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

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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*, 5623−5625). Several reversed chloroquines are now presented that 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.

Introduction

In terms of human suffering, malaria is clearly the most important parasitic disease. Furthermore, the worldwide burden of malaria is increasing in part because of the spread of resistance to most of the drugs that were once effective, inexpensive, and safe.[1] Among these drugs, chloroquine (CQ[a]) 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 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, 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-alkyl-aminoquinoline 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 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) compound and showed that our first prototype, **1**, has low nanomolar IC$_{50}$ against 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 Supporting Information. 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 the RA aromatics from diphenylamine to dibenzylamine

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[a] Abbreviations: CQ, chloroquine; CQR, chloroquine-resistant; CQS, chloroquine-sensitive; DV, digestive vacuole; PfCRT, *P. falciparum* chloroquine resistance transporter; RA, reversal agent; RCQ, reversed chloroquine.
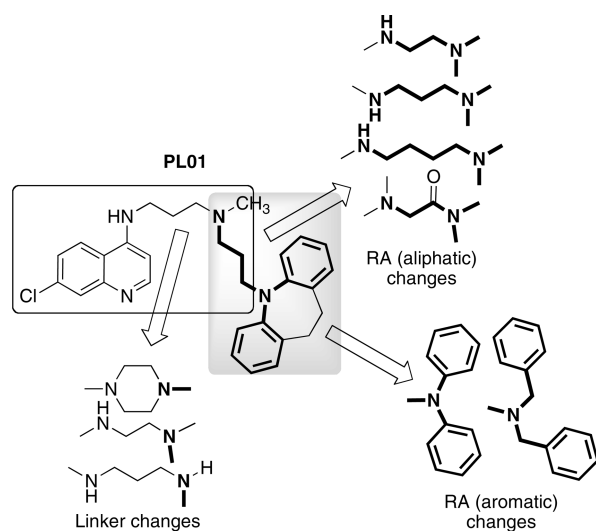
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Table 1. RCQ Compound Activities against *P. falciparum*

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
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
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
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 three runs (±15%). The uncertainties are estimated on the basis of weighing uncertainties for the various compounds (which are free bases and often oils) and on variability between determinations that were performed on different weeks. [c] Cytotoxicities are against mouse spleen lymphocytes. These values are estimated to be ±50% on the basis of weighing uncertainties for the various compounds (which are free bases and often oils) and of variability between determinations that were performed on different weeks. N.D.: not determined.

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 and 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 shortening and lengthening this appendage by one methylene and converting the amine proximal to the aromatic groups into an amide. In each case, the ethyl bridge between the aromatics in **1** was removed to give diphenylamino and dibenzylamino

Chart 1. Variations in the RCQ Structure[a]

[a] RA portions are shown in bold bonds.

Figure 1. IC$_{50}$ values for CQ and **1**−**13**. The gray 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).

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$_{50}$ ≤ 125 nM; see Table 1 and Figure 1) against 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$_{50}$ 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 CQ^S and CQ^R strains 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 RCQ molecules.[15]

However, there are significant differences among the compounds' effects on the CQ^S and CQ^R strains. From a comparison of **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 α to the RA phenyls to an amide is tolerable, giving IC$_{50}$ below those of CQ itself and reducing the ClogP to almost the same value as CQ. This helps substantially with water solubility, which is needed to develop 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, from a comparison of **2** to **9** or of **4** to **6**, the IC$_{50}$ values change by only a small amount when the linker is shortened from three to two methylenes, at least if the RA portion has a dibenzylamino moiety. With the linker fixed at three methylenes, varying the RA aliphatic length also does not make a large difference (IC$_{50}$: **4** < **5** < **2**) when the RA aromatic portion is dibenzylamino. **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
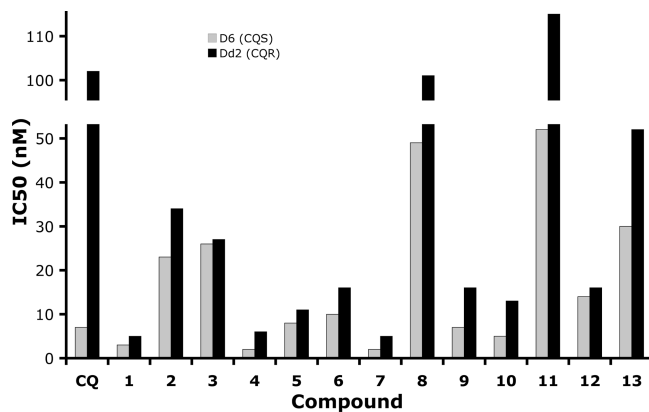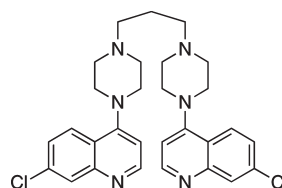
moieties. The reduced activity of **13** is surprising; the only change is the conversion of each RA nitrogen proximal to the aromatic groups into an amide, and this is seen not to be detrimental in the case of the superior activity of **10**. Both **7** and **13** are fairly large and complex molecules and 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 headgroup, a much lower ClogP than **13** (6.9 vs 8.2, respectively), and significantly better IC$_{50}$, comparable to those of its reduced analogue **6**.

8 and **11** each have a piperazine ring α 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 higher IC$_{50}$ 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, **10** has the combination of high efficacy and low cytotoxicity for a "therapeutic index" (cytotoxicity/efficacy) of 12 000 for D6 and of 4800 for Dd2. For comparison, these values are far superior to our calculated "therapeutic index" values for CQ: 1700 for D6 and only 120 for Dd2. Such numbers and the 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.

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

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