Analysis of the pharmacological properties of clozapine analogues using molecular electrostatic potential surfaces

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Molecular electrostatic potential surfaces have been used to study a series of neuroleptic compounds, clozapine and clozapine analogues, with similar structures but two rather different pharmacological profiles. Using the electrostatic potential surfaces, the compounds studied could be assigned to one of two distinct categories corresponding to the two pharmacological classes. The results of this study suggest that the molecular electrostatic potential surfaces may be useful in the a priori *prediction of pharmacological properties of untested clozapine analogues.*

Keywords: computer aided drug design, neuroleptic agents, clozapine, clozapine analogues, electrostatic potential surfaces

Clozapine (see Figure 1: structure (**A**) is a dibenzodiazepine derivative with potent neuroleptic activity[1]. It differs from many other neuroleptics (phenothiazines, butyrophenones) in that it does not display the usual array of side effects, most notably extrapyramidal side-effects[2]. However, in clinical use clozapine has displayed agranulocytosis as a serious toxic reaction[3]. Thus, there has been considerable interest in the development of analogues which retain clozapine's desirable properties without the toxicity. Some of the analogues (see Figure 1: structures (**B**) and (**C**), however do display a more traditional neuroleptic pharmacological profile even though they differ from clozapine only in degree and/or position of chlorine substitution in the aromatic ring system[2].

The application of molecular electrostatic potential surface calculations to problems in pharmacology and molecular biology is not a new procedure. Weinstein[4] Kaufman[5], and others have used quantum mechanically calculated electrostatic potentials to correlate electronic properties of molecules with pharmacological and/or physiological activity. Electrostatic potential surfaces have also been used to investigate DNA–protein interactions[6] and other ligand–macromolecule interactions[7]. The utility of electrostatic potential surfaces in the study of drug–receptor, substrate–enzyme, or other intermolecular interactions is based on the hypothesis that gross molecular electronic or electrostatic characteristics are of prime importance in the initial stages of association, namely molecular recognition and spatial orientation of the ligand for binding.

As the molecules studied are all rigid and X-ray crystallographic studies have shown all the molecules to be in the same conformation[8], the differences in pharmacological properties cannot be explained on the basis of conformational variations. Since the compounds differ only in degree and/or position of the chlorine substitution in the aromatic ring system, it seems reasonable that the pharmacological differences exhibited by these molecules might be correlated with the electrostatic potential surface characteristics. These may vary depending on the chlorine substitution pattern. Therefore, the molecular electrostatic potential surfaces for these molecules were calculated to examine this possibility.

METHODS

Several techniques were used to calculate the molecular electrostatic potential surfaces for the molecules studied. In the most primitive approach, the Mulliken electron density populations which had been determined within the CNDO/2 formalism were used to assign partial charges to each atom in the molecule of interest. A solvent-accessible molecular surface for the molecule was then calculated using an algorithm developed by Connolly[9]. The electrostatic potential at each surface point due to the partial charges centred on the atoms of the molecule was then computed. Finally, the computed molecular electrostatic potential surfaces were displayed with Midas[10], an interactive computer graphics molecular modelling program. Special colouring schemes, calculated and scaled by features within Midas,

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were used to achieve optimal colour differentiation corresponding to the electrostatic potential gradient over the molecular surface. With Midas, up to five molecules and molecular surfaces can be displayed simultaneously, thus making it easy to compare and contrast differences in the molecular electrostatic potential surfaces. A second approach in computing molecular electrostatic potential surfaces was quite similar to the first, differing only in that Mulliken electron density populations were calculated on an *ab initio* basis using an STO-3G basis set.

A third approach to computing molecular electrostatic potential surfaces followed a procedure used previously by Singh and Kollman[11]. First, *ab initio* wavefunctions for the molecules were calculated using an STO-3G basis set. Then, the electrostatic potential at each point of four contours of Connolly solvent-accessible surfaces (surfaces at the van der Waals radii, 1.2 × van der Waals radii, 1.4 × van der Waals radii, and 1.6 × van der Waals radii) was calculated using the STO-3G wavefunctions. Next, the quantum-mechanically calculated molecular electrostatic potential surfaces were used in a nonlinear least squares fitting algorithm to obtain an analytical atom-centred point charge model which optimally reproduced the quantum mechanical molecular electrostatic potential surfaces. The point charge models produced by this method were then used analogously to the Mulliken population charges in the two previous techniques to compute the molecular electrostatic potential surfaces for display by Midas.

Table 1. Atomic charges dipole moments, and minimum and maximum (min/max) e.s.p. values, of the tricyclic fragment of clozapine (X_1 = H, X_2 = Cl) as calculated by three methods: (i) CNDO/2, (ii) Gaussian 80, (iii) LS-fit of charges to Q.M. e.s.p.. (The nomenclature for atoms is shown in Figure 1)

Atom	Atomic charges (electrons)		
	CNDO/2	Gaussian 80	LS-fit to Q.M. e.s.p.
C(1)	−0.0148	−0.0684	−0.1073
X(1)=H	−0.0046	0.0667	0.0738
C(2)	0.0343	−0.0553	−0.0506
H(2)	−0.0093	0.0691	0.0709
C(3)	−0.0421	−0.0736	−0.1106
H(3)	0.0012	0.0692	0.0674
C(4)	0.1542	0.1247	0.2545
N(5)	−0.2528	−0.4044	−0.5651
H(4)	0.1216	0.2127	0.2928
C(6)	0.0951	0.0981	0.1776
C(7)	−0.0224	−0.0606	−0.1499
H(5)	0.0007	0.0718	0.1007
C(8)	−0.0030	−0.0629	−0.1662
H(6)	0.0095	0.0803	0.1120
C(9)	0.0927	0.0358	0.2586
X(7)=Cl	−0.1901	−0.1523	−0.2385
C(10)	−0.0286	−0.0573	−0.2519
H(8)	0.0145	0.0871	0.1340
C(11)	0.0980	0.0923	0.3234
N(12)	−0.2561	−0.3104	−0.6600
C(13)	0.2667	0.2638	0.7754
C(14)	−0.0611	−0.0302	−0.1999
C(15)	0.0236	−0.0522	−0.0071
H(9)	−0.0075	0.0721	0.0520
N(16)	−0.2434	−0.4296	−0.8316
H(16A)	0.1112	0.2017	0.3182
H(16B)	0.1124	0.2116	0.3275
Dipole (D)			
μ_x	−2.928	−3.069	−3.068
μ_y	4.379	3.913	3.906
μ_z	−1.672	−1.458	−1.487
$\lvert\mu\rvert$	5.527	5.182	5.185
e.s.p. on surface (kcal/mol)			
Min	−23.2	−28.7	−46.4
Max	21.1	27.9	38.3
e.s.p. on surface + 1.4Å (kcal/mol)			
Min	−10.6	−9.51	−12.6
Max	9.97	9.20	11.2

In some cases, notably the *ab initio* computations, calculations could not be performed on the entire molecule due to the large number of basis functions. Therefore, the molecules were divided into fragments with common overlap regions, calculations were carried out on the fragments, and the final point charge models for the molecules were derived by piecing together the fragments with adjustment of the partial charges in the common overlap regions to conserve the molecular charge. This approach was also used to derive point charge models for isomeric analogues so as to avoid the need for a full series of *ab initio* calculations of wavefunctions and quantum mechanical electrostatic potentials for each isomer. For example, the atomic partial charges were evaluated via the electrostatic potential using the approach of refill for cytidine-3′-phosphate. The atomic partial charges were then computed for dimethyl phosphate, 1-aminodeoxyribose, and *N*-methylcytosine fragments using the same procedure. The fragments were then pieced together to insure a net unit charge on the molecule. These two atomic partial charge models for cytidine-3′-phosphate displayed small differences (deviations in atomic charges were typically 5–10%)[12].

Point charge models were developed for all isomers listed in Figure 1 using the *ab initio* least squares fit, and CNDO/2 formalisms. Point charge models for the three-ring dibenzdiazepine fragments of each isomer were also developed. Additionally, point charge models for protonated species of each molecule were derived by the CNDO/2 method. Within a given point charge model, the molecular electrostatic potential surfaces were calculated both at the van der Waals surface and at a distance of 1.4Å above the van der Waals surface. The electrostatic potential beyond the van der Waals radius of a molecule is of interest as it more realistically represents the electrostatic potential that other molecules experience as they approach.

RESULTS

The tricyclic fragment of clozapine (Structure (**A**)) was chosen for a preliminary check to evaluate how well the resulting electrostatic potential (e.s.p.) surfaces compare, (calculated on the basis of the point charge models derived by the three methods described in the previous section). In Table 1, the point charge models are compiled for numerical comparison, and the resulting colour

coded e.s.p. Connolly surfaces are shown in Colour Plate 1 for visual comparison. Whereas the atomic point charges differ substantially from method to method (mainly in magnitude, but also in sign for a few atoms), the resulting e.s.p. surfaces appear to be surprisingly similar (as are dipole moments, see *Table 1*).

The e.s.p. surfaces obtained from the *ab initio* calculations (Colour Plate 1(b) and 1(c)) show well defined profiles with more detailed features, especially in the region of the amidine lone pair, than the semi-empirical CNDO/2 based surface (Colour Plate 1(a)). The computationally cheaper CNDO-calculation shows the same general distribution of the e.s.p. over the molecular surface. Colour Plate 1 also shows that the e.s.p. calculation from points on the Connolly surface, or from points 1.4Å above it, have only a marginal influence on the

Table 2. Atomic charges, dipole moments, and min/max e.s.p. values of the four clozapine analogues, calculated by the CNDO/22 method and Mulliken population analysis. The nomenclature of the atoms is shown by Figure 1

	Atomic point charges by CNDO/2–Mulliken population				
Atom	I	II	III	IV	STO-3G + LS-fit I
C(1)	−0.0165	0.0612	−0.0172	0.0634	−0.1073
X(1)	(=H) −0.0052	(=Cl) −0.1736	(=H) −0.0072	(=Cl) −0.1731	(=H) −0.0738
C(20)	0.0343	0.0379	0.0322	0.0397	−0.0507
H(2)	−0.0097	0.0069	−0.0106	0.0079	0.0709
C(3)	−0.0428	−0.0433	−0.0438	−0.0422	−0.1106
H(3)	0.0008	0.0067	−0.0007	−0.0082	0.0674
C(4)	0.1538	0.1609	0.1544	0.1603	0.2545
N(5)	−0.2527	−0.2522	−0.2534	−0.2515	−0.5651
H(4)	0.1212	0.1212	0.1182	0.1242	0.2928
C(6)	0.0946	0.0879	0.0880	0.0946	0.1776
C(7)	−0.0225	−0.0222	−0.0229	−0.0216	−0.1499
H(5)	0.0004	−0.0062	−0.0074	0.0016	0.1007
C(8)	−0.0039	−0.0113	−0.0103	−0.0047	−0.1662
H(6)	0.0093	−0.0063	−0.0089	0.0120	0.1120
C(9)	0.0931	0.0158	0.0122	0.0972	0.2586
X(7)	(=Cl) −0.1915	(=H) −0.0173	(=H) −0.0180	(=Cl) −0.1911	(=Cl) −0.2385
C(10)	−0.0294	−0.0348	−0.0342	−0.0300	−0.2519
H(8)	0.0137	−0.0024	−0.0047	0.0160	0.1340
C(11)	0.0986	0.0945	0.0946	0.0983	0.3234
N(12)	−0.2575	−0.2526	−0.2569	−0.2538	−0.6600
C(13)	0.2538	0.2498	0.2498	0.2542	0.7261
C(14)	−0.0628	−0.0605	−0.0630	−0.0600	−0.1999
C(15)	0.0245	0.0313	0.0252	0.0305	−0.0071
H(9)	−0.0089	−0.0049	−0.0120	−0.0081	0.0520
N(16)	−0.1681	−0.1695	−0.1690	−0.1682	−0.1932
C(17)	−0.0854	0.0898	0.0891	0.0862	−0.3273
H(10)	−0.0034	−0.0062	−0.0051	−0.0046	0.1289
H(11)	−0.0084	−0.0100	−0.0108	−0.0077	0.1526
C(18)	−0.0930	−0.0924	0.0928	0.0926	−0.0213
H(12)	−0.0191	−0.0167	−0.0180	−0.0178	0.0843
H(13)	−0.0130	−0.0133	−0.0156	−0.0108	0.0973
N(19)	−0.1466	−0.1461	−0.1457	−0.1470	−0.1979
C(20)	0.0931	0.0909	0.0906	0.0934	0.0174
H(14)	−0.0204	−0.0194	−0.0198	−0.0200	0.0767
H(15)	−0.0150	−0.0141	−0.0153	−0.0139	0.0748
C(21)	0.1009	0.0996	0.0992	0.1012	−0.2429
H(16)	−0.0178	−0.0177	−0.0182	−0.0173	0.1152
H(17)	−0.0007	−0.0007	−0.0003	−0.0012	0.1126
C(22)	0.0863	0.0820	0.0818	0.0866	−0.4859
H(18)	−0.0120	−0.0109	−0.0114	−0.0115	0.1592
H(19)	−0.0098	−0.0101	−0.0105	−0.0095	0.1652
H(20)	−0.0195	−0.0161	−0.0171	−0.0186	0.1484
Dipole (D)					
μ_x	2.54	0.31	−1.05	−1.18	–
μ_y	4.69	1.68	2.68	3.51	–
μ_z	−0.02	−0.45	1.13	−1.59	–
$\lvert\mu\rvert$	5.34	1.77	3.25	4.03	–
e.s.p. surface + 1.4Å (kcal/mol)					
Min	−10.70	−7.02	−5.38	−8.38	–
Max	8.74	8.95	6.79	11.33	–

appearance of the colour coded e.s.p. surface (which, of course, is mainly due to the adjustment of the colour range to the numerical e.s.p. range (see also caption of Colour Plate 1).

In summary, these preliminary calculations have convincingly shown that the CNDO/2-Mulliken method can produce e.s.p. surfaces for this type of molecule comparable to the much more elaborate *ab initio* computations (the CPU–time ratio on a VAX 11/780 is about 1:50). In Colour Plate 2 shows the e.s.p. surfaces of the pharmacologically interesting compounds, (structures (**A**) – (**D**)) calculated on the basis of a CNDO/2 point charge model. For comparison, the e.s.p. surfaces of clozapine (**A**) and analogues (**B**)–(**D**) based on the most elaborate point charge model (i.e., an *ab initio* STO-3G calculation, and LS-fit of charges) are given in Colour Plate 3. The point charges for these e.s.p. surfaces are summarized in Table 2.

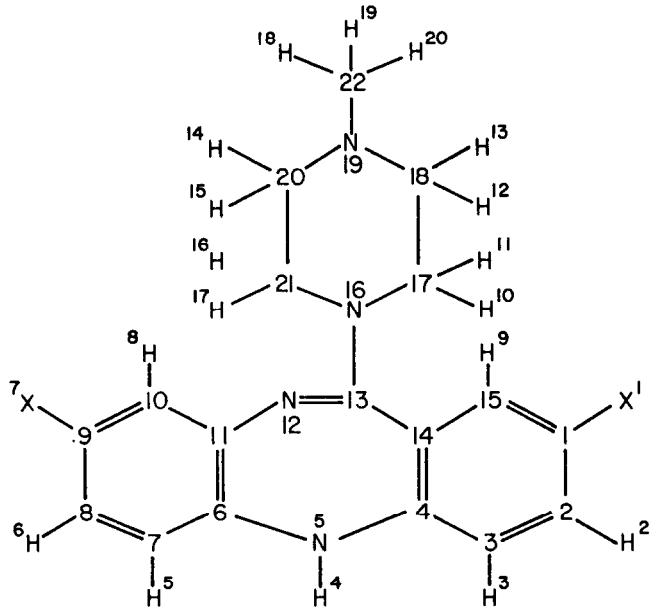

Figure 1. Atomic numbering scheme for clozapine and analogues

- **A** Clozapine X^7 = Cl, X^1 = H;
- **B** HUF-2046 X^7 = H, X^1 = Cl;
- **C** 1,9-'dichloroclozapine' X^7 = X^1 = Cl;
- **D** des-chloroclozapine X^7 = X^1 = H

DISCUSSION

The resultant molecular electrostatic potential surfaces could be correlated quite nicely with the pharmacological variance between molecules. Clozapine (Colour Plates 2(a) and 3(a)) and the des-chloro compound (Colour Plates 2(b) and 3(b)) both exhibited similar pharmacological profiles, each being devoid of extrapyramidal side effects and each exhibiting appreciable anticholinergic activity[2]. These two molecules also displayed the same qualitative molecular electrostatic surface characteristics. For example, the electrostatic surface gradient varies smoothly from a positive potential around C2 to relative neutrality in the central ring to relatively negative potential around C9. In contrast, HUF-2046 (Colour Plate 2(b) and 3(b)), which displays a different pharmacological profile (extrapyramidal side effects comparable to chlopromazine and little perceivable anitcholinergic activity[2]), exhibited an electrostatic potential surface gradient. This ranged from a slightly negative potential at C9 to a strongly positive potential in the central ring to strongly negative potential around C2. Finally, the dichloro compound has pharmacological properties common to both clozapine and HUF–2046 (extrapyramidal side effects and anticholinergic activity[2]). Also, the electrostatic potential surfaces' characteristics of both clozapine and HUF-2046 are seen in the potential surface of the dichloro compound. It exhibited a strongly negative potential around the C2 and C9 regions, with a strongly positive potential in the central ring region. As can be seen from the Colour Plates 2 and 3, the differences in electrostatic potential surface characteristics are quite distinctive and easily visualized with computer graphics.

The same qualitative results were obtained regardless of the technique used to develop the point charge model. The only difference between the CNDO/2 model electrostatic potential surfaces and those obtained by the *ab initio* least squares fit method was the magnitude of the gradient (degree of sharpness) between the regions of greatest positive and negative potential in the molecules. Since a scaled colouring scheme has been used to display the molecular surfaces, the same features are seen whether the CNDO/2 Mulliken population charges or the *ab initio* least squares fit charges are used. The protonated species were less informative because the formal positive charge tended to overwhelm most other aspects of the electrostatic potential surface.

Similar qualitative patterns were also obtained when the electrostatic potential was calculated on the van der Waals surface or at a distance of 1.4Å above the van der Waals surface. As mentioned previously, the potential above the molecular surface represents the electrostatic potential the receptor site would experience as the ligand molecule approached the receptor site. It should therefore play a key role in the processes of recognition and spatial orientation of the ligand at the receptor site. The potential on the molecular surface represents the electrostatic potential the receptor site experiences after a tightly bound complex has been formed. The degree of complementarity between the electrostatic potentials of the ligand and receptor site determines to a large extent the strength of the ligand–receptor complex.

CONCLUSIONS

In this study molecular electrostatic potential surfaces have been used to categorize a series of four structurally related compounds which have distinctive pharmacological properties. The results suggest that the molecular electrostatic potential surfaces might be quite useful in correlating pharmacological properties with chemical structure in a series of related compounds. This may be especially true with compounds such as clozapine and certain of its analogues which in many biological screening tests do not behave as traditional neuroleptic agents.

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