Synthesis, Stability, and Antimalarial Activity of New Hydrolytically Stable and Water-Soluble (+)-Deoxoartelinic Acid

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(+)-Deoxoartelinic acid (**13**), a new hydrolytically stable, water-soluble, and potent non-acetal-type antimalarial drug candidate, was successfully prepared from artemisinic acid by using sulfur ylide and photooxygenative cyclization in seven steps. This compound showed superior in vitro antimalarial activity against the chloroquine-resistant K1 strain of *Plasmodium falciparum* and higher suppression (98.7%) than arteether in vivo against *Plasmodium chabaudi* infected mice. (+)-Deoxoartelinic acid also showed remarkable stability with a half-life of 258.66 h, 23 times more stable than clinically useful arteether in simulated stomach acid, and improved solubility, 4 times more soluble than artemisinin in water.

Introduction

Artemisinin (**1**), a sesquiterpene lactone with the first natural 1,2,4-trioxane, isolated from *Artemisia annua* L, and its derivatives have been important as antimalarial drugs having the most effective activity against multidrug-resistant forms of *Plasmodium falciparum*.[1] Artemisinin has been the subject of a number of reviews[1−10] because of its novel molecular structure, clinically useful antimalarial activity, and interesting in vitro anticancer activity.[11−14] The rapid action, powerful effect, and good tolerance of artemisinin and its derivatives may be attributed to the 1,2,4-trioxane moiety in these molecules (Chart 1). Such a novel chemical structure from natural resources and interesting bioactivities has encouraged chemists and pharmacologists to attempt further exploration.

Most semisynthetic derivatization has involved replacing the C-12 acetal functionality, forming the first generation ether derivatives, most of which were poorly soluble and hydrolytically unstable in simulated stomach acid. Furthermore, evidence that acetal-type analogues at C-12 are more neurotoxic in animal studies than non-acetal-type analogues is also emerging[15] and may thus lead to the future abandonment of the currently clinically used acetal-type analogues (artemisinin, artemether, arteether, artesunate, and artelinic acid). Artelinic acid (**5**) was prepared to improve on artesunate (**4**), which has poor stability in aqueous solution because of the facile hydrolysis of ester linkage, but in previously in vitro antimalarial tests against the cloned D6 and W2 strains of *Plasmodium falciparum*, it was 1.4−2.1 times less potent than artemisinin.[16,17] Although many derivatives of artemisinin have been prepared and show good antimalarial activities,[2] most of them possess an acetal group at the C-12 position. Non-acetal-type analogues of deoxoartemisinin recently received attention owing to their better bioavailability and acid stability and their reduced neurotoxicity compared to acetal-type analogues. Deoxoartemisinin (**2**), prepared from either artemisinin (**1**) or artemisinic acid (**6**) by Jung et al.,[18] is the first non-acetal-type analogue of artemisinin and shows more antimalarial activity than artemisinin (**1**) both in vitro and in vivo.[19] Thus, we designed hydrolytically stable and water-soluble deoxoartelinic acid **13** as a C-12 non-acetal type that is expected not only to be considerably more stable in simulated stomach acid than clinically useful acetal-type drugs but also to be active against *P. falciparum* in vitro and in vivo. In this paper, we report the first preparation of deoxoartelinic acid **13** and its exceptionally high in vitro and in vivo antimalarial activity, acid stability, and water solubility.

Chemistry

Since the preparation of 12-*n*-butyldeoxoartemisinin (**3c**) as the first hydrolytically stable non-acetal type analogue[20] containing a C−C bond at C-12, a series of non-acetal-type derivatives including a few of heteroaryl and unsaturated substituents at C-12 have been prepared.[21−29] Because direct introduction of the C−C bond at C-12 of artemisinin for the preparation of novel analogues may cause destruction of the biologically essential endoperoxide, photooxygenative cyclization[20] of the dihydroartemisinol derivative (**11**) has been utilized as a key step.[30] Deoxoartelinic acid **13** was prepared from more abundant artemisinic acid (**6**) as a useful chiral synthon in seven steps as shown in Scheme 1. Thus, reaction of dihydroartemisinyl aldehyde (**9**), prepared from artemisinic acid (**6**) by a known procedure[31] (yield 88%), with trimethylsulfur iodide as a sulfur ylide gave the epoxide **10** ($12R/S = 7/1$) (yield 76%). The ring opening of epoxide **10** with 4'-vinylbenzylmagnesium chloride afforded alcohol **11** (yield 81%) with introduction of the C−C bond at C-12. Photooxygenative cyclization, as previously mentioned,[18] of alcohol **11** provided the 4'-vinylhomobenzyldeoxoartemisinin **12** (yield 35%) with natural β-configuration ($J =$
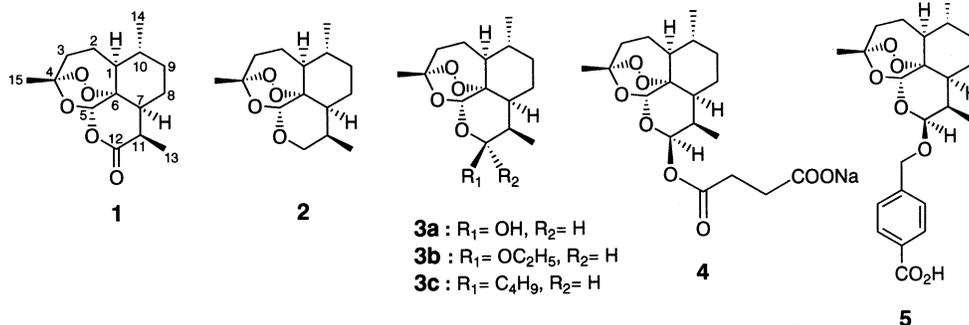
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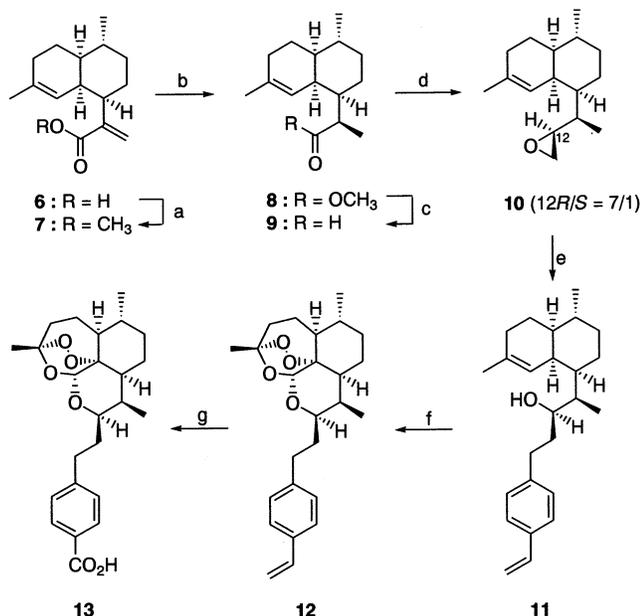† Yonsei University.
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Chart 1

**3a** : R$_1$= OH, R$_2$= H
3b : R$_1$= OC$_2$H$_5$, R$_2$= H
3c : R$_1$= C$_4$H$_9$, R$_2$= H

Scheme 1[a]

6 : R = H
7 : R = CH$_3$ a

8 : R = OCH$_3$
9 : R = H c

10 (12R/S = 7/1)

[a] Reagents and conditions: (a) CH$_2$N$_2$, Et$_2$O, room temperature for 3 h; (b) NaBH$_4$, NiCl$_2$·6H$_2$O, MeOH, 0 °C for 30 min; (c) DIBAL-H, CH$_2$Cl$_2$, −78 °C for 1 h; (d) (CH$_3$)$_3$S$^+$I$^−$, KOH, DMSO, room temperature for 2 h, 76%; (e) Mg, 4-vinylbenzy chloride, Et$_2$O, 0 °C for 12 h, 81%; (f) (i) O$_2$, rose bengal, 500 W tungsten lamp, CH$_2$Cl$_2$/CH$_3$CN (1/9), −40 °C for 4 h, (ii) TFA, O$_2$, CH$_3$CN, −40 °C for 2 h, 35%; (g) NaHCO$_3$, KMnO$_4$, acetone, room temperature for 1 h, 1 M HCl, room temperature for 5 h, 83%.

10.9 Hz) in a single step. No α-diastereomer at C-12 was detected. Although the yield for the photooxidative cyclization is low, this step represents the shortest synthetic route to compound **12**. Direct oxidation of **12** with KMnO$_4$ afforded deoxoartelinic acid **13** (yield 83%) in a single step.[31,40]

Stability

Artemisinin is stable in neutral solvent heated to 150 °C.[32] However, being a hemiacetal, dihydroartemisinin (**3a**) is chemically more vulnerable than its parent compound artemisinin (**1**), a lactone itself. Artemether, arteether, artesunate, and artelinic acid, which are acetal-type prodrugs under clinical use or trials are susceptible to moisture and acidic conditions.[32,33] From published data on other arteether and artesunate analogues, it would appear that any derivative at C-12 of the type −OCH$_2$CH$_2$R and −O(C=O)CH$_2$R may not have sufficient stability in aqueous solution to be clinically useful in intravenously injectable dosage
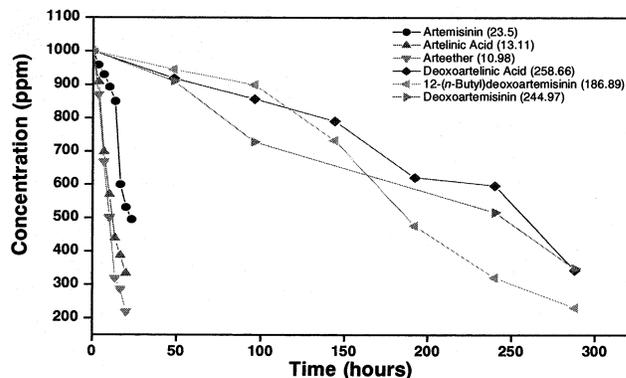
Figure 1. Kinetics of hydrolysis and decomposition of acetal (C−O) type and non-acetal-type prodrugs of artemisinin in simulated stomach acid (0.01 N HCl, pH 2.0, 37 °C).

form.[32] Therefore, we are still in urgent need of acid-stable and water-soluble antimalarial drugs from the series for oral and parenteral application. In bioavailability studies of orally administered arteether, for example, one should consider the effect of gastric pH and emptying time in the design of the study. To examine the effect of molecular structure of acetal- and non-acetal-type prodrugs of artemisinin on the stability in simulated stomach acid (pH 2.0, 37 °C), 12-(n-butyl)-deoxoartemisinin[20,34] (**3c**), deoxoartemsinin[18,19] (**2**), and artelinic acid[16] (**5**) have been prepared as comparative references by known procedures from either artemisinin or artemisinic acid.[16,18−20] To measure the stability of deoxoartelinic acid **13** and the other prodrugs in simulated stomach acid, the disappearance of prodrugs (1 mg/mL) on 0.01 N HCl, pH 2.0 at 37 °C, was followed by HPLC analysis. All prodrugs tested were found to disappear with pseudo-first-order kinetics, with a half-life of 10.98, 13.11, 23.5 h for arteether, artelinic acid, and artemisinin, respectively, and of 186.89, 244.97, and 258.66 h for 12-(n-butyl)deoxoartemisinin, deoxoarte-misinin, and deoxoartelinic acid, respectively (Figure 1 and Table 1). In general, it was found that the half-lives of non-acetal-type analogues were 15−23 times longer than those of acetal-type analogues under simulated stomach acid condition (Table 1). In the simulated stomach acid, artemisinin (**1**), arteether (**3b**), and artelinic acid (**5**) were found to be unstable and to easily undergo hydrolysis. On the other hand, deoxoartelinic acid **13** and its non-acetal-type derivatives were found to be very stable and to possess long half-lives under the same conditions, showing sufficient stability for oral administration. Deoxoartelinic acid **13** and its sodium salt are 4 and 6 times more soluble in water (5.0 mg/

Table 1. Stability of Acetal (C−O) Type and Non-Acetal (C−C) Type Prodrugs of Artemisinin in Simulated Stomach Acid (1 mg/mL, 0.01 N HCl, pH 2.0, 37 °C)

compound	half-life (h)
Acetal (C−O) Type	
artemisinin (**1**)	23.50
arteether (**3b**)	10.98
artelinic acid (**5**)	13.11
Non-Acetal (C−C) Type	
deoxoartemisinin (**2**)	244.97
deoxoartelinic acid (**13**)	258.66
12-(*n*-butyl)-deoxoartemisinin (**3c**)	186.89

Table 2. In Vitro Antimalarial Activity of Antimalarial Drugs

antimalarial drug	ED_{50} (ng/mL) clone of *P. falciparum*	
	3D7	K1
deoxoartelinic acid (**13**)	1.1	0.6
artemisinin (**1**)	2.9	0.7
artesunate (**4**)	0.2	0.6
artelinic acid (**5**)	4.0	1.4
arteether (**3b**)	0.2	0.9
chloroquine	8.0	220

mL for deoxoartelinic acid, 7.4 mg/mL for sodium salt of deoxoartelinic acid) when compared with artemisinin (1.2 mg/mL) and have the same solubility as artelinic acid.

Biological Evaluation

Deoxoartelinic acid **13** was tested in vitro against the chloroquine-sensitive 3D7 strain and the chloroqine-resistant K1 strain of *P. falciparum* (Table 2). Although deoxoartelinic acid **13** possesses lower antimalarial activity against 3D7 than artesunate (**4**), it is identical to artesunate against the chloroquine-resistant K1 strain. Deoxoartelinic acid **13** shows 4 and 2 times more activity than artelinic acid (**5**) against 3D7 and K1 strains of *P. falciparum*, respectively. Compound **13** shows almost the same level of activity as that of arteether (**3b**) against the K1 strain. Deoxoartelinic acid **13** was subjected to further testing in mice (Table 3). The results of the in vivo tests showed that there was a 98.7% suppression of parasitaemia in the *Plasmodium chabaudi* infected mice with deoxoartelinic acid and that the ED_{50} and ED_{90} values were both less than 10 mg/kg. The survival rate of the five *P. chabaudi* infected mice treated with deoxoartelinic acid is 100% compared to 60% with arteether treatment (Table 4). Therefore, deoxoartelinic acid is stimulating much interest as a new and potent non-acetal-type antimalarial drug candidate.

In conclusion, (+)-deoxoartelinic acid, a new and more active antimalarial drug candidate, was successfully prepared from more abundant artemisinic acid by using sulfur ylide and photooxygenative cyclization. (+)-Deoxoartelinic acid shows superior antimalarial activity in vitro against chloroquine-resistant malaria when compared with artemisinin and artelinic acid and shows higher suppression in vivo than either arteether or artelinic acid. Deoxoartelinic acid is 23 times more stable than clinically useful arteether and artelinic acid and is 10 times more stable than artemisinin in simulated stomach acid. Deoxoartelinic acid is 4 times more soluble than artemisinin in water. Thus, deoxoartelinic acid deserves further investigation as a new antimalarial drug candidate. Tests for bioavailabilty and antimalarial activity from oral administration will be performed and published in due course.

Table 4. In Vivo Antimalarial Activity of Deoxoartelinic Acid, Arteether, and Chloroquine (10 mg kg^{-1} day^{-1} ip × 4) against *P. chabaudi* in Mice

	deoxoartelnic acid	arteether	chloroquine
survival, mice	5/5	3/5	0/5
survival rate (%)	100	60	0

Experimental Section

Chemistry. NMR spectra were obtained on a Bruker AC250 spectrometer using Me_4Si as an internal standard. The GC−MS and direct mass were operated on an HP 5980II GC-HP 5988 and JMS-700 M station spectrometer in EI mode. Infrared spectra were taken on a Nicolet Impact 400 spectrometer. Specific rotations were recorded on a Rudolph AP III-589 polarimeter.

Preparation of Dihydroartemisinyl Epoxide (10) from Dihydroartemisinyl Aldehyde (9). Dihydroartemisinyl aldehyde (**9**) (184 mg, 0.84 mmol) was mixed with trimethylsulfur iodide (375 mg, 1.67 mmol) and KOH (94 mg, 1.67 mmol) in dry DMSO (10 mL), and this solution was stirred at room temperature. After 2 h, ice water (50 mL) was added to the reaction mixture and extracted with $CHCl_3$ (30 mL × 3). The extract was dried over $MgSO_4$ and concentrated in vacuo to give crude products, which were purified by a silica gel column (hexane/ethyl acetate = 5:1 as eluent) (R_f = 0.71) to give dihydroartemisinyl epoxide **10** (150 mg) in 76% yield as a colorless oil: ^{1}H NMR (CDCl$_3$, 250 MHz) δ 5.12 (1H, s), 2.87−2.78 (1H, m), 2.71−2.62 (1H, m), 2.58−2.53 (1H, m), 2.49 (1H, bs), 1.96−1.64 (4H, m), 1.61 (3H, s), 1.59−1.40(5H, m), 1.07 (3H, d, *J* = 5.9 Hz), 1.03−0.91 (2H, m), 0.89 (3H, d, *J* = 6.3 Hz), 0.85−0.82 (1H, m); ^{13}C NMR (CDCl$_3$, 63 MHz) δ 135.7, 120.4, 49.6, 44.9, 42.3, 38.7, 37.5, 37.2, 35.9, 28.1, 27.2, 27.0, 26.2, 24.1, 20.2, 15.5; IR ν_{max} 2920, 1454, 1382, 1214, 909, 822, 751 cm^{-1}; EIMS *m/z* 234 ([M]$^+$), 216 ([M − H_2O]$^+$), 201, 159, 145, 131, 105, 79, 55, 41, 29, 23, 14; HRMS (EI, 70 eV) *m/z* 234.1987 (obsd), 234.1985 (calcd for $C_{16}H_{26}O$).

Preparation of 12-(*p*-Vinyl)homobenzyldihydroartemisinyl Alcohol (11) from Dihydroartemisinyl Epoxide (10). Under a nitrogen atmosphere, the Grignard reagent, prepared by 4'-vinylbenzyl chloride (183.2 mg, 1.2 mmol) and magnesium metal, was slowly added dropwise to a solution of dihydroartemisinyl epoxide **10** (130 mg, 0.55 mmol) in dry diethyl ether (10 mL) at 0 °C. The mixture was stirred at 0 °C in an ice bath in the dark. After 12 h, the mixture was neutralized with 5% aqueous HCl and further diluted with cooled H_2O (50 mL). The mixture was extracted with diethyl ether (20 mL × 3) and was washed with brine (10 mL × 2). The extract was dried over $MgSO_4$ and concentrated in vacuo to give crude products, which were purified by a silica gel column (hexane/ethyl acetate = 5:1 as eluent) (R_f = 0.42) to give 12-(*p*-vinyl)homobenzyldihydroartemisinyl alcohol **11** (157

Table 3. Blood Schizontocidal Activity (% Infected Erythrocytes) of Deoxoartelinic Acid and Chloroquine (10 mg kg^{-1} day^{-1} ip × 4) against *P. chabaudi* in Mice

	mouse							
	1	2	3	4	5	group mean	% control	% suppression
deoxoartelinic acid (**5**)	0.5	0.5	2.9	0.5	0.3	0.9	1.3	98.7
chloroquine	65.0	71.3	62.1	82.4	81.3	72.4	100	

mg) in 81% yield as a colorless foam: $[\alpha]^{25}_D$ +120.8° (*c* 0.48, CHCl$_3$); ^{1}H NMR (CDCl$_3$, 250 MHz) δ 7.35 (2H, d, *J* = 7.8 Hz), 7.19 (2H, d, *J* = 7.9 Hz), 6.75 (1H, dd, *J* = 10.8, 17.6 Hz), 5.74 (1H, d, *J* = 17.6 Hz), 5.21 (1H, d, *J* = 10.9 Hz), 5.17 (1H, s), 3.91−3.86 (1H, m), 2.79−2.58 (2H, m), 2.48 (1H, bs), 1.92−1.81 (3H, m), 1.78−1.64(2H, m), 1.62 (3H, s), 1.56−1.46 (3H, m), 1.41−1.36 (2H, m), 1.30−1.24 (2H, m), 0.99−0.91 (2H, m), 0.88 (6H, d, *J* = 6.7 Hz), 0.85−0.83 (1H, m); ^{13}C NMR (CDCl$_3$, 63 MHz) δ 142.2, 136.8, 135.4, 131.0, 128.7, 126.4, 120.7, 113.1, 72.2, 43.7, 42.4, 42.1, 40.4, 40.1, 37.7, 35.8, 32.8, 27.8, 26.9, 26.0, 24.0, 19.9, 10.7; IR ν_{max} 3394, 2920, 2864, 1601, 1510, 1443, 1377, 990, 904, 822 cm^{-1}; HRMS (EI, 70 eV) *m/z* 352.2752 (obsd), 352.2766 (calcd for C$_{25}$H$_{36}$O). Because of the tendency of the styryl system to polymerize, compound **11** was used immediately in the next step.

Preparation of 12-(*p*-Vinyl)homobenzyldeoxoartemisinin (12) from 12-(*p*-Vinyl)homobenzyldihydroartemisinyl Alcohol (11). A pale-pink solution of alcohol **11** (120 mg, 0.34 mmol) and rose bengal (5 mg) in CH$_3$CN/CH$_2$Cl$_2$ (9/1, 25 mL) was irradiated with white light (500 W tungsten lamp) at −40 °C under oxygen. After 4 h, TLC analysis indicated that the majority of the starting material had disappeared. The mixture was poured onto a saturated NaHCO$_3$ solution (50 mL), and products were extracted into diethyl ether (20 mL × 3). The sensitizer remained in the aqueous phase. The combined ether extracts were washed with brine (20 mL × 2) and dried with MgSO$_4$. Removal of solvent under reduced pressure left a colorless foam. This was dissolved in CH$_3$CN (10 mL), and the resultant solution was cooled to −40 °C and was followed by in situ treatment of acidic catalyst TFA. The mixture was stirred at this temperature under an oxygen atmosphere for 2 h. The reaction mixture was quenched with saturated NH$_4$Cl solution (10 mL), and the products were extracted with diethyl ether (20 mL × 3). The extract was washed with water (30 mL × 2) and brine (30 mL × 2) and dried with MgSO$_4$. Concentration in vacuo gave crude products, which were purified by a silica gel column (hexane/ethyl acetate = 5:2 as eluent) (R_f = 0.53) to give 12-(*p*-vinyl)-homobenzyldeoxoartemisinin **12** (47 mg) in 35% yield as a colorless foam: $[\alpha]^{25}_D$ +18.2° (*c* 0.1, CHCl$_3$); ^{1}H NMR (CDCl$_3$, 250 MHz) δ 7.34 (2H, d, *J* = 8.1 Hz), 7.20 (2H, d, *J* = 8.0 Hz), 6.74 (1H, dd, *J* = 10.9, 17.6 Hz), 5.73 (1H, d, *J* = 17.6 Hz), 5.36 (1H, s), 5.21 (1H, d, *J* = 10.9 Hz), 4.24−4.17 (1H, m), 3.04−2.89 (1H, m), 2.79−2.53 (2H, m), 2.33 (1H, ddd, *J* = 3.8, 3.0, 3.8 Hz), 2.09−2.01 (2H, m), 1.94−1.78 (3H, m), 1.74−1.64 (2H, m), 1.57−1.46 (1H, m), 1.44 (3H, s), 1.42−1.20 (3H, m), 1.17−1.04 (1H, m), 0.96 (3H, d, *J* = 5.9 Hz), 0.85 (3H, d, *J* = 7.6 Hz); ^{13}C NMR (CDCl$_3$, 63 MHz) δ 142.4, 136.8, 135.4, 128.8, 126.4, 113.1, 103.4, 89.2, 81.3, 75.5, 52.6, 44.6, 37.6, 36.8, 34.6, 33.8, 31.9, 30.4, 26.3, 26.1, 24.9, 20.4, 13.2; IR ν_{max} 3373, 2925, 2864, 1627, 1510, 1448, 1382, 1179, 1128, 1056, 1011, 832., 756 cm^{-1}; HRMS (EI, 70 eV) *m/z* 398.2463 (obsd), 398.2457 (calcd for C$_{25}$H$_{34}$O$_4$).

Preparation of Deoxoartelinic Acid (13) from 12-(*p*-Vinyl)homobenzyldeoxoartemisinin (12). A solution of 12-(4'-*p*-vinylhomobenzyldeoxoartemisinin **12** (40 mg, 0.10 mmol) in HPLC grade acetone was slowly mixed with NaHCO$_3$ (4.2 mg, 0.05 mmol) and KMnO$_4$ (47 mg, 0.30 mmol). The reaction mixture was stirred at room temperature for 1 h and then treated with 10% aqueous HCl (3 mL) and stirred at room temperature for 5 h. The mixture was extracted with diethyl ether (10 mL × 3) and was washed with brine (10 mL × 2). The extract was dried over MgSO$_4$ and was concentrated in vacuo to give crude products, which were purified by a silica gel column (hexane/ethyl acetate = 1:2 as eluent) (R_f = 0.46) to give deoxoartelinic acid **13** (35 mg) in 83% yield as a white solid: mp 135−137 °C; $[\alpha]^{20}_D$ +58.3° (*c* 0.1, CHCl$_3$); ^{1}H NMR (CDCl$_3$, 250 MHz) δ 8.03 (2H, d, *J* = 8.2 Hz), 7.34 (2H, d, *J* = 8.1 Hz), 5.36 (1H, s), 4.26−4.20 (1H, m), 3.12−3.00 (1H, m), 2.75−2.65 (2H, m), 2.34 (1H, ddd, *J* = 3.8, 2.9, 3.8 Hz), 2.13−2.01 (1H, m), 1.96−1.89 (3H, m), 1.84−1.73 (3H, m), 1.68−1.48 (4H, m), 1.44 (3H, s), 1.38−1.17 (2H, m), 0.96 (3H, d, *J* = 5.6 Hz), 0.86 (3H, d, *J* = 7.5 Hz); ^{13}C NMR (CDCl$_3$, 63 MHz) δ 171.6, 149.2, 130.6, 128.9, 127.1, 103.5, 89.3, 81.3, 75.0, 52.5,

44.5, 37.7, 36.8, 34.6, 34.1, 31.6, 30.4, 26.3, 25.1, 24.9, 20.4, 13.2; IR ν_{max} 3424, 2903, 2864, 1693, 1611, 1428, 1377, 1291, 1184, 1128, 1016, 944, 751 cm^{-1}; HRMS (EI, 70 eV) *m/z* 416.2206 (obsd), 416.2199 (calcd for C$_{24}$H$_{32}$O$_6$).

Stability Tests of Artemisinin Analogues in Simulated Stomach Acid. Sample Preparation and Measurement of Stability. A 100 μL portion of a 1.0 mg artemisinin prodrug stock solution (in acetonitrile) was added to 1.0 mL of a freshly prepared 0.01 N HCl aqueous solution (preheated to 37 °C). The resulting mixture was sealed to prevent water evaporation and maintained at 37 °C in a water bath. Acetal-type samples (**1**, **3b**, **5**) of the reaction mixture were taken at time intervals of 0, 200, 400, 600, 800, 1000, 1200, 1400 min, and non-acetal-type samples (**2**, **3c**, **13**) were taken at 2, 4, 6, 8, 10, 12, and 14 days. The samples were stored in dry ice−acetone bath and analyzed (in triplicate) as quickly as possible. HPLC with UV detector (Waters 486 tunable absorbance detector, Millipore) was used to identify the decomposition of the prodrugs and quantitation. All prodrugs were detected with a wavelength of 210 nm except 250 nm for aromatic compounds **5** and **13**. The column was a Nova-Pak C-18 (4 μm particle size, 15.0−3.9 cm length) column used with a mobile phase (1.0 mL/min), which was completely deoxygenated by flowing argon gas. Two mobile-phase systems were used, where system 1 (for the assay of *n*-butyldeoxoartemisinin (t_r = 5.53 min) consisted of 0.1 M ammonium acetate with 80% acetonitrile in water. System 2, used for deoxoartelinic acid (t_r = 9.83 min) consisted of 0.1 M ammonium acetate with 30% acetonitrile in water. Two internal standards were used: arteether (t_r = 3.45 min) for HPLC system 1 and artelinic acid (t_r = 9.97 min) for system 2.

Biology. In Vitro Antimalarial Studies. Cultures of the 3D7 chloroquine sensitive strain of *P. falciparum*[35] and K1 chloroquine resistant[36] were maintained in RPMI 1640 medium (Sigma, U.K.), 37 °C, 5% CO$_2$ in 5% hematocrit in A+ erythrocytes. Test compounds were tested in a 96-well plate format using synchronized ring stage cultures prepared at 1% parasitemia. An amount of 100 μL of synchronized culture was added per well. Drugs were tested in triplicate over a 3-fold dilution series from a final top drug concentration of 30 μg/mL. Standard drug was chloroquine diphosphate. Control wells were infected erythrocytes with no drug, and blank wells were uninfected A+ erythrocytes. After 24 h of incubation at 37 °C, 5% CO$_2$, 20 μL of [^{3}H]-hypoxanthine was added to all wells (0.1 μCi /well)[37,38] and plates were shaken for 1 min and then incubated for a further 24 h. The plates were freeze−thawed rapidly, harvested onto a 96-well glass fiber filter plate (Canberra Packard), and dried at 42 °C. Incorporation of radioactive hypoxanthine was measured using a Canberra Packard TopCount scintillation counter. Results were analyzed using the Microsoft Excel based Ms*Xl*fit (IDBS, U.K.) to calculate ED$_{50}$ values.

Biology. In Vivo Antimalarial Studies. Female mice (BALB/c, specific pathogen free), 18−20 g, were infected intravenously (iv) with 1 × 10^7 *P. chabaudi* ANKA infected erythrocytes from donor mice on day 1 of the experiment. Blood was taken from donor mice, in serum, and diluted in RPMI (Sigma, U.K.) to a parasitemia of 1% (the equivalent of 1 × 10^7 infected erythrocytes), and 0.2 mL was administered to each mouse iv. Mice were randomly sorted into groups of five. Then 2 h of postinoculation dosing commenced. The control drug, chloroquine, was given ip every day for 4 days. The route of administration of experimental compounds was ip. Drugs were administered at 0.2 mL every day for 4 days in 10% DMSO in phosphate-buffered saline (Sigma, U.K.). On day 5, postinfection blood smears of all animals were prepared and stained with 10% Giemsa. Parasitemia was determined microscopically by counting a minimum of 1000 red cells. Results are reported as the percentage of infected erythrocytes and compared to the chloroquine control group and the uninfected control group.[39]

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