Structural Analysis of Chloroquine Resistance Reversal by Imipramine Analogs

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For imipramine, desipramine, and eight analogs of these well-known drugs, an N-5-aminoalkyl substitution was a minimum but insufficient structural feature associated with chloroquine resistance reversal. Although a second distal aliphatic nitrogen atom was unnecessary for resistance reversal, the direction of the dipole moment vector was critical.

The tricyclic antidepressants imipramine and desipramine possess modest antimalarial activity (8) and are two well-studied compounds known to reverse chloroquine (CQ) resistance in plasmodia in vitro (1, 4, 5, 7, 11). One report (8), however, noted that neither drug reversed CQ resistance in vitro, and in one clinical study (15) desipramine did not enhance the efficacy of CQ. Nonetheless, the identification of compounds that reverse CQ resistance is an important goal in malaria chemotherapy.

In order to probe the structural specificity of this phenomenon, we compared the abilities of imipramine (referred to as compound 1), desipramine (compound 2), clomipramine (compound 5), and six analogs of these well-known drugs (compounds 3, 4, 6, 7, 8, and 9) (Fig. 1) to reverse CQ resistance. In these experiments, the 50% inhibitory concentration (IC50) of CQ against the W2 clone of Plasmodium falciparum was determined in the presence and absence of 500 ng of test compound/ml (Table 1). A resistance modification index (RMI) was calculated by dividing the IC50 of CQ in the presence of the tricyclic antidepressant by the IC50 of CQ alone. The IC50s of CQ against the CQ-sensitive D6 and CQ-resistant W2 P. falciparum clones were 2.5 and 49 ng/ml, respectively.

The W2 clone was rendered fully sensitive to CQ in the presence of compounds 1 to 6, each of which contained a secondary or tertiary aliphatic aminoalkyl nitrogen atom with a two- or three-carbon bridge to the heteroaromatic nitrogen (N-5). Notably, compound 7, a regioisomer of compound 6, had almost no effect on altering the susceptibility of the W2 clone to CQ, suggesting that its methyl group sterically prevents a receptor binding interaction with the proximal piperazine nitrogen atom. The more rigid alkyne-bridged compound 8 and diazepine (compound 9) were somewhat less effective than compounds 1 to 6, but they still increased the CQ sensitivity of the W2 clone 4.9- and 4.1-fold, respectively. Consideration of these data reveals that N-5-aminoalkyl substitution is a minimum but insufficient structural feature associated with resistance reversal. This conclusion is also supported by the complete lack of activity of the N-5-acetamide derivative (data not shown). As illustrated for the data for compounds 6, 7, and 9, we can also infer that a second distal aliphatic nitrogen atom is unnecessary for resistance reversal. These results suggest that there is considerable latitude in the distance and geometry between the two nitrogen atoms in this series of imipramine analogs.

To better understand this situation, the structures for compounds 1 to 9 were entered into the molecular modeling software Spartan (Wavefunction, Inc.) and energy minimized with molecular mechanics. The resulting structures were then subjected to a conformational search (30° increments of the C-C bonds bridging the two nitrogen atoms) to determine the lowest energy conformer and the range of N-N distances present in conformers 2 kcal/mol within that of the lowest energy conformer. The N-N distances ranged from 3.294 to 5.963 Å for the nine analogs, and there was no correlation between this N-N distance range (minimum or maximum) and the RMI values.

The minimum energy conformers were then subjected to further geometry optimization using the AM1_mol model developed by Dixon et al. (6) to derive parameters such as dipole moment, HOMO (highest occupied molecular orbital) and LUMO (lowest unoccupied molecular orbital) energies, positive and negative electrostatic potentials, and molecular electrostatic (MEP) energies. Electrostatic potential characteristics largely reflect the location of π electrons in molecules, and

<table>
<thead>
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<th>Compound no.</th>
<th>IC50 (ng/ml)</th>
<th>RMI</th>
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<tr>
<td>1</td>
<td>2.3</td>
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</tr>
<tr>
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<td>7</td>
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</tr>
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<td>8</td>
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</tr>
<tr>
<td>9</td>
<td>12</td>
<td>0.25</td>
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</table>

* RMI was calculated by dividing the IC50 of CQ in the presence of the tricyclic antidepressant by the IC50 of CQ alone (49 ng/ml).

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it is through this characteristic that molecules recognize and bind to their receptors (9). There was no correlation between any of these parameters and the RMI values for this set of tricyclic antidepressant analogs (data not shown). However, the MEP surfaces (Fig. 2) for compounds 6 and 7, indicating a different orientation of the distal piperazine nitrogen atom lone pair in these two regioisomers, provided a potential clue for the striking lack of CQ resistance reversal by compound 7. In compound 6 (dipole moment, 0.97 debye), as in compounds 1 and 2 (dipole moments, 0.89 and 1.79 debye, respectively), the direction of the vector is toward one of the aromatic rings, whereas in compound 7 (dipole moment, 1.06 debye), the direction is toward the N-5 atom of the tricyclic ring system. Thus, for compounds 6 and 7, although there is little difference in the magnitude of dipole moment, the direction of the dipole moment vector appears to be critical for CQ resistance reversal. However, as illustrated by compounds 1 and 2, CQ resistance reversal seems to be relatively independent of the magnitude of the dipole moment.

It is interesting that several structurally related antihistaminic drugs including cyproheptadine (10), azatadine (2), and chlorpheniramine (13) potentiate the activity of CQ against resistant parasites, and in each of these antihistamines the direction of the dipole moment vector is similar to those of compounds 1, 2, and 6. Among these antihistamines, and indeed among all drugs known to reverse CQ resistance, chlorpheniramine appears to be the most promising. In combination with CQ, chlorpheniramine was effective in the treatment of mild-to-moderate CQ-resistant *P. falciparum* malaria (12). The relatively conformationally mobile chlorpheniramine may be the superior CQ resistance reversal agent because it can more easily access pharmacologically active conformations associated with CQ resistance reversal versus antihistaminic activity.

In summary, the common structural framework of these antihistamines and the tricyclic antidepressants imipramine (compound 1), desipramine (compound 2), and amitriptyline (8, 14), namely a diarylmethane linked by a three-atom bridge to a secondary or tertiary nitrogen atom, may point to a common pharmacological target associated with CQ resistance reversal. The electronic bioisosteresm of these compounds is illustrated (Fig. 3) by the similarity of their MEP profiles characterized by a localized negative-potential region by the side chain N atom and a large negative region covering the aromatic regions. Even though the identity of this target is still

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**FIG. 1.** Structures of compounds 1 to 9. Clomipramine (compound 5) is the 3-chloro analog of imipramine (compound 1).

**FIG. 2.** Isopotential contours of molecular electrostatic potentials at ~40 kcal/mol for compounds 6 and 7.
unknown (3), these data suggest that it should be possible to design well-tolerated compounds with greater potency and specificity as CQ resistance reversal agents.

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REFERENCES


FIG. 3. Isopotential contours of molecular electrostatic potentials at −10 kcal/mol for imipramine (compound 1), desipramine (compound 2), cyproheptadine, azatadine, and chlorpheniramine.