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Preliminary communication

Synthetic ferrocenic mefloquine and quinine analogues as potential antimalarial agents

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Abstract – Afew years ago we proposed a strategy for the synthesis of new ferrocene-chloroquine analogues replacing the carbon chain of chloroquine by hydrophobic ferrocenyl moieties. Now, this strategy has been applied to the antimalarial amino-alcohols class to afford new potentially active analogues of mefloquine and quinine bearing a substituted ferrocenic group. The pathway used for the synthesis of the mefloquine analogues includes the coupling of an aminomethyl substituted ferrocene carboxaldehyde with a lithio quinoline compound. On the other hand, the synthesis of quinine analogues was ensured by the 'inverse' reaction of a lithio aminomethyl ferrocene with a quinoline carboxaldehyde. The configurations of each diastereoisomer were unambiguously determined by spectroscopic data. The mechanistic interpretations were fully discussed. Ferrocenyl analogues of mefloquine and quinine exhibited a lower antimalarial activity than mefloquine and quinine themselves. Comparing optical isomers, those isomers dissimilar to ferrocenyl derivatives presented better antimalarial activities than those similar to ferrocenyl. © 2000 Éditions scientifiques et médicales Elsevier SAS

malaria / ferrocene / mefloquine / quinine / diastereomers

1. Introduction

The incidence of malaria in the world is estimated to be 300–500 million clinical cases annually. An estimated 1.5–2.7 million people die of malaria each year [1–4]. Approximately 1 million deaths among children under five years of age are attributed to malaria alone or in combination with other diseases. Countries in tropical Africa are estimated to account for more than 90% of the total malaria incidence and the great majority of malaria deaths [1–4]. So there is a great need for new antimalarials, with different structures and modes of action, in order to deal with the development of resistance to the drugs in current use.

Organometallic compounds offer exciting new possibilities in drug development due to their unique structure [5–8]. In our laboratory, we have previously synthesized a ferrocene-chloroquine analogue, i.e. ferrochloroquine (7-chloro-4-[(2-*N*,*N*-dimethylaminomethyl)ferrocenylmethylamino]quinoline) which proved to be active against chloroquine-resistant parasites [9–12]. In this new drug, the carbon chain of chloroquine was replaced by a ferrocenyl group [9–11]. A similar strategy may be achieved through application of new antimalarials with the structure of amino-alcohols (such as mefloquine or quinine) covalently linked to a substituted ferrocenyl unit [9–10]. It is expected that this modification should enhance the antimalarial activity. We report here the synthesis, determination structures and the in vitro antimalarial activity of synthetic ferrocenic mefloquine and quinine analogues.

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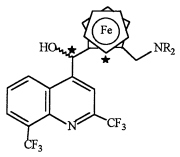


compd.	—NR_2	% Like **l**	% Unlike **u**
1	—N	44	56
2	—N	41	59
3	—N	32	68
4	—N NMe	19	81

Figure 1. [2-(*N*,*N*-substituted aminomethylferrocenyl)]-{4[(2,8-bistrifluoromethyl)quinolyl]}-methanol derivatives. The relative proportion of each diastereomer was determined by ^{1}H-NMR of the crude mixture.

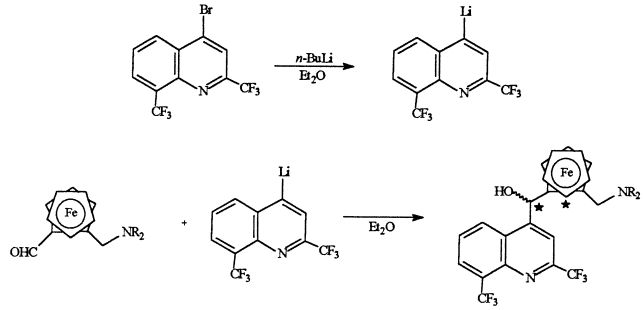


Figure 2. Synthesis of [2-(*N*,*N*-substituted aminomethyl-ferrocenyl)]-{4[(2,8-bistrifluoro-methyl)quinolyl]}-methanol derivatives.

2. Chemistry

The pathway used for the synthesis of the mefloquine analogues includes the addition of a prochiral carboxaldehyde function of a racemic metallocene to a lithio quinoline compound. On the other hand, the synthesis of quinine analogues was ensured by the addition of a prochiral carboxaldehyde quinoline derivative into racemic chelated metallocycle (*figure 1*).

2.1. Ferrocenic mefloquine analogues

The piperidinyl group of mefloquine was replaced by the hydrophobic ferrocenyl unit. 2,8-Bis(trifluoromethyl)-4-lithioquinoline was obtained by metalation with *n*-butyllithium in anhydrous diethylether of 4-bromo-2,8-bis(trifluoromethyl)quinoline at –65 °C under nitrogen atmosphere [13]. Subsequent addition of the 2-(*N*,*N*-substituted aminomethyl)ferrocenecarboxaldehyde to the lithiation mixture afforded the ferrocenic mefloquine analogues in 51–90% yields (*figure 2*).

Three products were isolated from the sequence shown in *figure 3* by chromatography over silica gel of the crude reaction mixture.

The first eluted product was 2,8-bis(trifluoromethyl)quinoline, resulting from an uncompleted addition. The second and third band to elute were the isomeric amino alcohols A and B. In solution ($CHCl_3$), compound A was found to exhibit an infrared absorption for the hydroxyl group (~3 000 cm^{-1}), indicating that the hydroxyl group participates in a strong hydrogen bond with the nitrogen lone pair [14]. Compound B was found to exhibit a broad, strong hydroxyl absorption (~3 000 cm^{-1}) characteristic of a hydroxyl group coordinated to an iron atom [15, 16].

The ^{1}H-NMR and MS spectra of the two amino-alcohols are similar, except for the resonance of the Fc–C*H*–OH proton [17]. In A, this resonance appears at δ ~6.5 ppm (singlet, 1H) and corresponds to a seven-membered ring. In B, the resonance appears at δ ~6.2 ppm due to a different anisotropic zone of the ferrocenic skeleton. A is a solid, whereas B is an oil (*figure 4*).

2.2. Ferrocenic quinine analogues

The ferrocenic analogues were prepared by replacement of the quinuclidinyl group by a substituted ferrocenyl moiety. 4-Carboxaldehyde-6-methoxyquinoline was obtained by selenium dioxide oxidation of 6-methoxy-4-methylquinoline in dioxane in 77% yield and 63% conversion, as described by Kwartler and Lindwall [18] and modified [19]. Then, the lithio ferrocenic derivative was condensed with the crude product (described above) in anhydrous THF/Et_2O giving, after work up, the ferrocenic quinine analogues (*figure 5*).

The amount of each diastereomer was deduced from the intensity of the ^{1}H-NMR resonance for the Fc–C*H*–OH proton (singlet) in the spectra of the crude product. The reaction mixture was purified by chromatography over silica gel. Compound A (minor, the less polar) was found to exhibit a singlet at δ ~6.1 ppm. For compound B (major, the more polar), the signal is shifted by c.a. ~0.4 ppm downfield (*figure 6*).

3. Results and discussion

3.1. Ferrocenic mefloquine analogues

The electronic and steric interactions between the aldehydic O atom and the *N*,*N*-substituted aminomethyl side chain are not prominent. This repulsion is compensated by the possibility for a chelation on the lithium atom [20]. The condensation step of the lithio derivative

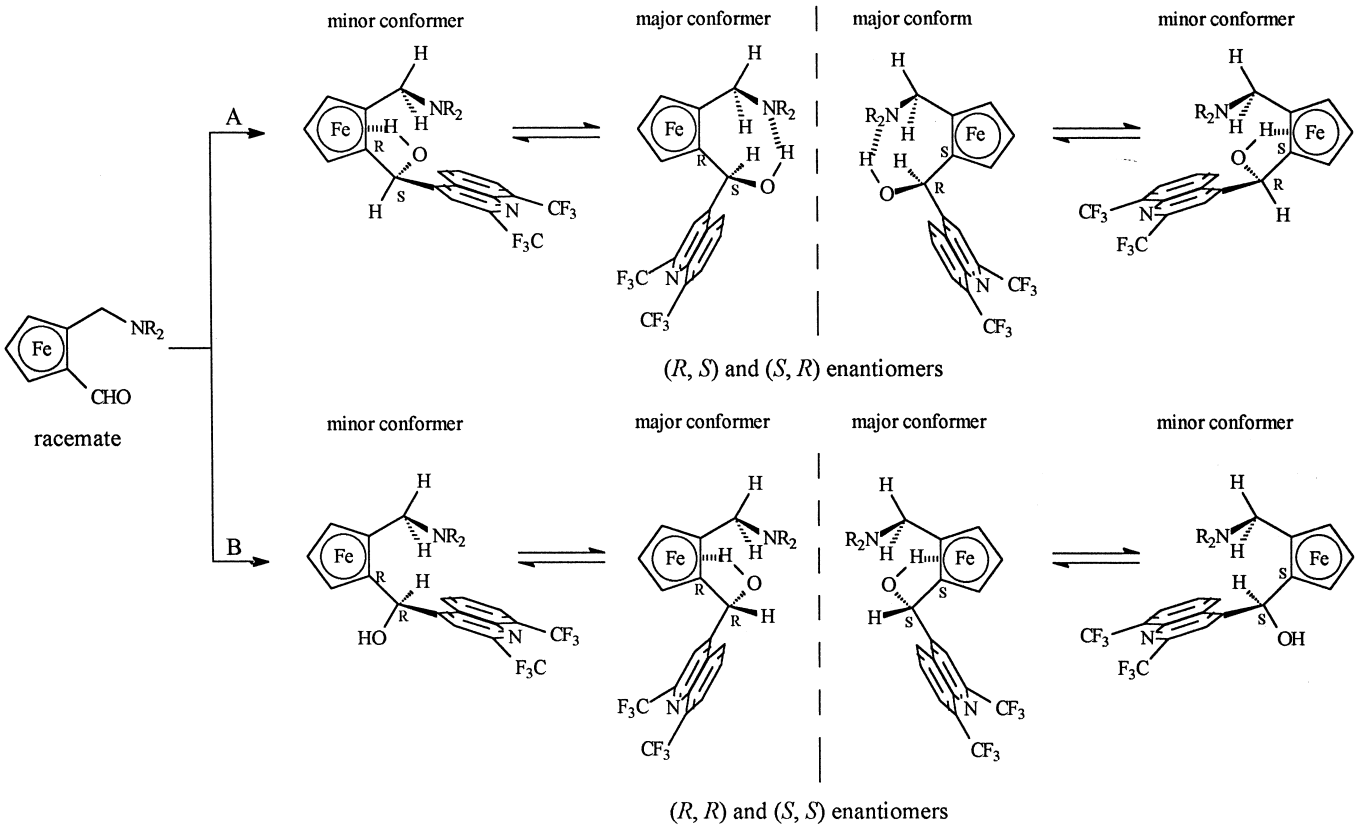


Figure 3. Diastereoselective synthesis of the ferrocenic mefloquine analogues.

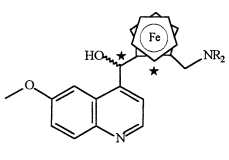
with induction by the *N,N*-substituted aminomethyl side chain allows for the formation of a stable cyclic complex (*figure 7*). During this stereoselective reaction, the **u** diastereomer, i.e. compound A: (*R, S*) and (*S, R*), is obtained.

The transition state relative to the other diastereomer (*figure 7*) does not present this stabilization and leads to the **l** form, i.e. compound B: (*R, R*) and (*S, S*), as an oil.

The relative proportion of the **u** and **l** diastereomers depends on the bulky group borne by the nitrogen atom (*figure 1*). The observed diastereomeric excess for the formation of **4** seems to be caused by the second nitrogen atom in the side chain which contributes to the decrease of the steric interactions.

3.2. Ferrocenic quinine analogues

The condensation step which produces the ferrocenic quinine analogues involves steric interaction [21]. The selectivity depends on the difference in the bulkiness of



Compd.	—NR_2	% Like **l**	% Unlike **u**
5	—N	63	37
6	—N	69	31

Figure 4. [2-(*N,N*-substituted aminomethylferrocenyl)]-(6-methoxyquinolyl)methanol derivatives. The relative proportion of each diastereomer was determined by ^{1}H-NMR of the crude mixture.

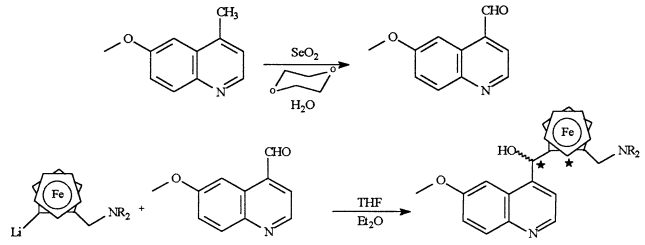


Figure 5. Synthesis of [2-(*N,N*-substituted aminomethyl-ferrocenyl)]-(6-methoxyquinolyl)-methanol derivatives.

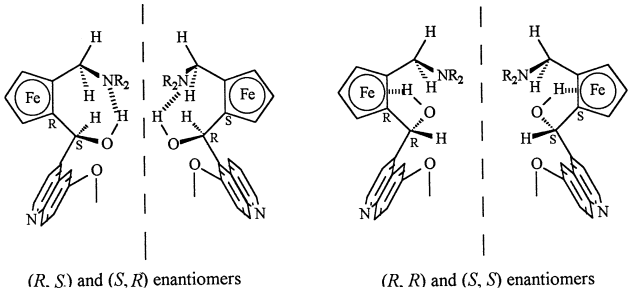


Figure 6. Diastereomers of the ferrocenic quinine analogues.

the groups on 6-methoxyquinoline and the hydrogen atom, and not on the substituents on the nitrogen atom. The *re*-face of the aldehyde is attacked in the proposed most stable transition state with an *exo*-orientation of the quinoline group of the aldehyde, leading to the **l** amino-alcohols (*figure 7* and *8*). The alternative transition state with an *endo*-quinoline group has severe interaction between the quinoline and the ferrocenic nucleus (*figure 8*). This addition leads to the minor **u** amino-alcohols (*figure 7*).

The relative proportion of the **u** and **l** diastereomers is not influenced by the nature of the substituents on the nitrogen atom (*figure 4*).

3.3. Biological activities

We compared the biological activity of the new ferrocenyl analogues to those of the parent compounds: mefloquine or quinine. The in vitro screening assays are briefly described in the experimental protocols section. Whichever *Plasmodium falciparum* strain used: sensitive (HB3) or chloroquine, mefloquine-resistant (Dd2), the ferrocenyl compounds exhibited a lower antimalarial activity than mefloquine or quinine themselves (*figures 9* and *10*). Though no precipitate was observed making pre-dilutions and dilutions in extemporane, all ferrocenyl

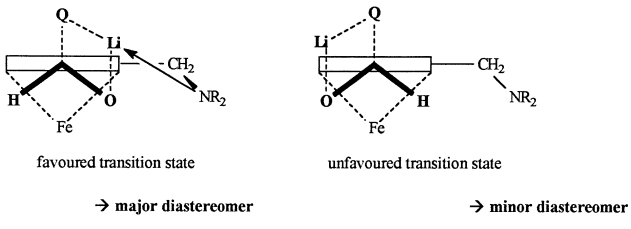


Figure 7. Mechanistic interpretation for the formation of the diastereomers of the mefloquine analogues. Q is 2,8-bis(trifluoromethyl)quinoline.

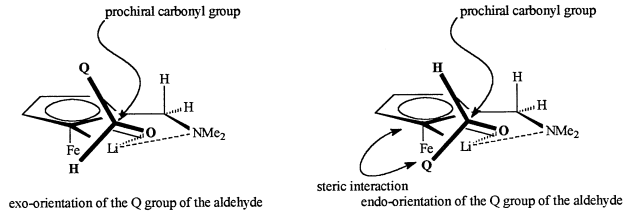


Figure 8. Mechanistic interpretation for the formation of the diastereoisomers of the quinine analogues. Q is 6-methoxyquinoline.

analogue solutions seemed to be unstable. Comparing isomers, the isomers dissimilar to ferrocenyl derivatives, (R, S) and (S, R), presented better antimalarial activities than corresponding similar isomers (*figures 9* and *10*). In spite of debated results [22–24], the structure of the aromatic ring system seems to be important to the differential activity exhibited by antimalarial agents containing a piperidine ring such as mefloquine and quinine. Moreover, stereoselective differences between the two mefloquine enantiomers have been reported in pharmacokinetic studies in human subjects [25].

4. Conclusion

To summarize, we have shown that it is possible to synthesize ferrocenic mefloquine and quinine analogues. The configurations of each diastereomer were determined by spectroscopic data. So, these compounds are close structural and stereochemical mimics of the parent drugs.

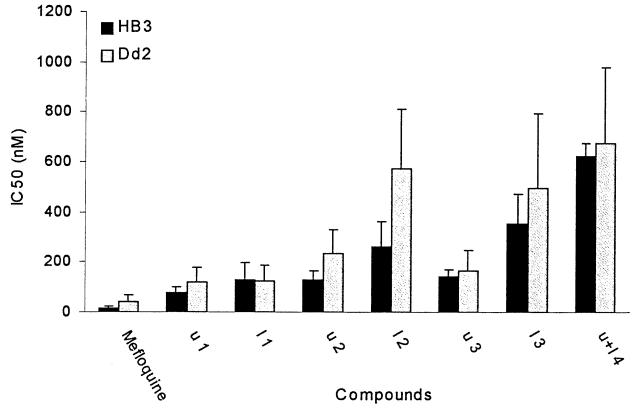


Figure 9. Mean of IC_{50} of mefloquine and ferrocenyl analogues for each *P. falciparum* strain: the chloroquine-sensitive strain HB3 and the mefloquine and chloroquine-resistant strain Dd2. Results are means of at least three independent experiments. Bars denote ± standard deviations.

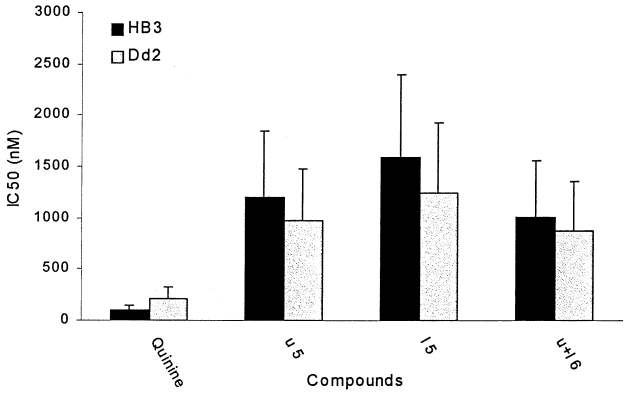


Figure 10. Mean of IC_{50} of quinine and ferrocenyl analogues for each *P. falciparum* strain: the chloroquine-sensitive strain HB3 and the mefloquine and chloroquine-resistant strain Dd2. Results are means of at least three independent experiments. Bars denote ± standard deviations.

These modifications were expected to result in our new potential antimalarial compounds with properties markedly different from mefloquine and quinine. But whichever *Plasmodium falciparum* strain used: sensitive (HB3) or chloroquine, mefloquine-resistant (Dd2) and whichever isomer (similar or dissimilar) tested, the ferrocenyl compounds exhibited a lower antimalarial activity than mefloquine or quinine themselves.

5. Experimental protocols

5.1. Chemistry

The ^{1}H-NMR spectra were recorded on a Bruker AC 300 spectrometer using tetramethylsilane (TMS) as the internal standard. δ are given in ppm, solvent: $CDCl_3$ + D_2O (95:5), s: singlet; d: doublet; t: triplet; q: quadruplet and m: multiplet. MS 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 the reflector mode were accumulated to obtain a good signal-to-noise ratio. The matrix used was 2,4,6-trihydroxyacetophenone (thap) or dihydroxybenzoic acid (dhb). EI mass spectra were acquired with a quadrupole instrument Nermag R 10-10 H. Melting points are uncorrected. Merck's Kieselgel 60 PF254 was used for the chromatography. Elemental analyses were performed at the 'Service Central d'Analyse', Vernaison and were within ± 0.4% of the theoretical values. The preparation of the ferrocenic aldehydes is analogous to that previously described [11].

5.1.1. Synthesis of ferrocenic mefloquine analogues

5.1.1.1. [2-(N,N-Diethylaminomethylferrocenyl)]-{4-[(2,8-bistrifluoromethyl)quinolyl]}methanol **1**

Under nitrogen, a stirred solution of 2,8-bis-(trifluoromethyl)-4-bromoquinoline (172 mg; 0.5 mmol) in 5 mL of anhydrous diethylether was treated with *n*-butyllithium in hexane (200 µL; 0.5 mmol) at –65 °C. Metalation was completed in 20 min at –65 °C. The crude solution was reacted with 2-(*N,N*-diethylaminomethyl)ferrocenecarboxaldehyde (140 mg; 0.5 mmol) in 5 mL of anhydrous diethylether. After 1 h at –65 °C, the solution was progressively warmed up to room temperature. The compound was hydrolysed by addition of water (10 mL). The organic layer was separated and the remaining aqueous phase was washed with small portions of diethylether (2 × 30 mL). The Et_2O extracts were combined, dried over Na_2SO_4 and evaporated to dryness. The resulting oil was purified by chromatography over silica gel (eluent: Et_2O/hexane/triethylamine, 30:60:10). Compound **1** was isolated as an oil (254 mg; 90%). The diastereomers were separated by TLC (silica gel: acetone/hexane, 40:60).

u diastereomer 56%. Yellow solid. Decomposed over 45 °C. ^{1}H-NMR: δ 8.87 (d, *J* = 8.50 Hz, 1H, H-5), 8.16 (d, *J* = 7.30 Hz, 1H, H-7), 7.97 (s, 1H, H-3), 7.66 (dd, *J* = 7.50, 8.10 Hz, 1H, H-6), 6.53 (s, 1H, CHOD), 4.23 (m, 1H, 1H Cp), 4.16 (d, *J* = 13.20 Hz, 1H, 1CHN), 4.02 (s, 5H, Cp′), 3.94 (m, 1H, 1H Cp), 3.22 (m, 2H, 1H Cp and 1CHN), 2.79 (q, *J* = 7.10 Hz, 2H, C**H**$_2$–CH_3), 2.46 (q, *J* = 7.10 Hz, 2H, C**H**$_2$–CH_3), 1.10 (t, *J* = 7.10 Hz, 6H, CH_3). MS (dhb): 564 M^+, 548 $(M.– O)^+$, 492 $(M – (NEt_2))^+$, 476 $(M – (ONEt_2))^+$, 388, 300.

l diastereomer 44%. Brown oil. ^{1}H-NMR: δ 8.42 (s, 1H, H-3), 8.30 (d, *J* = 9.0 Hz, 1H, H-5), 8.17 (d, *J* = 7.6 Hz, 1H, H-7), 7.69 (dd, *J* = 8.0, 8.3 Hz, 1H, H-6), 6.24 (s, 1H, CHOD), 4.14 (m, 1H, 1H Cp), 4.02 (m, 2H, 1H Cp and 1 CHN), 3.87 (s, 5H, Cp′), 3.48 (m, 1H, 1H Cp), 3,23 (d, *J* = 13.2 Hz, 1H, 1CHN), 2.77 (q, *J* = 7.1 Hz, 2H, C**H**$_2$–CH_3), 2.63 (q, *J* = 7.1 Hz, 2H, C**H**$_2$–CH_3), 1.13 CH_3 (t, *J* = 7.1 Hz, 6H, CH_3). MS (dhb): 564 M^+, 492, 326, 228, 199, 127.

5.1.1.2. [2-(N,N-Dimethylaminomethylferrocenyl)]-{4-[(2,8-bistrifluoromethyl)quinolyl]}methanol **2**

The preparation is analogous to the preparation of **1** (90% yield).

u diastereomer 59%. Yellow solid. M.p. 140–142 °C. ^{1}H-NMR: δ 8.87 (d, *J* = 8.60 Hz, 1H, H-5), 8.17 (d, *J* = 7.30 Hz, 1H, H-7), 7.96 (s, 1H, H-3), 7.66 (m, 1H, H-6), 6.54 (s, 1H, CHOD), 4.21 (m, 1H, 1H Cp), 4.13 (d, *J* = 12.70 Hz, 1H, 1CHN), 4.03 (s, 5H, Cp′), 3.94 (m, 2H, 2H

Cp), 2.97 (d, J = 12.70 Hz, 1H, 1CHN), 2.30 (s, 6H, $2CH_3$). MS (EI, m/e (%)): 536 M^+ (77), 492 (M – $N(CH_3)_2)^+$ (26), 491 (M – $HN(CH_3)_2)^+$ (100), 354 (27), 334 (16), 285 (15), 242 (16); (MALDI TOF (dhb)): 536, 520, 492.

l diastereomer 41%. Brown oil. ^{1}H-NMR: δ 8.39 (d, J = 8.90 Hz, 1H, H-5), 8.35 (s, 1H, H-3), 8.18 (d, J = 7.30 Hz, 1H, H-7), 7.71 (m, 1H, H-6), 6.25 (s, 1H, CHOD), 4.14 (m, 1H, 1H Cp), 4.03 (m, 1H, 1H Cp), 3.93 (d, J = 13.03 Hz, 1H, 1CHN), 3.76 (s, 5H, Cp′), 2.93 (d, J = 13.03 Hz, 1H, 1CHN), 2.35 (s, 6H, $2CH_3$). MS (EI, m/e (%)): 536 M^+ (42), 491 (M^+ –$HN(CH_3)_2)^+$ (100), 353 (13), 334 (13), 199 (11); (MALDI TOF (dhb)): 536, 492.

5.1.1.3. [2-(Piperidinomethylferrocenyl)]-{4-[(2,8-bistrifluoromethyl)quinolyl]}methanol **3**

The preparation is analogous to the preparation of **1** (85% yield).

u diastereomer 68%. Yellow solid. M.p. 157–159 °C. ^{1}H-NMR: δ 8.87 (d, J = 8.80 Hz, 1H, H-5), 8.17 (d, J = 7.30 Hz, 1H, H-7), 7.97 (s, 1H, H-3), 7.67 (dd, J = 7.60, 8.10 Hz, 1H, H-6), 6.53 (s, 1H, CHOD), 4.21 (m, 1H, 1H Cp), 4.07 (d, J = 13.00 Hz, 1H, 1CHN), 4.01 (s, 5H, Cp′), 3.94 (m, 1H, 1H Cp), 3.23 (m, 1H, 1H Cp), 3.08 (d, J = 13.00 Hz, 1H, 1CHN), 2.54 (m, 4H, $NC\mathbf{H}_2CH_2$), 1.57 (m, 6H, $CH_2(C\mathbf{H}_2)_3CH_2$). MS (EI, m/e (%)): 576 M^+ (46), 492 (37), 491 (100), 311 (14), 121 (36), 56 (21).

l diastereomer 32%. Brown oil. ^{1}H-NMR: δ 8.36 (d, J = 7.30 Hz, 1H, H-5), 8.35 (s, 1H, H-3), 8.18 (d, J = 7.10 Hz, 1H, H-7), 7.73 (dd, J = 7.80, 8.10 Hz, 1H, H-6), 6.23 (s, 1H, CHOD), 4.12 (m, 1H, 1H Cp), 4.01 ppm: (m, 1H, 1H Cp), 3.87 (d, J = 13.00 Hz, 1CHN), 3.75 (s, 1H, Cp′), 3.65 (m, 1H, 1H Cp), 3.00 (d, J = 13.00 Hz, 1H, 1CHN), 2.56 (m, 4H, $NC\mathbf{H}_2CH_2$), 1.66 (m, 4H, $NCH_2C\mathbf{H}_2$), 1.52 (m, 2H, $CH_2C\mathbf{H}_2CH_2$). MS (EI, m/e (%)): 576 M^+ (42), 492 (36), 491 (100), 334 (11), 121 (13), 86 (56), 56 (15); MS (MALDI TOF (dhb)): 576 M^+, 492; 491.

5.1.1.4. [2-(N-Methylpiperazinomethylferrocenyl)]-{4-[(2,8-bistrifluoromethyl)quinolyl]}methanol **4**

The preparation is analogous to the preparation of **1** (51% yield). We were unable to separate the diastereomers by chromatography column or by HPLC. MS (EI, m/e (%)): 591 M^+ (81), 492 (34), 491 (100), 354 (49), 334 (26), 285 (34), 101 (72), 99 (52), 83 (17), 56 (59).

u diastereomer 81% (NMR estimation). ^{1}H-NMR: δ 8.85 (d, J = 8.60 Hz, 1H, H-5); 8.18 (d, J = 7.12 Hz, 1H, H-7), 7.98 (s, 1H, H-3), 7.68 (m, 1H, H-6), 6.54 (s, 1H, CHOD), 4.24 (m, 1H, 1H Cp), 4.14 (d, J = 12.80 Hz, 1H, 1CHN), 4.05 (m, 2H, 2H Cp), 4.02 (s, 5H, Cp′), 3.15 (d, J = 12.79 Hz, 1H, 1CHN), 2.8–2.3 (m, 8H, CH_2), 2.28 (s, 3H, CH_3).

l diastereomer 19% (NMR estimation). ^{1}H-NMR: δ 8.38 (d, J = 7.89 Hz, 1H, H-5), 8.26 (s, 1H, H-3), 8.18 (d, J = 7.09 Hz, 1H, H-7), 7.72 (m, 1H, H-6), 6.23 (s, 1H, CHOD), 3.97–3,89 (m, 3H, 2H Cp and 1CHN), 3.84 (s, 5H, Cp′), 3.72 (m, 1H, 1H Cp), 3.62 (m, 1H, 1CHN), 2.8–2.3 (m, 8H, CH_2), 2.18 (s, 3H, CH_3).

5.1.2. Synthesis of ferrocenic quinine analogues

5.1.2.1. 4-Carboxaldehyde-6-methoxyquinoline

A solution of selenium dioxide (130 mg; 1.16 mmol) in a mixture of 2 mL of dioxane and 500 μL of water was added for 1 h to a well-stirred solution of 6-methoxy-4-methylquinoline (200 mg; 1.16 mmol) in 1 mL of dioxane kept at 65 °C. The mixture was stirred at 85 °C for 5 h and then filtered over celite. The dioxane was evaporated from the filtrate and the crude mixture was treated as described below (77% yield, 63% conversion).

^{1}H-NMR: δ 10.42 (s, 1H, CHO), 9.04 (d, J = 4.30 Hz, 1H, H-2), 8.47 (d, J = 2.80 Hz, 1H, H-5), 8.10 (d, J = 9.28 Hz, 1H, H-7), 7.76 (d, J = 4.30 Hz, 1H, H-3), 7.47 (dd, J = 2.83, 9.27 Hz, 1H, H-8), 4.00 (s, 3H, CH_3O).

5.1.2.2. [2-(N,N-Dimethylaminomethyl-ferrocenyl)]-(6-methoxyquinolyl)methanol **5**

The mixture described above was dissolved in anhydrous tetrahydrofuran (25 mL) and reacted with the 2-lithio-(*N*,*N*-dimethylaminomethyl)ferrocene (2 eq.) in anhydrous diethylether (20 mL) under nitrogen at room temperature. The resulting solution was stirred 4 h at room temperature, quenched by addition of water (50 mL) and extracted with portions of diethylether (2 × 30 mL). The combined extracts were dried over Na_2SO_4 and evaporated to dryness under reduced pressure. The resulting oil was purified by TLC (silica gel, eluent: methylacetate/hexane/triethylamine, 40:50:10) to obtain pure **5** (170 mg, 0.32 mmol, 37%).

u diastereomer 37%. Yellow oil (crystallised at 4 °C). ^{1}H-NMR: δ 8.80 (d, J = 4.40 Hz, 1H, H-2), 8.05 (d, J = 9.23 Hz, 1H, H-8), 7.80 (d, J = 2.76 Hz, 1H, H-5), 7.47 (d, J = 4.42 Hz, 1H, H-3), 7.36 (dd, J = 2.80, 9.23 Hz, 1H, H-7), 6.42 (s, 1H, CHOD), 4.16 (m, 1H, 1H Cp), 4.09 (d, J = 11.48 Hz, 1H, 1CHN); 4.02 (s, 5H, Cp′), 3.90 (m, 1H, 1H Cp), 3.81 (s, 3H, CH_3O), 3.45 (m, 1H, 1H Cp), 2.95 (d, J = 12.60 Hz, 1H, 1CHN), 2.28 (s, 6H, $2CH_3N$). MS (thap): 430 (M^+), 412 (M – $H_2O)^+$, 385 (M – $HNMe_2)^+$, 370, 369, 367 (M – (H_2O + $HNMe_2$))$^+$.

l diastereomer 63%. Yellow oil. ^{1}H-NMR: δ 8.90 (d, J = 4.35 Hz, 1H, H-2), 8.08 (d, J = 9.14 Hz, 1H, H-8), 7.80 (d, J = 4.40 Hz, 1H, H-3), 7.39 (dd, J = 2.54,

9.15 Hz, 1H, H-7), 7.29 (d, *J* = 2.47 Hz, 1H, H-5), 6.12 (s, 1H, CHOD), 4.11 (m, 1H, 1H Cp), 4.03 (m, 1H, 1H Cp), 3.94 (d, *J* = 12.17 Hz, 1H, 1CHN), 3.93 (s, 3H, CH_3O), 3.88 (m, 1H, 1H Cp), 3.70 (s, 5H, Cp′), 2.91 (d, *J* = 12.85 Hz, 1H, 1CHN), 2.33 (s, 6H, $2CH_3N$). MS (thap): 453 $(M+ Na)^+$, 430 M^+, 386 $(M - NMe_2)^+$, 385 $(M - HNMe_2)^+$, 367 $(M - (H_2O + HNMe_2))^+$.

5.1.2.3. [2-(Piperidinomethyl-ferrocenyl)]-(6-methoxyquinolyl)methanol **6**

The preparation is analogous to the preparation of **5** (58% yield). We were unable to separate the diastereoisomers by chromatography column or by HPLC. MS (thap): 493 $(M+ Na)^+$, 471 MH^+, 470: M^+, 386 $(M - N(CH_2)_5)^+$, 367: $(M - (H_2O + H\ N(CH_2)_5))^+$.

u diastereomer 31% (NMR estimation). ^{1}H-NMR: δ 8.80 (d, *J* = 4.40 Hz, 1H, H-2), 8.05 (d, *J* = 9.25 Hz, 1H, H-8), 7.80 (d, *J* = 2.82 Hz, 1H, H-5), 7.48 (d, *J* = 4.45 Hz, 1H, H-3), 7.36 (dd, *J* = 2.88, 9.25 Hz, 1H, H-7), 6.44 (s, 1H, CHOD), 4.15–3.85 (m, 4H, 3H Cp and 1CHN), 4.01 (s, 5H, Cp′), 3.79 (s, 3H, CH_3O), 3.05 (d, *J* = 12.80 Hz, 1H, 1CHN), 2.53 2 (m, 4H, CH_2), 1.63 2 (m, 4H, CH_2) 1.45 (m, 2H, CH_2).

l diastereomer 69% (NMR estimation). ^{1}H-NMR: δ 8,91 (d, *J* = 4.35 Hz, 1H, H-2), 8.09 (d, *J* = 9.20 Hz, 1H, H-8), 7.78 (d, *J* = 4.54 Hz, 1H, H-3), 7.40 (dd, *J* = 2.73, 9.22 Hz, 1H, H-7), 7.32 (d, *J* = 2.71 Hz, 1H, H-5), 6.12 (s, 1H, CHOD), 4.15–3.85 (m, 4H, 3H Cp and 1CHN), 3.96 (s, 3H, CH_3O), 3.63 (s, 5H, Cp′), 2.96 (d, *J* = 12.98 Hz, 1H, 1CHN), 2.53 (m, 4H, CH_2), 1.63 (m, 4H, CH_2), 1.45 CH_2 (m, 2H, CH_2).

5.2. Biology protocols

5.2.1. Parasite cultures

Two culture-adapted strains of *P. falciparum* were used: the chloroquine-sensitive strain HB3 (Honduras) and the mefloquine and chloroquine-resistant strain Dd2 (Indochina). All stock parasite cultures were maintained using Trager and Jensen's method [26, 27].

5.2.2. In vitro activity of mefloquine and quinine analogues

The assays were conducted in vitro using a modification of the semi-automated microdilution technique of Desjardins et al. based on radiolabelled [^{3}H]hypoxanthine incorporation [28]. Drug testing was carried out in 96-well microtitre plates. All the compounds were tested as free bases and dissolved in dimethyl sulphoxide (5 mg/mL). They were then pre-diluted 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 mefloquine concentration ranged from 1.4–90.4 nM for HB3 strain and from 2.8–180.7 nM for Dd2 strain. Concerning each mefloquine ferrocenyl derivative, they ranged from 16.9–1 084.2 nM for the two *P. falciparum* strains. The final concentration of quinine and ferrocenyl analogues ranged from 52.08–3 333.3 nM for both strains. All these concentrations contained less than 0.01% dimethyl sulphoxide which had no detectable effect on parasite multiplication [29]. 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.

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