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Novel metallocenic compounds as antimalarial agents. Study of the position of ferrocene in chloroquine

C. Biot [a,*], L. Delhaes [b,d], H. Abessolo [b], O. Domarle [c], L.A. Maciejewski [a], M. Mortuaire [b], P. Delcourt [b], P. Deloron [c], D. Camus [b,d], D. Dive [b], J.S. Brocard [a]

[a] *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, F-59652 Villeneuve d'Ascq Cedex, France*

[b] *INSERM U.42, 369 rue Jules Guesde BP 39, F-59651 Villeneuve d'Ascq Cedex, France*

[c] *Centre International de Recherches Médicales, BP 769 Franceville, Gabon*

[d] *Laboratoire de Parasitologie-Mycologie, Faculté de Médecine, 1 place de Verdun, F-59045 Lille Cedex 2, France*

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Abstract

The synthesis, characterization and antimalarial activity of two new ferrocene–chloroquine compounds are reported. One of them, 7-chloro-4-*N*-[(4-*N*′-ethyl-*N*′-ferrocenylmethyl)ammonio-1-methylbutylamino]quinolin-1-ium bi-tartrate (**2**) showed very promising antimalarial activity in vivo on mice infected with *Plasmodium berghei* N. and *Plasmodium yoelii* NS. and in vitro against chloroquine resistant-strains of *Plasmodium falciparum*. © 1999 Elsevier Science S.A. All rights reserved.

Keywords: Antimalarial agents; Chloroquine; Ferrocene; Metallocenes

1. Introduction

For the year 1994, it was estimated that 2.3 billion people (41% of the entire world population) lived in areas presenting malaria risks, and among them 1.5–2.7 million died every year [1–3]. The discovery of new antimalarial agents is important because *Plasmodium* parasites are unfortunately becoming increasingly resistant to chloroquine (CQ) and other traditional drugs used to treat patients with malaria.

The use of metal complexes capable of enhancing the activity of biological compounds has become a vibrant and growing area of research in the communities of both organometallic chemists and biologists [4]. As a matter of fact, the introduction of metals can have profound effects [5,6]. Indeed, recently many different metals have been incorporated into antimalarial agents [7–9].

In earlier patents [10,11] and articles [12,13] we have presented our strategy for the development of organometallic-based antimalarial drugs. We focused mainly on the ferrocene–chloroquine analogue: ferrochloroquine (i.e. FQ: 7-chloro-4-[(2-*N*,*N*-dimethylaminomethyl)ferrocenylmethylamino]quinoline) and tested its antimalarial activity [12,13]. From our observations we thought that introducing the ferrocenyl unit into another position of the CQ might provide interesting biological properties (Fig. 1).

2. Results and discussion

2.1. Chemistry

N,*N*-Dimethyl(ferrocenylmethyl)amine (**1a**) was reacted with methyliodide in acetonitrile to afford *N*,*N*-trimethyl(ferrocenylmethyl)ammonium iodide (**1b**). Treatment of **1b** with chloroquine in acetonitrile under reflux gave 7-chloro-4-[*N*-(4-*N*′,*N*′-diethylamino)-1-methylbutylamino]-1-ferrocenylmethylquinolinium io-

* Corresponding author. Tel.: +33-20-434893; fax: +33-20-436501.

E-mail address: thecatal@pop.univ-lille1.fr (C. Biot)

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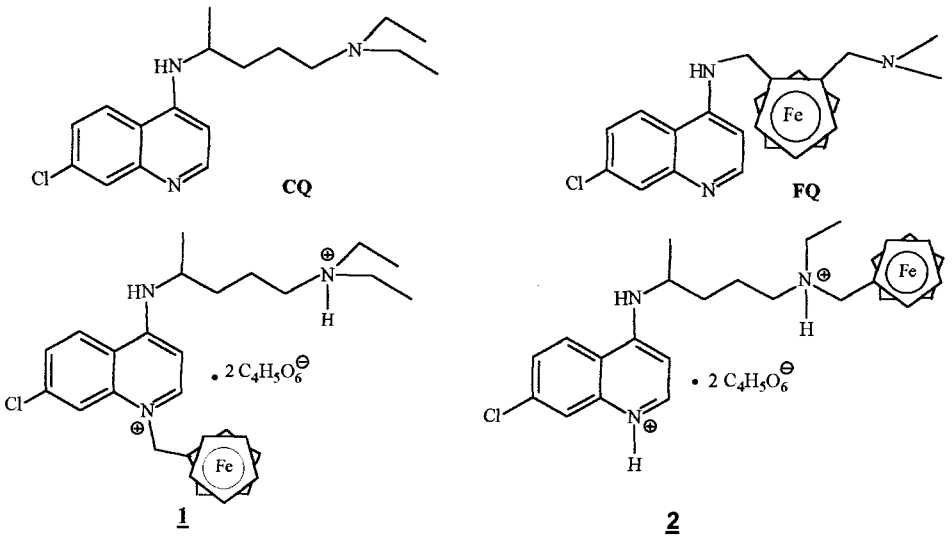


Fig. 1. Chemical structures of chloroquine (CQ), ferrochloroquine (FQ), 7-chloro-4-[*N*-(4-*N*′,*N*′-diethylamino)-1-methylbutylamino]-1-ferrocenylmethylquinolinium bi-tartrate **1**, and 7-chloro-4-*N*-[(4-*N*′-ethyl-*N*′-ferrocenylmethyl)ammonio-1-methylbutylamino]quinolin-1-ium bi-tartrate **2**.

dide (**1c**) in 53% yield [14]. This compound was reacted with L-(+)-tartaric acid in acetone to furnish the potent biologically active **1** (Scheme 1).

Assignment of structure to **1** was based on spectral evidence: the mass spectra (negative ions) have shown a molecular peak at $m/z = 149$ due to $C_4H_5O_6^-$ (exchange with I^-) and in the ^{1}H-NMR spectra the signals of quinoline protons (**1c**) are shifted relative to CQ (Table 1). This pattern indicates that CQ binds to the ferrocenylmethyl moiety through the quinoline N(1) atom.

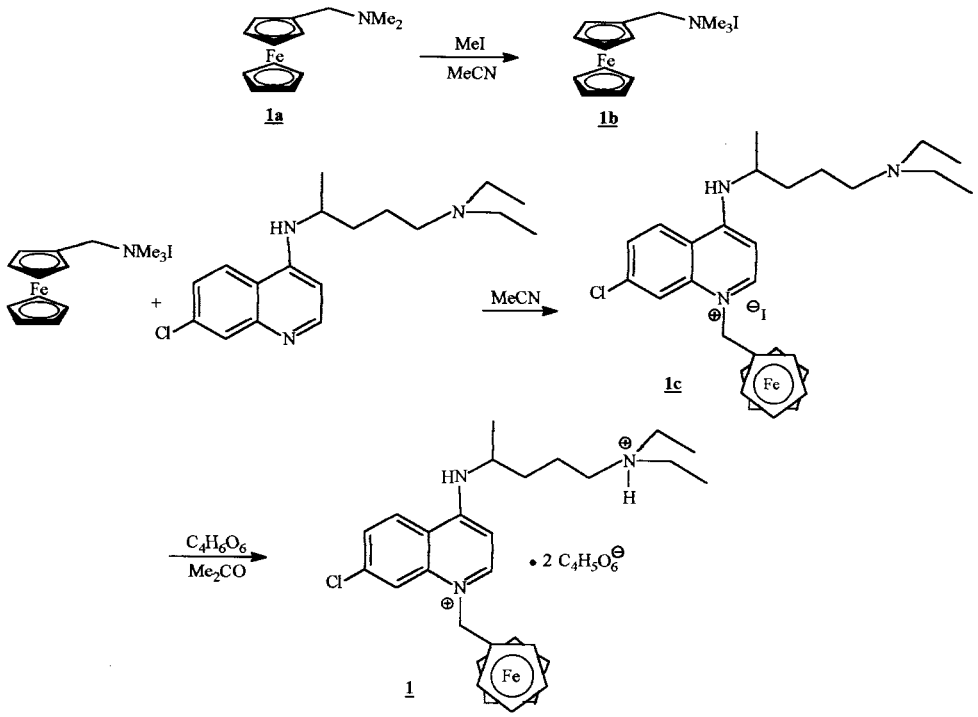
The synthesis of 7-chloro-4-*N*-[(4-*N*′-ethyl-*N*′-ferrocenylmethyl)ammonio-1-methylbutylamino]quinolin-1-ium bi-tartrate (**2**) was achieved as depicted in Scheme 2 [15,16]. The ferrocenecarboxaldehyde **2a** was condensed with ethylamine in anhydrous diethylether giving the imine **2b**. Reduction of **2b** by sodium borohydride in methanol gave the *N*-ethyl(ferrocenylmethyl)amine **2c** in 98% yield. Condensation of **2c** with 5-chloropentan-2-one in *N*-methylpyrrolidin-2-one produced 5-(*N*-ethyl-*N*-ferrocenylmethylamino)pentan-2-one **2d** in 39% yield [17]. Next, ketone **2d** was converted to the corresponding oxime **2e** (98%) through reaction with hydroxylamine hydrochloride and sodium hydroxide in ethanol under reflux. Reduction of the 5-(*N*-ethyl-*N*-ferrocenylmethylamino)pentan-2-one-oxime **2e** by $LiAlH_4$ in anhydrous tetrahydrofuran gave 5-(*N*-ethyl-*N*-ferrocenylmethylamino)pentan-2-amine **2f** (98%) [18,19], which was further reacted with 4,7-dichloroquinoline in *N*-methylpyrrolidin-2-one, leading to 7-chloro-4-*N*-[(4-*N*′-ethyl-*N*′-ferrocenylmethyl)-amino-1-methylbutylamino]quinoline (**2g**) in low unoptimized yield (10%). Finally, the conversion of the free base **2g** to the ammonium derivative **2** was achieved in acetone by acidification with two equivalents of L-(+)-tartaric acid, in 90% yield.

2.2. Biological activities

The need to synthesize novel antimalarials arises from the practical limitation of supplies based on natural antimalarials and from increased resistance to the currently used antimalarials, such as chloroquine and mefloquine. Although numerous quinoline-related agents have been prepared and screened for antimalarial activity by the Walter Research Institute, there have been relatively few studies reported on organometallic-chloroquine compounds [7,8]. For example, Sanchez-Delgado and co-workers reported that a gold–chloroquine complex $[Au(PPh_3)(CQ)]PF_6$ is active in vitro and in vivo against *Plasmodium berghei* probing that incorporation of the gold fragment produced marked enhancement of the efficacy of chloroquine [8].

Thus, we thought to study the new ferrocenyl derivatives described above, including the relationship between the site on chloroquine modified with ferrocene and the biological activity. The in vitro screening and in vivo assays are briefly described in Section 4. The antimalarial activities of the two compounds are summarized in Tables 2 and 3.

As can be seen in Table 2, if compared to CQ, **1** displayed only low antimalarial activity in vitro against the clones of *P. falciparum*. In fact, even if the architecture of CQ was present in derivative **1**, the activity was diminished compared to CQ. We suggest that the lack of protonation, at the quinolin nitrogen atom in the bridging unit, enabled **1** to penetrate into infected cells [20]. Now, even if **1** can enter infected cells, the quaternization might inhibit the interaction with the heme necessary for the efficiency of the agent [21]. These two hypotheses may account for the lack of antimalarial activity.



Scheme 1. Synthesis of 7-chloro-4-[*N*-(4-*N*′,*N*′-diethylamino)-1-methylbutylamino]-1-ferrocenylmethylquinolinium bi-tartrate **1.**

On the contrary, the new derivative **2** behaved differently when placed in biological conditions. In fact, the substitution of an ethyl substituent by a ferrocenyl methyl group in the bioactive chloroquine was expected to induce great changes in molecular properties, such as the solubility and lipophilicity. Indeed, we observed increased activity for analogue **2** (Tables 2 and 3). This enhancement can be attributed to increased cellular accumulation but also to increased lipophilicity brought about by the presence of the substituted ferrocene. Interestingly, the ferrocenic analogues reverse the CQ resistance; all of the analogues have equipotent activity against sensitive and resistant strains of *P. falciparum* (except for the FCR3 strain).

3. Conclusions

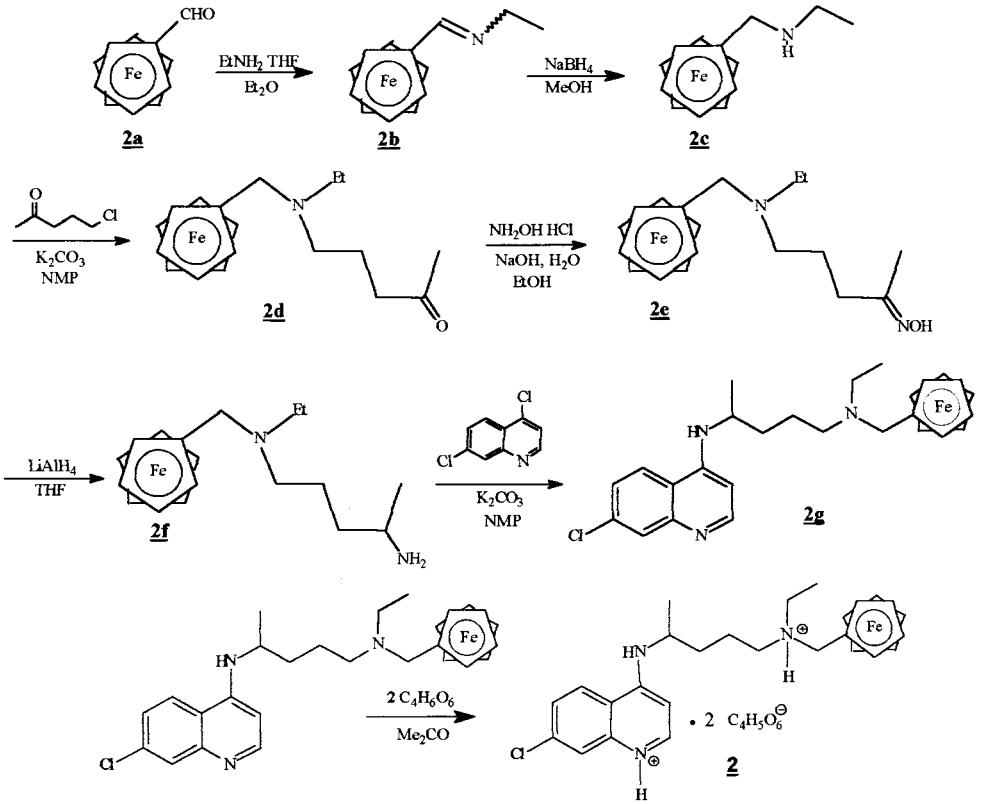
The antimalarial activity of **2**, reported here, compared to CQ and FQ, confirms our preliminary prediction, i.e. ferrocene-CQ compounds where a carbon chain of chloroquine is replaced by a ferrocenyl unit should be potent agents.

Both compound **2** and FQ [12,13] (reported earlier) exhibit greater antimalarial activity, in vitro and well as in vivo. Compared with compound **1**, bearing the ferrocenyl unit directly on the CQ nucleus, analogue **2** has the ferrocenyl group located on the lateral chain and thus maintained the quinoline moiety. Moreover, the length of the side chain (number of carbons) remained the same as in the original chloroquine, but the ferrocenyl group introduced a different steric crowding of the agent. However, as Ridley and co-workers [21] have already noticed, it is still surprising that such closely structurally related structures (compounds such as **2** or FQ), when compared to CQ, manage to overcome the current resistance to CQ. This observation suggests that the mechanism(s) contributing to CQ resistance is probably extremely structure specific. This hypothesis is corroborated on the one hand by the features of a carrier-mediated transport and a CQ import mechanism in *P. falciparum* demonstrated by Sanchez et al. [22], and on the other hand by the identification of a candidate gene *cg2* encoding a unique 330 kDa protein with complex polymorphism linked to chloroquine-resistant *P. falciparum* [23,24]. Based on these points, ferrocenyl analogues of CQ, such as **2** or FQ, might present reduced affinities of the quinoline compound for its transporter, restoring in parallel a potent antimalarial

Table 1
Selected ^{1}H-NMR data for **1c** and chloroquine (CQ)

δ [a] (ppm)	H2	H3	H5	H6	H8
CQ	8.51	7.35	7.69	7.35	7.94
1c	7.99	6.40	8.91	7.40	7.69

[a] Using $CDCl_3$ as the internal solvent and tetramethylsilane (TMS) as the internal standard. Complete NMR data in Section 4.



Scheme 2. Synthesis of 7-chloro-4-*N*-[(4-*N*′-ethyl-*N*′-ferrocenylmethyl)ammonio-1-methylbutylamino]quinolin-1-ium bi-tartrate **2**.

activity, especially against chloroquine-resistant *P. falciparum* strains.

4. Experimental

4.1. Chemistry

The ^{1}H-NMR spectra were recorded on a Brucker AC 300 spectrometer using tetramethylsilane (TMS) as the internal standard and $CDCl_3$, D_2O or DMSO-d_6 as the solvent. 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 dihydroxybenzoïc acid (dhb). Melting points are uncorrected. Merck's Kieselgel 60 PF254 was used for the chromatography.

4.1.1. *N,N-trimethyl(ferrocenylmethyl)ammonium iodide (**1b**)*

Methyliodide (1ml; 16 mmol) was added to a solution of *N,N*-dimethyl(ferrocenylmethyl)amine (1 g; 4.12 mmol) in 50 ml dry acetonitrile. The resulting mixture was stirred for 1 h at room temperature (r.t) and evaporated under reduced pressure and dried under vaccum. A total of 1.55 g (4.03 mmol) of compound **1b** was obtained and used without further purification. Yield: 98%. M.p.: 198–200°C (litt. 200°C). ^{1}H-NMR (DMSO-d^6) δ 4.49 (m, 2H, Cp), 4.40 (s, 2H, CH_2–Fc), 4.37 (m, 2H, Cp), 4.24 (s, 5H, Cp′), 2.91 (s, 9H, $N(CH_3)_3I$).

4.1.2. *7-Chloro-4-[N-(4-N′,N′-diethylamino)-1-methylbutylamino]-1-ferrocenylmethylquinolinium iodide (**1c**)*

A mixture of chloroquine (960 mg; 3 mmol), **1b** (1.1 g; 2.88 mmol) and K_2CO_3 (802 mg; 5,8 mmol) were heated in acetonitrile (20 ml) under reflux for 45 h. The

Table 2
Mean of IC_{50} of chloroquine, **1** and **2** for each *P. Falciparum* strain

Parasite strains	IC_{50} (nM) ± S.D. [a] of compounds [b]					
	CQ [c]	*n*	**1**	*n*	**2**	*n*
HB3	26 ± 3	16	143 ± 83	3	27 ± 15	6
Dd2	137 ± 22	9	789 ± 120	3	53 ± 5	3
FG3	154 ± 65	3	428 ± 107	3	58 ± 11	3
FG4	168 ± 93	4	477 ± 180	3	77 ± 33	3
FCR3	184 ± 130	11	294 ± 49	3	294 ± 49	3
FG1	288 ± 22	3	616 ± 126	3	86	2

[a] Values are the arithmetic mean ± standard deviation.
[b] Chloroquine (CQ) was applied as phosphate salt and **1**, **2** as tartrate salt.
[c] Chloroquine sensitive threshold adopted is 100 nM.

Table 3
Effect of chloroquine and **2** on *P. berghei* N and *P. yoelii* NS

	Inhibition of growth (mean in%)			
	CQ		**2**	
	1 mg^{-1} kg^{-1} d^{-1}	10 mg^{-1} kg^{-1} d^{-1}	1 mg^{-1} kg^{-1} d^{-1}	10 mg^{-1} kg^{-1} d^{-1}
P. berghei N	52.6	100	65.9	100
P. yoelii NS	17.6	100	1.9	100

heterogeneous mixture was filtered through a fritted funel and washed with small portions of CH_2Cl_2 (3 × 50 ml). The combined filtrates were evaporated under reduced pressure. The residual oil was diluted in a mixture of diethylether (30 ml), water (20 ml) and H_3PO_4 (3 ml). The aqueous phase was washed with diethylether (2 × 15 ml) and made alkaline by addition of K_2CO_3. The organic compounds were extracted with CH_2Cl_2 (2 × 40 ml). The organic layers were combined, dried over Na_2SO_4 and evaporated under reduced pressure. The red oil was purified by TLC (elution with 4:5:1 CH_2Cl_2–acetone–triethylamine) giving **1c** as an orange oil (1.023 g; 1.58 mmol; 53%). ^{1}H-NMR ($CDCl_3 + D_2O$) δ 8.91 (d, $J = 8.86$ Hz, 1H, H-5), 7.99 (m, 1H, H-2), 7.69 (m, 1H, H-8), 7.40 (dd, $J = 2.06$, 8.90 Hz, H-6), 6.40 (d, $J = 7.94$ Hz, 1H, H-3), 5.21 (s, 1H,: CH_2–Fc), 4.34 (m, 2H, Cp), 4.29 (s, 5H, Cp′), 4.26 (m, 2H, Cp), 3.78 (m, 1H, CHCH$_3$), 2.49 (q, $J = 7.30$ Hz, 4H, CH_2CH$_3$), 2.43 (t, $J = 7.30$ Hz, 2H, CH$_2$CH_2N), 1.92 (m, 1H, CHCH_2CH$_2$), 1.71 (m, 1H, CHCH_2CH$_2$), 1.56 (m, 2H, CH$_2$CH_2CH$_2$), 1.36 (d, $J = 6.38$ Hz, 3H, CH_3CH), 1.00 (t, $J = 7.00$ Hz, 3H, CH_3CH$_2$). MS-MALDI-TOF (thap) positive ions 520 ($M^{\bullet+}$ ^{37}Cl), 518 ($M^{\bullet+}$ ^{35}Cl), 398, 322, 320, 199, negative ions 127 (I^-).

4.1.3. 7-Chloro-4-[N-(4-N′,N′-diethylamino)-1-methylbutylamino]-1-ferrocenylmethylquinolinium bi-tartrate (1)

A solution of L-(+)-tartaric acid (300 mg, 2 mmol) in acetone (20 ml) was added dropwise to a solution of **1c** (520 mg, 1 mmol) in acetone (20 ml) at r.t. The resulting precipitate was collected by filtration and washed with Et_2O giving **1** (737 mg, 0.90 mmol, 90%). ^{1}H-NMR ($CDCl_3 + D_2O$) δ 8.36 (d, 1H, $J = 7.09$ Hz, H-2), 8.22 (d, 1H, $J = 9.07$ Hz, H-5), 8.14 (s, 1H, H-8), 7.63 (d, 1H, $J = 9.16$ Hz, H-6), 6.80 (d, 1H, $J = 7.35$ Hz, H-3), 5.34: (s, 2H, CH_2–Fc), 4.44 (s, 5H, Cp′), 4.31 (m, 4H, Cp, CHOD), 4.09 (m, 1H, CHCH$_3$), 3.13 (m, 6H, CH_2CH$_3$, CH$_2$CH_2N), 1.77 (m, 4H, CHCH_2, CH$_2$CH_2CH$_2$), 1.36 (d, 3H, $J = 6.41$ Hz, CH_3CH), 1.18 (td, 6H, $J = 2.80$, 7.30 Hz, CH_3CH$_2$). MS-MALDI-TOF (thap) positive ions 520($M^{\bullet+}$ ^{37}Cl), 518 ($M^{\bullet+}$ ^{35}Cl), 398, 322, 320, 199, negative ions 149(M–H)– $^{\bullet}C_4H_5O_6$. Found: C,54.25; H, 5.99; N, 5.13. $C_{37}H_{49}N_3O_{12}ClFe$. Anal. Calc.: C, 54.19; H, 6.15; N, 5.17.

4.1.4. N-ethyl(ferrocenylmethyl)amine (2c)

A solution of ethylamine (4.4 ml; 9.4 mmol) was added to a solution of ferrocenecarboxaldehyde (1 g; 4.4 mmol) in diethylether (20 ml) under a nitrogen atmosphere. After 1 h, the solvents were evaporated, and the imine **2b** was dissolved in methanol (20 ml). Small portions of $NaBH_4$ (total: 1g; 2.82 mmol) were added at 0°C. The resulting solution was stirred at r.t. for 1 h, quenched by the addition of water (50 ml), and extracted with portions of Et_2O (3 × 50 ml). The combined extracts were dried over Na_2SO_4, and evaporated to dryness to give **2c** (1.14 g; 4.7 mM; 98%). ^{1}H-NMR ($CDCl_3 + D_2O$) δ 4.19 (m, 2H, Cp), 4.12 (s, 5H, Cp′), 4.11 (m, 2H, Cp), 3.53 (s, 2H, FcCH_2), 2.68 (q, 2H, $J = 7.10$ Hz, CH_2CH$_3$), 1.12 (t, 3H, $J = 7,10$ Hz, CH_3CH$_2$). MS-MALDI-TOF (dhb) 243 ($M^{\bullet+}$), 199 $(FcCH_2)^+$.

4.1.5. 5-(N-ethyl-N-ferrocenylmethylamino)pentan-2-one (2d)

A mixture of **2c** (500 mg; 2.06 mmol), K_2CO_3 (285 mg; 2.06 mmol) and 5-chloropentan-2-one (240 µl; 2.06 mmol) was heated in *N*-methylpyrolidin-2-one (2 ml) at 125°C for 6 h. After cooling, diethylether (50 ml) was added and the solution was washed with brine (10 × 50 ml). The organic layer was dried over Na_2SO_4, and evaporated under reduced pressure. The resulting oil was purified by column chromatography (elution with 9:1 hexane–triethylamine) (223 mg; 0.69 mmol; 39%). ^{1}H-NMR ($CDCl_3$) δ 4.13 (m, 2H, Cp), 4.11 (s, 5H, Cp′), 4.09 (m, 2H, Cp), 3.46 (s, 2H, CH_2Fc), 2.41 (t, 2H, $J = 7.10$ Hz, CH_2CH$_3$), 2.41 (t, 2H, $J = 7.00$ Hz, CH$_2$CH_2N), 2.31 (t, 2H, $J = 7.10$ Hz, COCH_2), 2.12 (s, 3H, CH_3C=O), 1.72 (q, 2H, $J = 7,20$ Hz, CH$_2$CH_2CH$_2$), 1.00 (t, 3H, $J = 7,00$ Hz, CH_3CH$_2$). MS-MALDI-TOF (dhb) 299 ($M^{\bullet+}$), 315, 273, 199.

4.1.6. 5-(N-ethyl-N-ferrocenylméthylamino)pentan-2-one-oxime (**2e**)

A solution of sodium hydroxide (81 mg; 0.2 mmol) in water (3 ml) was added to a stirred mixture of **2d** (200 mg; 0.66 mmol), hydroxylamin hydrochloride (67 mg; 0.96 mmol) in EtOH (25 ml) at r.t. The resulting solution was further stirred under reflux for 2.5 h, quenched by the addition of water (50 ml) and extracted with CH_2Cl_2 (2×25 ml). The combined extracts were dried over Na_2SO_4, evaporated to dryness under reduced pressure giving **2e** (208 mg; 0.61 mmol; 98%). ^{1}H-NMR ($CDCl_3$) δ 4.14 (m, 2H, Cp), 4.11 (m, 7H, Cp′, Cp), 3.53–3.50 (2s, 2H, CH_2Fc *syn* and *anti*), 2.43 (m, 2H, CH_2CH_2N), 2.35 (m, 2H, CH_2CH_3), 2.15 (m, 2H, N=CCH_2), 1.87–1.86 (2s, 3H, CH_3C=N *syn* and *anti*), 1.69 (m, 2H, $CH_2CH_2CH_2$), 1.04 (m, 3H, CH_3CH_2). MS-MALDI-TOF (dhb) 342 ($M^{\bullet+}$), 199 $(FcCH_2)^+$.

4.1.7. 5-(N-ethyl-N-ferrocenylmethylamino)pentan-2-amine (**2f**)

$LiAlH_4$ (77 mg; 1.93 mmol) and **2e** (200 mg; 0.58 mmol) were combined in anhydrous THF (13 ml) under nitrogen at r.t. The reaction mixture was then heated under reflux for 3.5 h. After cooling, the solution was diluted with Et_2O, washed with brine, dried over K_2CO_3 and evaporated to dryness to obtain pure **2f** as an oil (187 mg; 0.57 mmol; 98%). ^{1}H-NMR ($CDCl_3 + D_2O$) δ 4.15 (m, 2H, Cp), 4.11 (m, 7H, Cp′, Cp), 3.50 (s, 2H, CH_2Fc), 2.86 (m, 1H, CHCH$_3$), 2.43 (q, 2H, $J = 7.10$ Hz, CH_2CH$_3$), 2.33 (t, 2H, $J = 7{,}60$ Hz, CH_2CH_2N), 1.45 (m, 2H, $CH_2CH_2CH_2$), 1.28 (m, 2H, CH_2CH), 1.04 (m, 6H, CH_3CH$_2$, CH_3CH). MS-MALDI-TOF (dhb) 328 ($M^{\bullet+}$), 203, 199.

4.1.8. 7-chloro-4-N-[(4-N′-ethyl-N′-ferrocenylmethyl)-amino-1-methylbutylamino]quinoline (**2g**)

A mixture of **2f** (505 mg; 1.5 mmol), 4,7-dichloroquinoline (595 mg; 3 mmol), triethylamine (2 ml; 14.4 mmol) and K_2CO_3 (415 mg; 3 mmol) in *N*-methylpyrrolidin-2-one (7 ml) was stirred under nitrogen at 115°C for 3 h. and, after cooling to r.t., the reaction mixture was diluted with CH_2Cl_2 (50 ml), washed with brine (10×50 ml) and dried over Na_2SO_4. The organic phase was then reduced under vacuum, and the resulting oil was purified by TLC (silica gel, using 5:4:1 MeOAc–hexane–triethylamine) (74 mg; 0.15 mmol; 10%). M.p. 117–118°C. ^{1}H-NMR ($CDCl_3 + D_2O$) δ 8.50 (d, 1H, $J = 5.40$ Hz, H-2), 7.95 (d, 1H, $J = 2.10$ Hz, H-8), 7.65 (d, 1H, $J = 8.90$ Hz, H-5), 7.32 (dd, 1H, $J = 2.00$, 8.90 Hz, H-6), 6.40 (d, 1H, $J = 5.50$ Hz, H-3), 4.10 (m, 9H, Cp, Cp′), 3.66 (m, 1H, CHCH$_3$), 3.46 (s, 2H, CH_2Fc), 2.40 (m, 4H, CH_2CH$_3$, CH_2CH_2N), 1.60 (m, 4H, CH_2CH_2CH$_2$N), 1.29 (d, 3H, $J = 6.30$ Hz, CH_3CH), 1.05 (t, 3H, $J = 6.40$ Hz, CH_3CH$_2$). MS-MALDI-TOF (dhb) 492 (MH$^+$ ^{37}Cl), 490 (MH$^+$ ^{35}Cl), 414, 341, 297, 292, 283, 243, 199. Found: C, 66.05; H, 6.78; N, 8.49. $C_{27}H_{32}N_3FeCl$. Anal. Calc.: C, 66.19; H,6.54; N, 8.58.

4.1.9. 7-chloro-4-N-[(4-N′-ethyl-N′-ferrocenylmethyl)-ammonio-1-methylbutylamino]quinolin-1-ium bi-tartrate (**2**)

A solution of L-(+)-tartaric acid (300 mg; 2 mmol) in acetone (20 ml) was added drop-wise to a solution of **2g** (490 mg; 1 mmol) in acetone at r.t. The resulting precipitate was collected by filtration and washed with Et_2O giving **2** (440 mg; 0.90 mmol; 90%). ^{1}H-NMR (D_2O) δ 8.33 (d, 1H, $J = 7.00$ Hz, H-2), 8.29 (d, 1H, $J = 9.70$ Hz, H-5), 7.94 (s, 1H, H-8), 7.72 (d, 1H, $J = 9.00$ Hz, H-6), 6.85 (d, 1H, $J = 6.60$ Hz, H-3), 4.31 (m, 4H, Cp, CHOD), 4.14 (s, 5H, Cp′), 4.49–4.10 (m, 7H, Cp, CH_2Fc, CHCH$_3$), 3.07 (m, 4H, CH_2CH$_3$, CH_2CH_2N), 1.75 (m, 4H, CH_2CH_2CH$_2$N), 1.38 (d, 3H, J = 5.70 Hz, CH_3CH), 1.30 (t, 3H, J = 6.10 Hz, CH_3CH$_2$). MS-MALDI-TOF (dhb) positive ions 492 (MH$^+$ ^{37}Cl), 490 (MH$^+$ ^{35}Cl), negative ions 149 ($C_4H_5O_6^-$). Found: C, 53.39, H, 5.48; N, 5.16. $C_{35}H_{44}N_3O_{12}FeCl$. Anal. Calc.: C, 53.20; H, 5.57; N, 5.32.

4.2. Biology

4.2.1. In vitro activity of chloroquine analogues

Three culture-adapted strains *of P. falciparum* were used: the chloroquine-resistant strain FCR3 (Gambia), the chloroquine/mefloquine-resistant strain Dd2 (Indochina) and the chloroquine-sensitive strain HB3 (Honduras). The FG1, FG3 and FG4 uncloned lineages were isolated from Gabonese individuals [25]. All stock parasite cultures were maintained using Trager and Jensen's method [26,27].

The assays were conducted in vitro using a modification of the semiautomated microdilution technique of Desjardins et al. [28] based on radiolabelled hypoxanthine. The parasites from asynchronous cultures with a majority of young trophozoites stages were treated by chloroquine, **1** and **2** for 48 h in appropriate conditions. Drug testing was carried out in 96-well microtiter plates. The compounds were dissolved in 70% methanol (5 mg ml^{-1}). They were then prediluted in complete culture medium (RPMI 1640 supplemented with 10% pooled human AB+ serum), and titrated in duplicate in serial two-fold dilutions. The final concentrations ranged from 5 to 10 000 nM ml^{-1}. After addition of the parasites from asynchronous culture with a majority of young trophozoites stages (0.5% of initial parasitaemia in a 6% erythrocyte suspension) and [H^3]hypoxanthine (0.5 μCu well^{-1}), the test plates were incubated at 37°C in an atmosphere of 5%O_2–5%CO_2–90%N_2, for 48 h. Growth of the parasites was measured in a liquid scintillation spectrometer (Beckman) by the incorpora-

tion of radiolabelled [H^3]Hypoxanthine into the nucleic acids of parasites. Fifty percent inhibitory concentrations (IC_{50}) refer to the molar concentrations of drug causing a 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.

4.2.2. In vivo antimalarial study

Chloroquine diphosphate (Sigma) and **2** were administrated in solution in PBS. Doses were calculated in chloroquine base equivalents for the two products. *P. berghei* N and *P. yoelii* NS were used for experiments. The blood schizontocidal activity of the compounds was assayed by the 4-day test [29]. Five female Swiss mice (Janvier), weighing 25 g in each group, were infected intraperitaneously at day 0 with 10^7 infected red blood cells from a donor exhibiting 20–30% parasitaemia. Infected mice received subcutaneously four single daily doses of drug (1 or 10 $mg^{-1}\ kg^{-1}\ d^{-1}$) for 4 consecutive days, or an identical volume of PBS for control mice. On the fifth day the level of parasitaemia in each animal was counted in thin films made from tail blood and stained with Giemsa stain. The percentages of growth inhibition were evaluated in comparison with control mice.

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