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New amine and urea analogs of ferrochloroquine: synthesis, antimalarial activity in vitro and electrochemical studies

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Abstract

Amine and urea analogs of ferrochloroquine with varying methylene spacer lengths were synthesised, studied by cyclic voltammetry and evaluated in vitro against a sensitive (D10) and resistant (K1) strain of *Plasmodium falciparum*. Most analogs were found to be more active than chloroquine in both strains. In D10 ureas were more active than amines and antimalarial activity in this strain correlated well with the length of the methylene spacer and redox potentials. The length of the methylene spacer was a major determinant of antimalarial activity in K1. © 2000 Elsevier Science Ltd. All rights reserved.

Malaria is a parasitic disease that afflicts 300–500 million people and kills up to 2 million worldwide. The chemotherapy of malaria has been undermined by the widespread development of resistance of the most dangerous form of the causative agent, *Plasmodium falciparum*, to clinically used antimalarial drugs such as chloroquine (CQ), mefloquine, quinine and sulfadoxine-pyrimethamine. As a result drugs such as CQ **1** (Fig. 1) have been rendered virtually useless and malaria is impossible to treat in some parts of the world. There is thus a clear need for new drugs with structures and mechanisms of action different from those that are presently used.

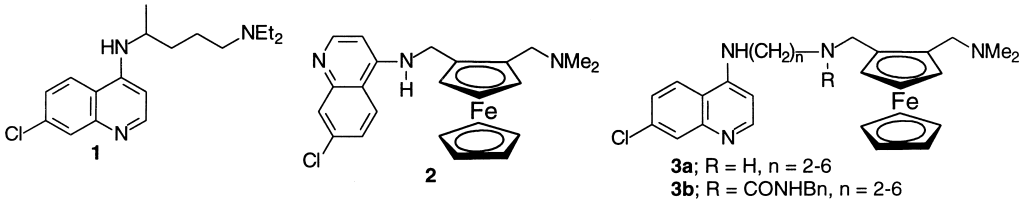

Figure 1. Chemical structures of chloroquine (**1**), ferrochloroquine (**2**) and new analogs **3a** and **3b**

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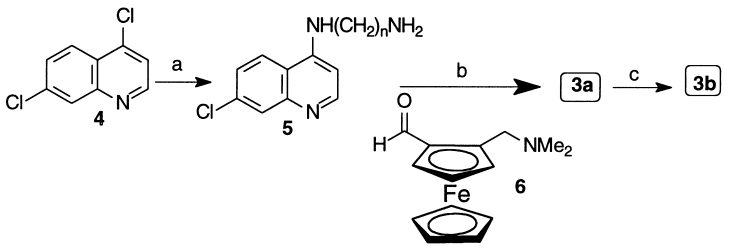
It has been shown throughout history that incorporation of metals into drugs can have a profound effect on the biological activity. Within the context of malaria, coordination of metal complexes to CQ is a relatively new approach to new antimalarial agents. So far, this approach has shown great potential with demonstrable enhancement of the efficacy of CQ against both sensitive and resistant strains of *Plasmodium falciparum* by coordination to ruthenium and gold complexes.[1,2] The disclosure of ferrochloroquine (FQ) **2** (Fig. 1) in which the carbon chain of CQ is replaced by the hydrophobic ferrocenyl group has been a welcome addition to organometallic antimalarial drugs.[3,4] This compound has been shown to be superior to CQ in both in vitro and in vivo tests. Related analogs of **2**, including its potential metabolites have also recently been reported.[5,6]

The recent[6] finding that unlike CQ metabolites monodesethylchloroquine (DECQ) and didesethylchloroquine (di-DECQ), potential metabolites of FQ are more active than CQ against *Plasmodium falciparum* suggests that the ferrocenyl moiety in FQ and related analogues plays an important role in the observed antimalarial activity. These compounds can thus be regarded as useful lead compounds which can serve as a basis for the design of further analogues and mimics. In this preliminary communication, we report on the synthesis, in vitro antimalarial activities and electrochemical studies of new amine (**3a**) and urea (**3b**) ferrocenic analogues of FQ.

The rationale behind the design of these new analogues and subsequent electrochemical studies is as follows. First, in order to explore extensive and detailed structure–activity relationships within the FQ class of organometallic antimalarial agents, we reasoned that new analogs (such as **3**, R = H) of FQ that would serve as scaffolds for the parallel synthesis of chemical libraries were required. We envisaged introducing chemical diversity at the more reactive nucleophilic secondary amino group adjacent to the ferrocenylmethyl moiety. In designing these new analogs we also took into account the recent disclosure that in 7-substituted 4-aminoquinolines, the length of the methylene spacer between two nitrogens in the side chain of CQ analogues is a major determinant of activity against CQ-resistant *Plasmodium falciparum*.[7,8] Second, we reasoned that although the ferrocenyl moiety is chemically stable in many media, the possibility exists that under appropriate conditions, in the presence of the malaria parasite in vitro and/or in vivo, the iron metal centre in **2** may undergo redox reactions. We reasoned that the ease of oxidation of Fe(II) in the ferrocenyl moiety of **3a** and **3b** might be influenced by chemical groups in the vicinity of this moiety. We thus wanted to explore a possible correlation between the redox potentials of initial compounds **3a** and **3b** and their in vitro antimalarial activities.

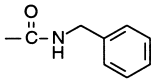
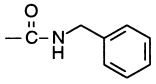
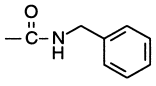
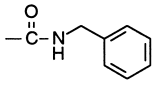
Derivatives **3a** and **3b** were prepared from commercially available 4,7-dichloroquinoline **4** and the corresponding diamines. Reaction of the resulting products **5** with ferrocenyl aldehyde **6**[3] gave compounds **3a** after reduction of the imine formed in situ using polymer-supported borohydride in moderate chemical yields. Reaction of compounds **3a** with benzyl isocyanate gave the corresponding urea derivatives **3b** in low to high yields, Scheme 1. All new compounds gave spectroscopic and analytical data consistent with their structures.

The antimalarial activities of derivatives **3a** and **3b** in vitro against both the CQ-sensitive (D10) and CQ-resistant (K1) strains of *Plasmodium falciparum* as well as their redox potentials are presented in Table 1.[9] Data for CQ are included for comparison purposes. Antimalarial activities of compounds **3a** were comparable to CQ in D10. Further in this series antimalarial activities in D10 correlated well with the chain length and ease of oxidation of the ferrocenyl group. The longer the chain length the greater the ease of oxidation and the lower the antimalarial activity as indicated by the decreasing values of the half-wave potential ($E_{1/2}$)[10] and increasing IC_{50} values, respectively, on moving from **3a** ($n = 2$) to **3a** ($n = 6$). In K1 no such correlation was revealed.



Scheme 1. *Reagents and conditions:* (a) 4.5 equiv. of $H_2N(CH_2)_nNH_2$, 80°C, 1 h, 82–90%; (b) 4.0 equiv. of Amberlite IRA-400 borohydride resin, 25°C, 18 h, 60–65%; (c) 1.2 equiv. of $PhCH_2NCO$, CH_2Cl_2, 25°C, 3 h, 34–75%

Table 1
Antimalarial activity in vitro and electrochemical data of compounds **3a** and **3b**[9]

Compound	n	R	IC_{50} in D10[a] (nM)	IC_{50} in K1[b] (nM)	E_{pa} (mV)[c]	E_{pc} (mV)[d]	$E_{1/2}$ (mV)[e]	E_{pa}-E_{pc} (mV)
CQ	N/A	N/A	41.86 ± 1.25	125.38 ± 4.53	N/A	N/A	N/A	N/A
3a	2	H	41.70 ± 2.60	73.46 ± 6.40	150	30	90	120
3a	3	H	51.37 ± 3.85	36.93 ± 1.70	120	13	66.5	107
3a	4	H	61.16 ± 1.53	111.5 ± 12.9	106	14	60	92
3a	6	H	86.92 ± 7.30	81.39 ± 5.57	Not found	12	N/A	N/A
3b	2		21.35 ± 1.99	37.50 ± 7.35	198	115	156.5	83
3b	3		16.20 ± 0.54	47.41 ± 2.41	171	91	131	80
3b	4		16.74 ± 4.25	75.23 ± 8.49	149	82	115.5	67
3b	6		19.01 ± 6.24	110.2 ± 9.46	164	81	122.5	83

[a]Chloroquine sensitive strain; [b]Chloroquine resistant strain; [c]anodic potential; [d]cathodic potential ; [e]half wave potential $(E_{pa} + E_{pc})/2$

However, it is noteworthy that good activity against K1 within the **3a** series was only observed for the compound with a 3-carbon methylene spacer ($n = 3$) which was found to be 3 times more active than CQ. This finding lends further support to earlier observations that in 7-substituted 4-aminoquinolines, the length of the methylene spacer between two nitrogens in the side chain of CQ analogues is a major determinant of activity against CQ-resistant *Plasmodium falciparum*.[7,8]

Generally speaking there was an improvement (up to fourfold in some cases) in the potency on moving from amines **3a** to the benzyl ureas **3b**. This was especially the case in D10. While no correlation was revealed between antimalarial activity, length of the methylene spacer and ease of oxidation in D10 within the urea series, a correlation was observed in K1 between the length of the methylene spacer and the antimalarial activity as evidenced by an increase (albeit modest) in IC_{50} values with increase in the length of the methylene spacer. With the exception of the 6-carbon methylene spacer, the ease of oxidation as evidenced by the decrease in the $E_{1/2}$ values correlated well with the antimalarial activity and length of the methylene spacer. In compounds

3b the length of the methylene spacer between the two secondary amine nitrogens in the side chain of CQ analogues is also a determinant of activity against CQ-resistant *Plasmodium falciparum*.[7,8] Higher $E_{1/2}$ values for the ureas relative to the amine precursors suggests that the presence of the electron-withdrawing urea moiety in close proximity to the cyclopentadiene system makes the urea compounds more difficult to oxidize.

In summary we have shown a correlation in D10 between in vitro antimalarial activity of FQ analogues and their redox potentials. Our results further support earlier findings that in 7-substituted 4-aminoquinolines, the length of the methylene spacer between two nitrogens in the side chain of CQ analogues is a major determinant of activity against CQ-resistant *Plasmodium falciparum*.[7,8] We have further demonstrated the potential benefits of utilizing analogs such as **3a** as scaffolds for the synthesis of new analogs exemplified by **3b**. Coupled with the simplicity of the chemistry, our approach to new analogs of FQ can potentially give rise to a large number of compounds for exploration of structure–activity relationships within this series of compounds in view of the commercial availability of a wide range of electrophilic reagents including acid and sulfonyl chlorides. This work is now in progress.

Acknowledgements

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9. In vitro antimalarial parasite lactate dehydrogenase assay was performed using a modification of the reported method (Makler, M. T.; Ries, J. M.; Williams, J. A.; Bancroft, J. E.; Piper, R. C.; Gibbons, B. L.; Hinrichs, D. J. *Am. J. Trop. Med. Hyg.* **1993**, *48*, 739–741). The assay was performed in 96-well microtitre plates. The blank for the assay was the unparasitized red blood cells without drug. The parasites were incubated at a 1% haematocrit and a 2–3% parasitaemia in a volume of 200 μl along with the particular drug at the appropriate defined concentration in DMSO. Each drug concentration was measured in duplicate. The parasites were incubated for 2 days in the plate in dessicator cabinets under a CO_2/air gas mixture. Once the incubation was complete, 100 μl of Malstat Reagent and 25 μl of NBT/PES solution containing equal volumes of Nitro Blue Tetrazolium (1.6 mg/ml) and phenazine etho sulphate (0.08 mg/ml) were added to each well of a new flat bottomed 96-well microtitre plate. The culture in each of the wells of the original plate was resuspended and 10 μl of the culture was taken from each

well and added to the corresponding well of the Malstat plate, thus initiating the lactate dehydrogenase reaction. Colour development was monitored at 620 nm as the reaction proceeded. The percentage viabilities were calculated using a SigmaPlot (Jandel Scientific) transformation. The dose–response curves were generated using a non-linear regression in Prism (Graphpad Software). Statistical analyses were performed also using Prism.

10. Cyclic voltammetry was performed on a BAS 100B Electrochemical Analyser using a three electrode system comprising a platinum disk working electrode, a platinum wire auxiliary electrode and a Ag/Ag^{+} reference electrode (0.01 M $AgNO_3$ and 0.1 M Bu_4NClO_4 in acetonitrile). Unless other stated, all measurements were made on acetonitrile solutions which were 1–2 mM in sample and 0.1 M Bu_4NClO_4 at a scan rate of 100 mV s^{-1}. Under these conditions the ferrocene/ferrocenium couple, which was used as a reference, had an $E_{1/2}$ value of 0.08 V. All solutions were purged with argon and voltammograms were recorded under a blanket of argon.