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Antimalarial Activity of Ferrocenyl Chalcones

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Abstract—A series of ferrocenyl chalcones were synthesized and evaluated for in vitro antimalarial activity against a chloroquine-resistant strain of *Plasmodium falciparum*. The most active compounds were 1-(3-pyridyl)-3-ferrocenyl-2-propen-1-one (**6**) and 1-ferrocenyl-3-(4-nitrophenyl)-2-propen-1-one (**28**) with IC_{50} of 4.5 and 5.1 μM, respectively. Differences in activity were not readily explained by the size and lipophilicity characteristics of these compounds.

Many reports of ferrocene-based antimalarial drugs have emerged in the past 5 years. The most outstanding compound reported so far is ferrochloroquine, the ferrocenyl analogue of chloroquine (CQ).[1–3] The antimalarial activity of ferrochloroquine is notable in that its in vitro activity is greater against CQ resistant *Plasmodium falciparum* strains than CQ susceptible strains. [1] Ferrochloroquine has a curative effect when given to mice infected with *Plasmodium vinckei* (CQ resistant and susceptible strains).[1] It has been proposed that the ferrocene ring in ferrochloroquine may interfere with the mode of resistance,[2] but it is not known how this is achieved. The ferrocene ring has also been incorporated into other known antimalarial agents but with variable outcomes. For example, only one member (an amine derivative) among a series of ferrocenic artemisinin derivatives has activity comparable to artemisinin against *P. falciparum* in vitro.[4] Ferrocenyl analogues of mefloquine and quinine are less active than the parent compounds.[5] An investigation of ellagitannins as antimalarial agents showed that derivatives with ferrocene and biphenic acid entities exhibit micromolar to submicromolar activity, but derivatives that lack either groups are inactive or have significantly less activity.[6] A novel series of benzylimidazolium compounds carrying ferrocenyl substituents have also been reported to have antimalarial activity.[7]

Ferrocene is a lipophilic, electron donating entity with no hydrogen bond donor or acceptor property.[8] Its π (2.46) and σ (σ_m −0.15, σ_p −0.18) values are close to those of the cyclohexyl ring (π 2.51, σ_m −0.15, σ_p −0.22), but the ferrocene ring has a significantly larger molar refractivity (48.26), possibly due to the presence of the ferrous ion sandwiched between two negatively charged cyclopentadienyl rings. The ferrous ion can undergo reversible oxidation–reduction and the nature of the substituents on the ferrocene ring has a marked influence on this process.[9]

Thus far, the role of the ferrocene ring in antimalarial activity remains uncertain. It may be related to the physicochemical properties of the ring (lipophilicity, electronic effects, size) which may be optimal for transport or delivery processes. It is also possible that the ring exerts a unique biological effect, not associated with other structural entities. In the design of the ferrocenic artemisinin derivatives, it was suggested that the ferrous ion in ferrocene may induce a homolytic cleavage of the peroxide linkage.[4]

In order to investigate the role of ferrocene in antimalarial activity, several ferrocenyl chalcones have been synthesized in this study. The choice of the ferrocenyl chalcones is two-fold. Firstly, their syntheses are readily achieved by conventional methods and a good number of members can be synthesized for evaluation. Secondly, we have investigated the antimalarial activities of alkoxylated and hydroxylated chalcones in an earlier study[10] and the results of these two classes of chalcones can be compared to give useful conclusions.

Two series of ferrocenyl chalcones were synthesized in this investigation (Table 1). Series A (**1**–**12**) is characterized by

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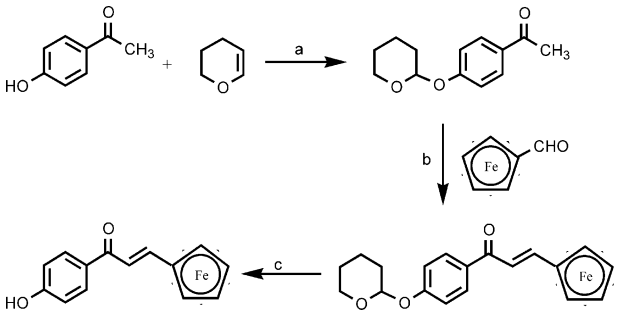
Table 1. Structure and in vitro antimalarial activity of ferrocenyl chalcones

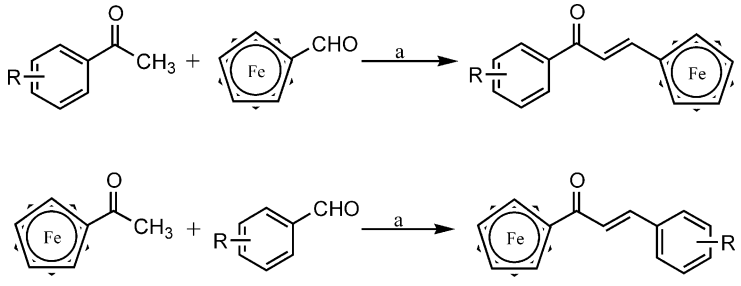


Series A				Series B			
No.	Ring B	Ring A	IC_{50}[a] (μm)	No.	Ring B	Ring A	IC_{50}[a] (μM)
1	Phenyl	Fc[b]	175	**13**	Fc	Phenyl	19
2	1-Methoxyphenyl	Fc	410	**14**	Fc	4-Methoxyphenyl	17
3	2,4-Dimethoxyphenyl	Fc	24.5[c]	**15**	Fc	2,4-Dimethoxyphenyl	18
4	2-Naphthalenyl	Fc	21	**16**	Fc	2-Naphthalenyl	73.5
5	1-Naphthalenyl	Fc	47.4	**17**	Fc	1-Naphthalenyl	48.3
6	3-Pyridinyl	Fc	4.6	**18**	Fc	3-Pyridinyl	17
7	4-Hydroxyphenyl	Fc	36[d]	**19**	Fc	4-Hydroxyphenyl	20.6
8	2,4-Dihydroxyphenyl	Fc	31.5	**20**	Fc	4-Methylphenyl	147
9	2-Hydroxyphenyl	Fc	73.5	**21**	Fc	4-Chlorophenyl	14.5
10	2,3,4-Trimethoxyphenyl	Fc	22.5[e]	**22**	Fc	3-Chlorophenyl	36.2
11	4-Ethoxyphenyl	Fc	200[f]	**23**	Fc	2-Chlorophenyl	42.4
12	4-Butoxyphenyl	Fc	80	**24**	Fc	2,4-Dichlorophenyl	29.4
				25	Fc	4-Fluorophenyl	12.1
29	Fc	4-Cyanophenyl	26.5	**26**	Fc	2,4-Difluorophenyl	23.2
30	Fc	3-Quinolinyl	18.2	**27**	Fc	4-Trifluoromethylphenyl	58.4
31	Fc	4-Quinolinyl	14	**28**	Fc	4-Nitrophenyl	5.1

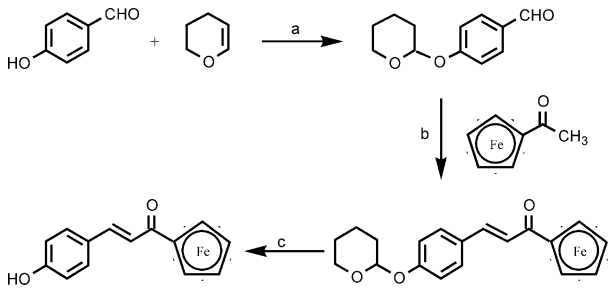
[a]IC_{50} values for inhibition of [^{3}H]hypoxanthine uptake into *P. falciparum* (K1) in the presence of drug, following the method described in ref. 10. IC_{50} for chloroquine = 0.250 μM. All readings are the average of two or more separate determinations.
[b]Fc, ferrocene.
[c]IC_{50} of Ring A = phenyl derivative of **3** is 55.5 μM.[10]
[d]IC_{50} of Ring A = phenyl derivative of **7** is 29.6 μM.[10]
[e]IC_{50} of Ring A = phenyl derivative of **10** is 15.8 μM.[10]
[f]IC_{50} of Ring A = phenyl derivative of **11** is 43.0 μM.[10]

the presence of ferrocene as Ring A, and various alkoxylated/hydroxylated phenyl substituents, heterocyclic or aromatic bicyclic rings as Ring B. Series B (**13**–**31**) has ferrocene as Ring B, and substituted phenyl, heterocyclic or aromatic bicyclic rings as Ring A. The substituents on the phenyl ring were chosen using the Craig Plot in order to ensure a reasonable coverage of lipophilic and electron donating/withdrawing properties. For compounds that do not have hydroxyl substituents on Ring A or B, synthesis was readily achieved by a base-catalyzed Claisen–Schmidt condensation between ferrocene aldehyde and an appropriately substituted aromatic ketone (for Series A), and acetylferrocene with an appropriately substituted aromatic aldehyde (for Series B) (Scheme 1).[11] In the case of compounds with hydroxyl substituents on Ring A or B, prior protection of the hydroxyl group using 2H-3,4-dihydropyran was necessary before reaction (Scheme 2). The protecting group was subsequently removed by acid hydrolysis.[12] No protection was necessary for hydroxyl groups at the *ortho* position of Ring A or B.

Table 1 gives the IC_{50} of the ferrocenyl chalcones for the inhibition of [^{3}H]hypoxanthine uptake into a CQ resistant strain of *P. falciparum* (K1).[10] It can be seen that chalcones with ferrocene as Ring B are generally more active than chalcones that have ferrocene as Ring A (the other ring being kept the same). For example, **13** (Ring



Scheme 1. (a) KOH, EtOH, rt. R are substituents listed in Table 1.



Scheme 2. (a) Pyridinium *p*-toluenesulfonate in CH_2Cl_2; (b) KOH, EtOH, rt; (c) 4 M HCl, rt.

B = ferrocene, Ring A = phenyl) is 9 times more active than **1** (Ring B = phenyl, Ring A = ferrocene). Other compound pairs, namely **2**/**14**, **3**/**15**, **7**/**19**, further attest to this observation. In all these cases, the other ring is a substituted phenyl ring. Interestingly, when the other ring is a heterocyclic or bicyclic ring, the compound with ferrocene as Ring A is now more active. The 2-naphthalenyl derivatives **4** and **16**, and the 3-pyridinyl derivatives **6** and **18** are such examples. However, an exception is seen in the 1-naphthalenyl derivatives **5** and **17.**

When compared with the previous series of chalcones,[10] the ferrocenyl chalcones are seen to be less active. For example, a comparison of chalcones **3**, **7**, **10**, **11** (Ring A = ferrocene) with previously investigated derivatives in which Ring A is phenyl shows that only **3** (IC_{50} 24.5 μM) is more active than its Ring A phenyl derivative (IC_{50} 55.5 μM[10]). The rest are less active than their phenyl counterparts.

Several Series B ferrocenyl chalcones (Ring B = ferrocene) have been synthesized in which Ring A is a phenyl ring substituted with groups of varying electronic and lipophilic characteristics (**13**–**15, 19**–**29**). A few interesting structure–activity observations can be made for this subset. It is evident that the unsubstituted phenyl ring (**13**) is quite active per se (IC_{50} 19 μM). In general, inclusion of electron donating or withdrawing groups adversely affects activity (4-methyl **20**, 4-CF_3 **27**) or leave it unchanged (2,4-dimethoxy **15**, 2,4-difluoro **26**). Only substitution with 4-nitro markedly enhanced activity (**28**, IC_{50} 5.1 μM). The 4-cyano derivative **29** is significantly less active, despite the fact that both nitro and cyano are polar, electron withdrawing groups. An attempt was made to quantify the structure–activity relationships using the π values of the Ring A phenyl substituent,[13] molecular weight and ^{13}C chemical shifts (to denote electronic effects of the Ring A substituent)[10] of these compounds using multiple linear regression.[14] However, no significant relationship could be obtained.

Another interesting observation is the reasonably good activity associated with the quinolinyl derivatives (**30**, **31**). This is in contrast to the poorer activities of naphthalenyl derivatives (**16, 17**), which have a comparable size but lack a basic center.

The results gathered from this fairly limited number of ferrocenyl chalcones suggest that the physicochemical properties of the ferrocene ring do not contribute significantly to antimalarial activity. The Ring B ferrocenyl chalcones like **13**, **14**, **15** and **19** have the same molecular weight (size) and comparable lipophilicity as their counterparts with ferrocene as Ring A (**1**–**3, 7**). Yet the Ring B ferrocenyl chalcones show greater activity. Similarly, it would be difficult to use physicochemical differences to explain the difference in activity between the structurally related analogues **4**/**16** and **6**/**18**. The preliminary QSAR study carried out with the Series B ferrocenyl chalcones also shows that size, lipophilicity and electronic factors have a limited role in activity but these results may be due to the small number of compounds investigated ($n = 14$) and the type of physicochemical parameters used in the quantification. If physicochemical properties are not important factors for activity, one may then query the biological character of the ferrocene ring and its role in antimalarial activity. This question remains unanswered but investigations in this direction would be useful in identifying the target of action for these compounds and, ultimately, the design of more effective antimalarial drugs.

Acknowledgements

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References and Notes

1. Delhaes, L; Abessiki, H.; Biot, C.; Berry, L.; Delcourt, P.; Maciejewski, L.; Brocard, L.; Camus, D.; Dive, D. *Parasitol. Res*. **2001**, *87*, 239.
2. Domarle, O.; Blampain, G.; Agnaniet, H.; Nzadiyabi, T.; Lebibi, J.; Delhaes, L; Abessiki, H.; Biot, C.; Berry, L.; Delcourt, P.; Maciejewski, L.; Brocard, J.; Maciejewski, L.; Biot, C.; Georges, A. J.; Millet, P. *Antimicrob. Agents Chemother*. **1998**, *42*, 540.
3. Biot, C.; Glorian, G.; Maciejewski, L.; Brocard, J. *J. Med. Chem*. **1997**, *40*, 3715.
4. Delhaes, L.; Biot, C.; Berry, L.; Maciejewski, L.; Camus, D.; Brocard, J.; Dive, D. *Bioorg. Med. Chem*. **2000**, *8*, 2739.
5. Biot, C.; Delhaes, L.; N'Diaye, C. M.; Maciejewski, L.; Camus, D.; Dive, D.; Brocard, J. *Bioorg. Med. Chem*. **1999**, *7*, 843.
6. Itoh, T.; Shirakami, S.; Ishida, N.; Yamashita, Y.; Yoshida, T.; Kim, H. S.; Wataya, Y. *Bioorg. Med. Chem. Lett*. **2000**, *10*, 1657.
7. Howarth, J.; Hanlon, K. *Tetrahedron Lett*. **2001**, *42*, 751.
8. Hansch, C.; Leo, A. *Substituent Constants for Correlation Analysis in Chemistry and Biology*; John Wiley and Sons: New York, 1979.
9. Liu, W. Y.; Xie, T.; Liang, Y. M.; Liu, W. M.; Ma, Y. X. *J. Organometallic Chem*. **2001**, *627*, 93.
10. Liu, M.; Wilairat, P.; Go, M. L. *J. Med. Chem*. **2001**, *44*, 4443.
11. Series A and B ferrocenyl chalcones (except hydroxyl containing analogues **7**, **8**, **19**) were synthesized in the following way: The substituted ketone (3 mmol) and KOH (0.2 g) were

dissolved in ethanol (5 mL) in a round bottomed flask and stirred at room temperature (28 °C) for 10 min. An ethanolic solution of the substituted aldehyde (3 mmol, 5 mL) was added dropwise and the mixture was stirred at room temperature. The reaction was monitored by TLC on silica gel sheets (with fluorescent indicator) using tetrahydrofuran (THF)–hexane (1:4) as eluting solvent. Reaction time may range from 1 to 24 h. The reaction was stopped by neutralizing the stirred solution with 2 M HCl. In most cases, the product was obtained as a dark red precipitate after neutralization. It was then removed by filtration, washed with water and some cold ethanol. In the absence of a precipitate on neutralization, the solution was extracted with ethyl acetate (20 mL×3). The organic layer was dried with anhydrous Na_2SO_4 and removed by evaporation under reduced pressure to give a liquid residue. The latter was passed through a column of silica gel (230–400 mesh ASTM) and eluted with THF–hexane (1:4). All compounds were characterized by ^{1}H NMR, accurate mass and elemental analyses.

12. Ferrocenyl chalcones **7**, **8** and **19** were synthesized following this procedure: 4-hydroxylacetophenone, 2,4-dihydroxylacetophenone or 4-hydroxylbenzaldehyde (3 mmol), pyridinium *p*-toluenesulfonate (0.2 mmol) and 2H-3, 4-dihydropyran (8 mmol) were dissolved in methylene chloride (10 mL) and stirred for 4 h at room temperature. The reaction mixture was then washed with Na_2CO_3 (1 M, 20 mL×2), the organic layer was separated, dried over anhydrous Na_2SO_4 and concentrated in vacuo to give the crude tetrahydropyranyl ether as a yellow colored product (solid or liquid). The latter was characterized by ^{1}H NMR to confirm its formation and was used without purification for the subsequent reaction with aldehyde or ketone. A methanolic solution of the aldehyde or ketone, protected as the tetrahydropyranyl ether (3 mmol), was added to a stirred solution of the aldehyde/ketone (3 mmol) in methanol (5 mL). Dropwise addition of a methanolic solution of sodium hydroxide (3% w/v, 5 mL) was made, stirring was continued for 24 h and the reaction mixture was worked up as described in ref. 11. Removal of the tetrahydropyranyl groups was effected with acid (4 M HCl, 2 mL), which was added to a stirred solution of the crude product in ethanol (10 mL), stirred (4 h, room temperature) and then diluted with water (40 mL). The mixture was extracted with ethyl acetate (50 mL×3) and the combined organic phases were concentrated in vacuo to give the crude hydroxylated chalcone. The crude product was purified by column chromatography using silica gel (230–400 mesh ASTM) and THF–hexane (1:4) as mobile phase. All compounds were characterized by ^{1}H NMR, accurate mass and elemental analyses.

13. Kubinyi, H. In *Burger's Medicinal Chemistry and Drug Discovery*; Wolff, M., Ed.; John Wiley and Sons: New York, 1995; Vol. 1, p 497.

14. Multiple linear regression analyses were carried out using SPSS 11 (SPSS Inc., Chicago, IL, USA).