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Relative binding orientations of adenosine A_1 receptor ligands – A test case for Distributed Multipole Analysis in medicinal chemistry

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Summary

The electrostatic properties of adenosine-based agonists and xanthine-based antagonists for the adenosine A_1 receptor were used to assess various proposals for their relative orientation in the unknown binding site. The electrostatic properties were calculated from distributed multipole representations of SCF wavefunctions. A range of methods of assessing the electrostatic similarity of the ligands were used in the comparison. One of the methods, comparing the sign of the potential around the two molecules, gave inconclusive results. The other approaches, however, provided a mutually complementary and consistent picture of the electrostatic similarity and dissimilarity of the molecules in the three proposed relative orientations. This was significantly different from the results obtained previously with MOPAC AM1 point charges. In the standard model overlay, where the aromatic nitrogen atoms of both agonists and antagonists are in the same position relative to the binding site, the electrostatic potentials are so dissimilar that binding to the same receptor site is highly unlikely. Overlaying the N^6-region of adenosine with that near C8 of theophylline (the N^6-C8 model) produces the greatest similarity in electrostatic properties for these ligands. However, N^6-cyclopentyladenosine (CPA) and 1,3-dipropyl-8-cyclopentylxanthine (DPCPX) show greater electrostatic similarity when the aromatic rings are superimposed according to the flipped model, in which the xanthine ring is rotated around its horizontal axis. This difference is mainly attributed to the change in conformation of N^6-substituted adenosines and could result in a different orientation for theophylline and DPCPX within the receptor binding site. However, it is more likely that DPCPX also binds according to the N^6-C8 model, as this model gives the best steric overlay and would be favoured by the lipophilic forces, provided that the binding site residues could accommodate the different electrostatic properties in the N^6/N7-region. Finally, we have shown that Distributed Multipole Analysis (DMA) offers a new, feasible tool for the medicinal chemist, because it provides the use of reliable electrostatic models to determine plausible relative binding orientations.

Introduction

Molecules which bind to the same receptor site are expected to have similar electrostatic properties, including similar possibilities for hydrogen bonding, for the electrostatic contribution to the binding energy to remain favourable. This hypothesis has been widely, and successfully [1], used in rational drug design. Indirect methods of determining binding orientations by comparing the properties of the ligands are designed for the common situation where the receptor structure has not yet been determined. Since this is done by finding a superposition of molecules in which a binding site for one ligand is likely to also bind the other, this also 'maps out' which features

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Abbreviations: DMA, distributed multipole analysis; SCF, self-consistent field; CPA, N^6-cyclopentyladenosine; DPCPX, 1,3-dipropyl-8-cyclopentylxanthine; *R*-PIA, *R*-1-phenyl-2-propyladenosine; *S*-PIA, *S*-1-phenyl-2-propyladenosine.

of the molecules are likely to be important for the binding. This information can then be used to design new, unrelated ligands.

Adenosine agonists and xanthine antagonists are expected to bind to the same binding site on the adenosine receptor [2,3], although this is not a phenomenon observed for all agonists and antagonists with competitive receptor binding. Therefore, the relative positions of the agonists and antagonists within the binding site are expected to define a relative orientation or superposition of the two ligands. Representative ligands for this binding site are: adenosine (Fig. 1A) and an adenosine-based agonist, N^6-cyclopentyladenosine (CPA, Fig. 1C) and two xanthine-like antagonists, theophylline (Fig. 1B) and 1,3-dipropyl-8-cyclopentylxanthine (DPCPX, Fig. 1D). At first sight a superposition of the four nitrogen atoms of both ring systems seems to be the most obvious relative orientation, as it emphasizes the closest chemical similarity of the two molecules. We have previously called this model the 'standard' model (Fig. 2A) [4]. However, two other models have been postulated. Van Galen et al. [5] proposed a model in which the positions of atoms N1, N3, N7 and N9 of the xanthine ring coincide with C2, C6, N9 and N7 of the adenine moiety, respectively, the so-called 'flipped' model (Fig. 2B). This model is based on two-dimensional electrostatic, steric and lipophilic similarities. Peet et al. [6] postulated a superposition in which the C8-substituents of xanthine analogues overlap with the N^6-substituents of adenosine analogues. In this model N1, N3 and N9 of the xanthine ring coincide with N9, N3 and N1, respectively, of adenosine (Fig. 2C). This model, which we called the 'N^6-C8' model [4], is based on analogous (stereo)chemical requirements of groups at the adenosine N^6-position and xanthine C8-position, and on a comparison of three-dimensional contour surfaces of the energy of interaction of the ligands with a water probe.

We have previously compared these models with respect to their steric and electrostatic properties, and found that they differed little in their electrostatic similarity [4]. This could have been an artefact of the approximate methods used to calculate and compare the electrostatic properties. To investigate this, we used accurate, ab initio-based, distributed multipoles [7,8] in the present study to assess the electrostatic similarity of the ligands in the three proposed relative orientations of the agonists and antagonists. We used various measures, emphasizing different aspects of the electrostatic interaction possibilities of the molecules, to establish whether there is sufficient correlation between the results to be convincing.

Thus, the main purpose of this study was to establish whether examining the electrostatic similarity of antagonists and agonists of the adenosine A_1 receptor can provide insight into the probable relative binding orientation. Therefore, we based the comparison on more accurate electrostatic calculations than are usually employed in modelling studies and applied various methods of assessing the electrostatic similarity.

Materials and Methods

The plausibility of various models for the relative binding orientation of two ligands within an unknown binding site can be tested only by making assumptions. Firstly, we assume that the two molecules occupy essentially the same region within the receptor, and hence that the relative binding orientation involves significant steric overlap of the two molecules. In comparing the overall (electrostatic) image of the ligands, the other main postulate is that the ligands are encompassed by the receptor residues.

Electrostatic model

Two pairs of molecules were compared with respect to their electrostatic similitude according to the three models for the relative binding of ligands: adenosine–theophylline and CPA–DPCPX. The first pair represents the unsubstituted ligands; the second pair represents compounds with a high (nanomolar) affinity for the adenosine A_1 receptor.

Van Galen et al. determined the conformation in which the adenosine N^6-substituents bind the adenosine receptor

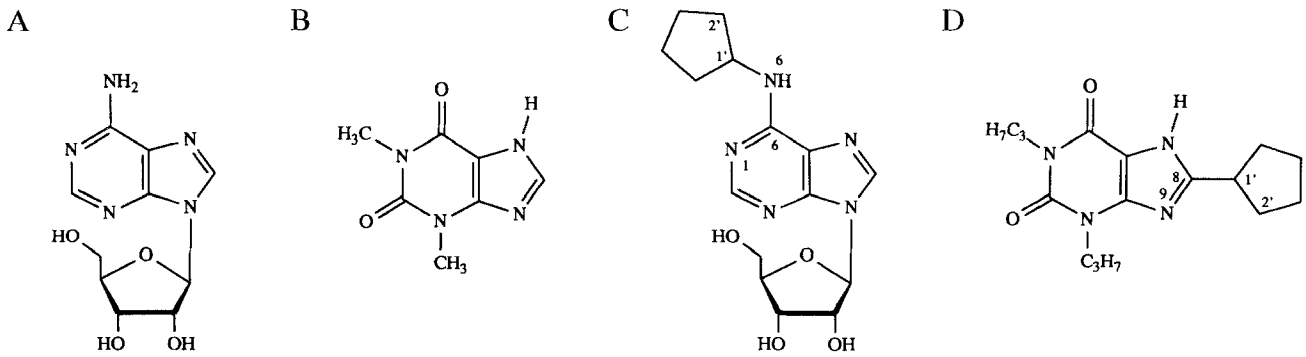

Fig. 1. Four ligands for the adenosine A_1 receptor: (A) adenosine; (B) theophylline; (C) N^6-cyclopentyladenosine (CPA); and (D) 1,3-dipropyl-8-cyclopentylxanthine (DPCPX).

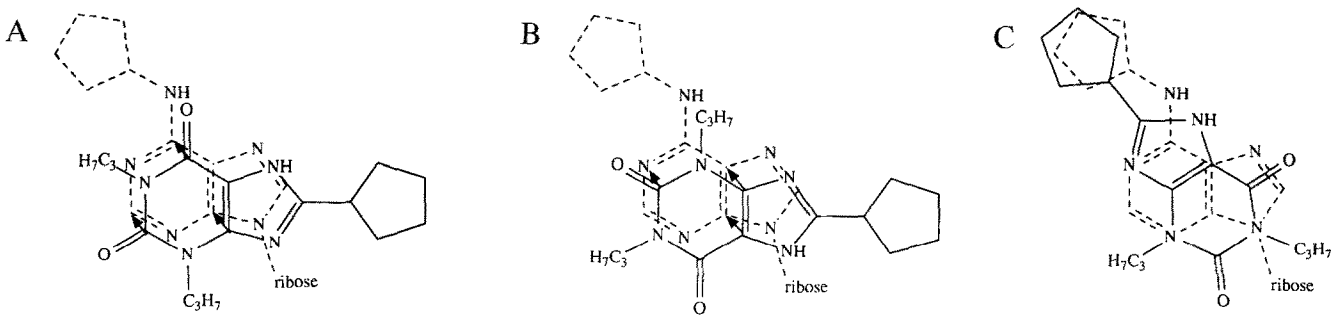

Fig. 2. The three models for the relative orientation of agonists and antagonists in the binding site: (A) standard; (B) flipped; and (C) N^6-C8, illustrated for CPA/DPCPX. (This figure is a slightly modified version of that published in Ref. 4.)

by molecular modelling of N^6-substituted adenosine analogues [9]. They concluded that the most probable conformation of the N1-C6-N^6-C^2 dihedral angle is + or −75 ± 10° and 60 ± 5° for the C6-N^6-C^2-C^1 dihedral angle. The −75° conformation for the N1-C6-N^6-C^2 dihedral angle was used as in the former study [4]. We determined the conformation of xanthine C8-cycloalkyl substituents in a similar way to be approximately 330° for the N9-C8-C1'-C2' dihedral angle [10]. Furthermore, the ribose moiety of adenosine analogues binds the adenosine receptor in an anti conformation [11]. These conformations were assumed in this study.

An important feature of this work is the use of accurate representations of ab initio charge densities, so that the comparison is not confused by errors in the calculated electrostatic properties. The self-consistent-field (SCF) wave function for a 3-21G basis set [12] was calculated for each molecule using the ab initio program suite

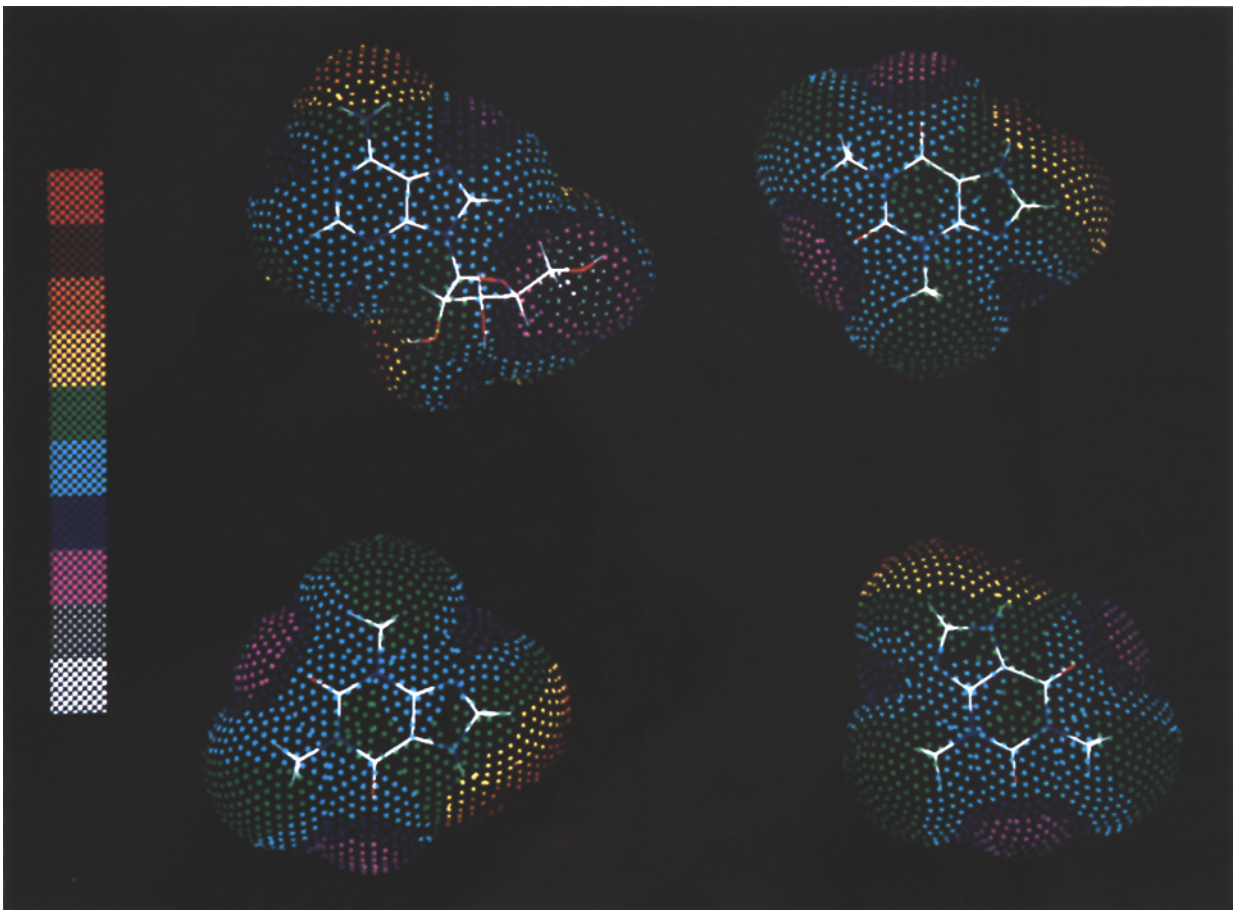

Fig. 3. Electrostatic potential (V) for adenosine (top left) and theophylline at 1.0 Å distance from the van der Waals surface. Theophylline is shown in the orientations corresponding to the three relative binding orientation models; top right: standard model; bottom left: flipped model; bottom right: N^6-C8 model. The value of the electrostatic potential V (kJ/mol) is colour coded red > +120 > brown > 90 > orange > 60 > yellow > 30 > green > 0 > cyan > −30 > blue > −60 > purple > −90 > grey > −120 > white.

CADPAC [13,14], issue 5.0. A Distributed Multipole Analysis (DMA) [7,8] was performed on each SCF wave function to derive a set of multipoles (charge, dipole, quadrupole, octopole and hexadecapole) on each atom. This provides a far more accurate description of the electron density than can be obtained by the use of atomic point charges, as the anisotropic multipoles automatically describe the electrostatic forces arising from lone pairs and π-bonds [15]. This has been shown to be crucial for the prediction of the structures of hydrogen bonded van der Waals complexes [16].

The electrostatic potential around the molecule, and other electrostatic interaction energies, were calculated by means of the usual expressions for the interactions of all point multipoles up to R^{-5} (in which R denotes the inter-site separation) [17], within the program ORIENT [18]. This ensures an accurate evaluation of the electrostatic properties corresponding to the wave function (excluding the effects of penetration within the charge distribution) which is so much more efficient than evaluation directly from the wave function that a wide range of methods of comparison are feasible.

The electrostatic properties were only calculated outside the molecule as defined by the Pauling–van der Waals radii (1.5 Å for N, 1.4 Å for O and 2.0 Å for C). The hydrogen atoms were assumed to lie within the hard sphere of the heavy atom to which they are bonded, as the hydrogen atom is included in a 'united atom' methyl radius, and polar hydrogen atoms effectively have no radius when involved in hydrogen bonding [14]. These radii were also used within ORIENT to define accessible orientations by a pseudo hard-sphere repulsion, for the calculations where the electrostatic energy was optimised.

Methods for comparison

Visual comparison

We have visualised the electrostatic potential 1 Å above the van der Waals surface of the molecules, since a polar, hydrogen bonding proton will normally be around

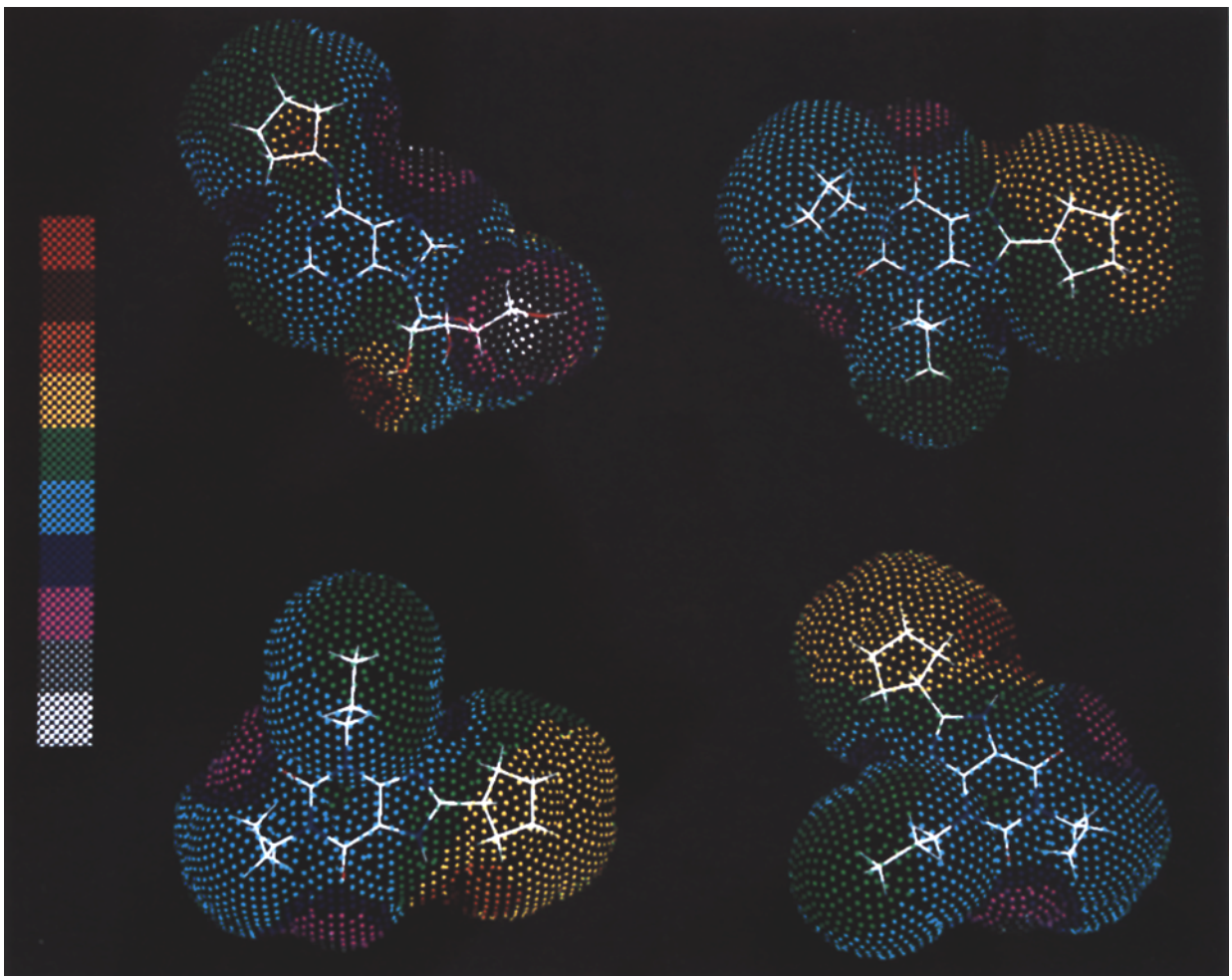

Fig. 4. Electrostatic potentials for CPA and DPCPX at 1.0 Å distance from the van der Waals surface. DPCPX is shown in the orientations corresponding to the three relative binding orientation models; top right: standard model; bottom left: flipped model; bottom right: N^6-C8 model. The value of the electrostatic potential V (kJ/mol) is colour coded red > +120 > brown > 90 > orange > 60 > yellow > 30 > green > 0 > cyan > –30 > blue > –60 > purple > –90 > grey > –120 > white.

0.5 Å from the van der Waals surface, and hydrogen bond acceptors and donors around 1.5 Å from it. The points were generated at intervals of ca. 0.25 Å, giving approximately 2400 points for theophylline up to 4000 points for DPCPX. The electrostatic potential was displayed, colour-coded by value using 10 colours, on a Personal Iris 4D/25GT, using a specially written program [19] to display the grid points generated by ORIENT superimposed on a Dreiding model of the molecule. (Examples of such pictures can be found in Figs. 3 and 4.)

Quantitative comparisons: Choice of region

The different models of the relative orientations of the ligands were compared for the points outside the van der Waals surface of the molecules, because only these electrostatic properties are relevant to its ability to interact with the receptor.

We have chosen as the outer limit of the region of comparison of the ligands a distance of 4 Å to include the region sampled by the receptor residues. This was implemented for the comparisons by producing a cubic grid of 0.5 Å spacing around the agonist, omitting all points within its van der Waals surface and more than 4 Å from any atom. For each model, a corresponding grid was produced around the antagonist in the appropriate relative orientation. The comparison criteria were only evaluated at the grid points in common to the two molecules.

Sign comparison

We have calculated the similarity of sign in the region defined above by counting the number of points where both potentials have the same sign and dividing it by the number of grid points used in the comparison.

Comparison of regions of significant electrostatic potential

Six limits were used to consider the regions of significant electrostatic potential: ≤ -75, ≤ -50, ≤ -25, $\geq +25$, $\geq +50$ and $\geq +75$ kJ/mol. The comparison of the agonists and antagonists for the three overlay models was carried out by a logical comparison, testing the limit for both molecules and accepting the grid point if the values for both molecules meet the requirement. This method is less sensitive to the outer limits of the region of comparison than the sign comparison, because the regions where the electrostatic potential is above the limit become smaller and are closer to the molecule as the limit increases in magnitude.

There are various methods for quantifying the similarity, S, using the numbers of grid points where the electrostatic potential for each molecule is in the given range, and the number of grid points in the region for comparison where the electrostatic potential for both molecules has this property. In our studies we used the molecular similarity as a quantitative nominator for the overlap:

$$S = \frac{2x}{A+B} \times 100\%$$

in which A = the total number of grid points corresponding to the specified range of the electrostatic potential for molecule **A**; B = the total number of grid points meeting this requirement for molecule **B**; and x = the number of grid points overlapping, i.e. meeting the requirement for both molecules.

In this method, the number of grid points overlapping is normalised by the average number of points for the molecules. This formula, which was also used in our previous comparison of agonists and antagonists for the adenosine receptor [4], is related to the similarity index formula of Hodgkin and Richards [20]. Other forms of normalisation were investigated, such as the maximum possible coincidence ($S = x/A \times 100\%$ if $A<B$ and $S = x/B \times 100\%$ if $A>B$), the weighted mean

$$S = \frac{x(A+B)}{2(A\times B)} \times 100\%$$

and the geometric mean

$$S = \frac{x}{\sqrt{A\times B}} \times 100\%$$

[23–25], but these did not affect the results qualitatively in this case. The reason for this is that the number of grid points within each range is similar for each pair of ligands, and very much larger than the number of overlapping grid points (i.e., $x \ll A \approx B$).

Matching minima and maxima

The regions where strong electrostatic interactions with the receptor can take place were also compared by locating the maxima and minima in the electrostatic potential around each molecule, at a fixed distance outside the van der Waals volume. To calculate the maxima in the electrostatic potentials, we optimised the electrostatic interaction of each ligand with an ion having a radius of 1.0 Å and a charge of −1. A similar ion with opposite charge was used to find the minima. The maxima and minima were located 1 Å from the van der Waals surface of the ligand as a compromise between the usual positions of protons involved in hydrogen bonds (≈0.5 Å), and the positions of hydrogen bond donors and acceptors (≈1.5 Å).

The optimisation of the matching of electrostatic extrema has been investigated as a method of determining relative overlays of phosphodiesterase III inhibitors by comparison with the natural substrate [21].

Results

Visual comparison

Adenosine and theophylline in the standard overlay show no similarities in the electrostatic potential pattern (Fig. 3), whereas the flipped overlay shows mainly similarities in the negative regions near theophylline O2 and

N9 and adenosine N1 and N7, respectively. The N^6-C8 model shows the largest similitude between these molecules, because it is similar in regions of negative potential near adenosine N1 and theophylline N9, and in those between adenosine N7 and C8 and theophylline O6, and also brings into coincidence the positive region near adenosine N^6 and that near theophylline C8. However, the positive region near theophylline N7-H overlays a rapidly changing potential near adenosine N^6/N7.

The electrostatic potentials of CPA and DPCPX, derived from the ab initio distributed multipoles (Fig. 4), also show no likeness at all in the standard model near the substituents of the purine ring systems. The flipped model yields a good matching of the negative potential region around CPA N1 with that of DPCPX O2, as for adenosine and theophylline, but the potential around N9 in DPCPX is less strong and, as a result, less similar to the region of negative potential around CPA N7. The N^6-C8 model causes the cyclopentyl moieties to coincide, giving an overlap of the weak electrostatic fields in this region. A major clash can be seen in the region of N^6 in CPA and N7 in DPCPX. It is clear from these pictures that there is no relative orientation which shows a strikingly good match of the electrostatic properties for this pair of molecules.

Sign comparison

The percentage of points with the same sign, calculated by dividing the number of grid points with equal sign of electrostatic potential by the number of grid points in the comparison, does not differ dramatically between the three models. The similarity of sign is approximately half of the grid points used in the comparison of adenosine and theophylline, 46, 56 and 54% for the standard, flipped and N^6-C8 models, respectively. The percentages were slightly less and more equal for CPA and DPCPX: 43% for the standard model, 42% for the flipped model and 44% for the N^6-C8 model.

Comparison of regions of significant electrostatic potential

The percentage of grid points that adenosine and theophylline have in common for the six ranges of potential are shown in Fig. 5A. The overlap is much smaller for the standard model than for the other two models. The N^6-C8 model has significantly more overlap of the regions of high positive potential than the other models, whereas the flipped model is fairly similar, and slightly better than the N^6-C8 model in the regions of significant negative potential. The comparisons change by up to 7% if performed in the region closer to the molecules, i.e. within 2 Å, with a step size of 0.25 Å (results not shown) making the difference between the flipped and N^6-C8 model for negative potentials within the uncertainties of the region of comparison. The overall conclusion for adenosine and theophylline is that the standard model is poor in overlapping regions of significant electrostatic potential, and that the N^6-C8 model is superior to the flipped model for matching regions of large (>50 kJ/mol) positive potential.

The results of the comparison of the three models for CPA and DPCPX (Fig. 5B) are surprisingly different. All three models give poor overlap in the positive potential regions (>50 kJ/mol), and the overlap for the flipped and N^6-C8 models is not markedly higher than for the standard model in the negative electrostatic potentials.

Matching minima and maxima

There are strong potential minima (< −50 kJ/mol) close to all hydrogen bond acceptors and strong maxima (>50 kJ/mol) close to all hydrogen bond donors (Fig. 6), with the exception of the minimum near N3 of adenosine and CPA, which is weaker because of its proximity to the strong maximum arising from a nearby sugar hydroxyl group. The strong electrostatic interaction points are virtually identical in theophylline and DPCPX, with the hydrocarbon substituents producing only fairly weak maxima. However, adenosine and CPA show differences

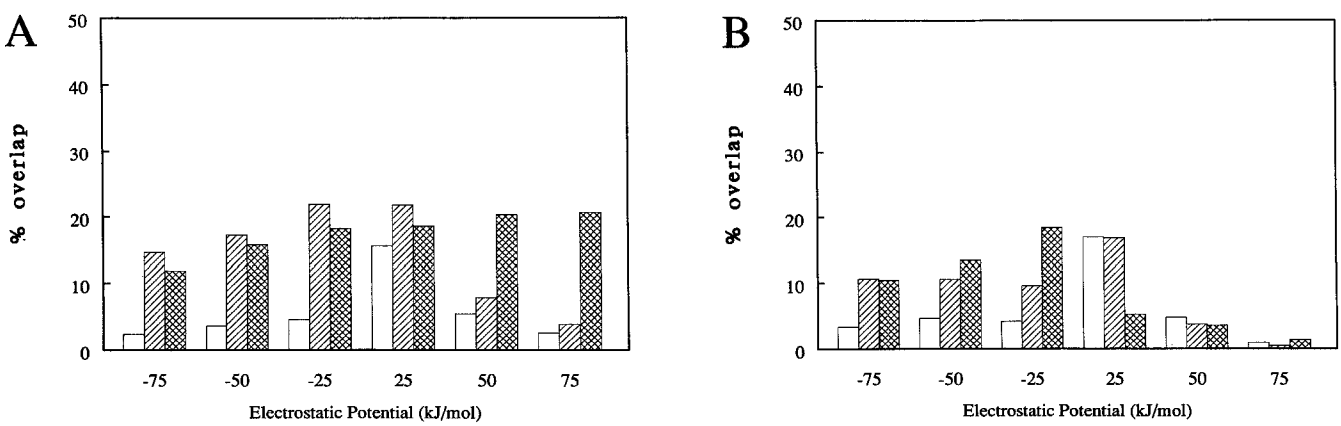

Fig. 5. Histograms showing the percentage overlap of regions of six significant ranges of electrostatic potential for three models: standard (white); flipped (shaded); and N^6-C8 (checked) for (A) adenosine and theophylline; and (B) CPA and DPCPX. The comparisons are carried out for a grid spacing of 0.5 Å, extending up to 4.0 Å from the van der Waals surface.

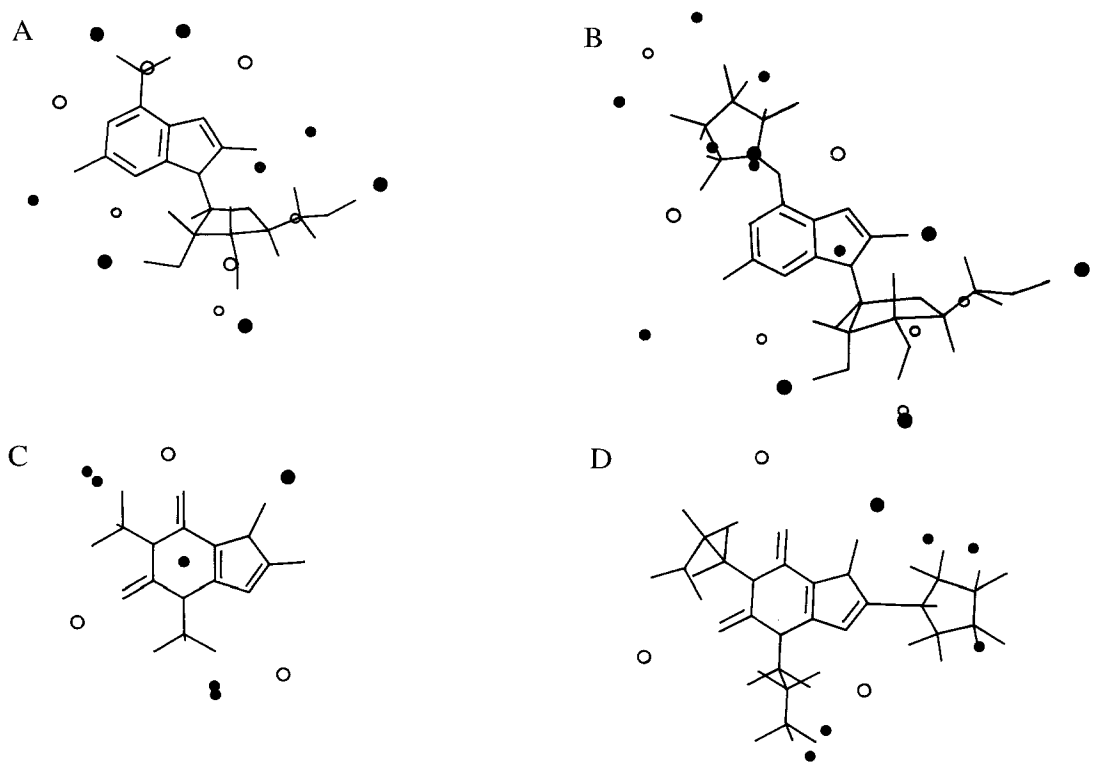

Fig. 6. Locations of minima and maxima in the electrostatic potential (V), 1 Å from the van der Waals surface, for (A) adenosine; (B) CPA; (C) theophylline; and (D) DPCPX, projected onto the plane of the ring system. Large filled circles represent maxima with V > 50 kJ/mol; small filled circles maxima of 50 kJ/mol > V > 0; large open circles minima of V < −50 kJ/mol; and small white dots minima of 0 > V > −50 kJ/mol. Only some of the electrostatic potential extrema are in the plane.

around the sugar rings and the N^6 region that can be attributed to differences in conformation. The rotation of the N^6-cyclopentyl substituent in the biologically active conformation, as determined by van Galen et al. [9], gives rise to an extremely deep minimum in the region around the lone pairs of N^6 and N7, as well as shifting the remaining NH potential maximum.

The results of the three overlays of adenosine and theophylline are shown in Table 1. All but one of the pairs of electrostatic extrema within 3 Å of each other in the standard model are of opposite sign and this suggests that both molecules could not bind to the same binding site in these relative orientations. There is a particularly strong repulsive overlay at the minimum of the adenosine N7 which is within 0.8 Å of the N7-H maximum of theophylline. The flipped overlay shows a close, strongly unfavourable correspondence between the adenosine sugar and the minimum near theophylline O6. The remaining unfavourable overlays mentioned are all at greater than 2 Å distance. The N^6-C8 model gives far fewer unfavourable comparison points than the other two models. The only unfavourable match within 2 Å is the overlay of a minimum near the ribose moiety with the very weak maximum of the C1-H of theophylline. Overlays involving weak extrema at least indicate the relative sign of the potential in the region, even if the interactions with the receptor are weak.

The comparison of the relative positions of the electrostatic extrema for the three overlay models for CPA and DPCPX can be found in Table 2. Again, there are significant differences in the matching of the extrema in comparison with those for adenosine and theophylline, mostly because of the effect of the conformations on the positions of the extrema. The standard overlay again causes regions of opposite potential to be overlaid. There are no correspondences of extrema within 3 Å which are not repulsive. In contrast, the signs of the potential match well in the flipped model, but the minima for the two molecules are quite widely separated. In the N^6-C8 overlay, the cyclopentyl rings of both molecules lie in the same region, so there is a good general overlay of maxima in this region. The hydrogen bond acceptors N1 and N9 are in a fairly similar position. However, potential hydrogen bonding sites at N^6 and N7 of CPA correspond to a region of opposite potential in DPCPX (N7-H) in this orientation. The magnitudes and proximity of the extrema classify this as a strongly unfavourable clash. This clash does not occur for the overlay of adenosine and theophylline, where the amino group is coplanar with the purine ring system.

Discussion

The purpose of this study was to evaluate the three

models for the agonist/antagonist binding site on the A_1 receptor regarding the similarity of their electrostatic properties, by different methods for comparing electrostatic potentials. The various methods of comparison focus on different aspects of the molecules' electrostatic properties, but a consistent picture emerges. Thus, after a discussion on the methods of comparison, we can compare the three models in the light of the total picture of the electrostatic interactions to emerge from the comparisons, and experimental structure–activity relationships. Finally, we analyze the differences between the results of these ab initio DMA-based methods and our previous electrostatic comparisons based on MOPAC AM1 semi-empirical point charges.

Methods of comparing the electrostatic potential

The major assumptions in comparing the overall charge distribution around two ligands are that they interact with the same polar receptor residues for favourable binding, and that the ligands are essentially surrounded by these receptor residues. Calculations on the electrostatic interactions between these ligands and the putative receptor binding site should provide insight into the various similarity methods, if these assumptions are valid. The information from structure–activity relationships and molecular biology [2,3], which was also used to derive a model for the adenosine A_1 receptor binding site [22], can be used to obtain some insight into the validity of the second assumption. Thus, it can be deduced that it is worthwhile assessing the overall electrostatic similarity, but that similarity in some regions will be more important than in others.

We have used several methods to study the similitude in electrostatic potentials of compounds. The method to quantify the comparison, comparing the signs of the potential on a grid, surveys the entire region, whereas the matching of minima and maxima concentrates on certain points, with the comparison of regions of significant potentials as an intermediate method. All three methods look at different aspects of the same 3D electrostatic field, and so have correlations with each other, and with a visual comparison; the methods have different strengths and weaknesses for assessing the electrostatic similarity in different relative binding orientations.

However, the results of the overall comparison (sign comparison) seem to be useless, even when considering a region as small as up to 2 Å from the van der Waals surface and a smaller step size (results not shown). The results are all approximately those expected from a random distribution of sign in the comparison region. Hence, the method does not indicate that there is some electrostatic similarity for some of the models. This false result arises probably because regions where both molecules have low potential magnitudes count as much as regions where strong electrostatic interactions with the receptor are possible. The other three methods to quantify the comparison and the visual comparison all provide a complementary and consistent picture of the electrostatic similarity.

Three models for the agonist/antagonist binding site

The standard model yields less electrostatic similarity than the other models. Indeed, it would be hard to devise a model for the receptor binding site that could bind both molecules according to the standard model, as there are very few regions of space where a polar residue could interact favourably with both molecules. Thus, this model can be dismissed on the grounds of poor electrostatic matching.

The difference between the flipped and N^6-C8 models depends on the molecules compared. The N^6-C8 model seems to be most favourable for the comparison of ade-

TABLE 1
OVERLAY OF THE MINIMA AND MAXIMA IN THE ELECTROSTATIC POTENTIAL 1 Å FROM THE VAN DER WAALS SURFACE OF ADENOSINE AND THEOPHYLLINE ACCORDING TO THE THREE OVERLAY MODELS

Adenosine	Theophylline	Sign	Strength	Distance (Å)
Standard overlay				
$N7_{min}$ (−75)	$N7\text{-}H_{max}$ (+154)	≠	s	0.7
$C2\text{-}H_{max}$ (+30)	$O2_{min}$ (−96)	≠	m	0.8
$N6\text{-}H'_{max}$ (+88)	$O6_{min}$ (−87)	≠	s	1.8
$N1_{min}$ (−72)	$C1\text{-}H'_{max}$ (+8)	≠	m	1.8
$N3_{min}$ (−28)	$C3\text{-}H''_{max}$ (+16)	≠	w	2.3
$N^6\text{-}H''_{max}$ (+82)	$O6_{min}$ (−87)	≠	s	2.3
$Sug2_{max}$ (+128)	$C3\text{-}H'_{max}$ (+17)	=	m	2.3
Flipped overlay				
$Sug2_{max}$ (+128)	$O6_{min}$ (−97)	≠	s	0.6
$N7_{min}$ (−75)	$N9_{min}$ (−63)	=	s	0.7
$N^6\text{-}H''_{max}$ (+82)	$C3\text{-}H'_{max}$ (+17)	=	m	1.2
$N1_{min}$ (−72)	$O2_{min}$ (−96)	=	s	1.9
N^6_{min} (−76)	$C3\text{-}H''_{max}$ (+16)	≠	m	2.0
$C2\text{-}H_{max}$ (+30)	$C1\text{-}H''_{max}$ (+7)	=	w	2.4
$C2\text{-}H_{max}$ (+30)	$C1\text{-}H'_{max}$ (+8)	=	w	2.6
$Sug4_{min}$ (−36)	$N7\text{-}H_{max}$ (+154)	≠	m	2.9
$Sug1_{min}$ (−126)	$N7\text{-}H_{max}$ (+154)	≠	s	3.0
N^6-C8 overlay				
$Sug1_{min}$ (−126)	$C1\text{-}H'_{max}$ (+8)	≠	m	1.1
$N^6\text{-}H''_{max}$ (+82)	$N7\text{-}H_{max}$ (+154)	=	s	1.2
$N1_{min}$ (−72)	$N9_{min}$ (−63)	=	s	2.0
$Sug4_{min}$ (−36)	$O2_{min}$ (−96)	=	m	2.1
$Sug2_{min}$ (−126)	$O6_{min}$ (−87)	=	s	2.2
$C2\text{-}H_{max}$ (+30)	$C3\text{-}H'_{max}$ (+17)	=	w	2.3
$C2\text{-}H_{max}$ (+30)	$C3\text{-}H''_{max}$ (+16)	=	w	2.5
$N7_{min}$ (−75)	$O6_{min}$ (−87)	=	s	2.7
$N3_{min}$ (−28)	$Purine2_{max}$ (+4)	≠	w	3.0
$Sug4_{max}$ (+48)	$Purine1_{max}$ (+3)	=	w	3.1

All cases where extrema of the two molecules are within approximately 3 Å of each other have been included. These are classified according to whether the potential has the same sign, denoted by =, or opposite sign, ≠, and the relative strength: s: strong, both extrema have |V| >50 kJ/mol; m: medium, only one extremum has |V| >50 kJ/mol; w: weak, both extrema have |V| <50 kJ/mol.

TABLE 2
OVERLAY OF THE MINIMA AND MAXIMA IN THE ELECTROSTATIC POTENTIAL 1 Å FROM THE VAN DER WAALS SURFACE OF CPA AND DPCPX ACCORDING TO THE THREE OVERLAY MODELS

CPA	DPCPX	Sign	Strength	Distance (Å)
Standard overlay				
C2-H_{max} (+30)	$O2_{min}$ (−100)	≠	m	1.0
N7/N^6_{min} (−152)	N7-H_{max} (+132)	≠	s	1.1
$Cpent1_{max}$ (+68)	$O6_{min}$ (−97)	≠	s	2.4
Flipped overlay				
N7/N^6_{min} (−152)	$N9_{min}$ (−57)	=	s	1.2
$Cpent3_{max}$ (+10)	$Prop2_{max}$ (+3)	=	w	2.1
$N1_{min}$ (−55)	$O2_{min}$ (−100)	=	s	2.1
$N3_{min}$ (−12)	$O6_{min}$ (−97)	=	m	3.1
N^6-C8 overlay				
$Cpent5_{max}$ (+9)	$Cpent2_{max}$ (+37)	=	w	0.6
N7/N^6_{min} (−152)	N7-H_{max} (+132)	≠	s	0.7
$N1_{min}$ (−55)	$N9_{min}$ (−57)	=	s	1.5
$Cpent4_{max}$ (+10)	$Cpent3_{max}$ (+32)	=	w	1.5

All cases where extrema of the two molecules are within approximately 3 Å of each other have been included. These are classified according to whether the potential has the same sign, denoted by =, or opposite sign, ≠, and the relative strength: s: strong, both extrema have |V| >50 kJ/mol; m: medium, only one extremum has |V| >50 kJ/mol; w: weak, both extrema have |V| <50 kJ/mol.

nosine and theophylline. However, this model appears less favourable for DPCPX in comparison with CPA, mainly because of a clash of the positive potential around N7/C8 of DPCPX with the minimum near N^6/N7 for CPA.

In discussing the relative binding orientation of the adenosine-based agonists and the xanthine-like antagonists, it seemed reasonable to assume that the hydrocarbon substituents would not significantly alter the electrostatic properties of the molecules. This is not the case when substitution alters the conformation of the polar groups. The lone pair of the N^6-amino group of adenosine produces an electrostatic minimum above N^6 when the amino group is in the plane of the purine ring as in adenosine. In contrast, the biologically active conformation of N^6-substituted adenosine analogues, as modelled by van Galen et al. [9] has an amino group which is not in the plane of the purine ring system, which causes the minimum near N7 to deepen significantly as well as changing the positions of the maxima associated with the amino group. We have investigated the effect of this conformational change, which is crucial to the electrostatic similarity in this important region, by transforming the multipoles to represent the other conformation. The minimum near N7 in CPA is approximately twice as deep for the rotated, biologically active conformation compared to the situation where the amino group is held planar. However, rotating the amino group the other way around can result in deepening the minimum near N1. For adenosine/theophylline, the greater similarity for the positive potentials shown by the N^6-C8 model in Fig. 5A is not drastically decreased when the amino group is rotated to the same conformation as in CPA, as the N7/N^6 minimum of adenosine, which is deepened by the rotation of the amino group, is further (1.5 Å) from the N7-H maximum of theophylline.

Another region which is very sensitive to conformational changes with respect to the potentials is the ribose moiety. Small differences in conformation of the ribose moiety can lead to large differences in the values of maxima and minima. We would expect that the ribose ring could adapt its structure on binding to optimise the electrostatic interactions with the binding site, as there are a variety of low-energy conformations. However, the electrostatic properties of the ribose group only have a minor effect on the comparison, because the xanthine-like antagonists have no polar substituents overlaying the ribose group in any of the models. Biochemical data confirm the importance of the ribose moiety for affinity, because removal of the sugar group results in a major loss of affinity [26]. None of the overlay models provide a good matching of the electrostatic properties in this region. However, studies on the influence of chemical modifications of the histidine receptor residues on the binding of ligands [27,28], and mutation of the bovine adenosine receptor [29] showed that probably different histidine residues are involved in agonist and antagonist binding. The overlay models are therefore not invalidated by the observation that the xanthine structures do not match the electrostatic potential around the ribose moiety, the major difference between agonists and antagonists.

Theophylline is small enough to bind whichever way is most favourable electrostatically, without extra steric hindrance. It will therefore be directed in the binding site by the electrostatic potentials. Therefore, it will probably bind according to the N^6-C8 model. There are, however, two possible conclusions regarding the positioning of DPCPX in the receptor binding site. The first possibility is that, if there is enough space in the binding site to accommodate DPCPX in the position according to the flipped model, then this position yields the most favourable electrostatic interactions in terms of matched extrema, and avoids the major clash in the N^6/N7-region. The second possibility is that DPCPX may be directed by its volume in the binding site according to the N^6-C8 model and the electrostatic interactions are not optimal, but still sufficient to produce binding. It is possible that the clash in electrostatic potentials in the N^6/N7-region for xanthine-like antagonists may be relieved by relatively small changes in the receptor conformation.

Evidence for the validity of the N^6-C8 model for substituted xanthines comes from receptor binding studies. The influence of xanthine C8-substituents on affinity is similar to that of the adenosine N^6-substituents. Peet et al. have substituted xanthine analogues at the 8-position

with a 1-phenyl-2-propyl group, analogous to *R*- and *S*-PIA to prove that the stereochemical requirements of the xanthine C8- and the adenosine N^6-positions are also similar [6]. The overlay of CPA and DPCPX according to the N^6-C8 model, with the substituents in the conformations as determined [9–11], shows that these groups coincide in these conformations.

Three attempts to add a ribose moiety to the xanthine ring have been described in the literature. Clanachan has tested theophylline-9-riboside, but found that it has no affinity for the adenosine receptors [30]. Therefore, it is not possible for the 9-ribose ring to accommodate the pocket to which the adenosine ribose ring binds; this is another argument against the standard model. Van Galen et al. substituted the xanthine ring at the 7-position [31], which coincides with the adenosine N9-position in their flipped model. The xanthine-7-ribosides appeared to bind the adenosine receptor, but with somewhat lower affinities than the corresponding xanthines. The effects of substituents at the 1- and 3-positions of the xanthine-7-ribosides agree with the structure–affinity relationships for xanthines. Obviously, the xanthine N7-region can accommodate such a relatively large ribose moiety. These conclusions seem to confirm that xanthine analogues can bind the receptor site according to the flipped model. Xanthine-1-ribosides are described to bind to the adenosine receptor with low affinities [32]. This would direct the xanthine ring more in the N^6-C8 overlay. The low affinity of these compounds would be the result of a less favourable fit of the ribose ring in the binding pocket than for the xanthine-7-ribosides. Thus, the experimental evidence implies that some xanthine analogues bind according to the N^6-C8 model, whereas others may well bind according to the flipped model. This is consistent with the electrostatic modelling, in that the difference in electrostatic similarity between the N^6-C8 and flipped models is sufficiently small that substituents can change which overlay appears more favourable.

Thus, there are clear limits to the conclusions on likely relative binding orientations that can be drawn by comparing the steric and electrostatic properties of the ligands in isolation. However, such calculations are a necessary preliminary to potentially more conclusive studies in which the interactions between the receptor and each ligand are optimised, as these calculations rely on sensible starting points for the optimisation.

Comparison with previous work

The conclusions of the former comparison [4] were mainly based on differences in volume overlap. Not much difference in electrostatic potential overlap could be found. Therefore, no choice could be made based on the electrostatic potentials. In the present study, the standard model can definitely be rejected, because it shows less resemblance between the potentials of agonists and xanthine antagonists than the other two models. However, the choice between the other models is still difficult and, as discussed above, cannot be made with any degree of confidence on the basis of electrostatic similarity alone. The conclusions of the two electrostatic studies differ sufficiently that we need to analyze the causes.

First, the quality of the molecular charge distribution and the accuracy of its representation are much higher in this study. We used distributed multipoles of ab initio SCF wave functions instead of a point charge representation of the MOPAC AM1 semiempirical wave functions [33,34]. There are significant differences between the predicted electrostatic properties. The AM1-derived atomic charges predict more moderate values for the electrostatic potential than the ab initio-based multipoles. For example, for adenosine the ab initio-based potential 1 Å from the van der Waals surface varies from −130 to +150 kJ/mol, whereas those calculated with AM1 range from −80 to +70 kJ/mol. The differences in potentials are not just a matter of scaling: the electrostatic potential over the purine ring system is far less negative for the DMA representation (between 0 and −30 kJ/mol) than for the semiempirical charges (< −60 kJ/mol). This probably reflects an inability of the AM1-derived atomic point charges to predict the electrostatic forces over aromatic rings, because they lack the flexibility to describe the anisotropic effects of the π-electrons [35,36].

The former comparison used Chem-X [37], a molecular modelling package, for the calculation and comparison of potentials; Chem-X creates contours which are calculated by interpolating between imaginary grid points and uses these maps for logical operations. Chem-X calculates the electrostatic potentials within the molecules as well, whereas in the present study we compared the potentials only outside the van der Waals surface; only the part outside the van der Waals volume is accessible for the receptor residues. This difference would be crucial if the method of comparison were dominated by the region within the molecule.

We have repeated the comparisons of significant electrostatic potential on the grids outside the molecule, using the MOPAC AM1 charges to calculate the electrostatic potential. The graphs are completely different from Figs. 5 and 6 and show little distinction between the models. Also, the MOPAC minimum around N^6/N7 is less significant. Hence, the approximation of the electrostatic potential by using the AM1 point charges was a major factor in the difficulty of the previous work to distinguish between the models on electrostatic grounds. The abberations in these AM1-derived charges are, therefore, sufficient to mask important features in the similarity and dissimilarity of molecules, and so accurate ab initio-based distributed multipole models must be preferred for confidence in the realism of the electrostatic properties being compared.

Conclusions

A comparison of electrostatic properties around pairs of ligands for the adenosine A_1 receptor is able to dismiss the standard model for the overlay of the ligands, and highlights some of the features which may distinguish between two other proposed overlays. The N^6-C8 model is most favourable for adenosine and theophylline, but the rotation of the substituted N^6-amino group makes this model less attractive for the comparison of CPA and DPCPX. This could result in a different binding orientation for theophylline and DPCPX, or in DPCPX being directed by its volume in the receptor binding site and thus binding also according to the N^6-C8 model. Conformational changes in the ligand and receptor could reduce the effect of the dissimilarity in electrostatic potential in the N^6/N7-region.

A variety of comparison methods have been applied to this problem. Since the electrostatic similarity of the pairs of ligands is quite low in all three models, the use of a range of complementary methods is definitely useful in this case. Little information can be gleaned by a comparison of the sign of the potential around the molecule. However, comparing the regions of strong electrostatic potential, by an overall comparison or a comparison of electrostatic extrema, is a useful way of quantifying the similarity and the extrema method locates the regions of similarity. These calculations clarify and quantify any visual comparison.

Thus, we have shown that Distributed Multipole Analysis offers a new, feasible tool for the medicinal chemist, because it provides the use of reliable electrostatic models to determine plausible relative binding orientations.

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