10
26	<10	>10
sodium artesunate	1.6	100

metabolized in vivo to dihydroartemisinin (DHA). This metabolite undergoes rapid phase II glucuronidation, which results in a polar conjugate that is excreted in bile.[10] It has been proposed that this metabolic pathway is responsible for the short half-life in vivo that results in the observed parasite recrudescence following treatment with ether derivatives.

The new carba analogues (**21**−**29**) were tested against the chloroquine-resistant parasites in vitro, and the results are shown in Table 3. All of the compounds had antimalarial activity similar to that of artemether. Several of these analogues were tested for in vivo antimalarial activity in mice. All of the compounds had an ED_{50} of less than 10 mg/kg (Table 4). The most potent analogue (**22**) also had an ED_{90} value of less than 10 mg/kg. Further testing of (**22**) versus *Plasmodium berghei* demonstrated that this compound was twice as active as artemether (Table 5).

Discussion

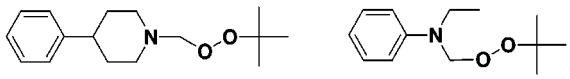
Following work by Homewood,[11] De Duve[12] reasoned that the weak base effect is based on two main assumptions: (i) the neutral (uncharged) forms of the weak bases readily cross both plasma and vesicle membranes;

Table 5. In Vivo Antimalarial Activity versus *Plasmodium berghei*

compd	ED_{50} (mg/kg)
22	3.12
artemether	6.02

(ii) these membranes are impermeable (or much less permeable) to the protonated forms of the bases. The logical consequences of these assumptions are consistent with the known effects of weak bases on both mammalian cells and plasmodia;[13] i.e., weak bases are concentrated by protonation in their nondiffusable form within acidic intracellular vesicles.

In addition to the wide variety of artemisinin analogues that have been examined to date,[14] simple peroxides such as *tert*-butyl hydroperoxide and hydrogen peroxide have been screened and found to have limited antimalarial potency.[15] Since both of these agents result in unwanted side effects, such as hemolysis of uninfected erythrocytes at parasiticidal concentrations, Vennerstrom synthesized a series of amine peroxides (e.g., (**30**) and (**31**)) with the idea that a selective drug delivery would circumvent toxicity to the host.[16] The



30 **31**

rationale for the intended specificity of action was the selective concentration of these weak base derivatives in the acidic digestive vacuoles of the parasite (pH ≥ 5.0) where the oxidant sensitivity of the malaria parasite could be exploited to maximum effect. The study revealed that linking *tert*-butyl peroxide to various amine groups enhanced antimalarial activity by 1 order of magnitude.

On the basis of this earlier work, we prepared a number of artemisinin analogues containing monoamine groups. It was anticipated that the incorporation of a moiety that can undergo protonation in the acidic food vacuole of the parasite would enhance cellular accumulation by ion trapping and thereby provide larger quantities of drug available for interaction with heme, the "cellular activator" of antimalarial peroxide drugs. Several of the compounds tested had potent activity against chloroquine-resistant and -sensitive parasites.[4]

In this work, we have attempted to enhance biological activity even further by covalent attachment of diamine functionality. On the basis of early models formulated for chloroquine accumulation, we anticipated that this structural modification would lead to enhanced intraparasitic accumulation. The two series of compounds investigated are shown in Chart 1. The first series of compounds are structurally related to derivatives synthesized in our earlier work. Members of this series all contain the metabolically susceptible C-10 ether linkage. Compounds of the second series were designed with this fact in mind, and all contain the metabolically more stable C-10 carba unit linked covalently to a variety of amino groups. The key features considered in the design of these analogues are summarized in Figure 1.

Critical examination of series 1 reveals a modest increase, 2- to 4-fold, in antimalarial activity versus the chloroquine-sensitive HB3 strain and chloroquine-resistant K1 strain. This suggests that the incorporation of two basic nitrogen atoms does not markedly increase the intracellular accumulation as anticipated with these analogues. This is illustrated by comparing the monoamine analogues in series 2 with their diamine counterparts. An important observation from this study is the lack of cross-resistance observed. Thus, the chloroquine resistance mechanism has no effect in reducing access to the proposed receptor heme. In terms of antimalarial activity versus the chloroquine-resistant strain, compounds of the second series were slightly more potent than artemether, with activities ranging from 3 to 8 nM.

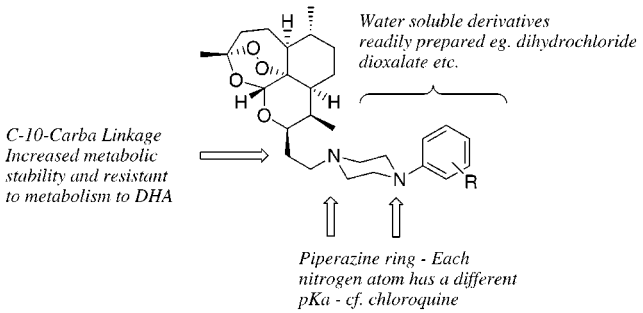
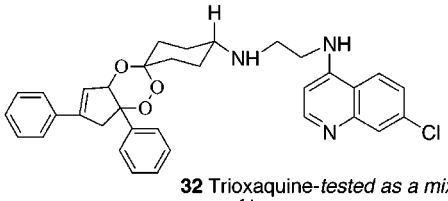


Figure 1. Design of series 2 trioxanes.

In preliminary in vivo testing carried out by Professor Peters in collaboration with the WHO, all of the compounds tested versus *P. yoelli* were active at less than 10 mg/kg. Our previous work[8a] and others have shown that the presence of a lipophilic fluorinated ring is beneficial in terms of in vivo antimalarial efficacy. This fact is compounded by compound (**22**), which not only is active at less than 10 mg/kg in terms of ED_{90} versus *P. yoelii* but also is active at 3 mg/kg versus *Plasmodium berghei* by oral administration. This makes this analogue a candidate worthy of further investigation.

Recent studies in the group suggest that the cellular uptake of chloroquine is dependent on binding to ferriprotoporphyrin IX, the so-called saturable element of chloroquine accumulation.[17] A number of studies have investigated the interaction of chloroquine with heme, and receptor-binding data are available for this interaction.[18−24] The main bonding interactions in the heme−4-aminoquinoline drug complex involves $\pi-\pi$ stacking interactions of the quinoline ring structure over the porphyrin ring with secondary electrostatic interactions of the carboxylate oxygen atoms with the charged ammonium side chain.[24] Since similiar $\pi-\pi$ stacking interactions are not available in these analogues, we suggest that accumulation within the acidic vacuole is governed by simple ion trapping or by a nonsaturable mechanism of accumulation. It is tempting to speculate that the accumulation of artemisinin analogues may be increased significantly by the incorporation of a structural motif with high affinity for heme and that is proven to aid cellular accumulation inside the food vacuole of the parasite. Such motifs are present in 4-aminoquinoline antimalarials that are active against chloroquine-resistant parasites and achieve a high degree of accumulation. Thus, covalent linkage of a 4-aminoquinoline unit, designed with the resistance mechanism in mind, may be more efficient at enhancing drug accumulation in the target vacuole.[25]

Recently, Meunier and co-workers prepared a hybrid analogue, known as a trioxaquine (**32**), that incorporates a 1,2,4-trioxane fenozan unit linked covalently to a 4-aminoquinoline.[26] Some of these compounds demonstrated good activity versus chloroquine-resistant strains. Although Fivelman and co-workers have demonstrated that chloroquine antagonizes trioxanes such as artemisinin,[27] this approach of combining a known "heme binder" to a peroxide-based unit is certainly worthy of further investigation and will be the subject of future work in our labs.



32 Trioxaquine-*tested as a mixture of isomers*

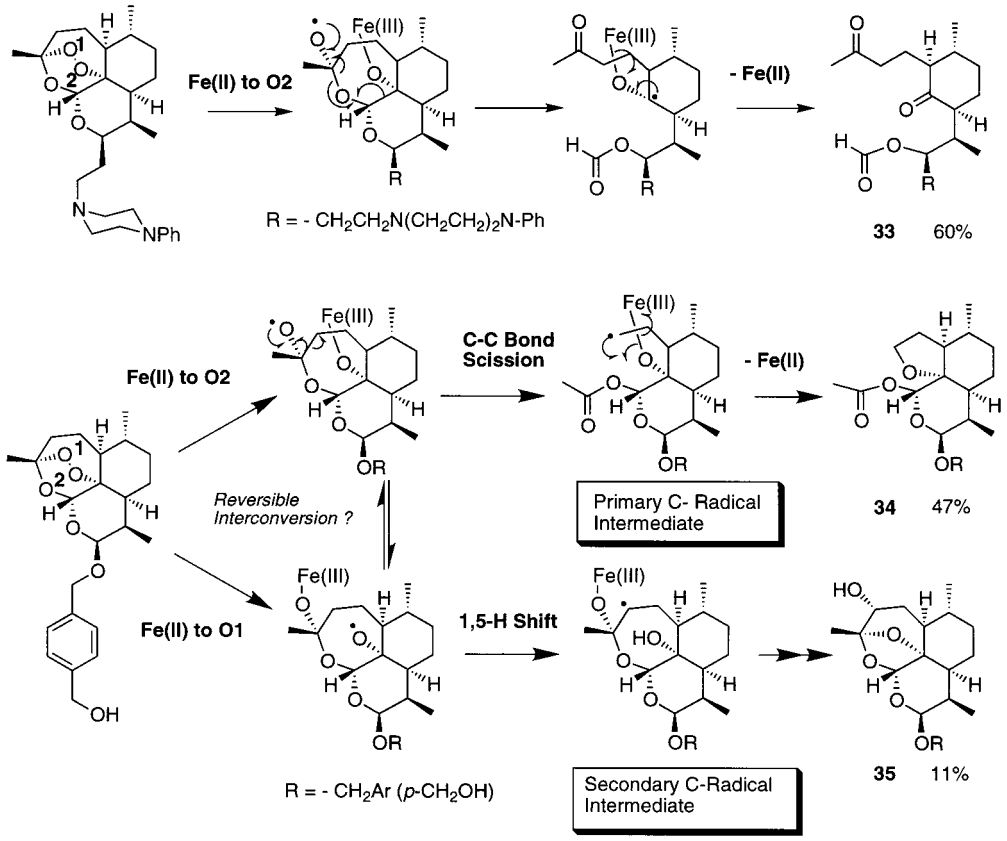
The generally accepted mechanism of action of peroxide antimalarials involves interaction of the peroxide-containing drug with heme, a hemoglobin degradation byproduct, derived from proteolysis of hemoglobin. This interaction is believed to result in the formation of a range of potentially toxic oxygen and carbon-centered radicals. To gain deeper mechanistic insight into the "actual" radical intermediates or cytotoxic end-products generated, several workers have employed biomimetic Fe(II)-mediated decompositions to simulate events in the ferrous-rich parasite food vacuole. Surprisingly, ferrous-mediated degradation of (**23**) provided the formate (**33**) as the main product in 60% yield. This type of product is not seen following identical degradation of artemisinin, artemether, or C-10 ether-linked alcohol (Scheme 4).

This result is surprising and tells us that simply modifying the substituent at the C-10 position of the artemisinin framework has a profound effect on the radical-mediated chemistry of these derivatives. A plausible mechanism for the formation of formate is shown in Scheme 4. (A mechanism involving initial heterolytic cleavage of the trioxane peroxide bridge (Haynes mechanism) followed by further radical-mediated processes can also be proposed.[28]) Whatever the "precise chemical mechanism", the release of a potentially reactive dicarbonyl selectively within the parasite may have a part to play in the actual "parasite kill". Indeed, from biomimetic Fe(II)-mediated reductions of related simplified C-3 aryltrioxane analogues (**36**), the resulting 1,5-diketone analogues of (**33**) were shown to have antimalarial activity (Scheme 5).

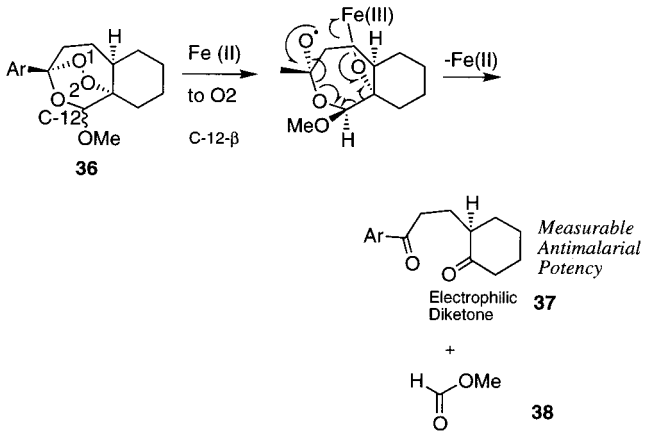
Summary and Conclusion

We have successfully designed and synthesized two new series of artemisinin analogues, some of which display good in vitro and in vivo biological activities. Although the antimalarial activity was not dramatically increased by this approach, the increased metabolic stability of series 2 coupled with the presence of amine functionality opens up the possibility of developing these new derivatives as new, potent, water-soluble analogues that are superior to sodium artesunate. Present work in our labs is focused on examining the cellular accumulation of radiolabeled peroxide analogues and the activity of these lead trioxane analogues against other chloroquine-resistant parasites.

Scheme 4. Biomimetic Fe(II) Degradation Chemistry Structures **30**−**32**



Scheme 5. 1,5-Diketone Generation from a Simplified C-3 Aryltrioxane Analogue of Artemisinin



Having critically assessed this approach to enhancing antimalarial potency, further studies in this area will involve the design and synthesis of artemisinin analogues bearing heme-binding templates. This approach should permit levels of drug to be achieved that are comparable to that observed with 4-aminoquinoline chloroquine analogues that are active against chloroquine-resistant parasites.

Experimental Section

1. Chemistry. Merck Kieselgel 60 F 254 precoated silica plates for TLC were obtained from BDH, Poole, Dorset, U.K. Column chromatography was carried out on Merck 938S silica gel. Infrared (IR) spectra were recorded in the range 4000−600 cm^{-1} using a Perkin-Elmer 298 infrared spectrometer. Spectra of liquids were taken as films. Sodium chloride plates (Nujol mull), solution cells (dichloromethane), and KBr disks were used as indicated.

^1H NMR spectra were recorded using Perkin-Elmer R34 (220 MHz) and Bruker (300 and 200 MHz) spectrometers. Solvents are indicated in the text, and tetramethylsilane was used as the internal reference. Mass spectra were recorded at 70 eV using a VG7070E mass spectrometer. The samples were introduced using a direct-insertion probe. In the text, the parent ion (M^+) is given, followed by peaks corresponding to major fragment losses with intensities in parentheses.

1.1. Synthesis of Ether Derivatives. Dihydroartemisinin (2.00 g, 7.04 mmol) was dissolved in anhydrous diethyl ether (200 mL) under N_2. $BF_3{\cdot}Et_2O$ (1.03 mL, 8.10 mmol) was added to the solution, followed by the appropriate (hydroxymethyl)-benzyl alcohol (1.46 g, 10.56 mmol). The mixture was allowed to stir at room temperature for 20 h, and then the reaction was quenched with water. The organic phase was washed with Na_2SO_4 solution (30% w/v), dried over $MgSO_4$, and filtered, and the solvent was removed under reduced pressure to give the crude product as an oil. Purification by silica gel chromatography using ethyl acetate/*n*-hexane (40/60) as the eluent gave the corresponding ether products.

10*β*-[[4-(Hydroxymethyl)benzyl]oxy]dihydroartemisinin (6c). This compound was prepared using the general procedure in section 1.1 to give the product as a colorless syrup (78% yield): ^1H NMR (300 MHz, $CDCl_3$) δ 7.20−7.08 (4 H, m, aromatic), 5.44 (1 H, s), 4.88 (1 H, d, J = 3.80 Hz), 4.85 (1 H, d, J = 12.19 Hz), 4.69 (2 H, s), 4.51 (1 H, d, J = 12.19 Hz), 2.67 (1 H, sex), 2.38 (1 H, dt, J = 13.46, 3.98 Hz), 2.07−1.20 (10 H, m), 1.46 (3 H, s), and 0.94 (6 H, d, J = 7.17 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 140.20, 137.88, 127.59, 127.05, 104.21, 101.45, 88.09, 81.18, 69.57, 65.11, 52.62, 44.45, 37.43, 36.46, 34.64, 30.94, 26.16, 24.69, 24.52, 20.32, and 13.06; IR (thin film)/cm^{-1} 3476, 2924, 1612, 1516, 1458, 1377, 1194, 1101, 1011, 876 (O−O), and 826 (O−O); MS m/z (CI) $[M + NH_4]^+$ 422 (8), 359 (100), 284 (39), 221 (96), and 138 (33).

10*β*-[[3-(Hydroxymethyl)benzyl]oxy]dihydroartemisinin. This compound was prepared using the general procedure in section 1.1 to give the product as a colorless solid (68% yield): mp 118−120 °C; ^1H NMR (300 MHz, $CDCl_3$) δ 7.34−7.26 (4 H, m, aromatic), 5.40 (1 H, s), 4.92 (1 H, d, J = 3.80 Hz), 4.88 (1 H, d, J = 12.20 Hz), 4.70 (2 H, s), 4.55 (1 H, d, J = 12.20 Hz), 2.67 (1 H, sex), 2.38 (1 H, dt, J = 13.50, 3.80 Hz), 2.07−1.20 (10 H, m), 1.45 (3 H, s), 0.96 (3 H, d, J = 6.00 Hz), and 0.88 (3 H, d, J = 7.60 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 141.05, 138.93, 128.64, 126.80, 126.15, 104.21, 101.65, 88.06, 81.17, 69.90, 65.37, 52.62, 44.47, 37.43, 36.46, 34.65, 30.97, 26.16, 24.70, 24.51, 20.31, and 13.07; IR (Nujol)/cm^{-1} 3507, 2924, 1611, 1462, 1378, 1227, 1192, 1104, 1011, 874 (O−O), and 823 (O−O). Anal. $C_{23}H_{32}O_6$ requires C 68.29%, H 7.97%; found C 68.01%, H 8.14%.

1.2. Synthesis of C-10 Oxo Derivatives. To a solution of the appropriate (hydroxymethyl)benzyl alcohol (0.20 g, 0.50 mmol) in anhydrous DCM (10 mL) under N_2 was added triethylamine (0.08 mL, 0.55 mmol), followed by mesyl chloride (0.06 mL, 0.74 mmol) at 0 °C. The mixture was stirred at 0 °C for 2 h and then quenched with water (10 mL). The organic phase was extracted with DCM (3 × 10 mL) and then dried over $MgSO_4$ and filtered, and the solvent was removed under reduced pressure. The crude mesylate and the appropriate substituted piperazine derivative (4.11 mmol) were dissolved in anhydrous benzene (10 mL) under N_2 atmosphere. The mixture was heated at reflux for 5 h. After cooling to room temperature, the mixture was quenched with saturated $NaHCO_3$ solution and the organic phase was extracted with diethyl ether (3 × 10 mL). The organic extracts were washed with brine, dried over $MgSO_4$, and filtered, and the solvent was removed under reduced pressure. Purification by silica gel chromatography using ethyl acetate/*n*-hexane (40/60) as the eluent gave the corresponding piperazine products.

10*β*-[[4-[Phenylpiperazyl)methyl]benzyl]oxy]dihydroartemisinin (7). This compound was prepared from 1-phenylpiperazine using the general procedure in section 1.2 to give the product as a yellow oil (84% yield): ^1H (300 MHz, $CDCl_3$) δ 7.30−7.28 (4 H, d, J = 4.5 Hz, aromatic), 7.00−6.90 (5 H, m, aromatic), 5.45 (1 H, s), 4.90 (1 H, d, J = 3.80 Hz), 4.86 (1 H, d, J = 12.20 Hz), 4.50 (1 H, d, J = 12.20 Hz), 3.53 (2 H, s, CH_2), 2.69 (1 H, m, CH), 2.51 (8 H, m, CH_2), 2.38 (1 H, dt, J = 13.32, 4.12 Hz), 2.07−1.20 (10 H, m), 1.45 (3 H, s, CH_3), 0.95−0.93 (6 H, 2 x CH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ 129.34, 128.28, 127.19, 104.16, 101.51, 88.08, 81.18, 69.67, 62.98, 62.68, 52.92, 52.66, 44.49, 37.43, 36.49, 34.67, 30.97, 26.19, 24.71, 24.52, 20.31, 13.06; LC/MS (NH_3) m/z 563[M + H^+, (100)], 279 (17).

10*β*-[[4-[(4-Nitrophenylpiperazyl)methyl]benzyl]oxy]-dihydroartemisinin (8). This compound was prepared from 1-(4-nitrophenyl)piperazine using the general procedure in section 1.2 to give the product as an orange solid (68% yield): ^1H NMR (300 MHz, $CDCl_3$) δ 8.12 (2 H, d, J = 9.48 Hz, aromatic), 7.35−7.27 (4 H, m, aromatic), 6.82 (2 H, d, J = 9.48 Hz, aromatic), 5.47 (1 H, s), 4.91 (1 H, d, J = 3.90 Hz), 4.90 (1 H, d, J = 12.50 Hz), 4.53 (1 H, d, J = 12.50 Hz), 3.58 (2 H, s), 3.47−3.42 (4 H, m), 2.68 (1 H, m), 2.64−2.59 (4 H, m), 2.38 (1 H, dt, J = 13.32, 4.12 Hz), 2.07−1.20 (10 H, m), 1.46 (3 H, s), and 0.95 (6 H, d, J = 6.70 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 154.96, 129.17, 127.34, 125.99, 112.70, 104.19, 101.43, 88.09, 81.16, 69.54, 62.61, 52.63, 52.48, 47.08, 44.46, 37.46, 36.47, 34.67, 30.94, 26.18, 24.71, 24.53, 20.31, and 13.06; IR (Nujol)/cm^{-1} 2927, 1597, 1493, 1462, 1377, 1327, 1251, 1231, 1099, 1010, 876 (O−O), and 828 (O−O); MS m/z (EI) $[M]^+$ 593 (1), 264 (98), 218 (34), 104 (100), and 56 (58).

10*β*-[[4-[(4-Fluorophenylpiperazyl)methyl]benzyl]oxy]-dihydroartemisinin (9). This compound was prepared from 1-(4-fluorophenyl)piperazine using the general procedure in section 1.2 to give the product as a yellow oil (64% yield): ^1H NMR (300 MHz, $CDCl_3$) δ 7.35−7.28 (4 H, m, aromatic), 6.99−6.85 (4 H, m, aromatic), 5.46 (1 H, s), 4.91 (1 H, d, J = 3.90 Hz), 4.90 (1 H, d, J = 12.45 Hz), 4.52 (1 H, d, J = 12.45 Hz), 3.58 (2 H, s), 3.16−3.11 (4 H, m), 2.68−2.63 (5 H, m), 2.38 (1 H, dt, J = 13.50, 3.90 Hz), 2.07−1.20 (10 H, m), 1.46 (3 H, s),

0.95 (3 H, d, $J = 6.00$ Hz), and 0.94 (3H, d, $J = 6.60$ Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 141.05, 138.93, 128.64, 126.80, 126.15, 104.21, 101.65, 88.06, 81.17, 69.90, 65.37, 52.62, 44.47, 37.43, 36.46, 34.65, 30.97, 26.16, 24.70, 24.51, 20.10, and 13.07; IR (thin film)/cm^{-1} 2945, 1633, 1510, 1455, 1374, 1359, 1240, 1142, 1099, 1011, 876 (O−O), and 825 (O−O). HRMS (EI) $C_{33}H_{43}FN_2O_5$ $[M]^+$ requires 566.316 51; found 566.314 93.

10β-[[4-[(4-Trifluoromethylphenylpiperazyl)methyl]benzyl]oxy]dihydroartemisinin (10). This compound was prepared from 1-(4-trifluoromethylphenyl)piperazine using the general procedure in section 1.2 to give the product as a yellow oil (64% yield). ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.35−7.28 (5 H, m, aromatic), 7.10−7.03 (3 H, m, aromatic), 5.46 (1 H, s), 4.92 (1 H, d, $J = 12.30$ Hz), 4.88 (1 H, d, $J = 3.80$ Hz), 4.52, (1 H, d, $J = 12.30$ Hz), 3.61 (2 H, m, CH_2), 3.27 (4 H, m, CH_2), 2.70−2.65 (5 H, m, CH_2), 2.39 (1 H, m, CH_2), 2.07−1.20 (10 H, m), 1.45 (3 H, s, CH_3), 0.97−0.94 (6 H, d, 2 × CH_3); ^{13}C NMR (75 MHz, $CDCl_3$) δ 129.59, 129.32, 127.33, 118.78, 112.29, 104.19, 101.50, 88.09, 81.17, 69.61, 62.58, 52.73, 48.55, 44.48, 37.46, 36.48, 34.67, 30.96,26.18, 24.71, 24.54, 20.31, 13.06; IR (thin film)/cm^{-1} (2925), (1454), (1136), (1011); LC/MS (NH_3) *m*/*z* 618 [M + H$^+$, (100)], 603 (100), 333 (7).

10β-[[4-[(Benzylpiperazyl)methyl]benzyl]oxy]dihydroartemisinin (11). This compound was prepared from 1-benzylpiperazine using the general procedure in section 1.2 to give the product as a yellow oil (72% yield): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.32−7.20 (9 H, m, aromatic), 5.45 (1 H, s), 4.91 (1 H, d, $J = 3.90$ Hz), 4.90 (1 H, d, $J = 12.50$ Hz), 4.52 (1 H, d, $J = 12.50$ Hz), 3.54 (4 H, br s), 2.68 (1 H, m), 2.54−2.49 (8 H, m), 2.38 (1 H, dt, $J = 14.10$, 3.90 Hz), 2.07−1.20 (10 H, m), 1.45 (3 H, s), 0.94 (3 H, d, $J = 5.70$ Hz) and 0.94 (3H, d, $J = 7.2$ Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 129.34, 128.28, 127.19, 104.16, 101.51, 88.08, 81.18, 69.67, 62.98, 62.68, 52.92, 52.66, 44.49, 37.43, 36.49, 34.67, 30.97, 26.19, 24.71, 24.52, 20.31, and 13.06; IR (thin film)/cm^{-1} 2938, 1609, 1495, 1457, 1374, 1344, 1227, 1099, 1010, 876 (O−O), and 826 (O−O).

10β-[[3-[(Phenylpiperazyl)methyl]benzyl]oxy]dihydroartemisinin (12). This compound was prepared from 1-phenylpiperazine using the general procedure in section 1.2 to give the product as a brown foam (59% yield): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.30−7.22 (4 H, m, aromatic), 6.94−6.85 (5 H, m, aromatic), 5.47 (1 H, s), 4.91 (1 H, d, $J = 3.70$ Hz), 4.90 (1 H, d, $J = 12.20$ Hz), 4.55 (1 H, d, $J = 12.20$ Hz), 3.59 (2 H, br s), 3.27−3.21 (4 H, m), 2.69−2.61 (5 H, m), 2.38 (1 H, dt, $J = 13.30$, 3.80 Hz), 2.07−1.20 (10 H, m), 1.46 (3 H, s), 0.95 (3 H, d, $J = 7.40$ Hz), and 0.94 (3H, d, $J = 6.00$ Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 139.10, 129.17, 128.38, 116.16, 104.19, 101.40, 88.10, 81.18, 69.68, 52.65, 44.47, 37.45, 36.48, 34.69, 30.96, 26.19, 24.71, 24.53, 20.32, and 13.10; IR (Nujol)/cm^{-1} 2925, 1601, 1504, 1455, 1375, 1228, 1101, 1013, 875 (O−O), and 825 (O−O). HRMS (EI) $C_{33}H_{44}N_2O_5$ $[M]^+$ requires 548.325 01; found 548.326 04.

10β-[[3-[(4-Nitrophenylpiperazyl)methyl]benzyl]oxy]dihydroartemisinin (13). This compound was prepared from 1-(4-nitrophenyl)piperazine using the general procedure in section 1.2 to give the product as an orange foam (85% yield): ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.13 (2 H, d, $J = 9.50$ Hz, aromatic), 7.36−7.25 (4 H, m, aromatic), 6.82 (2 H, d, $J = 9.50$ Hz, aromatic), 5.47 (1 H, s), 4.91 (1 H, d, $J = 5.00$ Hz), 4.90 (1 H, d, $J = 12.30$ Hz), 4.56 (1 H, d, $J = 12.30$ Hz), 3.61 (2 H, s), 3.49−3.43 (4 H, m), 2.71 (1 H, m), 2.69−2.63 (4 H, m), 2.39 (1 H, dt, $J = 13.87$, 3.98 Hz), 2.07−1.20 (10 H, m), 1.45 (3 H, s), and 0.94 (6H, d, $J = 7.40$ Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 151.00, 128.50, 128.00, 125.99, 112.79, 104.00, 101.00, 88.09, 81.50, 69.56, 60.38, 52.61, 52.20, 46.96, 44.20, 37.47, 36.45, 34.50, 30.93, 26.00, 24.70, 24.55, 20.32, and 13.00; IR (Nujol)/cm^{-1} 2923, 1598, 1506, 1456, 1378, 1328, 1248, 1099, 1010, 875 (O−O), and 826 (O−O); MS *m*/*z* (EI) $[M]^+$ 593 (1), 264 (43), 219 (18), 105 (100), and 56 (30).

10β-[[3-[(4-Chlorophenylpiperazyl)methyl]benzyl]oxy]dihydroartemisinin (14). This compound was prepared from 1-(4-chlorophenyl)piperazine using the general procedure in section 1.2 to give the product as a brown foam (64% yield): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.32−7.06 (6 H, m, aromatic), 6.83 (2 H, d, $J = 9.06$, aromatic), 5.47 (1 H, s), 4.92 (1 H, d, $J = 4.10$ Hz), 4.90 (1 H, d, $J = 12.01$ Hz), 4.55 (1 H, d, $J = 12.01$ Hz), 3.61 (2 H, br s), 3.22−3.17 (4 H, m), 2.69−2.61 (5 H, m), 2.39 (1 H, dt, $J = 13.50$, 3.85 Hz), 2.07−1.20 (10 H, m), 1.46 (3 H, s), and 0.94 (3H, d, $J = 6.60$ Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 138.50, 129.03, 128.44, 117.35, 104.19, 101.37, 88.09, 81.17, 69.64, 52.81, 49.03, 44.45, 37.45, 36.47, 34.68, 30.94, 26.19, 24.71, 24.53, 20.32, and 13.10; IR (Nujol)/cm^{-1} 2927, 1616, 1496, 1459, 1378, 1225, 1102, 1032, 874 (O−O), and 815 (O−O); MS *m*/*z* (EI) $[M]^+$ 422 (1), 300 (26), 193 (19), 131 (14), and 105 (100).

10β-[[3-[(3-Trifluoromethylphenylpiperazyl)methyl]benzyl]oxy]dihydroartemisinin (15). This compound was prepared from 1-[(3-trifluoromethyl)phenyl]piperazine using the general procedure in section 1.2 to give the product as a brown foam (66% yield): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.37−7.23 (4 H, m, aromatic), 7.10−7.06 (4 H, m, aromatic), 5.47 (1 H, s), 4.92 (1 H, d, $J = 3.98$ Hz), 4.90 (1 H, d, $J = 12.16$ Hz), 4.52 (1 H, d, $J = 12.16$ Hz), 3.58 (2 H, s), 3.16−3.11 (4 H, m), 2.68−2.63 (5 H, m), 2.38 (1 H, dt, $J = 13.48$, 4.02 Hz), 2.07−1.20 (10 H, m), 1.45 (3 H, s), 0.95 (3 H, d, $J = 7.20$ Hz), and 0.94 (3H, d, $J = 6.00$ Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 151.49, 138.65, 129.59, 128.40, 118.74, 115.81, 112.22, 104.19, 101.38, 88.09, 81.17, 69.68, 62.81, 52.80, 52.64, 48.64, 44.46, 37.46, 36.46, 34.69, 30.95, 26.19, 24.71, 24.53, 20.31, and 13.10; IR (Nujol)/cm^{-1} 2921, 1612, 1496, 1454, 1350, 1228, 1120, 1011, 875 (O−O), and 826 (O−O); MS *m*/*z* (EI) $[M]^+$ 616 (1), 334 (11), 227 (7), 105 (100), and 56 (10).

10β-[[3-[(4-Fluorophenylpiperazyl)methyl]benzyl]oxy]dihydroartemisinin (16). This compound was prepared from 1-(4-fluorophenyl)piperazine using the general procedure in section 1.2 to give the product as an off-white foam (69% yield): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.36−7.27 (4 H, m, aromatic), 6.98−6.84 (4 H, m, aromatic), 5.47 (1 H, s), 4.91 (1 H, d, $J = 3.43$ Hz), 4.90 (1 H, d, $J = 12.29$ Hz), 4.54 (1 H, d, $J = 12.29$ Hz), 3.58 (2 H, s), 3.12 (4 H, t, $J = 4.81$ Hz), 2.63 (1 H, m), 2.62 (4 H, t, $J = 13.80$ Hz), 2.38 (1 H, dt, $J = 13.40$, 3.90 Hz), 2.07−1.20 (10 H, m), 1.45 (3 H, s), 0.94 (3 H, d, $J = 6.00$ Hz), and 0.93 (3 H, d, $J = 6.00$ Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 148.04, 138.62, 128.40, 126.34, 117.89, 115.70, 115.40, 104.19, 101.36, 88.09, 81.18, 69.65, 62.82, 52.99, 52.64, 50.07, 44.46, 37.45, 36.47, 34.69, 30.95, 26.19, 24.71, 24.53, 20.32, and 13.10; IR (Nujol)/cm^{-1} 2924, 1510, 1460, 1377, 1229, 1160, 1102, 1012, 875 (O−O), and 826 (O−O). HRMS (EI) $C_{33}H_{43}FN_2O_5$ $[M]^+$ requires 566.315 61; found 566.314 37. Anal. $C_{33}H_{43}FN_2O_5$ requires C 69.97%, H 7.60%, N 4.97%; found C 69.67%, H 7.72%, N 4.82%.

Dihydroartemisinin 10α-Benzoate (20). Benzoyl chloride (3.17 mL, 27.29 mmol) was added to a solution of dihydroartemisinin (5.00 g, 17.61 mmol) in anhydrous DCM (54 mL) and anhydrous pyridine (9 mL) at 0 °C. The mixture was allowed to stir at room temperature for 16 h. The reaction mixture was then partitioned between 7% citric acid (50 mL) and ethyl acetate (2 × 50 mL). The combined organic extracts were washed with 7% citric acid, saturated $NaHCO_3$, and H_2O. The organic phase was then dried over $MgSO_4$, filtered, and concentrated under reduced pressure to afford a crude product that was recrystallized from a small volume of an ether/hexane mixture to give the product as a white crystalline solid (100% yield). Further product was obtained by purification of the mother liquors by silica gel chromatography using ethyl acetate/*n*-hexane (10/90) as the eluent: mp 111−112 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.13 (2 H, m, aromatic), 7.57 (1 H, m, aromatic), 7.45 (2 H, m, aromatic), 6.02 (1 H, d, $J = 9.89$ Hz), 5.53 (1 H, s), 2.76 (1 H, m), 2.40 (1 H, dt, $J = 13.99$, 4.26 Hz), 2.07−1.20 (10 H, m), 1.43 (3 H, s), 0.99 (3 H, d, $J = 5.91$ Hz), and 0.93 (3 H, d, $J = 7.14$ Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 165.41, 133.34, 130.20, 129.79, 128.36, 104.49, 92.62, 91.65, 80.23, 51.73, 45.42, 37.32, 36.32, 34.18, 32.03, 25.94, 24.62, 22.09, 20.22, 15.24, and 12.20; IR (Nujol)/cm^{-1} 2924, 1738, 1491, 1452, 1377, 1272, 1114, 1100, 1037, 877 (O−O), and 831 (O−O). HRMS (CI) $C_{23}H_{32}NO_6$ $[M + NH_4]^+$ requires 406.222 96; found 406.222 70. Anal. $C_{22}H_{28}O_6$ requires C 68.04%, H 7.22%; found C 68.17%, H 7.32%.

10β-Allyldeoxoartemisinin. A solution of dihydroartemisinin 10α-benzoate (7.02 g, 18.08 mmol) in anhydrous 1,2-dichloroethane (70 mL) was added via cannula to a mixture of allyltrimethylsilane (13.80 mL, 86.79 mmol) and $ZnCl_2$ (2.96 g, 21.70 mmol) in anhydrous 1,2-dichloroethane (70 mL), which was stirred over activated 4 Å molecular sieves under N_2 at 0 °C. The mixture was stirred at 0 °C for 1 h and then allowed to warm to room temperature. The mixture was diluted with ethyl acetate and washed with 5% citric acid solution, saturated $NaHCO_3$ solution, and brine. The organic layer was dried over $MgSO_4$, filtered, and concentrated under reduced pressure. The crude product was purified by silica gel chromatography using ethyl acetate/*n*-hexane (10/90) as the eluent to give the product as a white solid (90% yield): mp 76−78 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.93 (1 H, m), 5.33 (1 H, s), 5.12 (2 H, m), 4.31 (1 H, m), 2.68 (1 H, sex), 2.45−2.17 (3 H, m), 2.07−1.20 (10 H, m), 1.41 (3 H, s), 0.96 (3 H, d, J = 5.90 Hz), and 0.89 (3 H, d, J = 7.56 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 136.54, 116.06, 103.12, 89.24, 81.10, 74.58, 52.40, 44.36, 37.51, 36.66, 34.53, 34.31, 30.25, 26.07, 24.91, 24.74, 20.14, and 12.87; IR (Nujol)/cm^{-1} 2931, 1642, 1454, 1376, 1278, 1105, 1041, 879 (O−O), and 840 (O−O). HRMS (EI) $C_{18}H_{28}O_4$ $[M]^+$ requires 308.198 76; found 308.199 67. Anal. $C_{18}H_{28}O_4$ requires C 70.09%, H 9.15%; found C 69.73%, H 9.12%.

10β-(2-Hydroxyethyl)deoxoartemisinin. A solution of 10β-allyldeoxoartemisinin (0.30 g, 0.97 mmol) in anhydrous DCM (100 mL) was flushed with N_2 and then subjected to a steady stream of O_3 at −78 °C until the solution became saturated with O_3 and appeared blue. Residual O_3 was flushed from the solution with N_2, and the solvent was removed under reduced pressure. THF/methanol (100 mL, 9:1) was added, and the solution was treated with excess sodium borohydride (2.00 g) at 0 °C for 4 h. The resulting mixture was concentrated under reduced pressure, followed by addition of water and $CHCl_3$. The organic layer was dried over $MgSO_4$ and filtered, and the solvent was removed under reduced pressure. Purification by silica gel chromatography using ethyl acetate/*n*-hexane (40/60) as the eluent gave the product as a white solid (69% yield): mp 104−106 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.36 (1 H, s), 4.46 (1 H, m), 3.84 (2 H, m), 2.67 (1 H, sex), 2.57 (1 H, m), 2.33 (1 H, dt, J = 13.82, 3.88 Hz), 2.07−1.20 (12 H, m), 1.41 (3 H, s), 0.97 (3 H, d, J = 5.90 Hz), and 0.88 (3 H, d, J = 7.55 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 103.21, 89.28, 81.05, 74.96, 62.69, 52.29, 44.14, 37.50, 36.54, 34.43, 31.69, 30.33, 26.01, 24.74, 20.12, and 12.83; IR (Nujol)/cm^{-1} 3510, 2920, 1462, 1378, 1273, 1093, 1046, 881 (O−O), and 837 (O−O). HRMS (EI) $C_{17}H_{28}O_5$ $[M]^+$ requires 312.193 66; found 312.193 41. Anal. $C_{17}H_{28}O_5$ requires C 65.39%, H 9.04%; found C 65.17%, H 9.08%.

10β-(2-Methanesulfonylethyl)deoxoartemisinin. To a solution of 10β-(2-hydroxyethyl)deoxoartemisinin (0.19 g, 0.59 mmol) in anhydrous DCM (10 mL) under N_2 was added triethylamine (0.09 mL, 0.65 mmol), followed by mesyl chloride (0.07 mL, 0.89 mmol) at 0 °C. The mixture was stirred at 0 °C for 2 h and then quenched with water (10 mL). The organic phase was extracted with DCM (3 × 10 mL) and then dried over $MgSO_4$ and filtered, and the solvent was removed under reduced pressure. Purification by silica gel chromatography using ethyl acetate/*n*-hexane (40/60) as the eluent gave the product as a white crystalline solid (97% yield): mp 123−125 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.31 (1 H, s), 4.47−4.32 (3 H, m), 3.03 (3 H, s), 2.69 (1 H, sex), 2.33 (1 H, dt, J = 13.87, 3.84 Hz), 2.07−1.20 (12 H, m), 1.41 (3 H, s), 0.97 (3 H, d, J = 5.77 Hz), and 0.88 (3 H, d, J = 7.56 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 103.20, 89.24, 80.99, 70.85, 68.37, 52.23, 44.13, 37.29, 36.54, 34.40, 29.90, 29.64, 25.96, 24.79, 24.73, 20.08, and 12.72; IR (Nujol)/cm^{-1} 2926, 1462, 1378, 1350, 1279, 1167, 1100, 1037, 876 (O−O), and 824 (O−O). HRMS (EI) $C_{18}H_{30}O_7S$ $[M]^+$ requires 390.171 23; found 390.171 27. Anal. $C_{18}H_{30}O_7S$ requires C 55.39%, H 7.74%; found C 55.11%, H 7.81%.

1.3. Synthesis of C-10 Carba Derivatives. 10β-(2-Methanesulfonylethyl)deoxoartemisinin (0.20 g, 0.51 mmol) and the appropriate amine derivative (4.26 mmol) were dissolved in anhydrous benzene (10 mL) under N_2 atmosphere. The mixture was heated at 80 °C for 10 h. After cooling to room temperature, the mixture was quenched with saturated $NaHCO_3$ solution and the organic phase was extracted with diethyl ether (3 × 10 mL). The organic extracts were washed with brine, dried over $MgSO_4$, and filtered, and the solvent was removed under reduced pressure. Purification by silica gel chromatography using ethyl acetate/*n*-hexane (40/60) as the eluent gave the corresponding piperazine products.

10β-[2-(4-Chlorophenylpiperazyl)ethyl]deoxoartemisinin (21). This compound was prepared from 1-(4-chlorophenyl)piperazine using the general procedure in section 1.3 to give the product as an off-white foam (74% yield): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.20 (2 H, d, J = 9.00 Hz, aromatic), 6.83 (2 H, d, J = 9.00 Hz, aromatic), 5.33 (1 H, s), 4.20 (1 H, m), 3.19−3.13 (4 H, m), 2.75 (2 H, m), 2.68−2.62 (4 H, m), 2.44 (1 H, m), 2.33 (1 H, dt, J = 13.50, 3.90 Hz), 2.07−1.20 (12 H, m), 1.42 (3 H, s), 0.96 (3 H, d, J = 6.00 Hz), and 0.88 (3 H, d, J = 7.50 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 150.02, 128.99, 124.54, 117.23, 117.85, 103.33, 88.99, 81.11, 74.10, 56.86, 53.19, 52.46, 49.11, 44.48, 37.45, 36.59, 34.49, 30.23, 26.78, 26.16, 24.71, 20.19, and 13.14; IR (Nujol)/cm^{-1} 2926, 1597, 1456, 1377, 1301, 1229, 1116, 1041, 888 (O−O), 816 (O−O), 721. HRMS (EI) $C_{27}H_{39}ClN_2O_4$ (EI) $[M]^+$ requires 490.259 83; found 490.259 09.

10β-[2-(3-Trifluoromethylphenylpiperazyl)ethyl]deoxoartemisinin (22). This compound was prepared from 1-[(3-trifluoromethyl)phenyl]piperazine using the general procedure in section 1.3 to give the product as a white foam (74% yield): ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.34 (1 H, m, aromatic), 7.11−7.03 (3 H, m, aromatic), 5.33 (1 H, s), 4.22 (1 H, m), 3.25 (4 H, t, J = 5.01 Hz), 2.76 (2 H, m), 2.64 (4 H, t, J = 5.01 Hz), 2.43 (1 H, m), 2.33 (1 H, dt, J = 13.50, 3.90 Hz), 2.07−1.20 (12 H, m), 1.43 (3 H, s), 0.96 (3 H, d, J = 5.90 Hz), and 0.89 (3 H, d, J = 7.55 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 151.50, 129.56, 118.63, 115.78, 112.16, 103.35, 89.00, 81.12, 74.09, 56.84, 53.16, 52.48, 48.66, 44.49, 37.47, 36.60, 34.50, 30.24, 26.86, 26.16, 24.71, 20.19, and 13.14; IR (Nujol)/cm^{-1} 2926, 1611, 1459, 1377, 1311, 1279, 1238, 1128, 1041, 876 (O−O), and 827 (O−O). HRMS (EI) $C_{28}H_{39}F_3N_2O_4$ $[M]^+$ requires 524.286 19; found 524.285 28. Anal. $C_{28}H_{39}F_3N_2O_4$ requires C 64.12%, H 7.49%, N 5.34%; found C 63.89%, H 7.48%, N 5.25.

10β-[2-(Phenylpiperazyl)ethyl]deoxoartemisinin (23). This compound was prepared, using the general procedure in section 1.3, from 1-phenylpiperazine to give the product as a white foam (84% yield): ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.94−6.85 (5 H, m, aromatic), 5.33 (1 H, s), 4.18 (1 H, m), 3.21 (4 H, t), 2.75 (2 H, m), 2.64 (4 H, t), 2.37 (2 H, m), 2.07−1.20 (12 H, m), 1.42 (3 H, s), 0.99 (3 H, d, J = 5.78 Hz), and 0.88 (3 H, d, J = 7.68 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 151.44, 129.35, 129.15, 119.68, 116.07, 103.36, 88.95, 81.12, 74.29, 60.00, 57.01, 53.41, 52.51, 49.16, 44.53, 37.45, 36.60, 34.50, 30.24, 26.83, 26.17, 24.78, 24.71, 20.20, and 13.20; IR (Nujol)/cm^{-1} 2951, 1600, 1455, 1377, 1280, 1238, 1093, 1040, 876 (O−O), and 827 (O−O). HRMS (EI) $C_{27}H_{40}N_2O_4$ $[M]^+$ requires 456.298 77; found 456.297 46.

10β-[2-(4-Nitrophenylpiperazyl)ethyl]deoxoartemisinin (24). This compound was prepared from 1-(4-nitrophenyl)piperazine using the general procedure in section 1.3 to give the product as an orange foam (74% yield): ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.11 (2 H, d, J = 9.3 Hz, aromatic), 6.81 (2 H, d, J = 9.3 Hz, aromatic), 5.32 (1 H, s), 4.22 (1 H, m), 3.42 (4 H, t, J = 5.10 Hz), 2.73 (2 H, m), 2.62 (4 H, t, J = 5.10 Hz), 2.42 (1 H, m), 2.31 (1 H, dt, J = 13.50, 4.20 Hz), 2.07−1.20 (12 H, m), 1.41 (3 H, s), 0.96 (3 H, d, J = 6.00 Hz), and 0.88 (3 H, d, J = 7.50 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 154.97, 138.56, 125.99, 112.67, 103.33, 89.07, 81.11, 73.79, 56.63, 52.81, 52.41, 47.08, 44.40, 37.49, 36.58, 34.47, 30.22, 26.89, 26.14, 24.80, 24.72, 20.17, and 13.08; IR (Nujol)/cm^{-1} 2928, 1599, 1459, 1377, 1334, 1244, 1114, 1038, 876 (O−O), and 825 (O−O). HRMS (EI) $C_{27}H_{39}N_3O_6$ $[M]^+$ requires 501.283 87; found 501.283 57.

10β-[2-(4-Fluorophenylpiperazyl)ethyl]deoxoartemisinin (25). This compound was prepared, using the general procedure in section 1.3, from 1-(4-fluorophenyl)piperazine to give the product as a white foam (79% yield): ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.99−6.85 (4 H, m, aromatic), 5.33 (1 H, s), 4.20 (1 H, m), 3.13 (4 H, t, J = 5.02 Hz), 2.76 (2 H, m), 2.64 (4 H, t, J = 5.02 Hz), 2.43 (1 H, m), 2.34 (1 H, dt, J = 13.46, 3.91 Hz), 2.07−1.20 (12 H, m), 1.42 (3 H, s), 0.96 (3 H, d, J = 6.04 Hz), and 0.88 (3 H, d, J = 7.56 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 159.11, 155.66, 152.86, 148.12, 117.85, 117.75, 115.67, 115.37, 103.36, 88.97, 81.12, 74.22, 56.92, 53.39, 52.49, 50.19, 44.51, 37.46, 36.60, 34.50, 30.25, 26.85, 26.16, 24.71, 20.19, and 13.16; IR (Nujol)/cm^{-1} 2953, 1616, 1455, 1377, 1298, 1279, 1229, 1093, 1040, 874 (O−O), and 824 (O−O). HRMS (EI) $C_{27}H_{39}FN_2O_4$ (EI) $[M]^+$ required 474.28937; found 474.29030. Anal. $C_{27}H_{39}FN_2O_4$ required C 68.35%, H 8.28%, N 5.90%; found C 68.48%, H 8.37%, N 5.90%.

10β-[2-(Benzylpiperazyl)ethyl]deoxoartemisinin (26). This compound was prepared from 1-benzylpiperazine using the general procedure in section 1.3 to give the product as an off-white solid (70% yield): mp 107−109 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.31−7.21 (5 H, m, aromatic), 5.31 (1 H, s), 4.13 (1 H, m), 3.51 (2 H, s), 2.73 (2 H, m), 2.56−2.36 (8 H, m), 2.33 (2 H, m), 2.07−1.20 (12 H, m), 1.41 (3 H, s), 0.95 (3 H, d, J = 5.90 Hz), and 0.86 (3 H, d, J = 7.55 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 138.20, 129.24, 128.22, 127.04, 103.32, 88.86, 81.08, 74.40, 63.05, 56.10, 53.20, 53.04, 52.50, 44.52, 37.40, 36.59, 34.49, 30.21, 26.69, 26.14, 24.73, 24.68, 20.20, and 13.18; IR (Nujol)/cm^{-1} 2926, 1614, 1455, 1373, 1316, 1277, 1219, 1099, 1011, 883 (O−O), and 836 (O−O). HRMS (EI) $C_{28}H_{42}N_2O_4$ $[M]^+$ requires 470.314 45; found 470.313 56.

10β-[2-(*N*-Morpholino)ethyl]deoxoartemisinin (27). This compound was prepared, using the general procedure in section 1.3, from morpholine to give the product as a yellow foam (63% yield): ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.32 (1 H, s), 4.18 (1 H, m), 3.72 (4 H, t, J = 4.67 Hz), 2.71 (2 H, m), 2.48 (4 H, t, J = 4.67 Hz), 2.41−2.28 (3 H, m), 2.07−1.20 (11 H, m), 1.42 (3 H, s), 0.96 (3 H, d, J = 6.04 Hz), and 0.88 (3 H, d, J = 7.56 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 103.35, 88.93, 81.09, 74.08, 66.97, 57.30, 54.98, 53.83, 52.42, 44.42, 37.38, 36.52, 34.42, 30.14, 26.43, 26.06, 24.68, 24.62, 20.10, and 13.04; IR (Nujol)/cm^{-1} 2953, 1616, 1455, 1377, 1298, 1279, 1229, 1093, 1040, 874 (O−O), and 824 (O−O). HRMS (EI) $C_{27}H_{39}FN_2O_4$ (EI) $[M]^+$ required 474.28937; found 474.29030. Anal. $C_{27}H_{39}FN_2O_4$ required C 68.35%, H 8.28%, N 5.90%; found C 68.48%, H 8.37%, N 5.90%.

10β-[2-(Piperidinyl)ethyl]deoxoartemisinin (28). This compound was prepared, using the general procedure in section 1.3, from piperidine to give the product as a yellow foam (66% yield): ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.33 (1 H, s), 4.10 (1 H, m), 2.73 (2 H, m), 2.44 (4 H, m), 2.37−2.26 (3 H, m), 2.07−1.20 (17 H, m), 1.42 (3 H, s), 0.95 (3 H, d, J = 6.01 Hz), and 0.88 (3 H, d, J = 7.53 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 103.36, 89.10, 81.50, 75.29, 58.33, 55.10, 52.90, 44.94, 37.76, 36.94, 34.87, 30.59, 26.90, 26.59, 26.18, 25.11, 24.71, 23.05, 20.66, and 13.72; IR (Nujol)/cm^{-1} 2953, 1616, 1455, 1377, 1298, 1279, 1229, 1093, 1040, 874 (O−O), and 824 (O−O). HRMS (EI) $C_{27}H_{39}FN_2O_4$ (EI) $[M]^+$ required 474.28937; found 474.29030. Anal. $C_{27}H_{39}FN_2O_4$ required C 68.35%, H 8.28%, N 5.90%; found C 68.48%, H 8.37%, N 5.90%.

10β-[2-(Di-*N*-propylamino)ethyl]deoxoartemisinin (29). This compound was prepared using the general procedure in section 1.3 to give the product as a yellow foam (69% yield): ^{1}H NMR (300 MHz, $CDCl_3$) δ 5.33 (1 H, s), 4.15 (1 H, m), 2.88 (1 H, m), 2.68 (1 H, m), 2.55−2.40 (2 H, m), 2.54 (4 H, t, J = 7.50 Hz), 2.33 (1 H, dt, J = 14.70, 3.90 Hz), 2.07−1.20 (15 H, m), 1.41 (3 H, s), 0.96 (3 H, d, J = 6.30 Hz), 0.90 (6 H, t), and 0.88 (3 H, d, J = 7.50 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 103.25, 89.10, 81.15, 73.93, 55.82, 52.73, 52.46, 44.43, 37.39, 36.60, 34.50, 30.23, 26.63, 26.13, 24.76, 24.68, 20.19, 19.30, 13.05, and 11.79.

$FeCl_2$-Mediated Degradation of 10β-[2-(Phenylpiperazyl)ethyl]deoxoartemisinin (33). To a room-temperature stirring solution of 10β-[2-(phenylpiperazyl)ethyl]deoxoartemisinin (**23**) (175 mg, 0.32 mmol) in CH_3CN (10 mL) under N_2 atmosphere was added $FeCl_2{\cdot}4H_2O$ (63.5 g, 0.32 mmol). After 30 min, the reaction mixture was filtered over Celite and the filtrate concentrated under reduced pressure. Purification by silica gel chromatography using ethyl acetate as the eluent gave the corresponding formate derivative (**33**) as an orange oil (84.7 mg, 48% yield): ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.09 (1 H, s, *H*C=O), 7.29−7.20 (2 H, m, aromatic), 6.94−6.86 (3 H, m, aromatic), 5.20 (1 H, s), 3.21 (4 H, m), 2.76 (4 H, m), 2.55 (1 H, m), 2.36 (1 H, m), 2.12 (3 H, s), 2.06−1.20 (14 H, m), 1.06 (3 H, d, J = 6.00 Hz), and 0.98 (3 H, d, J = 6.90 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 213.33, 209.05, 161.06, 150.98, 129.23, 120.21, 116.35, 72.71, 57.43, 54.53, 54.41, 52.91, 48.66, 41.42, 41.21, 34.93, 34.71, 30.88, 29.83, 29.68, 29.20, 20.46, 20.32, and 12.46; IR (thin film)/cm^{-1} 2925, 1716, 1600, 1496, 1459, 1379, 1242, 1188, 1033, and 993; MS m/z (EI) $[M]^+$ 456 (2), 411 (8), 175 (100), 70 (14), and 43 (15).

$FeCl_2$-Mediated Degradation of 10β-[[4-(Hydroxymethyl)benzyl]oxy]dihydroartemisinin. To a room-temperature stirring solution of 10β-[[4-(hydroxymethyl)benzyl]oxy]dihydroartemisinin (2.80 g, 6.93 mmol) in CH_3CN (100 mL) under N_2 atmosphere was added $FeCl_2{\cdot}4H_2O$ (1.38 g, 6.93 mmol). After 30 min, the reaction mixture was filtered over Celite and the filtrate concentrated under reduced pressure. Purification by silica gel chromatography using ethyl acetate/*n*-hexane (40/60) as the eluent gave the corresponding ring-contracted THF acetate (1.33 g, 47% yield) and hydroxydeoxo derivatives (0.32 g, 11% yield) as white crystalline solids. Ring-contracted THF acetate (**34**): mp 100−103 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.35−7.28 (4 H, m, aromatic), 6.34 (1 H, s), 4.94 (1 H, d, J = 12.00 Hz), 4.80 (1 H, d, J = 4.50 Hz), 4.68 (2 H, s), 4.46 (1 H, d, J = 12.00 Hz), 4.28 (1 H, dt, J = 7.50, 2.40 Hz), 3.92 (1 H, q, J = 8.10 Hz), 2.43 (1 H, m), 2.15 (3 H, s), 2.06−1.20 (9 H, m), 0.93 (3 H, d, J = 6.30 Hz), and 0.92 (3 H, d, J = 7.50 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 169.42, 140.17, 137.79, 128.25, 127.02, 101.00, 88.51, 80.61, 69.31, 68.62, 65.24, 55.74, 47.02, 35.87, 33.35, 30.63, 27.75, 24.81, 21.59, 20.47, and 12.46; IR (Nujol)/cm^{-1} 3493, 2922, 1746, 1506, 1456, 1374, 1220, 1071, 1011, and 994. HRMS (CI) $C_{23}H_{36}NO_6$ $[M + NH_4]^+$ requires 422.254 26; found 422.253 93. Anal. $C_{23}H_{32}O_6$ requires C 68.32%, H 7.97%; found C 68.14%, H 8.07%. Hydroxydeoxo derivative (**35**): mp 91−94 °C; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.36−7.28 (4 H, m, aromatic), 5.28 (1 H, s), 4.84 (1 H, d, J = 4.39 Hz), 4.83 (1 H, d, J = 12.22 Hz), 4.68 (2 H, s), 4.50 (1 H, d, J = 12.50 Hz), 3.56 (2 H, m), 2.48 (1 H, m), 2.06−1.20 (9 H, m), 1.53 (3 H, s), 0.96 (3 H, d, J = 7.42 Hz), and 0.86 (3 H, d, J = 6.46 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 140.23, 137.97, 127.73, 127.06, 108.03, 99.36, 93.73, 84.23, 69.62, 65.08, 65.02, 42.51, 40.65, 35.01, 34.77, 30.41, 30.27, 24.98, 20.88, 18.43, and 12.34; IR (KBr)/cm^{-1} 3334, 2920, 1618, 1518, 1466, 1384, 1213, 1124, and 1013. HRMS (EI) $C_{23}H_{36}NO_6$ $[M + NH_4]^+$ requires 422.254 26; found 422.253 54. Anal. $C_{23}H_{32}O_6$ requires C 68.32%, H 7.97%; found C 68.33%, H 8.00%.

2. Biological Testing. Antimalarial Activity. Two strains of *P. falciparum* were used in this study: (a) the uncloned K1 strain, which is known to be chloroquine resistant, and (b) the HB3 strain, which is sensitive to chloroquine. Parasites were maintained in continuous culture using the method of Jensen and Trager.[29] Cultures were grown in flasks containing human erythrocytes (2−5%), with parasitemia in the range 1−10% suspended in RPMI 1640 medium supplemented with 25 mM HEPES and 32 mM $NaHCO_3$, and 10% human serum (complete medium). Cultures were gassed with a mixture of 3% O_2, 4% CO_2, and 93% N_2.

2.1. In Vitro Testing. Antimalarial activity was assessed with an adaptation of the 48 h sensitivity assay of Desjardins et al.[30] using [^{3}H]-hypoxanthine incorporation as an assessment of parasite growth. Stock drug solutions were prepared in 100% dimethylsulfoxide (DMSO) and diluted to the appropriate concentration using complete medium. Assays were performed in sterile 96-well microtiter plates; each plate contained 200 μL of parasite culture (2% parasitemia, 0.5% haematocrit) with or without 10 μL drug dilutions. Each drug

was tested in triplicate and parasite growth compared to control wells (which constituted 100% parasite growth). After 24 h of incubation at 37 °C, 0.5 μCi hypoxanthine was added to each well. Cultures were incubated for a further 24 h before they were harvested onto filter mats, dried for 1 h at 55 °C, and counted using a Wallac 1450 Microbeta Trilux liquid scintillation and luminescence counter. IC_{50} values were calculated by interpolation of the probit transformation of the logarithm of the dose−response curve.

2.2. In Vivo Testing. Male, random Swiss albino mice weighing 18−22 g were inoculated ip with 10^7 parasitized erythrocytes with *P. berghei* NS strain. Animals were then dosed daily via two routes (intraperitroneal or oral) for four consecutive days beginning on the day of infection. Compounds were dissolved or suspended in the vehicle solution consisting of methanol, phosphate-buffered saline, and DMSO (2:5:3 v/v). The parasitemia was determined on the day following the last treatment and the ED_{50} (50% suppression of parasites when compared to vehicle-only treated controls) calculated from a plot of the logarithm of dose against parasitemia. Data recorded versus *P. yoelii* were carried out in the laboratories of Professor Wallace Peter under WHO sponsorship.

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