Pergamon

Bioorganic & Medicinal Chemistry 8 (2000) 2739–2745

BIOORGANIC & MEDICINAL CHEMISTRY

Novel Ferrocenic Artemisinin Derivatives: Synthesis, In Vitro Antimalarial Activity and Affinity of Binding with Ferroprotoporphyrin IX

L. Delhaes, [a,b] C. Biot, [c,d] L. Berry, [a] L. A. Maciejewski, [c] D. Camus, [a,b] J. S. Brocard [c] and D. Dive [a,*]

[a]*INSERM U.42, 369 rue Jules Guesde, BP 39, 59651 Villeneuve d'Ascq Cedex, France*
[b]*Laboratoire de Parasitologie-Mycologie, Faculté de Médecine, 1 place de Verdun, 59045 Lille Cedex 2, France*
[c]*Laboratoire de Catalyse, Groupe de Synthèse Organométallique, UPRESA 8010, Ecole Nationale Supérieure de Chimie de Lille, Bâtiment C7 Université des Sciences et Technologies, BP 108, 59652 Villeneuve d'Ascq Cedex, France*
[d]*Current address: UMR 8525 CNRS-Université de Lille II- Institut de Biologie et Institut Pasteur de Lille, 1 rue du Professeur Calmette, BP 447, 59021 Lille, France*

Received 8 June 2000; received in revised form 11 July 2000; accepted 26 July 2000

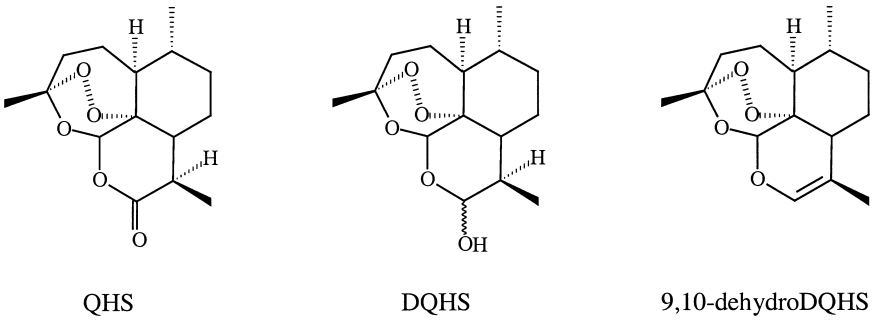
Abstract—Following our search for novel compounds with high antimalarial activity, a series of artemisinin (QHS) derivatives containing a ferrocenic nucleus was prepared and tested in vitro against *Plasmodium falciparum* strains. Two new metallocenic derivatives (**1** and **3**) were found as potent as QHS. All compounds showed a capacity to bind with ferroprotoporphyrin IX. A decrease in the Soret band absorbance of ferroprotoporphyrin IX, resulting from the addition of different drugs concentrations, was shown. The association stoichiometry of compounds to ferroprotoporphyrin IX appears to be 1:2 at equilibrium, with an intermediate 1:1 complexation. These results appear to strengthen the role of adducts between artemisinin derivatives and heme in generation of artemisinin radicals. Such interaction of artemisinin ferrocenyl derivatives with ferroprotoporphyrin IX and its biological significance could form a basis in future drug development.

Introduction

The incidence of malaria is dramatically increasing since many *Plasmodium falciparum* strains are now resistant to widely-used drugs like chloroquine.[1–4] The discovery of artemisinin, a potent Chinese antimalarial drug extract from *Artemisia annua*, which is an enantiomerically pure sesquiterpene lactone having an endoperoxide group, has drawn the attention to drugs having such function (Scheme 1). First- and second-generation artemisinin derivatives are being widely used in Asia.[5,6] In general, these endoperoxides have several advantages over existing antimalarial drugs. First, they are the most rapidly effective available drugs. Second, the sensitivity of *P. falciparum* is still high in spite of little cross-resistance with mefloquine.[7,8] However, the use of such endoperoxides is hampered by their short plasma half-life[2] and the rate of recrudescent infections, when they are used alone in short-course treatment.[5,8,9] On the basis of these findings, rational designs of potential next-generation endoperoxides have been synthesized.[10–15] For several years, we have proposed a strategy for the development of organometallic-based antimalarial drugs.[16–21] In this study, we report the incorporation of ferrocenyl moieties to artemisinin focusing attention on ethers and esters, with the hope of enhancing the antimalarial effect. As a close interaction between the Fe(II)-heme and the artemisinin peroxide function has been reported to induce a homolytic cleavage of the peroxidic bond,[13–15,22–24] we expected the drug to become more potent if an iron atom could be available within the vicinity of artemisinin derivatives. On the other hand, the binding of the artemisinin derivatives with ferroprotoporphyrin IX seemed to be a significant step to the antimalarial activity.[11,13–15,24,25] So, we studied such interaction for each analogue and compared antimalarial activity with their capacity of binding with ferroprotoprophyrin IX.

*Corresponding author. INSERM U.167, Institut Pasteur, 1 rue du Pr. Calmette BP 245, 59019 Lille Cedex, France. Tel.: +33-3208-77896; fax: +33-320877888; e-mail: daniel.dive@pasteur-lille.fr

0968-0896/00/$ - see front matter
PII: S0968-0896(00)00206-6



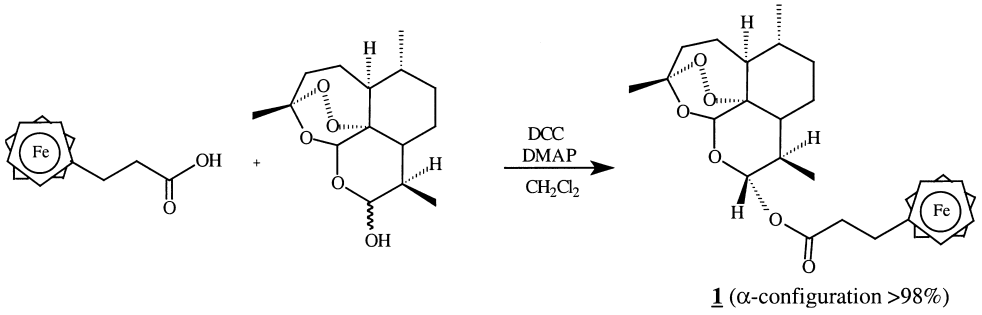
Scheme 1. Structures of artemisinin (QHS), dihydroartemisinin (DQHS) and 9,10-dehydrodihydroartemisinin (9,10-dehydroDQHS).

Results and Discussion

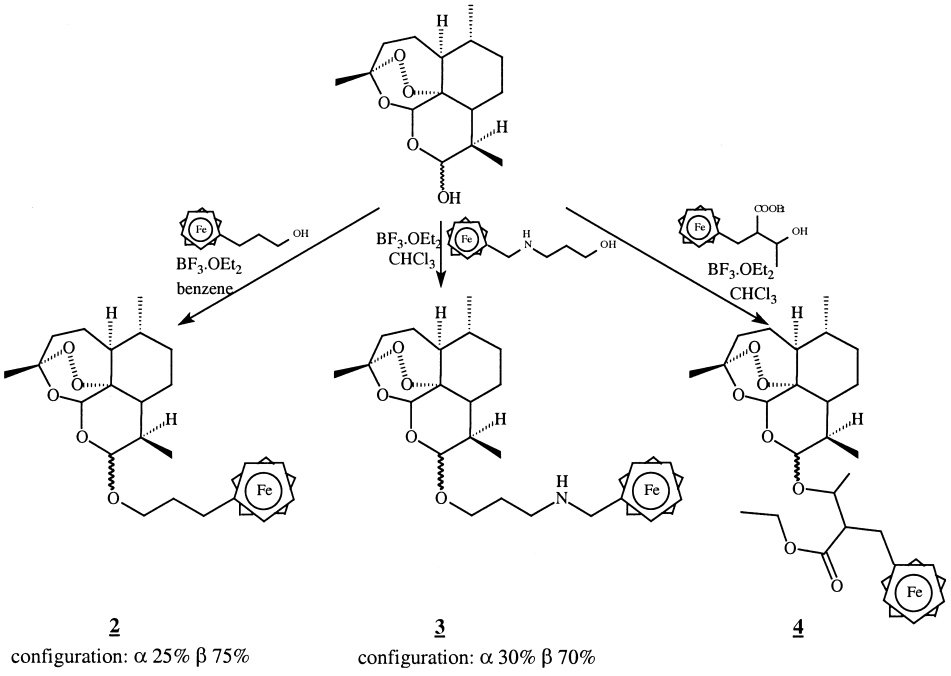
Chemistry

Ferrocenic artemisinin derivatives were prepared by reactions depicted in Schemes 2 and 3. Reduction of artemisinin (QHS) with an excess of sodium borohydride in methanol at 0 °C afforded dihydroartemisinin (DQHS) in 75% yields by a known procedure.[26] 10α-Dexoartemisinin 3-ferrocenylpropanoate **1** was obtained in 62% yields from DQHS with 3-ferrocenylpropanoic acid[27] by esterification in the presence of dicyclohexylcarbodiimide (DCC) and 4-dimethylaminopyridine (DMAP).[28,29] A large coupling constant ($J = 9.86$ Hz) measured between the vicinal protons, H9–H10, is indicative of a *trans* diaxial relationship, assigned to an α-configuration.[30,31]

Etherification of DQHS with 3-ferrocenylpropanol, in anhydrous benzene[27] in the presence of a catalytic amount of boron trifluoride at room temperature, led to



Scheme 2. Synthesis of 10α-dexoartemisinin 3-ferrocenylpropanoate **1**.



Scheme 3. Synthesis of ether ferrocenic artemisinin derivatives **2**, **3** and **4**.

Table 1. Chemical shifts (2D COSY 45) and analyses of compounds **1**, **2**, **3** and **4**

Compounds			^{1}H NMR (300 MHz, $CDCl_3$, δ)		Analyses (%)					
			C_{10}-H	C_{12}-H	Calcd			Found		
	S[a]	%	*J* (d, Hz)	(s)	C	H	N	C	H	N
1	α	>98	5.81 (9.9)	5.46	64.12	6.87	—	63.95	6.69	—
2	α	25	4.43 (9.2)	5.34	65.88	7.45	—	65.99	7.62	—
	β	75	4.81 (3.4)	5.42			—			—
3	α	30	4.42 (9.2)	5.33	64.56	7.61	2.60	64.22	7.38	2.72
	β	70	4.77 (3.4)	5.37						
4	*		4.94–4.89	5.52–5.42	64.43	7.38	—	64.84	7.56	—

[a]S = stereochemistry at C_{10}.
*__4__ was obtained as a mixture of eight stereoisomers.

(3-ferrocenylpropoxy)dihydroartemisinin **2** (84% yield).[32] Similarly, coupling of DQHS with 3-(*N*-ferrocenylmethylamino)propanol,[33] in dried chloroform,[34] afforded [3-*N*-ferrocenyl-methylamino(propyloxy)]dihydroartemisinin **3**. The yield of the purified condensation product **3** ranged from 28 to 36%. **2** and **3** were obtained as a mixture of α and β anomers whose ratio was determinated by ^{1}H NMR COSY experiments. Starting from **3**, a water-soluble salt by acidification (with formic acid in acetone, hydrochloric acid 1N in Et_2O or tartaric acid in acetone) was not achieved. The employed acidic conditions led to the formation of an α-ferrocenic carbenium, 9,10-dehydrodihydroartemisinin and unidentified CHO derivatives as evidenced by changes seen in their ^{1}H NMR and MS (MALDI-TOF) spectra. This may be due to the instability of the ketal function or the ferrocenic ammonium under acidic conditions.

Ethyl (2-dihydroartemisininoxy-3-ferrocenylmethyl)butanoate **4** was prepared by treatment of DQHS with ethyl (2-hydroxy-3-ferrocenylmethyl)butanoate[35] in dried $CHCl_3$ in the presence of $BF_3{\cdot}OEt_2$ (80% yield). Compound **4** has more than three chiral carbons. As the stereochemistry of carbon 10 on the ferrocenyl side chain was not controlled, eight stereoisomers are therefore possible and obtained. The ether **4** has been synthesized in order (a) to decrease the rate of oxidative dealkylation on the target compound and (b) to increase the lipophilia of the molecule. In fact, the introduction of the methyl substituent (CH_3–C18) should interfere with the rate of hydroxylation of the α-methylene group and should increase the plasma half-life. A high degree of lipophilia that should enhance the efficacy of the molecule was obtained by introduction of the COOEt group.

We were unable to separate the stereoisomers (Scheme 4) of **2**, **3** and **4** (by crystallization or column chromatography, Table 1). Each of these compounds was used as a mixture.

Biological Activities

In vitro activity of artemisinin derivatives

The assessments of in vitro antimalarial activity were made against two chloroquine-sensitive (HB3 and SGE2) and one chloroquine-resistant (Dd2) strains of *P. falciparum*. The screening procedure is briefly described in the Experimental. The choice of the ferrocenic group, despite its stable form, was governed by the importance of Fe(II)-heme to cleave the crucial peroxide linkage.[13–15,22–24] Modifications of dihydroartemisinin into compounds **1**–**4** by covalent linking with ferrocenic derivatives did not damage the endoperoxide function. Nevertheless they did not increase antimalarial potency of these organometallic compounds (Table 2). Compared to parent compounds, artemisinin and dihydroartemisinin, compound **3** stood out as the most active in vitro against *P. falciparum*, with activity similar to artemisinin (Table 2). Notably, it was the only compound to possess an amine function (Scheme 3). Such functions are known to improve solubility via ammonium salt. Recently, Li et al.[12] have reported in vivo antimalarial activity of artemisinin derivatives containing an amino group. The bases are combined with weak organic acid and this point may be interesting, improving the poor solubility of current endoperoxides.[12]

Interaction spectra of drug–ferroprotoporphyrin IX. Figure 1 shows the spectral changes observed when ferroprotoporphyrin IX was titrated with dihydroartemisinin and the more effective ferrocenyl derivatives: compounds **1** and **3**. There was a decrease in absorbance of the ferroprotoporphyrin IX Soret band resulting from different drug concentrations addition (Fig. 1(A)). These results are in agreement with published data.[11,25] Substantial changes in the Q-band region of the spectrum were observed (Fig. 1(B)), as previously described.[36] The absorption peak of ferroprotoporphyrin IX at 415 nm was strongly and immediately reduced not only by artemisinin and dihydroartemisinin as previously

Table 2. In vitro antimalarial activity of ferrocenic artemisinin derivatives and parent compounds against *P. falciparum* strains

Compounds	MW (g M^{-1})	IC_{50} (nM)		
		HB3	SGE2	Dd2
1	539	10 (±3)[a]	19 (±9)	32 (±7)
2	510	36 (±6)	49 (±13)	86 (±15)
3	524	12 (±2)	11 (±5)	14 (±2)
4	596	21 (±5)	31 (±9)	45 (±5)
QHS	282	7 (±1)	9 (±4)	13 (±3)
DQHS	284	5 (±1)	9 (±1)	5 (±2)

[a]Values are the arithmetic means of three independent experiments ± standard deviation.

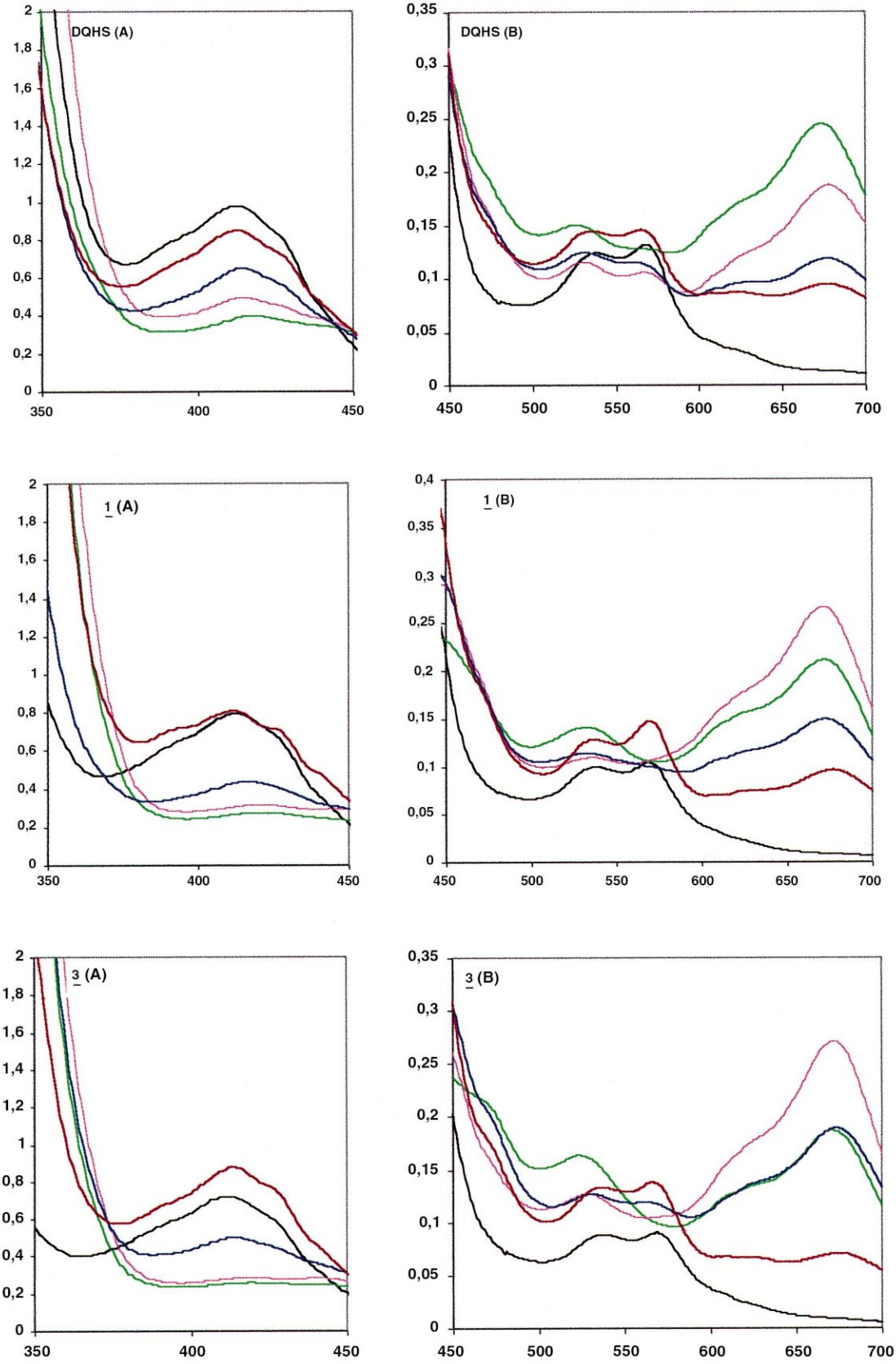


Figure 1. Spectroscopic changes observed when ferroprotoporphyrin IX is titrated with DQHS, compounds **1** and **3**. (A) Changes in the Soret band of ferroprotoporphyrin IX. (B) Changes in the Q-band region of ferroprotoporphyrin IX spectrum. Spectra of 10 μM ferroprotoporphyrin IX alone (–), 10 μM ferroprotoporphyrin IX in the presence of 1.1×10^{-5} M (–), 2.2×10^{-5} M (–), 4.4×10^{-5} M (–) and 8.9×10^{-5} M (–) of DQHS, compounds **1** and **3**, respectively, are represented. All spectra have been made in the presence of sodium dithionite.

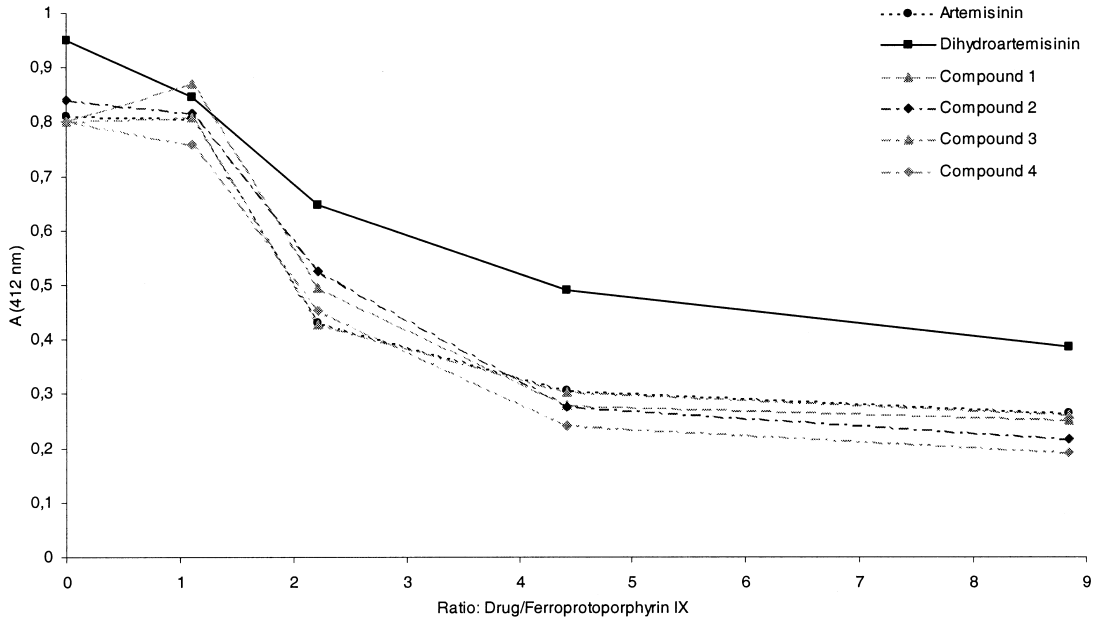
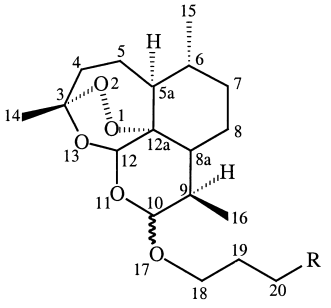


Figure 2. Variation in absorbance of ferroprotoporphyrin IX at 412 nm as function of drug concentration versus ferroprotoporphyrin IX (10 μM) ratio.



Scheme 4. Numbering of the carbon atoms of derivatives **2**, **3** and **4**.

described,[11] but also by all the ferrocenyl compounds (Fig. 2). Especially, compound **3** which exhibited the best antimalarial activity among ferrocenyl derivatives (Table 2), presented titration data similar to those observed in the presence of artemisinin (Fig. 2). The stoichiometry of association of compounds to ferroprotoporphyrin IX is deduced from Figure 2 and appeared to be 1:2 at equilibrium, with an intermediate 1:1 complexation. These results appear to strengthen the role of an adduct between artemisinin derivatives and heme in the mecanism of action of endoperoxide-based antimalarial drugs.[11,13–15,24,25,37]

Conclusion

Although the results from our study have not given any indication that the presence of ferrocene in the vicinity of artemisinin can potentiate its action, the fact that the linked compounds still remain effective antimalarials nevertheless encourages us to explore other derivatives. Furthermore, the interaction of artemisinin ferrocenyl derivatives with ferroprotoporphyrin IX and its biological significance could contribute to giving a better molecular basis for rational design of new synthetic endoperoxide-containing antimalarial drugs.

Experimental

Chemistry

The ^{1}H NMR spectra were recorded on a Bruker AC 300 spectrometer using tetramethylsilane (TMS) as the internal standard and $CDCl_3$ or DMSO-d_6 as the solvent. MALDI TOF spectra were obtained using a Vision 2000 time-of-flight instrument (Finnigan MAT, Bremen, Germany) equipped with a nitrogen laser operating at a wavelength of 337 nm. Between 20 and 30 single-shot spectra in either the reflector or linear mode were accumulated to obtain a good signal-to-noise ratio. The matrix used was dihydroxybenzoic acid (dhb) or trihydroxyacetophenone (thap). Merck's Kieselgel 60 PF254 was used for the chromatography.

10α-Dexoartemisinin 3-ferrocenylpropanoate (1). A mixture of dihydroartemisinin (122 mg, 0.43 mM), 4-dimethylaminopyridine (12 mg, 0.1 mM) and 3-ferrocenylpropanoic acid (112 mg, 0.43 mM) in dry CH_2Cl_2 (20 mL) was stirred in an ice bath. To the solution was added dicyclohexylcarbodiimide (100 mg, 0.49 mM) and was stirred at room temperature for 24 h. The mixture was filtered. The filtrate was washed with 2N HCl (2×25 mL), water (25 mL) and 5% aq $NaHCO_3$ (2×25 mL), dried with Na_2SO_4 and evaporated. The crude oil was chromatographed on a silica gel column using acetone:petroleum ether (1:9) to give the pure α anomer as an orange oil (139 mg, 0.27 mM, 62%). ^{1}H NMR ($CDCl_3 + D_2O$) δ 5.81: H 10 (d: $J = 9.86$ Hz), 5.46: H 12 (s), 4.12: Cp′ (s), 4.07: 4H Cp (m), 2.66: 2H

19 and 2H 20 (m), 2.54: H 9 (m), 2.37: H 4α (m), 2.02: H 4β (m), 1.89: H 5α (m), 1.74: H 7β (m), 1.57: 2H 8 (m), 1.48: H 5β (m), 1.46: H 8a (m), 1.44: CH_3 14 (s), 1.32: H 6 (m), 1.22: H 5a (m), 1.00: H 7α (m), 0.95: CH_3 15 (d: $J=5.87$ Hz), 0.84: CH_3 16 (d: $J=7.20$ Hz). MS (MALDI TOF, matrix: thap) *m/e* 563: $(M+K)^+$, 547: $(M+Na)^+$, 524: $M^{\cdot+}$ Anal. found: C, 63.95; H, 6.69. $C_{28}H_{36}O_6Fe$, calcd: C, 64.12; H, 6.87.

10α-(3-Ferrocenylpropoxy)dihydroartemisinin and 10β-(3-ferrocenylpropoxy)dihydroartemisinin (2). Dihydroartemisinin (65 mg, 0.23 mM) in dry benzene (10 mL) was treated with 3-ferrocenylpropanol (167 mg, 0.68 mM) under nitrogen in the presence of $BF_3{\cdot}Et_2O$ (50 μL) at room temperature for 18 h. The reaction mixture was washed with 5% aq $NaHCO_3$ (20 mL) and H_2O (20 mL), dried over Na_2SO_4 and evaporated. The resultant crude product was purified with silica gel column using EtOAc:petroleum ether (1:9) as eluant to give **2** as a mixture of the two anomers. The same procedure using dried Et_2O in place of benzene afforded only the β anomer (33%). ^{1}H NMR ($CDCl_3+D_2O$) δ β configuration 5.42: H 12 (s), 4.81: H 10 (d: $J=3.36$ Hz), 4.11: Cp′ (s), 4.07: 4H Cp (m), 3.85: H 18′ (dt: $J=6.30$ and 9.70 Hz), 3.41: H 18″ (dt: $J=6.32$ and 9.74 Hz), 2.64: H 9 (m), 2.39: 2H 20 (m), 2.37:H 4α (m), 2.04: H 4β (m), 1.87: H 5α (m), 1.80: 2H 19 (m) and 2H 8 (m), 1.69: H 7β (m), 1.56: H 5β (m) and H 8a (m), 1.48: CH_3 14 (s), 1.34: H 6 (m), 1.22: H 5a (m), 0.94: CH_3 15 (d: $J=6.25$ Hz), 0.92: H 7α (m), 0.91: CH_3 16 (d: $J=7.32$ Hz); α configuration 5.34: H 12 (s), 4.43: H 10 (d: $J=9.19$ Hz). ^{13}C NMR ($CDCl_3+D_2O$) δ β configuration 104.1, 101.9, 88.7, 81.1, 68.5, 68.1, 68.0, 67.1, 52.6, 44.5, 37.5, 36.5, 34.7, 31.0, 30.9, 26.2, 24.7, 24.5, 20.4, 13.1. MS (MALDI TOF, matrix: dhb) *m/e* 510: $M^{\cdot+}$, 460, 243: $Fc(CH_2)_3O^+$, 199: $FcCH_2^+$. Anal. found: C, 65.99; H, 7.62. $C_{28}H_{38}O_5Fe$, calcd: C, 65.88; H, 7.45.

10α-[3-*N*-Ferrocenylmethylamino(propyloxy)]dihydroartem-isinin and 10β-[3-*N*-ferro-cenylmethylamino(propyloxy)]dihydroartemisinin (3). The procedure described for the preparation of **2** was followed using $CHCl_3$ in place of benzene. ^{1}H NMR ($CDCl_3+D_2O$) δ β configuration 5.37: H 12 (s), 4.77: H 10 (d: $J=3.36$ Hz), 4.18: 2H Cp (m), 4.12: Cp′ (s), 4.10: 2H Cp (m), 3.91: H 18′ (dt: $J=6.02$ and 9.78 Hz), 3.52: 2H 22 (s), 3.43: H 18″ (dt: $J=6.16$ and 9.79 Hz), 2.70: 2H 20 (t: $J=7.15$ Hz), 2.61: H 9 (m), 2.36: H 4α (m), 2.04: H 4β (m), 1.87: H 5α (m), 1.78: 2H 19 (m) and 2H 8 (m), 1.61: H 7β (m), 1.50: H 5β (m) and H 8a (m), 1.44: CH_3 14 (s), 1.26: H 6 (m) and H 5a (m), 0.95: CH_3 15 (d: $J=6.02$ Hz), 0.88: CH_3 15 (d: $J=7.33$ Hz) and H 7a (m); α configuration 5.33: H 12 (s), 4.42: H 10 (d: $J=9.24$ Hz). MS (MALDI TOF, matrix: dhb) *m/e* 562: $(M+Na)^{\cdot+}$, 539: $M^{\cdot+}$, 516, 491, 199: $FcCH_2^+$. Anal. found: C, 64.22; H, 7.38; N, 2.72. $C_{29}H_{41}NO_5Fe$, calcd: C, 64.56; H, 7.61; N, 2.60.

Ethyl (2-dihydroartemisininoxy-3-ferrocenylmethyl)butanoate (4). The procedure described for the preparation of **2** was followed using $CHCl_3$ in place of benzene. ^{1}H NMR ($CDCl_3+D_2O$) δ 5.52–5.42: H 12 (8 s), 4.94–4.89: H 10 (m), 4.20–3.90: Fc ArtO–C*H* and O–CH_2–CH_3 (m), 2.72: H 9 and Fc-CH_2 (m), 2.52: H 4α (m), 2.34: H 4β (m), 2.03: H 5α (m), 1.87–0.85: 9H (m), 1.47: CH_3 14 (s), 1.13: CH_3–CH_2–O and CH_3–CH (m), 0.96: CH_3 Art (m), 0.87 CH_3 Art (m). MS (MALDI TOF, matrix: thap) *m/e* 635: $(M+K)^+$, 616: $(M+Na)^+$, 596: $M^{\cdot+}$, 548, 199. Anal. found: C, 64.84; H, 7.56. $C_{32}H_{44}O_7Fe$, calcd: C, 64.43; H, 7.38.

Biology

In vitro activity of artemisinin derivatives

Three culture-adapted strains of *P. falciparum* were used: the chloroquine-resistant strain Dd2 (Indochina) and the chloroquine-sensitive strains HB3 (Honduras) and SGE2 (Zaïre). All stock parasite cultures were maintained using Trager and Jensen's method.[38,39]

The assays were conducted in vitro using a modification of the semiautomated microdilution technique of Desjardins et al. based on radiolabelled [^{3}H]hypoxanthine incorporation.[40] Drug testing was carried out in 96-well microtitre plates. All the compounds were tested as free bases and dissolved in dimethyl sulphoxide (DMSO, 5 mg/mL). They were then prediluted in complete culture medium (RPMI 1640 supplemented with 10% pooled human AB+ serum), and titrated immediately in duplicate in serial 2-fold dilutions. The final concentration ranged from 0.39 to 200 nM, with less than 0.01% DMSO, which had no detectable effect on parasite multiplication.[41] After addition of a suspension of parasitized erythrocytes in complete culture medium (200 μL/well, 0.5% initial parasitaemia with a majority of ring stages, 2% haematocrit) and [H^3]hypoxanthine (Amersham, Little Chalfont, Buckinghamshire, UK, 0.5 μCu/well), the test plates were incubated at 37 °C in an atmosphere of 5% O_2, 5% CO_2 and 90% N_2, for 48 h. Growth of the parasites was estimated by the incorporation of radiolabelled [H^3]hypoxanthine into the parasites nucleic acids, measured in a liquid scintillation spectrometer (Beckman). Fifty percent inhibitory concentrations (IC_{50}) refer to molar concentrations of drug causing 50% reduction in [H^3]hypoxanthine incorporation compared to drug-free control wells. They were estimated by linear regression analysis of log-dose–response curves.

Interaction spectra of drug–ferroprotoporphyrin IX. The spectra measurements were conduced in vitro, as previously described.[11] Immediately before the experiment, hemin (Sigma, France) was dissolved in 0.1N NaOH, then diluted with 1% SDS in PBS (pH 7.2) to a final concentration of 10 μM. The tubes were then wrapped in aluminium foil to exclude light and stored at 4 °C. 17.9×10^{-4} M of each compound was dissolved in 120 μL of DMSO, followed by adding 1.08 mL of 1% SDS in PBS to obtain a final concentration of 17.9×10^{-5} M; 2-fold serial dilutions were then made. In a 1.0 mL cuvette was placed 0.5 mL of the hemin solution together with 0.5 mL of the drugs. In each sample, a few crystals of sodium dithionite were immediately added prior to measuring the spectra. UV–vis scans were performed on a Kontron Instruments Uvicon 930.

Acknowledgements

We express our sincere thanks to Dr. N'Guyen for providing the plant extract and to Dr. Bremard (Université de Sciences et Techniques de Lille 1, BP 108, 59652 Villeneuve d'Ascq Cedex, France) for his assistance and helpful discussions in spectrophotometric studies. This research was supported by the 'Ministère de l'Enseignement Supérieur et de la Recherche', the 'Centre national de la Recherche Scientifique', the World Heath Organization (contract: ID 980-140) and 'Pierre Fabre Médicament'.

References and Notes

1. WHO. *Weekly Epidemiol. Rep*. **1997**, *72*, 269.
2. WHO. *Weekly Epidemiol. Rep*. **1997**, *72*, 277.
3. WHO. *Weekly Epidemiol. Rep*. **1997**, *72*, 285.
4. Snow, R. W.; Craig, M.; Deichmann, U.; Marsh, K. *Bull. World Health Org*. **1999**, *77*, 624.
5. Meshnick, S. R.; Taylor, T. E.; Kamchonwongpaisan, S. *Microbiol. Rev*. **1996**, *60*, 301.
6. De Vries, P. J.; Bich, N. N.; Van Thien, H.; Hung, L. N.; Anh, T. K.; Kager, P. A.; Heisterkamp, S. H. *Antimicrob. Agents Chemother*. **2000**, *44*, 1302.
7. Wongsrichalai, C.; Wimonwattrawatee, T.; Sookto, P.; Laoboonchai, A.; Heppner, D. G.; Kyle, D. E.; Wernsdorfer, W. H. *Bull. World Health Org*. **1999**, *77*, 392.
8. Verdrager, J. *Lancet* **1999**, *354*, 1389.
9. Price, E.; Luxemburger, C.; van Vugt, M.; Nosten, F.; Kham, A.; Simpson, J.; Looareesuwan, S.; Chongsuphaisiddhi, T.; White, N. J.; Trans, R. *Soc. Trop. Med. Hyg*. **1998**, *92*, 207.
10. Posner, G. H.; McGarvey, D. J.; Ho Oh, C.; Kumar, N.; Meshnick, S. R.; Asawamahasadka, W. *J. Med. Chem*. **1995**, *38*, 607.
11. Paitayatat, S.; Tarnchompoo, B.; Thebtaranonth, Y.; Yuthavong, Y. *J. Med. Chem*. **1997**, *40*, 633.
12. Li, Y.; Zhu, Y. M.; Jiang, H. J.; Pan, J. P.; Wu, G. S.; Wu, J. M.; Shi, Y. L.; Yang, J. D.; Wu, B. A. *J. Med. Chem*. **2000**, *20*, 1635.
13. Robert, A.; Meunier, B. *Chem. Soc. Rev*. **1998**, *27*, 273.
14. Robert, A.; Meunier, B. *Chem. Eur. J*. **1998**, *4*, 1287.
15. Cazelles, J.; Camuzat-Dedenis, B; Provot, O.; Robert, A; Mayrargue, J.; Meunier, B. *J. Chem. Soc., Perkin Trans. 1* **2000**, 1265.
16. Brocard, J.; Maciejewski, L.; Lebibi, J. *Brevet France* 9505532, 1995.
17. Brocard, J.; Maciejewski, L.; Lebibi, J. *Brevet International* PCT/FR 96/00721, 1996. *Chem. Abstr*. **1997**, *126,* 60137E.
18. Biot, C.; Glorian, G.; Maciejewski, L. A.; Brocard, J. S.; Domarle, O.; Blampain, G.; Millet, P.; Georges, A. J.; Abessolo, H.; Dive, D.; Lebibi, J. *J. Med. Chem*. **1997**, *40*, 3715.
19. Domarle, O.; Blampain, G.; Agnanet, H.; Nzadiyabi, T.; Lebibi, J.; Brocard, J.; Maciejewski, L.; Biot, C.; Georges, A. J.; Millet, P. *Antimicrob. Agents Chemother*. **1998**, *42*, 540.
20. Biot, C.; Delhaes, L.; Abessolo, H.; Domarle, O.; Maciejewski, L. A.; Mortuaire, M.; Delcourt, P.; Deloron, P.; Camus, C.; Dive, D.; Brocard, J. S. *J. Organomet. Chem*. **1999**, *589*, 59.
21. Biot, C.; Delhaes, L.; N'Diaye, C. M.; Maciejewski, L. A.; Camus, C.; Dive, D.; Brocard, J. S. *Bioorg. Med. Chem*. **1999**, *7*, 2843.
22. Posner, G. H.; Cumming, J. N.; Ploypradith, P.; Ho Oh, C. *J. Am. Chem. Soc*. **1995**, *117*, 5885.
23. Posner, G. H.; Wang, D.; Cumming, J. N.; Ho Oh, C.; French, A. N.; Bodley, A. L.; Shapiro, T. A. *J. Med. Chem*. **1995**, *38*, 2273.
24. Robert, A.; Meunier, B. *J. Am. Chem. Soc*. **1997**, *119*, 5968.
25. Pandey, A. V.; Tekwani, B. L.; Singh, R. L.; Chauhan, V. S. *J. Biol. Chem*. **1999**, *274*, 19383.
26. Lin, A. J.; Klayman, D. L.; Milhous, W. K. *J. Med. Chem*. **1987**, *30*, 2147.
27. 3-Ferrocenylpropanoic acid was obtained from ethyl 3-ferrocenyl-3-oxo-propanoate: Rinehart, K. L.; Curby, R.J.; Gustafson, D. H.; Harrisson, K. G.; Bozak, R. E.; Bublitz, D. E. *J. Am. Chem. Soc*. **1962**, *84*, 3263.
28. Luo, X. D.; Yeh, H. J. C. *Heterocycles* **1984**, *22*, 2559.
29. Jung, M. *Bioorg. Med. Chem. Lett*. **1997**, *7*, 1091.
30. Luo, X. D.; Yeh, H. J. C.; Brossi, A.; Flippen-Anderson, J. L.; Gilardi, G. *Helv. Chim. Acta* **1984**, *67*, 1515.
31. Li, Y.; Yu, P. L.; Chen, Y. X.; Li, L. Q.; Gai, Y. Z.; Wang, D. S.; Zheng, Y. P. *Chem. Abstr*. **1982**, *97*, 92245n.
32. Etherification of DQHS with 3-ferrocenylpropanol, in anhydrous Et_2O in the presence of a catalytic amount of $BF_3{\cdot}OEt_2$ at room temperature, led to pure β-**2** (33% yield) and allowed complete ^1H NMR assignment.
33. 3-(*N*-Ferrocenylmethylamino)propanol was synthesized by (a) condensation between ferrocenecarboxaldehyde and 3-aminopropanol in dried THF in the presence of Al_2O_3 and (b) reduction of the imine in amine by $NaBH_4$ in dried methanol (two steps, 98% yield).
34. Venugopatan, B.; Karnik, P. J.; Bapat, C. P.; Chatterlee, D. K.; Iyer, N.; Lepcha, D. *Eur. J. Med. Chem*. **1995**, *30*, 697.
35. Ethyl (2-hydroxy-3-ferrocenylmethyl)butanoate was prepared by (a) condensation of *N,N,N*-trimethyl(ferrocenylmethyl)ammonium iodide and ethyl acetoacetate sodium salt in acetonitrile in the presence of K_2CO_3 under reflux and (b) reduction of the ketone in alcohol by $NaBH_4$ in dried methanol (two steps, 89% yield).
36. Egan, T. J.; Mavuso, W. W.; Ross, D. C.; Marques, H. M. *J. Inorg. Biochem*. **1997**, *68*, 137.
37. Meshnick, S. R.; Thomas, A.; Ranz, A.; Xu, C. M.; Pan, H. Z. *Mol. Biochem. Parasitol*. **1991**, *49*, 181.
38. Trager, W.; Jensen, J. B. *Science* **1976**, *193*, 673.
39. Trager, W.; Jensen, J. B. *Int. J. Parasitol*. **1997**, *27*, 989.
40. Desjardins, R. E.; Canfield, C. J.; Haynes, J. D.; Chulay, J. D. *Antimicrob. Agents Chemother*. **1979**, *16*, 710.
41. Elfort, B. C.; Roberts, M. F.; Phillipson, J. D.; Wilson, R. J. M. *Trans. R. Soc. Trop. Hyg*. **1987**, *81*, 434.