Mechanism-Based Development of New Antimalarials: Synthesis of Derivatives of Artemisinin Attached to Iron Chelators

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Various derivatives of artemisinin covalently linked to iron chelators were synthesized, and their antimalarial activities were evaluated. Although results show no indication that the presence of an iron chelator in the vicinity of artemisinin potentiates its action, the linked compounds prepared still retain comparable activities to that of artemisinin.

Malaria continues to be a major endemic infectious disease in many developing countries, requiring both global and national efforts in research toward its control and eradication. The disease is caused by *Plasmodium* spp., especially *Plasmodium falciparum*, the pathogen responsible for the life-threatening form of the disease. The problems have been further complicated by emergence of resistance to many drugs. Therefore there is a great need for new antimalarials, preferably with radically different structures and modes of action, in order to deal with development of resistance to the drugs in current use. Artemisinin offers exciting new possibilities in drug development due to its unique structure, mode of action, rapid action, and efficacy against chloroquine-resistant parasite.[1-3]

Artemisinin or qinghaosu had been isolated from *Artemisia annua* Linn., an ancient Chinese herbal used for treatment of fever and malaria. Its structure had been identified to be an endoperoxide-containing sesquiterpene lactone. The presence of endoperoxide linkage is essential for its action,[1,4] and many reports showed that the drug probably acts through generation and exertion of oxidative stress on the parasite.[5-7] Furthermore, the presence of free iron is likely to be important in the drug action, as simultaneous *in vitro* application of artemisinin or its derivatives, arteether and artesunate, and one of a number of iron chelators, which by themselves also have antimalarial property, leads to antagonism.[8,9] Antemisinin or its derivatives may thereore act, after reaching the site of action in the parasite, by exerting oxidative damage through combined action with Fe(III)/Fe(II) which generates reactive intermediates. The reactive intermediates may include reactive oxygen species and radical derivatives of the drug. If this is so, then it should be expected that the reactive intermediates will combine with parasite components at or very close to the site of their formation. It should furthermore be expected that if iron can be made available within the vicinity of artemisinin or its derivatives, the drug should become much more potent. Such strategy may be achieved through application of new drug agents with the structure of artemisinin covalently linked to a suitable iron chelator. Such a chelator should not bind iron too strongly since it must be able to deliver free iron to the site of drug action or allow reversible redox reactions of the iron and yet must have the capacity to carry iron to that site. In order to test this hypothesis, approaches to the synthesis of such linked compounds should be developed. In this report we show a general scheme for such synthesis, with a number of examples of linked compounds, some of which are shown to have *in vitro* antimalarial activities comparable to artemisinin.

Chemistry

Artemisinin **1** was reduced with sodium borohydride into dihydroartemisinin **2**.[4] Treatment of the benzene solution of **2** with bromopropanol at room temperature in the presence of boron trifluoride etherate catalyst[4] afforded a single β-isomer of the corresponding bromide **3**. Similar treatment of **2** with propanediol, on the other hand, gave both α- and β- isomers of artemisinin–propanol derivatives, **4** and **5**, in respectable yields (11% for α- and 30% for β-isomers), which can be crystallized from hexane at −20 °C (Scheme **1**). Elucidation of the stereochemistry of the above products was achieved by their NMR data whereupon the small coupling constant (J = 3.5 Hz) between the vicinal protons, H_9–H_{10}, was observed in the case of the β-isomer. The *trans* relationship of these two protons in the α-isomer was evident from a large coupling constant of 9 Hz.

Artemisinin–chelator derivatives were prepared by reactions depicted in Scheme 2. The iron chelator was dissolved in DMSO and subjected to sodium hydride (**1**.5 equiv) treatment to generate the corresponding anion for further reaction with artemisinin–bromide derivative **3**. The method provided a series of artemisinin hybrids whose structures were proved by high-resolution NMR. Linking of artemisinin to methyl caffeate regiospecifically afforded compound **6** as evidenced by the chemical shift of aromatic proton at C-2′ on the caffeate nucleus which remained unchanged upon linking. Alkoxylation of artemisinin to kojic acid provided compound **7** which resulted from the reaction of hydroxyl function at C-2′, causing an up field shift of the adjacent C-3′ proton by 0.4 ppm as compared to that of kojic acid itself. Reaction of artemisinin with pyrogallol yielded three products, **8**, **9**, and **10**. Signals of the equivalent (compound **8**) and unequivalent (compound **9**) aromatic protons at C-4′ and C-6′ on the pyrogallol nucleus indicated the linkage of artemisinin molecule to the hydroxyl group at C-2′ and C-1′, respectively. The NMR spectrum of the dialkylated product **10** not only showed the unequivalent protons at C-4′ and C-6′ but also the presence of two sets of signals of artemisinin

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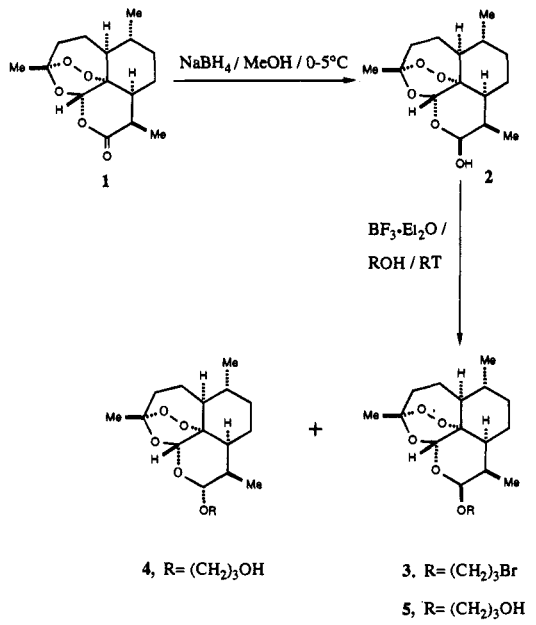
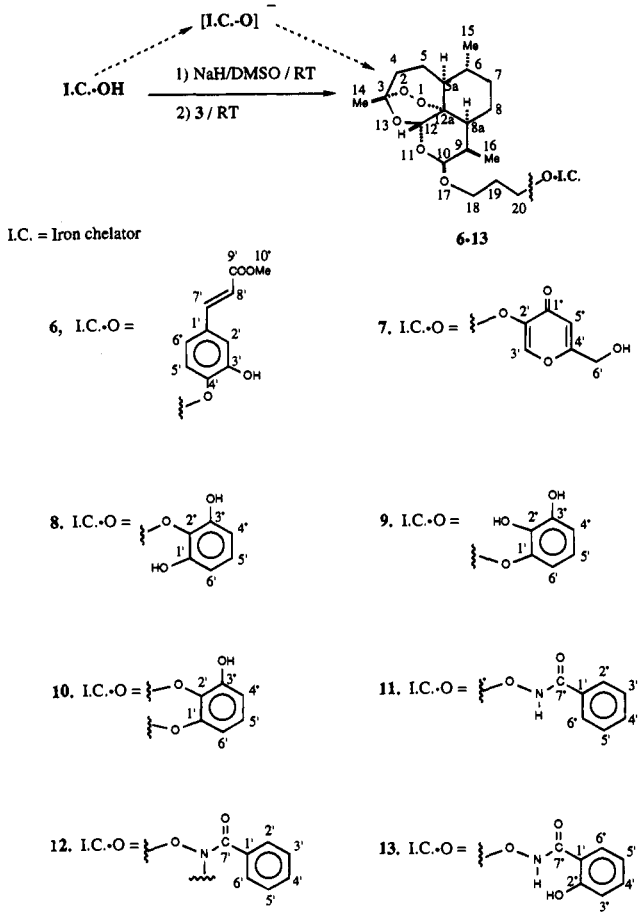
Scheme 1

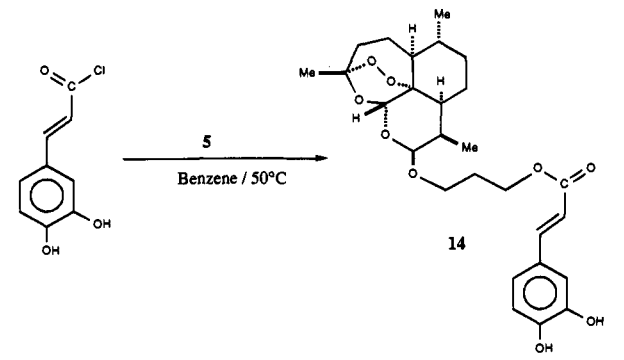


Scheme 2

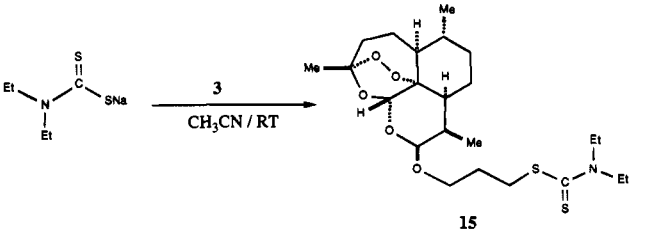


moieties, indicating the coupling of two artemisinin molecules to C-1′ and C-2′ hydroxyl groups. Reaction of artemisinin with benzohydroxamic acid gave the mono- and dialkylated products **11** and **12**, respectively. Salicylhydroxamic acid also linked to artemisinin *via* its hydroxamic OH giving rise to compound **13** as the sole product. Further experiment was performed to provide a better understanding of the site of covalent bonding between artemisinin and the hydroxamate.

Scheme 3



Scheme 4



Results from a model reaction between salicylhydroxamic acid and benzyl bromide indicated that the reactivity toward alkylation at each heteroatom of this chelator was in the order of hydroxamic OH > hydroxamic NH > phenolic OH.

It was worth noting that although reactions described above could be regarded as straightforward, manipulations were sometimes difficult due to the fact that dihydroartemisinin nucleus was unstable under the strong basic conditions employed in the study. Also, some of these products, bearing both oxidizing and reducing moieties, were rather unstable and underwent spontaneous decomposition.

An alternative method to construct artemisinin–caffeic acid hybrid was shown in Scheme 3. Caffeic acid was treated with thionyl chloride to afford the acid chloride which upon reaction with artemisinin–hydroxy derivative **5** provided the hybrid **14**, but in very low yield and the product very unstable. Scheme 4 also shows the construction method of compound **15**, a derivative of artemisinin coupled with diethyl dithiocarbamate, an iron chelator which is also a potent inhibitor of superoxide dismutase, an antioxidant enzyme present both in the erythrocyte and the parasite.[10]

Results and Discussions

Based on drug combination studies, we proposed[8,9] that artemisinin acts by binding with its target and exerting oxidative damage within its vicinity through combined action with redox metal ions, probably Fe(III)/Fe(II). Compounds with the structure of artemisinin cross-linked to suitable chelators can therefore have greater antimalarial activity, provided a number of important conditions can be fulfilled. The main criteria for effectiveness of such new compounds against the malarial parasite should be the ability to penetrate infected cells and bind to the same target as artemisinin, the ability to carry the iron to that target, and the ability to allow the iron to participate in reversible redox reactions. A number of iron chelators were chosen based on the presence of suitable groups which can be

Table 1. Antimalarial Activity of Iron Chelators, Diethyldithiocarbamate (DDC) and Their Derivatives against *P. falciparum in Vitro*

agents	IC_{50} (M)
caffeic acid	1.22×10^{-4}
methyl caffeate	4.65×10^{-5}
kojic acid	1.58×10^{-3}
pyrogallol	5.66×10^{-6}
benzohydroxamic acid	1.76×10^{-5}
salicylhydroxamic acid	1.07×10^{-4}

Table 2. Antimalarial Activity of Artemisinin and Its Novel Derivatives against *P. falciparum in Vitro*

compounds	IC_{50} (nM)	compounds	IC_{50} (nM)
artemisinin (**1**)	5.82 ± 1.4	compound **9**	12.7
dihydroartemisinin (**2**)	1.1	compound **10**	6.64
compound **3**	3.68 ± 0.55	compound **11**	23.64
compound **6**	4.78 ± 3.0	compound **12**	4.25
compound **7**	9.55 ± 5.0	compound **13**	52.45
compound **8**	7.67	compound **15**	7.67

Table 3. Effect of Iron (20 μM $FeCl_3$) on the Antimalarial Activity of Artemisinin and Its Novel Derivatives against *P. falciparum in Vitro*

	IC_{50} (nM)		
compounds	control (*a*)	with 20 μM $FeCl_3$ (*b*)	ratio of *b*/*a*
artemisinin 1	6.04	5.41	0.9
compound **6**	2.82	3.38	1.2
compound **7**	14.79	16.80	1.1
compound **8**	10.61	9.21	0.9
compound 11	19.36	32.91	1.7
compound **13**	45.53	48.71	1.1

used to link covalently with artemisinin. The iron chelators and the novel artemisinin derivatives were investigated for antimalarial activity against a multidrug-resistant *P. falciparum* K1 strain *in vitro*. The results are summarized in Tables **1** and 2. All the chosen iron chelators possessed intrinsic antimalarial activity. Derivatization of artemisinin into compound **3**, which is an ether derivative of dihydroartemisinin, exerted the antimalarial activity in the same range as its parent compound. Modifications of compound **3** into compounds **6**–**13** by covalent linking with iron chelators did not increase the antimalarial potency of these derivatives over that of artemisinin but did not diminish the potency remarkably either. Although compounds **9** and **14** structurally possess iron binding capability, they were impaired by their intrinsic instability and therefore might not be expected to show any superior antimalarial potency. Compound **15**, with a linked molecule of diethyl dithiocarbamate, which is both an iron chelator and an inhibitor of the antioxidant enzyme superoxide dismutase,[10] also has comparable activity with artemisinin.

In order to check whether the antimalarial acitivity might depend on previously chelated iron in the medium, $FeCl_3$ was added to antimalarial testing system. Table 3 shows that the presence of 20 μM $FeCl_3$ did not enhance the killing effect of these synthetic derivatives. The antimalarial activities of these derivatives therefore did not depend on the presence of exogenous iron.

In the testing of our hypothesis, it is important that the derivatives can chelate iron under the conditions of our experiment. We have ascertained, from the change in visible spectrum, that the salicylhydroxamate derivative **13** could indeed chelate with Fe(III) although not as effectively as the parent salicylhydroxamic acid.

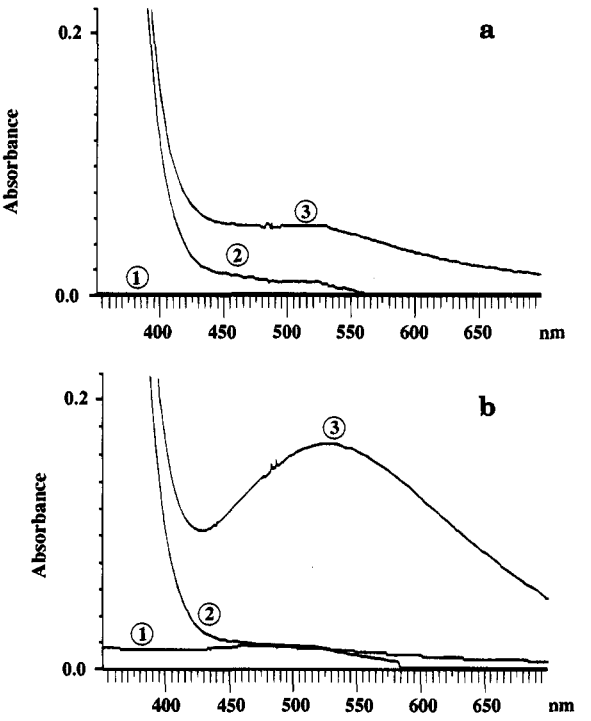


Figure 1. (A) Spectra of (1) 0.19 mM salicylhydroxamate derivative dissolved in 5 mM sulfuric acid, (2) 2.5 mM ferric nitrate dissolved in 5 mM sulfuric acid, and (3) 0.19 mM salicylhydroxamate derivative plus 2.5 mM ferric nitrate dissolved in 5 mM sulfuric acid. (B) Spectra of (1) 0.075 mM salicylhydroxamic acid dissolved in 5 mM sulfuric acid, (2) 2.5 mM ferric nitrate dissolved in 5 mM sulfuric acid, and (3) 0.075 mM salicylhydroxamic acid plus 2.5 mM ferric nitrate dissolved in 5 mM sulfuric acid.

Figure 1A shows the appearance of a peak at 515 nm when Fe(III) is present together with the derivative **13**. A similar, but stronger, peak also appears at 525 nm with the chelated parent salicylhydroxamate.

Although the results from our study have not given any indication that the presence of an iron chelator in the vicinity of artemisinin can potentiate its action, the fact that the linked compounds still retain antimalarial activities nevertheless encourages us to explore other derivatives which will meet the criteria anticipated for synergistic interaction.

Conclusions

Several novel artemisinin derivatives have been synthesized based on the requirement of iron in the mechanism of action of artemisinin. The general route for establishing a linkage of iron chelators to the C-10 lactol of artemisinin nucleus in this study is accomplished by a displacement reaction. Although the novel hybrids obtained did not show enhanced antimalarial activities, they were nontheless comparable to that of artemisinin. Hence they might be useful drugs if they exhibit less toxicity than artemisinin or other known derivatives. Furthermore, the study reveals the use of other iron chelators which might interact synergistically with artemisinin. Some of the linked derivatives reported here do not have high chelating ability (e.g., compounds **6**, **7**, **8**, **10**, **11**, **12** and **13**, and **15**). However, we have shown that compound **13** has a finite chelating ability, although not as high as the parent chelator salicylhydroxamate. Some of the compounds synthesized in this study (e.g., compounds **9** and **14**) turn out

to be unstable, possibly due to intramolecular redox reaction (between peroxide and iron chelator moieties). Consequently, more suitable chelators for optimum synergistic interaction with artemisinin still have to be found.

Experimental Section

Chemistry. NMR spectra were recorded either on a Varian EM 360L or on a Bruker AM 400 spectrometer. The IR spectra were determined on a JASCO model A-302 or a Perkin-Elmer 2000 NIR FT Raman spectrometer. Elemental analyses were carried out on a Perkin-Elmer elemental analyzer 2400 CHN, and the results are within 0.4% of the theoretical values. Mass spectra were recorded on a JMS-DX 300 JEOL mass spectrometer. Melting points were determined on an electrothermal melting point apparatus (Electrothermal 9100). Merck's Kiesegel 60 PF254 were used for preparative layer chromatography.

Conversion of Dihydroartemisinin to the Bromide or Hydroxy Derivatives (3–5). Dihydroartemisinin **2**[4] (500 mg, 1.76 mmol) in dry benzene (5 mL) was treated with bromopropanol (2 mL, 20 mmol) under nitrogen in the presence of freshly distilled boron trifluoride etherate ($BF_3{\cdot}Et_2O$, 1 mL of 5% (v/v) $BF_3{\cdot}Et_2O$ in dry benzene) at room temperature for 2 h. Purification of the bromo derivative **3** (90% yield) was performed by crystallization from hexane at −20 °C.

The reaction of dihydroartemisinin with propanediol gave the corresponding alcohols, **4** and **5**, which after purification by TLC (silica gel, using 50% ethylacetate in hexane as eluent) followed by crystallization from hexane at −20 °C provided pure samples in 11.4 and 30.11%, respectively.

Compound 3: white crystals; mp 85–86.5 °C (from hexane); IR ν_{max} (Nujol) 1100, 880, and 825 cm^{-1}; MS *m/e* (relative intensity) 388 ($M^+ - H_2O$, 0.4); ^1H NMR ($CDCl_3$) δ 0.90 (d, 3H, J = 7.36 Hz, H-16), 0.90 (m, 1H, H-7α), 0.95 (d, 3H, J = 6.25 Hz, H-15), 1.25 (m, 1H, H-5a), 1.33 (m, 1H, H-6), 1.44 (s, 3H, H-14), 1.48 (m, 1H, H-8a), 1.50 (m, 1H, H-5β), 1.64 (m, 1H, H-7β), 1.75 (m, 2H, H-8), 1.87 (m, 1H, H-5α), 2.03 (ddd, 1H, J = 14.32, 4.67, 2.92 Hz, H-4β), 2.10 (m, 2H, H-19), 2.37 (ddd, 1H, J = 14.32, 13.9, 3.94 Hz, H-4α), 2.63 (ddq, 1H, J = 2.8, 3.4, 7.36 Hz, H-9), 3.49 (t, 2H, J = 6.41 Hz, H-20), 3.49 (dt, 1H, J = 10.06, 5.73 Hz, H-18b), 4.00 (dt, 1H, J = 10.06, 5.73 Hz, H-18a), 4.80 (d, 1H, J = 3.4 Hz, H-10), 5.42 (s, 1H, H-12); ^{13}C NMR (δ in $CDCl_3$) 12.92, 20.31, 24.47, 24.62, 26.13, 30.53, 30.84, 32.50, 34.58, 36.38, 37.40, 44.34, 52.54, 65.66, 81.00, 87.88, 102.06, 104.06 $[\alpha]_D$ +122.1° (*c* 0.35, $CHCl_3$). Anal. ($C_{18}H_{29}O_5Br$) C, H.

Compound 4: white crystals; mp 106–109 °C (from hexane); IR ν_{max} (Nujol) 3500 (sharp), 1100, 880, and 830 cm^{-1}; MS *m/e* (relative intensity) 310 ($M^+ - 32$, 1); ^1H NMR ($CDCl_3$) δ 0.8–2.8 (m, 23H), 3.35 (s, 1H, OH, disappeared after D_2O), 3.45–4.3 (m, 4H, 2 × H-18, 2 × H-20), 4.50 (d, 1H, J = 9 Hz, H-10), 5.40 (s, 1H, H-12); $[\alpha]_D$ +73.3° (*c* 0.03, $CHCl_3$). Anal. ($C_{18}H_{30}O_6$) C, H.

Compound 5: white crystals; mp 72–73 °C (from hexane); IR ν_{max} (neat) 3500 (sharp), 1100, 880, and 830 cm^{-1}; MS *m/e* (relative intensity) 310 ($M^+ - 32$, 1); ^1H NMR ($CDCl_3$) δ 0.85–2.8 (m, 23H), 3.13 (s, 1H, OH, disappeared with D_2O), 3.3–4.3 (m, 4H, 2 × H-18, 2 × H-20), 4.75 (d, 1H, J = 3.5 Hz, H-10), 5.38 (s, 1H, H-12); $[\alpha]_D$ +141.1° (*c* 0.48, $CHCl_3$). Anal. ($C_{18}H_{30}O_6$) H; C: calcd, 63.14; found, 64.21.

Coupling of Artemisinin Derivative 3 with Iron Chelators 6–13. Typical Procedure. A mixture of 50% sodium hydride in oil (48 mg, 1 mmol) and methyl caffeate (1.5 mmol) in dimethyl sulfoxide (5 mL) was stirred under nitrogen at room temperature for 1 h. The reaction mixture was cooled down to 4 °C, and a solution of compound **3** (405 mg, 1 mmol) in dimethyl sulfoxide (5 mL) was introduced and the solution was left stirring at room temperature for 2 h. The reaction mixture was cooled down to 4 °C and acidified with dilute aqueous hydrochloric acid. The organic material was extracted into ethyl acetate, and the combined ethyl acetate extract was washed with water, dried ($MgSO_4$), filtered, and finally evaporated to dryness. The crude product was purified by TLC to obtain compound **6**. Compounds **7–13** were prepared accordingly.

Compounds 6: white crystals; mp 141–144 °C (from chloroform); IR ν_{max} ($CHCl_3$) 3550, 1700, 1640, 1620, 1590, 1515, 1100, 990, 880, and 830 cm^{-1}; MS *m/e* (relative intensity) 472 ($M^+ - 46$, 4); ^1H NMR ($CDCl_3$) δ 0.85 (m, 1H, H-7α), 0.85 (d, 3H, J = 6.27 Hz, H-15), 0.89 (d, 3H, J = 7.36 Hz, H-16), 1.02 (m, 1H, H-6), 1.19 (ddd, 1H, J = 11.47, 11.21, 6.54 Hz, H-5a), 1.42 (s, 3H, H-14), 1.43 (m, 1H, H-8a), 1.45 (m, 1H, H-5β), 1.50 (m, 1H, H-7β), 1.70 (m, 2H, H-8) 1.83 (dddd, 1H, J = 13.6, 3.08, 3.64, 6.84 Hz, H-5α), 2.02 (ddd, 1H, J = 14.48, 4.69, 3.05 Hz, H-4β), 2.11 (m, 2H, H-19), 2.35 (ddd, 1H, J = 14.48, 13.63, 3.95 Hz, H-4α), 2.64 (ddq, 1H, J = 7.36, 4.40, 3.36 Hz, H-9), 3.55 (dt, 1H, J = 9.97, 5.54 Hz, H-18b), 3.79 (s, 3H, H-10′), 4.07 (ddd, 1H, J = 9.97, 7.08, 5.82 Hz, H-18a), 4.19 (t, 2H, J = 6.41 Hz, H-20), 4.81 (d, 1H, J = 3.36 Hz, H-10), 5.33 (s, 1H, H-12), 5.82 (s, 1H, OH), 6.29 (d, 1H, J = 15.93 Hz, H-8′), 6.83 (d, 1H, J = 8.34 Hz, H-5′), 7.00 (dd, 1H, J = 8.34, 2.06 Hz, H-6′), 7.15 (d, 1H, J = 2.06 Hz, H-2′), 7.59 (d, 1H, J = 15.93 Hz, H-7′); ^{13}C NMR ($CDCl_3$) δ 12.94, 20.31, 24.54, 24.59, 26.09, 29.03, 30.82, 34.50, 36.37, 37.35, 44.28, 51.53, 52.44, 64.04, 65.82, 80.92, 87.82, 101.91, 104.15, 111.34, 113.36, 115.88, 121.61, 128.11, 144.59, 146.05, 147.73, 167.63; $[\alpha]_D$ +84.2° (*c* 0.30, $CHCl_3$). Anal. ($C_{28}H_{38}O_9$) C, H.

Compound 7: semisolid; IR ν_{max} (Nujol) 3370 (broad), 1650, 1620, 1600, 1110, 880 and 835 cm^{-1}; *m/e* (relative intensity) 420 ($M^+ - 46$, 0.2); ^1H NMR ($CDCl_3$) δ 0.81 (d, 3H, J = 7.35 Hz, H-16), 0.82 (m, 1H, H-7α), 0.87 (d, 3H, J = 6.00 Hz, H-15), 1.15 (m, 1H, H-5a), 1.21 (m, 1H, H-6), 1.35 (s, 3H, H-14), 1.38 (m, 1H, H-8a), 1.41 (m, 1H, H-5β), 1.53 (dq, 1H, J = 13.16, 3.10 Hz, H-7β), 1.68 (m, 2H, H-8), 1.80 (m, 1H, H-5α), 1.96 (ddd, 1H, J = 14.05, 4.42, 3.18 Hz, H-4β), 2.00 (quin, 2H, J = 6.29 Hz, H-19), 2.28 (ddd, 1H, J = 14.05, 13.91, 3.9 Hz, H-4α), 2.54 (ddq, 1H, J = 7.35, 4.10, 3.5 Hz, H-9), 3.47 (dt, 1H, J = 10.19, 6.06 Hz, H-18b), 3.88 (dt, 2H, J = 6.41, 1.76 Hz, H-20), 3.92 (dt, 1H, J = 10.19, 6.06 Hz, H-18a), 4.41 (s, 2H, H-6′), 4.71 (d, 1H, J = 3.5 Hz, H-10), 5.30 (s, 1H, H-12), 6.46 (s, 1H, H-5′), 7.55 (s, 1H, H-3′); ^{13}C NMR ($CDCl_3$) δ 12.87, 20.19, 24.35, 24.49, 25.95, 29.12, 30.72, 34.41, 36.22, 37.28, 44.18, 52.34, 60.29, 64.41, 66.61, 80.91, 87.70, 101.85, 104.01, 111.41, 139.37, 147.49, 168.04, 174.87; $[\alpha]_D$ +96.4° (*c* 0.29, $CHCl_3$). Anal. ($C_{24}H_{34}O_9$) C, H.

Compound 8: pale yellow needles; mp 140–141 °C (from a mixture of ethyl acetate–hexane); IR ν_{max} (Nujol) 3530, 3450, 1600, 1500, 1480, 1110, 875, and 820 cm^{-1}; MS *m/e* (relative intensity) 404 ($M^+ - 46$, 5), 266 (7), 249 (3), 221 (60), 184 (18), 180 (24), 163 (100), 149 (22), 137 (12), 126 (52), 93 (12), 81 (10), 71 (9), 55 (16), 46 (17), 43 (42); ^1H NMR ($CDCl_3$) δ 0.90 (m, 1H, H-7α), 0.93 (d, 3H, J = 7.39 Hz, H-16), 0.94 (d, 3H, J = 6.12 Hz, H-15), 1.25 (m, 1H, H-5a), 1.30 (m, 1H, H-6), 1.45 (s, 3H, H-14), 1.48 (m, 1H, H-8a), 1.50 (m, 1H, H-5β), 1.60 (dq, 1H, J = 13.19, 3.24 Hz, H-7β), 1.75 (m, 2H, H-8), 1.88 (m, 1H, H-5α), 2.04 (m, 2H, H-19), 2.06 (m, 1H, H-4β), 2.38 (ddd, 1H, J = 14.34, 13.70, 3.98 Hz, H-4α), 2.68 (ddq, 1H, J = 2.92, 3.49, 7.39 Hz, H-9), 3.76 (ddd, 1H, J = 10.89, 6.57, 4.32 Hz, H-18b), 4.06 (m, 1H, H-18a), 4.10 (m, 2H, H-20), 4.87 (d, 1H, J = 3.49 Hz, H-10), 5.44 (s, 1H, H-12), 5.72 (s, 1H, OH, disappeared with D_2O), 6.49 (d, 1H, J = 8.19 Hz, H-4′), 6.49 (d, 1H, J = 8.19 Hz, H-6′), 6.85 (t, 1H, J = 8.19 Hz, H-5′); ^{13}C NMR ($CDCl_3$) δ 12.83, 20.27, 24.43, 24.66, 26.07, 29.90, 30.89, 34.59, 36.42, 37.43, 44.36, 52.52, 65.23, 71.35, 81.06, 88.00, 101.95, 104.23, 108.10, 124.65, 133.69, 149.31; $[\alpha]_D$ +101.5° (*c* 0.62, $CHCl_3$). Anal. ($C_{24}H_{34}O_8$) C, H.

Compound 9: pale yellow oil; IR ν_{max} ($CHCl_3$) 3560 (sharp), 3460 (broad), 1620, 1510, 1470, 1100, 880, and 830 cm^{-1}; MS *m/e* (relative intensity) 404 ($M^+ - 46$, 2), 361 (1), 267 (5), 266 (4), 234 (1), 221 (67), 184 (21), 163 (100), 162 (59), 149 (17), 137 (14), 126 (41), 107 (90), 93 (15), 81 (12), 71 (9), 55 (17), 46 (20), 43 (45). Due to rapid decomposition in solution, no respectable NMR spectrum of **9** was obtained.

Compound 10: colorless oil; MS *m/e* (relative intensity) 502 ($M^+ - 272$, 1); ^1H NMR ($CDCl_3$) δ 0.88 (d, 3H, J = 6.02 Hz, H-15), 0.88 (m, 2H, H-7α), 0.89 (d, 3H, J = 7.30 Hz, H-16), 0.92 (d, 3H, J = 7.30 Hz, H-16), 0.93 (d, 3H, J = 6.02 Hz, H-15), 1.10 (m, 1H, H-6), 1.22 (m, 2H, H-5a), 1.29 (m, 1H, H-6), 1.42 (s, 3H, H-14), 1.44 (m, 2H, H-8a), 1.44 (s, 3H, H-14), 1.45 (m,

2H, H-5β), 1.51 (m, 2H, H-7β), 1.59 (dq, 1H, J = 13.14, 3.19 Hz, H-7β), 1.73 (m, 4H, H-8), 1.83 (m, 1H, H-5α), 1.87 (m, 1H, H-5α), 2.02 (m, 2H, H-4β), 2.04 (m, 2H, H-19), 2.09 (m, 2H, H-19), 2.36 (m, 2H, H-4α), 2.64 (m, 2H, H-9), 3.53 (dt, 1H, J = 9.90, 5.63 Hz, H-18b), 3.66 (m, 1H, H-18b), 4.04 (m, 2H, H-18a), 4.08 (m, 2H, H-20), 4.17 (m, 2H, H-20), 4.81 (d, 1H, J = 3.38 Hz, H-10), 4.84 (d, 1H, J = 3.44 Hz, H-10), 5.33 (s, 1H, H-12), 5.42 (s, 1H, H-12), 6.04 (s, 1H, OH, disappeared with D_2O), 6.44 (dd, 1H, J = 8.30, 1.15 Hz, H-6′), 6.58 (dd, 1H, J = 8.30, 1.15 Hz, H-4′), 6.88 (t, 1H, J = 8.30 Hz, H-5′); ^{13}C NMR ($CDCl_3$) δ 12.92, 12.97, 20.26, 20.28, 24.43, 24.50, 24.62, 24.65, 26.12, 26.14, 29.41, 30.22, 30.86, 34.55, 34.60, 36.40, 37.28, 37.35, 44.34, 44.39, 52.46, 52.51, 64.33, 65.25, 65.40, 70.81, 81.00, 81.05, 87.74, 87.89, 101.90, 101.93, 104.00, 104.09, 104.77, 108.13, 123.77, 134.76, 149.87, 151.84. No satisfactory elemental analysis was obtained.

Compound 11: pale yellow semisolid; IR ν_{max} (Nujol) 3550 (broad), 3250 (broad), 1740, 1605, 1580, 1520, 1490, 870, and 825 cm^{-1}; MS *m/e* (relative intensity), 415 (M^+ − 46, 1); 60-MHz ^1H NMR ($CDCl_3$) δ 0.7−2.9 (m, 23H), 3.32−4.33 (m, 4H, 2 × H-18, 2 × H-20), 4.77 (d, 1H, J = 3.5 Hz, H-10), 5.43 (s, 1H, H-12), 7.5 (m, 3H, H-3′, H-4′, H-5′), 7.75 (m, 2H, H-2′, H-6′), 9.47 (s, 1H, NH, disappeared with D_2O); $[\alpha]_D$ +103.4° (*c* 0.21, $CHCl_3$). Anal. ($C_{25}H_{35}O_7N$) C, H; N: calcd, 3.03; found, 3.46.

Compound 12: pale yellow semisolid; IR ν_{max} (Nujol) 1740, 1660, 1600, 1580, 1500, 1460, 1095, 870, and 820 cm^{-1}; MS *m/e* (relative intensity) 514 (M^+ − 271, 2); ^1H NMR ($CDCl_3$) δ 0.81 (d, 3H, J = 7.35 Hz, H-16), 0.88 (d, 3H, J = 7.32 Hz, H-16), 0.90 (m, 2H, H-7α), 0.94 (d, 3H, J = 6.21 Hz, H-15), 0.95 (d, 3H, J = 5.98 Hz, H-15), 1.22 (m, 1H, H-5a), 1.24 (m, 1H, H-5a), 1.27 (m, 1H, H-6), 1.33 (m, 1H, H-6), 1.43 (s, 6H, H-14), 1.45 (m, 2H, H-8α), 1.48 (m, 2H, H-5β), 1.60 (m, 2H, H-7β), 1.65 (m, 2H, H-8), 1.70 (m, 2H, H-8), 1.72 (m, 2H, H-19), 1.87 (m, 2H, H-5α), 2.01 (m, 2H, H-19), 2.05 (m, 2H, H-4β), 2.36 (ddd, 2H, J = 14.25, 13.77, 3.94 Hz, H-4α), 2.58 (m, 1H, H-9), 2.62 (m, 1H, H-9), 3.26 (dt, 1H, J = 10.01, 6.36 Hz, H-18b), 3.47 (dt, 1H, J = 10.12, 6.24 Hz, H-18b), 3.71 (dt, 1H, J = 10.01, 6.05 Hz, H-18a), 3.79 (m, 4H, H-20), 3.94 (dt, 1H, J = 10.12, 5.97 Hz, H-18a), 4.64 (d, 2H, J = 3.37 Hz, H-10), 4.78 (d, 1H, J = 3.40 Hz, H-10), 5.29 (s, 1H, H-12), 5.38 (s, 1H, H-12), 7.39 (m, 2H, H-3′, H-5′), 7.42 (m, 1H, H-4′), 7.64 (m, 2H, H-2′, H-6′); ^{13}C NMR ($CDCl_3$) δ 12.92, 12.97, 20.30, 24.42, 24.66, 26.14, 27.63, 28.44, 30.74, 30.85, 34.59, 34.64, 36.40, 36.43, 37.35, 37.46, 44.33, 44.42, 52.53, 52.58, 64.68, 65.61, 71.42, 80.95, 81.05, 87.81, 87.88, 101.85, 102.07, 104.03, 104.06, 127.93, 127.93, 128.11, 128.11, 130.48, 134.43, 169.88; $[\alpha]_D$ +128.3° (*c* 0.15, $CHCl_3$). Anal. ($C_{43}H_{63}O_{12}N$) C, H, N.

Compound 13: pale yellow semisolid; IR ν_{max} ($CHCl_3$) 3425 (sharp), 3250 (broad), 1650, 1605, 1590, 1495, 1450, 1305, 1240, 1095, 870, and 820 cm^{-1}; MS *m/e* (relative intensity) 431 (M^+ − 46, 1); ^1H NMR ($CDCl_3$) δ 0.90 (d, 3H, J = 7.32 Hz, H-16), 0.90 (m, 1H, H-7α), 0.93 (d, 3H, J = 6.19 Hz, H-15), 1.25 (m, 1H, H-5a), 1.32 (m, 1H, H-6), 1.43 (s, 3H, H-14), 1.50 (m, 1H, H-8a), 1.51 (m, 1H, H-5β), 1.61 (dq, 1H, J = 13.20, 3.11 Hz, H-7β), 1.75 (m, 2H, H-8), 1.88 (m, 1H, H-5α), 1.99 (m, 2H, H-19), 2.05 (m, 1H, H-4β), 2.37 (dd, 1H, J = 14.03, 13.97, 3.89 Hz, H-4α), 2.63 (ddq, 1H, J = 3.41, 4.12, 7.32 Hz, H-9), 3.55 (dt, 1H, J = 10.05, 5.86 Hz, H-18b), 3.99 (dt, 1H, J = 10.05, 6.20 Hz, H-18a), 4.14 (m, 2H, H-20), 4.80 (d, 1H, J = 3.41 Hz, H-10), 5.45 (s, 1H, H-12), 6.83 (m, 1H, H-5′), 7.00 (m, 1H, H-3′), 7.40 (m, 1H, H-6′), 7.41, (m, 1H, H-4′), 9.36 (s, 1H, NH, disappeared with D_2O), 11.58 (s, 1H, OH, disappeared with D_2O); ^{13}C NMR ($CDCl_3$) δ 12.96, 20.27, 24.51, 24.66, 26.14, 28.54, 30.90, 34.59, 36.44, 37.46, 44.40, 52.55, 65.09, 74.25, 81.11, 87.97, 102.21, 104.23, 112.33, 118.68, 118.85, 125.37, 134.66, 161.23, 169.10; $[\alpha]_D$ +96.3° (*c* 0.58, $CHCl_3$). Anal. ($C_{25}H_{35}O_8N$) C, H, N.

Preparation of Artemisinin−Caffeate Derivative 14. Compound **5** (100 mg, 0.3 mmol) in dry benzene (5 mL) was added to caffeyl chloride at 10 °C, and the mixture was left stirring at 50 °C for 7 h. The crude product was purified by TLC (silica gel, using 50% ethyl acetate in hexane as eluent) to obtain compound **14** (15.5 mg, 0.031 mmol, 10.05% yield).

Compound 14: yellow oil; IR ν_{max} ($CHCl_3$) 3550 (sharp), 3300 (broad), 1700, 1640, 1600, 1510, 1450, 1110, 980, 875, and 820 cm^{-1}; ^1H NMR ($CDCl_3$) δ 0.3−2.8 (m, 25H), 3.2−4.6 (m, 4H, 2 × H-18, 2 × H-20), 4.82 (d, 1H, J = 3.5 Hz, H-10) 5.50 (s, 1H, H-12), 6.30 (d, 1H, J = 16 Hz, H-8′), 7.08−7.22 (m, 3H, H-2′,5′,6′), 7.63 (d, 1H, J = 16 Hz, H-7′). Due to rapid decomposition, no mass ion or elemental analysis of this compound was obtained.

Preparation of Artemisinin−Diethyl Dithiocarbamate Derivative 15. Sodium diethyl dithiocarbamate (148 mg, 0.87 mmol) in acetonitrile (3 mL) was stirred with artemisinin bromide derivative **3** (350 mg, 0.87 mmol) in acetonitrile (3 mL) at room temperature for 1 h. The white precipitate of sodium bromide that formed in the reaction was filtered out. The filtrate was evaporated and diluted with ethyl acetate, washed with water followed by saturated aqueous sodium chloride solution, dried over $MgSO_4$, filtered, and evaporated to dryness. The crude product was purified by TLC (silica) using 30% ethyl acetate in hexane as eluent to obtain pure **15** as yellow oil (332 mg, 0.7 mmol, 80.68% yield).

Compound 15: yellow viscous oil; IR ν_{max} (neat) 1500, 1260, 1200, 1100, 880, 820, and 780 cm^{-1}; MS *m/e* (relative intensity) 473 (M^+, 1), 440 (3), 428 (0.2), 324 (6), 267 (3), 266 (2), 249 (2), 237 (6), 220 (17), 207 (14), 190 (76), 162 (100), 149 (66), 135 (15), 116 (92), 100 (15), 88 (51), 72 (30), 60 (36), 55 (23), 44 (74), 43 (59); ^1H NMR ($CDCl_3$) δ 0.7−3.0 (m, 29H), 3.2−4.4 (m, 8H, 2 × H-18, 2 × H-20, 2 × H-24, 2 × H-26), 4.77 (d, 1H, J = 3.5 Hz, H-10), 5.43 (s, 1H, H-12). Anal. ($C_{23}H_{39}O_5NS_2$) C, H; N: calcd, 2.96; found, 3.69.

Biology

Antimalarial Activity of the Novel Artemisinin Derivatives and Iron Chelators. The antimalarial activity of iron chelators and artemisinin novel derivatives against *P. falciparum*-infected red cells was measured by using a modification of the [^3H]hypoxanthine incorporation method reported by Desjardins.[11] Briefly, the drug was dissolved in dimethyl sulfoxide (DMSO) and diluted with the culture medium to the required concentration. The final concentration of DMSO was less than 0.1%, usually 0.001%, which had no effect on the parasite growth. A mixture of 25 μL of the medium containing the drug and 200 μL of 1.5% cell suspension with 1−2% parasitemia at immature schizont stage was cultured in triplicates for 24 h, after which 25 μL of 0.5 μCi [^3H]-hypoxanthine was added. After an additional 18 h in culture, the cells were harvested onto glass-fiber filters. The radioactivity was measured by a Beckman liquid scintillation counter mode LS-1801. The IC_{50}, the concentrations required for 50% reduction of the radioactivity as compared to control without the drug, of the drug against these infected cells were obtained from dose−response curves.

In testing for effect of iron on the antimalarial activity of the novel artemisinin derivatives, a sterilized solution of freshly prepared 2 mM $FeCl_3$ in double-distilled water was mixed with the parasitized cell suspension. The cell mixture was immediately used for testing the antimalarial activity of the prepared compounds as mentioned above. The final concentration of $FeCl_3$ in the test system was 20 μM.

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