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ScienceDirect
Bioorganic & Medicinal Chemistry 15 (2007) 6510–6516
Bioorganic & Medicinal Chemistry

Metallocene-based antimalarials: An exploration into the influence of the ferrocenyl moiety on in vitro antimalarial activity in chloroquine-sensitive and chloroquine-resistant strains of *Plasmodium falciparum*

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Received 10 April 2007; revised 6 July 2007; accepted 12 July 2007
Available online 1 August 2007

Abstract—To establish the role of the ferrocenyl moiety in the antiplasmodial activity of ferroquine, compounds in which this moiety is replaced by the corresponding ruthenium-based moieties were synthesized and evaluated. In both the sensitive (D10) and resistant (K1) strains of *Plasmodium falciparum*, ruthenoquine analogues showed comparable potency to ferroquine. This suggests that a probable role of the ferrocenyl fragment is to serve simply as a hydrophobic spacer group. In addition, ferroquine analogues with different aromatic substituents were synthesized and evaluated. Unexpectedly high activity for quinoline compounds lacking the 7-chloro substituent suggests the ferrocenyl moiety may have an additive and/or synergistic effect.

1. Introduction

Chloroquine (**1**) (Fig. 1) has been an effective antimalarial agent since the late 1940s. Unfortunately, in most affected areas the principal causative agent, *Plasmodium falciparum*, has developed resistance to **1** and other related quinoline antimalarials.[1] Since malaria affects between 300 and 500 million people each year and is responsible for 1.5–2.7 million fatalities,[2] the emergence of quinoline drug resistance is a major problem. To circumvent resistance, numerous aminoquinolines and aminoquinoline metal complexes[3] have been screened against resistant strains of *P. falciparum*. Of these complexes, ferroquine **2a**[4] shows the greatest promise, and clinical trials are currently in progress with this drug.

Numerous papers have been published exploring the efficacy of a variety of ferroquine analogues. The position of the ferrocenyl moiety has been altered;[5] the nature of the alkyl groups attached to the terminal tertiary amine has been explored;[4,6] the addition of a reactive secondary amine centre and the functionalisation of that centre have been carried out.[7] From all of this research it seems, in general, that the presence of a ferrocenyl moiety in the alkyl side chain of chloroquine analogues has a positive effect on the efficacy of these compounds in chloroquine resistant strains of *P. falciparum*. The primary mechanism of ferroquine has been reported to be similar to that of chloroquine,[8] in as far as binding to haem and preventing the formation of haemozoin are concerned. However, studies of ruthenoquine **2b** show accumulation of this compound in the parasitic membrane.[5] As yet, such accumulation has not been demonstrated to occur with chloroquine.[9]

In view of earlier structure–activity relationship studies in chloroquine and related 4-aminoquinolines,[10] and in order to differentiate between any toxicity associated with the ferrocenyl moiety, we have synthesized a series of organometallic chloroquine analogues containing the ferrocenyl[6,7] and ruthenocenyl[11,12] moieties. We have

Keywords: Antimalarial activity; Ferroquine; 4-Aminoquinolines; Metallocene; Bioorganometallic.
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doi:10.1016/j.bmc.2007.07.012

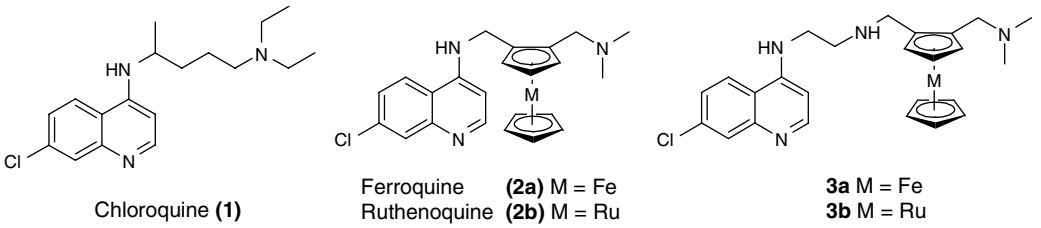


Figure 1. Chemical structures of chloroquine, ferroquine, ruthenoquine and related derivatives.

also screened the synthetic intermediates in this endeavour. It is not the intention of this preliminary study to re-examine features necessary for the antimalarial activity of 4-aminoquinolines which have already been identified from previous studies[8,10] but rather to complement these and previous studies on ferrocenyl and ruthenocenyl compounds.[4,13] From this study we hoped to ascertain whether the ferrocenyl moiety affords some synergistic or other beneficial effect. In addition, we wished to confirm that there is little difference in efficacy between ferrocenyl and ruthenocenyl analogues.[11,12] It has been observed that there is a significant difference in efficacy between ferrocifen and its ruthenocenyl analogue.[14] This has been attributed to the difference in stability of the ferrocenium and ruthenocenium ions. The relatively stable ferrocenium ion affords a significant change in the chemical reactivity of the highly conjugated ferrocifen molecule.

2. Synthesis

Ferroquine **2a** and ruthenoquine **2b** were prepared using established literature procedures.[4a,11] The final step of this synthetic strategy is shown in Scheme 1.

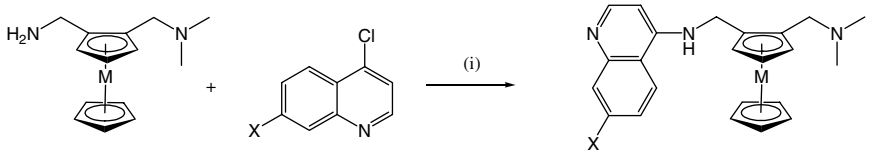
The ferroquine and ruthenoquine analogues **4a** and **4b** lacking the chlorine on the 4-aminoquinoline were synthesized in a similar manner using 4-chloroquinoline as a starting material in the place of 4,7-dichloroquinoline. The compounds bearing the reactive secondary amine centre were synthesized using the strategy we have previously reported[7,11] from the appropriate metallocene carboxaldehyde, Scheme 2.

The aromatic 1,2-diamines were synthesized using the method described for the synthesis of *N*-(7-chloro-quinolin-4-yl)-ethane-1,2-diamine.[15] The urea derivatives **8**–**10** were then synthesized from the secondary amine, Scheme 3, by the addition of 1.2 equiv of phenyl isocyanate in dichloromethane as previously reported.[7]

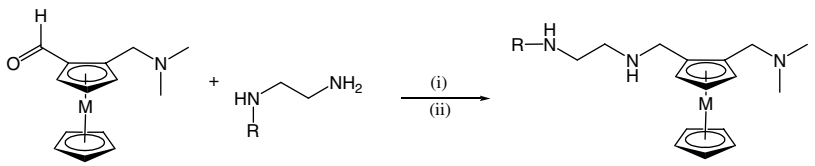
3. Results and discussion

To establish the role of the ferrocenyl moiety in ferroquine, a series of ruthenocene analogues were synthesized. To differentiate between effects consistent with structure–activity relationships previously reported for 4-aminoquinolines, and any secondary effects associated with the ferrocenyl moiety, a series of analogues including quinoline and pyridine complexes were also synthesized and screened for antiplasmodial activity. The results are presented in Tables 1 and 2.

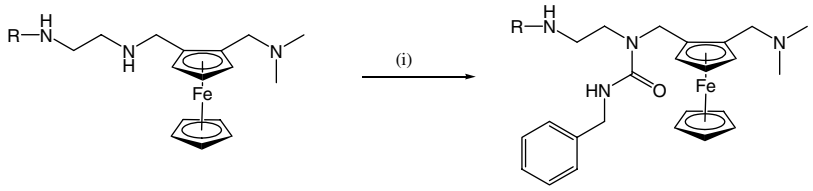
The synthetic intermediates of the ferrocene series were also screened but these showed little or no antimalarial activity. These compounds may not accumulate at the presumed site of action, the food vacuole, and some of the intermediates exhibited significantly lower stability than ferroquine. To overcome this problem, we attempted to make a series of compounds that retained the features necessary for accumulation in the parasite food vacuole but not necessarily for high antiplasmodial



Scheme 1. Reagents: (i) 5 equiv of 4-chloroquinoline or 4,7-dichloroquinoline, Et_3N, K_2CO_3 in 1-methylpyrrolidinone.



Scheme 2. Reagents: (i) MeOH; (ii) $NaBH_4$.



Scheme 3. Reagents: phenylisocyanate, dichloromethane.

Table 1. Comparative results of in vitro antiplasmodial testing of ferrocene and ruthenocene analogues conducted on chloroquine-sensitive (D10) and chloroquine-resistant (K1) strains of *P. falciparum*

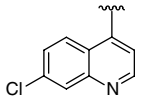
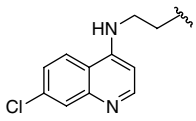
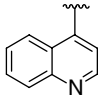
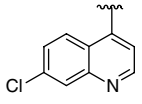
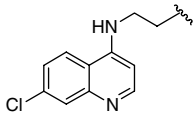
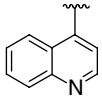
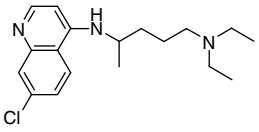
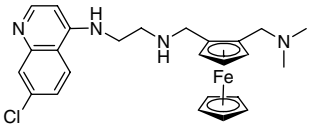
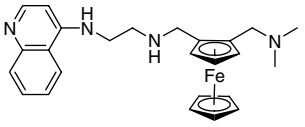
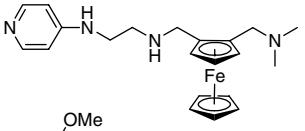
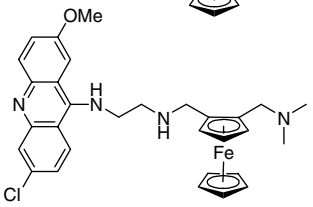
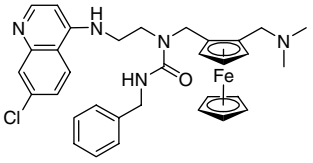
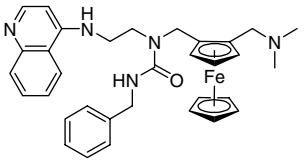
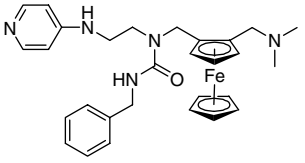
Compound No.	Compound	R	IC_{50} (nM)	
			D10	K1
1	Chloroquine	—	22.9	352.3
2a	M = Fe		17.7	13.5
3a	M = Fe		33.2	37.1
4a	M = Fe		13.5	75.9
2b	M = Ru		18.6	12.7
3b	M = Ru		20.3	19.5
4b	M = Ru		11.6	50.5

Table 2. Comparative results from in vitro antimalarial testing of ferrocene analogues conducted on chloroquine sensitive (3D7) and resistant (K1) strains of *P. falciparum*

Compound	Structure	ED_{50} (nM)	
		3D7	K1
1 (CQ)		8.52	290
3a		<200	65
5		<230	927
6		4300	38,200
7		1	8
8		7	290
9		12.1	590
10		837	21,700

activity [Tables 1 (**4a** and **4b**) and 2 (**5**, **6**, **9** and **10**)]. The ferrocene analogues without the 7-chloro group retain the 4-aminoquinoline moiety which is the minimum requirement for association with haematin, but alone is insufficient to facilitate antiplasmodial activity.[10b] The pyridine complexes possess the amine functionalities required for accumulation in the food vacuole of the parasite but do not associate with the haem.

It is evident from Tables 1 and 2 that no significant difference in antimalarial activity was observed between the ferrocene and ruthenocene analogues (Table 1). The steric bulk of ferrocene and ruthenocene is almost identical; both complexes have very similar properties, although they differ in their chemical reactivity and redox chemistry. This single comparison of course does little to establish the role of the metal in ferroquine, but it does seem to suggest that the physical characteristics of the metallocene moiety are more significant than the chemical reactivity. It is noteworthy that this is contrary to results of the ruthenocene analogue of ferrocifen.[14] In that case, the metallocene moiety is part of a highly conjugated molecule and it is postulated that the difference

in redox chemistry facilitates a difference in chemical reactivity at different sites in the molecule. This is facilitated by the relatively high stability of the ferrocenium cation in comparison with the unstable ruthenocenium cation. In ferroquine, ruthenoquine and the analogues discussed here no such conjugation exists in the molecule. Previous investigations have shown that there is no observed correlation between the redox potential of ferroquine analogues and antiplasmodial activity.[3g,7]

The pyridine analogue of ferroquine was found to be significantly less active compared to the 4-aminoquinoline counterparts. However, the ferroquine and ruthenoquine analogues lacking the 7-chloro substituent exhibited high activity in the chloroquine sensitive D10 strain of the malaria parasite. Lower activity has been observed for chloroquine[10c] analogues lacking the 7-chloro group. Since the ferroquine analogue lacking the 7-chloro group does not inhibit the formation of β-haematin[16] the metallocene in the side chain may have an additive and/or synergistic effect. The absence of the 7-chloro group in all derivatives resulted in decreased antiplasmodial activity in the chloroquine resistant K1 strain (Table 1, **4a** and **4b**). Given that ferroquine and ruthenoquine analogues lacking the 7-chloro group display higher activity against the sensitive D10 strain of malarial parasite but not for the resistant strain, this may suggest that inhibition of β-haematin formation is not the primary mechanism in the aforementioned analogues. This is in view of the fact that previous studies have shown that 7-chloro group in chloroquine is crucial in determining the ability of 4-aminoquinolines to inhibit β-haematin formation.[10c] It is also noteworthy that correlation between antimalarial activity in vitro and inhibition of β-haematin formation should only be considered in chloroquine-sensitive strains due to other factors at play in chloroquine-resistant strains that disallow an accurate comparison. Substitution with the acridine moiety of quinacrine leads to a more potent derivative (Table 2, **7**). This may be due to multiple mechanisms often associated with acridine moieties[17] including intercalation with the parasite DNA,[18] inhibition of the enzyme topoisomerase II[19] and inhibition of β-haematin.[20] We observed loose correlation (in some cases lack of correlation) between the antimalarial ED_{50} of the compounds and their ability to inhibit β-haematin formation. Nevertheless this correlation may be improved by normalising for cellular accumulation.[10b] The importance of cellular accumulation in the antimalarial activity of 4-aminoquinoline antimalarials has previously been highlighted.[21]

4. Conclusions

The ferrocene and ruthenocene analogues of ferroquine reported in this paper exhibit high antiplasmodial activity against a resistant strain of the *P. falciparum*. This observation is consistent with the hypothesis that the mechanism for drug resistance in the *Plasmodium* parasites is compound specific. It has been suggested that ferroquine has reduced affinity for the *P. falciparum* chloroquine resistance transporter (PfCRT).[8] This has previously been attributed to the lipophilicity of the ferrocenyl moiety.[8] The fact that there was no significant difference in efficacy between analogous ferrocenyl and ruthenocenyl compounds suggests that the difference in chemical behaviour of these moieties is insignificant. However, the lipophilicity and size of these moieties may be important. The increased lipophilicity may aid passage through membranes and lead to a greater affinity for haematin. However, it is noted that any correlation between the inhibition of haemazoin formation and efficacy is only relevant in chloroquine-sensitive strains of the parasite. In chloroquine-resistant strains other factors play a significant role thereby preventing accurate correlation. Whether the ferrocenyl moiety has a secondary function is not clear; nevertheless some of the results included in this paper indicate that there is an inherent antiplasmodial toxicity associated with the metallocene fragment. Further study into these compounds and their analogues may yield a greater understanding of the effect of the metallocene moiety on antiplasmodial activity. However, more detailed studies are required to confirm that the ferrocene functions as a hydrophobic group, and to establish the scope and limitations of this strategy.

5. Experimental

The syntheses were performed using standard Schlenk techniques; ferroquine **2a**,[4] compounds **3a**,[7] **2b**[11] and **3b**[11] were prepared according to literature methods. Diethyl ether and THF were distilled from Na/benzophenone, methanol was distilled from magnesium activated by iodine, 1-methylpyrrolidinone was purified by an azeotropic distillation from toluene and DMF was distilled from $CaSO_4$ (76 °C/39 mm Hg). The concentration of alkyl lithium reagents was determined by the Gilman double titration method prior to use.[22] All other chemicals were used as supplied by Aldrich. ^1H and ^{13}C NMR spectra were recorded at room temperature on Varian EM 400 or 300 MHz spectrometers. ^1H NMR spectra were recorded in $CDCl_3$ and referenced internally using the residual protons in the deuterated solvent (δ 7.27) and are reported relative to tetramethylsilane (δ 0.00). ^{13}C NMR spectra were referenced internally to the solvent resonance (δ 77.0) and are reported relative to tetramethylsilane (δ 0.0). Infrared spectra were recorded on a Perkin-Elmer Paragon 1000 FT-IR spectrometer. Melting points were performed on a Kofler hot-stage microscope (Reichert-Thermovar). Mass spectra were determined by Dr. P. Boshoff of the mass spectrometry unit at the Cape Technikon. Elemental analyses were performed using a Carlo Erba EA1108 elemental analyser in the microanalytical laboratory of the University of Cape Town. For results included in Table 1 the parasite viability was determined using the parasite lactate dehydrogenase assay according to Makler and Hinrichs.[23] For results included in Table 2 the whole cell growth inhibition assay of *P. falciparum* growth in human red blood cells was carried out in a 48 h [^3H]-hypoxanthinine incorporation assay.[24,25]

5.1. (2-Dimethylaminomethyl-ferrocenyl)-quinolin-4-yl-amine (4a)

Compound prepared in the manner described for **2a**[4a] using appropriate starting materials.

Deep orange crystals, 70%; mp 148–150 °C; R_f (silica/ethyl acetate:hexane:Et_3N, 45:50:5) 0.11; ^1H NMR (400 MHz; $CDCl_3$) δ: 8.56 (d, $^3J_{HH}$ 5, 1H), 7.94 (dd, $^4J_{HH}$ 1 and $^3J_{HH}$ 8, 1H), 7.70 (dd, $^4J_{HH}$ 1 and $^3J_{HH}$ 8, 1H), 7.58 (m,1H), 7.33 (m, 1H), 6.48 (d, $^3J_{HH}$ 5, 1H), 4.38 (d, $^2J_{HH}$ 13, 1H), 4.27 (m, 1H), 4.20–4.14 (m, 2H), 4.13 (s, 5H), 4.07 (t, $^3J_{HHH}$ 3, 1H), 3.79 (d, $^2J_{HH}$ 13, 1H), 2.89 (d, $^2J_{HH}$ 13, 1H), 2.22 (s, 6H); ^{13}C (100.6 MHz; $CDCl_3$) δ: 151.2, 150.4 (^{IV}C), 148.8 (^{IV}C), 129.7, 128.9, 124.2, 120.7, 119.6 (^{IV}C), 98.7, 84.4 (^{IV}C), 84.2 (^{IV}C), 71.5, 70.6, 69.3 (5C), 66.0, 58.2, 45.1 (2C), 42.5; IR (KBr) ν_{max} 3185br m, 1617m, 1572vs, 1540s, 1467s, 1117s, 1103m, 1005m, 838m, 822s, 497m, 480s; HRMS (EI) *m/z* 399.14058 (M^+ requires $C_{23}H_{25}N_3Fe$, 399.13979), 354.1, 255.1, 121.0, 91.1, 58.1; found: C, 69.18; H, 6.17; N, 10.10 (calcd for $C_{23}H_{24}N_3ClFe$: C, 69.18; H, 6.31; N, 10.52%.).

5.2. (Quinolin-4-yl)-(2-dimethylaminomethyl-ruthenocen-1-ylmethyl)-amine (4b)

Analogous procedure to **2b**.[11]

White crystals, 52%; mp 153–154 °C (recryst from acetonitrile and diethyl ether); δ_H (400 MHz; $CDCl_3$) 8.54 (d $^3J_{HH}$ 5, 1H), 7.94 (d, $^3J_{HH}$ 8, 1H), 7.71 (d, $^3J_{HH}$ 8, 1H), 7.58 (t, $^3J_{HH}$ 8, 1H), 7.35 (t, $^3J_{HH}$ 8 Hz, 1H), 7.31 (t br, NH $^3J_{HH}$ 7, 1H), 6.43 (d, $^3J_{HH}$ 5, 1H), 4.07 (m, 1H), 4.60 (m, 1H) 4.55 (s, 5H), 4.44 (m, 1H), 4.00–4.16 (m, 2H), 3.55 (d, $^2J_{HH}$ 13, 1H), 2.77 (d, $^2J_{HH}$ 13, 1H), 2.56 (s, 6H); δ_C (100 MHz; $CDCl_3$) 151.2, 150.1, 148.7 (C^{IV}), 129.7, 128.7, 124.1, 120.3, 119.4 (C^{IV}), 98.7, 88.4, 87.9 (C^{IV}), 73.7, 72.8 (5C), 68.6, 57.9, 44.9 (2C), 41.8; *m/z* (FAB) 446 (M + H), 401 (72), 302 (27), 286 (14), 245 (26), 167 (28), 145 (24); found C, 62.04; H, 5.67; N, 9.63 (calcd for $RuC_{23}H_{24}N_3$: C, 62.14; H, 5.67; N, 9.45%).

5.3. *N*-(2-Dimethylaminomethyl-ferrocenyl)-*N*-quinolin-4-yl-ethane-1,2-diamine (5)

Compound prepared in the manner described for **3a**[7] using appropriate starting materials. Yellow crystals, 60%; mp 128–129 °C; R_f (silica/CH_2Cl_2:MeOH:Et_3N, 80:20:1) 0.22; δ_H (400 MHz; $CDCl_3$) 8.46 (d, $^3J_{HH}$ 5, 1H), 7.90 (d, $^3J_{HH}$ 8, 1H), 7.79 (d, $^3J_{HH}$ 8, 1H), 7.52 (t, $^3J_{HHH}$ 7, 1H), 7.30 (t, $^3J_{HH}$ 8, 1H), 6.29 (d, $^3J_{HH}$ 5, 1H), 6.14 (s, 1H), 4.05 (m, 1H), 4.00 (m, 1H), 3.94 (s, 6H), 3.75 (d, $^2J_{HH}$ 13, 1H), 3.57 (d, $^2J_{HH}$ 12, 1H), 3.31 (d, $^2J_{HH}$ 13, 1H), 3.26–3.12 (m, 2H), 2.87–2.77 (m, 2H), 2.69 (d, $^2J_{HH}$ 12, 1H), 1.93 (s, 6H); δ_C (100.6 MHz; $CDCl_3$) 151.1, 150.1 (^{IV}C), 148.6 (^{IV}C), 129.7, 128.9, 124.5, 120.3, 119.2 (^{IV}C), 98.8, 85.7 (^{IV}C), 83.9 (^{IV}C), 71.1, 70.1, 69.0 (5C), 66.0, 58.2, 47.4, 46.6, 45.0 (2C), 42.2 (5′); IR (KBr) ν_{max} 3235br m, 1619m, 1577vs, 1550s, 1460s, 1104s, 1000m, 828m, 807s, 490m; HRMS (EI) *m/z* 442.18181 (M^+ $C_{25}H_{30}N_4Fe$ requires 442.18199), 255.0, 240.0, 91.0, 58.1; found: C, 69.19; H, 6.84; N, 12.33 (calcd for $C_{23}H_{24}N_3ClFe$, C, 67.88; H, 6.84; N, 12.66%).

5.4. *N*-(2-Dimethylaminomethyl-ferrocenyl)-*N*-pyridin-4-yl-ethane-1,2-diamine (6)

The compound was prepared in the same manner described for **3a**[7] using appropriate starting materials. An additional 1 mol equiv of triethylamine was added as 4-chloropyridine hydrochloride was used. Deep red oil; Yield: 515 mg (60%); R_f (silica/CH_2Cl_2:MeOH:Et_3N, 80:20:1) 0.22; δ_H (400 MHz; $CDCl_3$) 8.10 (d, $^3J_{HH}$ 5, 2H), 6.40 (d, $^3J_{HH}$ 6, 2H), 4.12 (m, 1H), 4.07 (m, 1H), 4.00 (s, 5H), 3.98 (m, 1H), 3.86 (d, $^2J_{HH}$ 13, 1H), 3.66 (d, $^2J_{HH}$ 12, 1H), 3.37 (d, $^2J_{HH}$ 13, 1H), 3.24–3.10 (m, 2H), 2.79–2.72 (m, 3H), 2.04 (s, 6H); δ_C (100.6 MHz; $CDCl_3$) 153.6 (^{IV}C), 149.9 (2C),107.6 (2C), 84.1 (^{IV}C), 83.8 (^{IV}C), 71.3, 70.4, 69.2 (5C), 66.2, 58.2, 47.3, 46.6, 44.6 (2C), 41.5 (4′); IR (thin film) ν_{max} 3251br m, 1605vs, 1466m, 1104m (ferrocene), 811s (ferrocene); HRMS (EI) *m/z* 392.16500 (M^+requires $C_{21}H_{28}N_4Fe$, 392.16634), 255.1, 240.0, 163.0, 121.0, 91.0, 58.1; found: C, 64.10; H, 7.20; N, 14.23 (calcd for $C_{21}H_{28}N_4Fe$, C, 64.29; H, 7.19; N, 14.28%).

5.5. *N*-(6-Chloro-2-methoxy-acridine-9-yl)-*N*′-[2-(*N*″,*N*″-dimethylaminomethyl)ferrocenylmethyl]-ethane-1,2-diamine (7)

Prepared in the same manner as that described for **3a**[7] using appropriate starting materials.

Deep orange crystals, 74%; mp 158–159 °C; R_f (silica/CH_2Cl_2:MeOH:Et_3N, 80:20:1) 0.25; δ_H (400 MHz; $CDCl_3$) 8.14 (d, $^3J_{HH}$ 9, 1H), 8.05 (d, $^4J_{HH}$ 2, 1H), 7.99 (d, $^3J_{HH}$ 10, 1H), 7.41–7.37 (m, 2H), 7.28 (dd, $^4J_{HH}$ 2 and $^3J_{HH}$ 9, 1H), 4.13–4.12 (m, 2H), 4.05 (s, 5H), 4.04–4.03 (m, 1H), 3.91 (d, $^2J_{HH}$ 13, 1H), 3.90 (s, 3H), 3.79–3.67 (m, 2H), 3.73 (d, $^2J_{HH}$ 12, 1H), 3.42 (d, $^2J_{HH}$ 13, 1H), 2.87–2.84 (m, 2H), 2.83 (d, $^2J_{HH}$ 12, 1H), 2.11 (s, 6H); δ_C (100.6 MHz; $CDCl_3$) 131.4, 128.3, 124.9, 124.6, 124.3, 99.8, 71.3, 70.1, 69.1 (5C), 66.0, 58.4, 55.7, 48.9, 48.4, 47.3, 46.4 (2C); IR (KBr) ν_{max} 3304br m, 1631s, 1606m, 1561s, 1519m, 1467s, 1104m, 1030m, 1001m, 488m; HRMS (EI) *m/z* 556.1685 (M^+ $C_{30}H_{33}N_4OClFe$ requires 556.4647), 511.1, 446.1, 255.1, 240.0, 121.0, 91.1, 58.1, 55.9; found: C, 64.10; H, 7.20; N, 14.23 (calcd for $C_{21}H_{28}N_4Fe$, C, 64.29; H, 7.19; N, 14.28%).

5.6. 3-Benzyl-1-[2-(7-chloro-quinolin-4-ylamino)-ethyl]-1-[2-(*N*″,*N*″-dimethylaminomethyl)-ferrocenylmethyl]urea (8)

Compound **3a** (1 equiv) was dissolved in anhydrous dichloromethane (10 mL). The phenyl isocyanate (1.2 equiv) was added and reaction vessel placed on a shaker at 200 rpm for 3 h at 25 °C. The product was then purified by silica gel chromatography eluting with 10% methanol in dichloromethane. The product **8** was isolated as a yellow crystalline solid. Yellow crystals,

90%; mp 95–96 °C; R_f (silica/CH_2Cl_2:MeOH, 80:20) 0.51; δ_H (400 MHz; $CDCl_3$) 8.45 (d, $^3J_{HH}$ 6, 1H), 8.12 (t, $^3J_{HH}$ 6, 1H), 7.96 (d, $^4J_{HH}$ 2, 1H), 7.75 (d, $^3J_{HH}$ 9, 1H), 7.15 (dd, $^4J_{HH}$ 2 and $^3J_{HH}$ 9, 1H), 7.13–7.07 (m, 5H), 6.32 (d, $^3J_{HH}$ 6, 1H), 4.24–4.16 (m, 7H), 4.08 (s, 5H), 3.82 (d, $^2J_{HH}$ 13, 1H), 3.73–3.68 (m, 2H), 3.58–3.40 (m, 2H), 2.82 (d, $^2J_{HH}$ 13, 1H), 1.98 (s, 6H); δ_C (100.6 MHz; $CDCl_3$) 160.4 (^{IV}C), 151.7 (^{IV}C), 149.7, 146.7 (^{IV}C), 140.2 (^{IV}C), 135.7 (^{IV}C), 128.2 (2C), 126.6, 126.5 (2C), 126.2, 125.7, 123.1, 116.9 (^{IV}C), 97.5, 83.9 (^{IV}C), 70.6, 69.5 (5C), 69.0, 67.7, 57.8, 47.0, 45.6, 44.6, 44.4 (2C), 43.7 (5'); IR (KBr) ν_{max} 3268br w (NH), 1611s, 1582vs, 1539s, 1452m, 1332m, 1105m, 1005m, 842m, 808m, 487m; HRMS (FAB) *m/z* 610.2030 (M+H $C_{33}H_{36}N_5ClOFe$ requires 610.2034), 565.1, 432.2, 409.1, 255.1, 191.0, 154.0, 134.1,91.1; found: C, 65.01; H, 5.83; N, 11.50. (calcd for $C_{23}H_{24}N_3ClFe$, C, 64.98; H, 5.95; N, 11.48%).

5.7. 3-Benzyl-1-(2-dimethylaminomethyl-ferrocenyl)-1-[2-(quinolin-4-ylamino)-ethyl]-urea (9)

Compound prepared in the manner described for **8**[7] using appropriate starting materials. Bright yellow crystal, 80%; mp 88–89 °C; R_f (silica/CH_2Cl_2:MeOH, 80:20) 0.47; δ_H (300 MHz; $CDCl_3$) 8.52 (d, $^3J_{HH}$ 5, 1H), 8.03 (t, $^3J_{HH}$ 5, 1H), 7.96 (d, $^3J_{HH}$ 8, 1H), 7.82 (d, $^3J_{HH}$ 8, 1H), 7.63–7.52 (m, 1H), 7.27 (t, $^3J_{HHH}$ 7, 1H), 7.14–7.09 (m, 5H), 6.34 (d, $^3J_{HH}$ 5, 1H), 4.49–4.16 (m, 7H), 4.12 (t, $^3J_{HH}$ 2, 1H), 4.06 (s, 5H), 4.05–4.03 (m, 1H), 3.76 (d, $^2J_{HH}$ 13, 1H), 3.71–3.63 (m, 2H), 3.58–3.37 (m, 2H), 2.74 (d, $^2J_{HH}$ 13, 1H), 1.94 (s, 6H); δ_C (75.5 MHz; $CDCl_3$) 160.3 (^{IV}C), 150.9 (^{IV}C), 150.2, 147.6 (^{IV}C), 140.3 (^{IV}C), 129.0, 128.6, 128.2 (2C), 126.6 (2C), 126.5, 124.7, 121.1, 118.8 (^{IV}C), 97.4, 84.0 (^{IV}C), 82.0 (^{IV}C), 70.4, 69.4 (5C), 68.8, 67.4, 57.9, 47.0, 45.4, 44.7 (2C), 44.6, 43.6 (5′); IR (KBr) ν_{max} 3280br m, 1618s, 1584vs, 1538s, 1456m, 1004m, 810m, 487w; HRMS (EI) *m/z* 575.23467 (M^+ $C_{33}H_{37}N_5OFe$ requires 575.23467), 255.1, 240.0, 121.0, 91.0, 58.1; found: C, 69.01; H, 6.50; N, 12.04 (calcd for $C_{33}H_{37}N_5OFe$, C, 68.86; H, 6.48; N, 12.16%).

5.8. 3-Benzyl-1-(2-dimethylaminomethyl-ferrocenyl)-1-[2-(pyridine-4-ylamino)-ethyl]-urea (10)

Prepared in the same manner described for**8**[7] using appropriate starting materials. Bright yellow crystals, 71%; mp 70–72 °C; R_f (silica/CH_2Cl_2:MeOH, 80:20) 0.05; δ_H (300 MHz; $CDCl_3$) 8.13 (d, $^3J_{HH}$ 6, 2H), 7.68 (br s, 1H), 7.25–7.08 (m, 5H), 6.40 (d, $^3J_{HH}$ 6, 2H), 6.28 (br s, 1H), 4.43 (d, $^2J_{HH}$ 16, 1H), 4.35–4.32 (m, 1H), 4.23 (d, $^2J_{HH}$ 16, 1H), 4.23–4.25 (m, 1H), 4.13 (t, $^3J_{HH}$ 3, 1H), 4.06 (s, 5H), 4.00–3.89 (m, 2H), 3.78 (d, $^2J_{HH}$ 13, 1H), 3.60–3.50 (m, 2H), 3.40–3.20 (m, 2H), 2.76 (d, $^2J_{HH}$ 13, 1H), 1.96 (s, 6H); δ_C (75.5 MHz; $CDCl_3$) 159.7 (^{IV}C), 154.2 (^{IV}C), 148.3 (2C), 140.4 (^{IV}C), 128.3 (2C), 126.7 (2C), 126.6, 107.2, 84.2 (^{IV}C), 82.1 (^{IV}C), 70.5, 69.4 (5C), 69.0, 67.4, 57.9, 47.2, 45.6, 44.7 (2C), 44.6, 42.6; IR (KBr) ν_{max} 3263m, 1604vs, 1455m, 1105m, 813m, 487m; HRMS (EI) *m/z* 525.21853 (M^+ requires $C_{29}H_{35}N_5OFe$, 525.21910), 255.1, 240.0, 91.1, 58.1.

Acknowledgments

We thank Johnson-Matthey for the loan of ruthenium trichloride, the Claude Harris Leon Foundation, the University of Cape Town and the National Research Foundation (South Africa) for financial support. We thank Pete Smith, Cailean Clarkson and Donelly van Schalkwyk (Division of Pharmacology, University of Cape Town) for preliminary antimalarial studies. H.K. and S.L.C. received financial support from the UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases.

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