

## Novel, Potent, Semisynthetic Antimalarial Carba Analogues of the First-Generation 1,2,4-Trioxane Artemether

Paul M. O'Neill,<sup>\*,†</sup> Natalie L. Searle,<sup>†</sup> Ka-Wing Kan, Richard C. Storr,<sup>†</sup> James L. Maggs, Stephen A. Ward, Kaylene Raynes, and B. Kevin Park

Departments of Chemistry and of Pharmacology and Therapeutics, The University of Liverpool, P.O. Box 147, Liverpool L69 7ZD, U.K.

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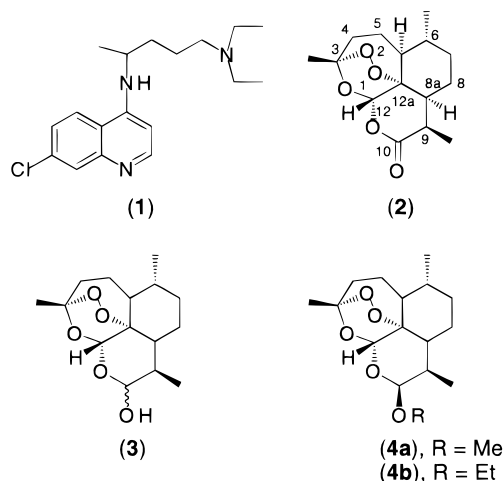
Ten novel, second-generation, fluorinated ether and ester analogues of the potent first-generation analogues artemether (**4a**) and arteether (**4b**) have been designed and synthesized. All of the compounds demonstrate high antimalarial potency in vitro against the chloroquine-sensitive HB3 and -resistant K1 strains of *Plasmodium falciparum*. The most potent derivative **8** was 15 times more potent than artemisinin (**2**) against the HB3 strain of *P. falciparum*. In vivo, versus *Plasmodium berghei* in the mouse, selected derivatives were generally less potent than dihydroartemisinin with ED<sub>50</sub> values of between 5 and 8 mg/kg. On the basis of the products obtained from the in vitro biomimetic Fe(II)-mediated decomposition of **8**, the radical mediator of biological activity of this series may be different from that of the parent drug, artemisinin (**2**).

### Introduction

Despite efforts to eradicate malaria, the disease still affects approximately 2 billion people per year. The development of drug resistance by many strains of *Plasmodium falciparum* to the traditional alkaloid drugs chloroquine (**1**) (Chart 1) and quinine has enhanced the prevalence of the disease throughout the world.<sup>1</sup> The frightening spread of parasite resistance has led the WHO to predict that without new antimalarial drug intervention, the number of cases of malaria will have doubled by the year 2010.<sup>2</sup> Artemisinin (**2**) (qinghaosu) is an unusual 1,2,4-trioxane which has been used clinically in China for the treatment of multidrug-resistant *P. falciparum* malaria. Reduction of artemisinin to dihydroartemisinin (**3**) (DHA) has led to the preparation of a series of semisynthetic first-generation analogues which include artemether (**4a**, R = Me) and arteether (**4b**, R = Et).

However poor bioavailability and rapid clearance are observed with these analogues, principally as a result of the poor chemical and metabolic instability of the acetal function present in these derivatives. One of the principal routes of metabolism of artemether, for example, involves oxidative dealkylation to DHA, a compound associated with toxicity<sup>3</sup> and short half-life.<sup>4</sup> Replacement of the oxygen at the C-10 position with carbon would be expected to produce compounds not only with greater hydrolytic stability but also with a longer half-life and potentially lower toxicity. Consequently, several groups have developed synthetic and semisynthetic approaches to C-10 carba analogues.<sup>5–8</sup> In this paper, we report for the first time the synthesis and in vitro biological activity of a new series of carba ether (**8–12**) and ester (**13–17**) analogues. In addition, we also describe details of the biomimetic Fe(II)-medi-

Chart 1



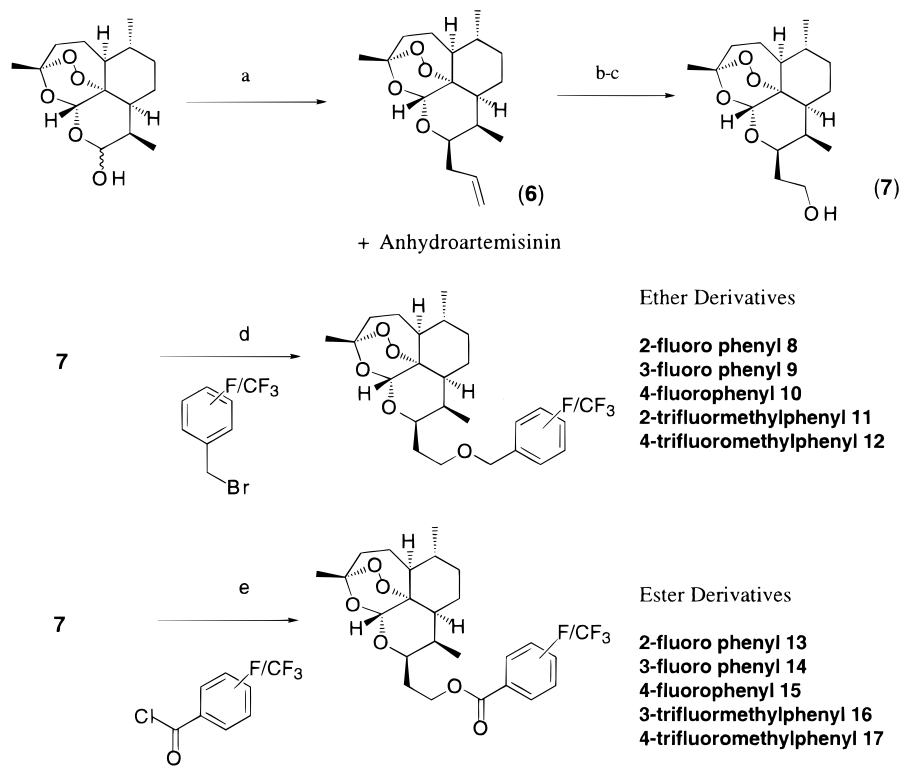
ated decomposition of our most potent derivative, which provides some insight into the possible radical species that may be responsible for the high antimalarial potency of this class of derivatives.

### Chemistry

Since several groups had previously observed that the presence of a lipophilic fluorine-containing aromatic group promotes high antimalarial activity in this class of drugs, we decided to incorporate a fluorinated aromatic ring into our novel derivatives.<sup>9</sup> A similar strategy for producing potent derivatives was reported earlier by Posner and co-workers, who demonstrated that the antimalarial potency of the simplified trioxane alcohol **5a** could be significantly improved by conversion to the benzyl fluoro ether derivative **5b** (Chart 2).<sup>10</sup> This compound was several times more potent than artemisinin. The fluorinated aromatic ring systems selected were linked to alcohol (**7**) by either an ester linkage (**8–12**) or an ether linkage (**13–17**) as shown in Scheme

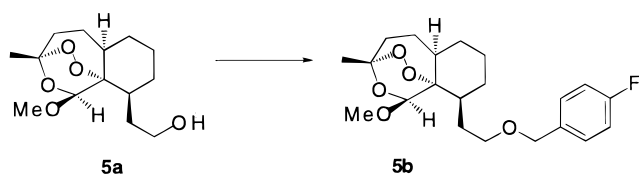
\* Corresponding author. Tel: 0151-794-3553. Fax: 0151-794-3588. E-mail: p.m.oneill01@liv.ac.uk.

<sup>†</sup> Department of Chemistry.

Scheme 1<sup>a</sup>

<sup>a</sup> Reagents: (a) allyltrimethylsilane/BF<sub>3</sub>·Et<sub>2</sub>O; (b) O<sub>3</sub>, CH<sub>2</sub>Cl<sub>2</sub>, -78 °C; (c) NaBH<sub>4</sub>, EtOH; (d) NaH, DMF; (e) Et<sub>3</sub>N.

## Chart 2. 1,2,4-Trioxanes 5a,b



1. The key intermediate required for the synthesis of targets was the allyl deoxy derivative **6** (Scheme 1). Coupling of dihydroartemisinin with allyltrimethylsilane in the presence of BF<sub>3</sub> etherate gave the required derivative **6** as a white crystalline product. The observed stereochemistry at the C-10 position was β, in line with the previous observation of Ziffer et al.<sup>11</sup> The modest yield of allyldeoxyartemisinin **6** was attributed to the competing dehydration reaction and subsequent formation of anhydroartemisinin. The resulting alkene was subjected to a stream of ozone at -78 °C for 30 min in CH<sub>2</sub>Cl<sub>2</sub>, and the subsequent ozonide was then treated with NaBH<sub>4</sub> for 2 h at 0 °C in THF/methanol (9:1) (Scheme 1). The alcohol **7** was then deprotonated and reacted with a range of benzyl bromides to give the targets **8–12** (Scheme 1). Synthesis of the ester derivatives was achieved by standard esterification with the appropriate fluorinated acid chlorides to give the crystalline ester derivatives **13–17**.

## Biology

The new synthetic derivatives were tested in vitro against the chloroquine-sensitive HB3 strain of *P. falciparum*. Apart from the trifluoromethyl derivative **12**, all of the new derivatives were more potent than the parent drug artemisinin. The two most potent derivatives, the 2- and 3-fluorophenyl ethers **8** and **9**,

**Table 1.** In Vitro Antimalarial Activity of New Fluorinated Carba Analogues versus the Chloroquine-Sensitive HB3 Strain of *P. falciparum*<sup>a</sup>

compd	IC <sub>50</sub> (nM)	std dev (±)
<b>7</b>	3.51	0.20(3)
<b>8</b>	0.22	0.10(2)
<b>9</b>	0.32	0.05(3)
<b>10</b>	0.73	0.32(2)
<b>11</b>	1.64	0.52(2)
<b>12</b>	4.24	0.62(3)
<b>13</b>	0.53	0.50(3)
<b>14</b>	0.35	0.52(3)
<b>15</b>	0.69	0.07(1)
<b>16</b>	0.64	0.33(3)
<b>17</b>	0.59	0.15(2)
artemisinin ( <b>2</b> )	3.04	0.54(2)
DHA ( <b>3</b> )	1.04	0.10(2)

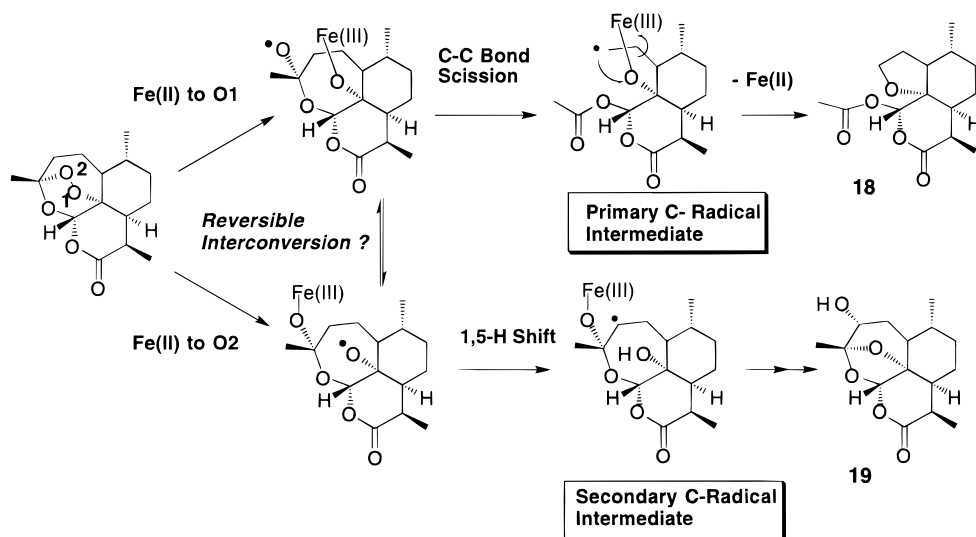
<sup>a</sup> Antimalarial activity was assessed by an adaptation of the 48-h sensitivity assay of Desjardins et al. which uses [<sup>3</sup>H]hypoxanthine incorporation as an assessment of parasite growth.<sup>12</sup>

demonstrated outstanding in vitro antimalarial potency (Table 1). Compound **8** is 15 times more potent than artemisinin and 5 times more potent than DHA in this screen. It is also clear that the presence of the fluorinated ring system is beneficial to antimalarial activity in both series of compounds. (For example, ethers **8** and **9** and ester derivative **14** are between 10 and 15 times more potent than alcohol **7**.)

All of the compounds were also highly potent against the chloroquine-resistant K1 strain, although the IC<sub>50</sub> values in this strain were slightly higher than against the HB3 strain. Compound **8** was again the most potent of the derivatives tested (Table 2).

Having obtained these promising results, we then selected key compounds for assessment in the standard mouse model of malaria (*Plasmodium berghei*-infected mice). The compounds were compared with artemether

## Scheme 2



**Table 2.** In Vitro Antimalarial Activity of New Fluorinated Carba Analogues versus the Chloroquine-Resistant K1 Strain of *P. falciparum*<sup>a</sup>

compd	IC <sub>50</sub> (nM)	std dev (±)
<b>7</b>	6.67	1.1(2)
<b>8</b>	1.02	1.85(2)
<b>9</b>	6.43	4.96(3)
<b>10</b>	6.08	4.64(3)
<b>11</b>	3.60	1.30(3)
<b>12</b>	3.75	1.12(3)
<b>13</b>	1.12	0.56(3)
<b>14</b>	3.78	1.46(2)
<b>15</b>	1.92	0.23(3)
<b>16</b>	4.70	1.91(2)
<b>17</b>	14.24	2.56(3)
artemisinin ( <b>2</b> )	3.60	0.80(3)
DHA ( <b>3</b> )	2.20	0.24(2)

<sup>a</sup> Antimalarial activity was assessed by an adaptation of the 48-h sensitivity assay of Desjardins et al. which uses [<sup>3</sup>H]hypoxanthine incorporation as an assessment of parasite growth.<sup>12</sup>

**Table 3.** In Vivo Antimalarial Activity of DHA, Artemether, Ethers **7** and **8**, and Ester **14** versus *P. berghei* in the Mouse Model

compd	ED <sub>50</sub> (mg/kg)	std dev (±)
<b>7</b>	7.81	0.20(3)
<b>8</b>	5.08	0.10(2)
<b>14</b>	6.99	0.05(3)
DHA	1.15	0.32(2)
artemether	3.15	0.52(2)

and the principal in vivo metabolite of artemether, dihydroartemisinin (DHA, **3**). Ether **8** was again the most potent analogue with an ED<sub>50</sub> value of 5.08 mg/kg, a value comparable to that of artemether (3.15 mg/kg) in this in vivo screen of antimalarial drug action. None of the compounds tested were as active as dihydroartemisinin.

## Discussion

In this work, we have identified several potent carba analogues of the clinically used antimalarial artemether. The potent in vitro and in vivo antimalarial activity of these derivatives makes them good candidates for further development. Since these compounds cannot form DHA (**3**), a metabolite associated with short half-life and neurotoxicity, these compounds should

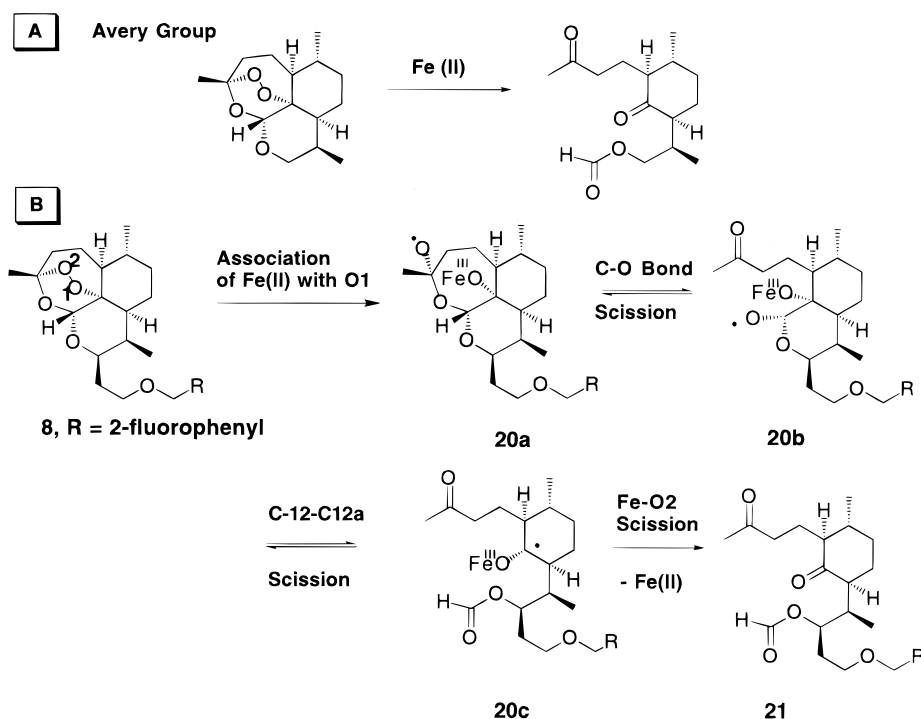
have a clear advantage over the currently available derivatives.

To gain insight into the potential mechanism of peroxide-containing antimalarials, several workers have investigated the biomimetic Fe(II)-mediated degradation of a range of 1,2,4-trioxane artemisinin derivatives and synthetic *endo*-peroxides.<sup>13–15</sup> These studies provide surrogate chemical markers of radical formation. The two major products obtained from the Fe(II)-mediated degradation of artemisinin have been shown to be a ring-contracted THF acetate **18** and a hydroxy deoxo derivative **19**.<sup>15</sup> The formation of **18** indicates the formation of a primary carbon-centered radical, whereas the formation of **19** indicates the formation of a secondary C-centered radical intermediate.

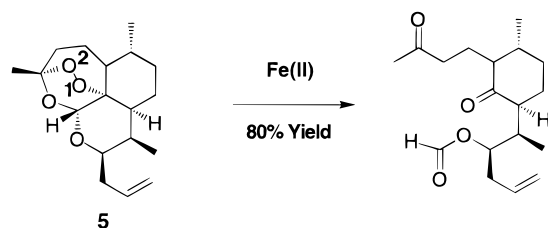
The most potent ether derivative **8** was treated with FeCl<sub>2</sub>·4H<sub>2</sub>O in aqueous acetonitrile. In contrast to the Fe(II)-mediated degradation of artemisinin, the reaction was complete within 5 min. Column chromatography revealed that the major product was the formate **21** (80% following chromatography). This observation indicates these derivatives degrade via a different mechanism to artemisinin and is in line with the recent work of Avery et al. who demonstrated that the main product of ferrous-mediated degradation of *C*-10-deoxoartemisinin was the formate (Scheme 3A).<sup>16</sup> On the basis of this earlier work, Wu et al.<sup>17</sup> proposed a plausible mechanism for this transformation; association of oxygen 1 with Fe(II) gives the oxyl radical **20a**, which fragments with C-12–O-11 scission resulting in **20b**. Further scission gives **20c**, which then loses Fe(II) to provide the observed aldehyde derivative **21**.

It is interesting to note that, by varying the substituent on the D-ring of the parent molecule, these new derivatives isomerize by an entirely different radical mechanism to artemisinin. Furthermore, the pathway may not involve formation of a secondary C-centered radical, which has been postulated to be crucial to the biological activity of the parent drug and other simplified 1,2,4-trioxane analogues.<sup>13,17</sup> Degradation of allyl-deoxoartemisinin **5** proceeds in a similar manner with an 80% yield of the corresponding formate (Scheme 4). The diketone products, liberated from **8** and **5**, could

## Scheme 3



## Scheme 4



in theory alkylate proteins, and further work is required to investigate this potential mechanism of action.

Although biomimetic studies of this type are mechanistically interesting, a degree of caution must be taken into consideration when using biomimetic Fe(II) studies to probe potential mechanisms of antimalarial action. Since many of the radical intermediates may be formed reversibly, it is difficult to pin point the exact radical species responsible for mediating the ultimate "parasite kill". In addition, the products obtained in the case of artemisinin are dependent not only on the type of iron salt used but also on the solvent.<sup>17</sup> It is apparent that more detailed work in this field is required to determine what solvent/Fe(II) salt type most accurately represents the decomposition products of artemisinin and other peroxides in the food vacuole of the parasite.

## Summary

Efficient synthetic routes have been developed for the synthesis of 10 new semisynthetic carba analogues of dihydroartemisinin. All of these compounds demonstrate outstanding *in vitro* antimalarial activity. On the basis of the Fe(II)-mediated decomposition of one of these derivatives, the radical mediator of biological activity is proposed to be different from that of the parent drug, artemisinin. Further work is in progress to determine the *in vivo* biological activity, metabolism,

and pharmacokinetics of these new antimalarial lead compounds.

## Experimental Section

**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  $\text{cm}^{-1}$  using a Perkin-Elmer 298 infrared spectrometer. Spectra of liquids were taken as films. Sodium chloride plates (Nujol mull) and solution cells (dichloromethane) were used as indicated.

Proton NMR spectra were recorded using Perkin-Elmer R34 (220 MHz) and Bruker (400 and 200 MHz) NMR spectrometers. Solvents are indicated in the text and tetramethylsilane was used as an 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.

The ether series of analogues was prepared by general procedure 1. The ester analogues were prepared by general procedure 2.

**General Procedure 1: Synthesis of Ether Derivatives.**

In a flame-dried vial NaH (60% in mineral oil, 64 mg, 1.62 mmol) under  $\text{N}_2$  was washed with anhydrous hexane (1 mL  $\times$  2) and dried *in vacuo*. The gray powder was cooled to 0  $^\circ\text{C}$  and a solution of 10 $\beta$ -(2-hydroxyethyl)deoxoartemisinin (**7**) (100 mg, 0.32 mmol)<sup>11</sup> in DMF (1.50 mL) was added via cannula directly onto the NaH. Vigorous release of gas occurred immediately. The flask containing the 10 $\beta$ -(2-hydroxyethyl)deoxoartemisinin was washed with DMF (0.30 mL) and cannulated into the reaction mixture. The mixture was removed from ice after 10 min and allowed to warm to room temperature with stirring for 45 min, by which time the bubbling had ceased. The dark beige turbid solution was cooled to 0  $^\circ\text{C}$  and the appropriate fluoro/trifluorobenzyl bromide (0.2 mL/0.26 mL, 1.62 mmol) was added dropwise via syringe. The mixture was allowed to stir at 0  $^\circ\text{C}$  for 10 min and warmed to room temperature over 3 h. The reaction was then cooled to 0  $^\circ\text{C}$ , quenched with dropwise additions of  $\text{H}_2\text{O}$  (1 mL), and diluted with  $\text{H}_2\text{O}$  (2 mL) and diethyl ether (5 mL). The organic phase was separated and the aqueous phase was extracted



with ether (5 mL  $\times$  3). The organic layer was washed with saturated aqueous NaCl, dried over MgSO<sub>4</sub>, filtered, and concentrated under reduced pressure. The oil was purified by silica gel chromatography with dichloromethane to give the corresponding ether products.

**General Procedure 2: Synthesis of Ester Derivatives.** Anhydrous DCM (16 mL) and fluoro/trifluorobenzoyl chloride (0.16 mL/21 mL, 1.28 mmol), respectively, were injected into a dry two-necked round-bottom flask under N<sub>2</sub> and were allowed to stir at 0 °C for 30 min. 10 $\beta$ -(2-Hydroxyethyl)-deoxoartemisinin (100 mg, 0.32 mmol) in anhydrous DCM (20 mL) and triethylamine (0.35 mL, 2.56 mmol) were injected into the solution mixture. After being stirred for a further 30 min, the reaction mixture was slowly warmed to room temperature and stirred for 2 h. It was concentrated to yield a crude product which was purified by silica gel chromatography with hexane/acetone (95:50) to produce the corresponding ester products.

**10 $\beta$ -[2-(2-Fluorobenzoyloxy)ethyl]deoxoartemisinin, 8.** This compound was prepared using general procedure 1 to give the product as a clear oil (32% yield): <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>)  $\delta$  7.73 (1 H, d,  $J$  = 7.69 Hz, aromatic), 7.62 (1 H, d,  $J$  = 8.10 Hz, aromatic), 7.54 (1 H, t,  $J$  = 7.56 Hz, aromatic), 7.35 (1 H, t,  $J$  = 7.69 Hz, aromatic), 5.31 (1 H, s), 4.59 (2 H, s), 4.28 (1 H, m), x (2 H, m), 2.73 (1 H, sex), 2.33 (1 H, dt), 1.40 (3 H, s), 0.96 (3 H, d,  $J$  = 5.91 Hz), 0.87 (3 H, d,  $J$  = 7.55 Hz); <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>)  $\delta$  130.09, 130.04, 129.25, 125.84, 125.65, 124.10, 124.06, 115.31, 115.03, 103.32, 88.86, 81.04, 72.71, 69.12, 66.49, 66.43, 52.42, 44.45, 37.39, 36.52, 34.42, 29.98, 29.68, 25.99, 24.61, 20.07, 12.98; IR (thin film)/cm<sup>-1</sup> 2926, 1616, 1375, 1011; MS (CI, NH<sub>3</sub>)  $m/z$  438.2 (M + NH<sub>4</sub><sup>+</sup>, 9), 375.2 (100), 361.1 (15); HRMS C<sub>25</sub>H<sub>34</sub>O<sub>5</sub>F [M + H]<sup>+</sup> required 421.23903, found 421.32910.

**10 $\beta$ -[2-(3-Fluorobenzoyloxy)ethyl]deoxoartemisinin, 9.** This compound was prepared using general procedure 1 to give the product as a clear oil (52% yield): <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>)  $\delta$  7.73 (1 H, d,  $J$  = 7.69 Hz, aromatic), 7.62 (1 H, d,  $J$  = 8.10 Hz, aromatic), 7.54 (1 H, t,  $J$  = 7.56 Hz, aromatic), 7.35 (1 H, t,  $J$  = 7.69 Hz, aromatic), 5.32 (1 H, s), 4.51 (2 H, s), 4.29 (1 H, m), 3.72 (2 H, m), 2.73 (1 H, sex), 2.39 (1 H, dt), 1.40 (3 H, s), 0.96 (3 H, d,  $J$  = 5.70 Hz), 0.87 (3 H, d,  $J$  = 7.50 Hz); <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>)  $\delta$  141.38, 129.90, 129.80, 122.96, 114.43, 114.15, 103.33, 88.88, 81.04, 72.39, 72.31, 69.02, 52.40, 44.43, 37.40, 36.52, 34.41, 29.98, 25.99, 24.63, 24.61, 20.07, 12.97; IR (thin film)/cm<sup>-1</sup> 2944, 1615, 1375, 1110, 1041; HRMS (CI, NH<sub>3</sub>) C<sub>25</sub>H<sub>34</sub>O<sub>5</sub>F [M + H]<sup>+</sup> required 421.23903, found 421.3287.

**10 $\beta$ -[2-(4-Fluorobenzoyloxy)ethyl]deoxoartemisinin, 10.** This compound was prepared using general procedure 1 to give the product as a clear oil (42% yield): <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>)  $\delta$  7.32 (2 H, t,  $J$  = 8.83 Hz, aromatic), 7.02 (2 H, t,  $J$  = 8.65 Hz, aromatic), 5.31 (1 H, s), 4.48 (2 H, s), 4.28 (1 H, m), 3.71 (2 H, m), 2.72 (1 H, sex), 2.33 (1 H, dt), 1.40 (3 H, s), 0.96 (3 H, d,  $J$  = 5.90 Hz), 0.87 (3 H, d,  $J$  = 7.70 Hz); <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>)  $\delta$  129.51, 129.41, 115.35, 115.06, 103.35, 88.92, 72.75, 72.53, 68.91, 52.48, 44.51, 37.49, 36.60, 34.50, 30.07, 29.83, 26.12, 24.72, 20.18, 13.09; IR (thin film)/cm<sup>-1</sup> 2940, 1604, 1110, 1041; HRMS (CI, NH<sub>3</sub>) C<sub>25</sub>H<sub>34</sub>O<sub>5</sub>F [M + H]<sup>+</sup> required 421.23903, found 421.23897.

**10 $\beta$ -[2-(2-Trifluoromethylbenzyloxy)ethyl]deoxoartemisinin, 11.** This compound was prepared using general procedure 1 to give the product as a clear oil (52% yield): <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>)  $\delta$  7.73 (1 H, d,  $J$  = 7.69 Hz, aromatic), 7.62 (1 H, d,  $J$  = 8.1 Hz, aromatic), 7.54 (1 H, t,  $J$  = 7.56 Hz, aromatic), 7.35 (1 H, t,  $J$  = 7.69 Hz, aromatic), 5.33 (1 H, s), 4.71 (2 H, s), 4.32 (1 H, m), 3.76 (2 H, m), 2.73 (1 H, m), 2.34 (1 H, dt,  $J$  = 3.9 Hz, 13.5 Hz), 1.39 (3 H, s), 0.96 (3 H, d,  $J$  = 5.91 Hz), 0.89 (3 H, d,  $J$  = 7.55 Hz); <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>)  $\delta$  131.94, 128.86, 127.16, 125.67, 103.31, 88.97, 81.09, 72.52, 69.23, 68.87, 52.46, 44.50, 37.50, 36.61, 34.52, 30.10, 29.82, 26.07, 24.74, 20.16, 13.03; IR (thin film)/cm<sup>-1</sup> 2943, 1610, 1118, 1038; MS (CI, NH<sub>3</sub>)  $m/z$  471.1 (14), 380.1 (12), 159.0 (100), 36.0 (36), 89.0 (18), 55.1 (20 5); HRMS (CI, NH<sub>3</sub>) C<sub>25</sub>H<sub>34</sub>O<sub>5</sub>F<sub>3</sub> [M + H]<sup>+</sup> required 471.23583, found 471.32699.

**10 $\beta$ -[2-(4-Trifluoromethylbenzyloxy)ethyl]deoxoartemisinin, 12.** This compound was prepared using general procedure 1 to give the product as a clear oil (52% yield): <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>)  $\delta$  7.59 (2 H, d,  $J$  = 8.1 Hz, aromatic), 7.47 (2 H, d,  $J$  = 5.1 Hz aromatic), 5.32 (1 H, s), 4.58 (2 H, s), 4.31 (1 H, m), 3.73 (2 H, m), 2.72 (1 H, sex), 2.34 (1 H, dt), 1.40 (3 H, s), 0.96 (3 H, d,  $J$  = 6.00 Hz), 0.88 (3 H, d,  $J$  = 7.50 Hz); <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>)  $\delta$  142.90, 127.62, 125.37, 125.33, 125.26, 103.31, 88.98, 81.07, 72.47, 72.33, 69.09, 52.40, 44.41, 37.44, 36.54, 34.43, 30.01, 29.81, 26.06, 24.67, 24.65, 20.07, 12.93; IR (thin film)/cm<sup>-1</sup> 2933, 1658, 1375, 1118, 1042; HRMS (CI, NH<sub>3</sub>) C<sub>25</sub>H<sub>34</sub>O<sub>5</sub>F<sub>3</sub> [M + H]<sup>+</sup> required 471.23583, found 471.23515.

**10 $\beta$ -[2-(2-Fluorobenzoyloxy)ethyl]deoxoartemisinin, 13.** This compound was prepared using general procedure 2 to give the product as a white crystalline product (72% yield): <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>)  $\delta$  7.76 (1 H, td,  $J$  = 7.50 Hz, 1.80 Hz aromatic), 7.50 (1 H, m, aromatic), 7.15 (2 H, m, aromatic), 5.35 (1 H, s), 4.57 (1 H, m), 4.57 (1 H, m), 1.37 (3 H, s), 0.97 (3 H, d,  $J$  = 6.00 Hz), 0.90 (3 H, d,  $J$  = 7.50 Hz); <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>)  $\delta$  165.54, 134.39, 134.27, 132.23, 123.94, 123.89, 117.11, 116.82, 103.23, 89.08, 81.08, 71.76, 63.43, 52.37, 44.33, 37.49, 36.60, 34.48, 30.08, 28.90, 25.98, 24.80, 24.75, 20.13, 12.84; IR (CHCl<sub>3</sub>)/cm<sup>-1</sup> 2957.0, 1718.0, 1302.0; LC/MS  $m/z$  552 [M + NH<sub>4</sub><sup>+</sup> (100)], 435 (15.2), 417 (32.5), 399 (6.3), 331 (5.2), 277 (25.4), 259 (15.6), 241 (19.6), 241 (7.3), 201 (6.7), 191 (13.5).

**10 $\beta$ -[2-(3-Fluorobenzoyloxy)ethyl]deoxoartemisinin, 14.** This compound was prepared using general procedure 2 to give the product as a white crystalline product (86% yield): <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>)  $\delta$  7.83 (1 H, d,  $J$  = 7.69 Hz, aromatic), 7.74 (1 H, d,  $J$  = 9.2 Hz, aromatic), 7.41 (1 H, m, aromatic), 7.23 (1 H, aromatic), 5.36 (1 H, s), 4.57 (1 H, m), 4.47 (2 H, m), 1.36 (3 H, s), 0.97 (3 H, d,  $J$  = 5.77 Hz), 0.90 (3 H, d,  $J$  = 7.56 Hz); <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>)  $\delta$  165.49, 130.01, 129.91, 125.43, 120.02, 119.75, 116.75, 116.44, 103.17, 89.25, 81.10, 71.45, 63.44, 52.31, 44.23, 37.51, 36.60, 34.46, 30.14, 29.08, 25.97, 24.83, 24.76, 20.11, 12.78; IR (CDCl<sub>3</sub>)/cm<sup>-1</sup> 2957.0, 1718.0, 1284.0, 1270.0, 1205; LC/MS (NH<sub>3</sub>)  $m/z$  552 [M + NH<sub>4</sub><sup>+</sup> (100)], 435 (26.3), 417 (46.7), 399 (9.4), 331 (7.5), 277 (15.6), 259 (15.6), 241 (9.2), 201 (9.4), 191 (11.7).

**10 $\beta$ -[2-(4-Fluorobenzoyloxy)ethyl]deoxoartemisinin, 15.** This compound was prepared using general procedure 2 to give the product as a white crystalline product (81% yield): <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>)  $\delta$  8.07 (2 H, dd,  $J$  = 8.70 Hz, 5.52 Hz, aromatic), 7.10 (2 H, d,  $J$  = 9.2 Hz, aromatic), 5.35 (1 H, s), 4.57 (1 H, m), 4.50 (3 H, m), 1.35 (3 H, s), 0.97 (3 H, d,  $J$  = 5.91 Hz), 0.89 (3 H, d,  $J$  = 7.55 Hz); <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>)  $\delta$  165.67, 132.29, 132.17, 115.60, 115.30, 103.15, 89.27, 81.13, 71.40, 63.12, 52.30, 44.22, 37.51, 36.60, 34.46, 30.16, 29.13, 25.97, 24.83, 24.76, 20.11, 12.77; IR (CDCl<sub>3</sub>)/cm<sup>-1</sup> 2957.0, 1712.0, 1280.0; LC/MS (NH<sub>3</sub>)  $m/z$  552 [M + NH<sub>4</sub><sup>+</sup> (100)], 435 (44.4), 417 (68.8), 399 (8.8), 331 (9.2), 277 (32.1), 259 (13.3), 241 (12.3), 201 (11.3), 191 (17.1).

**10 $\beta$ -[2-(3-Trifluorobenzoyloxy)ethyl]deoxoartemisinin, 16.** This compound was prepared using general procedure 2 to give the product as a white crystalline product (82% yield): <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>)  $\delta$  8.32 (1 H, s, aromatic), 8.25 (1 H, d,  $J$  = 7.83 Hz, aromatic), 7.81 (1 H, d,  $J$  = 7.82 Hz, aromatic), 7.58 (1 H, m, aromatic), 5.36 (1 H, s), 4.60 (1 H, m), 4.49 (2 H, m), 1.36 (3 H, s), 0.97 (3 H, d,  $J$  = 5.91 Hz), 0.89 (3 H, d,  $J$  = 7.78 Hz); <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>)  $\delta$  165.67, 132.29, 132.17, 115.60, 115.30, 103.15, 89.27, 81.13, 71.40, 63.12, 52.30, 44.22, 37.51, 36.60, 34.46, 30.16, 29.13, 25.97, 24.83, 24.76, 20.11, 12.77; IR (CHCl<sub>3</sub>)/cm<sup>-1</sup> 2957.0, 1720.0, 1336.0, 1256.0, 1171.0, 1135.0; LC/MS (NH<sub>3</sub>)  $m/z$  502 [M + NH<sub>4</sub><sup>+</sup> (100)], 485 (24.9), 467 (46.4), 449 (16.8), 439 (6.8), 381 (7.2), 277 (13.8), 259 (16.2), 241 (11.4), 201 (5.8), 191 (8.8).

**12 $\beta$ -[2-(4-Trifluorobenzoyloxy)ethyl]deoxoartemisinin, 17.** This compound was prepared using general procedure 2 to give the product as a white crystalline product (85% yield): <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>)  $\delta$  8.18 (2 H, d,  $J$  = 7.96 Hz, aromatic), 7.70 (2 H, d,  $J$  = 8.10 Hz, aromatic), 5.36 (1 H, s), 4.54 (3 H, m), 1.35 (3 H, s), 0.97 (3 H, d,  $J$  = 5.76 Hz), 0.90 (3 H, d,  $J$  = 7.42 Hz); <sup>13</sup>C NMR (75 MHz, CDCl<sub>3</sub>)  $\delta$  165.39, 134.60,

134.15, 133.75, 130.51, 130.06, 125.37, 1125.32, 121.88, 103.10, 89.29, 81.08, 71.255, 63.56, 52.22, 44.13, 37.48, 36.54, 34.40, 30.13, 29.07, 25.91, 24.80, 24.72, 20.05, 12.67; IR (CHCl<sub>3</sub>)/cm<sup>-1</sup> 2958.0, 1718.0, 1325.0, 1280.0, 1136.0, 1098.0; LC/MS (NH<sub>3</sub>) *m/z* 502 [M + NH<sub>4</sub><sup>+</sup> (100)], 485 (25.0), 467 (44.2), 449 (16.9), 439 (6.2), 381 (8.9), 277 (28.8), 259 (25.5), 241 (11.5), 201 (9.6), 191 (14.9).

**FeCl<sub>2</sub>-Mediated Degradation of 10β-Allyldeoxoartemisinin, 5.** 10β-Allyldeoxoartemisinin (**5**) (95 mg, 0.030 mmol) and FeCl<sub>2</sub>·4H<sub>2</sub>O (0.012 g, 0.614 mmol) were added to CH<sub>3</sub>CN (2 mL) and allowed to stir at room temperature for 5 min. The reaction was extracted with ethyl acetate (3 × 10 mL), dried over MgSO<sub>4</sub>, filtered, and concentrated in vacuo. The crude oil was purified by silica gel column chromatography with hexane/ethyl acetate (80/20) as eluent to give the product (0.076 g, 80%): <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ 8.06 (1 H, s, HC=O), 5.73 (1 H, m, HC=C), 5.13 (1 H, m, C=CH), 5.08 (1 H, m, C=CH), 2.12 (3 H, s, CH<sub>3</sub>), 1.57 (2 H, s), 1.06 (3 H, d, *J* = 6.0 Hz, CH<sub>2</sub>), 0.97 (3 H, d, *J* = 6.6 Hz, CH<sub>3</sub>); <sup>13</sup>C NMR (300 MHz, CDCl<sub>3</sub>) δ 160.79, 133.54, 118.12, 73.40, 57.47, 54.45, 41.40, 41.23, 37.17, 34.78, 34.37, 30.96, 29.82, 20.46, 20.35, 12.35; MS *m/z* (CI) 326 (62.39), 309 (29.05), 263 (100).

**FeCl<sub>2</sub>-Mediated Degradation of 10β-[2-(3-Fluorobenzoyloxy)ethyl]deoxoartemisinin, 8.** 10β-[2-(3-Fluorobenzoyloxy)ethyl]deoxoartemisinin (**8**) (95 mg, 0.225 mmol) and FeCl<sub>2</sub>·4H<sub>2</sub>O (89 mg, 0.044 mmol) were added to CH<sub>3</sub>CN (2 mL) and allowed to stir at room temperature for 10 min. The reaction was extracted with ethyl acetate (3 × 10 mL), dried over MgSO<sub>4</sub>, filtered, and concentrated in vacuo. The crude oil was purified by silica gel column chromatography with ethyl acetate/hexane (20/80) as eluent affording one major product (0.076 g, 80%): <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>) δ 8.06 (1 H, s, HC=O), 7.02 (2 H, t, *J* = 9.0 Hz, aromatic), 7.29 (2 H, aromatic), 5.29 (1 H, m, CH), 4.43 (2 H, s, CH<sub>2</sub>), 3.48 (2 H, q, CH<sub>2</sub>), 2.11 (3 H, s, CH<sub>3</sub>), 1.06 (3 H, d, *J* = 6.3 Hz, CH<sub>3</sub>), 0.96 (3 H, d, *J* = 6.6 Hz, CH<sub>3</sub>); <sup>13</sup>C NMR (300 MHz, CDCl<sub>3</sub>) δ 160.93, 129.57, 129.46, 72.47, 71.93, 66.84, 57.53, 54.50, 41.39, 35.35, 34.75, 32.81, 31.21, 29.80, 20.45, 20.37, 12.55; MS *m/z* (CI) 317 (100).

**Antimalarial Activity.** Two strains of *P. falciparum* from Thailand were used in this study: (a) the uncloned K1 strain which is known to be CQ-resistant and (b) the HB3 strain which is sensitive to all antimalarials. Parasites were maintained in continuous culture using the method of Trager and Jensen.<sup>21</sup> Cultures were grown in culture flasks containing human erythrocytes (2–5%) with parasitemia in the range of 1–10% suspended in RPMI 1640 medium supplemented with 25 mM HEPES, 32 mM NaHCO<sub>3</sub>, and 10% human serum (complete medium). Cultures were gassed with a mixture of 3% O<sub>2</sub>, 4% CO<sub>2</sub>, and 93% N<sub>2</sub>.

**(a) In Vitro Testing.** Antimalarial activity was assessed using an adaption of the 48-h sensitivity assay of Desjardins et al.<sup>12</sup> using [<sup>3</sup>H]hypoxanthine incorporation as an assessment of parasite growth. Stock drug solutions were prepared in 100% dimethyl sulfoxide (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% hematocrit) with or without 10-μL drug dilutions. Each drug was tested in triplicate and parasite growth compared to control wells (which consisted 100% parasite growth). After 24 h of incubation at 37 °C, 0.5 μCi of hypoxanthine was added to each well. Cultures were incubated for a further 24 h before they were harvested onto filtermats, dried for 1 h at 55 °C, and counted using a Wallac 1450 Microbeta Trilux liquid scintillation and luminescence counter. IC<sub>50</sub> values were calculated by interpolation of the probit transformation of the log dose–response curve.

**(b) In Vivo Testing.** Male, random Swiss albino mice weighing 18–22 g were inoculated intraperitoneally with 10<sup>7</sup> parasitized erythrocytes with *P. berghei* NS strain. Animals were then dosed daily via two routes (ip) 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). The parasitemia was determined on the day following the last treatment and the ED<sub>50</sub> (50% suppression of parasites when compared to vehicle-only-treated controls) determined from a plot of log dose against parasitemia.

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