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Development of piperazine-tethered heterodimers as potent antimalarials against chloroquine-resistant *P. falciparum* strains. Synthesis and molecular modeling

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Abstract—The design, synthesis, and antiplasmodial activity of antimalarial heterodimers based on the 1,4-bis(3-aminopropyl)piperazine linker is reported. In this series key structural elements derived from quinoline antimalarials were coupled to fragments capable of coordinating metal ions. Biological evaluation included determination of activity against chloroquine-sensitive and chloroquine-resistant *Plasmodium falciparum* strains. Some of the novel compounds presented high activity in vitro against chloroquine-resistant strains, more potent than chloroquine and clotrimazole. Computational studies revealed that the activity is likely due to the ability of the compounds to assume a multisite iron coordinating geometry.

Malaria is a disease caused by *Plasmodium falciparum* (*Pf*) and represents one of the most widespread and deadly parasitic diseases in man. For over 50 years, chloroquine (CQ (**1**), Fig. 1) has been the drug of choice for malaria chemotherapy, until its efficacy was hampered by the spread of CQ-resistant (CQ-R) *Pf* strains. Consequently, new therapeutic strategies are needed.[1] During the intraerythrocytic phase of *Pf* life cycle, hemoglobin is metabolized within the parasite food vacuole (FV) and the released toxic-free heme is detoxified through formation of hemozoin in the FV or by the glutathione system in the cytoplasm.[2,3] Both the widely used classes of antimalarial drugs, namely 4-aminoquinolines and endoperoxides,[4] are thought to interfere with the heme detoxification process killing *Plasmodium* parasites via free-radical intermediates.

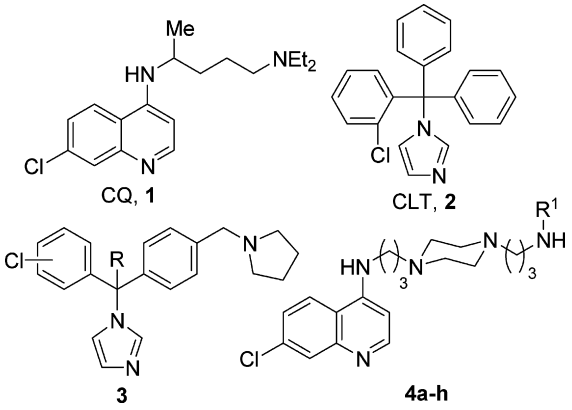

Figure 1. Reference and title compounds.

Keywords: Malaria; Chloroquine; Iron-complexing agents.
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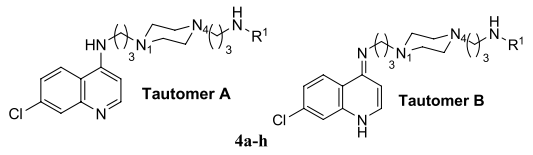
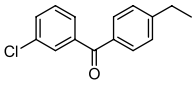
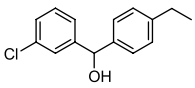
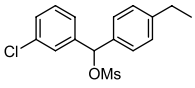
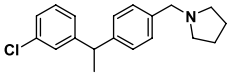
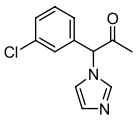
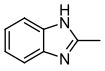
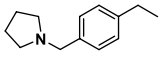
Table 1. Antiplasmodial activity of compounds **4a–h**



Compound	R^1	Prevalent ionic form tautomer A[a]			Prevalent ionic form tautomer B[a]			IC_{50}[d] (μM)			
		pH 7.4	pH 7.2	pH 5.5	pH 7.4	pH 7.2	pH 5.5	D10[b]	W2[c]	3D7[b]	K1[c]
4a		P (56)	DP (48)	TP (85)	DP (79)	DP (73)	TP (94)	26	33	1.6	16
4b		P (56)	DP (50)	TP (85)	DP (81)	DP (73)	TP (94)	145	269	28	135
4c		P (56)	DP (50)	TP (85)	DP (81)	DP (73)	TP (94)	110	98	7.8	447
4d		DP (55)	DP (49)	TeP (84)	TP (67)	TP (68)	TeP (93)	663	495	nt	nt
4e		P (47) N (47)	P (55)	TP (68)	P (69)	P (58)	TP (76)	221	540	40	200
4f		P (51)	DP (51)	TP (54)	DP (66)	DP (58)	TP (57)	1610	1440	nt	nt
4g		P (49)	P (44)	TP (85)	DP (54)	DP (54)	TP (95)	407	535	nt	nt
4h		DP (56)	TP (49)	TeP (85)	TP (80)	TP (73)	TeP (94)	260	207	nt	nt
2 (CLT)	—	N (95)	N (92)	P (81)	—	—	—	550	490	60	250
1 (CQ)	—	P (92)	P (88)	DP (87)	DP (100)	DP (100)	DP (100)	22	280	10	260

nt, not tested.

[a] TeP, tetraprotonated form; TP, triprotonated form; DP, diprotonated form; P, protonated form; N, neutral form (ACD/p*K*a DB version 10.00 software (Advanced Chemistry Development Inc., Toronto, Canada)). Percentage of prevalent ionic form in brackets.

[b] CQ-sensitive clone.

[c] CQ-resistant clone.

[d] Values are means of three experiments, standard deviation is within 10% of the mean.

Iron is an essential nutrient for the asexual erythrocytic phase of the parasite and a considerable number of iron chelators showed antimalarial activity in vitro, apparently through the mechanism of withholding iron from vital metabolic pathways of the intraerythrocytic parasite and through the formation of toxic complexes.

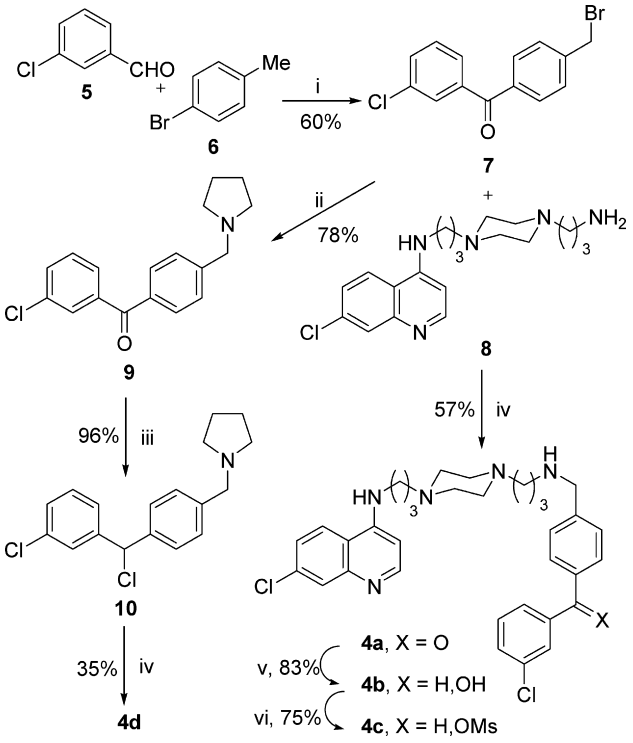
There is evidence that iron chelation therapy with desferrioxamine has clinical activity in both uncomplicated and severe malaria in humans.[5] A promising polycyclic pharmacophore to generate antimalarials is held in clotrimazole (CLT, **2**), a well-known antimycotic drug, endowed with low in vitro antimalarial activity (Table 1). We recently exploited the scaffold of CLT and developed potent antimalarial agents of the general structure **3** characterized by specific electronic features and by improved penetration into the FV.[6]

In order to identify novel antimalarial lead compounds with the potential to interfere with iron metabolic and detoxification pathways, disrupting the peculiar redox equilibria necessary for *Pf* survival, we designed a new series of compounds (**4a**–**h**) in which key structural elements derived from CQ were linked to specific aromatic residues through a piperazine tether.

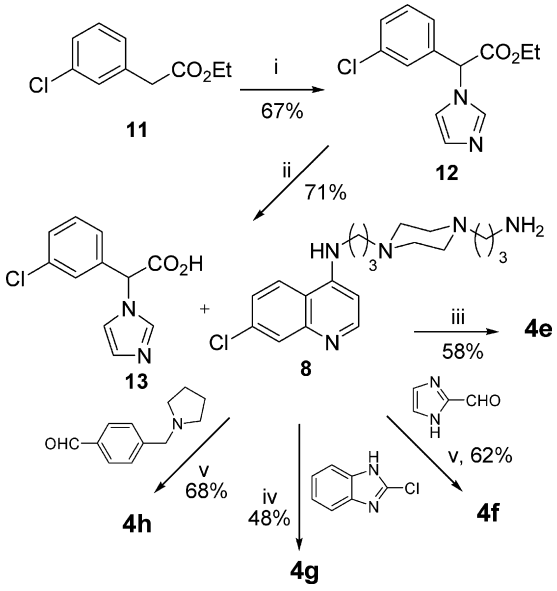
Previously, quinolyl-1,4-bis(3-aminopropyl)piperazines were reported to have in vitro and in vivo antimalarial activity showing a site of action different from that of CQ (cytoplasm and not FV) although their mechanism of action was uncertain.[7,8] Moreover, during the preparation of this report Link and co-workers exploited the same quinolin-1,4-bis(3-aminopropyl)piperazine template for the synthesis of carboxamide antimalarials.[9] In this communication we report the development of new antimalarial heterodimers, typified by **4a** (Table 1), a potent antiplasmodial agent, specifically designed to generate multisite iron coordination antimalarials. The *N*1-(7-chloro-4-quinolyl)-1,4-bis(3-aminopropyl)piperazine was the key structural support of choice to link a panel of aromatic and heteroaromatic groups, characterized by different metal ion coordination and protonatability properties (**4a**–**h**).

The synthesis of compounds **4a**–**d** is described in Scheme 1. The aldehyde **5** was reacted with the Grignard reagent obtained from *p*-bromotoluene **6** to afford the corresponding ketone which was brominated to **7** in good overall yield. Alkylation of *N*1-(7-chloro-4-quinolyl)-1,4-bis(3-amino propyl)piperazine **8**[10] with the bromo-derivative **7** provided the desired compound **4a**. This latter was further modified by reduction to **4b** and subsequently esterified to **4c** by means of methanesulfonyl chloride. To functionalize the bridged methylene of the diphenylmethane moiety (**4d**), compound **7** was transformed to the pyrrolidine derivative **9**, which was converted into the chloro derivative **10** by reduction to the alcohol intermediate followed by chlorination using thionyl chloride. Finally, compound **10** was coupled to **8** providing the desired final derivative **4d**. In Scheme 2 is described the synthesis of compounds **4e**–**h**. Intermediate **8** was coupled with the carboxylic acid derivative **13** to afford amide **4e**. Compound **13** was in turn prepared starting from 3-chlorophenyl acetic acid ethyl ester **11** which was transformed into the imidazole derivative **12** by exposing **11** first to *N*-bromosuccinimide and then to imidazole. Finally, alkaline hydrolysis using LiOH in aqueous THF afforded acid **13**. The synthesis of **4f** and **4h** was accomplished by a reductive amination of the appropriate aldehyde as shown in Scheme 2, by using sodium borohydride as the reducing agent. Finally, compound **4g** was obtained by reacting amine **8** with 2-chlorobenzimidazole in refluxing pentanol.

Compounds **4a**–**h** were tested in vitro against the CQ-S D10 and the CQ-R W2 *Pf* strains and selected compounds were also tested against the CQ-S 3D7 and CQ-R K1 *Pf* strains.[6] The results are reported in Table 1. Coupling of *N*1-(7-chloro-4-quinolyl)-1,4-bis(3-aminopropyl)piperazine to a diphenyl ketone moiety (**4a**), a reported iron chelating group (ConQuest 1.9, CSDS), had beneficial effect on the antimalarial potency, highly improving the activity against both CQ-S and CQ-R strains with respect to CQ and CLT. Transformation of the carbonyl group of **4a** to the



Scheme 1. Reagents and conditions: (i) a—Mg turnings, THF, reflux, 6 h; b—NBS, AIBN, CCl_4, reflux; (ii) pyrrolidine, Et_3N, MeCN, 0 °C, 1 h; (iii) a—$NaBH_4$, EtOH, rt, 2 h; b—$SOCl_2$, DCM, 0 to 45 °C, 4 h; (iv) **8**, Et_3N, MeCN, 80 °C, 18 h; (v) $NaBH_4$, THF/H_2O 2:1, 80 °C, 2 h; (vi) $MeSO_2Cl$, Et_3N, DCM, 0 °C, 2 h.



Scheme 2. Reagents and conditions: (i) a—NBS, AIBN, CCl_4, reflux, 4 h; b—imidazole, K_2CO_3, acetone, reflux, 6 h; (ii) LiOH, H_2O/THF, 1:2, reflux, 30 min; (iii) HOBt, NMM, EDC, *N*,*N*-DMF, rt, overnight; (iv) DIEA, *n*-pentanol, 160 °C, 24 h; (v) Et_3N, 3 Å molecular sieves, MeOH, then $NaBH_4$, rt, 6 h.

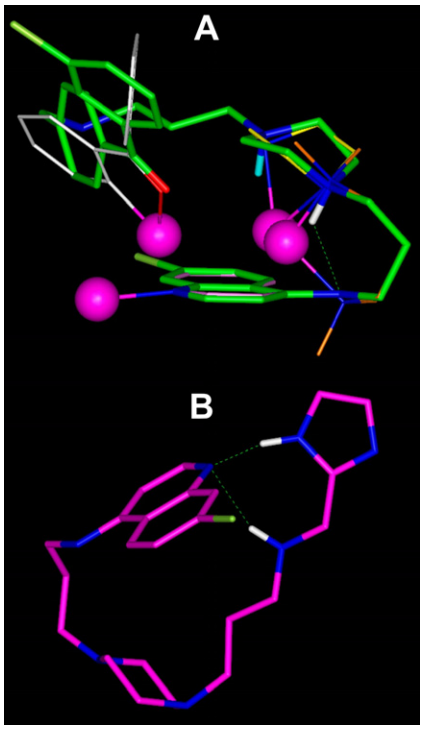


Figure 2. Resulting conformers at pH 7.2 of **4a** (A, green) and **4f** (B, magenta) are shown. In (A) **4a** was superimposed on the X-ray structure of iron complexes containing the following moieties: diphenylketone (white; CSD code: VESCEB), piperazine (yellow; CSD code: HAVNUO), *N*1,*N*3-dimethylpropane-1,3-diamine (orange; CSD code: METGIB), and quinoline (pink; CSD code: FOFROH). The superimposition was made fitting the putative iron coordinating atoms. Iron atoms' *vdW* volumes (magenta) are scaled (0.3) for clarity. Displayed lone pair of piperazine nitrogen is colored in cyan. Heteroatoms are colored: O, red; N, blue; Fe, magenta; and Cl, light green. All hydrogens, except those involved in hydrogen bonds (green dashed lines), have been omitted for clarity.

alcoholic group (**4b**) or to the corresponding sulfonate (**4c**) determined a decrease in activity against CQ-S and CQ-R strains (**4b** and **4c** vs **4a**). In order to define the role played by iron chelating groups in antimalarial activity (R^1, Table 1), we introduced at R^1 aromatic and heteroaromatic systems endowed with different metal chelating and protonatability properties. Consequently, as R^1 residue, we selected fragments potentially able to coordinate iron (ConQuest 1.9, CSDS), such as a fragment of the CLT/**3** polycyclic scaffold (**4e**), the 2-imidazolylmethyl group (**4f**), and the 2-benzimidazole bicyclic system (**4g**); on the other hand, compounds **4d** and **4h** were characterized by a pyrrolidinyl(di)phenylmethylene system, two residues unable to chelate iron (pyrrolidine chelates iron when a carbonyl function is at C-2—ConQuest 1.9, CSDS). Compounds **4d** and **4h** as well as **4e**–**g** resulted less potent than **4a** against D10 and W2 strains, confirming the importance of the iron chelating properties of the R^1 substituent. Recently, we have reported the potent antimalarial activity against CQ-S and CQ-R strains of quinolines bearing an imidazole moiety, a known Fe(III)–FPIX complexing group.[11] Surprisingly, introduction of an imidazole system in this series (R^1, **4f**) dropped the activity against both strains. The effect of imidazole of **4f**, as well as of the diphenylcarbonyl group of **4a**, was investigated as reported below. To analyze structure–activity relationships (SAR), we calculated for all compounds the prevalent ionic forms of the two possible tautomers at pH 7.4 (physiological), 7.2 (cytoplasm), and 5.5 (FV) (Table 1) and, accordingly, performed a comprehensive computational analysis (Insight2005, Accelrys, San Diego) on the resulting forms of **4a** and **4f**, in order to investigate the role played by electronic and conformational parameters on the antimalarial activity. According to the results reported by Ryckebusch et al.[7], the presence of several nitrogens protonated at cytoplasmic pH in the 1,4-bis(3-aminopropyl)piperazine linker of **4a**–**h** (Table 1) probably prevents the access to the FV. Consequently the compounds elicit their antimalarial activity at the cytoplasm level. Taking into account this hypothesis, we focused on the effect of the imidazole (**4f**) and polycyclic (**4a**) terminal fragments on electronic and conformational properties at pH 7.2 (cytoplasm).

At pH 7.2 the presence of the diphenylketone moiety of **4a** allows the formation of an intramolecular hydrogen bond between the *N*1 of piperazine and the aniline nitrogen of the quinoline ring. This, in turn, increases the electronic density at the quinoline heterocyclic nitrogen and, consequently, improves its iron coordination properties, thus reproducing the geometry of a multisite iron chelator (Fig. 2A) (ConQuest 1.9, CSDS). Moreover the diphenylketone moiety represents a further iron chelation site (Fig. 2A). On the contrary, the 2-imidazolylmethyl group (**4f**), protonated at pH 7.2 (Table 1), establishes hydrogen bond interactions with the quinoline heterocyclic nitrogen thus decreasing the iron coordination properties of **4f** and consequently, its antimalarial activity (Fig. 2B).

In summary, we identified novel CQ-based heterodimers characterized by a 1,4-bis(3-aminopropyl)piperazine linker as potent antimalarials against CQ-S and CQ-R *Pf* strains. Among the compounds presented in this study, **4a** was the most potent antimalarial, being 16- and 8-fold more active than CQ against K1 and W2 strains, respectively. A putative mechanism of action of **4a** was investigated, paving the way for the development of new multisite iron complexing antimalarials. Further studies are in progress to assess the in vivo biological properties of this class of antimalarial agents.

Acknowledgments

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