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Articles

Correlation of Antimalarial Activity of Artemisinin Derivatives with Binding Affinity with Ferroprotoporphyrin IX

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The antimalarial activity of a number of artemisinin derivatives, both newly synthesized and currently used as drugs, against *Plasmodium falciparum* in culture shows a correlation with their affinity of binding with ferroprotoporphyrin IX, as measured from the spectral change of the latter. The new C-16-functionalized artemisinin derivatives were obtained through a novel one-pot synthesis of artemisitene (**2**) from naturally abundant artemisinin (**1**), followed by Michael addition with nucleophiles. The correlation points to the biological significance of the interaction of these derivatives with ferroprotoporphyrin IX and may provide a basis for primary screening of peroxidic antimalarials of similar structures.

The prevalence of malaria in many regions of the world, together with the lack of vaccine and the emergence of resistance to antimalarials in use, makes it necessary to search for new antimalarials.[1] Artemisinin derivatives form a group of such new drugs currently under development and already in use in some countries.[2−6] They share a common sesquiterpenoid structure with an endoperoxide function which is essential for the activity. Due to synergism with other oxidant drugs and oxygen and reduction of activity by agents which lower oxidative stress, we and others proposed that the mechanism of their action involves exertion of oxidative stress on the parasite.[7−11] Although the specific nature of the oxidative stress is as yet unclear, the action probably involves desequestration of iron, as the drugs are strongly antagonistic with such iron chelators as desferrioxamine, pyridoxal benzoylhydrazone, and 1,2-dimethyl-3-hydroxypyrid-4-one.[11]

Meshnick *et al.*[12−14] showed that artemisinin interacts strongly with hemin (ferriprotoporphyrin IX) and its ferrous form, ferroprotoporphyrin IX. In parasite metabolism, hemin is left after digestion of hemoglobin by the parasite. A potentially toxic compound, it is converted to hemozoin (malarial pigment), which has been shown to be essentially β-hematin, a polymerized form of heme devoid of immediate toxicity.[15] Some antimalarials, such as chloroquine, interfere with the polymerization, probably accounting for the generation of toxicity to the parasite.[16] The interaction of artemisinin with hemin, which leads to adduct formation and presumably interferes with the normal conversion of hemin to hemozoin, could therefore form the basis of its antimalarial action. Hemin or its immediate precursor, ferroprotoporphyrin IX, could therefore be the biological target of artemisinin. Alternatively, the interaction could be similar to, and provide a model for, the interaction of artemisinin with its real, as yet unidentified target within the parasite. It is therefore important to understand the mode of interaction of artemisinin and its derivatives with hemin and ferroprotoporphyrin IX and its relation with the antimalarial action of the drugs. A correlation between a parameter

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of such interaction and the antimalarial activity would provide strong evidence for its biological significance and could furthermore give a convenient method for primary screening of new artemisinin derivatives.

In this paper, we show that a number of artemisinin derivatives, many of which are those with modifications at C-16, interact strongly with ferroprotoporphyrin IX. The dissociation constants for the interaction of the artemisinin derivatives with ferroprotoporphyrin IX, calculated from spectral change due to binding, show a correlation with the antimalarial activity of the derivatives. We also demonstrate that artemisitene, **2**, can be obtained from artemisinin, **1**, by a one-pot selenoxide elimination reaction. Compound **2** readily undergoes a 1,4-addition reaction with nucleophiles; hence it serves as a convenient precursor for the C-16-functionalized artemisinin derivatives **3**.

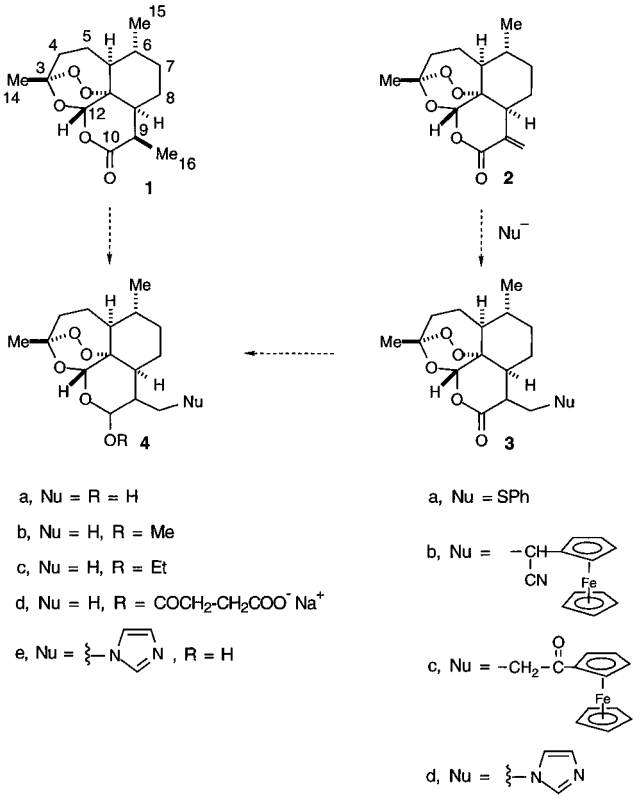
Chemistry

Artemisitene, **2**, the oxidized form of artemisinin, **1**, is reported to be a minor constituent in an American variant of *Artemisia annua*.[17] The unique structural feature of **2**, bearing an exocyclic α,β-unsaturated ketone moiety, chemically presents an opportunity for nucleophilic attack *via* a (Michael) 1,4-addition reaction. The overall process, finally providing the Michael adduct, *e.g.*, **3**, is equivalent to a straightforward derivatization of the artemisinin molecule at the C-16 methyl carbon (see numbering in **1**). Artemisinin derivatives such as **3** (and also **4** where Nu ≠ H) would be highly attractive for the structure−activity relationship study due to the fact that they are structurally different from other artemisinin derivatives which are now in use clinically, *e.g.*, artemether, arteether, and artesunate, **4b**−**d**, respectively. The latter are products obtained from derivatization of **1** at the C-10 carbonyl moiety and are known to readily metabolize *in vivo* giving dihydroartemisinin, **4a**.[18]

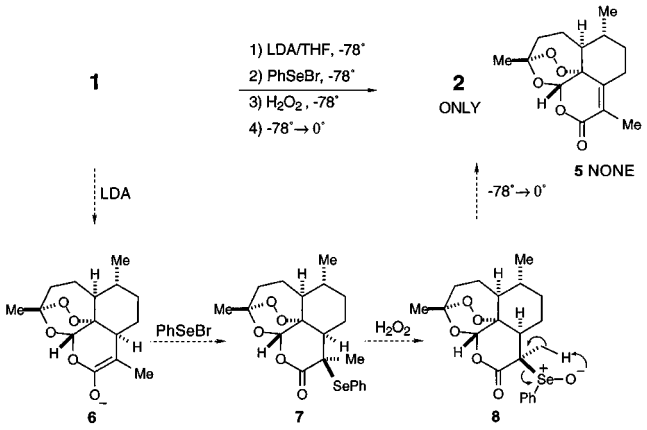
However, owing to the fact that artemisitene (**2**) coexists with **1** in the plant in rather low and variable quantities and also that its isolation and purification from the crude extract are not straightforward,[17] artemisitene has so far been underutilized.[19] Obviously, a semisynthesis of artemisitene from the cheap, naturally abundant artemisinin would provide the solution to overcome this drawback. Indeed, following a number of unsuccessful attempts,[20] artemisitene was recently finally synthesized from artemisinin.[21] Upon inspection of the molecular model of **1** we concluded that the short and convenient method for conversion of **1** to **2** should involve the selenoxide elimination reaction.[22,23] This mentioned approach has already been examined but gave a negative result from which the starting material and its C-9 stereoisomer, 9-*epi*-artemisinin, were obtained.[20] Therefore it was of importance to reinvestigate the method of conversion of **1** to **2**.

Here we report a straightforward, regiospecific, one-pot, high-yield conversion of artemisinin (**1**) to artemisitene (**2**) involving selenoxide elimination and the reaction of **2** with various nucleophiles in a Michael addition fashion. Upon treatment of **1** with lithium diisopropylamide (LDA) in tetrahydrofuran at −78 °C followed by sequential additions of phenylselenenide bromide[24] and 30% aqueous hydrogen peroxide solution followed by raising the reaction temperature to 0 °C,

Scheme 1



Scheme 2



artemisitene (**2**) was obtained as the sole product in 73% purified yield.

The origin of reaction regiospecificity, providing only **2** without any trace of its double-bond isomer, isoartemisitene **5**, can be explained in terms of involving the formation of lithium ester enolate **6** which stereospecifically reacts with phenylselenide bromide on the β-face to provide **7** and finally **2** as shown in Scheme 2.

Artemisitene (**2**) readily reacts with sulfur, carbon, and nitrogen nucleophiles to give the corresponding Michael adducts **3** (**3a**−**d** in Scheme 1). In some cases lithium anions derived from ferrocene derivatives were deliberately employed as carbon nucleophiles (**3b**,**c**). While only one (β-stereochemistry at C-9) isomer was detected in the case of adduct **3a**, isomeric mixtures were obtained in the other cases.

Results and Discussion

If the binding of artemisinin with ferroprotoporphyrin IX is a biologically significant step, leading to killing of

Table 1. Dissociation Constants (K_d) and IC_{50} Values of Artemisinin and Its Derivatives against *P. falciparum in Vitro*

compd	K_d ($\times 10^{-4}$ M^{-1})	IC_{50} (M)
1	0.24	5×10^{-9}
2	0.26	3×10^{-8}
3a	0.34	2×10^{-8}
3b	0.14	1×10^{-9}
3c (isomer II)	0.25	2.1×10^{-9}
3d	0.20	3.2×10^{-8}
4a	0.21	1.1×10^{-9}
4b	0.27	4.1×10^{-9}
4c	0.32	2.4×10^{-8}
4d	0.38	6.2×10^{-10}
4e	0.05	3.9×10^{-10}

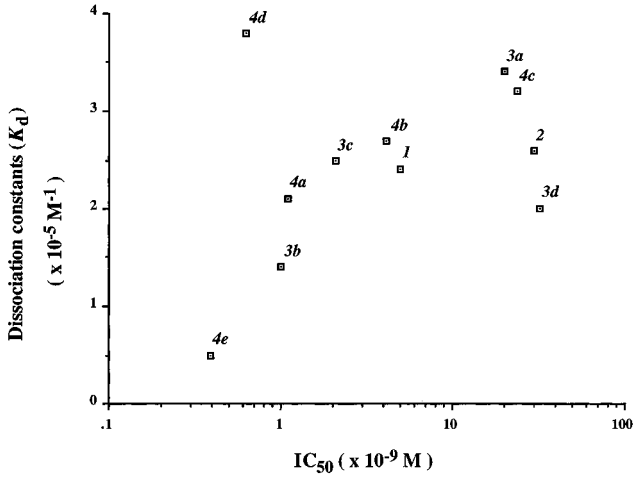


Figure 2. Relation between dissociation constants (K_d) and antimalarial activity of artemisinin and its derivatives against *P. falciparum in vitro.*

the parasite, or reflects an as yet unknown step leading to such killing, the strength of binding may have correlation with the killing effect of artemisinin derivatives. The absorption peak of ferroprotoporphyrin IX at 415 nm was strongly and immediately reduced by a number of artemisinin and dihydroartemisinin derivatives which have antimalarial activity (see Figure 1 in the Supporting Information). In contrast, deoxyartemisinin, which is devoid of antimalarial activity, did not give rise to a similar spectral change. From the dependence of spectral change on the concentration of the artemisinin derivatives, the dissociation constants (K_d) between these derivatives and ferroprotoporphyrin IX can be calculated based on a simple binding model (Table 1). With the exception of artesunate (**4d**), the values of K_d show a correlation with log IC_{50} (concentration of the drug required for killing 50% of *Plasmodium falciparum* in culture) (Figure 2).

A similar spectral change has been shown to occur with hemin (ferriprotoporphyrin IX) in the presence of artesunate, albeit over a much longer period.[25] We also confirm a similar interaction in our experimental system, when the incubation was made in the absence of the reducing agent dithionite (Figure 3 in the Supporting Information). The change can be observed after several hours, instead of immediately as in the presence of dithionite.

The correlation between antimalarial activity of artemisinin derivatives and their dissociation constants for the binding interaction with ferroprotoporphyrin IX (Figure 2) indicates that this interaction may be biologically significant. Ferroprotoporphyrin IX is released from hemoglobin in the food vacuole after the parasite has digested the globin component. Normally, it would proceed to become ferriprotoporphyrin IX and subsequently hemozoin. Interaction with artemisinin or its derivatives disrupts this normal process and may give rise to potentially toxic agents. Meshnick *et al.* showed that, after binding of artemisinin with hemin and hemozoin, an adduct is formed with a molecular weight almost equal to the sum of the two components.[12] However, the biological significance of such adduct formation is unclear. Asawamahasakda *et al.*[26,27] showed later that artemisinin and derivatives bind covalently with parasite protein components, and the extent of this binding reflects the antimalarial activities of the derivatives. It was therefore postulated that artemisinin acts in two steps: firstly, an artemisinin radical is formed after interaction with suitable parasite component(s), and secondly, the radical alkylates other essential parasite component(s) thereby interfering with the normal function of the component(s). Hemin or ferroprotoporphyrin IX could, according to this scenario, play a role in the generation of the artemisinin radical, but although it is also alkylated by this radical, this alkylation was not considered vital for the killing of the parasite. Our finding strengthens the role of ferroprotoporphyrin IX in the initial interaction with artemisinin or its derivatives. It is presently unclear whether this interaction leads to generation of artemisinin-derived free radicals or to the interruption of the normal process of hemozoin formation, thereby leaving the heme−artemisinin complex in the form which causes damage to the parasite. Since the relation between the binding of ferroprotoporphyrin IX and artemisinin derivatives and the IC_{50} as shown in Figure 2 is an exponential one, the binding must lead to, or reflect a process which leads to, amplification of the parasite damage leading to killing.

Although artemisinin is active against chloroquine-resistant parasites and must therefore act differently in the killing process, the interaction of artemisinin and derivatives with heme draws some parallels with chloroquine−hemin interaction, which presumably forms the basis for its antimalarial action.[16,28] Another similarity noted earlier is that a normally drug-sensitive malaria parasite becomes resistant to both artemisinin and chloroquine on infection of α-thalassemic erythrocytes.[29] It is therefore possible that early events in the interaction of the drug with the parasite are similar for artemisinin and chloroquine.

In our previous search for new artemisinin-based derivatives with good antimalarial activity, we linked various moieties to artemisinin or its dihydro derivative. In an earlier approach,[30] we linked the hydroxyl group at C-10 of dihydroartemisinin to an alkyl chain which is in turn linked to other functional groups. The ether derivatives of dihydroartemisinin, which fall into the same group of other artemisinin derivatives under clinical development or already in clinical use, are unstable *in vivo*, and all break down to dihydroartemisinin which is presumably the major active form of all these drugs.[18,31,32] This group of compounds would not be expected to yield much information regarding the requirement for interaction between artemisinin derivatives with their biological targets. Nevertheless, they

can still provide some structural information in binding studies with isolated components.

The deviation shown by artesunate from the relationship between the dissociation constants for ferroprotoporphyrin IX binding and the antimalarial activity can be explained from the fact that the active form of the drug is probably dihydroartemisinin formed from the decomposition of the hemisuccinate. The intact form of the drug, if any exists in the test system, would be less active as predicted from the relationship found from other derivatives. This observation applies to all artemisinin derivatives which give rise to dihydroartemisinin *in vivo* or *in vitro*. The relationship between the biological activity of artemisinin derivatives and their chemical property, such as interaction with ferroprotoporphyrin IX, is obtained with more confidence by studying stable derivatives, such as those based on modification at C-16 as presented here.

In conclusion, we have shown that a number of artemisinin derivatives, many of which are those with modifications at C-16, interact strongly with ferroprotoporphyrin IX, causing a major immediate decrease in the Soret band. The spectrum of hemin is also similarly affected, albeit much more slowly. The dissociation constants for the interaction of the artemisinin derivatives with ferroprotoporphyrin IX, calculated from a model of simple, reversible binding, show a correlation with the antimalarial activity of the derivatives, as assessed from *P. falciparum in vitro* culture. However, artesunate shows a stronger antimalarial activity than would be expected from the correlation with other artemisinin derivatives. This correlation provides a rationale for further investigation of the nature of the interaction of artemisinin-derived compounds with ferroprotoporphyrin IX and its biological significance and could form a basis for a convenient assay for the screening of peroxidic antimalarials of similar structure and mode of action. This would be very beneficial in future drug development, considering the fact that no such convenient assay exists at present.

Experimental Section

Chemistry. NMR spectra were recorded on a Bruker DPX 300 spectrometer. The IR spectra were determined on a JASCO model A-302 or a Perkin-Elmer 2000 NIR FT Raman spectrometer. UV spectroscopy was studied with a JASCO UVIDEC-650 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. Artemisinin was purchased from the National Center of Natural Science and Technology, Vietnam. β-Arteether was obtained as a gift from Artecef BV, The Netherlands.

Conversion of Artemisinin (1) to Artemisitene (2). To a solution of lithium diisopropylamide, LDA (2.2 mmol), prepared by the conventional method from diisopropylamine and *n*-butyllithium, in THF (5 mL) at −78 °C was slowly added a solution of artemisinin (**1**) (564 mg, 2.0 mmol), and the reaction mixture was left stirring at −78 °C for 10 min. A solution of freshly distilled phenylselenenide bromide (519 mg, 2.2 mmol) in THF (2 mL) was introduced, and the solution was again left stirring at −78 °C for 15 min, after which glacial acetic acid (0.3 mL) was added followed by a solution of 30% aqueous hydrogen peroxide (1.5 mL). The reaction temperature was raised to 0 °C, and the mixture was stirred for 30 min. Water (50 mL) was added and the organic material extracted into methylene chloride. The methylene chloride solution was successively washed with saturated aqueous sodium bicarbonate solution and water, then dried ($MgSO_4$), filtered, and evaporated to dryness. The crude product was purified by silica gel column chromatography using a mixture of hexane/ethyl acetate/methylene chloride (8:2:0.1) as eluant to give, after crystallization from methylene chloride/hexane, artemisitene (**2**) (409 mg, 73.21% yield).

2: white crystals; mp 163−164 °C (lit.[17] mp 161−162 °C); IR ν_{max} (KBr) 3027, 2933, 1725, 1457, 1380, 1223, 1170, 1112, 1034, 999, 880, 833 cm^{-1}; MS *m/e* (rel intensity) 248 (M^+ − 32, 2), 230 (32), 220 (4), 205 (6), 190 (68), 93 (74), 55 (100); ^1H NMR ($CDCl_3$) δ 0.94 (d, J = 5.6 Hz, 3H, H-15), 1.16 (m, 1H, H-8β), 1.38 (s, 3H, H-14), 1.33−1.53 (m, 4H, H-5a, H-6, H-5β, H-7α), 1.67 (m, 1H, H-8α), 1.72 (m, 1H, H-7β), 1.91 (m, 1H, H-5α), 2.00 (ddd, J = 14.89, 4.67, 2.29 Hz, 1H, H-4β), 2.33 (m, 1H, H-4α), 2.49 (dd, J = 13.46, 4.52 Hz, 1H, H-8a), 5.60 (d, J = 0.68 Hz, 1H, H-16), 5.93 (s, 1H, H-12), 6.47 (s, 1H, H-16); ^{13}C NMR ($CDCl_3$) δ 19.68, 24.43, 25.26, 31.41, 33.50, 35.68, 37.55, 45.86, 49.93, 79.28, 93.36, 105.23, 130.19, 134.84, 162.61; $[\alpha]_D$ +125.97° (*c* 0.996, $CHCl_3$). Anal. ($C_{15}H_{20}O_5$)C,H.

Nucleophilic Addition of Nucleophiles to 2. Preparation of 3. Typical procedure for the preparation of **3a**−**c**: To a solution of lithium phenylthiolate (1.1 mmol), prepared *in situ* from phenylthiol and *n*-butyllithium, in THF (1 mL) at −78 °C was added a solution of artemisitene (**2**) (280 mg, 1 mmol) in THF (2 mL), and the mixture was left stirring at −78 °C for 30 min. Saturated aqueous ammonium chloride solution workup followed by extraction with methylene chloride, drying ($MgSO_4$), and evaporation gave the crude product which upon crystallization from ethyl acetate/hexane provided the adduct **3a** (280 mg, 71.8% yield) as a single (β, C-9) isomer.

3a: white crystals, mp 99−100 °C; IR ν_{max} ($CHCl_3$) 2933, 2877, 1728, 1584, 1440, 1379, 1106, 1010, 979, 878, 835, 692 cm^{-1}; MS *m/e* (rel intensity) 392 (M^+ + 2, 4), 391 (M^+ + 1,12), 390 (M^+, 53), 376 (0.2), 375 (0.9), 374 (1), 361 (0.6), 360 (0.1), 358 (0.2), 285 (16), 267 (7), 257 (3), 123 (100), 109 (51), 67 (28), 55 (68); ^1H NMR ($CDCl_3$) δ 0.99 (d, J = 6 Hz, 1H, H-15), 1.20 (m, 1H, H-8β), 1.35 (m, 1H, H-7α), 1.45 (s, 3H, H-14), 1.39−1.50 (m, 3H, H-8a, H-6, H-5β), 1.54−1.60 (m, 1H, H-7β), 1.66 (ddd, J = 12.96, 6.37, 3.16 Hz, 1H, H-8α), 1.90−1.98 (m, 1H, H-5α), 2.06 (ddd, J = 14.82, 4.41, 2.82 Hz, 1H, H-4β), 2.21 (dd, J = 13.53, 4.61 Hz, 1H, H-5a), 2.27 (ddd, J = 11.65, 3.34, 0.95 Hz, 1H, H-9), 2.39 (m, 1H, H-4α), 3.13 (dd, J = 13.8, 11.67 Hz, 1H, H-16), 3.86 (dd, J = 13.8, 3.34 Hz, 1H, H-16), 5.92 (s, 1H, H-12), 7.19 (dddd, J = 7.23, 7.23, 1.40, 140 Hz, 1H, H-20), 7.29 (m, 2H, Ar-H), 7.38 (m, 2H, Ar-H); ^{13}C NMR ($CDCl_3$) δ 19.78, 24.65, 25.40, 31.19, 33.78, 35.78, 37.50, 37.93, 41.07, 43.35, 50.27, 80.67, 94.00, 105.36, 126.43, 129.12, 129.35, 134.43, 170.49; $[\alpha]_D$ +185.57° (*c* 0.499, $CHCl_3$). Anal. ($C_{21}H_{26}SO_5$)C,H.

Anions of the ferrocene derivatives were prepared by treatment of the corresponding ferrocenes with lithium diisopropylamide.

3b: obtained as a mixture of two (C-17) stereoisomers in a ratio of 1:1 (NMR analysis); yellow crystals; mp 139−140 °C; 86.05% yield; IR ν_{max} ($CHCl_3$) 3025, 2931, 2243, 1733, 1453, 1379, 1136, 1107, 1002, 882 cm^{-1}; MS *m/e* (rel intensity) 506 (M^+ + 1, 26), 505 (M^+, 100), 489 (18), 463 (6), 445 (2), 389 (4), 250 (3), 237 (71), 224 (49), 165 (17), 121 (66); ^1H NMR ($CDCl_3$) δ 1.08 [m, 2H, H-8β (isomers I and II)], 1.27 [br s, 6H, H-15 (isomers I and II)], 1.41 [s, 6H, H-14 (isomers I and II)], 1.32−1.49 [m, 9H, H-16 (isomer II), H-7α (isomers I and II), H-5β (isomers I and II), H-6 (isomers I and II), H-5a (isomers I and II)], 1.59−1.72 [m, 5H, H-5α (isomers I and II), H-8α (isomer I), H-8a (isomers I and II)], 1.84−2.04 [m, 6H, H-16 (isomer I), H-16 (isomer II), H-4β (isomers I and II), H-7β (isomers I and II)], 2.22−2.56 [m, 6H, H-16 (isomer I), H-9 (isomers I and II), H-4α (isomers I and II), H-8α (isomer II)], 2.63 [ddd, J = 13.97, 7.13, 7.13 Hz, 1H, H-16 (isomer I)], 3.92 [t, J = 7.88, 7.88 Hz, 1H, H-17 (isomer I)], 4.18−4.24 [m, 1H, H-17 (isomer II)], 4.19 [s, 5H, Ar-H (isomer II)], 4.22 [s, 5H, Ar-H (isomer I)], 4.13−4.24 [m, 8H, Ar-H (isomers I and II)], 5.87 [s, 1H, H-12 (isomer I)], 5.90 [s, 1H, H-12 (isomer II)]; ^{13}C NMR ($CDCl_3$) δ 19.68 (isomers I and II), 24.53 (isomers I and II), 25.27 (isomers I and II), 30.32 (isomer I), 30.58 (isomer II), 30.75 (isomers I and II), 32.13 (isomer II), 33.65 (isomer I),

35.72 (isomer II), 37.34 (isomer I), 37.37 (isomer II), 39.77 (isomer I), 41.56 (isomer II), 42.70 (isomers I and II), 43.17 (isomer I), 45.40 (isomer II), 50.17 (isomers I and II), 66.22 (isomers I and II), 66.46 (isomers I and II), 67.44 (isomers I and II), 68.13 (isomers I and II), 68.35 (isomers I and II), 68.44 (isomers I and II), 69.13 (isomer II), 69.26 (isomer I), 79.78 (isomer II), 79.89 (isomer I), 82.48 (isomer I), 83.34 (isomer II), 93.71 (isomer I), 93.88 (isomer II), 105.31 (isomers I and II), 120.29 (isomer I), 120.66 (isomer II), 170.68 (isomer I), 170.95 (isomer II); $[\alpha]_D$ +44.97° (*c* 0.378, $CHCl_3$). Anal. ($C_{27}H_{31}NFeO_5$)C,H.

3c: obtained as two separable (α and *β*) C-9 stereoisomers (by PLC using silica gel/20% ethyl acetate in hexane) in a ratio of 1:29, respectively (IC_{50}) = 2.1×10^{-9} M for isomer I and $IC_{50} = 3.15 \times 10^{-9}$ M for isomer II).

Isomer I: orange crystals; mp 155−156 °C; 3% yield; IR ν_{max} ($CHCl_3$) 3014, 2932, 1736, 1663, 1456, 1380, 1264, 830, 761, 732 cm^{-1}; MS *m/e* (rel intensity) 508 (M^+, 100), 492 (20), 462 (8), 448 (3), 420 (3), 391 (3), 282 (8), 251 (2), 240 (8), 228 (69), 213 (21), 186 (35), 121 (77), 67 (13); ^1H NMR ($CDCl_3$) *δ* 0.94 (d, *J* = 5.42 Hz, 3H, H-15), 0.99−1.11 (m, 2H, H-7α, H-8*β*), 1.38 (s, 3H, H-14), 1.28−1.47 (m, 3H, H-6, H-5a, H-5*β*), 1.65−1.70 (m, 2H, H-7*β*, H-16), 1.73−1.80 (m, 2H, H-8α, H-8a), 1.92−2.02 (m, 2H, H-4*β*, H-5α), 2.10 (m, 1H, H-16), 2.35 (m, 1H, H-4α), 2.80 (ddd, *J* = 16.9, 8.83, 6.72 Hz, 1H, H-17), 3.16 (ddd, *J* = 16.9, 9.1, 5.19 Hz, 1H, H-17), 3.22 (ddd, *J* = 8.78, 8.78, 4.16 Hz, 1H, H-9), 4.15 (s, 5H, Ar-H), 4.44 (t, *J* = 1.76, 1.76 Hz, 2H, Ar-H), 4.74 (m, 1H, Ar-H), 4.78 (m, 1H, Ar-H), 5.82 (s, 1H, H-12); ^{13}C NMR ($CDCl_3$) *δ* 19.68, 22.69, 23.50, 24.69, 25.04, 33.42, 35.75, 37.58, 38.31, 44.67, 49.79, 69.16, 69.20, 69.69, 72.12, 78.73, 79.18, 93.59, 105.25, 171.56, 203.77; $[\alpha]_D$ +38.84° (*c* 0.515, $CHCl_3$). Anal. ($C_{27}H_{32}FeO_6$)C,H.

Isomer II: red crystals; mp 120−121 °C; 87.05% yield; IR ν_{max} ($CHCl_3$) 3014, 2932, 1733, 1661, 1456, 1380, 1226, 1107, 1032, 831, 772, 737 cm^{-1}; MS *m/e* (rel intensity) 508 (M^+, 100), 492 (15), 474 (2), 448 (2), 420 (0.8), 391 (1), 282 (4), 251 (1), 240 (6), 228 (40), 213 (15), 186 (30), 121 (65), 67 (13); ^1H NMR ($CDCl_3$) *δ* 1.00 (d, 3H, *J* = 6 Hz, H-15), 1.17 (m, 1H, H-8*β*), 1.43 (s, 3H, H-14), 1.37−1.58 (m, 4H, H-6, H-5a, H-5*β*, H-7α), 1.69−1.74 (m, 1H, H-8α), 1.76−1.82 (m, 1H, H-8a), 1.87−2.00 (m, 2H, H-5α, H-7*β*), 2.04−2.11 (m, 1H, H-4*β*), 2.13−2.22 (m, 1H, H-16), 2.29 (ddd, *J* = 14.5, 7.74, 5.71 Hz, 1H, H-16), 2.33−2.46 (m, 2H, H-9, H-4α), 2.90 (m, 1H, H-17), 3.15 (m, 1H, H-17), 4.22 (s, 5H, Ar-H), 4.50 (t, *J* = 1.96, 1.96 Hz, 2H, Ar-H), 4.81 (dd, *J* = 3.22, 1.89 Hz, 1H, Ar-H), 4.84 (dd, 1H, *J* = 3.22, 1.89 Hz, Ar-H), 5.93 (s, 1H, H-12); ^{13}C NMR ($CDCl_3$) *δ* 19.75, 24.50, 25.37, 28.99, 31.21, 33.78, 35.79, 37.07, 37.37, 43.76, 44.47, 50.32, 69.15, 69.22, 69.69, 72.04, 78.81, 79.98, 93.79, 105.08, 171.59, 204.36; $[\alpha]_D$ +42.69° (*c* 0.56, $CHCl_3$). Anal. ($C_{27}H_{32}FeO_6$)C,H.

Compound **3d** was prepared by stirring a solution of artemisitene (280 mg, 1 mmol) and imidazole (74.9 mg, 1.1 mmol) in THF (3 mL) at room temperature for 24 h. The reaction mixture was evaporated to dryness and the crude product recrystallized from ethyl acetate/hexane to yield **3d** as a mixture of two inseparable (α and *β*) isomers (269 mg, 77.44% yield).

3d: white crystals; mp 137−138 °C; IR ν_{max} ($CHCl_3$) 2962, 2877, 1737, 1508, 1455, 1394, 1380, 1283, 1135, 1116, 1001, 972, 835 cm^{-1}; MS *m/e* (rel intensity) 348 (M^+, 20), 332 (0.6), 319 (15), 305 (9), 289 (4), 277 (9), 259 (16), 230 (12), 209 (5), 190 (26), 140 (15), 121 (23), 95 (30), 82 (75), 69 (100), 55 (45); ^1H NMR ($CDCl_3$) *δ* 1.15 [m, 1H, H-8*β* (isomer I)], 1.35 [s, 3H, H-14 (isomer I)], 1.38 [s, 3H, H-14 (isomer II)], 1.21−1.41 [m, 8H, H-6 (isomers I and II), H-5*β* (isomers I and II), H-5a (isomers I and II), H-8*β* (isomer II), H-8α (isomer II)], 1.46 [m, 1H, H-8a (isomer II)], 1.53 [m, 1H, H-8a (isomer I)], 1.70 [ddd, *J* = 13.70, 6.78, 3.21 Hz, 2H, H-7*β* (isomers I and II)], 1.77 [ddd, *J* = 13.19, 7.05, 3.56 Hz, 1H, H-8α (isomer I)], 1.85−2.03 [m, 4H, H-4*β* (isomers I and II), H-5α (isomers I and II)], 2.26−2.38 [m, 2H, H-4α (isomers I and II)], 2.58 [ddd, *J* = 10.96, 5.00, 0.68 Hz, 1H, H-9 (isomer II)], 3.65 [ddd, *J* = 9.79, 5.44, 5.02 Hz, 1H, H-9 (isomer I)], 3.95 [dd, *J* = 14.45, 9.7 Hz, 1H, H-16 (isomer I)], 4.23 [dd, *J* = 13.90, 10.96 Hz, 1H, H-16 (isomer II)], 4.51 [dd, *J* = 13.90, 5.11 Hz, 1H, H-16 (isomer II)], 4.56 [dd, *J* = 14.45, 5.44 Hz, 1H, H-16 (isomer I)], 5.82 [s, 1H, H-12 (isomer I)], 5.88 [s, 1H, H-12 (isomer II)], 6.89 [t, *J* = 1.41 Hz, 1H, Ar-H (isomer I)], 6.91 [t, *J* = 1.03 Hz, 1H, Ar-H (isomer II)], 6.98 [s, 1H, Ar-H (isomer I)], 7.00 [s, 1H, Ar-H (isomer II)], 7.43 [s, 1H, Ar-H (isomer I)], 7.46 [s, 1H, Ar-H (isomer II)]; ^{13}C NMR ($CDCl_3$) *δ* 19.45 (isomer I), 19.49 (isomer II), 23.18 (isomer I), 24.40 (isomer II), 24.51 (isomer I), 24.82 (isomer I), 25.15 (isomer II), 30.80 (isomer II), 32.81 (isomer II), 33.46 (isomer II), 35.54 (isomer II), 35.57 (isomer I), 37.13 (isomer I), 37.19 (isomer II), 39.43 (isomer II), 39.74 (isomer I), 41.23 (isomer I), 43.44 (isomer I), 46.41 (isomer II), 49.44 (isomer II), 49.49 (isomer I), 50.00 (isomer I), 78.66 (isomer I), 80.04 (isomer II), 93.82 (isomer I), 93.90 (isomer II), 105.45 (isomers I and II), 118.65 (isomers I and II), 129.83 (isomer I), 130.04 (isomer II), 137.28 (isomer I), 137.70 (isomer II), 168.33 (isomer II), 168.49 (isomer I); $[\alpha]_D$ +98.06° (*c* 0.21, $CHCl_3$). Anal. ($C_{18}H_{24}N_2O_5$)C,H.

Compound **4e** was obtained from the reduction of **3d** with sodium borohydride employing the known standard conditions.[33] NMR analysis (300 MHz) of the product revealed the presence of two *cis*-isomers (C-9 and C-10).

4e: white crystals; mp 154−155 °C; IR ν_{max} (KBr) 2938, 1653, 1519, 1457, 1377, 1284, 1129, 1095, 978, 846, 634 cm^{-1}; MS *m/e* (rel intensity) 352 ($M^+ + 2$, 0.5), 351 ($M^+ + 1$, 0.7), 350 (M^+, 2), 332 ($M^+ - H_2O$, 36), 303 (56), 289 (9), 261 (44), 247 (13), 233 (24), 218 (34), 193 (19), 178 (50), 95 (27), 82 (100), 68 (97), 55 (44); ^1H NMR ($CDCl_3 + CD_3OD$) *δ* 0.81−0.91 [m, 2H, H-8*β* (isomers I and II)], 0.86 [d, *J* = 6.38 Hz, 3H, H-15 (isomer II)], 0.89 [d, *J* = 6.38 Hz, 3H, H-15 (isomer I)], 1.21 [m, 2H, H-5a (isomers I and II)], 1.28−1.36 [m, 2H, H-6 (isomers I and II)], 1.33 [s, 3H, H-14 (isomer I)], 1.35 [s, 3H, H-14 (isomer II)], 1.37 [m, 2H, H-7α (isomers I and II)], 1.52−1.68 [m, 6H, H-5*β* (isomers I and II), H-8α (isomers I and II), H-8a (isomers I and II)], 1.82 [m, 2H, H-7*β* (isomers I and II)], 1.95−2.12 [m, 4H, H-4*β* (isomers I and II), H-5α (isomers I and II)], 2.29 [ddd, *J* = 14.52, 14.52, 3.79 Hz, 2H, H-4α (isomers I and II)], 2.69 [m, 1H, H-9 (isomer II)], 2.74 [m, 1H, H-9 (isomer I)], 3.74 [m, 1H, H-16 (isomer II)], 3.78 [dd, *J* = 13.6, 5.95 Hz, 1H, H-16 (isomer I)], 4.10 [dd, *J* = 13.6, 10.44 Hz, 1H, H-16 (isomer I)], 4.23 [dd, *J* = 14.21, 5.03 Hz, 1H, H-16 (isomer II)], 4.88 [d, *J* = 3.34 Hz, 2H, H-10 (isomers I and II)], 5.35 [s, 1H, H-12 (isomer II)], 5.56 (s, 1H, H-12 (isomer I)], 6.87 [s, 1H, Ar-H (isomer II)], 6.89 [s, 1H, Ar-H (isomer I)], 6.93 [s, 2H, Ar-H (isomers I and II)], 7.39 [s, 1H, Ar-H (isomer II)], 7.43 [s, 1H, Ar-H (isomer I)]; ^{13}C NMR ($CDCl_3 + CD_3OD$) *δ* 19.86 (isomer II), 20.04 (isomer I), 21.94 (isomer II), 24.44 (isomer I), 24.85 (isomers I and II), 25.49 (isomer II), 25.68 (isomer I), 33.47 (isomer II), 34.18 (isomer I), 36.11 (isomers I and II), 37.03 (isomers I and II), 37.57 (isomers I and II), 40.98 (isomer II), 41.71 (isomer I), 44.16 (isomer II), 46.34 (isomer I), 51.12 (isomer II), 52.20 (isomer I), 79.61 (isomer II), 80.37 (isomer I), 87.79 (isomer I), 90.70 (isomer II), 91.23 (isomer II), 91.81 (isomer I), 104.14 (isomer I), 104.40 (isomer II), 119.06 (isomers I and II), 128.67 (isomers I and II), 137.33 (isomers I and II); $[\alpha]_D$ + 154.55° (*c* 0.15, $CHCl_3$). Anal. ($C_{18}H_{26}N_2O_5$)C,H.

General Procedures To Evaluate the Interaction Spectra of Drug−Ferroprotoporphyrin IX. Hemin (0.4 mg, 6.13×10^{-4} mmol) was initially dissolved in 100 *μ*L of 0.1 M sodium hydroxide; then 29.9 mL of 1% SDS in PBS (pH 7.2) was added. Various amounts of compounds (**1**−**4e**) were dissolved in 120 *μ*L of either MeOH or DMSO followed by adding 1.08 mL of 1% SDS in PBS to obtain required concentrations; 2-fold serial dilutions were then made. In a 1.5 mL cuvette was placed 0.5 mL of the hemin solution together with 0.5 mL of the drugs. A few crystals of sodium dithionite were added prior to measuring the spectra.

Biology. *In Vitro* Assessment of Antimalarial Activity. The detailed protocol for *in vitro* antimalarial testing of drugs against *P. falciparum*-infected red cells using a modification of the [^3H]hypoxanthine incorporation has been previously published by Desjardins *et al.*[34] Briefly, the drugs were initially dissolved in dimethyl sulfoxide (DMSO) and diluted with RPMI 1640 culture medium supplemented with 25 mM Hepes buffer, 32 mM $NaHCO_3$, and 10% human plasma to the required concentrations with a final concentration of 0.01% DMSO. A 200 *μ*L portion of the 1.5% cell suspension with

1−2% parasitemia at early ring stage was pre-exposed to the 25 μL of the medium containing the drugs for 24 h and incubated at 37 °C with 5% O_2, 5% CO_2, and 90% N_2 prior to the addition of 25 μL of 0.5 μCi of [^{3}H]hypoxanthine. After further incubation for 18−24 h, the parasites' DNA was harvested from each microtiter well onto glass filters. The [^{3}H]hypoxanthine incorporation in each well was determined by a Beckman liquid scinillation countermode LS-1801. The IC_{50} value was obtained from a logistic dose−response curve.

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Supporting Information Available: Figures 1 and 3 (3 pages). Ordering information is given on any current masthead page.

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