Inhibition of trypanothione reductase and glutathione reductase by ferrocenic 4-aminoquinoline ureas

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In honour of Prof Torbjorn Norin on the occasion of his 75th anniversary

Abstract

New ferrocenic 4-aminoquinoline urea compounds have been tested for inhibition of trypanothione reductase (TryR) and human glutathione reductase (GR). Several compounds were also tested *in vitro* against *Leishmania donovani, Trypanosoma cruzi* and *Trypanosoma brucei rhodesiense.* All compounds showed significant improvement in inhibition of TryR relative to chloroquine with the best compound showing an IC_{50} value of 2.4μM (Chloroquine IC_{50} = 47.6 μM).

Keywords: Ferroquine, 7-chloroquinoline, urea, *in vitro*, trypanothione reductase

Introduction

The causative agents of Chagas' disease (*Trypanosoma cruzi*), African sleeping sickness (*Trypanosoma brucei spp.*) and Leishmaniasis (*Leishmania spp.*) are all parasitic protozoa of the order Kinetoplastida.[1] It has been established that trypanothione (T[SH]$_2$ or N^1,N^8-bis(glutathionyl)spermidine) is crucial for the maintenance of an intracellular redox balance of all of the parasitic protozoa of this order. As this mechanism is absent in host cells, the key enzyme in this process, trypanothione reductase (TryR) is an ideal target for antiprotozoal drugs.[2] In mammals, the analogous process is mediated by glutathione and the key enzyme is glutathione reductase. Whilst TryR and glutathione reductase carry out related functions, the difference in the structure of the respective active sites of these enzymes affords specificity for their respective substrates.[3] Hence, selective inhibition of TryR is possible as has been demonstrated in numerous studies.[4] As TryR is common to the parasitic protozoa of the order

Kinetoplastida, the possibility of a common therapeutic agent effective against multiple parasites is potentially possible.[5]

Some known antimalarial agents, for example, the tricyclic quinacrine, have shown efficacy against the causative agents of Chagas' disease, African Sleeping Sickness and Leishmaniasis.[6,7] To date, the focus in the context of TryR inhibitors has been primarily on tricyclics as this moiety interacts with the polyamine hydrophobic binding site in the active site of TryR.[8] The co-crystal structure of quinacrine mustard in complex with TryR has clearly shown this interaction.[9] It has been established that the chloro, methoxy and alkylamino groups present in quinacrine are all involved in the binding of this tricyclic to the active site of TryR.[4d] The methoxy group has been found to be within hydrogen bonding distance of the side chain oxygen of Ser109 and has a kinetic influence on the binding of the compound to the enzyme. The chloro group is in the vicinity of the ring nitrogen of Trp21 and its presence has a positive effect on the kinetics of the inhibitor binding. The alkylamino group is thought to have an influence on the orientation of binding of the tricyclic.[9] In the light of this, it was deemed reasonable to investigate the efficacy of ferrocenic analogues of the related chloroquine compounds as inhibitors of TryR, Figure 1. In addition to this, recent preliminary studies on 2-iminobenzimidazoles have shown that in these systems the presence of basic and phenyl substituents have proved crucial for TryR inhibition.[10] The efficacy of polyamines as TryR inhibitors has been established.[11] The presence of basic amine centres in ferroquine analogues together with the aromatic quinoline, ferrocenyl and urea substituent groups that suggested these molecules may indeed exhibit TryR inhibition even though they lack the tricyclic acridine moiety. Finally, the promising *in vitro* efficacy of previously disclosed urea analogues of quinacrine[12] coupled with the potentially interesting impact of the ferrocenyl group on the redox chemistry involved led us to explore the use of ferroquine urea derivatives as potential antiprotozoal inhibitors of TryR.
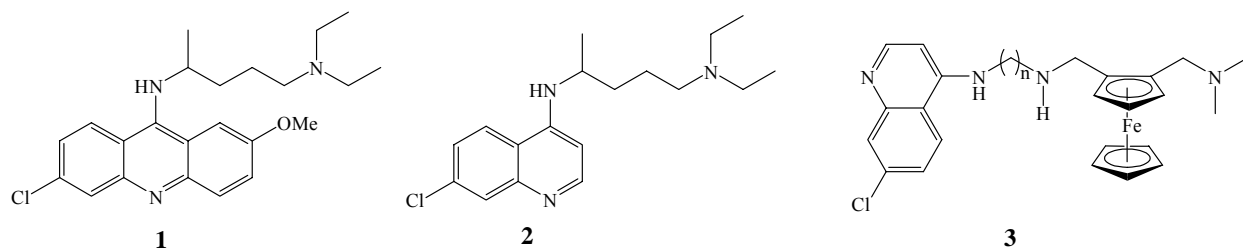

Figure 1. Chemical structures of quinacrine **1**, chloroquine **2** and ferroquine analogues **3**.

Results and Discussion

N-(7-Chloroquinolin-4-yl)alkyl-1,n-diamine, **10**[13] and 2-[(*N*,*N*-dimethylamino)methyl]ferrocenecarboxaldehyde **11**[14] and were synthesised according to literature procedures. The synthesis of the ferroquine analogues was achieved via reductive amination of **11** with the

appropriate alkyl-1,n-diamine, **10**.[15,16] Products were isolated via column chromatography. A series of aromatic urea derivatives were synthesised from the appropriate ferroquine analogue compound and the relevant isocyanates, Scheme 1. No work up was necessary, but purification over silica gel was essential. Compounds **3a-d** and **4a-d** have been previously reported,[15] whilst compounds **5-9** are novel. All new compounds gave ^{1}H NMR, ^{13}C NMR, IR, FABMS and microanalysis data consistent with their structures. The full characterisation data and crystal structure of the parent ferroquine analogue with the two-carbon methylene spacer **3a** has been reported elsewhere.[17]
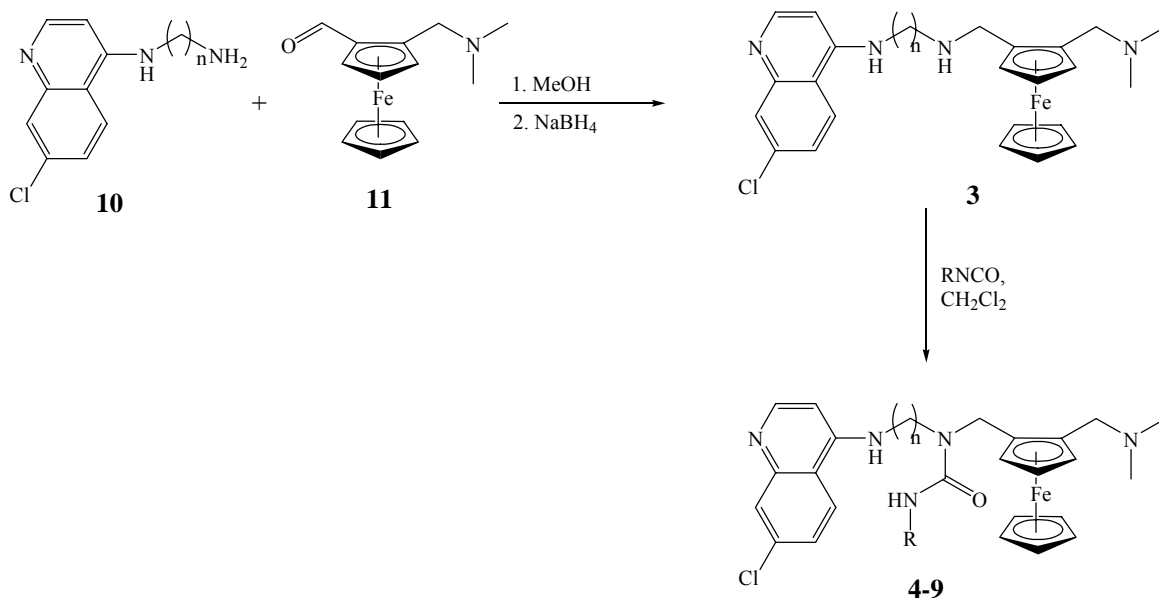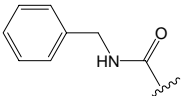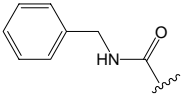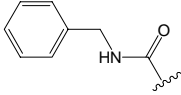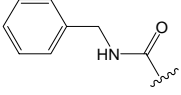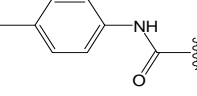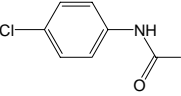

Scheme 1. Synthesis of ferroquine analogues and the urea derivatives (n = 2, 3, 4 and 6).

The enzyme assays were performed as previously described.[18] Recombinant *T. cruzi* trypanothione reductase (128 mU) was assayed using a Beckman DU640 spectrophotometer in 40mM HEPES, pH 7.5, 1mM EDTA and 200μM NADPH at 25°C followed by the addition of 100μM Try[SH]$_2$. Human glutathione reductase, purified from human erythrocytes (42.3mU), was analysed in a similar manner and under identical 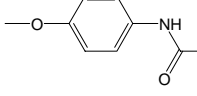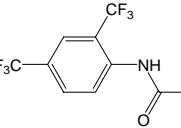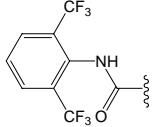conditions followed by the addition of glutathione disulfide (100μM). Enzyme mixtures were preincubated with NADPH (10 min at 25°C) before the addition of varying concentrations of the inhibitor added in DMSO (1% v/v final concentration).

Based on their selectivity and potency indices, several compounds were selected for *in vitro* testing against *Leishmania donovani* (cultured as murine macrophages), amastigote forms of *Trypanosoma cruzi* (cultured as murine macrophages), and the bloodstream form trypomastigote *Trypanosoma brucei rhodesiense*. Experimental details have previously been described.[19]

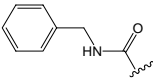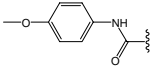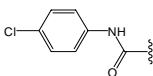**Table 1.** Inhibition of Trypanothione Reductase and Glutathione Reductase by the ferroquine and urea compounds

Compound	n^a	Secondary amine substituent	IC$_{50}$ TryR (µM)	IC$_{50}$ GR (µM)	Selectivity[b]	Relative Potency[c]
CQ			47.6 ± 3.8	$> 2000^d$	> 42	N/A
3a	2	H	6.8 ± 0.7	14.1 ± 1.4	2.1	7
3b	3	H	2.7 ± 0.2	39.6 ± 2.2	14.6	18
3c	4	H	2.1 ± 0.1	20.0 ± 1.8	9.5	23
3d	6	H	15.0 ± 2.0	8.5 ± 0.8	0.6	3
4a	2		3.2 ± 0.3	25.0 ± 2.3	7.8	15
4b	3		3.3 ± 0.2	83.8 ± 3.1	25.2	14
4c	4		5.4 ± 0.3	18.4 ± 0.9	3.4	9
4d	6		2.4 ± 0.1	16.8 ± 0.9	7.0	20
5	2		6.4 ± 0.4	143 ± 39	22.1	7
6	2		2.3 ± 0.2	76.3 ± 7.8	32.9	21
7	2		10.5 ± 0.2	144 ± 30	13.8	5
8	2		2.0 ± 0.2	15.2 ± 1.0	7.5	24
9	2		2.5 ± 0.2	34.43 ± 5.45	13.8	19

[a] n refers to the number of carbons in the methylene spacer; [b] IC$_{50}$ in GR / IC$_{50}$ in TryR; [c] IC$_{50}$ of CQ (TryR) / IC$_{50}$ of ferroquine analogue (TryR); [d] no significant inhibition at 2000µM.

Table 2. Results of *in vitro* testing against *L. donovani*, *T. cruzi* and *T. brucei*

Compound	Secondary amine substituent	ED$_{50}$ (ED$_{90}$) μM			Toxicity ED$_{50}$
		L. donovani	*T. cruzi*	*T. brucei*	
Podophyllotoxin					0.00024
Pentostam		144.2			
Benznidazole			47.7		
Pentamidine				0.025	
3a	H	20.9 (26.5)	>63	5.36	133
4a		9.5 (10.8)	6.1 (>49)[a]	2.54	24
6		22.4 (26.5)	>48	2.73	nd
9		22.2 (26.3)	>48	7.00	nd

[a] This result was repeated as it was anomalous. The second time an ED$_{50}$ of 2.5 μM was reported

Discussion

All compounds tested showed a significant improvement in activity against TryR relative to chloroquine, with compounds **3c**, **4d**, **6** and **8** showing > 20-fold increase in potency, Table 1. Perusal of the results of the ferroquine analogues and their benzyl urea derivatives with varying lengths of methylene spacer suggests that the three-methylene spacer compounds inhibit GR to a lesser degree than the analogues with either longer or shorter chains. The variance of the urea side chain also has a significant impact on the selectivity indices. However, the selectivity of the ferroquine compounds was significantly lower than that shown by chloroquine. Several urea compounds were chosen, on the basis of their potency and selectivity, for *in vitro* testing against *L. donovani*, *T. cruzi* and *T. b. rhodesiense*. The ferroquine scaffold with the 2-carbon spacer was also tested.

At first glance, the results against *L. donovani*, Table 2, appear quite promising as all compounds show an efficacy which is significantly better than the standard drug, Pentostam. Unfortunately, these compounds were found to be toxic to macrophages. As *L. donovani* exists as intracellular amastigotes, this toxicity is problematic. Efficacy against *T. cruzi* was poor with the exception of the benzyl urea. However, *T. cruzi*, like *L. donovani* exists as intracellular

amastigotes so the problems associated with macrophage toxicity are again significant. The series of compounds shows good activity against *Trypanosoma brucei rhodesiense*. Furthermore, this parasite exists in the bloodstream as extracellular trypomastigotes, so the drug is more likely to reach the parasite intact than in the case of *T. cruzi* and *L. donovani*. However, their activity requires at least a hundred fold increase in concentration when compared to the standard drug, Pentamidine. There appears then, to be no strong correlation between *in vitro* efficacy against the parasite and inhibition of TryR by the ferroquine analogues or their urea derivatives.

Experimental Section

General Procedures. The syntheses were performed using standard Schlenk techniques; Compounds **3a-d** and **4a-d** were prepared as previously described.[15,17] Full characterisation data for compounds **3b-d** and **4a-d** is given as supplementary information. All other chemicals were used as supplied by Aldrich. ^{1}H and ^{13}C NMR spectra were recorded at room temperature on Varian EM 400 or 300 MHz spectrometers. ^{1}H 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.

General synthesis of urea compounds
Compound **3** was dissolved in anhydrous dichloromethane in a small sample vial. 1.2 mol equivalents of the required isocyanate was added and the sample vial was placed on a shaker at 200 rpm for 2 h at 25°C. The reaction mixture was placed directly onto the column. The product was then purified by silica gel chromatography eluting with 10% methanol in dichloromethane. The product was isolated as a yellow crystalline solid.
1-[2-(7-Chloro-quinolin-4-ylamino)-ethyl]-1-[2-(*N''*,*N''*-dimethylaminomethyl)-ferrocenylmethyl]-3-*p*-tolyl-urea (5). Yellow crystalline solid; Yield: 145mg (75%); mp: 81-84°C; R$_f$ (silica/CH$_2$Cl$_2$: MeOH = 80:20) 0.51; δ_H (400 MHz; CDCl$_3$) 8.51 (1H, d, $^3J_{HH}$ = 6), 7.91 (1H, d, $^4J_{HH}$ = 2), 7.69 (1H, d, $^3J_{HH}$ = 9), 7.15-7.08 (5H, m), 6.31 (1H, d, $^3J_{HH}$ = 6), 4.43 (1H, d, $^2J_{HH}$ = 16), 4.40-4.39 (1H, m), 4.36-4.34 (1H, m), 4.28 (1H, d, $^2J_{HH}$ = 16), 4.15 (1H, t, $^3J_{HHH}$ = 3), 4.10 (5H, s), 3.80 (1H, d, $^2J_{HH}$ = 13), 3.71-3.68 (2H, m), 3.55-3.40 (2H, m), 2.77 (1H, d, $^2J_{HH}$ = 13), 1.98 (9H, s); $\delta_{C\{H\}}$ (100.6 MHz; CDCl$_3$) 160.5 (^{IV}C), 151.5 (^{IV}C), 150.8, 140.3 (^{IV}C), 134.9 (^{IV}C), 128.3 (2C), 127.7 (^{IV}C), 126.6, 126.5 (2C), 125.3, 122.7, 117.3 (^{IV}C), 97.6, 85.0 (^{IV}C), 82.1 (^{IV}C), 70.5, 69.5 (5C), 68.9, 67.6, 57.9, 47.2, 45.6, 44.7 (2C), 44.7, 43.7; IR

(KBr) ν_{max} 3266br m, 1610s, 1582vs 1537s, 1452m, 1332m, 1105m, 1005s, 841m, 808s, 488m; HRMS (FAB) m/z 610.2025 [M$^+$ + H, $C_{33}H_{36}N_5ClFeO$ + H requires 610.2034], 565.1, 432.1, 409.0, 304.0, 255.1, 213.1, 134.1, 91.0; Found: C, 65.11; H, 5.88; N, 11.61. Calc. for $C_{33}H_{36}N_5ClOFe$: C, 64.98; H, 5.95; N, 11.48%.

1-[2-(7-Chloro-quinolin-4-ylamino)-ethyl]-1-[2-(*N″,N″*-dimethylaminomethyl)-ferrocenylmethyl]-3-*p*-methoxyphenyl-urea (6). Yellow crystalline solid; Yield: 101mg (51%); mp: 99-100°C; R$_f$ (silica/CH$_2$Cl$_2$: MeOH = 80:20) 0.49; δ_H (400 MHz; CDCl$_3$) 8.53 (1H, d, $^3J_{HH}$ = 5), 7.94 (1H, d, $^4J_{HH}$ = 2), 7.81 (1H, d, $^3J_{HH}$ = 9), 7.29 (1H, dd, $^4J_{HH}$ = 2 and $^3J_{HH}$ = 9), 6.99 (2H, d, $^3J_{HH}$ = 9), 6.84 (2H, d, $^3J_{HH}$ = 9), 6.31 (1H, d, $^3J_{HH}$ = 5), 4.48 (1H, d, $^2J_{HH}$ = 16), 4.43 (1H, m), 4.34 (1H, d, $^2J_{HH}$ = 16), 4.17 (1H, t, $^3J_{HH}$ = 2), 4.13-4.12 (1H, m), 4.11 (5H, s), 3.87 (1H, d, $^2J_{HH}$ = 13), 3.81 (3H, s), 3.71-3.67 (2H, m), 3.60-3.40 (2H, m), 2.82 (1H, d, $^2J_{HH}$ = 13), 2.05 (6H, s); $\delta_{C\{H\}}$ (100.6 MHz; CDCl$_3$) 159.8 (^{IV}C), 157.1 (^{IV}C), 151.5, 150.8 (^{IV}C), 132.2 (^{IV}C), 127.8 (^{IV}C), 126.8 (2C), 125.3, 122.9, 117.4 (^{IV}C), 113.9 (2C), 98.0, 83.8 (^{IV}C), 82.1 (^{IV}C), 70.7, 69.5 (5C), 69.0, 67.6, 58.0, 55.5, 46.7, 45.7, 45.0 (2C), 43.4; IR (KBr) ν_{max} 3284br m, 1636m, 1610m, 1583vs, 1510vs, 1458m, 1332m, 1232s, 1105m, 1032m, 1006m, 824s, 492m; HRMS (FAB) m/z 626.1995 [M$^+$ + H, $C_{33}H_{36}N_5ClFeO_2$ + H requires 626.1983], 581.1, 530.9, 477.0, 432.0, 365.9, 334.0, 270.1, 213.0, 191.0, 134.1, 91.0; Found: C, 63.52; H, 5.83; N, 11.38. Calc. for $C_{33}H_{36}N_5ClO_2Fe$: C, 63.29; H, 5.79; N, 11.23%.

1-[2-(7-Chloro-quinolin-4-ylamino)-ethyl]-1-[2-(*N″,N″*-dimethylaminomethyl)-ferrocenylmethyl]-3-[2,4-bis-(trifluoromethyl)-phenyl]-urea (7). Yellow crystalline solid; Yield: 138mg (65%); mp: 94-96°C; R$_f$ (silica/ CH$_2$Cl$_2$: MeOH = 80:20) 0.63; δ_H (400 MHz; CDCl$_3$) 8.52 (1H, d, $^3J_{HH}$ = 5), 7.91 (1H, d, $^4J_{HH}$ = 2), 7.63 (1H, d, $^3J_{HH}$ = 9), 7.38 (1H, dd, $^4J_{HH}$ = 2 and $^3J_{HH}$ = 9), 7.24-7.14 (3H, m), 6.33 (1H, d, $^3J_{HH}$ = 5), 4.52 (1H, d, $^2J_{HH}$ = 16), 4.42-4.40 (1H, m), 4.34 (1H, d, $^2J_{HH}$ = 16), 4.20 (1H, t, $^3J_{HHH}$ = 3), 4.14 (1H, m), 4.13 (5H, s), 3.85 (1H, d, $^2J_{HH}$ = 13), 3.76-3.70 (2H, m), 3.61-3.39 (2H, m), 2.80 (1H, d, $^2J_{HH}$ = 13) and 1.94 (6H, s); $\delta_{C\{H\}}$ (100.6 MHz; CDCl$_3$) 159.8 (^{IV}C), 151.6, 150.6 (^{IV}C), 133.3, 133.2, 127.9, 125.0, 122.6, 119.5 (^{IV}C), 117.3 (^{IV}C), 114.3 (^{IV}C), 114.0 (^{IV}C), 97.8, 83.5 (^{IV}C), 82.0 (^{IV}C), 71.0, 69.5 (5C), 69.3, 67.7, 57.7, 46.2, 45.6, 44.6 (2C), 43.1 (5'); IR (KBr) ν_{max} 3312br w, 1636m, 1612m, 1583vs, 1470m, 1319s, 1280s, 1163s, 1136s, 1047m, 1005m, 820m, 488m; HRMS (FAB) m/z 682.1669 [M$^+$ + H, $C_{33}H_{32}N_5ClF_4FeO$ + H requires 682.1658], 636.9, 597.0, 531.0, 477.0, 431.9, 367.0, 321.9, 256.1, 213.0, 191.0, 134.1, 91.0 [CH$_2$-Cp-CH$_2$]; Found: C, 58.02; H, 4.85; N, 10.17. Calc. for $C_{33}H_{32}N_5ClF_4OFe$: C, 58.10; H, 4.73; N, 10.31%.

1-[2-(7-Chloro-quinolin-4-ylamino)-ethyl]-1-[2-(*N″,N″*-dimethyl-aminomethyl)ferrocenyl methyl]-3-[2,6-bis-(trifluoromethyl)-phenyl]-urea (8). Yellow crystalline solid; Yield: 96mg (43%); mp: 113-115°C; R$_f$ (silica/CH$_2$Cl$_2$: MeOH = 80:20) 0.79; δ_H (400 MHz; CDCl$_3$) 8.50 (1H, d, $^3J_{HH}$ = 6), 7.98 (1H, d, $^4J_{HH}$ = 2), 7.65 (1H, d, $^3J_{HH}$ = 9), 7.51-7.32 (3H, m), 7.17 (1H, dd, $^4J_{HH}$ = 2 and $^3J_{HH}$ = 9), 6.35 (1H, d, $^3J_{HH}$ = 6), 4.64 (1H, d, $^2J_{HH}$ = 16), 4.45-4.44 (1H, m), 4.38 (1H, d, $^2J_{HH}$ = 16), 4.21 (1H, t, $^3J_{HHH}$ = 2), 4.15 (1H, m), 4.13 (5H, s), 3.91 (1H, d, $^2J_{HH}$ = 13), 3.74-3.72 (2H, m), 3.66-3.41 (2H, m), 2.81 (1H, d, $^2J_{HH}$ = 13), 1.95 (6H, s); $\delta_{C\{H\}}$ (100.6 MHz; CDCl$_3$) 159.9 (^{IV}C), 151.6 (^{IV}C), 149.8, 146.9 (^{IV}C), 135.6 (^{IV}C), 128.5, 126.3, 125.3, 123.1,

122.0, 119.8, 119.6, 116.9 (^{IV}C), 97.7, 83.3 (^{IV}C), 81.8 (^{IV}C), 71.1, 69.7 (5C), 67.8, 66.1, 57.6, 53.0 (2C), 46.0, 45.8, 44.1 (2C), 43.0; IR (KBr) ν_{max} 3296br w, 1637m, 1616m, 1582vs, 1522s, 1476s, 1323vs, 1276m, 1166s, 1136s, 1039w, 1005m, 844m, 804m, 488w; HRMS (FAB) m/z 682.1666 [M^+ + H, $C_{33}H_{32}N_5ClF_4FeO$ + H requires 682.1658], 637.0, 477.1, 432.1, 367.1, 322.0, 213.1, 205.0, 134.1, 91.0; Found: C, 57.96; H, 4.79; N, 10.23. Calc. for $C_{33}H_{32}N_5ClF_4OFe$: C, 58.10; H, 4.73; N, 10.31%.

1-[2-(7-Chloro-quinolin-4-ylamino)-ethyl]-1-[2-(N'',N''-dimethyl-aminomethyl)-ferrocenylmethyl]-3-p-chloro-phenyl-urea (9). Yellow crystalline solid; Yield: 115mg (56%); mp: 115-116°C; R$_f$ (silica/CH$_2$Cl$_2$: MeOH = 80:20) 0.62; δ_H (400 MHz; CDCl$_3$) 8.52 (1H, d, $^3J_{HH}$ = 6), 7.95 (1H, d, $^4J_{HH}$ = 2), 7.79 (1H, d, $^3J_{HH}$ = 9), 7.31 (1H, dd, $^3J_{HH}$ = 2 and $^3J_{HH}$ = 9), 7.28-7.24 (4H, m), 6.35 (1H, d, $^3J_{HH}$ = 6), 4.54 (1H, d, $^2J_{HH}$ = 16), 4.42-4.41 (1H, m), 4.38 (1H, d, $^2J_{HH}$ = 16), 4.19 (1H, t, $^3J_{HH}$ = 3), 4.16-4.14 (1H, m), 4.12 (5H, s), 3.89 (1H, d, $^2J_{HH}$ = 13,), 3.74-3.72 (2H, m), 3.66-3.41 (2H, m), 2.80 (1H, d, $^2J_{HH}$ = 13),1.97 (6H, s); $\delta_{C\{H\}}$ (100.6 MHz; CDCl$_3$) 159.1 (^{IV}C), 151.5 (^{IV}C), 150.7, 138.0 (^{IV}C), 135.5 (^{IV}C), 128.7 (2C), 127.9 (2C), 125.9, 125.4, 122.6, 117.3 (^{IV}C), 97.9, 83.4 (^{IV}C), 82.0 (^{IV}C), 70.8, 69.6 (5C), 69.0, 67.7, 58.1, 46.7, 45.8, 45.3 (2C), 43.2 (5'); IR (KBr) ν_{max} 3306s, 1634s, 1608m, 1579vs, 1528s, 1482s, 844m, 820m; HRMS (FAB) m/z 630.1479 [M^+ + H, $C_{32}H_{33}N_5Cl_2FeO$ + H requires 630.1489], 585.0, 477.1, 432.0, 366.0, 307.1, 274.1, 256.1, 213.0, 91.0; Found: C, 61.10; H, 5.17; N, 11.20. Calc. for $C_{32}H_{33}N_5Cl_2$ OFe: C, 60.94; H, 5.27; N, 11.15%.

Supplementary Information Available

Full characterisation data for compounds **3b-d** and **4a-d.**

Acknowledgements

References and Notes

1. Manson-Bahr, P. E. C; Bell, D. R.; *Manson's Tropical Diseases*, 19th Edn., Baillière Tindall: London, 1987; pp54-113.
2. Fairlamb, A. H; Blackburn, P.; Ulrich, P.; Chait, B. T.; Cerami, A. *Science* **1985**, *227*, 1485.
3. Henderson, G. B; Fairlamb, A. H.; Ulrich, P.; Cerami, A. *Biochemistry* **1987**, *26*, 3023.
4. (a) Werbovetz, K. A. *Curr. Med. Chem.* **2000**, *7*, 835. (b) Chibale, K.; Musonda, C. C. *Curr. Med. Chem.* **2003**, *10*, 1863. (c) Krauth-Siegel, R. L.; Meiering, S. K.; Schmidt, H. *Biol. Chem.* **2003,** *384*, 539. (d) Bonse, S.; Santelli-Rouvier, C.; Barbe, J.; Krauth-Siegel, R. L. *J.*

Med. Chem. **1999**, *42*, 5448. (e) Martyn, D. C.; Jones, D. C.; Fairlamb, A. H.; Clardy, J. *Bioorg. Med. Chem. Lett.* **2007**, *7*, 1280.

5. Chibale, K. *Arkivoc* **2002**,*(ix)*, 93.

6. Neal, R. A. In *The Leishmaniases*; Peters, W.; Killick-Kendrick, R.; Eds.; Academic: London, 1987, pp793-845.

7. Hammond, D. J.; Cover, B.; Gutteridge, W. E. *Trans. R. Soc. Trop. Med. Hyg.* **1984**, *78*, 91.

8. Garforth, J.; Yin, H.; McKie, J. H.; Douglas, K. T.; Fairlamb, A. H. *J. Enzyme Inhib.* **1997,** *12*, 161.

9. Saravanamuthu, A.; Vickers, T. J.; Bond, C. S.; Peterson, M. R.; Hunter, W. N.; Fairlamb, A. H. *J. Biol. Chem.* **2004**, *279*, 29493.

10. Holloway, G. A.; Baell, J. B.; Fairlamb, A. H.; Novello, P. M.; Parisot, J. P.; Richardson, J.; Watson, K. G.; Street, I. A.; *Bioorg. Med. Chem. Lett.* **2007**, *17*, 1422.

11. Ponasik, J. A.; Strickland, C.; Faerman, C.; Savvides, S.; Karplus, P. A.; Ganem, B. *Biochem. J.* **1995**, *311*, 371.

12. Chibale, K.; Haupt, H.; Kendrick H.; Yardley V.; Saravanamuthu, A.; Fairlamb, A. H.; Croft, S. L. *Bioorg. Med. Chem. Lett.* **2001**, *11*, 2655.

13. De, D.; Byers, L. D.; Krogstad, D. J. *J. Heterocyclic Chem.* **1997**, *34,* 315.

14. Picart-Goetgheluck, S.; Delacroix O.; Maciejewski L.; Brocard, J. *Synthesis* **2000**, *10,* 1421.

15. Chibale, K.; Moss, J. R.; Blackie, M.; Van Schalkwyk, D.; Smith, P. J. *Tetrahedron Lett.* **2000**, *41*, 6231.

16. Beagley, P.; Blackie, M. A. L.; Chibale, K.; Clarkson, C.; Moss, J. R.; Smith, P. J. *J. Chem. Soc., Dalton Trans.* **2002**, 4426.

17. Beagley, P.; Blackie, M. A. L.; Chibale, K.; Clarkson, C.; Meijboom, R.; Moss, J. R.; Smith, P. J.; Su, H. *J. Chem. Soc., Dalton Trans.* **2003**, 3046.

18. Borges, A.; Cunningham, M. L.; Tovar, J.; Fairlamb, A. H. *Eur. J. Biochem.* **1995**, *228*, 745.

19. Chibale, K.; Visser, M.; Yardley, V.; Croft, S. L.; Fairlamb, A. H. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 1147.