Synthesis, Antimalarial Activity, Biomimetic Iron(II) Chemistry, and in Vivo Metabolism of Novel, Potent *C*-10-Phenoxy Derivatives of Dihydroartemisinin

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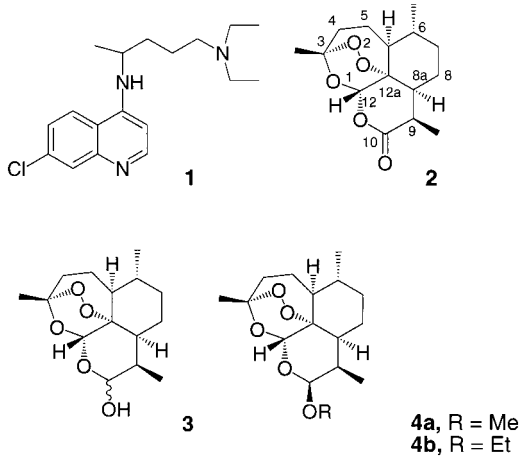
The combination of TMSOTf and $AgClO_4$ promotes the efficient C-10-phenoxylation of dihydroartemisinin (**3**) in good chemical yield and excellent stereoselectivity. All of the new phenoxy derivatives have potent in vitro antimalarial activity. On the basis of the excellent yield and stereoselectivity obtained for the *p*-trifluoromethyl derivative **7b**, this compound and the parent phenyl-substituted derivative **5b** were selected for in vivo biological evaluation against *Plasmodium berghei* in the mouse model and for metabolism studies in rats. Compound **7b** demonstrated excellent in vivo antimalarial potency with an ED_{50} of 2.12 mg/kg (cf. artemether = 6 mg/kg) versus *P. berghei*. Furthermore, from preliminary metabolism studies, this compound was not metabolized to dihydroartemisinin; suggesting it should have a longer half-life and potentially lower toxicity than the first-generation derivatives artemether and arteether. From biomimetic Fe(II)-catalyzed decomposition studies and ESR spectroscopy, the mechanism of action of these new lead antimalarials is proposed to involve the formation of both primary and secondary C-centered cytotoxic radicals which presumably react with vital parasite thiol-containing cellular macromolecules.

Introduction

The spread of the malaria parasite's resistance to chloroquine (**1**) 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.[1,2] Artemisinin (**2**, qinghaosu) is an unusual 1,2,4-trioxane which has been used clinically in China for the treatment of multidrug-resistant *Plasmodium falciparum* malaria. However the therapeutic value of **2** is limited to a great extent by its low solubility in both oil and water. Consequently, in the search for more effective and soluble drugs, a number of derivatives of the parent drug have been prepared. Reduction of artemisinin produces dihydroartemisinin (**3**, DHA), which has in turn led to the preparation of a series of semisynthetic first-generation analogues which include artemether (**4a**, R = −Me) and arteether (**4b**, R = −Et) and the water-soluble derivative sodium artesunate (**4c**, R = $-C(O)CH_2CH_2CO_2Na$).

Although these derivatives are potent antimalarial agents in vitro, they have poor bioavailability principally as a result of the metabolic instability of the acetal function. One of the principal routes of metabolism of arteether (**4b**), for example, involves oxidative dealkylation to DHA (**3**), a compound associated with toxicity[3]



and short half-life (Scheme 1).[4] Replacement of the oxygen at the C-10 position with carbon would be expected to produce compounds not only with greater metabolic 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.[5−8] An alternative approach to increasing the metabolic stability of artemisinin derivatives involves incorporation of a phenyl group in place of the alkyl group (in the ether linkage) of first-generation analogues, e.g. **4a** and **4b**.

This modification would be expected to block oxidative metabolic formation of DHA in vivo (e.g. Scheme 1). This paper deals with the synthesis, in vitro and in vivo biological activity, metabolism, and biomimetic Fe(II) reactions of a new series of *C*-10-phenoxy derivatives

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Scheme 1

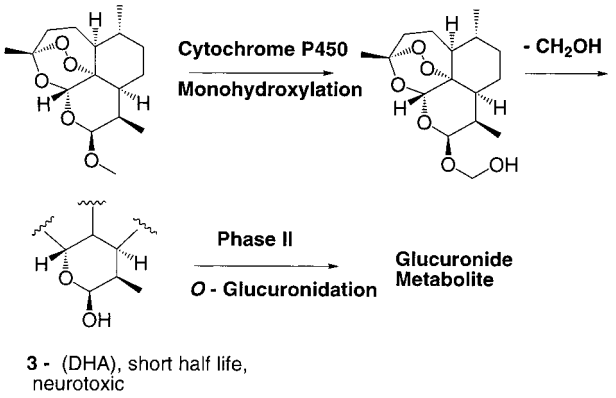
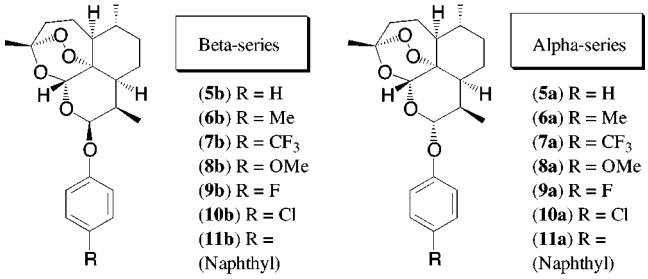
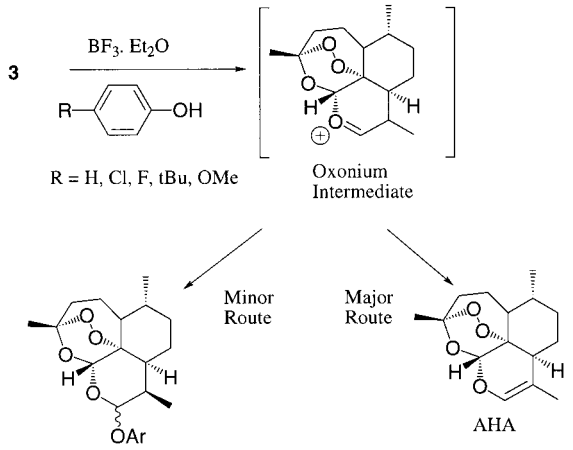


Chart 1. Structures of *C*-10-Phenoxy Derivatives of DHA



Scheme 2

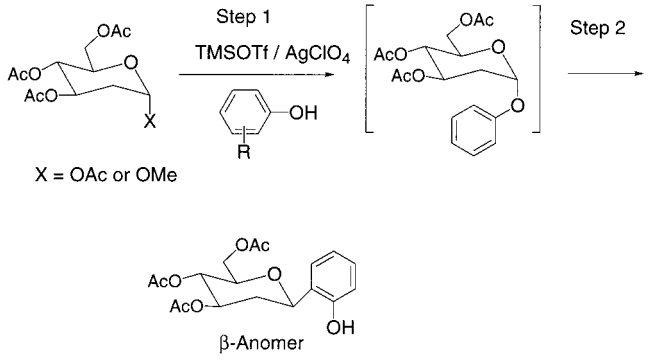


(β-series **5b**−**11b** and α-series **5a**−**11a**; Chart 1). ESR spectroscopy has also been utilized to provide evidence for the existence of potentially toxic C-centered radicals.

Chemistry

Several synthetic approaches have been investigated for the production of target C-10 derivatives. The initial approach was to couple DHA with various phenols using boron trifluoride diethyl etherate catalysis. Many researchers have used this method to form first-generation endoperoxides. The method involved reacting 1 equiv of DHA with 4 equiv of the phenol in anhydrous ether at room temperature in the presence of BF_3 etherate. In every case, the major product obtained was the anhydro derivative (AHA) in high yield. This suggests the involvement of an oxonium ion (Scheme 2) intermediate. The oxonium ion, being a chemically reactive intermediate either reacts with a phenol to give the phenyl ether or loses a proton to give the byproduct, anhydroartemisinin (AHA).

Scheme 3



The reactions compete for the intermediate oxonium ion. Similar results were obtained using either TMSOTf or TMSCl as Lewis acid with phenol as nucleophile. Further studies involving the use of the Mitsunobu reaction[10,11] (DEAD, Ph_3P, phenol) on **3** gave high stereoselectivity (12.5/1 β/α) but low overall yield (27.5%).

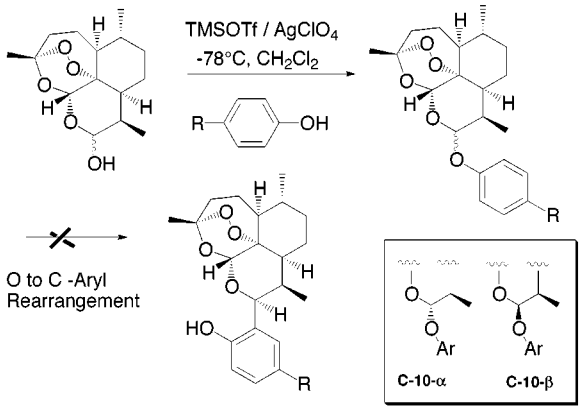
The dihydroartemisinin lactol can be recognized as a relative of a pyranose sugar with a free anomeric hydroxyl group.[12] There are many papers detailing reactions of glycosides, including C−C bond formation at the anomeric site.[13,14] Recently, studies by Suzuki investigated the O to C glycoside rearrangement of phenoxy glycosides.[15] Different Lewis acid promoters have been used, including BF_3 etherate, $SnCl_4$, and Cp_2HfCl_2−$AgClO_4$ with varying success. In 1992, Toshima discovered the efficient β-stereoselective *C*-aryl glycosidation of 1-*O*-methyl sugars by the use of a TMSOTf−$AgClO_4$ catalyst system.[16] This procedure gives excellent yields and diastereoselectivity in favor of the β-isomer by rearrangement of the preformed phenoxy glycoside as shown (Scheme 3).

Such intriguing results led us to explore the use of TMSOTf−$AgClO_4$ catalysis in our DHA−phenol coupling reactions. This approach involves dissolving 1 equiv of DHA, approximately 2 equiv of the desired phenol and 0.20 equiv of $AgClO_4$ in anhydrous dichloromethane, under nitrogen at −78 °C. TMSOTf (1 equiv) is then added to the reaction mixture. In every case, the reaction provided excellent yields with good diastereoselectivity in favor of the β-isomers (Scheme 4). Notably, only minor quantities of AHA were observed (Scheme 3) and no *O*- to *C*-aryl glycoside rearrangment was noted for any of the phenoxy derivatives obtained (in contrast to the situation depicted in Scheme 3).

As can be seen, compared with earlier methods described above, the increase in yield upon using the new catalyst combination was dramatic. Noticeably, when R = OMe, the yield increased by 55% (the yield was only 18% using $BF_3{\cdot}Et_2O$ catalysis). There is no obvious explanation for the success of this reaction. It may be that under these conditions the oxonium intermediate is stabilized. Alternatively the conditions may catalyze slow oxonium ion formation. Hence at any given time there are larger quantities of nucleophile available to react with the intermediate oxonium species (Figure 1).

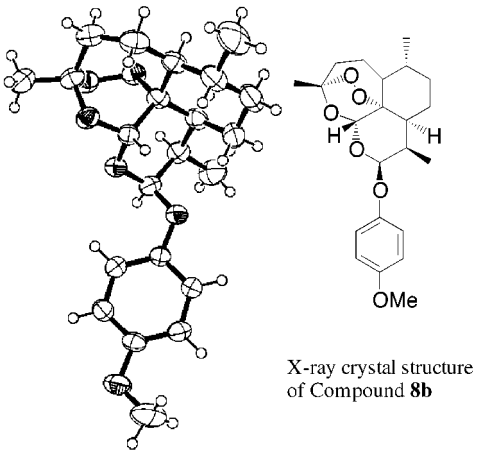
The reaction was repeated for a range of phenols, and yields are recorded in Scheme 4. The diastereoselectivity ratios were calculated from NMR data and the stereo-

Scheme 4



R	Compounds	Yield	α/β Ratio
H	**5a** & **5b**	68	1:4
Me	**6a** & **6b**	84	1:5
CF_3	**7a** & **7b**	68	1:8
OMe	**8a** & **8b**	73	1:4
F	**9a** & **9b**	60	1:3
Cl	**10a** & **10b**	62	1:5
Napthyl	**11a** & **11b**	84	1:5

Compounds **5a-12a** (C-10 alpha series)
Compounds **5b-11b** (C-10 beta series)



X-ray crystal structure of Compound **8b**

Figure 1. X-ray crystal structure of phenoxy compound **8b**.

chemistry of the phenoxy derivatives was confirmed by X-ray crystallography.[17]

Recent studies by Wu et al. have described the boron trifluoride-catalyzed Friedel Crafts alkylation of a *C*-10-acetate derivative of **3** with naphth-2-ol.[18] The reaction is proposed to proceed through the intermediacy of the *β*- and α-naphthoxy derivatives. The fact that no O to C rearrangement was observed in our system may reflect the much lower temperatures (−78 °C) employed compared with those required to induce glycosidic rearrangement. Currently, we are investigating the use of the TMSOTf−$AgClO_4$ activating system and higher temperatures to induce O to C rearrangement of these phenoxy derivatives.

Biology

In Vitro and in Vivo Antimalarial Activity. The *C*-10-phenoxy derivatives were tested in vitro against the K-1 chloroquine-resistant strain of *P. falciparum*. The IC_{50} values are shown in Table 1.

It is immediately clear that compounds in this class are potent antimalarials. The parent derivative (**5a** and **5b**) and naphthyl derivative (**11a**), for instance, have similar antimalarial activity to artemether. In general the α-series have similar biological activity to their *β*-diastereomers against this strain. The phenoxy derivatives were also tested against the chloroquine-sensitive HB3 strain (Table 2). The most potent *β*-isomers were the phenyl (**5b**), 4-methylphenyl (**6b**), and 4-fluorophenyl (**9b**) DHA derivatives. These were all slightly more potent than artemether. In the α-series, the phenyl (**5a**), 4-methylphenyl (**6a**), 4-methoxyphenyl (**8a**), and naphthoxy derivatives of DHA were all more potent than artemether.

Table 1. In Vitro Antimalarial Activity of *C*-10-α-Phenoxy (**5a**−**12a**) and *C*-10-*β*-Phenoxy (**5b**−**11b**) Derivatives of DHA versus *P. falciparum* (K1 strain)

compd	IC_{50} (nM)	SE	compd	IC_{50} (nM)	SE
5a	2.97	0.02	**5b**	3.66	1.88
6a	3.18	0.05	**6b**	3.92	0.11
7a	4.62	0.06	**7b**	5.29	0.08
8a	3.86	0.24	**8b**	4.58	0.04
9a	5.70	1.01	**9b**	4.58	0.04
11a	2.88	0.04	**11b**	8.89	0.05
artemisinin	11.15	0.32	artemether	4.55	0.12

Table 2. In Vitro Antimalarial Activity of *C*-10-α-Phenoxy (**5a**−**12a**) and *C*-10-*β*-Phenoxy (**5b**−**11b**) Derivatives of DHA versus *P. falciparum* (HB3 strain)

compd	IC_{50} (nM)	SE	compd	IC_{50} (nM)	SE
5a	2.61	0.31	**5b**	3.24	0.05
6a	2.87	0.10	**6b**	3.24	0.16
7a	3.42	0.05	**7b**	3.90	0.07
8a	3.32	0.14			
9a	4.04	0.03	**9b**	2.88	0.21
11a	2.88	0.04	**11b**	7.06	0.04
artemisinin	9.67	0.24	artemether	3.42	1.14

Table 3. In Vivo Antimalarial Activity of DHA, Artemether, **5b**, and **7b** versus *P. berghei*

compd	ED_{50} (mg/kg)	SD (±)
5b	3.09	0.30 (3)
7b	2.12	0.20 (2)
artemether	6.02	0.15 (3)
DHA	1.95	0.32 (2)

On the basis of the excellent chemical yield and stereoselectivity obtained for the *p*-trifluoromethyl derivative **7b**, this compound along with the parent phenyl-substituted derivative **5b** were selected for in vivo biological evaluation against *Plasmodium berghei* in the mouse model of malaria.

Table 3 lists the in vivo biological activity of lead compounds **5b** and **7b**. Trioxane **7b** has outstanding in vivo antimalarial activity: equal to that of dihydroartemisinin and superior to that of artemether. Further studies conducted by the WHO also demonstrate that **7b** is active orally with an ED_{50} of 2.7 mg/kg and ED_{90} of 5.4 mg. The data shown in Table 4, which also has data versus *Plasmodium yoelli ssp*. (NS), confirm that

Scheme 5

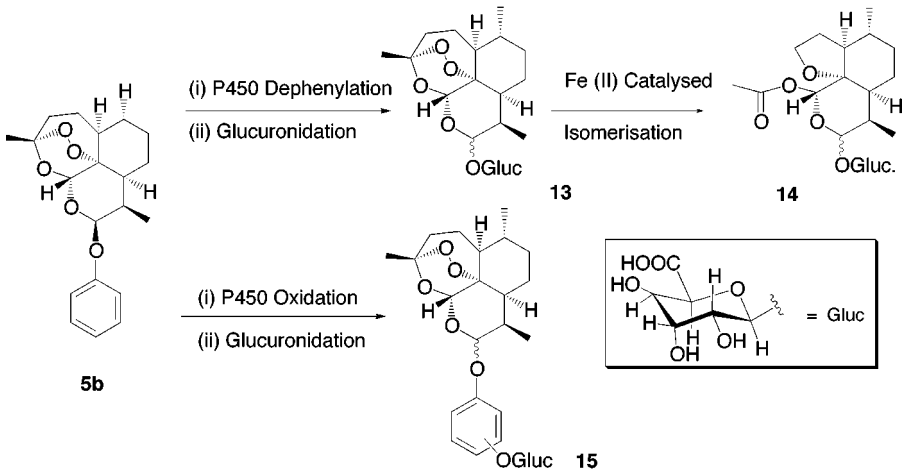


Table 4. In Vivo Comparison of **7b** with Sodium Artesunate versus *P. berghei* and *P. yoelli ssp.* NS

	P. berghei (po)		*P. yoelli* (sc)	
compd	ED_{50} (mg/kg)	ED_{90} (mg/kg)	ED_{50} (mg/kg)	ED_{90} (mg/kg)
7b	2.7	5.4	2.2	3.1
sodium artesunate	4.0	13.0	3.6	13.5

this new lead trioxane is superior to the clinically used derivative, sodium artesunate.

Drug Metabolism Studies

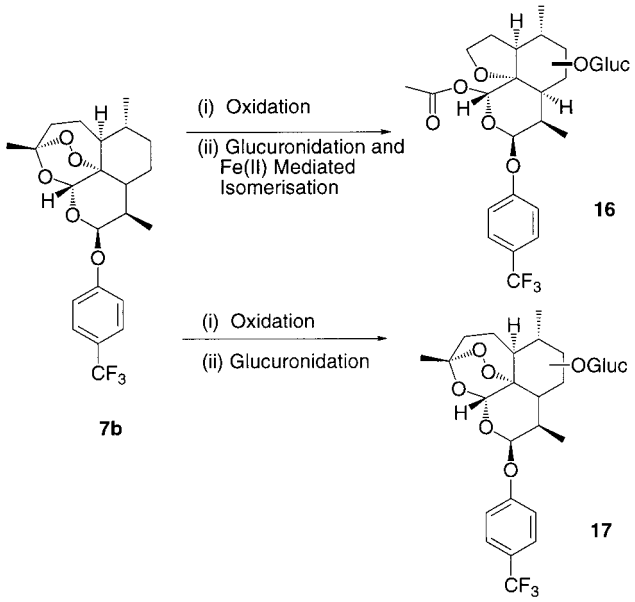
The structure−metabolism relationships of the selected phenoxy derivatives were investigated in the bile-duct-cannulated rat. Cannulated and anesthetized male Wistar rats were administered DHA and the following *O*-ether derivatives of DHA (35 μmol/kg) by either iv injection or gastric intubation: phenylDHA (iv) (**5b**), *p*-trifluoromethylphenylDHA (iv and po) (**7b**), and *p*-methoxyphenylDHA (iv) (**8b**).

Compound 5b. Early bile fractions (hourly or half-hourly) from iv-dosed animals contained DHA glucuronide (**13**) and highly variable proportions of the furano acetate glucuronide (**14**) (both identified by coelution with the previously identified biliary metabolites of DHA in rats), and also a metabolite (t_R 21 min; it was absent from the bile collected immediately before injection of the drug) corresponding to a glucuronide of the hydroxylated drug (**15**) (*m*/*z* 570 for ammonium adduct) (Scheme 6). The proportions of both metabolites in sequential half-hourly bile collections decreased rapidly, but they were still present in the 1.5−2.0 half-hourly collection. Dearylation of phenylDHA to give DHA should also yield phenol, which, in part, will be conjugated with glucuronic acid. However, no phenol glucuronide was located in rat bile following administration of **5b**.

The hydroxyphenylDHA (hpDHA) glucuronide fragmented to the same lower mass ions as DHA glucuronide, i.e. *m*/*z* 267, 221, and 163. From this, it is deduced that the hydroxyl substituent is located on the phenyl ring; the interpreted fragmentations of DHA glucuronide and the hpDHA glucuronide are listed in Table 5.

An aliquot (100 μL) of 0−1 h bile was diluted (1:1, v/v) with sodium acetate buffer (0.1 M, pH 5.0) and

Scheme 6



incubated with *Helix pomatia* (H-2) β-glucuronidase−arylsulfohydrolase preparation (12.5 μL) at 37 °C for 5 h. This incubation was performed in parallel with an incubation of bile from a rat given DHA. The incubations were analyzed by LC−MS without processing. The prominent peaks of *m*/*z* 478 representing the two metabolites of DHA (DHA glucuronide and its furano acetate isomer) were absent after 5 h. The peaks corresponding to DHA glucuronide and hpDHA glucuronide in bile from the animal given phenylDHA were likewise absent. The conversion of **13** to **14** may occur in vivo, but isomerization followed by conjugation cannot be excluded.

Compound 7b. Bile fractions (0−0.5 h to 1.5−2.0 h) from iv-dosed rats contained three major (I, II, III on Ultracarb column chromatograms) and two minor metabolites (ion current peaks were absent from bile collected immediately before administration of the drug) corresponding to glucuronides of the hydroxylated drug (*m*/*z* 638 for ammonium adduct). Superior resolution of these compounds was obtained at shorter retention times with the Nucleosil column. The proportions of the major metabolites varied considerably with time but not

Table 5. Electrospray Mass Spectra[a] of Biliary Metabolites of **5b** in the Rat

proposed metabolite	MS *m/z*
DHA glucuronide (**13**)	478 ($[M + NH_4]^+$, 100), 267 ($[M + NH_4 - NH_3 - DHG - H_2O]^+$, 11), 221 ($[267 - CO - H_2O]^+$, 4), 163 ($[221 - (CH_3)_2CO]^+$, 22)
OH-phenylDHA glucuronide (**15**)	570 ($[M + NH_4]^+$, 100), 267 ($[M + NH_4 - NH_3 - DHG -$ dihydroxybenzene$]^+$, 37), 221 ($[267 - CO - H_2O]^+$, 6), 163 ($[221 - (CH_3)_2CO]^+$, 36)

[a] Spectra were acquired at a cone voltage of 50 V; DHG = dehydroglucuronic acid.

Table 6. Electrospray Mass Spectra[a] of Biliary Metabolites of Compound **7b** in the Rat

proposed metabolite	MS *m/z*
II (**16**) (furano acetate gluc)	638 ($[M + NH_4]^+$, 100), 384 ($[M + NH_4 - NH_3 - CH_3CO_2H - DHG - H_2O]^+$, 60), 338 ($[384 - CO - H_2O]^+$, 55)
III (**17**) (OH-TFMphenylDHA gluc)	638 ($[M + NH_4]^+$, 57), 381 ($[M + NH_4 - NH_3 - DHG - H_2O - CO - H_2O]^+$, 100), 219 ($[381 -$ (p-TFMphenol)$]^+$, 81)

[a] Spectra were acquired at a cone voltage of 50 V; gluc = glucuronic acid.

consistently from one animal to another. *DHA glucuronide was not seen*.

Cone-voltage fragmentation of metabolite II **(16)** (t_R 23.5 min) at 50 V included loss of acetic acid and thereby suggested a furano acetate structure (Table 6). The fragmentation of III **(17)** (24 min) was indicative of a conjugate with an intact endoperoxide function. The parent ion was notable for the ease with which it fragmented extensively even at very low cone voltages (<30 V). Bile (80 μL) which contained a very high proportion of metabolite II **(16)** was diluted (1:1, v/v) with sodium acetate buffer and incubated with H-2 preparation (12 μL) at 37 °C for 24 h. The incubation was analyzed by LC−MS after 3 and 24 h. The minor *m/z* 638 peaks disappeared within 3 h, but metabolite II was still present at a considerable intensity after 24 h. As in the case of **5b**, isomerization of the hydroxylated parent drug may precede glucuronylation.

Compound 8b. Bile fractions (hourly collections) from an iv-dosed animal contained a peak in the mass chromatogram for *m/z* 570 which was not present in predosing bile and coeluted with the putative glucuronide of hydroxyphenylDHA excreted by the rats given phenylDHA. It was present, in diminishing amounts, in the first and second hourly fractions but not in the third. Another possible metabolite (t_R 20.0 min), which again was absent from the predose bile, yielded *m/z* 600, corresponding to the glucuronide of the hydroxylated drug. *DHA glucuronide was not found*.

Bile (0−1 h collection) was incubated with H-2 β-glucuronidase−arylsulfohydrolase preparation in the same manner as bile from the rats administered either DHA or phenylDHA. The peaks of *m/z* 570 and 600 corresponding, respectively, to the putative hpDHA and hydroxylated *p*-methoxyphenylDHA glucuronides were absent from the enzymic incubations after 5 h.

Discussion

Artemether (Paluther, **4a**; Rhone-Poulenc-Rorer) is the *O*-methyl ether of DHA and a first-generation derivative of artemisinin (quinghaosu), the principal antimalarial constituent of the medicinal plant *Artemisia annua*.[21,22]Artemether is active against the erythrocytic stage of multidrug-resistant *P. falciparum*.[22] Oral, rectal, and intramuscular regimens are generally rapidly effective and well-tolerated for both severe and uncomplicated falciparum malaria, though short-term monotherapy has been associated with high rates of recrudesence. Combinations of artemether with nonendoperoxide drugs such as mefloquine and lumefantrine have been investigated.[23]

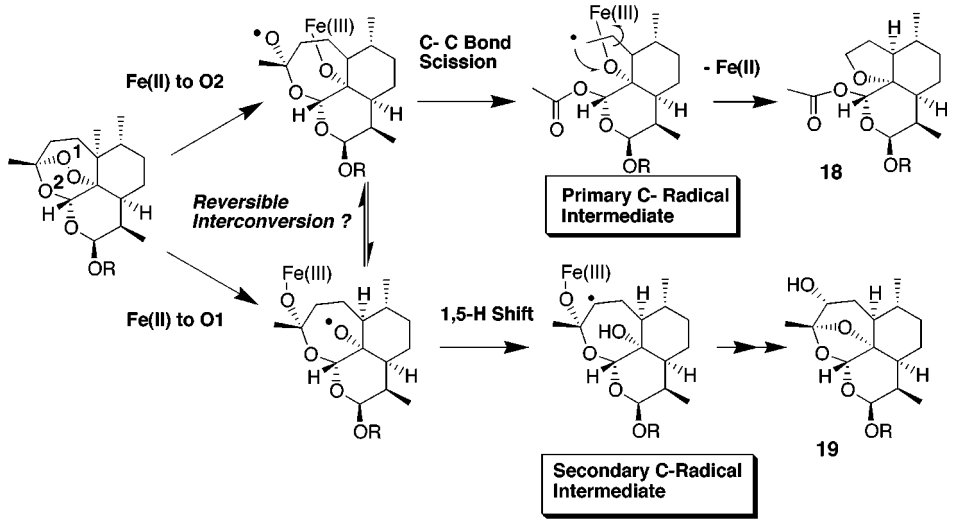
Artemether is regarded as a prodrug of DHA in humans though the two endoperoxides have similar antiparasitic activities in vitro. Oral doses are absorbed rapidly and converted extensively to DHA in humans and rats. Systemic drug exposure is generally greater when artemether is given intramuscularly, but the bioavailability in severe malaria may be highly variable.[24] DHA in plasma is the only known human metabolite of artemether, and plasma DHA has been the only identified metabolite in rats.[25]

Since oxidative metabolism of artemether yields DHA, a metabolite associated with neurotoxicity in high doses and rapid O-glucuronidation to an inactive conjugate, several groups have investigated the syntheses and biological activity of *C*-10-carba analogues of the parent drug. For example, Jung[5,26] and Haynes and Vonwiller[27] have shown that several *C*-10-alkyl and -aryl anlogues of *C*-10-deoxoartemisinin have significant in vitro antimalarial activity. Pu and Ziffer, using allylsilane chemistry, were able to prepare *C*-10-deoxoalkyl derivatives which had potent antimalarial activity in vivo.[28] Further studies have improved the synthesis of allyldeoxo artemisinin, and we have recently prepared 10 fluorinated *C*-10-carba analogues, some of which were more than 15 times as active as the parent drug.[29] Further major advances in the preparation of *C*-10-deoxo derivatives were recently reported by Posner.[30]

On the basis of the mechanism for the metabolic conversion of artemether to DHA, we proposed that the incorporation of an aryl group in place of the methyl in **4a** would prevent oxidative formation of DHA in vivo.[31] For this approach to be considered a viable alternative to the incorporation of carbon at the C-10 position, in terms of retarding metabolism to DHA and thereby increasing the drug's half-life, it was necessary to demonstrate (i) that phenoxy derivatives are potent antimalarials both in vitro and in vivo and (ii) that these compounds are resistant to oxidative dearylation.

The data shown in Tables 1 and 2 confirm that all of the phenoxy derivatives of **3** are potent antimalarials with IC_{50} values comparable to that of artemether. The parent derivatives (**5a** and **5b**) and naphthyl derivative (**11a**), for instance, are slightly more potent than artemether. In general compounds in the α-series have similar biological activity to their β-diastereomers against the K1 strain. The phenoxy derivatives were also tested against the chloroquine-sensitive HB3 strain (Table 2).

Scheme 7



The most potent β-isomers were the phenyl (**5b**), 4-methylphenyl (**6b**), and 4-fluorophenyl (**9b**) DHA derivatives which were all more potent than artemether. In the α-series, the phenyl (**5a**), 4-methylphenyl (**6a**), 4-methoxyphenyl (**8a**), and naphthoxy derivatives of DHA were all more potent than artemether. On the basis of the excellent chemical yields and excellent stereoselectivities obtained for the β-phenoxy trioxane **7b**, this compound and the parent β-substituted analogue **5b** were selected for evaluation versus *P. berghei* in the mouse model. Compound **7b** demonstrated potent activity, with an ED_{50} value directly comparable to that of DHA and superior to that of artemether (Table 3). Good activity was also displayed by the parent derivative **5b**. In in vivo screens performed by the WHO this derivative was significantly more potent than the clinically used sodium artesunate, regardless of the route of administration (Table 4).

To gain insight into the potential mechanism of peroxide antimalarials, several workers have investigated the biomimetic Fe(II)-mediated degradation of a range of 1,2,4-trioxanes, artemisinin derivatives, and synthetic endoperoxides.[32−34] These studies provide surrogate chemical markers of radical formation. The two major products obtained from the Fe(II)-mediated degradation of artemisinin and artemether **18** (R = Me) are a ring-contracted THF acetate **18** (R = Me for artemether) and the hydroxydeoxo derivative **19** (R = Me for artemether).[34] The formation of the former product is indicative of the formation of a primary C-centered radical intermediate, whereas the formation of **19** (R = Me) indicates a secondary C-centered radical intermediate (Scheme 7).

Significantly in the presence of very low concentrations of non-heme Fe(II), the primary radical derived from **1** forms a covalent adduct with cysteine.[35] It was suggested that this mode of reactivity may be responsible for alkylation of parasite proteins by artemisinin.[36] Alternatively, based on mechanistic work of Posner and Oh, the radical at the C-4 position of the carbon framework of artemisinin has been implicated as a potential mediator of antimalarial activity.[37] Recently, evidence for the formation of this radical during ferrous-mediated reductive activation was provided by Wu, who

Table 7. In Vitro Fe(II)-Mediated Degradation of Phenoxy Derivative **8b**

Fe(II) salt	solvent	*T* (°C)	ratio **18:19**	reaction time	yield of products (%)
$FeSO_4 \cdot 7H_2O$	CH_3CN	rt	3:1	5 days	80[a]
$FeSO_4 \cdot 7H_2O$	CH_3CN/H_2O	37	1:1	48 h	65[a]
$FeCl_2 \cdot 4H_2O$	CH_3CN	rt	9:1	30 min	78

[a] Starting material was recovered in these reactions.

demonstrated that the C-4 radical can be efficiently spin-trapped by MNP.[38]

The *p*-methoxy analogue **8b** was allowed to react with $FeCl_2 \cdot 4H_2O$, and in line with the previous work of Jefford on artemether, the two main products obtained from the reaction were **18** (R = *p*-MeO-Ar) and **19** (R = *p*-MeO-Ar). Notably, when $FeCl_2 \cdot 4H_2O$ was utilized, **18** was always the major product with a typical ratio of 9:1 (**18**:**19**). In contrast, degradation of **8b** with $FeSO_4$ resulted in a much slower reaction: with 20% of the starting material still present after 5 days at ambient temperature. Heating the reaction to 37 °C produced 65% turnover after 48 h. Interestingly, in this latter reaction, the ratio of products was 1:1 (Table 7), indicating that both primary and secondary radicals are formed as intermediates.

To obtain evidence for the generation of the radical intermediates shown in Scheme 7, we investigated the electron-transfer reactions with ESR spin-trapping techniques using conditions recently reported by Butler and co-workers for artemether.[34] Using ferrous sulfate and the water-soluble spin-trap DBNBS (sodium 3,5-dibromo-4-nitrosobenzenesulfonate), the ESR signal observed after 4 h indicated two spin-trapped radicals. From the intensities of the signals, the secondary C-spin-trapped radical **19a** was the major product (Scheme 8).

However, in contrast to the study by Wu et al. on artemisinin, where MNP was used as the spin-trapping agent, the spin-trapped secondary radical obtained here was always accompanied by quantities of the corresponding primary spin-trapped product **18a**. This difference could be a result of subtle differences in the reactivity of MNP (2-methyl-2-nitrosopropane) and DBNBS with carbon radical species. The measured hyperfine constants are listed in Table 8 and are compared

Scheme 8

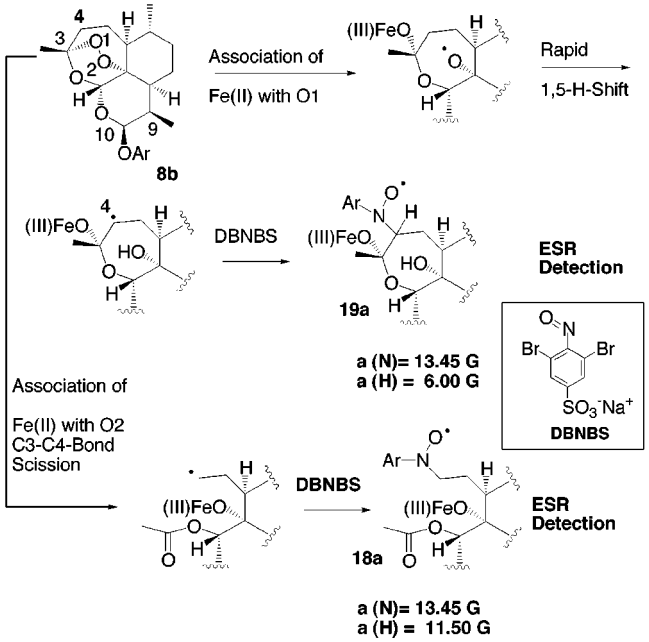


Table 8. ESR Parameters of the Carbon Radicals Formed on Reaction of Phenoxy Derivative **8b** with $FeSO_4$ in the Presence of DBNBS in Aqueous Acetonitrile (1:1)[a]

endoperoxide	carbon radical adduct	*a* N (G)	*a* H (G)
compound **8b**	primary **18a**	13.45	11.50
	secondary **19a**	13.45	6.00
artemisinin (**2**)	primary	13.6	12.0
	secondary	13.30	6.80
artemether (**4a**)	primary	13.60	12.20
	secondary	13.50	7.50

[a]Values are compared with those of artemether and artemisinin.

with those obtained for artemether and artemisinin. Thus, it is likely that these phenoxy derivatives behave in a manner similar to artemether and artemisinin, and we propose that their potent antimalarial activity is mediated by the formation of carbon radical species in the parasite's food vacuole.

The structure−metabolism relationships of the selected phenoxy derivatives were investigated in the bile-duct-cannulated rat with particular reference to the following considerations: (i) the blocking of *O*-glucuronylation, (ii) the redirection of hydroxylation from the artemisinin ring system to the substituent, and (iii) the stability of the endoperoxide bridge. With respect to proof of concept particular importance was attached to the ability of these derivatives to form DHA. The glucuronylation of DHA, reduction of the endoperoxide bridge, and with few exceptions hydroxylation of the artemisinin ring system (in arteether) abolish parasiticidal activity in vitro. Since our previous studies[25] have shown that the glucuronide of DHA is excreted exclusively in bile, the model used in this study was considered a suitable means of investigating the capacity of the phenoxy derivatives to resist metabolism to DHA.

Studies on **5b** established that this compound underwent dephenylation. The formation of DHA glucuronide from **5b** in vivo implies that the C-10 position is readily hydroxylated which is, presumably, the necessary preliminary step to dearylation. Substitution of a phenyl ring for the *C*(*O*)-10-methyl of artemether not only redirects hydroxylation from the endoperoxide ring system to the C-10 substituent but also restricts the regioselectivity of hydroxylation to the extent that only one hydroxyphenyl DHA glucuronide (**15**) was located in bile. These results are in sharp contrast to the metabolism of artemether in vivo, in which several hydroxylated metabolites were observed.

A *p*-trifluoromethyl group on the phenyl ring not only blocks dearylation entirely but also has a profound effect on hydroxylation, which now occurs at several sites (at least five) on the molecule. The most plausible interpretation is that the hydroxylation reactions have been redirected to the artemisinin ring system; thereby the oxidative metabolism of **5b** resembles that of artemether and arteether. A notable feature of this derivative's fate in the rat is the complete absence of metabolites from bile following oral administration. This appears to be a biological phenomenon rather than an artifact of the experimental system since DHA administered in the same manner (as well as by iv injection) is eliminated extensively in bile as its glucuronide.

A *p*-methoxy group on the phenyl ring also precludes dearylation but does not have such a marked facilitating effect on hydroxylation because a competing reaction, namely, O-demethylation, appears to unmask an hydroxyl function which is readily glucuronylated in vivo. The metabolite obtained by this process was also formed from **5b**, presumably in this case by seqeuential oxidation at the *para* position and glucuronidation.

The fact that no dearylation of either **7b** or **8b** occurred in these studies is significant since it indicates that *para*-substituted phenoxy derivatives in general may not form DHA metabolically in vivo. Consequently, compounds such as **7b** would be expected to have longer half-lives and potentially lower neurotoxicity. Further work is required to determine the in vivo pharmacokinetics and hydrolytic stability[41] of these lead phenoxy derivatives of dihydroartemisinin which appear to be promising new candidates with potential advantages over existing derivatives.

Summary

We have investigated the synthesis, in vitro and in vivo antimalrial activity, and metabolism of a new series of *C*-10-phenoxy derivatives of artemisinin. Biomimetic degradation with Fe(II) salts and ESR spectroscopy suggest that these derivatives readily form both secondary and primary C-centered radicals. Intraparasitic formation of these potentially cytotoxic radicals *might be* responsible for the derivatives high antimalarial activity. Drug metabolism studies have shown that chemical substitution at the *para* position of the phenyl ring can prevent DHA formation in the rat. This finding, coupled with the ease of synthesis and potent oral antimalarial potency, makes this class of antimalarials worthy of further investigation as next-generation 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 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.

^{1}H NMR spectra were recorded using Perkin-Elmer R34 (220 MHz) and Bruker (400 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.

General Procedure 1. To a stirred solution of dihydroartemisinin (1.0 g, 3.54 mmol) in anhydrous dichloromethane (25 mL) were added phenol (10.5 mmol) and silver perchlorate (0.15 g, 0.72 mmol). The resulting solution was cooled to −78 °C (CO_2/acetone) and trimethylsilyl triflate (1.0 mL, 5.00 mmol) added. The reaction was allowed to stir for 1 h. After this time the reaction was quenched with triethylamine (1.5 mL) and allowed to warm to room temperature. The suspension was filtered, and the solvent removed in vacuo. Purification by column chromatography gave the required products as β- and α-isomers.

10β-Phenoxydihydroartemisinin (5b) and 10α- Phenoxydihydroartemisinin (5a). These compounds were prepared by general procedure 1 to give the products as white cystalline products in 68% yield with a β/α ratio of 4:1. Data for β-isomer **5b:** mp 104−106 °C; $[\alpha]_D$ +204° (*c* 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.29 (2H, t, *J* = 8.81 Hz), 7.22 (2H, d, *J* = 8.81 Hz), 6.99 (1H, t, *J* = 7.9 Hz), 5.50 (1H, d, *J* = 3.30 Hz), 2.81 (1H, m), 2.39 (1H, dt), 1.23−2.07 (10H, m), 1.44 (3H, s), 1.13 (3H, d, *J* = 7.40 Hz), 0.96 (3H, d, *J* = 6.0 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 157, 129, 122, 116, 104, 100, 88, 81, 52, 44, 37, 36, 34, 31, 26, 25, 24, 20, 12; IR (Nujol mull)/cm^{-1} 2926(CH), 1463, 1193, 875(O−O), 832 (O−O); MS (EI) 361 (M^+, 85), 267 (M^+ − OPh, 33), 221 (25).

Data for α-isomer **5a:** mp 143−145 °C; $[\alpha]_D$ −54.5° (*c* 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.25 (2H, t, *J* = 4.4 Hz), 7.11 (2H, d, *J* = 5.8 Hz), 7.00 (1H, t, *J* = 7.1 Hz) 5.49 (1H, s), 5.05 (1H, d, *J* = 9.5 Hz), 2.73 (1H, m), 2.42 (1H, dt), 1.26−2.10 (10H, m), 1.43 (3H, s), 0.99 (3H, d, *J* = 3.2 Hz), 0.97 (3H, d, *J* = 6.0 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 145, 129, 122, 117, 104.5, 100, 99, 91, 52, 45, 37, 36, 33, 26, 25, 22, 20, 12.5; IR (Nujol mull)/cm^{-1} 2925 (CH), 1040, 881 (O−O), 825 (O−O); MS (CI, NH_3) 378 ($[M + NH_4]^+$, 44), 332 (90), 301 ($[M + NH_4]^+$ − Ph, 5), 221 (100); HRMS (CI, NH_4^+) *m/z* calcd for $C_{21}H_{32}NO_5$ $[M + NH_4]^+$ 378.22805, found 378.22777

10β-(*p*-Methylphenoxy)dihydroartemisinin (6b) and 10α-(*p*-Methylphenoxy)dihydroartemisinin (6a). These compounds were prepared by general procedure 1 to give the products as white cystalline solids in 84% yield with a β/α ratio of 5:1. Data for β-isomer **6b**: mp 78−80 °C; $[\alpha]_D$ +175° (*c* 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.01 (4H, m, aromatic), 5.5 (1H, s), 5.43 (1H, d, *J* = 3.4 Hz), 2.78 (1H, m), 2.37 (1H, dt), 2.27 (3H, s, Me), 1.20−2.07 (10H, m), 1.40 (3H, s), 0.99 (3H, d, *J* = 5.7 Hz), 0.95 (3H, d, *J* = 6.0 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 156, 131, 129, 117, 104, 100, 88, 81, 77, 60, 53, 44.5, 37, 36, 35, 31, 26, 24, 21, 20, 14, 13; IR (Nujol mull)/cm^{-1} 2925 (CH), 1509, 1226, 1036, 979, 959, 876 (O−O); MS (FAB) 375 ($[M + H]^+$, 45), 329 (100), 267 ($[M + H]^+$ − OC_6H_4-Me, 22).

Data for α-isomer **6a**: mp 160−162 °C; $[\alpha]_D$ −59° (*c* 1.0, $CHCl_3$); ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.05 (4H, m, aromatic), 5.46 (1H, s), 4.98 (1H, d, *J* = 9.0 Hz), 2.71 (1H, m), 2.40 (1H, dt), 2.28 (3H, s, Me), 1.23−2.07 (10H, m), 1.42 (3H, s), 0.97 (6H, d, *J* = 4.7 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 156, 131.5, 130, 117, 104, 99, 91, 80, 52, 45, 37, 36, 34, 33, 26, 25, 22, 20.5, 20, 12.5; IR (Nujol mull)/cm^{-1} 2925 (CH), 1509, 1225, 1038, 878 (O−O), 818 (O−O); MS (FAB) 375 ($[M + H]^+$, 18), 329 (100), 267 ($[M + H]^+$ − OC_6H_4Me, 30). Anal. C 70.62, H 8.11, requires C 70.55, H 8.09.

10β-(*p*-Trifluoromethylphenoxy)dihydroartemisinin (7b) and 10α-(*p*-Trifluoromethylphenoxy)dihydroartemisinin (7a). These compounds were prepared by general procedure 1 to give the products as white cystalline solids in 68% yield with a β/α ratio of 8:1. Data for β-isomer **7b**: mp 141−143 °C; $[\alpha]_D$ +167° (*c* 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.55 (2H, d, *J* = 8.0 Hz); 7.20 (2H, d, *J* = 8.0 Hz), 5.57 (1H, d, *J* = 3.0 Hz), 5.45 (1H, s), 2.86 (1H, m), 2.36 (1H, dt), 1.21−2.05 (10H, m), 1.43 (3H, s), 1.02 (3H, d, *J* = 8.0 Hz), 0.95 (3H, d, *J* = 6.0 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 160, 127, 117, 104, 100, 88, 80, 52.5, 44, 37, 36, 35, 31, 26, 25, 24, 20, 13; IR (Nujol mull)/cm^{-1} 2925 (CH), 1461, 1377, 1328, 1240, 1151, 1108, 981, 876 (O−O); MS (CI, NH_3) 429 ($[M + H]^+$, 64), 411 ($[M + H]^+$ − F, 34), 289 (74), 221 (18); HRMS $[M + H]^+$ *m/z* calcd for $C_{22}H_{28}O_5F_3$ 429.18888, found 429.18818.

Data for α-isomer **7a**: mp 161−163 °C; $[\alpha]_D$ −39.5° (*c* 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.54 (2H, d, *J* = 9.0 Hz), 7.17 (2H, d, *J* = 8.0 Hz), 5.51 (1H, s), 5.10 (1H, d, *J* = 9.0 Hz), 2.78 (1H, m), 2.41 (1H, dt, *J* = 4.0 Hz), 1.26−2.09 (10H) 1.50 (3H, s), 0.98 (6H, d, *J* = 5.0 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 127, 117, 105, 98.5, 91, 80, 52, 45, 37, 36, 34, 32, 26, 22, 24, 20, 12; IR (Nujol mull)/cm^{-1} 2926 (CH), 1462, 1120, 1037, 879 (O−O), 838 (O−O); MS (CI, NH_3) 429 ($[M + H]^+$, 26), 383 (85), 289 (100), 221 (25), 43 (100).

10β-(*p*-Methoxyphenoxy)dihydroartemisinin (8b) and 10α-(*p*-Methoxyphenoxy)dihydroartemisinin (8a). These compounds were prepared by general procedure 1 to give the products as white cystalline solids in 73% yield with a β/α ratio of 4:1. Data for β-isomer **8b**: mp 118−120 °C; $[\alpha]_D$ +141.1° (*c* 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.03 (2H, m); 6.83 (2H, m), 5.53 (1H, s), 5.37 (1H, d, *J* = 3.30 Hz), 3.76 (3H, s, OMe), 2.78 (1H, m), 2.38 (1H, dt), 1.21−2.07 (10H, m), 1.43 (3H, s), 1.02 (3H, d, *J* = 7.2 Hz,), 0.95 (3H, d, *J* = 3.60 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 155, 152, 118, 115, 104, 102, 88, 81, 56, 53, 44, 37, 36, 35, 31, 26, 25, 24, 20, 13; IR (Nujol mull)/cm^{-1} 2990 (CH), 1421, 1262, 895 (O−O); MS (CI, NH_3) 408 ($[M + NH_4]^+$, 7%), 373 (24%), 348 (51%), 345 (100%), 221 (100%); HRMS $[M + NH_4]^+$ *m/z* calcd for $C_{22}H_{34}NO_6$ 408.23861, found 408.23798.

Data for α-isomer **8a**: mp 147−149 °C; $[\alpha]_D$ −43.5° (*c* 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.08 (2H, m), 6.80 (2H, m), 5.44 (1H, s), 4.92 (1H, d, *J* = 9.3 Hz), 3.76 (3H, s, OMe), 2.70 (1H, m), 2.40 (1H, dt), 1.22−2.07 (10H, m), 1.42 (3H, s), 1.02 (3H, d, *J* = 3.5 Hz), 0.96 (3H, d, *J* = 4.86 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 155, 152, 118.5, 114.5, 104, 100, 91, 80, 56, 52, 45, 37, 36, 34, 33, 26, 25, 22, 20, 12.5; IR (Nujol mull)/cm^{-1} 2934 (CH), 1468, 1423, 1379, 1224, 1131, 1029, 879 (O−O), 825 (O−O); MS (CI, NH_3) 390 ($[M + H]^+$, 16%), 221 (28%), 124 (80%).

10β-(*p*-Fluorophenoxy)dihydroartemisinin (9b) and 10α-(*p*-Fluorophenoxy)dihydroartemisinin (9a). These compounds were prepared by general procedure 1 to give the products as white cystalline solids in 60% yield with a β/α ratio of 4:1. Data for β-isomer **9b**: mp 75−77 °C; $[\alpha]_D$ +186° (*c* 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 6.91−7.10 (4H, m, aromatic), 5.51 (1H, s), 5.39 (1H, d, *J* = 3.3 Hz), 2.79 (1H, m), 2.38 (1H, dt), 1.21−2.07 (10H, m), 1.42 (3H, s, Me at C-3), 1.02 (3H, d, *J* = 7.2 Hz, Me at C-6), 0.97 (3H, d, *J* = 6.0 Hz, Me at C-9);^{13}C NMR (75 MHz, $CDCl_3$) δ 160, 156.5, 154, 118, 116, 104, 101, 86, 81, 52.5, 44, 37, 36, 35, 31, 26, 25, 20, 13; IR (Nujol mull)/cm^{-1} 2932 (CH), 1503, 1378, 1139, 1121, 1095, 877 (O−O), 832 (O−O); MS (EI), 379 (M^+, 39%), 361 (M^+ − F, 61%), 333 (69%), 289 (74%), 221 (18%).

Data for α-isomer **9a**: mp 154−156 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.05−7.09 (2H, m, aromatic), 6.93−6.98 (2H, m, aromatic), 5.46 (1H, s), 4.95 (1H, d, *J* = 9.3 Hz), 2.70 (1H, m), 2.40 (1H, dt, *J* = 13.8, 3.8 Hz), 1.25−2.07 (10H, m), 1.44 (3H, s, Me at C-3), 1.02 (3H, d, *J* = 3.0 Hz, Me at C-6), 0.98 (3H, d, *J* = 1.8 Hz, Me at C-9); ^{13}C NMR (75 MHz, $CDCl_3$) δ 160, 157, 154, 118.5, 118, 116, 115.5, 104.5, 100, 91, 80, 51.5, 45, 37, 36, 34, 32, 26, 25, 22, 20, 12; IR (Nujol mull)/cm^{-1} 2923 (CH), 1505, 1377, 1134, 1094, 881 (O−O), 833 (O−O). Anal. Calcd for $C_{21}H_{27}FO_5$: C 66.67, H 7.19. Found: C 66.49, H 7.19.

10β-(*p*-Chlorophenoxy)dihydroartemisinin (10b) and 10α-(*p*-Chlorophenoxy)dihydroartemisinin (10a). This mixture of compounds was prepared by general procedure 1. NMR analysis revealed a diastereomeric mixture of 4:1 (β/α). However, attempted separation of the mixture by silica gel chromatography failed.

10β-(Naphthyloxy)dihydroartemisinin (11b) and 11α-(Naphthyloxy)dihydroartemisinin (11a). These compounds were prepared by general procedure 1 to give the products as white cystalline solids in 84% yield with a β/α ratio of 4:1: Data for β-isomer **11b:** mp 138−140 °C; $[\alpha]_D$ +240° (*c* 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.75 (1H, d, *J* = 9.0 Hz, 1 × aromatic), 7.56 (1H, s, 1 × aromatic), 7.40 (3H, m, 3 × aromatic), 7.24 (2H, d, *J* = 9.0 Hz, 2 × aromatic), 5.66 (1H, d, *J* = 3.0 Hz), 5.55 (1H, s), 2.86 (1H, m), 2.40 (1H, dt), 1.46 (3H, s, Me at C-3), 1.23−2.12 (10H, m), 1.06 (3H, d, *J* = 8.0 Hz, Me at C-6), 0.97 (3H, d, *J* = 6.0 Hz, Me at C-9); ^{13}C NMR (75 MHz, $CDCl_3$) δ 155, 135, 129, 127, 126, 124, 119, 111, 100, 88, 81, 53, 44.5, 37, 36, 35, 31, 26, 25, 24, 20, 13; IR (Nujol mull)/cm^{-1} 2926 (CH), 1464, 1211, 977, 839 (O−O); MS (EI) 410 (M^+, 22%), 364 (100%), 221 (25%), 163 (61%), 144 (100%), 43 (72%). Found, M^+ 410.20913, $C_{25}H_{30}O_5$ requires 410.20932. Anal. Calcd for $C_{25}H_{30}O_5$: C 73.15, H 7.37. Found: C 72.92, H 7.41,

Data for α-isomer **11a**: mp 148−150 °C; $[\alpha]_D$ +16° (*c* 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.75 (2H, m, 2 × aromatic), 7.40 (5H, m, 5 × aromatic), 5.58 (1H, s), 5.33 (1H, d, *J* = 9.30 Hz), 2.78 (1H, m), 2.43 (1H, dt), 1.56 (3H, s, Me at C-3), 1.26−2.08 (10H, m), 1.06 (3H, d, *J* = 12.9 Hz, Me at C-6), 1.00 (3H, d, *J* = 7.2 Hz, Me at C-9); ^{13}C NMR (75 MHz, $CDCl_3$) δ 129, 128, 127, 126, 124, 119.5, 111, 99, 80, 52, 45, 37, 36, 34, 32.5, 26, 22, 20, 12.5; IR (Nujol mull)/cm^{-1} 2926 (CH), 1465, 1377, 1038, 879 (O−O), 839 (O−O); MS (EI) 410 (M^+, 100%), 364 (97%), 346 (82%), 321 (38%), 304 (54%), 221 (24%), 163 (93%), 144 (100%), 115 (58%), 43 (87%). Calcd for $C_{25}H_{30}O_5$ 410.20932, found 410.20913.

Ferrous-Mediated Degradation Reactions. To a room temperature stirred solution of **8b** (0.200 g, 0.51 mmol) in acetonitrile and water (1:1, 10 mL) under nitrogen atmosphere was added ferrous sulfate (0.140 g, 0.51 mmol, 1 equiv). The reaction mixture was heated to 37 °C and left for 2 days. The reaction mixture was then diluted with water (10 mL) and extracted with ethyl acetate (3 × 20 mL). The combined organic extracts were dried over anhydrous magnesium sulfate and concentrated in vacuo. Flash column chromatography using 25% ethyl acetate/75% *n*-hexane as the eluent afforded the THF acetate **18** (0.045 g, 22%), **19** (0.046 g, 23%) and recovered starting material **8b** (0.080 g, 40%).

Data for THF acetate **18**: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.07−7.02 (2H, m, Ar), 6.82−6.79 (2H, m, Ar), 6.47 (1H, s, 12-H), 5.20 (1H, d, *J* = 4.5 Hz, 10-H), 4.28 (1H, ddd, *J* = 9.3 Hz, 8.1 Hz, 2.1 Hz), 3.94 (1H, ddd, *J* = 16.5 Hz, 7.8 Hz, 7.8 Hz), 3.76 (3H, s, OMe), 2.55 (1H, m), 2.12 (3H, s, Me), 2.11 (1H, m), 1.96−1.73 (5H, m), 1.57 (1H, m), 1.43−1.26 (2H, m), 1.04 (3H, d, *J* = 7.5 Hz, Me), 0.94 (3H, d, *J* = 6.3 Hz, Me); ^{13}C NMR (75 MHz, $CDCl_3$) δ 168.8, 155.3, 152.2, 119.4, 114.5, 103.1, 88.9, 80.6, 68.7, 55.8, 55.6, 47.1, 35.9, 33.5, 30.6, 27.8, 24.7, 21.5, 20.5, 12.6; IR (Nujol mull)/cm^{-1} 2925, 1759, 1506, 1457, 1368, 1222, 1065, 1038, 969, 940. Anal. C 67.40, H 7.50; requires C 67.67, H 7.74.

Data for **19**: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.05−7.00 (2H, m, Ar), 6.84−6.80 (2H, m, Ar), 5.37 (1H, s, 12-H), 5.34 (1H, d, *J* = 4.2 Hz, 10-H), 3.76 (3H, s, OMe), 3.60 (1H, m, C*H*OH), 2.62 (1H, m), 2.02−1.75 (6H, m), 1.55 (3H, s, Me), 1.45 (1H, m), 1.28−1.23 (2H, m), 1.07 (3H, d, *J* = 7.2 Hz, Me), 0.89 (3H, d, *J* = 6.3 Hz, Me); ^{13}C NMR (75 MHz, $CDCl_3$) δ 154.9, 151.8, 118.0, 114.3, 108.2, 99.6, 94.2, 84.2, 69.8, 55.7, 42.4, 40.8, 34.8, 34.7, 30.5, 30.4, 25.0, 20.9, 18.4, 12.2; IR (Nujol mull)/cm^{-1} 3504, 2929, 1462, 1378, 1225, 1032, 979 and 871. Anal. C 67.45, H 7.38; requires C 67.67, H 7.74.

The reaction was repeated in acetonitrile using **8b** (0.200 g, 0.51 mmol) and ferrous sulfate (0.140 g, 0.51 mmol, 1 equiv). After 5 days, the reaction mixture was filtered through Celite and the filtrate concentrated in vacuo. Flash column chromatography using 25% ethyl acetate/75% *n*-hexane as the eluent afforded **18** (0.133 g, 66%), **19** (0.028 g, 14%) and recovered starting material (0.020 g, 10%). All analytical data agreed with those obtained for the products of the above reaction. The reaction was also carried out using the conditions of Jefford[32c] to afford a 9:1 mixture of **18:19**.

EPR Spectroscopy. All spectra were recorded at ambient temperatures. Typical spectrometer conditions were: center field 3368 G; field sweep 70 G; field modulation amplitude 1 G; time constant 320 ms; microwave power 10 mW; microwave frequency 9.45 GHz. Experiments involved the mixing of solutions of **8b** (typically 2 mM, concentrations throughout being after mixing), the spin-trap (typically, 40 mM for DBNBS) and finally the metal ion (typically 2 mM) in unbuffered deoxygenated aqueous acetonitrile (1:1) at pH 7.

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 Jenson.[39] Cultures were grown in flasks containing human erythrocytes (2−5%) with parasitemia in the range of 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.

(a) In Vitro Testing. Antimalarial activity was assessed with an adaption of the 48-h sensitivity assay of Desjardins et al.[40] using [^{3}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 consituted 100% parasite growth). After 24-h 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 log dose−response curve.

(b) In Vivo Testing. Male, random Swiss albino mice weighing 18−22 g were inoculated intraperitoneally with 10^7 parasitized erythrocytes with *P. berghi* NS strain. Animals were then dosed daily via two routes (intraperitoneal or oral) for 4 consecutative 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 log dose against parasitemia. The data shown in Table 4 were obtained from the WHO.

Drug Metabolism Studies. The structure−metabolism relationships of the selected phenoxy derivatives were investigated in the bile-duct-cannulated rat. Cannulated and anesthetized male Wistar rats were administered DHA and the following *O*-ether derivatives of DHA (35 μmol/kg) by either iv injection or gastric intubation. Aliquots of the bile (hourly or half-hourly collections) were analyzed by LC−electrospray MS using (i) a Columbus or Ultracarb 5-μm C-8 column (0.46 × 25 cm; Phenomenex, Macclesfield, Cheshire) with gradients of acetonitrile in 0.1 M ammonium acetate, pH 6.9: 20−35% over 15 min and 35−70% over 10 min (the gradient employed unless otherwise indicated); or 20−35% over 15 min, at 35% for 10 min and 35−70% over 10 min; (ii) a Nucleosil 5-μm C-8 column (0.32 × 25 cm; Phenomenex) with a gradient of acetonitrile, 20−35% over 15 min, 35−70% over 10 min (for **7b** metabolites only). The flow rate in all cases was 0.9 mL/min.

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