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Design, synthesis, and antimalarial activity of structural chimeras of thiosemicarbazone and ferroquine analogues

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Abstract—The design, synthesis, and antimalarial activity of chimeras of thiosemicarbazones (TSC) and ferroquine (FQ) is reported. Key structural elements derived from FQ were coupled to fragments capable of coordinating metal ions. Biological evaluation was conducted against four strains of the malaria parasite *Plasmodium falciparum* and against the parasitic cysteine protease falcipain-2. To establish the role of the ferrocenyl moiety in the antiplasmodial activity of this series, purely organic parent compounds were also synthesized and tested. The presence of the aminoquinoline structure, allowing transport of the compounds to the food vacuole of the parasite, seems to be the major contributor to antimalarial activity.

Following the discovery of the antimalarial drug candidate ferroquine (FQ, SR97193, Fig. 1),[1,2] there has been an intense interest in the synthesis and evaluation of its analogues and/or derivatives.[3–5] For the last several years, we have been working in this bioorganometallic area with the aim of developing "back-up" 4-aminoquinolines.[6]

On the other hand, derivatives of thiosemicarbazones (TSC, Fig. 1) have shown potent antimalarial activities.[7,8] Due to their intrinsic metal (e.g. iron) chelating properties, the mechanism of action of TSCs is believed to result from the generation of reactive oxygen radicals.[9] Nevertheless, the toxicity of the TSCs in *Plasmodium berghei* infected mice precluded their development as antimalarial therapeutics.[10] Renewed interest in TSCs has arisen following the discovery of new leads that kill species of protozoan parasites through the inhibition of cysteine proteases as well as other novel targets.[11,12]

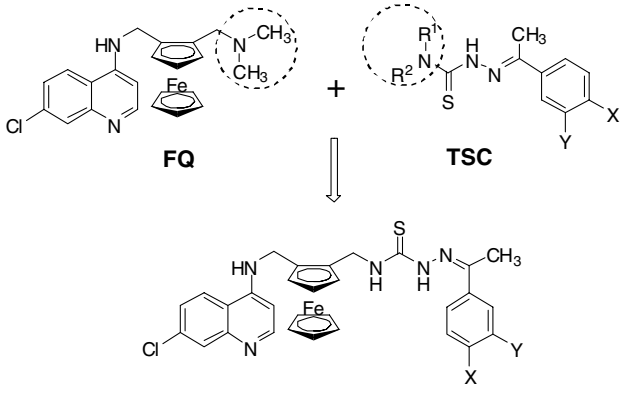

Figure 1. Development of structural chimeras based on ferroquine (FQ) and thiosemicarbazones (TSC). The dashed circles indicate the merged groups.

Keywords: Malaria; Antimalarial agents; Chimeras; Ferroquine; Thiosemicarbazones; Falcipain-2; Bioorganometallics.
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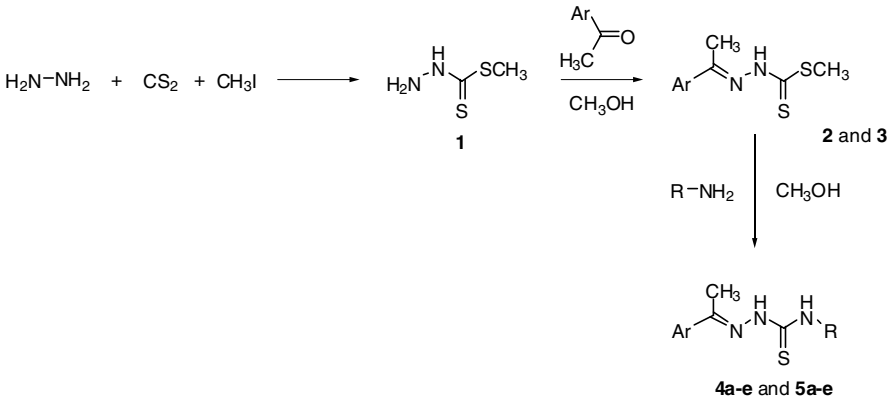


Scheme 1. Typical synthetic procedure of thiosemicarbazone-ferrocene conjugates. Ar is 1-(3,4-dichlorophenyl)ethanone or 1-(4-bromophenyl)ethanone.

In this study, a chimeric ligand approach (Fig. 1) was used to combine the properties of FQ (accumulation in the food vacuole of the parasite via the 4-aminoquinoline nucleus and high antimalarial activity due to the metallocenic fragment) and of the selected TSC.

In principle this strategy is aimed at either preventing or slowing down the emergence of drug resistance and is based on covalent binding between two active fragments. Potent antimalarial activities have been reported for hybrid molecules based on trioxanes and chloroquine,[13,14] amodiaquine and naphthoquinone,[15,16] trifluoromethylartemisinin and mefloquine,[17] as well as artemisinin and quinine.[18]

In order to compare the contribution of each fragment, the corresponding ferrocenic derivatives **4a**, **4b** and **5a**, **5b** (without the 4-aminoquinoline moiety) and the purely organic compounds **4d–e** and **5d–e** were also synthesized.

The target molecules were synthesized according to the general synthetic procedure as shown in Scheme 1.[19] The methyl hydrazinecarbodithioate **1** was synthesized from commercially available hydrated hydrazine, carbon disulfide, and methyl iodide.[7] Condensation of **1** with the appropriate acetophenone in methanol afforded the thiosemicarbazone thioesters **2** and **3**. The choice of the acetophenone was based on a previous study.[20] Finally, nucleophilic substitution with the primary amines R-NH_2 under conditions previously reported[21] provided the thiosemicarbazones **4a–e** and **5a–e** (Table 1).

The new compounds were evaluated in vitro against four different *Plasmodium falciparum* strains (Table 2) from different geographical and antimalarial resistance backgrounds (3D7 originates from Africa, W2 from Indochina, FCR3 from Gambia, and Bre1 from Brazil).[22]

In order to determine potential targets for this class of compounds in *P. falciparum*, selected compounds were also tested against the parasitic cysteine protease falcipain-2 (Table 3).[23] As described previously, potent inhibitors of falcipain-2 block hemoglobin hydrolysis,

Table 1. Chemical structures of the hybrid molecules and reference compounds

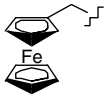
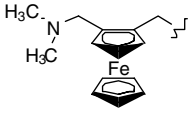
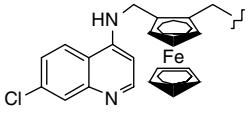
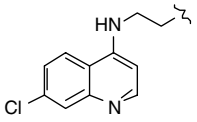
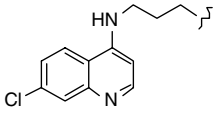
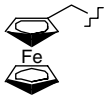
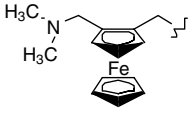
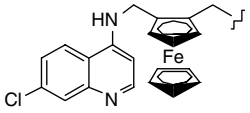
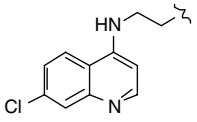
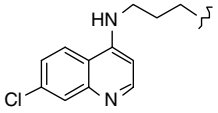
Compound	Ar		R	Yields (%)
	X	Y		
4a	Cl	Cl		62
4b	Cl	Cl		18
4c	Cl	Cl		15
4d	Cl	Cl		94
4e	Cl	Cl		97
5a	Br	H		75
5b	Br	H		37
5c	Br	H		15
5d	Br	H		99
5e	Br	H		99

Table 2. In vitro susceptibilities of *P. falciparum* strains in nM (gray) and a μM (white)

Strain	3D7		W2		FCR3		Bre1	
Compound	IC_{50} SD[a]	IC_{90} SD	CI_{50} SD	CI_{90} SD	CI_{50} SD	CI_{90} SD	CI_{50} SD	CI_{90} SD
CQ	23.9	41.7	540.0	991.8	645.0	1175.0	396.2	721.8
	4.6	9.6	87.5	188.9	63.1	154.9	54.2	73.9
FQ	3.5	6.5	7.1	13.2	1.8	3.5	3.0	5.9
	0.5	0.9	1.8	2.1	0.3	0.6	1.0	2.1
4a	30.2	52.5	95.8	149.2	74.0	133.2	46.0	85.8
	6.1	7.2	20.4	14.4	15.4	17.5	14.4	26.4
4b	33.0	49.7	28.3	46.2	31.2	49.7	34.0	52.5
	6.4	8.5	9.9	13.4	5.8	9.3	8.1	16.8
4c	0.2	2.0	0.8	16.2	0.2	1.6	1.0	4.9
	0.1	0.9	0.2	4.5	0.1	0.7	0.4	2.6
4d	0.3	2.4	0.3	1.3	0.6	1.7	0.3	1.6
	0.2	0.6	0.1	0.5	0.1	0.5	0.1	0.5
4e	0.1	1.4	0.2	2.2	0.8	3.5	0.6	3.5
	0.0	0.5	0.1	0.7	0.2	1.8	0.2	1.8
5a	64.2	117.8	68.2	119.3	30.2	49.5	33.0	55.0
	12.8	18.7	10.6	22.0	5.6	7.9	8.5	23.3
5b	20.7	45.5	21.8	35.7	22.2	32.0	17.0	31.5
	6.3	10.3	7.9	11.6	5.9	7.2	3.5	5.9
5c	0.2	1.3	0.2	0.8	0.8	2.3	0.8	2.2
	0.1	0.6	0.1	0.2	0.2	1.0	0.2	0.4
5d	0.3	26.3	0.3	13.3	0.5	24.2	0.4	21.5
	0.1	7.7	0.1	5.2	0.1	6.3	0.1	7.1
5e	0.6	25.8	2.0	48.3	0.5	2.4	0.7	12.0
	0.1	8.1	0.4	22.3	0.2	0.6	0.1	3.2

[a]SD, standard deviation.

Table 3. Effects on the activity of falcipain-2 and FV of *P. falciparum* W2 strain

Compound	falcipain-2 IC_{50} (nM)	SD[a]	Food vacuole abnormality
4a	8130	1307	ND
4b	14,250	877	ND
4c	2415	212	—
4d	4416	1870	741
4e	>20,000		—
5a	>20,000		—
5b	4250	1628	—
5c	1072	95	—
5d	12,932	5697	—
5e	>20,000		—

[a] SD, standard deviation.

leading to swollen, dark staining parasite food vacuoles.[24]

Compounds **4b–e** and **5b–e** were much more active than the first ferrocenyl TSC hits,[20] **4a** and **5a**, with activity in the low micromolar range. Introduction of the amino side chain, in compounds **4b** and **5b** compared to compounds **4a** and **5a**, slightly increased the antimalarial activity. These data suggest that the basic amino group may add potency, probably by assisting transport to the acidic food vacuole of the parasite. The low inhibitory potency against falcipain-2 (see Table 3), which did not correlate with the antimalarial activity, suggests that other mechanisms of inhibition are involved, as previously suggested.[25]

As expected, chimeras of TSC and FQ analogues (**4c** and **5c**) were the most active derivatives of this series against the different strains of *P. falciparum*. Nevertheless, the corresponding purely organic derivatives (**4d**, **4e**, **5d**, and **5e**) showed comparable potency. Contrary to previous results,[1–6,26] introduction of the ferrocene moiety did not increase antimalarial activity.

Interestingly, the rigid metallocenic compounds (**4c** and **5c**) derived from FQ showed better inhibition of falcipain-2 compared to the corresponding flexible alkyl analogues (**4d**, **4e**, **5d**, and **5e**).

It is intriguing that the typical food vacuole abnormality associated with inhibitors of falcipain-2 was observed with only compound **4d**. These results suggest that the inhibitors may have two different modes of action, one hitting falcipain-2 and another acting independently. At this point, we can speculate that these compounds preferentially concentrate in the food vacuole, explaining better parasite than enzyme IC_{50}s.

Previously, it has been demonstrated that FQ has reduced affinity for mutant forms of the *P. falciparum* chloroquine resistance transporter (PfCRT), a protein located in the parasite digestive vacuole and involved in drug transport and chloroquine resistance.[27–29] Specific mutations in PfCRT mediate resistance to chloroquine.[30] However, once again, there was no significant difference in the activity of ferrocenyl compounds between chloroquine susceptible and chloroquine-resistant parasites.

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19. Details of the typical synthetic procedure and spectroscopic data of the representative conjugates **4c** and **5c** are displayed as follows. The thiosemicarbazone thioester **2** (76 mg, 0.18 mM) (or **3**, 173 mg, 0.57 mM) was refluxed with the corresponding amine (R-NH_2) in methanol (5 mL) for 4 h. After cooling, the solvent was removed under reduced pressure. *N*-{2-[(7-Chloroquinolin-4-ylamino)methyl]ferrocenyl}-2-[1-(3,4-dichlorophenyl)ethylidene]-hydrazinecarbothioamide **4c**. The crude product was purified by silica-gel chromatography (6:2:1, ethyl acetate:petroleum ether: triethylamine) to give **4c** (18 mg, 0.03 mM, 15%) as a yellow solid. Mp 98–102 °C. ^{1}H NMR (300 MHz; $CDCl_3$) δ 8.43 (d, 1H, J = 5.37 Hz), 7.83 (d, 1H, J = 1.98 Hz), 7.74 (d, 1H, J = 2.13 Hz), 7.60 (d, 1H, J = 8.85 Hz), 7.40 (d. 1H, J = 8.46 Hz), 7.25 (d, 1H, J = 8.55 Hz), 7.06 (dd, 1H, J = 2.01 and 8.97 Hz), 6.45 (d, 1H, J = 5.43 Hz), 4.40 (m, 1H), 4.32 (m, 1H), 4.18 (br s, 6H). 4.15 (m, 1H). 4.14 (m, 1H), 3.41 (s, 2H). 2.01 (s, 3H); ^{13}C NMR (75 MHz; $CDCl_3$) δ 208.0, 176.7, 145.2, 136.8, 134.8, 133.9, 132.8, 130.4, 127.9, 125.3, 124.9, 121.8 (2C), 117.0, 98.9, 83.1 (2C), 70.7, 70.3, 69.4 (5C), 67.4, 41.1, 29.7, 13.4; HRMS (electrospray) *m/z* 649: $M^{\circ+}$ $^{35}Cl^{35}Cl^{35}Cl$, 650: MH^+ $^{35}Cl^{35}Cl^{35}Cl$, 651: $M^{\circ+}$ $^{35}Cl^{35}Cl^{37}Cl$, 652: MH^+ $^{35}Cl^{35}Cl^{37}Cl$, 653: $M^{\circ+}$ $^{35}Cl^{37}Cl^{37}Cl$, 655: $M^{\circ+37}Cl^{37}Cl^{37}Cl$. *N*-{2-[(7-Chloroquinolin-4-ylamino)methyl]ferrocenyl}-1-[1-(3-bromophenyl)ethylidene]-hydrazinecarbothioamide **5c**. The crude product was purified by silica-gel chromatography (6:2:1. ethyl acetate:petroleum ether: triethylamine) to give **5c** (60 mg, 0.09 mM, 15%) as a yellow solid. Mp 103–105 °C. ^{1}HNMR (300 MHz; $CDCl_3$) δ 8.40 (d, 1H, J = 4.99 Hz), 7.78 (d, 1H, J = 2.34 Hz), 7.66 (m, 1H), 7.61 (s, 1H), 7.60 (d, 1H, J = 7.51 Hz), 7.40 (d, 1H, J = 7.99 Hz), 7.32 (d, 1H, J = 7.52 Hz), 7.09 (dd, 1H, J = 1.88 and 9.45 Hz), 6.45 (d, 1H, J = 5.30 Hz), 4.46 (m, 1H). 4.30 (m, 1H), 4.16 (br s, 6H), 4.15 (m, 1H), 4.14 (m, 1H), 3.65. (s, 2H), 2.01 (s, 3H); ^{13}CNMR (75 MHz; $CDCl_3$) δ 211.7,176.7 (2C), 151.8, 149.1 (2C), 146.1, 138.9, 132.7, 130.0, 129.1, 124.9, 122.7, 121.7, 117.0, 99.0, 83.3 (2C), 70.6, 70.1, 69.5 (5C), 67.4, 46.1, 41.0, 29.7, 13.5; HRMS (electrospray) *m/z* 659: $M^{\circ+}$ $^{35}Cl^{79}Br$, 661: $M^{\circ+}$ $^{35}Cl^{81}Br$ and $^{37}Cl^{79}Br$, 663: $M^{\circ+}$ $^{37}Cl^{81}Br$.
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22. Chloroquine diphosphate was purchased from Sigma. Ferroquine base (SR97193) was obtained from Sanofi-Aventis (France). The antimalarial activity of all the synthesized compounds was tested against *P. falciparum* strains 3D7 (Africa), W2 (Indochina), FCR3 (Gambia), and Bre1 (Brazil) (one chloroquine-susceptible and three chloroquine-resistant strains, respectively) and compared with that of chloroquine and ferroquine. Parasites were maintained in culture in RPMI 1640 (Invitrogen, Paisley, United Kingdom) supplemented with 10% human serum (Abcys S.A., Paris, France) and buffered with 25 mM Hepes and 25 mM $NaHCO_3$. Parasites were grown in A-positive human (Etablissement Français du Sang, France) under a controlled atmosphere (10% O_2, 5% CO_2, and 85% N_2 at 37 °C, humidity 95%). FQ and thiosemicarbazone-ferroquine hybrids were dissolved in DMSO 1% (v/v) in RPMI. Twofold serial dilutions with final concentrations ranging from 0.01 to 200 μM were prepared in DMSO 1% in RPMI and distributed into Falcon 96-well plates just before use. For in vitro isotopic microtests 25 μL/well of the molecules and 200 μL/well of the suspension of parasitized erythrocytes (final parasitemia 0.5–1%, mostly young trophozoites; final hematocrit 1.5%) were distrib-

uted in 96-well plates. Parasite growth was assessed by adding 1 μCi of [^{3}H]hypoxanthine with a specific activity of 14.1 Ci/mmol (Perkin-Elmer, Courtaboeuf, France) to each well. The plates were then incubated for 42 h in the controlled atmosphere previously described. Immediately after incubation plates were frozen and then thawed to lyse erythrocytes. The content of each well was collected on filter microplates (Unifilter GF/B; Perkin-Elmer) and washed by using a cell harvester (Filter-Mate Cell Harvester; Perkin-Elmer). Filter microplates were dried, and 25 μL of scintillation cocktail (Microscint O; Perkin-Elmer) was placed in each well. Radioactivity incorporated by the parasites was measured with a scintillation counter (Top Count; Perkin-Elmer). The IC_{50} (the drug concentration leading to 50% of the uptake of [^{3}H]hypoxanthine by parasites in drug-free control wells) was determined by nonlinear regression analysis of log-dose/response curves.

23. Assays of recombinant falcipain-2 activity and of abnormal food vacuole morphology were performed as previously described. Shenai, B. R.; Lee, B. J.; Alvarez-Hernandez, A.; Chong, P. Y.; Emal, C. D.; Neitz, R. J.; Roush, W. R.; Rosenthal, P. J. *Antimicrob. Agents Chemother.* **2003**, *47*, 154.
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