Journal of Medicinal Chemistry Article

J. Med. Chem. **2010,** *53,* 633–640 **633**
DOI: 10.1021/jm901216v

Modular Synthesis and in Vitro and in Vivo Antimalarial Assessment of C-10 Pyrrole Mannich Base Derivatives of Artemisinin

Bénédicte Pacorel,[†] Suet C. Leung,[†] Andrew V. Stachulski,[†] Jill Davies,[§] Livia Vivas,[∥] Hollie Lander,[∥] Stephen A. Ward,[§] Marcel Kaiser,[∥] Reto Brun,[∥] and Paul M. O'Neill*[,†,‡]

[†]*Department of Chemistry, University of Liverpool, Liverpool L69 7ZD, U.K.,* [‡]*MRC Centre for Drug Safety Science, Department of Pharmacology, School of Biomedical Sciences, University of Liverpool, Liverpool L69 3GE, U.K.,* [§]*Liverpool School of Tropical Medicine, Pembroke Place, Liverpool L3 5QA, U.K.,* [∥]*Department of Infectious and Tropical Diseases, London School of Hygiene and Tropical Medicine, Keppel Street, London WC1E 7HT, U.K., and Swiss Tropical Institute, Parasite Chemotherapy, Socinstrasse 57, P.O. Box CH-4002, Basel, Switzerland*

Received August 14, 2009

In two steps from dihydroartemisinin, a small array of 16 semisynthetic C-10 pyrrole Mannich artemisinin derivatives (**7a**−**p**) have been prepared in moderate to excellent yield. In vitro analysis against both chloroquine sensitive and resistant strains has demonstrated that these analogues have nanomolar antimalarial activity, with several compounds being more than 3 times more potent than the natural product artemisinin. In addition to a potent antimalarial profile, these molecules also have very high in vitro therapeutic indices. Analysis of the optimal Mannich side chain substitution for in vitro and in vivo activity reveals that the morpholine and *N*-methylpiperazine Mannich side chains provide analogues with the best activity profiles, both in vitro and in vivo in the Peter's 4 day test.

The therapeutic value of artemisinin (**1**), *qinghaosu*, is limited to a great extent by its low solubility in both oil and water. Therefore, a number of more soluble derivatives have been developed, such as DHA[a] (**2**), artemether (**3**),[1] arteether (**4**),[2] and sodium artesunate (**5**)[3,4] (Figure 1). Although artemisinins (**1**−**5**) are the most efficient and fast acting antimalarial drugs used in malaria chemotherapy, the current first-generation semisynthetics (**2**−**5**) are cleared from blood within 2 h and parasites that are not eliminated within this time can re-emerge, resulting in parasite recrudescence. To prevent recrudescence,[5] the artemisinins are used in combination therapies with drugs that have longer half-lives (e.g., amodiaquine, mefloquine, piperaquine, and lumefantrine).[6−9]

To treat advanced cases of *Plasmodium falciparum* malaria, a water-soluble derivative of artemisinin is required, which can be delivered quickly by intramuscular injection.[3] The water-soluble sodium artesunate is currently the drug of choice[10] and is administered in combination therapy most often with mefloquine.[11]

In terms of semisynthetic analogues, the major challenge in this field is to prepare analogues from DHA in only one or two high-yield steps to provide analogues with a log *P* of <4 (ideally 3.25−3.75) and enhanced metabolic and chemical stability compared to artesunate (**5**) or artemether (**3**).[12] Artemisone (**6**) is a molecule that fulfils both of these criteria available in only three steps from DHA, an excellent in vitro and in vivo antimalarial activity profile with enhanced metabolic stability with acceptable exposure profiles and half-life following oral administration.[13]

In this paper, we describe a modular approach to new, more water-soluble analogues of DHA based on the C-10 pyrrole template **7**. Several "mechanism-based approaches" already have been investigated for improving the antimalarial activity of artemisinin-based trioxane derivatives; these include the incorporation of groups to enhance the stability of proposed "parasiticidal intermediates"[14] and the covalent attachment of "iron chelator functionality"[15] to enhance the interaction of the peroxide bridge with available "chelatable iron"[16] in the food vacuole of the parasite. From our earlier work, we proposed that the incorporation of protonatable amino functionality into an artemisinin derivative would enhance drug activity by increasing the level of cellular accumulation within the acidic (pH 4.7) parasite food vacuole by "ion trapping".[17] The higher concentration of drug available for interaction with heme or non-heme iron may have been responsible for the increased antimalarial activity observed in this work. The aim of this work was to prepare C-10 pyrrole analogues with alkylamine side chains. The C-10 pyrrole analogues were chosen for the following reasons. (i) C-10 aryl artemisinins or C-10 heteroaryl systems cannot generate DHA by hydrolysis or metabolism. (ii) The Mannich side chain provides molecules that have the potential to be formulated as salts, and as discussed, amine analogues should accumulate to higher concentrations than nonbasic derivatives such as artemether by an ion trapping mechanism.

*To whom correspondence should be addressed: Department of Chemistry, University of Liverpool, Liverpool L69 7ZD, U.K. Phone: +44 (0)151 794 3553. Fax: +44 (0)151 794 3588. E-mail: p.m.oneill01@liv.ac.uk.

[a] Abbreviations: AcOH, acetic acid; DCM, dichloromethane; DHA, dihydroartemisinin; DMAP, 4-(dimethylamino)pyridine; ED_{50}, effective dose; MSD, mean survival time in days; IC_{50}, half-maximal inhibitory concentration; NMO, *N*-methylmorpholine *N*-oxide; NMR, nuclear magnetic resonance; rt, room temperature; TI, Therapetic Index; TLC, thin layer chromatography; TPAP, tetrapropylammonium perruthenate.

Published on Web 12/03/2009

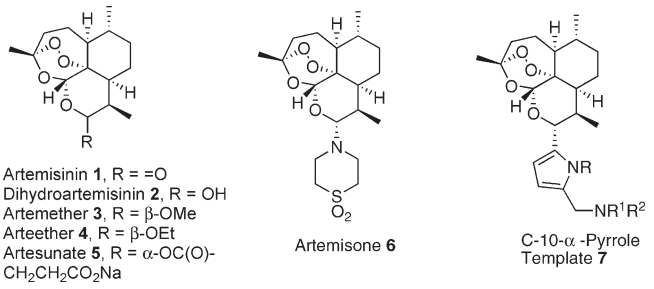


Figure 1. Artemisinin and semisynthetic analogues **2**−**7**.

Results and Discussion

Chemistry. Three different approaches were explored for the synthesis. First, C-10 analogues **8** and **9** were synthesized directly from **2** in the presence of a Lewis acid using the pyrrole and *N*-methylpyrrole (Scheme 1A). This particular heterocycle was chosen as the electron rich ring provides scope for the introduction of electrophiles at the C-5 position following incorporation into the artemisinin scaffold at the C-2 position.

The stereochemistry at the C-10 position was determined by ^{1}H NMR spectroscopy. The signal due to H10 appears as a doublet at 4.49 ppm with a $^3J_{H10-H9}$ value of 10.8 Hz, which is indicative of a *trans*−*trans* diaxial relationship between H10 and H9 (Scheme 1B).[18] This stereochemistry results from the bulky pyrrole nucleophile attacking the oxonium intermediate in an equatorial manner to minimize steric interaction with the C-9 β-methyl group. We also examined the synthesis of **8** via acetate **11** formed by treatment of DHA with acetic anhydride in DMAP.[19] The $BF_3 \cdot Et_2O$-catalyzed reaction of **11** with the pyrrole led to the formation of product **8** (via oxonium **13**) in good yield with minor amounts of side product **10** as shown (Scheme 2). Our observations are in accordance with the results obtained by the Posner group where a C-10β anomeric fluoride was employed in this Friedel−Crafts type chemistry.[20] In their approach, *N*-methylpyrrole was produced in 84% yield. We have also previously used the C-10 anomeric benzoate as a leaving group in the synthesis of C-10 carba analogues of artemisinin.[21] Thus, we also examined the reactivity of benzoate **12** prepared from benzoyl chloride, dihydroartemisinin, and pyridine as the nucleophilic catalyst.[19] In our hands, **12** proved to be a worse substrate for this reaction with a lower yield for target molecule **8**. It can be concluded that there is no benefit from synthesis via C-10α acetate or C-10α benzoate derivatives as the overall yields were not as good as that for the direct route from **2** (77% yield).

Having developed a high-yielding route to **8** and **9**, we decided to employ the Mannich reaction to incorporate the requisite amino alkyl side chain (Scheme 3). As noted via the amino functionality in these analogues and expected enhanced water solubility, we anticipated improved in vitro and in vivo antimalarial activities based on the observations made with other semisynthetic amino alkyl artemisinin derivatives.[13,19,22] We chose to conduct this reaction on the pyrrole ring under acidic conditions using formaldehyde and several secondary amines. The reaction was allowed to proceed by sequentially dissolving **8** or **9** at room temperature in ethanol, followed by addition of secondary amine (3.2 equiv), formaldehyde (3.2 equiv), and acetic acid (1.0 mL in 5.0 mL of EtOH). The reaction mixture was then left for 0.5 h and the reaction quenched with sodium hydroxide. The crude product was extracted with dichloromethane and the organic phase washed with brine.

The conditions described above were used to prepare **7a**−**g** (purified by flash column chromatography) from **8** in acceptable yields (Table 1). The Mannich reactions gave lower yields with amines diethylamine, piperidine, and pyrrolidine. Similarly, **7h**−**p** were synthesized from **9** in very good yields (Table 1). The higher yields are probably explained by the fact that *N*-methylpyrrole **9** is slightly more electron rich than its N-H counterpart **8**. Repetition of the Mannich reaction with commercial Eschenmoser's salt (Scheme 3) gave **7g** and **7o** in 70 and 86% yields, respectively. As one can see from Table 1, a range of clogP values are encompassed within this array from 2.89 for **7p** to 4.53 for **7k**.

Biology. The antimalarial activity of the Mannich-based artemisinin derivatives was first evaluated in vitro against the chloroquine sensitive 3D7 strain of *P. falciparum*, and then selected compounds were examined against the chloroquine resistant K1 isolate with artemisinin and chloroquine as positive controls (Tables 2 and 3). For the Mannich analogues derived from **8**, in vitro testing versus the 3D7 strain revealed that the most potent analogue was morpholine derivative **7b** which was almost 5 times more potent than artemisinin. This molecule also had an excellent in vitro therapeutic index with an IC_{50} in the high micromolar region versus the control KB mammalian cell line. Two additional parent pyrrole analogues had slightly better potencies than artemisinin against 3D7, **7c** and **7d**. Surprisingly, compounds **7a**, **7e**, and **7g** had higher-than-anticipated IC_{50} values, and analysis of the testing ethanol stock solution by TLC revealed some decomposition of the samples with the appearance of several minor impurities. For this reason, these compounds were not selected further for biological evaluation; the remaining compounds (derived from *N*-methylpyrrole **9**) were shown to be stable in ethanol for 1 week at room temperature. For the *N*-methyl series, the compounds were all more potent than artemisinin and more stable apart from trioxane **7o** which was found to have poor stability in ethanol and other protic solvents.

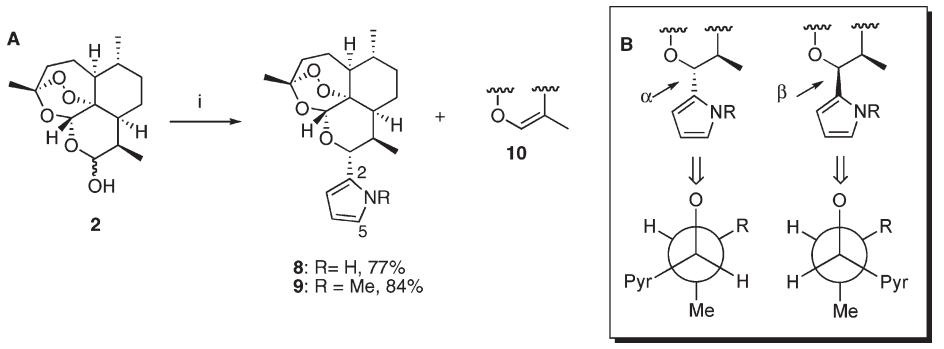
Selected compounds from both series were then assessed against the K1 strain. The best compounds from each series were morpholine derivatives **7b** and **7h**; the latter compound has an IC_{50} of 1.9, making it nearly 4 times more potent than artemisinin and 2 times as active as artemether. As a series, it is clear that these molecules have a very high in vitro therapeutic window with values ranging from 2000 to 25000.

On the basis of the chemical yield and in vitro performance, a selection of the compounds was screened for their in vivo activity against *Plasmodium berghei*. First, Peter's 4 day suppressive test was performed using 30 mg/kg over days 1−3 post-infection (Table 4). All the compounds tested displayed good activity, with **7b**, **7h**, and **7i** achieving 100% elimination of parasites according to this protocol.

Encouraged by the experiments, we conducted dose−response experiments with compounds **7b**, **7h**, and **7i** to determine ED_{50} and ED_{90} values which are definitive measurements of reduction of parasitemia by 50 and 90%, respectively, following oral dosing. All three compounds clearly outperformed the water-soluble sodium artesunate and the oil-soluble control trioxane artemether (Table 5). Data are also included for the activity of **9** from the previous work of Posner and co-workers.[20]

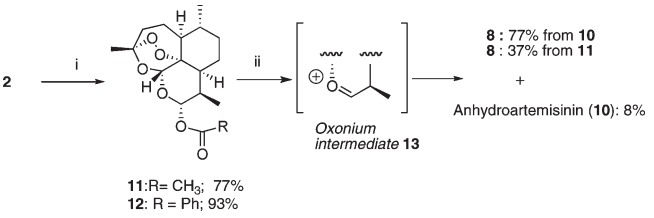
Finally, given the potential to formulate the piperazine analogues such as **7i**, some additional in vivo work was

Scheme 1. (A) Synthesis of **8**−**10**[a] and (B) Newman Projections of C-10α and C-10β Pyrrole Analogues



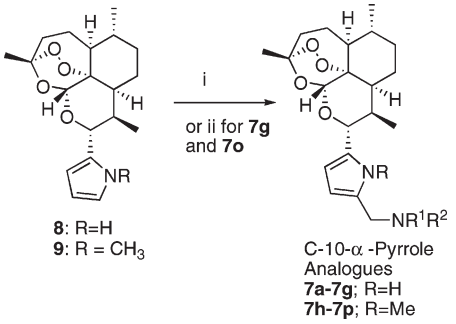
[a] (i) $BF_3 \cdot Et_2O$, pyrrole or *N*-methylpyrrole, CH_2Cl_2, −50 °C, 30 min. In the synthesis of both **8** and **9**, 15 and 10% of the side product anydroartemisinin (**10**), respectively, was produced.

Scheme 2[a]



[a] (i) DMAP, pyridine, acetic anhydride or benzoyl chloride, DCM; (ii) pyrrole, $BF_3 \cdot Et_2O$, DCM, −50 °C, 30 min.

Scheme 3[a]



[a] (i) CH_2O, secondary amine, AcOH, EtOH, rt, 30 min; (ii) $[CH_2N(CH_3)_2]^+I^-$, acetonitrile, rt, 24 h.

conducted at the Swiss Tropical Institute, and the data are compiled in Table 6. In these experiments, groups of three female NMRI mice (20−22 g) were intravenously infected with parasitized erythrocytes (2×10^7) on day 0 with GFP-transfected *P. berghei* strain ANKA.[24] The three Mannich derivatives were formulated in a standard suspending vehicle (SSV) and were administered orally on four consecutive days (4, 24, 48, and 72 h post-infection). Parasitemia was determined on day 4 post-infection (24 h after last treatment) by FACS analysis. Activity was calculated as the difference between the mean percent parasitemia for the control (n = 5 mice) and treated groups expressed as a percent relative to the control group.

In this assay, **7i** was the most potent, achieving 100% clearance of parasitemia 24 h after the last treatment and extending mouse survival to 9 days compared with 8 days for the artesunate control.

In terms of SAR within this series of molecules, it is clear that the morpholine, *N*-methylpiperazine, and sulfonylmorpholine heterocyles provide molecules with improved efficacy both in vitro and in vivo (for morpholine and *N*-methylpiperazine). These heterocycles also appear in some of the most potent semisynthetic analogues prepared to data Figure 2 (see 6, 14 and 17) and in totally synthetic tetraoxanes[25] being developed at Liverpool Figure 2 (see 15 and 16).

Artemisinins act via mechanisms that are distinct from those of other antimalarial classes. Antimalarial activity may arise from alkylation of vital intraparasitic biomolecules by free radicals generated within the malaria parasite through an iron(II)-induced degradation process.[26−28] The parasite death that ensues in the presence artemisinin is more likely to involve specific radicals and targets rather than nonspecific cell damage caused by freely diffusing oxygen- and carbon-centered radical species.

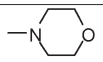
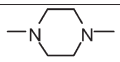
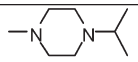
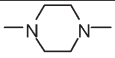
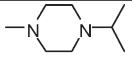
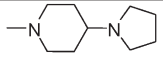
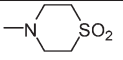
To determine the impact of the polar Mannich side chain on the iron-mediated degradation process, we examined the ferrous iron-mediated degradation of **7h** with iron(II) sulfate and iron(II) chloride. As anticipated, iron(II)-mediated degradation of **7h** gave two main products as shown in Scheme 4. Isolation of **18** and **19**, surrogate markers of primary and secondary C radicals, indicates that carbon-centered radical intermediates can be readily produced during iron-dependent cleavage of morpholine **7h** and that these species may play a role in the mechanism of action of these semisynthetic artemisinins (Table 7).

In summary, a small library of weak base and polar C-10 pyrrole analogues has been prepared with modular chemistry amenable to parallel synthesis methods. The in vitro antimalarial results have revealed that morpholine **7h** and *N*-methylpiperazine **7i** analogues have biological profiles superior to those of clinically used sodium artesunate. The in vivo profiles of **7h** and **7i** warrant further investigation, including combination and pharmacokinetic and preclinical toxicological evaluation to fully assess the potential of these compounds. Initial studies employing ferrous(II) salts indicate that members of this class of semisynthetic artemisinin generate both primary and secondary carbon-centered radicals in a manner similar to that of artemisinin. Further work is required to establish the role of these intermediates in the mechanism of action.

Experimental Section

Chemistry. Air- and moisture-sensitive reactions were conducted in oven-dried glassware sealed with rubber septa under a positive pressure of dry nitrogen or argon from a manifold or balloon. Similarly sensitive liquids and solutions were transferred via syringe. Reaction mixtures were stirred using

Table 1. Semisynthetic Artemisinins Prepared

Compound	Structure -R	Structure –R'	Clog P[a]	Yield (%)
8	-H	-	3.53	77
9	-Me	-	3.77	84
7a	-H		4.13	24
7b	-H		3.01	70
7c	-H		3.00	60
7d	-H		3.80	70
7e	-H		4.24	35
7f	-H		3.83	24
7g	-H		3.40	70
7h	-Me		3.33	75
7i	-Me		3.38	83
7j	-Me		4.12	76
7k	-Me		4.53	88
7l	-Me		4.14	97
7m	-Me		4.22	90
7n	-Me		4.46	54
7o	-Me		3.98	86
7p	-Me		2.89	35

[a] clogP values were calculated using AlogPS version 2.1. [23]

Teflon-coated magnetic stir bars. Organic solutions were concentrated using a Buchi rotary evaporator with a diaphragm vacuum pump.

(i) Purification of Reagents and Solvents. Anhydrous solvents were either obtained from commercial sources or dried and distilled immediately prior to use under a constant flow of dry nitrogen. DCM was distilled from CaH_2. All other reagents were used as received from commercial sources unless otherwise indicated.

(ii) Purification of Products. Analytical thin layer chromatography was performed with 0.25 mm silica gel 60F plates with 254 nm fluorescent indicator-coated aluminum sheets from Merck. Plates were visualized with ultraviolet light or by treatment with iodine, *p*-anisaldehyde, ninhydrin, or potassium permanganate followed by gentle heating. Chromatographic purification of products was accomplished by flash chromatography, as described by Still and co-workers.[29]

(iii) Analysis. Melting points were determined in open tubes in a Gallenkamp melting point apparatus and are uncorrected. NMR spectra were recorded on Bruker AC 200 (^{1}H, 200 MHz) and Bruker AMX 400 (^{1}H, 400 MHz; ^{13}C, 100 MHz) spectrometers. Chemical shifts are described in parts per million downfield from an internal standard of trimethylsilane. Multiplicities are recorded as broad peaks (br), singlets (s), doublets (d), triplets (t), quartets (q), doublets of doublets (dd), doublets of triplets (dt), and multiplets (m). Coupling values are in hertz. Mass spectra were recorded on a VG analytical 7070E machine and Frisons TRIO spectrometers using electron ionization (EI),

Table 2. In Vitro Antimalarial Activity of Pyrrole Artemisinin Derivatives versus Chloroquine Sensitive 3D7 *P. falciparum*[a]

compound	IC_{50} (nM) for 3D7	IC_{50} (μM) for KB	TI (KB/3D7)	relative IC_{50} for artemisinin/ analogue IC_{50}
artemisinin	12.8	> 354	> 27656	1.00
artemether	4.2	ND[c]	ND[c]	ND[c]
chloroquine	29.6	112.5	3784	0.43
8	7.4	62.5	8446	1.73
9	5.2	60.3	11596	2.46
7a	75.8[b]	ND[c]	ND[c]	0.17
7b	2.5	56.5	22600	2.84
7c	6.5	85.2	13108	1.97
7d	8.5	45.3	5329	1.51
7e	59.4[b]	143.4	2414	0.22
7f	16.9	47.0	2781	0.76
7g	20.5[b]	43.6	2127	0.62
7h	3.5	86.2	24628	3.66
7i	6.5	96.5	14846	1.97
7j	5.1	ND[c]	ND[c]	2.51
7k	6.1	39.2	6426	2.10
7l	3.7	42.2	11405	3.46
7m	7.2	137.8	19139	1.78
7n	2.5	ND[c]	ND[c]	5.12
7o	31.6[b]	ND[c]	ND[c]	0.40
7p	2.2	68.32	31054	5.82

[a] Chloroquine was tested as the diphosphate salt. All other compounds were all tested as free bases. 3D7 is a chloroquine sensitive strain of *P. falciparum*. [b] IC_{50} values may underestimate potency due to compound degradation. [c] Not determined.

Table 3. In Vitro Antimalarial Activity of Pyrrole Mannich Analogues versus Chloroquine Resistant K1[a] *P. falciparum*

compound	IC_{50} (nM) for K1	relative IC_{50} for artemisinin/ analogue IC_{50}
artemisinin	8.2	1.00
artemether	3.4	2.41
chloroquine[b]	150.2	0.05
7b	3.2	2.56
7c	5.0	1.64
7d	3.5	2.34
7h	1.9	4.32
7i	3.8	2.16
7j	8.3	0.99
7n	7.3	1.12
7p	3.57	2.30

[a] K1 is a chloroquine resistant strain of *P. falciparum*. [b] Chloroquine was tested as the diphosphate salt. All other compounds were tested as free bases.

Table 4. Results of Peter's 4 Day Suppressive Test at a Dose of 3 × 30 mg/kg

compound	% inhibition for 30 mg/kg
artemether (**3**)	100
artesunate (**5**)	100
7b	100
7c	97.7
7d	90.5
7h	100
7i	100

chemical ionization (CI), or electron spray (ES). Infrared spectra were recorded on a PerkinElmer RX1 FT-IR spectrometer and are reported in wavenumbers (cm^{-1}). Microanalyses (%C, %H, %N) were performed in the University of Liverpool Microanalysis laboratory. Reported atomic percentages

Table 5. In Vivo Antimalarial Activities versus the *P. berghei* N Strain

endoperoxide	ED_{50} (mg/kg)	ED_{90} (mg/kg)
7b	2.11	4.3
7h	1.77	5.20
7i	1.99	5.34
9	4.50	8.50
artemether	5.88	10.57
artesunate	3.23	> 10

Table 6. In Vivo Antimalarial Activities versus the *P. berghei* ANKA Strain

endoperoxide	% inhibition[a] (10 mg/kg × 4)	MSD[b]
7c	98	7
7d	90	6
7i	100	9
artesunate	98	8

[a] Parasitemia was determined on day 4 post-infection (24 h after last treatment) by FACS analysis. Activity was calculated as the difference between the mean percent parasitemia for the control (n = 5 mice) and treated groups expressed as a percent relative to the control group. [b] The mean survival time in days (MSD) is recorded up to a maximum of 30 days after infection. A compound was considered curative if the animal survived to day 30 after infection with no detectable parasites.

are within error limits of ±0.4%. The artemisinin numbering scheme used by Oh was employed throughout this analysis.[30]

10α-(1*H*-Pyrrol-2-yl)artemisinin (8). A solution of DHA (**2**) (250 mg, 0.88 mmol) in DCM (15 mL) was treated sequentially with pyrrole (295 mg, 4.40 mmol) and $BF_3 \cdot Et_2O$ (187 mg, 1.32 mmol) and stirred at −50 °C for 1 h. The reaction was quenched with saturated $NaHCO_3$, and the mixture was extracted with DCM, washed with brine, dried with $MgSO_4$, and concentrated in vacuo. The crude product was purified by flash chromatography (10% EtOAc/*n*-hexanes) to give a clear oil (125 mg, 77%): R_f = 0.36 (25% EtOAc/*n*-hexanes); ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 8.64 (1H, s, NH), 6.76 (1H, dd, J = 2.5, 1.6, N-CH), 6.07 (1H, dd, J = 2.5, 5.5, pyr CH), 6.03 (1H, m, pyr CH), 5.40 (1H, s, H12), 4.49 (1H, d, J = 10.8, H10), 2.57 (1H, m, H9), 2.39 (1H, dt, J = 13.7, 4.1, H4α), 1.42 (3H, s, H14), 2.08−1.20 (10H, m), 0.97 (3H, d, J = 6.3, H15), 0.63 (3H, d, J = 7.1, H16); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 130.2, 117.7, 107.39, 106.8, 104.3, 92.1, 80.7, 72.1, 51.9, 45.9, 37.4, 36.4, 34.2, 33.1, 31.6, 26.1, 24.8, 22.6, 21.3, 20.3, 14.1, 14.0; HRMS calcd for $C_{19}H_{28}NO_4$ $[M + H]^+$ 334.2018, found 334.2012. Anal. ($C_{19}H_{27}NO_4$) C, H, N.

10α-(1-Methyl-pyrrol-2-yl)artemisinin (9). A solution of dihydroartemisinin (300 mg, 1.05 mmol) in DCM (25 mL) at room temperature was treated sequentially with *N*-methylpyrrole (0.47 mL, 5.29 mmol) and $BF_3 \cdot Et_2O$ (0.19 mL, 1.51 mmol), stirred for 10 min at rt, and then cooled at −50 °C for 20 min. The reaction was quenched with saturated $NaHCO_3$, and the mixture was extracted with DCM, washed with brine, dried with $MgSO_4$, and concentrated in vacuo. The crude product was purified by flash chromatography (10% EtOAc/*n*-hexanes) to give a colorless crystal (378 mg, 84%): mp 95 °C; R_f = 0.42 in 25% EtOAc/*n*-hexanes; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 6.54 (1H, t, J = 2.2, N-CH), 5.90 (2H, m, pyr CH), 5.38 (1H, s, H12), 4.50 (1H, d, J = 11.3, H10), 3.84 (3H, s, N-CH_3), 2.83 (1H, m, H9), 2.39 (1H, dt, J = 14.0, 4.1, H4α), 1.39 (3H, s, H14), 2.08−1.20 (10H, m), 0.98 (3H, d, J = 6.3, H15), 0.61 (3H, d, J = 7.2 Hz, H16); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 130.2, 124.2, 109.9, 106.6, 104.6, 92.3, 81.1, 72.9, 52.4, 46.3, 37.8, 36.7, 35.4, 34.6, 31.3, 26.4, 25.2, 21.3, 20.7, 14.8; HRMS calcd for $C_{20}H_{30}NO_4$ $[M + H]^+$ 348.2175, found 348.2174. Anal. ($C_{20}H_{29}NO_4$) C, H, N.

General Procedure for 7a−o. Formaldehyde (0.1 mL, 3.2 equiv) and a secondary amine (3.2 equiv) solution were added to **8** or **9** (150 mg, 1 equiv) in anhydrous ethanol (5 mL). Then

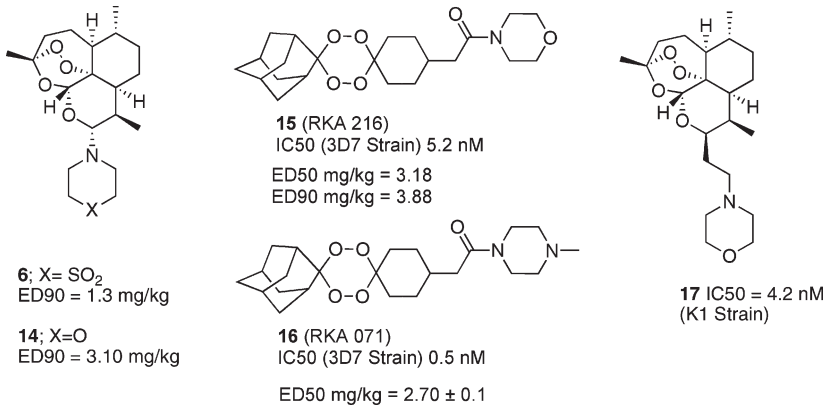
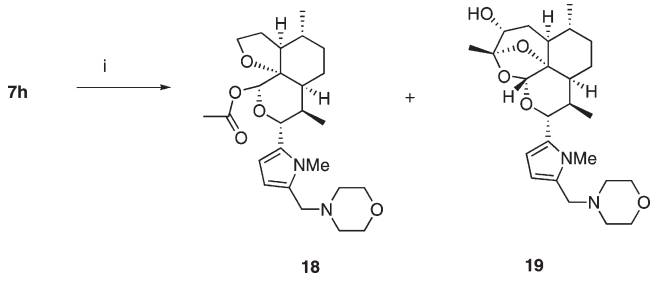


Figure 2. Potent semisynthetic and synthetic endoperoxides containing the morpholine,[13,23] sulfonylmorpholine,[13] and piperazine side chains.[18,23] Key data from original publications are included; ED_{50} and ED_{90} are for oral dosing in mice infected with *P. berghei*.

Scheme 4[a]



[a] (i) $FeSO_4$, 1/1 acetonitrile/water, 1 h, rt, or $FeCl_2 \cdot 4H_2O$, acetonitrile, 30 min, rt.

Table 7

iron(II)	yield for **18** (%)	yield for **19** (%)
$FeSO_4 \cdot 6H_2O$	72	21
$FeCl_2 \cdot 4H_2O$	42	23

glacial acetic acid (1.0 mL) was added to the reaction mixture, which was left at rt for 30 min. The reaction mixture was basified (pH 8) with a 2 M sodium hydroxide solution (5 mL). The mixture was extracted with EtOAc (3 × 25 mL), and combined organic extracts were washed with brine. The organic phase was dried over $MgSO_4$, filtered, and concentrated under reduced pressure to afford a crude product that was purified by flash chromatography using a 5% methanol/dichloromethane mixture.

10α-{5-[(Dimethylamino)methyl]-1*H*-pyrrol-2-yl}artemisinin (7g). Eschenmoser's salt (124 mg) was dissolved in the minimum amount of anhydrous acetonitrile and added dropwise over a period of 30 min to a solution of **8** (140 mg) in anhydrous acetonitrile (10 mL). The mixture was left to stir at room temperature for 24 h. The mixture was then basified (pH 8) with a 2 M NaOH solution (3.0 mL). The organic layer was then separated and the aqueous layer extracted with EtOAc (3 × 25 mL). The combined organic layers were washed with saturated H_2O and brine. The organic phase was then dried over $MgSO_4$, filtered, and concentrated under reduced pressure to afford a crude product that was purified by silica gel chromatography using a 10−30% MeOH/DCM mixture as the eluent. This gave **7g** (70% yield): colorless sticky solid; mp 60 °C; R_f = 0.13 in 10% MeOH/DCM; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 9.70 (1H, s, NH), 6.02 (2H, m, CH-CH [pyrrole]), 5.37 (1H, s, H12), 4.43 (1H, d, J = 10.8, H10), 3.78−3.69 (2H, AB quartet, J = 13.4, CH_2-N), 2.63 (1H, m, H9), 2.46 [6H, s, N-$(CH_3)_2$], 2.39 (1H, dt, J = 13.7, 4.1, H4α), 1.42 (3H, s, H14), 2.08−1.20 (10H, m), 0.97 (3H, d, J = 6.3, H15), 0.63 (3H, d, J = 7.1, H16); HRMS calcd for $C_{22}H_{35}N_2O_4$ $[M + H]^+$ 391.2597, found 391.2598. Anal. ($C_{22}H_{34}N_2O_4$) C, H, N.

10α-[1-Methyl-5-(sulfonylmorpholinomethyl)pyrrol-2-yl]artemisinin (7p). To a solution of **7m** (100 mg, 0.22 mmol), prepared as previously described, in DCM at rt under nitrogen were added NMO (76 mg, 0.65 mmol), powered molecular sieves (500 mg), and TPAP (10 mg, catalytic). The mixture was stirred at rt overnight and then filtered through a pad of silica, and the residue was washed with EtOAc (3 × 15 mL). The filtrate was concentrated in vacuo. The residue was then purified by flash chromatography (SiO_2; 35% EtOAc/*n*-hexanes) to give **7p** as a yellow solid (38 mg, 35% yield): mp 77 °C; R_f = 0.92 in 10% MeOH/DCM; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 5.90 (1H, d, J = 3.5, pyr CH), 5.88 (1H, d, J = 3.5, pyr CH), 5.38 (1H, s, H12), 4.48 (1H, d, J = 11.3, H10), 3.82 (3H, s, N-CH_3), 3.59−3.49 (2H, AB quartet, J = 13.7, CH_2), 3.00 (4H, m, thiomorpholine CH_2), 2.95 (4H, m, thiomorpholine CH_2), 2.85 (1H, m, H9), 2.39 (1H, dt, J = 14.0, 4.1, H4α), 1.39 (3H, s, H14), 2.08−1.20 (10H, m), 0.98 (3H, d, J = 6.3, H15), 0.57 (3H, d, J = 7.2, H16); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 131.2, 129.8, 109.2, 108.6, 104.6, 92.3, 81.0, 73.2, 55.5, 54.8, 52.4, 46.3, 37.8, 36.7, 34.6, 32.1, 31.2, 28.3, 26.4, 25.2, 21.3, 20.6, 14.8; HRMS calcd for $C_{25}H_{38}N_2O_6$-Na $[M + Na]^+$ 517.2348, found 517.2344.

$FeSO_4$-Mediated Degradation of 10α-[1-Methyl-5-(morpholinomethyl)-1*H*-pyrrol-2-yl]artemisinin with Iron(II) Sulfate. To a solution of **7h** (110 mg, 0.25 mmol) in acetonitrile (5 mL) and water (5 mL) was added $FeSO_4 \cdot 7H_2O$ (86 mg, 0.31 mmol). The reaction mixture was left stirring at rt for 1 h before being filtered through Celite and washed with acetonitrile. Concentration under reduced pressure and flash column chromatography using a 9/1 DCM/MeOH mixture as the eluent yielded the products **18** as a yellow oil (0.08 g, 72%) and **19** as a yellow oil (0.05 g, 21%).

(i) $FeCl_2$-Mediated Degradation of 10α-[1-Methyl-5-(morpholinomethyl)-1*H*-pyrrol-2-yl]artemisinin with Iron(II) Chloride. To a solution of **7h** (0.15 g, 0.34 mmol) in acetonitrile (13 mL) was added $FeCl_2 \cdot 4H_2O$ (74 mg, 0.34 mmol) under a nitrogen atmosphere. The reaction mixture was left stirring at room temperature for 30 min before being filtered through Celite and washed with acetonitrile. Concentration under reduced pressure and flash column chromatography using a 5/95 EtOAc/*n*-hexane mixture as the eluent yielded **18** (0.09 g, 42%) and **19** (0.03 g, 23%).

(ii) Furano acetate (18): yellow oil; R_f = 0.58 in 9/1 DCM/MeOH; ^{1}H NMR (400 MHz) δ_H 6.15 (1H, s, H12), 6.05 (1H, d, J = 3.6, pyr CH), 5.88 (1H, d, J = 3.6, pyr CH), 4.59 (1H, d, J = 11.1, H10), 4.27 (1H, t, J = 9.5, H4), 3.91 (1H, q, J = 8.0, H4), 3.68 (4H, m, morph CH_2), 3.61 (3H, s, N-CH_3), 3.36 (2H, AB quartet, J = 13.5, CH_2), 2.74 (1H, m, H9), 2.38 (4H, m, morph CH_2), 2.09 (3H, s, H14), 2.00−1.00 (9H, m), 0.95 (3H, d, J = 6.6, H15), 0.87 (3H, d, J = 7.0, H16); ^{13}C NMR (400 MHz) δ_C 169.8,

130.8, 108.8, 106.9, 92.9, 80.6, 71.8, 68.9, 67.5 (2C), 55.7, 55.5, 53.7 (2C), 48.5, 35.9, 32.4, 31.0, 30.9, 30.4, 28.1, 22.5, 22.0, 21.0, 15.0; HRMS calcd for $C_{25}H_{38}N_2O_5Na$ $[M + Na]^+$ 469.2678, found 469.2687.

(iii) 3α-Hydroxydeoxyartemisinin (19): yellow oil; $R_f = 0.48$ in 9/1 DCM/MeOH; ^1H NMR (400 MHz) δ_H 5.92 (1H, d, J = 3.5, pyr CH), 5.87 (1H, d, J = 3.5, pyr CH), 5.33 (1H, s, H12), 4.53 (1H, d, J = 10.8, H10), 3.72 (3H, s, N-CH_3), 3.66 (4H, m, morph CH_2), 3.57 (1H, brs, -OH), 3.38 (2H, AB quartet, J = 16.4, CH_2), 2.79 (1H, m, H9), 2.37 (4H, m, morph CH_2), 2.1−1.1 (9H, m), 1.55 (3H, s, H14), 0.90 (3H, d, J = 6.44, H15), 0.65 (3H, d, J = 7.2, H16); ^{13}C NMR (400 MHz) δ_C 131.8, 130.0, 108.8, 108.6, 107.7, 95.7, 84.5, 77.1, 71.8, 70.1, 67.5 (2C), 55.5, 53.7 (2C), 42.9, 35.3, 34.8, 31.8, 30.8, 30.3, 22.5, 21.4, 21.0, 14.6; HRMS calcd for $C_{25}H_{38}N_2O_5Na$ $[M + Na]^+$ 469.2678, found 469.2691.

Biology. (i) *P. falciparum* in Vitro Culture and Parasite Growth Inhibition Assays. All parasite clones, isolates, and strains were acquired from MR4 (Malaria Research and Reference Reagent Resource Center, Manassas, VA). Strains and isolates used in this study were the drug sensitive 3D7 clone of the NF54 isolate (unknown origin) and the chloroquine, pyrimethamine, and cycloguanyl resistant K1 strain (Thailand). In vitro culture of *P. falciparum* was conducted following standard methods[31] with modifications as described previously.[32] In vitro parasite growth inhibition was assessed by the incorporation of [^{3}H]hypoxanthine based on the method used by Desjardins[33] and modified as described previously.[34] Briefly, stock drug solutions were dissolved in 100% dimethyl sulfoxide (Sigma, Dorset, U.K.), and 50 μL of a 3-fold dilution series (10.0, 3.33, 0.111, 0.0370, 0.0123, and 0.0041 μg/mL) of the drugs prepared in assay medium [RPMI 1640 supplemented with 0.5% Albumax II (Invitrogen), 0.2% (w/v) glucose, 0.03% L-glutamine, and 5 μM hypoxanthine] was added to each well of 96-well plates in triplicate. Fifty microlitres of asynchronous (65−75% ring stage) *P. falciparum* culture (0.5% parasitemia) or uninfected erythrocytes (blank) were added to each well, reaching a final volume of 100 μL per well, a final hematocrit of 2.5%, and final dimethyl sulfoxide concentrations of ≤0.01%. Plates were incubated at 37 °C in a 5% CO_2/95% air mixture for 24 h, at which point 10 μL (0.2 μCi/well) of [^{3}H]hypoxanthine (Perkin-Elmer, Hounslow, U.K.) was added to each well. After an additional 24 h incubation period, the experiment was terminated by placing the plates in a −80 °C freezer. Plates were thawed and harvested onto glass fiber filter mats using a 96-well cell harvester (Harvester 96, Tomtec, Oxon, U.K.) and left to dry. After the addition of MeltiLex solid scintillant (Perkin-Elmer), the incorporated radioactivity was counted using a Wallac 1450 Betalux scintillation counter (Wallac).

Data acquired by the Wallac BetaLux scintillation counter were exported into a Microsoft Excel spreadsheet, and the IC_{50} values of each drug were calculated by using XLFit line fitting software (ID Business Solution). Chloroquine diphosphate, as a standard drug, and control wells with untreated infected and uninfected erythrocytes were included in all assays.

(ii) In Vitro Cytotoxicity Assay. The AlamarBlue (Accumed International) method was used to assess cytotoxicity to KB cells as previously described.[32] BrieXy, microtiter plates were seeded at a density of 4×10^4 KB cells/mL in RPMI 1640 culture medium supplemented with 10% heat-inactivated fetal calf serum (complete medium) (Seralab). Plates were incubated at 37 °C in a 5% CO_2/95% air mixture for 24 h followed by addition of the compound to triplicate wells in a dilution series in complete medium. The positive control drug was podophyllotoxin (Sigma). Plates were incubated for a further 72 h followed by the addition of 10 μL of AlamarBlue (AccuMed International) to each well and incubation for 2−4 h at 37 °C in a 5% CO_2/95% air mixture. Fluorescence emission at 585 nm was measured in a SPECTRAMAX GEMINI plate reader (Molecular Devices) after excitation at 530 nm. IC_{50} values were calculated using XLFit (ID Business Solutions) line fitting software.

Peter's Fully Suppressive 4 Day Test.[35] In vivo tests were performed under UK Home Office Animals (Scientific Procedures) Act 1986. The rodent malaria line used was the *P. berghei* ANKA (drug susceptible). Swiss outbred 20 g male CD-1 mice (Charles River) were kept in specific pathogen-free conditions and fed ad libitum. For oral administration, compounds were dissolved in standard suspending formula (SSV) [0.5% sodium carboxymethylcellulose, 0.5% benzyl alcohol, 0.4% Tween 80, and 0.9% NaCl (all from Sigma)]. Mice were infected intravenously with 4×10^6 infected red cells (day 0), randomized, and divided into groups of five mice for each dose. Oral treatment started after 3 h and continued for up to 3 days postinfection once a day. Artesunate doses were 30, 10, 3, and 1 mg/kg. Parasitemias were determined by microscopic examination of Giemsa-stained blood films taken on day 4.

Microscopic counts of blood films from each mouse were processed using Microsoft Excel and expressed as percentages of inhibition from the arithmetic mean parasitemias of each group in relation to the untreated group.

In Vivo Antimalarial Mean Survival Time Studies. All efficacy studies were approved by the institutional animal experimentation ethics committee. In vivo antimalarial activity was assessed basically as previously described.[36] Groups of three female NMRI mice (20−22 g) were intravenously infected with 2×10^7 parasitized erythrocytes on day 0 with GFP-transfected *P. berghei* strain ANKA.[24] Compounds were formulated in 100% DMSO, diluted 10-fold in distilled water, and administered intraperitoneally in a volume of 10 mL/kg on four consecutive days (4, 24, 48, and 72 h post-infection). Parasitemia was determined on day 4 post-infection (24 h after the last treatment) by FACS analysis. Activity was calculated as the difference between the mean percent parasitemia for the control (n = 5 mice) and treated groups expressed as a percent relative to the control group. The survival time in days was also recorded up to 30 days after infection. A compound was considered curative if the animal survived to day 30 after infection with no detectable parasites.

Acknowledgment. We thank Romark and the EPSRC for studentships to B.P. and S.C.L. and J. C. Janse (Leiden University, Leiden, The Netherlands) for providing the GFP-transfected *P. berghei* strain.

Supporting Information Available: Additional spectroscopic data for **7a**−**o**, **8**, and **9** and details of the synthesis of acetate **11** and benzoate **12** along with C,H,N analyses. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Shuhua, X.; Chollet, J.; Weiss, N. A.; Bergquist, R. N.; Tanner, M. Preventive effect of artemether in experimental animals infected with *Schistosoma mansoni*. *Parasitol. Int.* **2000**, *49*, 19–24.
(2) Valecha, N.; Gupta, D.; Usha, D.; Biswas, S.; Sharma, A.; Adak, T.; Asthana, O. P.; Sharma, V. P. Efficacy of α,β-arteether in acute uncomplicated *P. falciparum* malaria. *Int. J. Clin. Pharmacol. Res.* **1997**, *17*, 11–15.
(3) Lin, A. J.; Lee, M.; Klayman, D. L. Antimalarial activity of new water-soluble dihydroartemisinin derivatives. 2. Stereospecificity of the ether side chain. *J. Med. Chem.* **1989**, *32*, 1249–1252.
(4) Haynes, R. K.; Chan, H.-W.; Cheung, M.-K.; Lam, W.-L.; Soo, M.-K.; Tsang, H.-W.; Voerste, A.; Williams, I. D. C-10 ester and ether derivatives of dihydroartemisinin-10-α artesunate, preparation of authentic 10-β artesunate, and of other ester and ether derivatives bearing potential aromatic intercalating group at C-10. *Eur. J. Org. Chem.* **2002**, 113–132.
(5) Olliaro, P. L.; Taylor, W. R. J. Developing artemisinin based drug combinations for the treatment of drug resistant falciparum malaria: A review. *J. Postgrad. Med.* (*Bombay*) **2004**, *50*, 40–44.
(6) Adjei, G. O.; Kurtzhals, J. A. L.; Rodrigues, O. P.; Alifrangis, M.; Hoegberg, L. C. G.; Kitcher, E. D.; Badoe, E. V.; Lamptey, R.; Goka,

B. Q. Amodiaquine-artesunate vs artemether-lumefantrine for uncomplicated malaria in Ghanaian children: A randomized efficacy and safety trial with one year follow-up. *Malar. J.* **2008**, *7*, 127.
(7) German, P. I.; Aweeka, F. T. Clinical pharmacology of artemisinin-based combination therapies. *Clin. Pharm.* **2008**, *47*, 91–102.
(8) Tangpukdee, N.; Krudsood, S.; Srivilairit, S.; Phophak, N.; Chonsawat, P.; Yanpanich, W.; Kano, S.; Wilairatana, P. Gametocyte clearance in uncomplicated and severe *Plasmodium falciparum* malaria after artesunate-mefloquine treatment in Thailand. *Korean J. Parasitol.* **2008**, *46*, 65–70.
(9) Penali, L. K.; Jansen, F. H. Single-day, three-dose treatment with fixed dose combination artesunate/sulfamethoxypyrazine/pyrimethamine to cure *Plasmodium falciparum* malaria. *Int. J. Infect. Dis.* **2008**, *12*, 430–437.
(10) Awad, M. I.; Alkadru, A. M.; Berhens, R. H.; Baraka, O. Z.; Eltayeb, I. B. Descriptive study on the efficacy and safety of artesunate suppository in combination with other antimalarials in the treatment of severe malaria in Sudan. *Am. J. Trop. Med. Hyg.* **2003**, *68*, 153–158.
(11) Barradell, L. B.; Fitton, A. Artesunate. A review of its pharmacology and therapeutic efficacy in the treatment of malaria. *Drugs* **1995**, *50*, 714–741.
(12) Haynes, R. K. Artemisinin and derivatives: The future for malaria treatment? *Curr. Opin. Infect. Dis.* **2001**, *14*, 719–726.
(13) Haynes, R. K.; Fugmann, B.; Stetter, J.; Rieckmann, K.; Heilmann, H.-D.; Chan, H.-W.; Cheung, M.-K.; Lam, W.-L.; Wong, H.-N.; Croft, S. L.; Vivas, L.; Rattray, L.; Stewart, L.; Peters, W.; Robinson, B. L.; Edstein, M. D.; Kotecka, B.; Kyle, D. E.; Beckermann, B.; Gerisch, M.; Radtke, M.; Schmuck, G.; Steinke, W.; Wollborn, U.; Schmeer, K.; Romer, A. Artemisone: A highly active antimalarial drug of the artemisinin class. *Angew. Chem., Int. Ed.* **2006**, *45*, 2082–2088.
(14) Posner, G. H.; Wang, D.; Cumming, J. N.; Oh, C. H.; French, A. N.; Bodley, A. L.; Shapiro, T. A. Further evidence supporting the importance of and the restrictions on a carbon-centred radical for high antimalarial activity of 1,2,4-trioxanes like artemisinin. *J. Med. Chem.* **1995**, *38*, 2273–2275.
(15) Kamchonwongpaisan, S.; McKeever, P.; Hossler, P.; Ziffer, H.; Meshnick, R. Artemisinin neurotoxicity: Neuropathology in rats and mechanistic studies *in vitro*. *Am. Soc. Trop. Med. Hyg.* **1997**, *56*, 7–12.
(16) Stocks, P. A.; Bray, P. G.; Barton, V. E.; Al-Helal, M.; Jones, M.; Araujo, N. C.; Gibbons, P.; Ward, S. A.; Hughes, R. H.; Biagini, G. A.; Davies, J.; Amewu, R.; Mercer, A. E.; Ellis, G. L.; O'Neill, P. M. Evidence for a common non-heme chelatable-iron-dependent activation mechanism for semisynthetic and synthetic endoperoxide antimalarial drugs. *Angew. Chem., Int. Ed.* **2007**, *46*, 6278–6283.
(17) Homewood, C. A.; Warhurst, D. C.; Peters, W.; Baggaley, V. C. Lysosomes, pH and the anti-malarial action of chloroquine. *Nature* **1972**, *235*, 50–52.
(18) O'Neill, P. M.; Searle, N. L.; Kan, K.-W.; Storr, R. C.; Maggs, J. L.; Ward, S. A.; Raynes, K.; Park, B. K. Novel, potent, semisynthetic antimalarial carba analogues of the first generation 1,2,4-trioxane artemether. *J. Med. Chem.* **1999**, *42*, 5487–5493.
(19) Hindley, S.; Ward, S. A.; Storr, R. C.; Searle, N. L.; Bray, P. G.; Park, B. K.; Davies, J.; O'Neill, P. M. Mechanism-based design of parasite-targeted artemisinin derivatives: Synthesis and antimalarial activity of new diamine containing analogues. *J. Med. Chem.* **2002**, *45*, 1052–1063.
(20) Posner, G. H.; Parker, M. H.; Northrop, J.; Elias, J. S.; Ploypradith, P.; Xie, S.; Shapiro, T. A. Orally active, hydrolytically stable, semisynthetic, antimalarial trioxanes in the artemisinin family. *J. Med. Chem.* **1999**, *42*, 300–304.
(21) O'Neill, P. M.; Pugh, M.; Stachulski, A. V.; Ward, S. A.; Davies, J.; Park, B. K. Optimisation of the allylsilane approach to C-10 deoxo carba analogues of dihydroartemisinin: Synthesis and in vitro antimalarial activity of new, metabolically stable C-10 analogues. *J. Chem. Soc., Perkin Trans. 1* **2001**, 2682–2689.
(22) Haynes, R. K.; Ho, W.-Y.; Chan, H.-W.; Fugmann, B.; Stetter, J.; Croft, S. L.; Vivas, L.; Peters, W.; Robinson, B. L. Highly antimalaria-active artemisinin derivatives: Biological activity does not correlate with chemical reactivity. *Angew. Chem., Int. Ed.* **2004**, *43*, 1381–1385.
(23) Tetko, I. V.; Tanchuk, V. Y. Application of associative neural networks for prediction of lipophilicity in ALOGPS 2.1 program. *J. Chem. Inf. Comput. Sci.* **2002**, *42*, 1136–1145.
(24) Franke-Fayard, B.; Trueman, H.; Ramesar, J.; Mendoza, J.; Van Der, K. M.; Van Der, L. R.; Sinden, R. E.; Waters, A. P.; Janse, C. J. A *Plasmodium berghei* reference line that constitutively expresses GFP at a high level throughout the complete life cycle. *Mol. Biochem. Parasitol.* **2004**, *137*, 23–33.
(25) (a) For studies with weak base 1,2,4,5-tetraoxanes see Amewu, R.; Stachulski, A. V.; Ward, S. A.; Berry, N. G.; Bray, P. G.; Davies, J.; Labat, G.; Vivas, L.; O'Neill, P. M. Design and synthesis of orally active dispiro 1,2,4,5-tetraoxanes; synthetic antimalarials with superior activity to artemisinin. *Org. Biomol. Chem.* **2006**, *4*, 4431−4436. (b) For studies with weak base 1,2,4-trioxolanes with excellent antimalarial activity profiles see Tang, Y.; Dong, Y.; Wittlin, S.; Charman, S. A.; Chollet, J.; Chiu, F. C.; Charman, W. N.; Matile, H.; Urwyler, H.; Dorn, A.; Bajpai, S.; Wang, X.; Padmanilayam, M.; Karle, J. M.; Brun, R.; Vennerstrom, J. L. Weak base dispiro-1,2,4-trioxolanes: potent antimalarial ozonides. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 1260−1265.
(26) Posner, G. H.; Oh, C. H. A regiospecifically O-18 labeled 1,2,4-trioxane, a simple chemical-model system to probe the mechanism-(s) for the antimalarial activity of artemisinin (Qinghaosu). *J. Am. Chem. Soc.* **1992**, *114*, 8328–8329.
(27) O'Neill, P. M.; Posner, G. H. A medicinal chemistry perspective on artemisinin and related endoperoxides. *J. Med. Chem.* **2004**, *47* (12), 2945–2964.
(28) Posner, G. H.; Oh, C. H.; Wang, D.; Gerena, L.; Milhous, W. K.; Meshnick, R. S.; Asawamahasadka, W. Mechanism-based design, synthesis, and in vitro antimalarial testing of new 4-methylated trioxanes structurally related to artemisinin: The importance of a carbon-centered radical for antimalarial activity. *J. Med. Chem.* **1994**, *37*, 1256–1258.
(29) Still, W. C.; Kahn, M.; Mitra, A. Rapid chromatographic technique for preparative separations with moderate resolution. *J. Org. Chem.* **1978**, *43*, 2923–2925.
(30) Oh, S.; Jeong, I. H.; Ahn, C. M.; Shin, W.-S.; Lee, S. Synthesis and antiangiogenic activity of thioacetal artemisinin derivatives. *Bioorg. Med. Chem.* **2004**, *12*, 3783–3790.
(31) Trager, W.; Jensen, J. B. Human malaria parasites in continuous culture. *Science* **1976**, *193*, 673–675.
(32) Cameron, A.; Read, J.; Tranter, R.; Winter, V. J.; Sessions, R. B.; Brady, R. L.; Vivas, L.; Easton, A.; Kendrick, H.; Croft, S. L.; Barros, D.; Lavandera, J. L.; Martin, J. J.; Risco, F.; Garcia-Ochoa, S.; Gamo, F. J.; Sanz, L.; Leon, L.; Ruiz, J. R.; Gabarro, R.; Mallo, A.; de las Heras, F. G. Identification and activity of a series of azole-based compounds with lactate dehydrogenase-directed anti-malarial activity. *J. Biol. Chem.* **2004**, *279*, 31429–31439.
(33) Desjardins, R. E.; Canfield, C. J.; Haynes, J. D.; Chulay, J. D. Quantitative assessment of anti-malarial activity invitro by a semiautomated microdilution technique. *Antimicrob. Agents Chemother.* **1979**, *16*, 710–718.
(34) Vivas, L.; Easton, A.; Kendrick, H.; Cameron, A.; Lavandera, J. L.; Barros, D.; de las Heras, F. G.; Brady, R. L.; Croft, S. L. *Plasmodium falciparum*: Stage specific effects of a selective inhibitor of lactate dehydrogenase. *Exp. Parasitol.* **2005**, *111*, 105–114.
(35) Vivas, L.; Rattray, L.; Stewart, L. B.; Robinson, B. L.; Fugmann, B.; Haynes, R. K.; Peters, W.; Croft, S. L. Antimalarial efficacy and drug interactions of the novel semi-synthetic endoperoxide artemisone in vitro and in vivo. *J. Antimicrob. Chemother.* **2007**, *59*, 658–665.
(36) Peters, W.; Robinson, B. L. *Malaria*; Academic Press: San Diego, 1999.