

Enhancement of the Antimalarial Activity of Ciprofloxacin Using a Double Prodrug/Bioorganometallic Approach

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Abstract: The derivatization of the fluoroquinolone ciprofloxacin greatly increases its antimalarial activity by combining bioorganometallic chemistry and the prodrug approach. Two new achiral compounds **2** and **4** were found to be 10- to 100-fold more active than ciprofloxacin against *Plasmodium falciparum* chloroquine-susceptible and chloroquine-resistant strains. These achiral derivatives killed parasites more rapidly than did ciprofloxacin. Compounds **2** and **4** were revealed to be promising leads, creating a new family of antimalarial agents.

The burden of malaria to health and the economy in endemic areas^{1,2} and the emergence of parasite strains resistant to several antimalarials^{2,3} underscore the importance of developing new and/or improved therapeutic strategies against this infection. An initial study reported that quinolones and fluoroquinolones were active against *P. falciparum*.⁴ Their mode of action was first studied in bacteria and mycobacteria, and they were shown to affect bacterial DNA by targeting topoisomerases II and IV.^{5,6} The subsequent discovery of an apicoplastic prokaryotic-like organelle in *Toxoplasma* and *Plasmodium*⁷ increased interest in elucidating further the mode of action of ciprofloxacin (CIPRO[®]) on these parasites. In this context, it has been shown that, as with *T. gondii*, CIPRO (Figure 1) affects *P. falciparum* by causing the formation of abnormal apicoplasts and a “delayed death” of treated parasites.^{8,9} The (fluoro)quinolones products tested against *P. falciparum* strains (3D7 or NF54-R) all exhibited IC₅₀ ≥ 10 μM (the most active were grepafloxacin, gatifloxacin, and moxifloxacin; Figure 1).¹⁰ Additional studies have shown that halogenated alkyl-, alkoxy-4(1*H*)-, and 1-hydroxy-2docecyl-4(1*H*)-quinolones were active in vitro against chloroquine-susceptible (D6) and chloroquine-resistant (Dd2) *P. falciparum* strains.¹¹ Unlike classical fluoroquinolones that

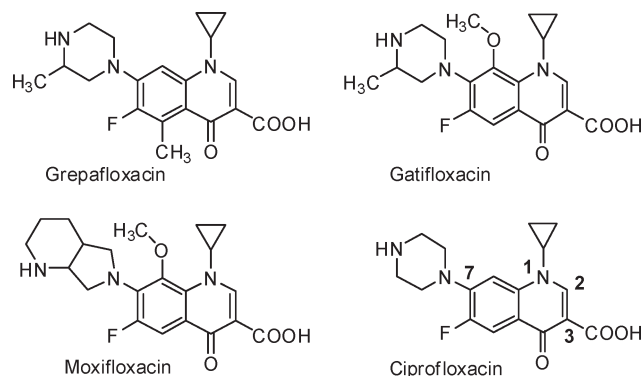


Figure 1. Chemical structures of grepafloxacin, gatifloxacin, moxifloxacin, and ciprofloxacin.

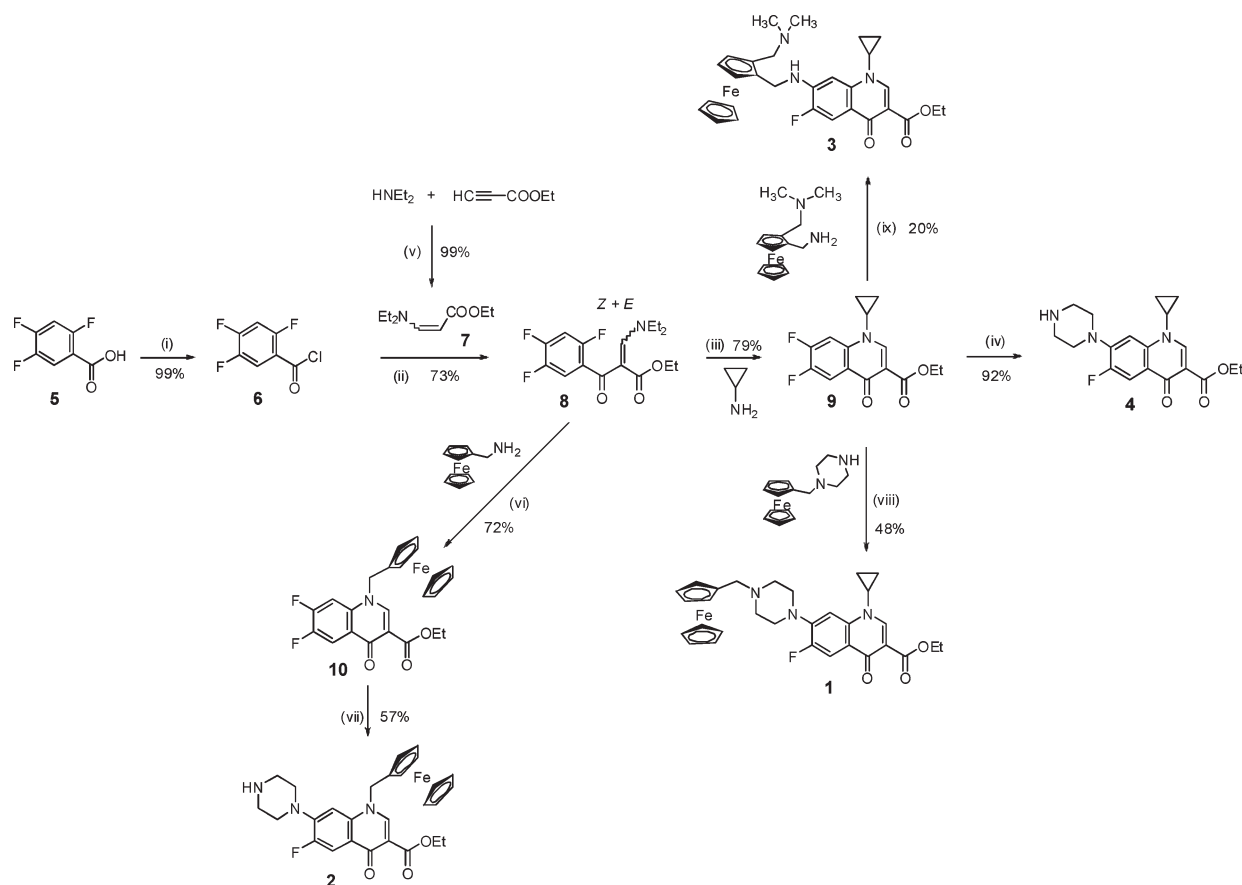
are thought to inhibit the plastid gyrase, these classes of quinolones target the parasite's cytochrome bc1 complex and NADH dehydrogenase, respectively.^{11b}

In the search for new therapeutic agents, our previous studies have shown that the incorporation of a ferrocene core into chloroquine (CQ) can lead to ferrocenyl complexes with a strong antiplasmodial effect on CQ-resistant strains of *P. falciparum*.¹² This approach led to the development of a ferrocene CQ conjugate (ferroquine, FQ, SR97193), currently in phase IIb clinical trials.¹³ From the structure–activity studies of FQ, it appeared that the increase in the hydrophobicity of the drug has a predominant role in the enhancement of its antimalarial activity. In the case of CIPRO, the strategy of increasing its hydrophobicity, and consequently its activity toward *P. falciparum*, has not been tested so far. Hence, we designed new CIPRO derivatives bearing a ferrocenyl substituent at position N(1) or at C(7) of the quinolone ring. In parallel, to enhance the hydrophobic capacity of these products, we adopted a prodrug strategy consisting of the ethyl esterification of the carboxylic acids.¹⁴ In this report, we compare the antimalarial activity and cytotoxicity of these derivatives to those of CIPRO and CIPRO ethyl ester to identify an effective strategy to improve the antimalarial activity of CIPRO.

The ferrocenic fluoroquinolones **1–3** were synthesized according to a procedure adapted from the method of Cecchetti et al. (Scheme 1).¹⁵ Commercially available 2,4,5-trifluorobenzoic acid **5** reacts with Vilsmeier–Haack reagent (oxalyl chloride and dimethylformamide (DMF)) in dichloromethane to afford **6** in quantitative yield. The acyl chloride **6** is then condensed with ethyl 3-(diethylamino)acrylate **7** (freshly obtained from ethyl propiolate and *N,N*-diethylamine) in a mixture of toluene/triethylamine to provide the cetoester **8** in 73% yield. ¹H and ¹³C NMR spectra revealed that **8** exists in a 1:9 ratio of *E* to *Z* stereoisomers. Transaminolysis of **8** with cyclopropylamine and successive cyclization with potassium carbonate in DMF gave the key intermediate **9** in 79% yield. The fluoroquinolones **1**, **3**, and **4** were then obtained by coupling the difluoroquinolone **9** in acetonitrile with the corresponding amines¹⁶ (1 week, under reflux) in yields varying from 20% to 92%. Target **2** was synthesized in a similar fashion. Transaminolysis of **8** with ferrocenylmethylamine and cyclization with K₂CO₃ in DMF gave the difluoroquinolone **10** in 72% yield. The slow nucleophilic aromatic

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^a Abbreviations: CIPRO, ciprofloxacin; CQ, chloroquine; FQ, ferroquine; DMF, dimethylformamide; ASU, artesunate; DOX, doxycycline; QN, quinine; IC₅₀, inhibitory concentration at 50%; MQ, mefloquine.

Scheme 1. Synthesis of Target Compounds 1–4^a

^a Reagents and conditions: (i) (COCl)₂, DMF, dichloromethane, N₂ atm, room temp, 24 h; (ii) toluene, Et₃N, N₂ atm, reflux, 24 h; (iii) EtOH/Et₂O (1/2), room temp, 3 h, then DMF, K₂CO₃, reflux, 12 h; (iv, vii, viii, and ix) CH₃CN, reflux, 48 h to 1 week; (v) CH₃CN, N₂ atm, room temp, 24 h; (vi) EtOH/Et₂O (1/2), room temp, 3 h, then DMF, K₂CO₃, reflux, 4 h.

Table 1. In Vitro Antimalarial Activity against CQ-Susceptible (3D7) and CQ-Resistant (W2) *P. falciparum* Strains after Exposures of 48 and 96 h and in Vitro Cytotoxicity in Mice Spleen Cells^a

compd	IC ₅₀ (μM) after 48 h		IC ₅₀ (μM) after 96 h		cytotoxicity (μM)	therapeutic index	
	3D7	W2	3D7	W2		a	b
1	2.7 ± 0.4	3.8 ± 0.7	2.6 ± 0.2	2.9 ± 0.4	> 40	> 14.8	> 10.5
2	1.7 ± 0.2	1.5 ± 0.2	1.0 ± 0.2	0.8 ± 0.2	12.9 ± 2.2	7.6	8.6
3	2.8 ± 0.5	2.2 ± 0.9	3.2 ± 0.3	2.5 ± 0.3	13 ± 2.8	4.6	5.9
4	3.1 ± 0.4	3.9 ± 0.6	1.6 ± 0.4	3.1 ± 0.4	> 40	> 12.9	> 10.2
CIPRO	45.9 ± 3.8	119.3 ± 17.8	10.1 ± 3.4	14.1 ± 4.3	> 40	> 0.8	> 0.3
DOX	12.0 ± 1.4	10.7 ± 2.1	1.7 ± 0.5	1.2 ± 0.3	n.d.	n.d.	n.d.
compd	IC ₅₀ (nM) after 48 h		IC ₅₀ (nM) after 96 h		cytotoxicity (μM)	therapeutic index	
	3D7	W2	3D7	W2		a	b
CQ	19 ± 4	480 ± 50	21 ± 3	462 ± 57	3.46	182	7.2
ASU	1.7 ± 0.4	2.4 ± 0.7	2 ± 0.5	2.1 ± 0.8	nd	nd	nd
QN	186 ± 20	520 ± 42	162 ± 22	456 ± 61	nd	nd	nd

^a IC₅₀ values are the mean of five experiments for each strain ± standard deviation. DOX = doxycycline; CQ = chloroquine, ASU = artesunate, QN = quinine; therapeutic index a = cytotoxicity/3D7 IC₅₀; therapeutic index b = cytotoxicity/W2 IC₅₀ after 48 h; nd = not determined.

substitution of **10** by piperazine in acetonitrile at reflux produced **2** in 57% yield.

Antiplasmodial activities were first determined against the CQ-susceptible (3D7) and the CQ-resistant (W2) *P. falciparum* strains. The antiplasmodial activities of **1–4** are shown in Table 1, along with the values for the standard drug CIPRO, CQ, artesunate (ASU), doxycycline (DOX), and quinine (QN). Compounds **1–4** showed micromolar antiplasmodial activity against both strains. The activities of **1–4** are remark-

ably constant whatever the strains' level of resistance to CQ. This is not surprising, as the molecular targets of CQ and of the (fluoro)quinolone derivatives are very likely to be different. From the above data, three main conclusions can be drawn: (1) ethyl esterification of CIPRO (**4**) resulted in a dramatic increase in activity after 48 h of contact (15-fold for 3D7 and 30-fold for W2); (2) the exchange of the cyclopropyl group by a ferrocenylmethyl moiety (comparison of **2** and **4**) resulted in an additional gain of about 2-fold in activity,

Table 2. In Vitro Antimalarial Activity against Nine *P. falciparum* Strains after Exposure for 48 h

strains	IC ₅₀ ^a					
	2 (μM)	CIPRO (μM)	DOX (μM)	CQ (nM)	QN (nM)	ASU (nM)
3D7	1.7 ± 0.2	45.9 ± 3.8	12.0 ± 1.4	19 ± 4	186 ± 20	1.7 ± 0.4
W2	1.5 ± 0.2	119.3 ± 17.8	10.7 ± 2.1	480 ± 50	520 ± 42	2.4 ± 0.7
IMT 10500	2.36 ± 1.1	74.8 ± 9.4	13.3 ± 3.8	51 ± 18	314 ± 101	3.8 ± 0.4
IMT L1	2.0 ± 0.4	41.3 ± 6.4	8.4 ± 1.7	250 ± 48	543 ± 71	1.7 ± 0.6
IMT Vol	0.92 ± 0.1	51.6 ± 6.8	9.1 ± 2.1	267 ± 26	428 ± 69	3.0 ± 0.7
PA	1.32 ± 0.1	45.5 ± 2.4	12.9 ± 4.0	284 ± 46	571 ± 158	2.0 ± 0.5
FCR3	0.72 ± 0.1	74.3 ± 2.5	9.7 ± 2.3	501 ± 155	650 ± 143	2.1 ± 1.1
FCM29	0.44 ± 0.2	45.6 ± 1.5	11.06 ± 2.5	508 ± 63	5.8 ± 125	1.9 ± 0.5
IMT K2	1.35 ± 0.3	80.8 ± 5.8	14.9 ± 3.6	523 ± 89	6.7 ± 59	1.8 ± 0.7

^a Values are the mean IC₅₀ of five experiments for each strain ± standard deviation.

provoking a decrease in the IC₅₀ of product **2** to the sub-micromolar range; (3) all of the new compounds are more active than CIPRO and DOX against both strains (Table 1). These data also show that there was no significant difference in the IC₅₀ for products **1** and **4**. This suggests that **1** could be metabolized to **4** by loss of the stable metallocenylcarbenium ion. Further studies will be required to validate this pathway.

Clearly, modulation of the antimalarial activity of CIPRO is a consequence of its “double” derivatization as a prodrug and as a ferrocene conjugate. More importantly, it seems that the prodrug strategy makes the main contribution to the improvement in activity. Under their prodrug forms, all these compounds are more lipophilic than the parent molecule and are therefore more able to traverse the multiple membranes present in the intracellular parasite and its plastid to reach their potential targets. It is noted that potential targets for (fluoro)quinolones, principally gyrase and topoisomerase IV, have recently been annotated in the *Plasmodium* genome.^{17,18} Thus, we can speculate that the target could be gyrase, which would lead to a rapid toxic effect of the prodrug molecules on parasite replication. It is noted, however, that topoisomerase IV was considered the preferential target of CIPRO, which may explain the delay in parasite death¹⁷ that we noted. Hydrophobicity is increased in **2** by replacement of the cyclopropyl group by a ferrocenylmethyl moiety and contributes to a higher activity of **2** compared to **4**.

Next, the most active derivative, **2**, was further tested against a larger panel of well characterized *P. falciparum* laboratory strains or strains obtained from isolates grown in culture for an extended period of time (Table 2). Compound **2** exhibited a higher level of activity than the parent compound. The IC₅₀ for **2** ranged from 0.44 to 3.90 μM, with a mean of 1.79 μM (standard deviation of ±1.16 μM). In vitro cross-resistance was measured by the pairwise correlation of IC₅₀ for all nine strains. The data presented in Table 2 did not reveal any significant correlation between **2** and CIPRO ($r^2 = 0.014$), between **2** and DOX ($r^2 = 0.052$), between **2** and QN ($r^2 = 0.015$), or between **2** and ASU ($r^2 = 0.074$). However, CQ and **2** were slightly correlated ($r^2 = 0.421$).

As many compounds have been identified and reported to demonstrate chemosensitization against malaria parasites,¹⁹ the lead fluoroquinolone **2** was tested in association with quinoline-containing antimalarials such as CQ, QN, MQ, monodesethylamodiaquine (MDAQ), or dihydroartemisinin (DHA) against the nine *P. falciparum* strains.

In an earlier study, Andrade et al. claimed to have found a synergistic effect between CIPRO and, respectively, artemisinin and MQ in in vitro assays on *P. falciparum* and in vivo tests on *P. berghei*.¹⁹ Their conclusions were based on data obtained with single combinations of drugs. In the present

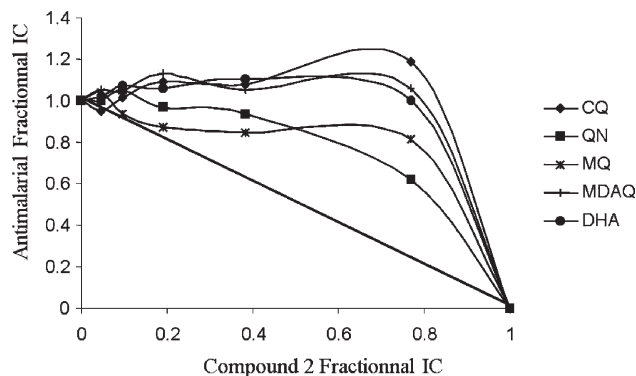


Figure 2. Isobolograms of the in vitro interaction between **2** and CQ, QN, MQ, MDAQ, and DHA against the W2 strain of *P. falciparum*. The diagonal line indicates the hypothetical additive effect. A concave curve indicates synergism, a straight line shows an additive response, and a convex curve is evidence of antagonism.

study, derivative **2** was tested with known antimalarials according to the isobologram method, which enables the exploration of a wide range of drug combinations.²⁰

Surprisingly, isobologram analysis showed that **2** exerts a marked antagonistic effect on the two *P. falciparum* strains 3D7 (resistant in vitro to mefloquine, MQ) (Supporting Information) and W2 (resistant in vitro to CQ, QN, and MDAQ) (Figure 2). The same data were observed with the seven other *P. falciparum* strains (data not shown). It can be postulated that **1–4** could be active upon plastid functions whereas CQ, QN, MQ, MDAQ, and DHA are involved in inhibition of pigment biocrystallization in the digestive vacuole of the parasite. Since the targets are distinct, an additive effect would be expected. This was not observed under our experimental conditions. Hence, it could be speculated that the observed antagonism may be related to a possible relationship between metabolic pathway(s), yet to be determined, between the apicoplast and the digestive vacuole which could in turn influence the mode of action of the drugs tested in combination. The link between the two organelles is illustrated by the synthesis of the iron sulfur clusters in the apicoplast,²¹ known to be involved in the active site of aconitase, and the presence of an aconitase activity in the digestive vacuole.²¹

Next, the in vitro toxicity of **1–4** was tested using mouse spleen cells (Table 1). Compound **2** was found to be more toxic than the parent drug CIPRO but less toxic than CQ. Moreover, the selectivity index of **2** remained at about 8 independent of the resistance level of the *P. falciparum* strains used, while the selectivity index of CQ dropped from 182 to 7 for CQ-susceptible and CQ-resistant *P. falciparum* strains.

Compound **4** also appears very interesting, as it is less toxic than **2** for an activity only 2-fold lower.

In conclusion, the first series of ferrocenic fluoroquinolones related to CIPRO has been successfully prepared and characterized. Our approach shows that the prodrug strategy resulted in a major increase of CIPRO antimalarial activity and that the bioorganometallic strategy led to an additional improvement in activity, allowing the identification of two promising hits, **2** and **4**. In vitro results have to be confirmed in vivo to determine the bioavailability of these two molecules and their potential interest as new antimalarials. Because of the potential cytotoxicity of these compounds, a program will be initiated to identify new derivatives at least as active but less toxic. Our ciprofloxacin derivatives represent a promising family to mine new potential antimalarials.

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Supporting Information Available: Details of the synthesis and characterization of conjugates **1–3**; protocols for in vitro experiments; isobolograms of the in vitro interaction between **2** and CQ, QN, MQ, MDAQ, and DHA against the 3D7 strain of *P. falciparum*. This material is available free of charge via the Internet at <http://pubs.acs.org>.

References

- (1) Hay, S. I.; Guerra, C. A.; Gething, P. W.; Patil, A. P.; Tatem, A. J.; Noor, A. M.; Kabaria, C. W.; Manh, B. H.; Elyazar, I. R.; Brooker, S.; Smith, D. L.; Moyeed, R. A.; Snow, R. W. A world malaria map: *Plasmodium falciparum* endemicity in 2007. *PLoS Med.* **2009**, *6*, No. e1000048.
- (2) Price, R. N.; Douglas, N. M.; Anstey, N. M. New developments in *Plasmodium vivax* malaria: severe disease and the rise of chloroquine resistance. *Curr. Opin. Infect. Dis.* **2009**, *22*, 430–435.
- (3) (a) Andrianantoanina, V.; Ratsimbao, A.; Bouchier, C.; Jahevitra, M.; Rabearimanana, S.; Radrianjafy, R.; Andriananjaka, V.; Randrianjato, T.; Rason, T.; Tichit, M.; Rabarijaona, M.; Mercereau-Puijalon, O.; Durand, R.; Ménard, D. *P. falciparum* drug resistance in Madagascar: facing the spread of unusual pfdhfr and pfmdr-1 haplotypes and the decrease of dihydroartemisinin susceptibility. *Antimicrob. Agents Chemother.* **2009**, *53*, 4588–4597. (b) Dondorp, A. M.; Nosten, F.; Yi, P.; Das, D.; Phyto, A. P.; Tarning, J.; Lwin, K. M.; Ariey, F.; Hanpithakpong, W.; Lee, S. J.; Ringwald, P.; Silamut, K.; Imwong, M.; Chotivanich, K.; Lim, P.; Herdman, T.; An, S. S.; Yeung, S.; Singhasivanon, P.; Day, N. P.; Lindegardh, N.; Socheat, D.; White, N. J. Artemisinin resistance in *Plasmodium falciparum* malaria. *N. Engl. J. Med.* **2009**, *361*, 455–467.
- (4) Divo, A. A.; Sartorelli, A. C.; Patton, C. L.; Bia, F. J. Activity of fluoroquinolone antibiotics against *Plasmodium falciparum* in vitro. *Antimicrob. Agents Chemother.* **1988**, *32*, 1182–1186.
- (5) Drlica, K.; Malik, M. Fluoroquinolones: action and resistance. *Curr. Top. Med. Chem.* **2003**, *3*, 249–282.
- (6) Hooper, D. C. Mechanisms of action and resistance of older and newer fluoroquinolones. *Clin. Infect. Dis.* **2001**, *32*, S24–S28.
- (7) McFadden, G. I.; Reith, M.; Munholland, J.; Lang-Unnasch, N. Plastid in human parasites. *Nature* **1996**, *482*, 381.
- (8) Fichera, M. E.; Roos, D. S. A plastid organelle as a drug target in apicomplexan parasites. *Nature* **1997**, *390*, 407–409.
- (9) Dahl, E. L.; Rosenthal, P. J. Multiple antibiotics exert delayed effects against the *Plasmodium falciparum* apicoplast. *Antimicrob. Agents Chemother.* **2007**, *51*, 3485–3490.
- (10) Anquetin, G.; Greiner, J.; Vierling, P. Quinolone-based drugs against *Toxoplasma gondii* and *Plasmodium* spp. *Curr. Drug Targets: Infect. Disord.* **2005**, *5*, 227–245.
- (11) (a) Winter, R. W.; Kelly, J. X.; Smilkstein, M. J.; Dodean, R.; Hinrichs, D.; Riscoe, M. K. Antimalarial quinolones: synthesis, potency, and mechanistic studies. *Exp. Parasitol.* **2008**, *118*, 487–497. (b) Saleh, A.; Friesen, J.; Baumeister, S.; Gross, U.; Bohne, W. Growth inhibition of *Toxoplasma gondii* and *Plasmodium falciparum* by nanomolar concentrations of 1-hydroxy-2-dodecyl-4(1H)quinolone, a high-affinity inhibitor of alternative (type II) NADH dehydrogenases. *Antimicrob. Agents Chemother.* **2007**, *51*, 1217–1222.
- (12) (a) Dive, D.; Biot, C. Ferrocene conjugates of chloroquine and other antimalarials: the development of ferroquine, a new antimalarial. *ChemMedChem* **2008**, *3*, 383–391. (b) Dubar, F.; Khalife, J.; Brocard, J.; Dive, D.; Biot, C. Ferroquine, an ingenious antimalarial drug: thoughts on the mechanism of action. *Molecules* **2008**, *13*, 2900–2907.
- (13) <http://clinicaltrials.gov/ct2/show/NCT00563914>.
- (14) Sissi, C.; Perdoni, E.; Domenici, E.; Feriani, A.; Howells, A. J.; Maxwell, A.; Palumbo, M. Ciprofloxacin affects conformational equilibria of DNA gyrase A in the presence of magnesium ions. *J. Mol. Biol.* **2001**, *311*, 195–203.
- (15) (a) Cecchetti, V.; Tabarrini, O.; Sabatini, S.; Miao, H.; Filippini, E.; Fravolini, A. Studies on 6-aminoquinolones: synthesis and antibacterial evaluation of 6-amino-8-ethyl- and 6-amino-8-methoxyquinolones. *Bioorg. Med. Chem.* **1999**, *7*, 2465–2471. (b) Cecchetti, V.; Fravolini, A.; Palumbo, M.; Sissi, C.; Tabarrini, O.; Termini, P.; Xin, T. Potent 6-desfluoro-8-methylquinolones as new lead compounds in antibacterial chemotherapy. *J. Med. Chem.* **1996**, *39*, 4952–4957. (c) Cecchetti, V.; Fravolini, A.; Lorenzini, M. C.; Tabarrini, O.; Termini, P.; Xin, T. Studies on 6-aminoquinolones: synthesis and antibacterial evaluation of 6-amino-8-methylquinolones. *J. Med. Chem.* **1996**, *39*, 436–445.
- (16) Primary ferrocenyl amines were synthesized according to the following: (a) Biot, C.; Pradines, B.; Sergeant, G.; Gut, J.; Rosenthal, P. Design, synthesis, and antimalarial activity of structural chimeras of thiosemicarbazone and ferroquine analogues. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 6434–6438. (b) Biot, C.; Glorian, G.; Maciejewski, L.; Brocard, J.; Domarle, O.; Blampain, G.; Millet, P.; Georges, A. J.; Abessolo, H.; Dive, D.; Lebibi, J. Synthesis and antimalarial activity in vitro and in vivo of a new ferrocene-chloroquine analogue. *J. Med. Chem.* **1997**, *40*, 3715–3718.
- (17) Dahl, E. L.; Rosenthal, P. J. Apicoplast translation, transcription and genome replication: targets for antimalarial antibiotics. *Trends Parasitol.* **2008**, *24*, 279–284.
- (18) Dar, M. A.; Sharma, A.; Mondal, N.; Dhar, S. K. Molecular cloning of apicoplast-targeted *Plasmodium falciparum* DNA gyrase genes: unique intrinsic ATPase activity and ATP-independent dimerization of PfGyrB subunit. *Eukaryotic Cell* **2007**, *6*, 398–412.
- (19) Andrade, A. A.; de Pilla Varotti, F.; Oliveira de Freitas, I.; Nora de Souza, M. V.; Alves Vasconcelos, T. R.; Boechat, N.; Krettli, A. U. Enhanced activity of mefloquine and artesunate against *Plasmodium falciparum* in vitro and *P. berghei* in mice by combination with ciprofloxacin. *Eur. J. Pharmacol.* **2007**, *558*, 194–198.
- (20) (a) Pradines, B.; Rogier, C.; Fusai, T.; Mosnier, J.; Daries, W.; Baret, E.; Parzy, D. In vitro activities of antibiotics against *Plasmodium falciparum* are inhibited by iron. *Antimicrob. Agents Chemother.* **2001**, *45*, 1746–1750. (b) Pradines, B.; Spiegel, A.; Rogier, C.; Tall, A.; Mosnier, J.; Fusai, T.; Trape, J. F.; Parzy, D. Antibiotics for prophylaxis of *Plasmodium falciparum* infections: in vitro activity of doxycycline against Senegalese isolates. *Am. J. Trop. Med. Hyg.* **2000**, *62*, 82–85.
- (21) Hodges, M.; Yikilmaz, E.; Patterson, G.; Kasvosve, I.; Rouault, T. A.; Gordeuk, V. R.; Loyevsky, M. An iron regulatory-like protein expressed in *Plasmodium falciparum* displays aconitase activity. *Mol. Biochem. Parasitol.* **2005**, *143*, 29–38.