Published in final edited form as:
J Med Chem. 2010 January 28; 53(2): 916–919. doi:10.1021/jm900972u.

Reversal Agent and Linker Variants of Reversed Chloroquines: Activities against *Plasmodium falciparum*

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Abstract

We have shown that "Reversed Chloroquine molecules" constructed from chloroquine-like and resistance "reversal agent"-like cores, can be powerful drugs against malaria (*J. Med. Chem.*, 2006, **49**, 5623–5). Several Reversed Chloroquines are now presented which probe parameters governing the activities against chloroquine-resistant and chloroquine-sensitive malaria strains. The design is tolerant to linker and reversal agent changes, but a piperazinyl group adjacent to the quinoline, at least for the group of compounds studied here, may be detrimental.

Keywords

Antimalarials; Dose-Response Relationship; Drug Evaluation; Erythrocytes/parasitology; *Plasmodium falciparum*; drug effects

INTRODUCTION

In terms of human suffering, malaria is clearly the most important parasitic disease. Furthermore, the worldwide burden of malaria is increasing – in part due to the spread of resistance to most of the drugs that were once effective, inexpensive, and safe.[1] Among these drugs, chloroquine (CQ) had been the prime therapy for nearly half a century. CQ was safe, effective, remarkably inexpensive, and could be administered to pregnant women and infants. Unfortunately, *P. falciparum*, the cause of the most deadly malaria, is now CQ-resistant (CQ^R) in most endemic regions. The continuing spread of CQ^R, as well as resistance to alternative drugs has helped fuel a strong increase in incidence and consequence of malaria worldwide.[1]

In considering new antimalarial drug candidates, it seemed to us that CQ's safety and economic advantages are simply too strong to abandon. Others have sought CQ modifications to combat drug resistance.[2, 3] We began a program to use CQ's quinoline core, but linked to entities that are known to overcome CQ^R, postulating that the resulting hybrid molecules might give enough physicochemical flexibility to allow such hybrids to be tailored to produce compounds that retain the beneficial qualities of CQ and that can be combined with a wide range of other drugs,

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Supporting Information Available: General Experimental Methods; Compound characterizations: ^1H & ^{13}C NMR data. This material is available free of charge via the Internet at http://pubs.acs.org.

in current use or in development, for combination therapies. This is an important point because it has become generally accepted that combination therapy should be used to delay the emergence of resistance to new antimalarial agents.[1, 4, 5]

CQ resistance in *P. falciparum* malaria is strongly associated with mutations in a parasite digestive vacuole (DV) membrane protein, PfCRT; these mutations have been found to be correlated with enhanced CQ export from the DV.[6–9] Various molecules, termed reversal agents (RA), have been identified that inhibit this CQ export from the DV in CQ^R parasites.[10–13] One RA pharmacophore may be described as a pair of aromatic rings, often with an aliphatic nitrogen atom a few angstroms removed from the aromatic rings.[14]

In an earlier publication,[15] we showed that it is possible to synthesize a molecule that, conceptually, is a 7-chloro-4-alkylamino-quinoline linked to a reversal agent (RA) *via* an alkyl group. The RA was envisioned as inhibiting the *P. falciparum* chloroquine resistance transporter (PfCRT) -associated CQ export from the DV in CQ^R parasites.[10–13] If such an effect were 'perfect' (i.e., no drug export, and no other resistance mechanism), then there should be equal efficacy against both CQ^S and CQ^R strains. Such a construct would deliver the RA in a 1:1 ratio with the quinoline, lowering the RA dose required if the two were given separately. We termed this conjugate drug a "reversed chloroquine" (RCQ), and showed that our first prototype, **1**, has low-nM IC_{50} values against both CQ^S (e.g., D6) and CQ^R (e.g., Dd2) strains of *P. falciparum* malaria in red cell culture. Further, **1** was able to clear parasitemia from a mouse model to <1% via oral dosing. Encouraging as all this was, **1** is quite lipophilic (ClogP ~ 8.9), and there had been no intentional effort to optimize it against *P. falciparum*. We therefore undertook to modify the RCQ structure in an effort to delineate the factors that govern efficacy against *P. falciparum*. Others have also taken up the RCQ approach.[19] Herein we report initial variations in the RCQ structure, beginning with the linker and aromatic groups of the RA moiety. The RCQ features evaluated include bridging between the two RA aromatic rings (conversion of the diphenyl to dibenzyl) in addition to some evaluation of the linker length and flexibility between the RA-end and the quinoline (Chart 1).

RESULTS AND DISCUSSION

A set of molecules, shown in Table 1, was synthesized as outlined in the Supplementary Material. This set was chosen to be a minimal representation of variants including the linker between the chloroquinoline ring and the RA, as well as varying both the RA aromatics from diphenylamine to dibenzylamine or dibenzylamide, as well as the length of the aliphatic appendage. Thus, the linker was shortened from the imipramine propyl in **1** to an ethyl group, as well as to a piperazine (formally two ethyl linkers). In addition, the methyl attached to the aliphatic N of the RA aliphatic appendage was deleted. Other changes made to the RA moiety included both shortening and lengthening this appendage by one methylene, as well as converting the amine proximal to the aromatic groups into an amide. In each case, the ethyl bridge between the aromatics was removed to give diphenylamino- and dibenzylamino-functionalities. Although we did not produce every possible combination of all these changes, the set was sufficient to examine effects of these structural changes on the activities against CQ^S and CQ^R *P. falciparum* malaria.

All of the compounds have significant activity ($IC_{50} \leq 125$ nM; see both Table 1 and Figure 1) against both D6 (CQ^S) and Dd2 (CQ^R) *P. falciparum* malaria strains. The CQ^R strain generally gives a higher IC_{50} than the CQ^S strain, but with the ratio of $IC_{50}(CQ^R)/IC_{50}(CQ^S)$ ranging only from about 1 to 3. In fact, of the 11 compounds presented here, 6 have lower IC_{50} values than does CQ against CQ^S *P. falciparum*, and all have a lower ratio of $IC_{50}(CQ^R)/IC_{50}(CQ^S)$ than does CQ, by at least a factor of 5. Although the low IC_{50} values against both types of strain (CQ^S and CQ^R) are probably the most important factor, the low strain-sensitivities also

contribute to the drug-development process. We conclude that the RCQ design is more general than the single molecule, **1**, which was presented in our earlier manuscript on RCQs.[15]

However, there are significant differences among the compounds' effects on the CQ^S and CQ^R strains. Comparing **4** to **3**, it is seen that the dibenzylamino moiety can be advantageous relative to the diphenylamino group, although this does not infer that the diphenylamino group is inherently bad. **10** is an interesting case, demonstrating that changing the amino alpha to the RA phenyls to an amide is tolerable, giving IC_{50} values below those of CQ itself, and reduces the ClogP value to almost the same value as CQ. This helps substantially with water solubility, which is needed in developing orally effective drug candidates. Also, the diphenyl to dibenzyl amine advantage noted above (e.g., **3** to **4**) is not found for the amides, going from **10** to **12**. Also, comparing **2** to **9**, or **4** to **6**, the IC_{50} values change by only a small amount when the linker is shortened from 3 to 2 methylenes, at least if the RA portion has a dibenzylamino moiety. With the linker fixed at 3 methylenes, varying the RA aliphatic length also does not make a large difference (IC_{50}s: **4** < **5** < **2**) when the RA aromatic portion is dibenyzlamino. **7** and **13** were obtained as side-products during the syntheses of **6** and **10**. They constitute an unusual pair, in that they give the lowest and nearly the highest IC_{50} values, respectively. It was unsurprising that **7** was so effective, insofar as the PfCRT is presumed to be unable to export it to a significant extent, having two RA moieties. The reduced activity of **13** is surprising; the only change is to covert each RA nitrogen proximal to the aromatic groups into an amide, this is seen not to be detrimental in the case of the superior activity of **10**. Both **7** and **13** are fairly large and complex molecules, so likely would not be preferred drugs in the context of the Developing World where they are most needed. Compound **12** has a single RA head group, has a much lower ClogP than **13** (6.9 vs. 8.2, respectively), and significantly better IC_{50} values, comparable to those of its reduced analog, **6**.

8 and **11** each have a piperazine ring alpha to the 7-chloroquinoline ring, as does piperaquine (Figure 2), a drug that has been in use for some time and has been reported to have an IC_{50} < 10 nM against the D6 strain.[16] This appears to be at odds to the rather higher IC_{50} values against even D6 presented by **8** and **11**. Others have explored arylpiperazines as an antimalarial scaffold, but focused on CQ^S/CQ^R cross-reactivity rather than maximizing potency.[17] However, piperaquine is a 'bisquinoline', and the presence of two 7-chloroquinoline moieties is perhaps the major contributor to its stronger efficacy. This may be balanced by the lack of proton on the nitrogen at the quinoline 4-position, as has been pointed out by others.[18]

Toxicity is an important consideration in any drug development program, and so we provide cytotoxicity data in Table 1. Given the strong potencies of the compounds against malaria, the cytotoxicities are encouraging, especially for **10** – **12**. In fact, compound **10** has the combination of high efficacy and low cytotoxicity for a 'therapeutic index' (cytotoxicity/efficacy) of 12,000 for D6, and 4800 for Dd2. For comparison, these values are far superior to our calculated 'therapeutic index' values for CQ: 1,700 for D6, and only 120 for Dd2. Such numbers, in addition to its low ClogP (approximating that of CQ, and indicating relatively high water solubility) suggest that **10** and **12** could prove to be possible starting points, leading to further progress in the drug development process.

In conclusion, linking any of several reversal agent-like moieties to a 4-amino-7-chloroquinoline yields good activity against CQ^S or CQ^R *P. falciparum* malarias, so that there is considerable flexibility available to the drug designer.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Abbreviations

CQ	chloroquine
CQR	chloroquine-resistant
CQS	chloroquine-sensitive
DV	digestive vacuole
PfCRT	P. falciparum chloroquine resistance transporter
RA	reversal agent
RCQ	reversed chloroquine

Acknowledgments

The authors thank the following for supporting this research: The Medical Research Foundation of Oregon (Grant 0530), and the National Institutes of Health (Grants AI067837 & AI072923) to DHP, as well as a grant from the Murdock Charitable Trust for the NMR instruments.

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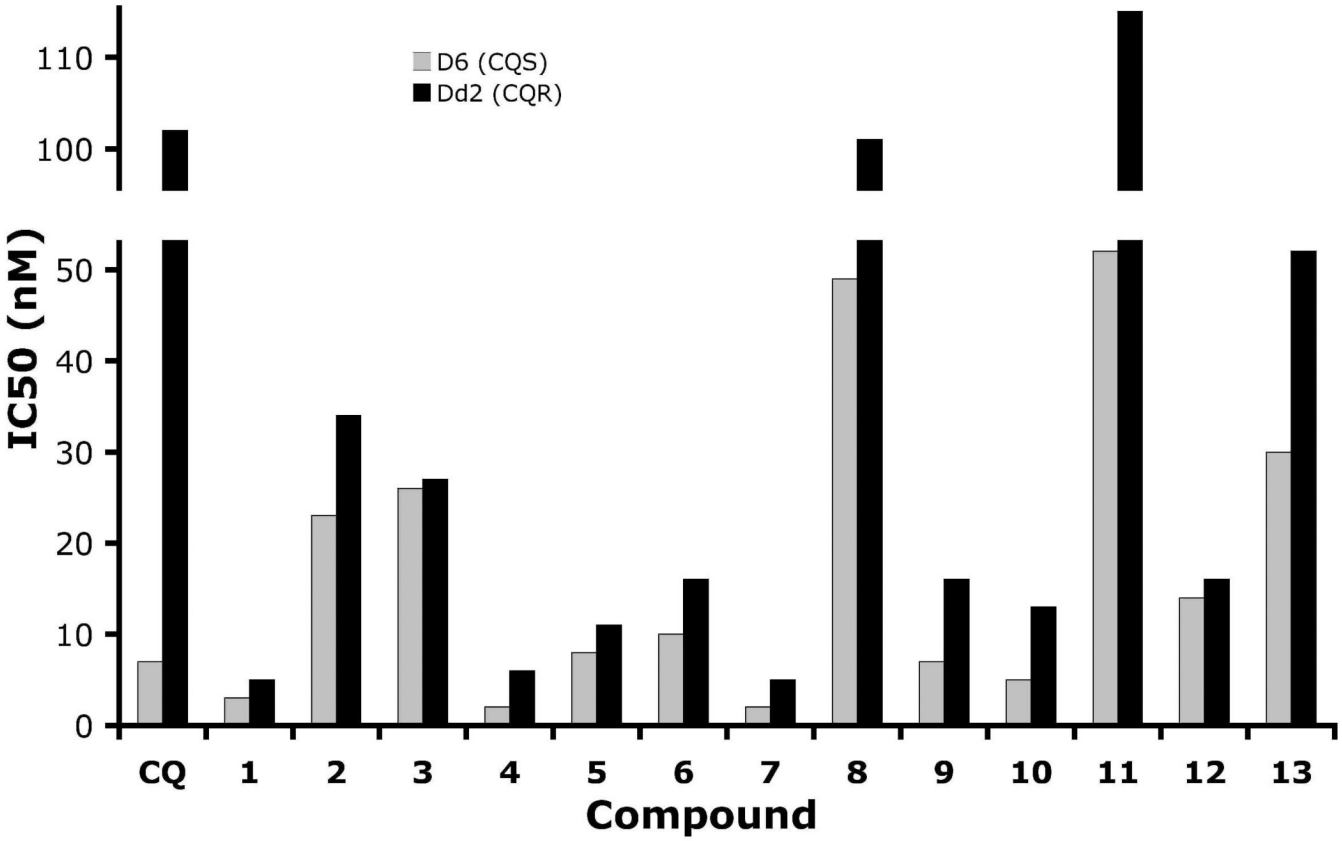


Figure 1.
The IC_{50} values for CQ and compounds **1** – **13**. The grey bars are for the CQS D6 strain, and the black bars are for the CQR Dd2 strain, of *P. falciparum* malaria.

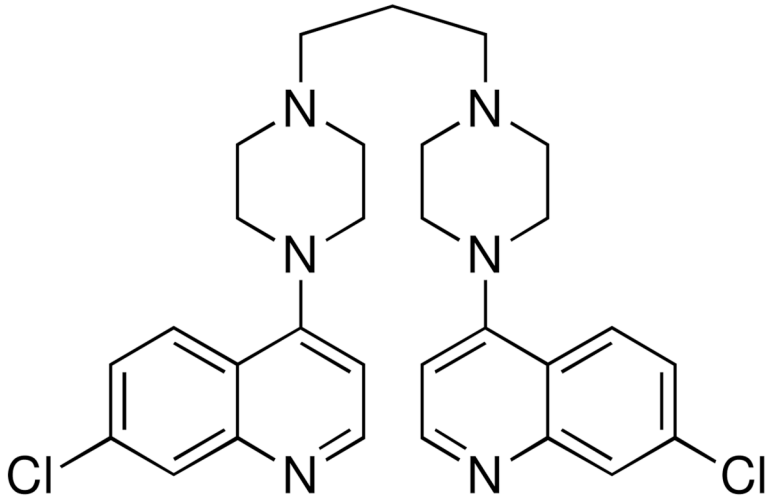


Figure 2.
Piperaquine (1,3-bis-[4-(7-chloroquinolyl-4)-piperazinyl-1]-propane).

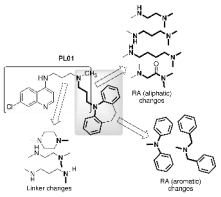
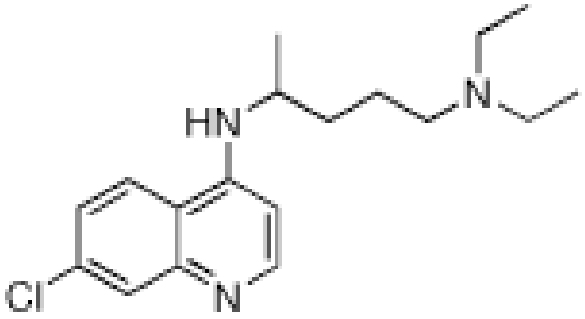
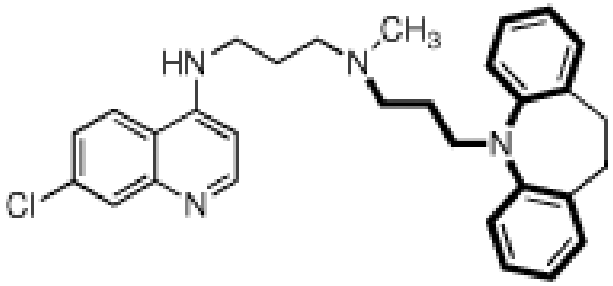
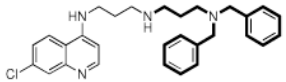
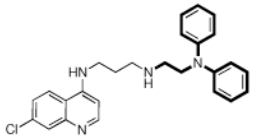


Chart 1.
Variations in the RCQ structure; the RA portions are shown in bold bonds.

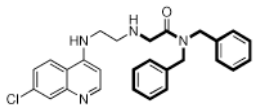
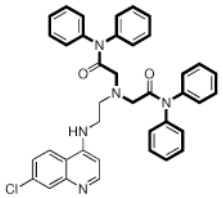
Table 1

RCQ activities against *P. falciparum*.

Compound		ClogP[a]	IC50[b] (n*M*)		IC_{50} Ratio Dd2/D6	Cytotoxicity[c] (n*M*)
			D6 (CQ^S)	Dd2 (CQ^R)		
CQ		5.1	7	102	15	12000
1		8.9	3	5	1.7	700
2		7.6	23	34	1.5	700
3		7.3	26	27	1.0	3700

Compound	ClogP[a]	IC50[b] (nM)		IC50 Ratio Dd2/D6	Cytotoxicity[c] (nM)
		D6 (CQ^S)	Dd2 (CQ^R)		
4	7.3	2	6	3.0	N.D.
5	7.4	8	11	1.4	1300
6	7.0	10	16	1.6	2200
7	11.7	2	5	2.5	6200

Compound	ClogP[a]	IC50[b] (nM)		IC_{50} Ratio Dd2/D6	Cytotoxicity[c] (nM)
		D6 (CQ^S)	Dd2 (CQ^R)		
8	5.6	49	101	2.1	30000
9	7.3	7	16	2.3	N.D.
10	5.2	5	13	2.6	62000
11	7.3	52	115	2.2	61000

Compound		ClogP[a]	IC50[b] (n*M*)		IC$_{50}$ Ratio Dd2/D6	Cytotoxicity[c] (n*M*)
			D6 (CQ^S)	Dd2 (CQ^R)		
12		5.9	14	16	1.1	22000
13		8.2	30	52	1.7	4300

[a] Evaluated using ChemDraw software.

[b] Averages of at least 3 runs (± 15%). The uncertainties are estimated based on weighing uncertainties for the various compounds (which are free-bases and often oils), as well as on variability between determinations that were performed on different weeks.

[c] Cytotoxicities are against mouse spleen lymphocytes. These values are estimated to be ±50%, based on weighing uncertainties for the various compounds (which are free-bases and often oils), as well as on variability between determinations that were performed on different weeks. N.D.: not determined.