Pergamon

Bioorganic & Medicinal Chemistry 6 (1998) 1927–1933

BIOORGANIC & MEDICINAL CHEMISTRY

Functional Correlation of Molecular Electronic Properties with Potency of Synthetic Carbinolamine Antimalarial Agents

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Received 17 March 1998; accepted 22 June 1998

Abstract—Specific calculated molecular electronic properties of structurally diverse synthetic aromatic carbinolamines containing phenanthrene, quinoline, and *N*-substituted biphenyl rings are associated with antimalarial potency allowing use of these electronic features in the prediction of antimalarial efficacy, thus aiding the design of new antimalarial agents. These electronic features include the magnitude and location of 3-dimensional molecular electrostatic potentials, lowest unoccupied molecular orbitals, and highest occupied molecular orbitals. Stereoelectronic properties were calculated using quantum chemical AM1 methods on the optimized geometry of the lowest energy or most populated conformer in both gaseous and aqueous environments. In the phenanthrene carbinolamines, the aliphatic nitrogen atom and the hydroxyl proton are intrinsically more nucleophilic and less electrophilic, respectively, than in the non-phenanthrene compounds. Hydrogen bonding ability and the electrophilic nature of the aromatic ring appear to be two important features responsible for interaction with receptor molecules. Published by Elsevier Science Ltd.

Introduction

Plasmodium falciparum malaria is increasingly more difficult to treat worldwide primarily due to parasite resistance to most currently available drugs.[1,2] Thus, new drug therapies to treat resistant malaria are increasingly urgent. Determining molecular electronic properties responsible for antimalarial potency should aid in the designing of new drugs and toward a better understanding of their mechanism of action. Interactions between molecules are the consequence of stereoelectronic interactions.[3] Thus, it is the molecule's stereoelectronic properties that govern strength of bonds, strength of nonbonded interactions, and molecular reactivity. Molecular electronic properties affect the strength of interaction with receptor proteins and transport across cell membranes. Structural parameters alone, such as the intramolecular N···O nonbonded distance of the carbinolamines, are insufficient to ensure antimalarial potency.[4]

A previous study of 41 mefloquine (Fig. 1, compound **2c**) analogues showed that these analogues were most potent when they possessed a large lateral negative potential region across the C2 and C8 carbon atoms of the quinoline ring, no negative potential over the quinoline ring, a positive potential adjacent to the hydroxyl hydrogen atom of at least 36 kcal/mol, and a negative potential on the van der Waals surface of the molecule in the range of −64 to −55 kcal/mol.[4] Less active mefloquine analogs did not possess each of these attributes. In the previous study, the structural variation of the analogs was limited such that all of the carbinolamines had both a quinoline ring and a piperidine ring.

The present study expands the structural diversity of the carbinolamine antimalarial agents (Fig. 1) to determine how ring type and cyclic versus acyclic aliphatic amine groups affect their molecular electronic properties. The results show that all of these structurally diverse compounds share specific electronic properties and are very similar to the most potent mefloquine analogs studied previously. The phenanthrene compounds are slightly different than the non-phenanthrene compounds with respect to the nucleophilicity of their amine and the

Key words: Antimalarial agent; mefloquine; halofantrine; stereoelectronic properties; computational chemistry.
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0968-0896/98/$—see front matter Published by Elsevier Science Ltd.
PII: S0968-0896(98)00146-1

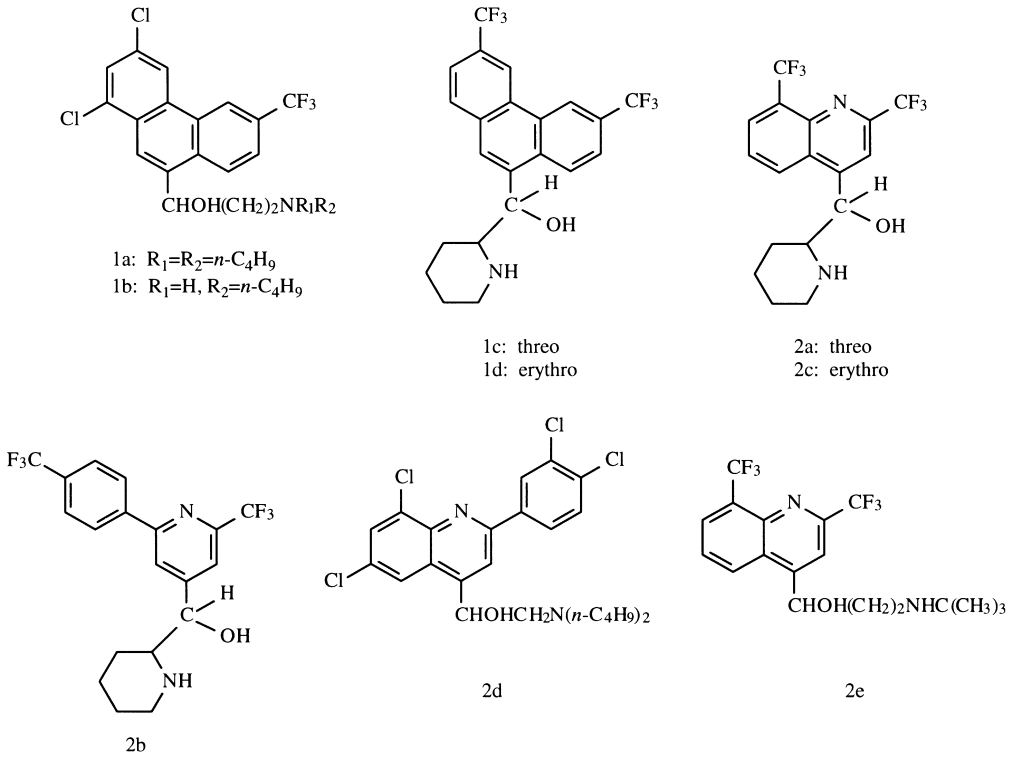

Figure 1. Chemical structures of halofantrine (**1a**), desbutylhalofantrine (**1b**), WR 165,355 (**1c**), WR 122,455 (**1d**), WR 177,602 (**2a**), enpiroline (**2b**), mefloquine (**2c**), WR 30,090 (**2d**), and WR 184,806 (**2e**).

electrophilicity of their hydroxyl group. These differences may indicate a different strength of interaction with receptors.

Results and Discussion

Antimalarial efficacy

All of the molecules in this study are relatively efficacious antimalarial agents in vitro (Table 1) with IC_{50} values ranging from 1.30 to 15.9 nM against the D6 chloroquine-sensitive *P. falciparum* strain and 0.63 to 4.52 nM against the W2 chloroquine-resistant *P. falciparum* strain.

Molecular conformation

The low-energy–high-abundance conformations of the molecules were very similar with the $C_{aromatic}$-C-C-$N_{aliphatic}$ dihedral angle equal to 152.3–171.2° for compounds **1c** to **2d** and the C_{OH}-C-C-$N_{aliphatic}$ dihedral angle equal to 176.0–177.8° for compounds **1a, 1b**, and **2e**. This resulted in aliphatic N···O distances of 2.76 Å to 4.29 Å which are within 0.15 Å of the distances found in crystal structures.[5–10] These conformations represent both the most abundant conformation and the lowest energy conformation obtained by rotational search of the rotatable bonds between the aromatic ring and the aliphatic nitrogen atom. The only exception is for compounds **1a** and **1b** (halofantrine and desbutylhalofantrine) whose conformational search calculations resulted in two low energy structures, one in which the hydroxyl hydrogen atom points towards the aromatic ring plane making the hydroxyl group available for intermolecular hydrogen bonding (open form) and another in which the hydroxyl hydrogen atom points towards the side-chain nitrogen atom making an intramolecular hydrogen bond (closed form). Crystallographic studies[6,7] of either racemic halofantrine hydrochloride or (−)-halofantrine hydrochloride demonstrate that in salt form **1a** is in the open conformation. Although the closed form of **1a** and **1b** are 1.4 to 2.0 kcal/mol more stable than the open form both in vacuo and in aqueous medium, the abundance of the open form was 3–4 times higher than the closed form. Since the open form permits intermolecular hydrogen bonding with receptor molecules and is the most abundant conformer, the open form conformations of **1a** and **1b** were chosen for analysis of the electronic properties.

Molecular electrostatic potential (MEP) profiles

The negative electrostatic potential extending beyond the surface of the molecule provides a measure of charge distribution from the point of view of an approaching molecule (Fig. 2). These electrostatic features are important for recognition interactions with the receptor occurring when the molecule and the receptor are at a relatively large distance of separation.[11,12] All of the molecules have similar features including the lack of −5 kcal/mol potential above or below the aromatic rings, large negative potential zones by the CF_3 groups, and negative potential regions around the hydroxyl and aliphatic amine groups. These features remain almost unchanged in the aqueous environment. Lack of negative potential regions above and below the aromatic ring indicates nucleophilic susceptibility of the aromatic ring plane.

MEPs plotted onto essentially the van der Waals surface of the molecule (Fig. 3) show that the site for the most negative potential is by the aliphatic nitrogen atom and the site for the most positive potential is by the hydroxyl hydrogen atom. The exception is compound **2d** in which the site for the most negative potential is by the

Table 1. Selected electronic properties and IC_{50} values of the compounds listed by ring type with compounds **1a** to **1d** having a phenanthrene ring and compounds **2a** to **2e** having a non-phenanthrene aromatic ring system

Compd	Aqueous stabilization energy[a]	Most negative potential[a]	Negative potential by aliphatic N[a]	Most positive potential[a]	HOMO eigenvalue[b]	LUMO eigenvalue[b]	Dipole moment[c]	IC_{50} D6 clone[d]	IC_{50} W2 clone[d]
1a	26.2	−67.7	−67.7	35.4	−9.06	−1.31	2.10	1.30	0.63
1b	24.7	−68.9	−68.9	36.1	−9.18	−1.34	1.87	3.30	1.31
1c	26.1	−67.6	−67.6	36.1	−9.51	−1.51	3.56	3.32	1.41
1d	26.5	−65.8	−65.8	38.0	−9.32	−1.47	4.20	6.25	3.51
2a	26.5	−54.5	−54.5	42.7	−9.78	−1.59	6.19	4.90	2.34
2b	26.3	−60.7	−60.7	43.3	−9.76	−1.21	4.38	6.19	2.09
2c	22.5	−58.6	−58.6	43.4	−9.80	−1.60	6.44	8.39	3.43
2d	25.6	−63.1	−57.9	36.7	−9.13	−1.22	3.36	11.4	2.36
2e	23.8	−64.2	−64.2	43.8	−9.63	−1.65	6.37	15.9	4.52

[a]kcal/mol
[b]eV
[c]debye
[d]nmol

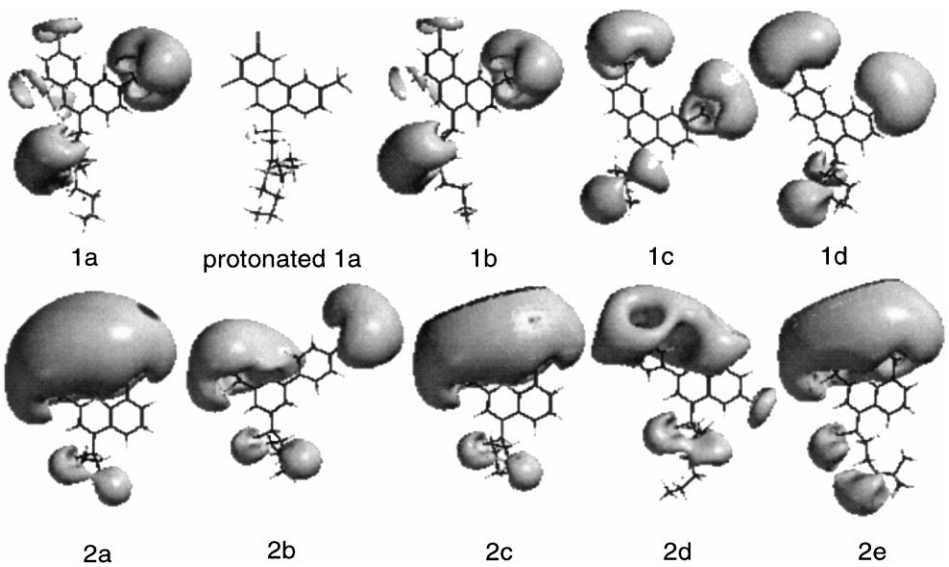

Figure 2. Three-dimensional isopotential contours of MEP at −5 kcal/mol showing negative potential regions around the CF_3, chlorine, hydroxyl, and amine groups. Disappearance of nearly all of the negative potential upon protonation of the carbinolamines is illustrated by protonated **1a**.

quinoline nitrogen atom. The magnitude of the MEP by the aliphatic nitrogen atom ranges from −68.9 to −54.5 kcal/mol with the three most efficacious compounds (**1a** to **1c**) having the most negative values and the compounds with a phenanthrene ring having more negative values than the non-phenanthrene compounds (Table 1). The value of the MEP by the hydroxyl hydrogen atom ranges from 35.4 to 43.8 kcal/mol with the three most efficacious compounds having the lowest values (Table 1). Very little change in the intrinsic nucleophilicity and acidity of the compounds occurs while going from the gaseous to the aqueous medium as the values of the most positive and negative potentials generally change less than 10% (data not shown).

Lowest unoccupied and highest occupied molecular orbitals (LUMOs and HOMOs)

LUMO sites plotted onto the molecular surface for all molecules irrespective of ring type are scattered over the aromatic ring plane (Fig. 4) implying the susceptibility of the aromatic ring toward nucleophilic attack. HOMO sites plotted onto the molecular surface show only one prominent site by the aliphatic nitrogen atom for all molecules (Fig. 4). This single HOMO site by the nitrogen atom most likely facilitates intermolecular hydrogen bond formation. The location of the LUMOs and HOMOs are the same for the gaseous and the aqueous environment.

Mechanistically, the electron acceptor ability of the LUMO may play a greater role than the electron donation of the HOMO. The HOMO eigenvalues (Table 1) are consistently quite negative indicating that the electrons are firmly bound to the nuclei. However, the LUMO eigenvalues (Table 1) are also consistently negative, but far less negative compared to the HOMO eigenvalues still indicating a strong affinity for electrons.

Dipole moment

The dipole moment of the compounds varies widely from 1.87 to 6.44 debye and does not have any apparent relationship with antimalarial potency (Table 1).

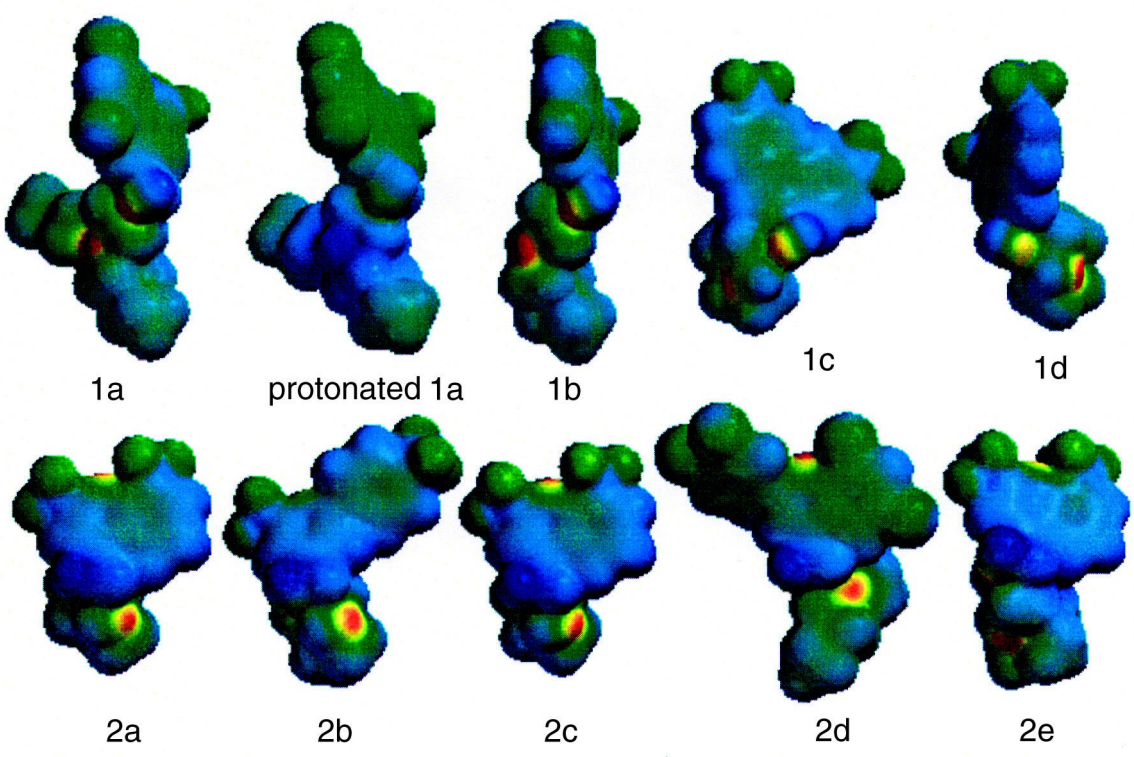

Figure 3. MEP superimposed onto a surface of constant electron density (0.002 e/au^3) showing the most positive potential region (deepest blue color) by the hydroxyl proton and the most negative potential region (deepest red color) by the aliphatic amine lone electron pair except for compound **2d** where the deepest red region is by the quinoline nitrogen atom. Disappearance of the negative potential upon protonation of the carbinolamines is illustrated by protonated **1a** where the most positive potential is located by the amine group. Each compound was color-coded using a range of MEP from −69 to 44 kcal/mol except for protonated **1a** which was drawn using an MEP range of −69 to 140 kcal/mol.

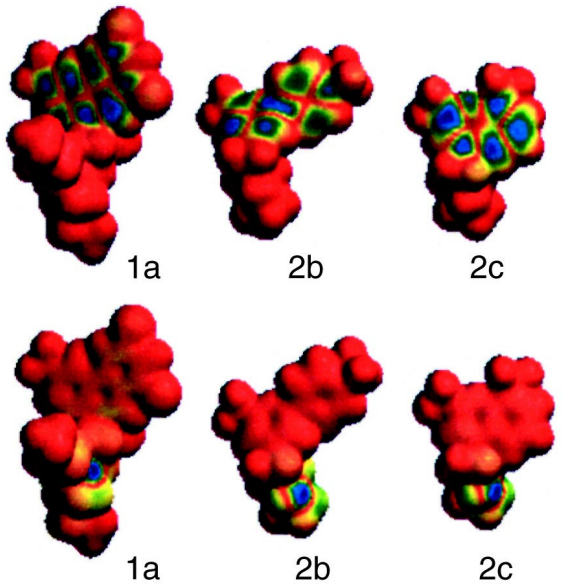

Figure 4. LUMO (top row) and HOMO (bottom row) encoded onto a surface of constant electron density (0.002 e/au^3) for halofantrine (**1a**), enpiroline (**2b**), and mefloquine (**2c**) showing scattered LUMO sites over the aromatic rings and a single dominant HOMO site adjacent to the aliphatic nitrogen atom. The dominant LUMO and HOMO sites are colored deepest blue.

Aqueous stabilization

All compounds are stabilized in aqueous medium from 22.5 to 26.5 kcal/mol (Table 1), most likely due to an interaction of the hydroxyl and amine groups with the medium. Other than small changes in the position of the hydroxyl group, the conformational features of the molecules remain virtually unaltered.

Protonation

Protonation at the aliphatic nitrogen atom results in a destabilization of all of the compounds by 91.5–153 kcal/mol indicating that the free base form is more thermodynamically stable than the protonated form. In the protonated compounds, the MEP on the van der Waals molecular surface shows no negative potential region (Fig. 3). Similarly, the isopotential profile at −5 kcal/mol shows only a small patch by the hydroxyl oxygen atoms (Fig. 2), indicating that when protonated, the compounds lack negative potential for recognition interactions with receptor proteins when several angstroms apart. The calculated proton affinities[13] of the compounds are very similar, ranging between 217 and 221 kcal/mol.

Conclusion

This study shows that potent carbinolamine antimalarial agents share specific molecular electronic properties regardless of aromatic ring type and whether the aliphatic amine is cyclic or acyclic. The values of the MEP by the hydroxyl and aliphatic amine groups and the shape and distribution of the isopotential surfaces of all the compounds in this study are essentially the same as those found in a previous study only for the most potent antimalarial agents.[4] Thus, carbinolamines whose negative potential by the aliphatic amine ranges from −68.9 to −54.5 kcal/mol, whose positive potential by the hydroxyl proton is at least 35.4 kcal/mol, and who have a laterally extended negative potential region adjacent to, but not over the aromatic ring system, should be relatively potent antimalarial agents. These calculable values provide a guide to the chemist as to which compounds are worthy of expensive chemical synthesis.

Particular electronic properties indicate that intermolecular hydrogen bonding is an important element in the interaction of the carbinolamines with receptors. These properties include the location of the most positive potential by the hydroxyl proton, the large negative potential by the aliphatic nitrogen atom, the negative potentials beyond the surface of the molecules adjacent to the hydroxyl and aliphatic amine groups, and the location of the HOMO by the aliphatic nitrogen atom. A measure of likely strength of hydrogen bonding is given by the value of the MEP being at least 35.4 kcal/mol and −54.5 kcal/mol at the hydroxyl hydrogen and amine nitrogen atoms, respectively. Crystallographic studies[5–9] of compounds **1a, 2b** and **2c** have shown that these compounds can participate in intermolecular hydrogen bonding.

The relative electrophilicity of the aromatic ring also appears important for antimalarial potency. Lack of negative potential regions above the aromatic ring is associated with antimalarial potency suggesting a requirement for interaction with electron rich regions of the receptor. The large potential surfaces extending from the molecule lateral to the aromatic ring may be an important recognition element for the receptors. These large, extended negative potential regions are an indication of regions with higher electron density and are likely to enhance the hydrophobicity/lipophilicity of the compounds.[14] Lipophilicity has been shown to be an important contributing factor toward potent antimalarial activity.[15] Substitution of electron withdrawing groups like CF_3 or halogens in the aromatic ring in the compounds facilitates the rings being devoid of negative potential and also increases the lipophilicity lateral to the aromatic ring.

Only a couple of relationships with aromatic ring type were found. Both the negative potential by the aliphatic nitrogen atom and the positive potential by the hydroxyl proton were lower for the phenanthrene carbinolamines than for the non-phenanthrene carbinolamines. The sample size is small, so generalizations to all carbinolamines need to be made with caution. When the compounds in this study were grouped by the acyclic/cyclic nature of the amine group rather than by aromatic ring type, no significant relationships between amine group type and electronic properties were found. Except for **1a** and **1b**, all the compounds, irrespective of an acyclic or cyclic aliphatic amine group, have distinctly localized negative potential regions by the hydroxyl oxygen and the aliphatic nitrogen atoms. This difference in the electrostatic profiles of **1a** and **1b**, which have a single negative potential region extending from the hydroxyl group to the amine group, from the rest of the compounds may indicate a different recognition interaction with receptors and a different manner in which the compounds bind to receptors. However, the similar LUMO locations on the aromatic ring plane and HOMO location by the aliphatic nitrogen atom as well as similar proton affinity of the amine may be linked to the observance of common resistance patterns[16–18] by the parasite.

The methodology employed in this study of calculating molecular electronic properties, correlating these properties to biological potency, and compiling a set of electronic properties that are necessary for potent biological activity should be applicable to other classes of compounds, enabling educated decisions on the design of new compounds.

Experimental

Calculation of electronic properties

The conformers with lowest energy and maximum abundance were identified through conformational search and population density calculations. Their geometry was optimized using semiempirical quantum chemical AM1[19] methods as implemented in Spartan 4.0[20] running on a Silicon Graphics Indigo Extreme R4000 workstation. Molecular electronic properties were then calculated on the optimized geometry of the molecules using Spartan and displayed using Spartan's graphics.

Aqueous solvation

Polarizing effects due to aqueous solvation were calculated using a quantum mechanical continuum, the self-consistent reaction field method of Dixon et al.[21] In this method solvent is represented by a continuous dielectric characterized by a given dielectric constant $\in$. The molecule is embedded into a spherical cavity surrounded by a medium. The permanent dipole of the molecule induces a dipole in the medium, which in turn interacts with the molecular dipole. This solute–solvent interaction is introduced as a perturbation operator in the Hamiltonian of the isolated molecule. The reaction field is iteratively updated until the intramolecular electric field is self-consistent.[22] The method has been modified in Spartan based upon the SMx model of Cramer and Truhlar[23] where the numerical integration required for calculation of the polarization energy in the SMx model has been replaced by a scaled two-center Coulomb integral.

Three-dimensional display of molecular electrostatic potentials

Electrostatic potentials were sampled over the entire accessible surface of a molecule (corresponding roughly to a van der Waals contact surface) and into space extending beyond the molecular surface. Regions of positive electrostatic potential indicate excess positive charge, i.e. repulsion for the positively-charged test probe, while regions of negative potential indicate areas of excess negative charge, i.e. attraction of the positively charged test probe. Color-coded electrostatic potentials were superimposed onto a surface of constant electron density ($0.002\,e/au^3$) to provide an indication of overall molecular size and of location of negative or positive electrostatic potentials. Isopotential contours were also calculated to provide a measure of charge distribution from the point of view of an approaching molecule.

Experimental activity data

The antimalarial activity data, expressed as IC_{50} values in nanomolar concentrations, were obtained from reference 24 and the Chemical Information System, Division of Experimental Therapeutics, Walter Reed Army Institute of Research, a databank of the results of the in vitro screen performed by the Department of Parasitology. The IC_{50} values were determined as described[24] using the chloroquine-sensitive D6 or chloroquine-resistant W2 *P. falciparum* strains. Each compound was assayed multiple times ($n = 2$–9) concurrently with mefloquine, and its IC_{50} value was normalized to the IC_{50} value of mefloquine.

Acknowledgement

We thank the National Research Council, Washington, DC, for their assistance in the support of Dr Bhattacharjee.

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