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Easily synthesized antimalarial ferrocene triazacyclononane quinoline conjugates

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Abstract

Starting from triazacyclononane, easily accessible ferrocenic quinoline derivatives were synthesized. Their antiplasmodial properties were investigated against chloroquine sensitive (HB3) and chloroquine-resistant (Dd2) *Plasmodium falciparum*. One of them, 7-chloro-4-[4-(7-chloro-4-quinolyl)-7-ferrocenylmethyl-1,4,7- triazacyclononan-1-yl]quinoline (**4**) showed potent antimalarial activity in vitro against the chloroquine-resistant strain Dd2 and therefore revealed to be the most promising lead from the present work for new organometallic antimalarial agents.

Keywords: Bioorganometallics; Ferroquine; Triazacyclononane; Malaria; *Plasmodium falciparum*

1. Introduction

Malaria is by far the world's most important tropical parasitic disease. It kills over a million people a year and is second only to tuberculosis in its impact on world health [1–3]. Malaria is present in 90 countries and infects one in 10 of the world's population. There are four main types of malaria, all spread via mosquitoes. The most dangerous parasite *Plasmodium falciparum* is becoming resistant to almost traditional treatments like chloroquine, CQ (Chart 1). In some areas (e.g., South-East Asia), none of the major drugs is effective in fighting malaria. And in spite of intensive efforts, no vaccine is currently available [4].

Some years ago, a new strategy, based on incorporation of a metallocenic moiety (with well-known cytotoxic properties) into a "standard" drug (which enables to vectorize the drug to the selected target), was developed [5–7]. The resulting compounds offer new possibilities in reversal of resistance and therapeutic application [8,9].

In this exciting context of bioorganometallic chemistry, the rationale of our approach was to synthesize new, rapidly accessible drugs based not only on the structure–activity relationship proposed for CQ [10] but also on the discovery of ferroquine (FQ, Chart 1) [6,11] and related analogs [7,12,13]. That means: (1) a 4-aminoquinoline moiety for vacuolar accumulation through pH trapping and haematin association, (2) a side chain including a basic amino group for strong antiplasmodial activity, (3) a ferrocenyl group for its lipophilic and redox properties and (4) few steps with easily performed chemical reactions. Taking into account these ideas, we reasoned that the use of a ferrocenic-derived TACN would rapidly lead to bisquinoline with potent antimalarial activity, since bisquinolines have already been found to be active on *P. falciparum* [14–16].

Abbreviations: TACN 1,4,7-triazacyclononane; TEA, triethylamine.

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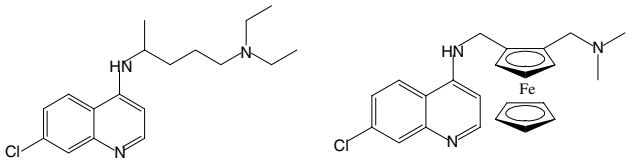


Chart 1.

2. Experimental

2.1. Chemistry

Melting points were uncorrected. The ^{1}H NMR spectra were recorded on a Brucker AC 300 spectrometer using tetramethylsilane (TMS) as the internal standard and $CDCl_3$ as the solvent. MS MALDI TOF spectra were obtained using a Vision 2000 TOF instrument (Finnigan MAT, Bremen, Germany) equipped with a nitrogen laser operating at 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 2,5-dihydroxybenzoic acid (dhb). Merck's Kieselgel 60 PF254 was used for the chromatography. Analytical HPLC was performed on a Shimadzu system equipped with a UV detector set at 254 nm. Compounds were dissolved in EtOH and injected through a 50-μL loop on a Macherey-Nagel C_{18} Nucleosil column (4 × 300 mm, 5 μm, 100 Å). The following eluent system was used: A (H_2O/TFA, 99.95:0.05) and B (CH_3CN/H_2O/TFA, 79.95:20:0.05). Conditions: a linear gradient run from 100% A during 5 min, then to 100% B over the next 30 min.

2.1.1. 7-Chloro-4-[4-(7-chloro-4-quinolyl)-7-ferrocenylmethyl-1,4,7-triazacyclononan-1-yl]quinoline (4) and 7-chloro-4-(4,7-diferrocenylmethyl-1,4,7-triazacyclo-nonan-1-yl)quinoline (5)

A two-steps procedure was used: first, a solution of TACN · 3HCl (1 g, 4.2 mM) dissolved in deoxygenated water (20 ml) is added drop-wise to a mixture of *N*,*N*,*N*-trimethyl(ferrocenylmethyl)ammonium iodide (**1**) (539 mg, 1.4 mM) and K_2CO_3 (966 mg, 7 mM) under nitrogen atmosphere at room temperature. The solution was then refluxed at 100 °C for 3 h. The products (**2** and **3**) were extracted with Et_2O (2 × 50 ml) and dried over Na_2SO_4. After evaporation of the solvent, a crude orange oil (275 mg) was obtained. The ^{1}H NMR spectrum was similar to this previously reported by Poggi et al. (for more information, see Section 3).

Then, the mixture of **2** and **3** (60 mg, 20 mM), 4,7-dichloroquinoline (198 mg, 1 mM) and K_2CO_3 (70 mg, 0.5 mM) were heated in *N*-methyl-2-pyrrolidinone (5 ml) at 135 °C under in nitrogen atmosphere for 4 h. After cooling to r.t., the reaction mixture was diluted with Et_2O (50 ml), washed with brine (10 × 50 ml) and extracted with a solution of citric acid (5%, 50 ml). The organic layer were then dried over Na_2SO_4, and evaporated under reduced pressure. The resulting oil was purified by column chromatography (elution with 9:1 EtOAc/triethylamine) to give **4** (yellow oil, 50 mg, 0.08 mM, 40%) and **5** (yellow oil, 5 mg, 0.02 mM, 7%). Compound **4**: ^{1}H NMR (δ ppm, $CDCl_3$) 8.58 (d, $J = 5.34$ Hz, 2H), 8.01 (d, $J = 2.23$ Hz, 2H), 8.01 (d, $J = 9.00$ Hz, 2H), 7.27 (dd, $J = 9.12$ and 2.20 Hz, 2H), 6.72 (d, $J = 5.41$ Hz, 2H), 4.08 (m, 7H), 4.06 (m, 2H), 3.97 (brs, 4H), 3.63 (m, 4H), 3.50 (s, 2H), 2.95 (m, 4H). MS MALDI TOF (dhb) 654 ($MH^{\cdot+\,37/37}Cl$), 652 ($MH^{\cdot+35/37}Cl$), 650 ($MH^{\cdot+35/35}Cl$). Anal. Calc. for $C_{35}H_{33}N_5Cl_2Fe$: C, 64.61; H, 5.08; N, 10.77. Found: C, 64.82; H, 5.39; N, 10.53%. Compound **5**: ^{1}H NMR (δ ppm, $CDCl_3$) 8.49 (d, $J = 5.46$ Hz, 1H), 8.05 (m, 1H), 7.95 (d, $J = 2.20$ Hz, 1H), 7.27 (dd, $J = 9.09$ and 2.25 Hz, 1H), 6.61 (d, $J = 5.51$ Hz, 1H), 4.09 (m, 4H), 4.07 (m, 10H), 4.05 (m, 4H), 3.65 (m, 4H), 3.50 (s, 4H), 2.86 (m, 4H), 2.63 (m, 4H). MS MALDI TOF (dhb) 689 ($MH^{\cdot+\,37}Cl$), 687 ($MH^{\cdot\,35}Cl$). Anal. Calc. for $C_{37}H39N_4ClFe_2$: C, 64.68; H, 5.68; N, 8.16. Found: C, 64.87;H, 5.72;N, 7.91%.

2.2. In vitro activity studies

Two culture-adapted strains of *P. falciparum* were used: the chloroquine-resistant strain Dd2 (Indochina) and the chloroquine-sensitive strain HB3 (Honduras). All stock parasite cultures were maintained using Trager and Jensen's method [17,18].

The assays were conducted in vitro using a modification of the semi-automated microdilution technique of Desjardins et al. [19] based on radiolabeled hypoxanthine incorporation in parasites. Chloroquine diphosphate was supplied by Sigma. Dihydrochloride salt of FQ was prepared according to the reported procedure [6]. CQ stock solutions were prepared at 5 mg/mL in 70% ethanol and stored at −20 °C until the assays were performed. The other compounds were dissolved in DMSO. Then, all the serial dilutions were realized in complete culture medium (RPMI 1640 supplemented with 10% pooled human A+ serum). The final concentrations ranged from 4.5 to 581.5 nM for CQ and FQ and from 9.1 to 1160 nM for **4** and **5**. The parasites from asynchronous cultures with a majority of young trophozoite stages were treated with chloroquine (CQ), ferroquine (FQ), **4** and **5** for 48 h under appropriate conditions. Parasite growth was monitored by the incorporation of radiolabeled [G-H^3]Hypoxanthine (Amersham) into the nucleic acids of the parasites measured with a liquid scintillation spectrometer (Beckman). Fifty percent inhibitory concentrations (IC_{50}) refer to molar concentrations of drug causing 50% reduction in [G-H^3]Hypoxanthine incorporation

compared to drug-free control wells. These were estimated by linear regression analysis of probability–log–dose–response curves.

3. Results and discussion

Synthesis of the compounds **4** and **5** was carried out as shown in Scheme 1. The commercially available *N*,*N*-dimethyl(ferrocenylmethyl)amine was reacted with methyl iodide in dry acetonitrile to afford *N*,*N*,*N*-trimethyl(ferrocenylmethyl)ammonium iodide (**1**) (100% yield) [20]. Treatment of **1** with TACN · 3HCl in deoxygenated water in the presence of K_2CO_3 under nitrogen atmosphere led to **2** and **3** (60% yield). Production of compound **3** was surprising since this compound including two ferrocenyl moieties was not previously reported by Poggi and co-workers [21]. Due to their high polarity, we were unable to separate these compounds by column chromatography or crystallization. Moreover, the ^{1}H NMR spectra showed no specific attribution between **2** and **3** due to overlapping signals. Nevertheless, the presence of **3** was confirmed by MS MALDI TOF ($MH^+ = 526\ g\,mol^{-1}$) and HPLC[14] (**2**: t_R, 17.3 min (86%) and **3**: t_R, 22.8 min (14%)). Condensation of this mixture with 4,7-dichloroquinoline in *N*-methyl-2-pyrrolidinone under a nitrogen atmosphere produced **4** (68%) and **5** (32%) which were isolated by column chromatography on silica gel using EtOAc:TEA (9:1) with a moderate yield (40%).

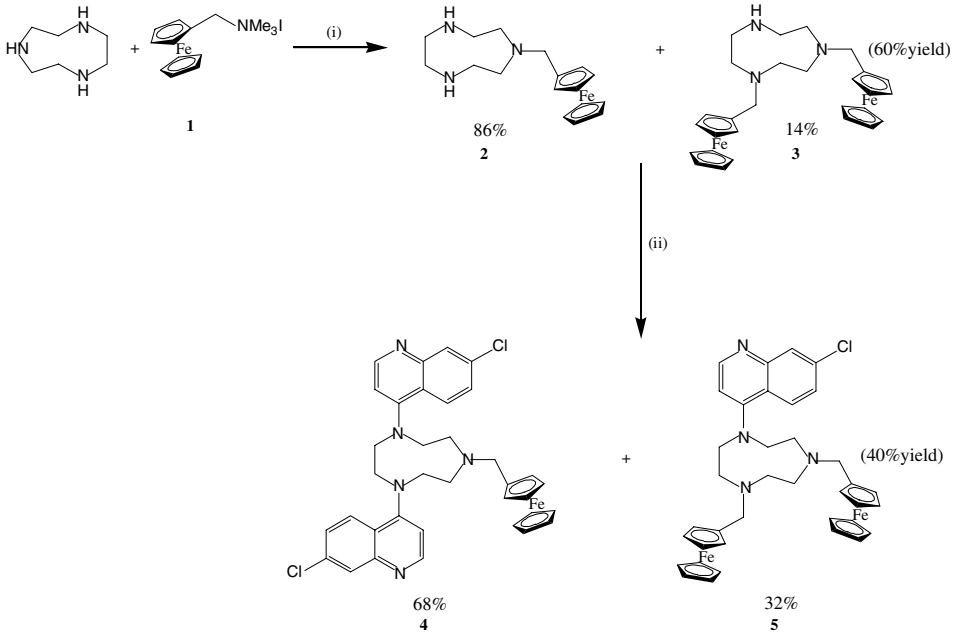
The in vitro activity of the ferrocenic compounds **4** and **5** was monitored against two *P. falciparum* strains selected for their sensitivity to chloroquine: the CQ-sensitive strain HB3 and the moderately CQ-resistant strain Dd2 (Table 1). Magnitude (expressed as log (IC_{50}CQ/IC_{50} product)) facilitated comparison of the product's efficiency towards both strains (Fig. 1). $M = 0$ indicates that the product is as effective as chloroquine, $M > 0$ that it is more effective and $M < 0$ that it is less effective.

Table 1
Mean of IC_{50} of CQ, FQ, **4** and **5** for each *P. falciparum* strains

Compounds	IC_{50} (nM) ± SD[a] of compounds[b]			
	HB3	*n*	Dd2	*n*
CQ	19 ± 9	6	94 ± 8	3
FQ	20 ± 5	6	13 ± 1	3
4	110 ± 27	6	62 ± 12	3
5	754 ± 170	6	1027 ± 95	3

[a] Values are the arithmetic mean ± standard deviation.
[b] Chloroquine (CQ) was tested as the phosphate salt, ferroquine (FQ) as the hydrochloride salt, **4** and **5** as the free bases.

Compound **4** remained more efficient on the Dd2 strain than chloroquine although this compound was less active on the CQ-sensitive strain HB3, than ferroquine. Note that the electrochemical behavior of ferrocene as a one-electron redox system and its high lipophilicity may partially explain the increased activity of compound **4**. An alternative hypothesis could also be proposed: the presence of the metallocenic moiety in compounds like **4** or FQ should lead to a reduced affinity for the putative transporter (*P. falciparum* chloroquine resistance transporter, PfCRT), conferring



Scheme 1. Reagents and conditions: (i) 5 equiv K_2CO_3, H_2O, reflux, 3 h; (ii) 5 equiv 4,7-dichloroquinoline, 2.5 equiv K_2CO_3, NMP, 135 °C, 4 h.

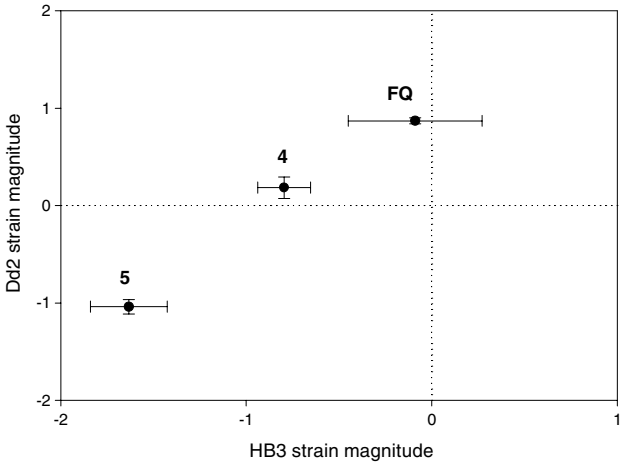


Fig. 1. Magnitude calculated for FQ, **4** and **5**.

resistance to chloroquine [22]. But other effects could also contribute to the activity of compound **4**: for example a combination of **4** and iron could generate in situ a potent inhibitor of *P. falciparum* superoxide dismutase [23]. So, it should be stressed that an end to these debates is not currently in sight due to the complexity of the subject.

Compound **5** was much less active on both strains. Although the rule-of-five established by Lipinski [24,25] invites exceptions (e.g., erythromycin), the high molecular weight (MW 688) and the high lipophilicity of **5** render it less drug-like [26] which should perturb its transport to the food vacuole of the parasite. The presence of two bulky ferrocenyl moieties in compound **5** could also destabilize the interaction (steric hindrance) of the quinoline cycle with ferriprotoporphyrin IX leading to a weaker antiparasitic activity than for compound **4**.

Magnitude analysis confirmed these results and showed that compound **4** was a little less potent than FQ, but could remain useful on CQ-resistant strains, contrary to previously tested R_0 47–7737 (WR 268,668) where a significant positive correlation between the IC_{50} values of R_0 47–7737 and CQ was noted [27]. However, the present results have to be confirmed on a larger sample of strains and isolates.

4. Conclusions

In the course of investigations aimed at developing new antimalarial organometallic derivatives, the use of 1,4,7-triazacyclononane allowed construction of the chloroquine-like bisquinoline **4** and bisferrocene **5**. Bisquinoline **4** remained more efficient on the Dd2 strain than chloroquine although this compound was less active on the CQ-sensitive strain HB3, than ferroquine. Bisferrocene **5** was much less active on both strains. These preliminary results confirmed the interest of ferrocenic bisquinolines as antimalarial agents.

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