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Synthesis of 2-ferrocenylquinoline derivatives and evaluation of their antimalarial activity

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ABSTRACT

Some quinoline-based compounds bearing a ferrocenyl unit in the 2-position of the heterocyclic system were synthesized from ferrocenyl-*o*-nitrochalcones through a simple hydrogenation/intramolecular cyclization sequence and fully characterized. The obtained ferrocenyl derivatives were evaluated *in vitro* as antimalarial agents against chloroquine-susceptible D10 and chloroquine-resistant W2 strains of *Plasmodium falciparum* and a beneficial effect of the organometallic moiety was evidenced in comparison with the phenyl-substituted analogues. All the ferrocenyl heterocycles inhibited the parasite growth in μM range and the lowest values of IC_{50} were determined for derivatives containing a dimethylamino group as additional substituent.

1. Introduction

Organometallic analogues of biologically active compounds have attracted growing interest since the presence of metals can markedly influence the physical and chemical properties of a drug leading to increased or new medicinal activity with respect to the parent molecules [1]. The high stability and lipophilicity as well as the low toxicity and the redox activity of ferrocene have made it an interesting pharmacophore for drug design and the introduction of a ferrocenyl moiety into a drug molecule has been recognized as a useful approach for the development of more effective therapeutic applications [2].

As selected examples, ferrocenyl-conjugates of commercial antiestrogen tamoxifen [3] and antiandrogen nilutamide [4] exhibited higher cytotoxicity on breast or prostate cancer cells with respect to the reference drugs while ferroquine, the ferrocenyl derivative of antimalarial drug chloroquine, is currently at the phase II clinical trial stage as the best promise against the chloroquine-resistant strains of *Plasmodium falciparum* [5].

Since quinolines and structurally related heterocycles display a large spectrum of biological activities [6] exploitable in the development of new therapies for different human diseases [7] we were interested in the preparation of quinoline-based ferrocenyl derivatives as a potentially useful class of bioactive organometallics. Several quinolines bearing in the side chain a ferrocene unit linked to the heterocycle through different spacers have been reported and many of them have showed interesting antimalarial [8] or antitubercular [9] activities. Derivatives in which the metallocene is directly connected to the heteroaromatic ring have been less investigated [10] and, in this context, herein we describe the preparation and characterization of some 2-ferrocenylquinoline derivatives with structures **1**–**3**. As a first evaluation of their biological activity, the obtained ferrocenes were tested as antimalarial agents against two selected strains of *P. falciparum* parasite.

2. Results and discussion

Some ferrocenylquinolines have been prepared by direct coupling of ferrocenyllithium with azaheterocycles or condensation of suitable ferrocenes with carbonyl compounds [11] whereas the corresponding 1,2,3,4-tetrahydroquinolines have not been reported up to date. In order to prepare some representative examples of such ferrocenyl derivatives we resorted on the reductive cyclization of *o*-nitrochalcones by hydrogenation over Pd/C (Scheme 1) for the mild conditions required and the possibility to selectively drive the reaction outcome toward the formation of 2-substituted quinolines or 1,2,3,4-tetrahydroquinolines by simple variation of the solvent and hydrogen source [12].

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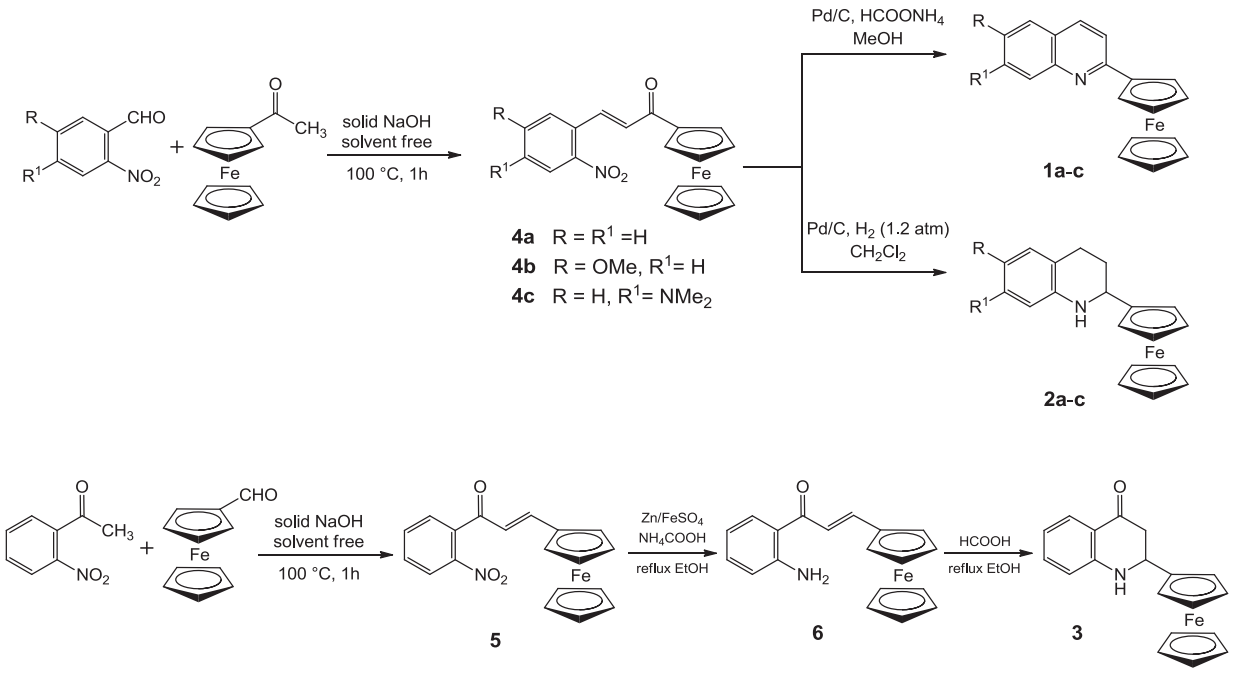

Scheme 1. Synthesis of 2-ferrocenylquinoline derivatives.

The Claisen–Schmidt condensation of acetylferrocene and the suitable 2-nitrobenzaldehyde in the presence of solid NaOH was carried out without any solvent at 100 °C to give the expected ferrocenyl-*o*-nitrochalcones **4a**–**c** as deep-red solids in 68–74% isolated yield after chromatographic purification. The same procedure was also applied to the condensation of 2-nitroacetophenone with ferrocene-carboxaldehyde to give chalcone **5** in which the carbonyl group is shifted near the phenyl ring with respect to the isomeric compound **4a**. In comparison with the reported synthesis of **4a** and **5a** using KOH in refluxing EtOH [13] our solvent-free method provided higher yields of the target chalcones (68% *vs*. 28% for **5**) and in shorter reaction times.

For all the ferrocenyl chalcones **4a**–**c** and **5** the exclusive formation of the *trans*-isomers was deduced from their ^{1}H NMR spectra that displayed a large coupling constant ($J = 15.6$ Hz) for the doublets of the ethylenic protons. In consequence of the electron-withdrawing effect exerted by the nitro-substituted phenyl ring the resonances for H-3 ethylenic protons of **4a**–**c** were found at more than 1 ppm downfield with respect to the H-2 counterpart whereas the corresponding signals in the spectrum of **5** are closer together and both shifted upfield.

Ferrocenyl-*o*-nitrochalcones **4a**–**c** were subjected to hydrogenation reactions in MeOH in the presence of Pd/C catalysts and ammonium formate as hydrogen source to afford the corresponding 2-ferrocenylquinolines **1a**–**c** in moderate to satisfactory yields. In analogy with reported data for other reducing systems [14], the substrates were smoothly converted into the corresponding aminochalcones that *in situ* underwent the subsequent intramolecular Friedländer condensation.

On the contrary, the use of Pd/C and gaseous hydrogen as reducing system in CH_2Cl_2 allowed to selectively achieve the 2-ferrocenyl-1,2,3,4-tetrahydroquinolines **2a**–**c**, such reaction outcome being in agreement with a preferential hydrogenation of the ethylenic bonds with respect to the reduction of the nitro-groups present in the substrates or before the cyclization step.

For the preparation of quinolone derivative **3** the cyclization of **5** through aminochalcone **6** as suitable intermediate substrate for intramolecular Michael addition was considered, but different reaction conditions needed to be developed in order to selectively reduce the nitrogroup of **5** saving the ethylenic bond. Hydrogenation reactions of **5** with ammonium formate in the presence of Pd/C or Ni Raney catalysts gave complex mixtures of reduced compounds (alcohols, saturated and unsaturated aminoketones) with small amounts of the target product **4** so indicating the occurrence of different concurrent reactions that could not be controlled by varying the experimental conditions. In a screening of more selective reducing systems, activated iron generated *in situ* by Zn/$FeSO_4$ [15] allowed us to obtain aminochalcone **6** in nearly quantitative yield. Compound **6** was then subjected to cyclization reaction by refluxing in 10% HCOOH/EtOH to give the quinolone **3** in 86% overall yield.

The Zn/$FeSO_4$ system could be effectively applied also for the preparation of 2-ferrocenylquinolines and higher yields with respect to the hydrogenation/cyclization reaction with Pd/C and NH_4COOH were obtained for derivative **1c** from nitrochalcone **4c** (67% *vs*. 39%).

In the course of the continuous and intense search for new effective antimalarials aimed to overcome the emerging resistance of *Plasmodium* parasites against the most used drugs [16] as quinine, chloroquine or artemisinin, a large chemical diversity has been explored and novel pharmacophores have been identified [17]. However, quinolines continue to be an important source of antimalarial agents and many active compounds have been prepared by chemical modifications of the heterocyclic scaffold [18].

As a novel class of substituted quinolines, the obtained ferrocenes **1a**–**c**, **2a**–**c** and **3** were screened for their activity in inhibiting the growth of chloroquine-susceptible D10 and chloroquine-resistant W2 strains of *P. falciparum* using chloroquine (CQ) as reference drug. The phenyl analogues of **1a** and **2a**, i.e. 2-phenylquinoline **7** and 2-phenyl-1,2,34-tetrahydroquinoline **8**, were also tested in order to evaluate the contribution of the organometallic moiety to the overall activity. A modified parasite lactate dehydrogenase (pLDH) method as described by Makler et al. [19] was chosen as *in vitro* antiplasmodial assay.

From the results summarized in Table 1 it was shown that all the ferrocenyl derivatives gave positive growth-inhibition in μM range

Table 1
Antimalarial activity of ferrocenylquinoline derivatives.[a]

Entry	Compound	Antimalarial activity (IC_{50}, μM)	
		D10 strain (mean ± SD)	W2 strain (mean ± SD)
1	**1a**	36.3 ± 3.3	39.3 ± 1.3
2	**1b**	35.6 ± 3.2	33.5 ± 1.3
3	**1c**	14.8 ± 1.4	13.8 ± 2.4
4	**2a**	29.3 ± 4.6	28.3 ± 1.1
5	**2b**	38.5 ± 2.8	36.4 ± 3.3
6	**2c**	24.8 ± 7.1	24.1 ± 4.3
7	**3**	39.5 ± 2.1	41.5 ± 0.7
8	**7**	50.3 ± 3.2	54.0 ± 3.8
9	**8**	66.0 ± 2.8	66.8 ± 1.6
10	CQ	0.030 ± 3.2	0.398 ± 9.8

[a] Each compound was tested at doses from 1.5 μM to 100 μM. IC_{50} values correspond to means ± SD from three independent experiments and each concentration was tested in triplicate.

with comparable IC_{50} values against both the parasite strains and the best results were obtained for derivative **1c** (entry 3). In the 1,2,3,4-tetrahydroquinoline series only compound **2a** was slightly more active than its heteroaromatic counterpart (compare entries 1 and 4) and the lower IC_{50} value determined for **2c** with respect to **2a** and **2b** (entries 4–6), in parallel with data observed for quinolines, confirmed the beneficial influence of dimethylamino substituent in the 7-position of the heterocycles in hand. The replacement of the ferrocenyl substituent in the 2-position of quinoline or 1,2,3,4-tetrahydroquinoline scaffold with a phenyl group gave rise to about halved parasitic activity, so evidencing a positive effect strictly related with the presence of the organometallic moiety in the molecules (compare entries 1 and 8, 4 and 9).

3. Conclusions

Nitrochalcones deriving from a solvent-free Claisen–Schmidt condensation of 2-nitrobenzaldehydes and acetylferrocene have been shown useful starting material for the preparation of 2-ferrocenylsubstituted aza-heterocycles through a reduction-cyclization sequence. By simple variation of the reducing system the reaction outcome was selectively driven toward the formation of quinolines or tetrahydroquinolines in satisfactory yields. The obtained products were tested for their antimalarial activity on two different strains of *P. falciparum* but they were not found active against the parasite. However, contributes of ferrocenyl and dimethylamino substituents on the heterocyclic system to the overall inhibition of parasite growth were evidenced.

On the basis of such preliminary data, the design of different ferrocenylquinoline derivatives to increase their antimalarial potency and the evaluation of other biological activities are currently in progress.

4. Experimental

4.1. Materials and methods

Microprilled sodium hydroxide was purchased from Riedel-de-Haën. Palladium 10% wt on activated carbon was available from Aldrich and used as received. Column chromatography was performed on Si 60 (230–400 mesh) silica gel using the specified eluents. ^1H and ^{13}C NMR spectra were registered in $CDCl_3$ at 400.13 and 100.69 MHz respectively on a Bruker Avance™ 400 instrument. 2D-NMR experiments were performed using standard Bruker microprograms. Chemical shifts (δ) are given as ppm relative to the residual solvent peak and coupling constants (J) are in Hz. In the NMR assignments, Cp and Cp′ refer to substituted and unsubstituted cyclopentadienyl rings, respectively. UV spectra were recorded in CH_3CN on Agilent 8453 UV–visible spectrophotometer. ESI-MS spectra were acquired on Waters Micromass ZQ2000 instrument using a 20 V cone voltage and 150 °C source temperature. Elemental analyses were obtained from the Department of Pharmaceutical Sciences, University of Catania. Melting points are uncorrected.

4.2. General procedure for the synthesis of nitrochalcones

A 10-mL sealed vial charged with the suitable aldehyde (0.5 mmol) and ketone (0.5 mmol) was placed in a bath oil at 100 °C and solid NaOH (1.0 mmol) was added. The mixture was stirred vigorously and left to react until the TLC analysis showed complete disappearance of substrates (1–3 h). After addition of CH_2Cl_2 (10 mL) the mixture was partitioned with satd. NH_4Cl solution (3 × 5 mL) and the organic layer washed with brine. The organic solvent was then removed *in vacuo* and the residue purified by column chromatography on silica gel (*n*-hexane:CH_2Cl_2:AcOEt 4:1:1) to give pure chalcones that were recrystallized from EtOH.

4.2.1. 1-Ferrocenyl-3-(2-nitro)phenyl-2-propen-1-one (*4a*)

Obtained in 68% yield from 2-nitrobenzaldehyde and acetylferrocene, compound **4a** displayed physical and spectroscopic properties in agreement with literature data [13].

4.2.2. 1-Ferrocenyl-3-(2-nitro-5-methoxy)phenyl-2-propen-1-one (*4b*)

Following the general procedure acetylferrocene and 2-nitro-5-methoxybenzaldehyde were reacted to give **4b** in 71% yield as a deep-red solid, mp = 149–150 °C; ^1H NMR: δ 3.96 (s, 3H, OMe), 4.24 (s, 5H, Cp′H), 4.61 (t, 2H, J = 1.6, CpH), 4.91 (t, 2H, J = 1.6, CpH), 6.87 (d, 1H, J = 15.6, H-2), 6.99 (dd, 1H, J = 2.4 and 9.2, ArH), 7.08 (d, 1H, J = 2.48, ArH), 8.15 (d, 1H, J = 9.2, ArH), 8.22 (d, 1H, J = 15.6, H-3); ^{13}C NMR: δ 56.0 (OMe), 69.9 (CpH), 70.2 (Cp′H), 73.1 (CpH), 79.7 (Cp), 114.0 (CH), 114.6 (CH), 127.7 (ArH), 128.2 (ArH), 134.9 (ArH), 137.4 (ArH), 141.4 (Ar), 163.3 (Ar), 192.7 (CO); UV (λ_{max} nm) 272, 318, 503; Anal. Calcd. for $C_{20}H_{17}FeNO_4$: C, 61.39; H, 4.38; N, 3.58. Found: C, 61.45; H, 4.40; N, 3.60.

4.2.3. 1-Ferrocenyl-3-(2-nitro-4-dimethylamino)phenyl-2-propen-1-one (*4c*)

Following the general procedure acetylferrocene and 2-nitro-4-dimethylaminobenzaldehyde were reacted to give **4c** in 74% yield as a deep-red solid, mp = 150–151 °C; ^1H NMR: δ 3.09 (s, 6H, NMe_2), 4.23 (s, 5H, Cp′H), 4.57 (t, 2H, J = 2.0, CpH), 4.90 (t, 2H, J = 2.0, CpH), 6.88 (dd, 1H, J = 2.8 and 8.8, ArH), 6.90 (d, 1H, J = 15.6, H-2), 7.14 (d, 1H, J = 2.8, ArH), 7.64 (d, 1H, J = 8.8, ArH), 8.03 (d, 1H, J = 15.6, H-3); ^{13}C NMR: δ 40.1 (NMe_2), 69.8 (CpH), 70.1 (Cp′H), 72.6 (CpH), 80.4 (Cp), 106.7 (CH), 115.4 (CH), 116.8 (Ar), 123.7 (ArH), 129.5 (ArH), 135.8 (ArH), 150.6 (Ar), 151.0 (Ar), 192.8 (CO); UV (λ_{max} nm) 258, 287, 379, 503; Anal. Calcd. for $C_{21}H_{20}FeN_2O_3$: C, 62.38; H, 4.99; N, 6.93. Found: C, 62.29; H, 4.97; N, 6.89.

4.2.4. 1-(2-Nitro)phenyl-3-ferrocenyl-2-propen-1-one (*5*)

Following the general procedure ferrocene carboxaldehyde and 2-nitroacetophenone were reacted to give **5** in 68% yield as a deep-red solid with physical and spectroscopic properties in agreement with literature data [13].

4.3. 2-Ferrocenylquinoline (*1a*)

To a solution of the nitrochalcone **4a** (145 mg, 0.4 mmol) in MeOH (10 mL), solid NH_4COOH (250 mg, 4.0 mmol) and Pd/C (Pd/C:substrate 1:5 w/w) were added and the suspension maintained at

65 °C under stirring for 20 h. The reaction mixture was then filtered over a short plug of Celite and the solution taken to dryness to give a residue that was purified by column chromatography on silica gel (*n*-hexane:Et_2O 9:1). Pure **1a** (88 mg, 0.28 mmol, 70% yield) was obtained as an orange solid whose characterization data were in agreement with those reported in literature [20].

4.4. 2-Ferrocenyl-6-methoxyquinoline (**1b**)

Chalcone **4b** (120 mg, 0.3 mmol) was reacted as above described to give **1b** as an orange solid (71 mg, 0.2 mmol, 68% yield), mp = 145–146 °C; ^{1}H NMR: δ 3.94 (s, 3H, OMe) 4.07 (s, 5H, Cp′H), 4.45 (t, 2H, J = 2.0, CpH), 5.04 (t, 2H, J = 2.0, CpH), 7.05 (d, 1H, J = 2.8, H-5), 7.33 (dd, 1H, J = 2.8 and 9.2, H-7), 7.56 (d, 1H, J = 8.4, H-3), 7.95–7.97 (m, 2H, H-8 and H-4); ^{13}C NMR: δ 55.5 (OMe), 67.6 (CpH), 69.5 (Cp′H), 70.1 (CpH), 84.3 (Cp), 105.4 (ArH), 119.8 (ArH), 121.7 (ArH), 127.5 (Ar), 130.4 (ArH), 134.3 (ArH), 144.3 (Ar), 156.9 (Ar), 157.1 (Ar); ESI-MS: 344.2 $(M + 1)^+$. Anal. Calcd. for $C_{20}H_{17}FeNO$: C, 69.98; H, 5.00; N, 4.08. Found: C, 69.88; H, 4.98; N, 4.00.

4.5. 2-Ferrocenyl-7-dimethylaminoquinoline (**1c**)

To a solution of **4c** (120 mg, 0.3 mmol) in EtOH (2.0 mL), H_2O (0.25 mL), $FeSO_4\cdot 7H_2O$ (225 mg, 0.8 mmol), NH_4COOH (113 mg, 1.8 mmol) and activated Zn powder (58 mg, 0.9 mmol) were sequentially added and the mixture stirred vigorously at 70 °C. After 2 h, the reaction mixture was filtered over a Celite pad and the solution concentrated under reduced pressure. The residue was partitioned between CH_2Cl_2 and satd. NH_4Cl and the organic layer washed with brine. After drying over Na_2SO_4 the organic solution was taken to dryness under vacuo and the residue purified by column chromatography (Si gel, *n*-hexane:Et_2O 9:1). Pure **1c** was obtained in 70% yield (71 mg, 0.2 mmol) as an orange solid, mp = 159–161 °C; ^{1}H NMR: δ 3.11 (s, 6H, NMe_2) 4.07 (s, 5H, Cp′H), 4.44 (br s, 2H, CpH), 5.04 (br s, 2H, CpH), 7.11 (d, 1H, J = 8.8, H-6), 7.15 (s, 1H, H-8), 7.33 (d, 1H, J = 8.4, H-3), 7.59 (d, 1H, J = 8.8, H-5), 7.87 (d, 1H, J = 8.4, H-4); ^{13}C NMR: δ 40.5 (NMe_2), 69.4 (Cp′H), 70.4 (CpH), 83.9 (Cp), 119.5 (ArH), 125.4 (ArH), 126.7 (Ar), 127.5 (ArH), 129.0 (ArH), 129.3 (ArH), 135.4 (ArH), 148.3 (Ar), 159.5 (Ar); ESI-MS: 357.3 $(M + 1)^+$. Anal. Calcd. for $C_{21}H_{20}FeN_2$: C, 70.78, H, 5.66; N, 7.87. Found: C, 70.83; H, 5.67; N, 7.91.

4.6. General procedure for the synthesis of 2-ferrocenyl-1,2,3,4-tetrahydroquinolines

The required nitrochalcone **4a**–**4c** (0.4 mmol) was dissolved in CH_2Cl_2 (10 mL) in a 100 mL flask equipped with a Teflon stopcock and Pd/C catalyst in a 1:4 w/w ratio with the substrate was added. The flask was filled with H_2 (1.2 atm) and the mixture stirred overnight at room temperature. The suspension was then filtered over a short plug of Celite and the solution taken to dryness. The residue was purified by column chromatography on silica gel (*n*-hexane:Et_2O 95:5) to give pure **2a**–**2c**.

4.6.1. 2-Ferrocenyl-1,2,3,4-tetrahydroquinoline (**2a**)

Yellow oil, 85% yield, ^{1}H NMR: δ 1.74–1.84 (m, 1H, H-3a), 2.10–2.16 (m, 1H, H-3b), 2.75 (dt, 1H, J = 4.3, 4.3 and 16.2, H-4a), 2.90 (ddd, 1H, J = 5.6, 11.2 and 16.2, H-4b), 4.12–4.14 (m, 1H, CpH), 4. 15 (s, 1H, CpH), 4.22 (br s, 6H, Cp′H and H-2), 4.24 (s, 1H, CpH), 6.58 (d, 1H, J = 7.9, H-8), 6.65 (t, 1H, J = 7.9, H-6), 6.99–7.03 (m, 2H, H-5 and H-7); ^{13}C NMR: δ 26.8 (C-4), 30.9 (C-3), 51.1 (CH), 65.7 (CpH), 66.6 (CpH), 67.5 (CpH), 67.8 (CpH), 68.3 (Cp′H), 92.8 (Cp), 113.8 (ArH), 117.0 (ArH), 121.0 (Ar), 126.8 (ArH), 129.2 (ArH), 144.5 (Ar); ESI-MS: 340.3 $(M\cdot Na)^+$. Anal. Calcd. for $C_{19}H_{19}FeN$: C, 71.92; H, 6.04, N, 4.42. Found: C, 71.85; H, 6.02; N, 4.39.

4.6.2. 2-Ferrocenyl-6-methoxy-1,2,3,4-tetrahydroquinoline (**2b**)

Yellow oil, 80% yield, ^{1}H NMR: δ 1.77–1.84 (m, 1H, H-3a), 2.09–2.14 (m, 1H, H-3b), 2.73 (dt, 1H, J = 4.4, 4.4 and 16.4, H-4a), 2.89 (ddd, 1H, J = 5.6, 11.2 and 16.4, H-4b), 3.76 (s, 3H, OMe) 4.08 (dd, 1H, J = 2.8 and 10.0), 4.14 (s, 1H, CpH), 4. 18 (s, 1H, CpH), 4.21 (s, 5H, Cp′H), 4.22 (s, 1H, CpH), 4.25 (s, 1H, CpH), 6.55 (d, 1H, J = 8.4, H-8), 6.61 (d, 1H, J = 2.8, H-5), 6.66 (dd, 1H, J = 2.8 and 8.4, H-7); ^{13}C NMR: δ 27.0 (C-4), 30.9 (C-3), 51.4 (CH), 55.8 (OMe), 65.9 (CpH), 66.5 (CpH), 67.5 (CpH), 67.7 (CpH), 68.3 (Cp′H), 92.8 (Cp), 113.0 (ArH), 114.7 (ArH), 115.0 (ArH), 122.3 (Ar), 138.9 (Ar) 151.8 (Ar); ESI-MS: 370.6 $(M\cdot Na)^+$. Anal. Calcd. for $C_{20}H_{21}FeNO$: C, 69.16; H, 6.10; N, 4.04. Found: C, 69.22; H, 6.12; N, 4.06.

4.6.3. 2-Ferrocenyl-7-dimethylamino-1,2,3,4-tetrahydroquinoline (**2c**)

Yellow oil, 72% yield, ^{1}H NMR: δ 1.73–1.81 (m, 1H, H-3a), 2.07–2.13 (m, 1H, H-3b), 2.65 (dt, 1H, J = 4.4, 4.4 and 16.0, H-4a), 2.80 (ddd, 1H, J = 5.2, 11.2 and 16.0, H-4b), 2.91 (s, 6H, NMe_2) 4.09–4.13 (m, 2H, CpH and H-2), 4.17 (s, 1H, CpH), 4.22 (br s, 6H, Cp′H and CpH), 4.26 (s, 1H, CpH), 5.99 (d, 1H, J = 2.4, H-8), 6.16 (dd, 1H, J = 2.4 and 8.0, H-6), 6.97 (d, 1H, J = 8.0, H-5); ^{13}C NMR: δ 25.9 (C-4), 31.5 (C-3), 41.1 (NMe_2), 51.3 (CH), 65.8 (CpH), 66.6 (CpH), 67.5 (CpH), 67.7 (CpH), 68.3 (Cp′H), 93.0 (Cp), 98.5 (ArH), 103.4 (ArH), 110.5 (Ar), 129.7 (ArH), 145.1 (Ar) 150.5 (Ar); ESI-MS: 383.4 $(M\cdot Na)^+$. Anal. Calcd. for $C_{21}H_{24}FeN_2$: C, 69.99; H, 6.72; N, 7.78. Found: C, 69.87; H, 6.70; N, 7.74.

4.7. 2-Ferrocenyl-1,2,3,4-tetrahydro-4-quinolone (**3**)

To a solution of chalcone **5** (145 mg, 0.4 mmol) in EtOH (2.5 mL), H_2O (0.35 mL), $FeSO_4\cdot 7H_2O$ (310 mg, 1.1 mmol), NH_4COOH (150 mg, 2.4 mmol) and activated Zn powder (78 mg, 1.2 mmol) were sequentially added and the mixture stirred vigorously at 50 °C. After 2 h, the reaction mixture was filtered over a Celite pad and the solution concentrated under reduced pressure. The residue was partitioned between CH_2Cl_2 and satd. NH_4Cl and the organic layer washed with brine. After drying over Na_2SO_4 the organic solution was taken to dryness under vacuo and the residue purified by column chromatography (Si gel, *n*-hexane:AcOEt:CH_2Cl_2 3:1:1) to give pure 1-(2-amino)phenyl-3-ferrocenyl-2-propen-1-one, **6** (129 mg, 0.39 mmol, 97% yield) as deep-red solid, mp 134–135 °C; ^{1}H NMR: δ 4.18 (s, 5H, Cp′H), 4.46 (br s, 2H, CpH), 4.59 (br s, 2H, CpH), 6.30 (br s, 2H, NH_2), 6.70 (d, 1H, J = 8.0, ArH), 7.23 (d, 1H, J = 15.6, H-2), 7.27–7.30 (m, 2H, ArH), 7.68 (d, 1H, J = 15.6, H-3), 7.81 (d, 1H, J = 8.0, ArH); ^{13}C NMR: δ 68.7 (CpH), 69.6 (Cp′H), 71.0 (CpH), 79.6 (Cp), 115.7 (ArH), 117.2 (ArH), 119.2 (Ar), 119.9 (CH), 130.7 (ArH), 133.8 (ArH), 144.5 (CH), 150.8 (Ar), 191.1 (CO); UV (λ_{max} nm) 237, 272, 318, 501. Anal. Calcd. for $C_{19}H_{17}FeNO$: C, 68.89; H, 5.18; N, 4.23. Found: C, 68.93; H, 5.21; N, 4.26.

Compound **6** was then dissolved in 10% HCOOH/EtOH (2.5 mL) and the solution maintained at 80 °C for 3 h. The solution was concentrated under reduced pressure and the residue dissolved in CH_2Cl_2. After extraction with satd. aqueous solution of $NaHCO_3$ the organic layer was washed with brine and dried over Na_2SO_4. The solution was taken to dryness and the residue purified by column chromatography (Si gel, *n*-hexane:AcOEt:CH_2Cl_2 3:1:1) to give pure **3** (115 mg, 0.35 mmol, 89% yield) as a dark yellow solid whose physical and spectroscopical properties are in agreement with reported data [10].

4.8. Biological studies

4.8.1. Parasite cultures

A chloroquine-susceptible (D10) and a chloroquine-resistant (W2) strains of *P. falciparum* were used in this study. All cultures

were sustained *in vitro* as described by Trager and Jensen [21]. Parasites were maintained at 5% haematocrit (human type A-positive red blood cells) in RPMI 1640 (Gibco-BRL; 24 mM $NaHCO_3$) medium with the addition of 10% heat-inactivated A-positive human plasma, 20 mM HEPES (Euroclone) and 2 mM glutamine (Euroclone). All cultures were placed in a humidified incubator at 37 °C in a standard 1% O_2, 5% CO_2 and 94% N_2 gas mixture. When parasitaemia exceeded 5%, subcultures were taken; the culture medium was changed every second day.

4.8.2. In vitro antiplasmodial assay

The compounds were assessed for antiplasmodial activity *in vitro* using modified parasite lactate dehydrogenase (pLDH) method as described by Makler et al. [19]. The assay was made using asynchronous cultures with parasitaemia of 1–1.5% and 1% final haematocrit. Parasitaemia was determined by light microscopy using Giemsa-stained thin smears by counting at least 1000 cells. In all the experiments standard chloroquine (Sigma) was used as control. The ferrocenylquinoline derivatives were dissolved in DMSO and further diluted with the culture medium (final DMSO concentration <1%, which is non-toxic to the parasite), while chloroquine was dissolved in sterile distilled water. Seven two-fold dilutions were carried out starting from 100 μM for the ferrocenylquinoline derivatives, while for chloroquine the initial concentration was 1938 nM for W2 strain and 193.8 nM for D10 strain. Drug solutions were placed in 96-well flat-bottom microplates (Costar) and parasite culture was added. The plates were then incubated for 72 h at 37 °C. In each plate drug-free un-parasitized erythrocytes (RBC) as blank control and parasitized erythrocytes (pRBC) as positive control were also placed in triplicate. All drug concentrations were tested in triplicate for each experiment and three independent experiments were performed for each drug tested.

4.8.3. LDH measurements

Parasite growth was determined spectrophotometrically at 650 nm by measuring the activity of the parasite lactate dehydrogenase. The pLDH activity is distinguishable from human LDH using the 3-acetyl pyridine adenine dinucleotide (APAD) as co-factor. At the end of incubation, the cultures were resuspended, and aliquots of 20 μL were removed and added to 100 μL of the Malstat reagent in a 96-well microtiter plate. The spectrophotometric assessment of pLDH activity was obtained by adding 25 μL of a solution of 1.9 mM NBT (Nitro Blue Tetrazolium) and 0.24 mM PES (Phenazine Ethosulphate) to the Malstat reagent. As APADH is formed, the NBT is reduced and forms a blue formazan product that can be measured at the spectrophotometer.

4.8.4. IC_{50} determination and dose–response curve construction

The antimalarial activity of the test compounds was expressed as 50% inhibitory concentration (IC_{50}); each IC_{50} value is the mean ± S.D. of the three separate experiments performed in triplicate. The IC_{50} values were obtained from the dose–response curves using Log-Logit method with SoftMax software (Molecular Devices).

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