Journal of Computer-Aided Molecular Design, 9 (1995) 33–43
ESCOM

J-CAMD 280

The matching of electrostatic extrema: A useful method in drug design? A study of phosphodiesterase III inhibitors

Robert P. Apaya, Baldo Lucchese, Sarah L. Price* and J.G. Vinter

Department of Chemistry, University College London, 20 Gordon Street, London WC1H 0AJ, U.K.

Received 5 April 1994
Accepted 13 September 1994

Keywords: Distributed multipoles; Electrostatic similarity; Relative binding orientation; Phosphodiesterase inhibitors

Summary

Ligands which bind to a specific protein binding site are often expected to have a similar electrostatic environment which complements that of the binding site. One method of assessing molecular electrostatic similarity is to examine the possible overlay of the maxima and minima in the electrostatic potential outside the molecules and thereby match the regions where strong electrostatic interactions, including hydrogen bonds, with the residues of the binding site may be possible. This approach is validated with accurate calculations of the electrostatic potential, derived from a distributed multipole analysis of an ab initio charge density of the molecule, so that the effects of lone pair and π-electron density are correctly included. We have applied this method to the phosphodiesterase (PDE) III substrate adenosine-3',5'-cyclic monophosphate (cAMP) and a range of nonspecific and specific PDE III inhibitors. Despite the structural variation between cAMP and the inhibitors, it is possible to match three or four extrema to produce relative orientations in which the inhibitors are sufficiently sterically and electrostatically similar to the natural substrate to account for their affinity for PDE III. This matching of extrema is more apparent using the accurate electrostatic models than it was when this approach was first applied, using semiempirical point charge models. These results reinforce the hypothesis of electrostatic similarity and give weight to the technique of extrema matching as a useful tool in drug design.

Introduction

A common early stage in drug design is to find new molecules which will bind in the same protein binding site as a known natural substrate or 'lead compound'. The 'lock and key' analogy for these processes emphasises that the geometry of the binding site, and the passage into it, place steric requirements on the ligands. The requirement of surface complementarity for binding is often unnecessary. Medicinal chemists have produced molecules of widely varying sizes which bind at a particular site, implying that many protein active sites are much larger than the ligands they attract. Some have very little surface contact as they are bound by hydrogen bonds, which are primarily electrostatic. The attractive r^{-6} dispersion forces can only dominate the binding when the surface complementarity is high. The repulsion forces affect binding when the ligand is too large to fit the site, i.e., when the steric clashes outweigh the attractive binding forces. Thus, in cases where the surface complementarity is low, and the ligands are polar and capable of hydrogen bonding, the major driving force for binding would seem to be electrostatic. The binding process is rather late in the overall recognition process, and the ligand must be attracted into the binding site from some distance. This long-range attraction is likely to be electrostatic in nature. These arguments lead to the hypothesis that electrostatic interactions are always of major importance in the ligand/protein binding mechanism, and may be dominant in many cases.

This hypothesis has been recognised for some time [1,2], but in practice, the difficulty in applying it is in defining electrostatic similarity. Various quantitative methods have been proposed, for example similarity indices introduced by Carbo et al. [3], which measure molecular similarity in terms of integrals over the superimposed charge densities. Hodgkin and Richards [4] use various integrals over the electrostatic potentials outside the entire region around the two molecules. Alternative methods

*To whom correspondence should be addressed.

have been developed by van der Wenden et al. [5] for comparing the electrostatic potentials only within the regions where there could be binding site atoms in close contact with both ligand molecules. However, even this method only partially recognises that in most binding sites there are some regions around the ligand that are not in van der Waals contact with the binding site atoms. Sanz et al. [6] have developed several different algorithms to analyse electrostatic potentials, calculated directly from the wave function, and generate various comparisons. Clearly, we need to develop a range of methods of assessing electrostatic similarity before it will be possible to determine which method, or combination of methods, will be most effective in drug design.

In this paper we develop an alternative approach to electrostatic similarity, first used by Davis et al. [7], which focuses on the regions where particularly strong electrostatic interactions with the unknown binding site are possible, namely the maxima and minima in the electrostatic potential in the accessible region outside the van der Waals surface of the molecule. Davis et al. [7] found that the overlay of potential minima between various inhibitors and the natural substrate of the phosphodiesterase (PDE) III system was more important than steric factors in determining the biological activity of the inhibitors. This confirms the picture of the binding site which emerges from the experimentally observed effects of substitution on the binding of inhibitors, namely that the binding site is very open, and the major binding force is electrostatic. Hence, this is a suitable system for developing methods to test for electrostatic similarity.

The aim of this study is to establish whether focusing on the relative disposition of electrostatic extrema is a useful method of assessing possible relative binding orientations of ligands. We use a rigorously defined accurate electrostatic model, that is a distributed multipole (DMA) [8] representation of an ab initio wave function of each molecule, to test whether the similarity observed by Davis et al. [7] is an artefact of their approximate method of generating electrostatic properties. If electrostatic similarity is important in determining binding, the similarity should be more obvious from accurate electrostatic properties. We also examine all extrema to assess overall similarity. Davis et al. [7] used semiempirical point charges to find the minima and maxima, but the maxima did not appear to play a role in determining the binding because they were inaccurately determined. We provide a detailed analysis of the electrostatic properties of the substrate and a diverse sample of inhibitors, to establish the degree of similarity that is found in this system. We do not study all inhibitors, since Davis et al. [7] have already established sufficient matching of extrema. Our qualitative conclusions will not be affected by a larger sample, and it is not our intention in this paper to comment or expand on the biological content and conclusions of the earlier paper [7].

Distributed multipole electrostatic models differ fundamentally from atomic point charge models, in that the atomic dipoles, quadrupoles, etc. represent the electrostatic effects of lone pair and π-electron density, which play a major role in hydrogen bonding. Indeed, the structures of many hydrogen-bonded van der Waals complexes can be predicted by optimising the electrostatic interactions (calculated from a DMA) within accessible orientations [9]. The strong link between electrostatic forces and hydrogen bonding results in most of the electrostatic maxima and minima being associated with hydrogen bond donor and acceptor sites, respectively. Hence, there are strong similarities between our approach and that used by Kato et al. [10] based on matching expected hydrogen bonding sites, although our method makes no presumptions about hydrogen bonding and also includes other electrostatic extrema, such as those associated with aromatic rings.

To be compatible with the original work [7] we compared the electrostatic potential around a selection of known inhibitors of PDE III with that of the natural substrate, adenosine 3',5'-cyclic monophosphate (cAMP) (Fig. 1). For each molecule, we located all the maxima and minima in the electrostatic potential, as calculated from a Distributed Multipole Analysis of an ab initio wave function, at fixed distances outside the van der Waals surface of the molecule. In each case it was possible to find a relative orientation of the inhibitor and substrate in which four of the extrema of the inhibitor were within an Ångstrom or so of an extremum of the same sign of the substrate. These overlays produced an overall match of the electrostatic and steric properties which is consistent with the inhibitor binding to the enzyme in the same site as cAMP. The distribution of the potential extrema around the substrate is sufficiently complex and sensitive, that it is highly unlikely that such a matching would occur by chance. Thus, empirically, a

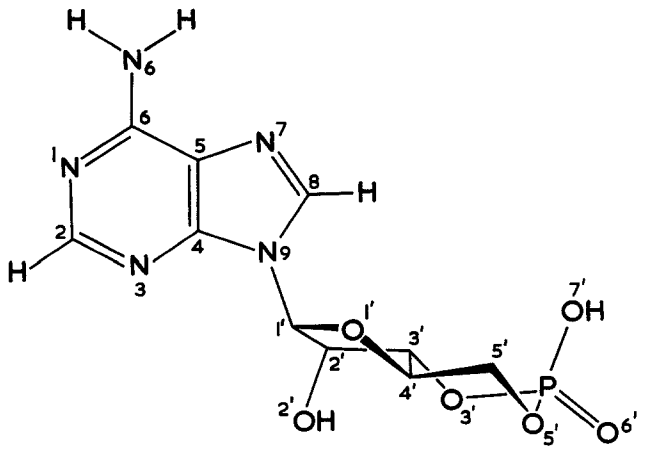


Fig. 1. Adenosine-3',5'-cyclic monophosphate (cAMP), the natural substrate for PDE III.

TABLE 1
STRUCTURE AND PROPOSED FOUR-POINT RELATIVE BINDING ORIENTATION OF THE SPECIFIC PDE III INHIBITORS

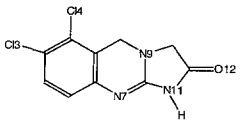
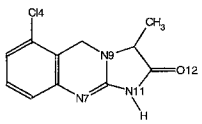
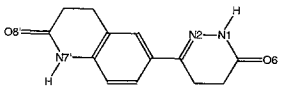
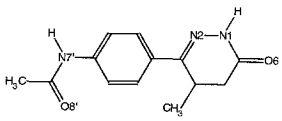
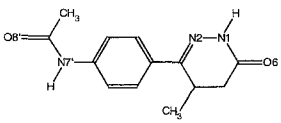
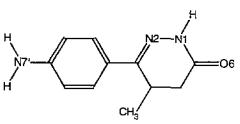
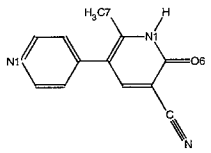
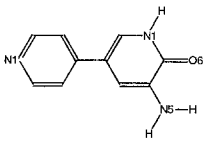
Inhibitor	IC_{50} (μM)	Corresponding maxima and minima				Same sign	Strength[a]	Separation (Å)	Rmsd of points in match[b]	
		Nearest inhibitor atom	V (kJ mol^{-1})	Nearest cAMP atom	V (kJ mol^{-1})				4-point (Å)	(3-point) (Å)
Anagrelide	0.13	$O12_{min}$	−73.3	$O6'_{min}$	−206.8	y	s	0.688	0.602	(0.602)
		$N7_{min}$	−116.4	$O2'_{min}$	−105.0	y	s	0.377		
		Ar^{A}_{min}	−88.2	$O1'_{min}$	−73.5	y	s	0.833		
		$Cl3_{min}$	−46.6	$N6^{B}_{min}$	−16.7	y	w	0.376		
		$Cl4_{min}$	−93.6	$N7_{min}$	−102.2	y	s	1.332	Additional close extrema	
		$N7_{min}$	−116.4	$N3_{min}$	−132.1	y	s	1.945		
		C13-$H1_{max}$	+62.5	O7'-H_{max}	+126.9	y	s	2.739		
		$C13^{A}_{max}$	+58.5	C3'-H_{max}	+86.5	y	s	3.013		
		Ar^{A}_{min}	−88.2	C2'-H_{max}	+56.4	n	s	2.723		
Dazonone	1.68	$O12_{min}$	−143.9	$O6'_{min}$	−206.8	y	s	0.423	0.590	(0.552)
		$N7_{min}$	−112.1	$O2'_{min}$	−105.0	y	s	0.545		
		Ar^{B}_{min}	−54.3	$O1'_{min}$	−73.5	y	s	0.851		
		$Cl4^{B}_{min}$	−40.4	$N7_{min}$	−102.2	y	m	0.439		
		$C13_{max}$	+21.1	C2'-H_{max}	+56.4	y	m	2.049	Additional close extrema	
		$N7_{min}$	−112.1	$N3_{min}$	−132.1	y	s	2.313		
		C13-$H2_{max}$	+46.3	C8-H_{max}	+86.5	y	m	2.798		
		C3-H_{max}	+29.5	$N6^{B}_{min}$	−16.7	n	w	1.847		
SK&F95800	0.76	$O6_{min}$	−152.3	$O6'_{min}$	−206.8	y	s	1.123	1.038	(0.673)
		$O8'_{min}$	−167.0	$N1_{min}$	−118.1	y	s	0.984		
		Ar^{B}_{min}	−49.7	$O1'_{min}$	−73.5	y	m	1.380		
		C3'-H_{max}	+63.9	C8-H_{max}	+86.5	y	s	0.425		
		$N2_{min}$	−50.1	$O2'_{min}$	−105.0	y	s	2.218	Additional close extrema	
		N7'-H_{max}	+84.7	N6-$H2_{max}$	+65.8	y	s	2.608		
		C10'-H_{max}	+33.9	$O2'_{min}$	−105.0	n	m	2.192		
		C10'-H_{max}	+33.9	$N3_{min}$	−132.1	n	m	2.629		
		C5'-H_{max}	+33.9	$O2'_{min}$	−105.0	n	m	1.537		
		C5'-H_{max}	+33.9	$N3_{min}$	−132.1	n	m	2.585		
		N7'-H_{max}	+84.7	$N7_{min}$	−102.2	n	s	1.986		
		N7'-H_{max}	+84.7	$N6^{B}_{min}$	−16.7	n	m	2.333		
		Ar^{A}_{min}	−44.7	C2'-H_{max}	+56.4	n	m	2.159		
		$N2_{min}$	−50.1	O2'-H_{max}	+64.3	n	s	1.913		
SK&F93741g	0.90	$O6_{min}$	−162.0	$O6'_{min}$	−206.8	y	s	0.357	0.781	(0.611)
		$O8'_{min}$	−164.8	$N7_{min}$	−102.2	y	s	1.019		
		$N2_{min}$	−85.3	$O2'_{min}$	−105.0	y	s	0.662		
		C3'-H_{max}	+33.4	C2'-H_{max}	+56.4	y	m	0.913		
		Ar^{A}_{min}	−64.6	$N3_{min}$	−132.1	y	s	1.726	Additional close extrema	
		C7-H_{max}	+30.6	O7'-H_{max}	+126.9	y	m	3.098		
		N7'-H_{max}	+113.5	$N1_{min}$	−118.1	n	s	3.083		
SK&F93741ac	0.90	$O6_{min}$	−149.8	$O6'_{min}$	−206.8	y	s	0.603	0.815	(0.659)
		$O8'_{min}$	−176.3	$N1_{min}$	−118.1	y	s	1.028		
		$N2_{min}$	−99.5	$O2'_{min}$	−105.0	y	s	0.986		
		C3'-H_{max}	+61.1	C8-H_{max}	+86.5	y	s	0.518		
		C5-$H1_{max}$	+44.4	O7'-H_{max}	+126.9	y	m	1.859	Additional close extrema	
		C5-$H2_{max}$	+44.4	O2'-H_{max}	+64.3	y	m	2.570		
		N7'-H_{max}	+65.4	$N6^{A}_{min}$	−11.8	n	m	1.500		
		Ar^{A}_{max}	+26.0	$N6^{A}_{min}$	−11.8	n	m	2.054		
SK&F93505	37.00	$O6_{min}$	−168.5	$O6'_{min}$	−206.8	y	s	1.156	1.145	(0.558)
		N1-H_{max}	+47.4	O2'-H_{max}	+64.3	y	m	0.259		
		N7'-$H1_{max}$	+77.5	N6-$H2_{max}$	+65.8	y	s	1.061		
		N7'-$H2_{max}$	+74.3	N6-$H1_{max}$	+69.2	y	s	1.648		
		$N7'^{B}_{min}$	−52.0	$N6^{B}_{min}$	−16.7	y	m	1.041	Additional close extrema	
		$N7'^{A}_{min}$	−54.0	$N6^{A}_{min}$	−11.8	y	m	1.493		
		C3'-H_{max}	+41.7	C8-H_{max}	+86.5	y	m	1.913		
		$N2_{min}$	−102.4	C2'-H_{max}	+56.4	n	s	2.154		
		N7'-$H2_{max}$	+74.3	$N1_{min}$	−118.1	n	s	2.221		
		Ar^{A}_{min}	−87.8	C2'-H_{max}	+56.4	n	s	2.604		

TABLE 1 (continued)

Inhibitor	IC_{50} (μM)	Corresponding maxima and minima				Same sign	Strength[a]	Separation (Å)	Rmsd of points in match[b]	
		Nearest inhibitor atom	V (kJ mol^{-1})	Nearest cAMP atom	V (kJ mol^{-1})				4-point (Å)	(3-point) (Å)
Milrinone 	2.20	$O6_{min}$	−170.0	$O6'_{min}$	−206.8	y	s	0.674	0.749	(0.497)
		C7-H_{max}	+88.2	C8-H_{max}	+86.5	y	s	0.626		
		N1-H_{max}	+117.2	O7'-H_{max}	+126.9	y	s	0.527		
		C4-H_{max}	+49.0	O2'-H_{max}	+64.3	y	m	1.058		
		$N1'_{min}$	−128.2	$N6^{A}_{min}$	−11.8	y	m	1.729	Additional close extrema	
Amrinone 	51.00	$O6_{min}$	−142.8	$O6'_{min}$	−206.8	y	s	1.786	1.406	(0.858)
		$N1'_{min}$	−147.4	$N1_{min}$	−118.1	y	s	0.479		
		N1-H_{max}	+94.2	O2'-H_{max}	+64.3	y	s	0.550		
		Ar^{A}_{min}	−40.7	$N6^{A}_{min}$	−11.8	y	w	2.045		
		$C3^{B}_{min}$	−29.4	$N3_{min}$	−132.1	y	m	1.449	Additional close extrema	
		$C4^{B}_{min}$	−32.2	$O1'_{min}$	−73.5	y	m	1.975		
		Ar^{B}_{min}	−41.1	$N3_{min}$	−132.1	y	m	2.023		
		$C4^{A}_{min}$	−31.6	$N3_{min}$	−132.1	y	m	2.422		
		$C5^{B}_{min}$	−33.7	$O1'_{min}$	−73.5	y	m	2.848		
		$C4^{B}_{min}$	−32.2	C2'-H_{max}	+56.4	n	m	1.313		
		$N5^{A}_{min}$	−58.4	C8-H_{max}	+86.5	n	s	1.360		
		N5-$H1_{max}$	+86.0	$O1'_{min}$	−73.5	n	s	1.953		
		$C3^{A}_{min}$	−28.8	C2'-H_{max}	+56.4	n	m	1.956		
		$C5^{A}_{min}$	−33.4	C2'-H_{max}	+56.4	n	m	2.066		
		C3'-H_{max}	+66.9	$O1'_{min}$	−73.5	n	s	2.353		

[a] The relative strength of each pair of corresponding extrema is classified according to the following definitions: s: strong, both extrema have $|V| > 50$ kJ mol^{-1}; m: moderate, only one extremum has $|V| > 50$ kJ mol^{-1}; w: weak, both extrema have $|V| < 50$ kJ mol^{-1}.

[b] Relative binding orientations quantified by the minimum rmsd of the separation of the first four (three) corresponding pairs of matched points. All other pairs of contacts within 3 Å are also given as additional close extrema. Separations of extrema pairs are given for the four-point match.

good matching of the extrema appears to pick out molecules which can bind to the same site, and indeed there are some qualitative correlations with the experimental inhibitory activity. These observations could be readily rationalised by assuming that the matched extrema are close to polar binding groups of the opposite sign in the binding site. However, the positions of the binding site atoms may not be that crucial to the success of the method, as the matching of the extrema does produce a general similarity in the steric and electrostatic properties of the ligands.

Materials and Methods

The anti conformer of the natural substrate adenosine 3',5'-cyclic monophosphate (cAMP, Fig. 1) has been determined to give an explanation of inhibitory effectiveness which is sterically consistent with the size and conformation of the selective PDE III inhibitors [11]. The protonated form was used in this study, following the consideration of both the protonated and unprotonated forms by Davis et al. [7]. Their conclusion that electrostatic similarity is more important in determining inhibitory action than structural similarity was based on a study of five nonspecific and 54 specific PDE III inhibitors. To confirm the degree of similarity in this work, four nonspecific and eight specific PDE III inhibitors have been chosen. The specific inhibitors are a sub-set of the original data set which includes pairs of molecules which are structurally similar, but have very different inhibitory effectiveness. The structure of each specific PDE III inhibitor is given in Table 1, and the nonspecific inhibitors are shown in Table 2.

The assumed anti geometry of cAMP and the geometries of the specific and nonspecific PDE III inhibitors studied here have been obtained by energy minimisation with the software package MacroModel [12] using the AMBER [13] molecular mechanics force field, running on a Silicon Graphics workstation. Initial modelling and minimisation of the specific PDE III inhibitors showed that most of them had essentially planar conformations. This is in agreement with previous studies [14] which propose a generally flat topology for specific PDE III inhibitors, and the molecular geometry in the crystal of many inhibitors of phosphodiesterase III [15].

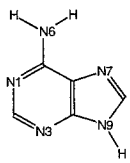
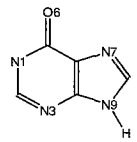
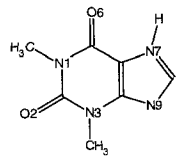
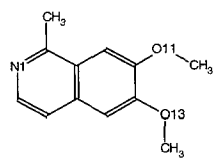
Minimisation of milrinone within MacroModel gave a nonplanar structure with an inter-ring torsion angle of 49.5°, in agreement with X-ray data [16]. The milrinone 2-methyl group cannot adopt a pseudoaxial conformation, and as a result milrinone is markedly nonplanar due to steric repulsion between the 2-methyl group and the 3' and 5' hydrogens. Despite a general requirement for

planarity in specific PDE III inhibitors, milrinone is still an effective compound.

Two pyridazinone compounds have some flexibility in their inter-ring torsion angle. Unlike milrinone, the compound SK&F93505 has greater flexibility in the pyridazinone ring, allowing the two rings to be approximately coplanar (inter-ring torsion angle 11.7°) when the methyl group adopts a pseudoaxial conformation. In the related compound SK&F93741, the minimum energy conformer has the C1'-N7' and C8'=O8' bonds of the acetyl group in a cis conformation, with an inter-ring torsion angle of 11°. An alternative conformation, with the C1'-N7' and C8'=O8' bonds in a trans arrangement and the acetyl group rotated at 50° to the aromatic ring plane has also been studied. The barrier to rotation for the acetyl C=O group was approximately 30 kJ mol^{-1}. The acetyl-rotated structure of SK&F93741 mimics the structure of a third pyridazinone inhibitor, SK&F95800, in which the conformation of the C8'=O8' bond is fixed as part of a ring closure. To investigate the effect of the acetyl conformation on the electrostatic similarity of these inhibitors to cAMP, both the global energy minimum, labelled SK&F93741g, the acetyl-rotated structure, SK&F93741ac, and SK&F95800 have been used in these calculations.

For this work, SCF wave functions were obtained for each molecule using the CADPAC [17] suite of ab initio programs, and a 3-21G basis set [18] in all cases. This basis set was found to give essentially the same location (to within 0.01 Å) and relative strengths of the electrostatic extrema as a 6-31G** basis set for adenine and theophylline (B. Lucchese, unpublished results). This is in agreement with the observation by Price et al. [19] that

TABLE 2
STRUCTURE AND PROPOSED THREE- OR FOUR-POINT RELATIVE BINDING ORIENTATION OF THE NONSPECIFIC PDE III INHIBITORS

Inhibitor	Corresponding maxima and minima				Same sign	Strength[a]	Separation (Å)	Rmsd of points in match[b]	
	Nearest inhibitor atom	V (kJ mol^{-1})	Nearest cAMP atom	V (kJ mol^{-1})				4-point (Å)	(3-point) (Å)
Adenine 	$N7_{min}$	−120.2	$N7_{min}$	−102.2	y	s	0.009	0.038	(0.014)
	$N3_{min}$	−138.6	$N3_{min}$	−132.1	y	s	0.013		
	$N1_{min}$	−131.4	$N1_{min}$	−118.1	y	s	0.018		
	$N6^{B}_{min}$	−35.9	$N6^{B}_{min}$	−16.7	y	w	0.095		
	$N6^{A}_{min}$	−35.9	$N6^{A}_{min}$	−11.8	y	w	0.098	Additional close	
	N6-$H1_{max}$	+54.2	N6-$H1_{max}$	+69.2	y	s	0.160	extrema	
	N6-$H2_{max}$	+45.7	N6-$H2_{max}$	+65.8	y	m	0.204		
Guanine 	$O6_{min}$	−234.6	$N7_{min}$	−102.2	y	s	0.387	–	(0.994)
	$N3_{min}$	−79.2	$N3_{min}$	−132.1	y	s	1.138		
	N2-$H1_{max}$	+151.2	N6-$H1_{max}$	+69.2	y	s	1.109		
	N2-$H1_{max}$	+151.2	$N1_{min}$	−118.1	n	s	2.782	Additional close extrema	
Theophylline 	N7-H_{max}	+110.1	N6-$H2_{max}$	+65.8	y	s	0.144	–	(0.227)
	$O2_{min}$	−151.0	$O2'_{min}$	−105.0	y	s	0.261		
	$N9_{min}$	−112.0	$N1_{min}$	−118.1	y	s	0.256		
	$O2_{min}$	−151.0	$N3_{min}$	−132.1	y	s	1.889	Additional close	
	$O6_{min}$	−137.0	$N7_{min}$	−102.2	y	s	2.253	extrema	
Papaverine 	$O11_{min}$	−105.4	$N7_{min}$	−102.2	y	s	0.636	1.023	(0.727)
	$N1_{min}$	−160.0	$N1_{min}$	−118.1	y	s	0.752		
	C14-$H1_{max}$	+59.8	O2'-H_{max}	+64.3	y	s	0.785		
	$Ar1^{A}_{min}$	−60.5	$N3_{min}$	−132.1	y	s	1.962		
	C14-$H2_{max}$	+46.1	O7'-H_{max}	+126.9	y	m	2.401	Additional close	
	$Ar2^{A}_{min}$	−57.0	$O1'_{min}$	−73.5	y	s	2.719	extrema	
	$Ar1^{B}_{min}$	−60.5	$N6^{A}_{min}$	−11.8	y	m	2.873		
	$O13^{B}_{min}$	−30.2	C2'-H_{max}	+56.4	n	m	1.446		
	$O13^{A}_{min}$	−30.2	C8-H_{max}	+86.5	n	m	2.003		

[a] The relative strength of each pair of corresponding extrema is classified according to the following definitions: s: strong, both extrema have |V| > 50 kJ mol^{-1}; m: moderate, only one extremum has |V| > 50 kJ mol^{-1}; w: weak, both extrema have |V| < 50 kJ mol^{-1}.

[b] Relative binding orientations quantified by the minimum rmsd of the separation of the first three or four corresponding pairs of matched points. All other pairs of contacts within 3 Å are also given as additional close extrema. Separations of extrema pairs are given for the three-point match if a four-point match was not possible.

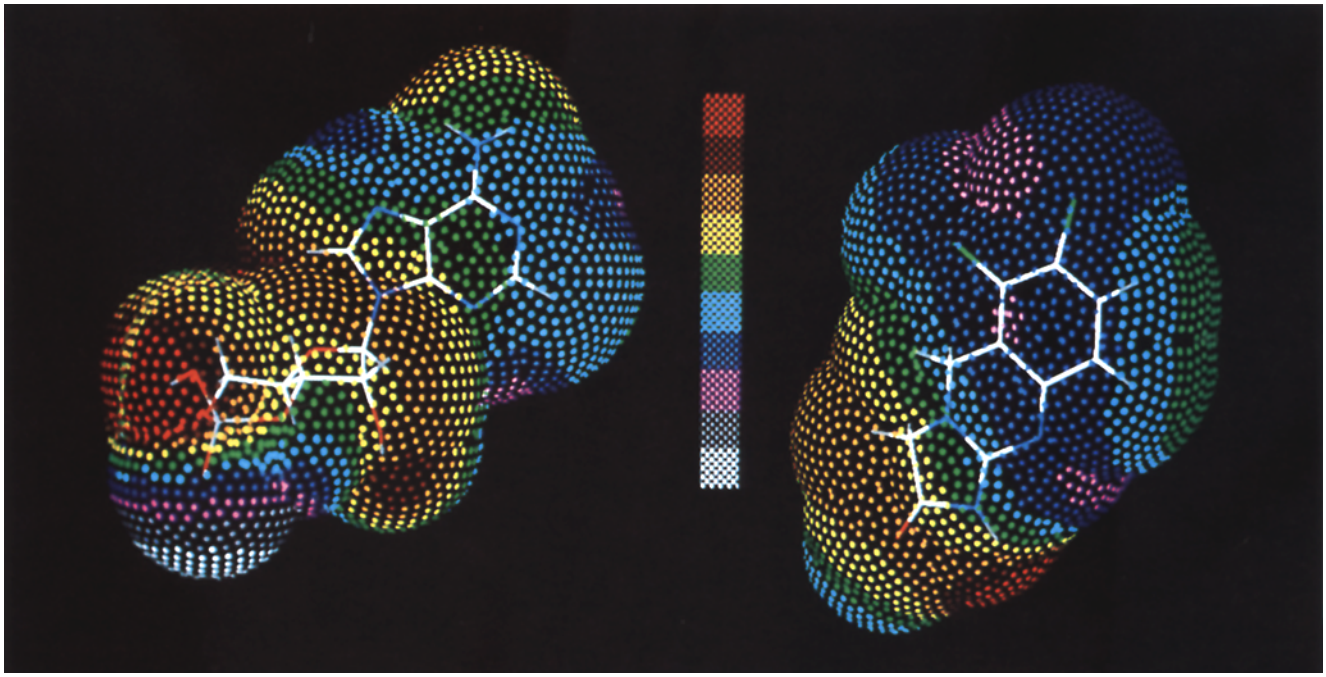


Fig. 2. The electrostatic potential at 1.0 Å from the van der Waals surface around cAMP and anagrelide in their proposed four-point relative binding orientation. The potential V (kJ mol^{-1}) is calculated from the DMA of the 3-21G SCF wave function, colour coded as follows: white < −120 < grey < −90 < magenta < −60 < blue < −30 < cyan < 0 < green < 30 < yellow < 60 < orange < 90 < brown < 120 < red.

the electrostatic potential around a dipeptide only changes by a scaling factor with basis set, within the range of reasonable-quality basis sets. The Distributed Multipole Analysis [20] of each wave function was calculated to represent the charge density by a charge, dipole, quadrupole, octupole and hexadecapole moment at every nuclear position. The inclusion of the anisotropic multipole moments gives a more accurate representation of the nonspherical features of charge distributions. Hydrogen bonds form to regions of lone pair and π-electron density, and it is precisely these features of charge density which are accurately represented by distributed multipole models, unlike atomic charges.

The electrostatic potential surface was calculated from the DMA model of the charge distribution employing the program ORIENT2 [21,22], using all terms in the multipole series up to r^{-5} between each atom in the molecule and a point charge. The electrostatic potential surface was first calculated on a grid of points at a distance of 1.0 Å from the molecular van der Waals surface for visualisation. The molecular van der Waals volume was defined using the Pauling radii, i.e., C: 2.0 Å, O: 1.4 Å, N: 1.5 Å, Cl: 1.8 Å and P: 1.9 Å. All hydrogens were treated as having a zero van der Waals radius, due to the lack of repulsion between the proton and its acceptor atom in hydrogen bonds. Nonpolar hydrogens, such as methyl hydrogens, are included in a 'united atom' carbon radius.

The positions and strengths of minima (maxima) in the electrostatic potential energy were determined by minimising the interaction energy of a single positive (negative) point charge with the molecule under examination, using pseudo hard-sphere repulsion between sites with

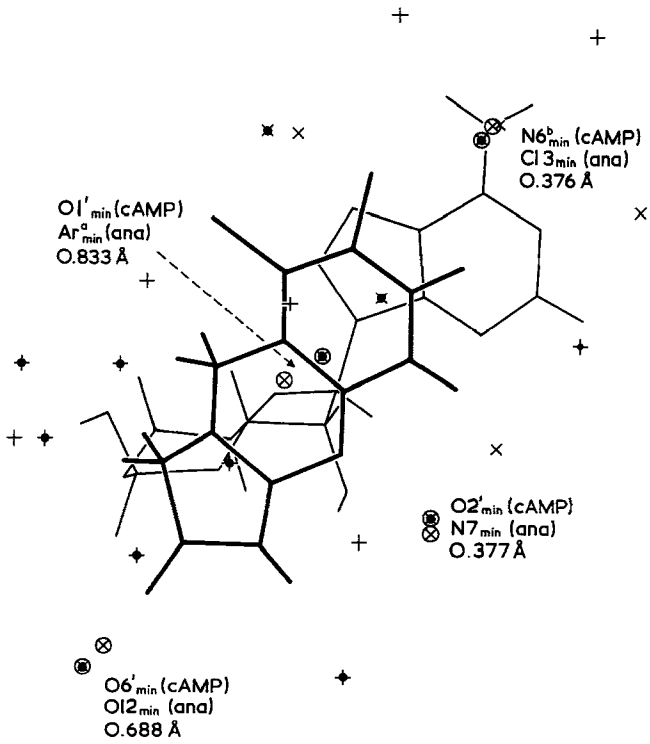


Fig. 3. 2D projection of the four-point relative binding orientation of cAMP (thin lines) and anagrelide (bold lines). The four explicitly matched pairs of extrema are encircled, and their separation is indicated. Minima: × (cAMP), × (anagrelide); maxima: + (cAMP), + (anagrelide).

non-zero van der Waals radii. Initially, all work was performed using point charges of 1.0 Å van der Waals radius. It was found that most of the extrema in the molecular electrostatic potential were located near potential hydrogen bonding groups, with others arranged above and below the plane of aromatic systems. The extrema close to hydrogen bonding groups were generally of greater magnitude than the other extrema.

The receptor atoms most likely to be near the polar hydrogen atoms in the ligand are hydrogen bond acceptors, which would be sampling the electrostatic potential about 1.4 Å from the van der Waals surface of the ligand. Similarly, any receptor protons that are hydrogen bonded to the ligand acceptors will be approximately 0.5 Å from its surface. We wish to study the electrostatic similarity of the ligands in the regions most likely to be occupied by the protein atoms in van der Waals contact with the ligand, particularly those which hydrogen bond to it. Thus, the positions and strengths of the potential maxima and minima were found by minimising the electrostatic interaction energy of a negative charge of radius 1.4 Å and a positive charge of radius 0.5 Å, respectively. This identified the points around cAMP and each ligand where the strongest interactions with polar binding site atoms could occur. We then sought relative orientations of cAMP and the ligand where the maxima around the ligand are close to maxima in cAMP. This implies that both molecules could interact favourably with any

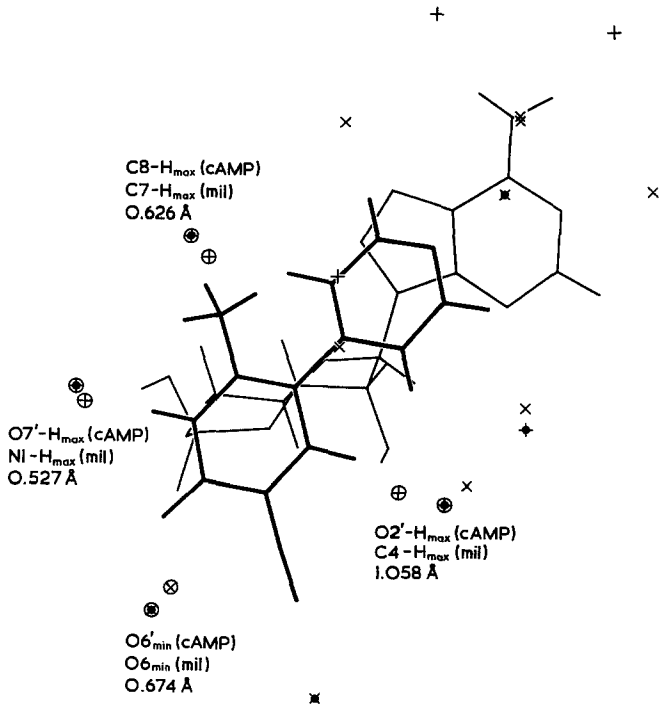


Fig. 4. 2D projection of the four-point relative binding orientation of cAMP (thin lines) and milrinone (bold lines). The four explicitly matched pairs of extrema are encircled, and their separation is indicated. Note that the apparent potential clash of cAMP $N3_{min}$ and milrinone C4-H_{max} is an artefact of the 2D projection, as these extrema are separated by 4.46 Å. Minima: × (cAMP), ✕ (milrinone); maxima: + (cAMP), ✦ (milrinone).

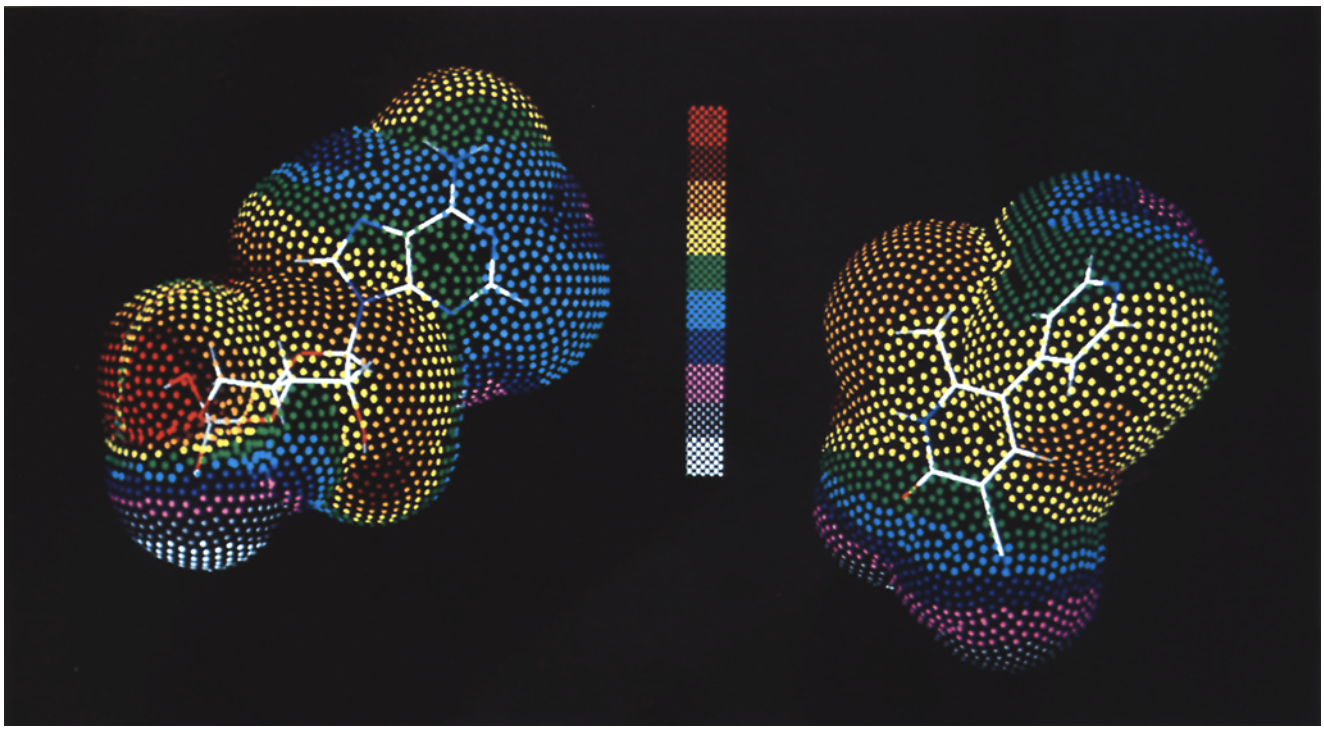


Fig. 5. The electrostatic potential at 1.0 Å from the van der Waals surface around cAMP and milrinone in their proposed four-point relative binding orientation. The potential V (kJ mol^{-1}) is calculated from the DMA of the 3-21G SCF wave function, colour coded as follows: white < −120 < grey < −90 < magenta < −60 < blue < −30 < cyan < 0 < green < 30 < yellow < 60 < orange < 90 < brown < 120 < red.

electronegative atom in the binding site at the positions of the maxima. Minima were overlaid in a similar way. Orientations with the greatest number of approximately overlaid pairs of maxima or minima have the greatest electrostatic similarity and so are most likely to represent the relative binding orientation. However, steric constraints will ensure that the polar binding site atoms will not be located exactly at the maxima or minima and that not all possible interaction sites will be occupied. Indeed, since many of the inhibitors are much smaller than cAMP, it is likely that different inhibitors will interact with different binding site residues, in which case we would expect their electrostatic extrema to overlay with a different subset of cAMP extrema.

The superposition of the ligand molecules and their maxima and minima was carried out using the 3D molecular graphics software MacroModel version 3.0, running on an Evans and Sutherland PS330 terminal hosted by a MicroVAX II computer. The electrostatic minima and maxima of each compound were input as dummy atoms. For each inhibitor, three or four extrema were chosen which would provide a visually plausible overlay with cAMP. The sets of extrema were superimposed by means of the standard MacroModel options for molecular superpositions using atomic positions, but in this case the superpositioned dummy atoms represented electrostatic extrema. The quality of the resulting three- or four-point overlay was quantified by the minimised root-mean-square deviation (rmsd) of the chosen pairs of extrema.

The rmsd error in the position of the overlay extrema only indicates the degree of similarity at the chosen points, and so the overall electrostatic similarity was also qualitatively assessed. A probable overlay model would be expected to align areas of similar electrostatic properties at more than just the three or four pairs of points chosen. With this in mind, the separations of all maxima and minima between the superimposed molecules, which were less than 3 Å apart, were calculated. Shorter separations of extrema of the same sign between molecules were assumed to be more favourable, since this makes it more probable that a binding site could form optimum hydrogen bonds with both molecules. Clashes were defined as situations where a maximum of one molecule is within 3 Å of a minimum of the other molecule. These were assumed to be unfavourable, as a polar residue in the binding site could not stabilize a region of similar potential in a ligand.

The effect of any clashes which occur will depend not only on the separation of the corresponding extrema, but also on their relative strengths. We defined a strong correspondence as one where both extrema have an electrostatic potential of magnitude greater than 50 kJ mol^{-1}. It was assumed that such an overlay of extrema was highly favourable if the electrostatic potentials were of the same sign, since regions of high potential may be involved in strong electrostatic interactions with the binding site. If the potentials were of opposite sign, then such a clash is considered to be highly destabilising. An overlay was classed as moderate when only one extremum has a potential of magnitude greater than 50 kJ mol^{-1}. Weak overlays were those where both extrema have a magnitude less than 50 kJ mol^{-1}. Thus, the degree of similarity was assessed on the basis of the distance between corresponding maxima and minima and their relative strengths.

For each inhibitor, a variety of superpositions relative to cAMP were attempted, involving different combinations of extrema. These were assessed by eye, and through the use of rmsd separations. Any relative superpositions which gave poor fitting at the chosen maxima and minima (and therefore high rmsd errors), or which resulted in poor steric overlay of the superimposed molecules were disregarded. Visual inspection was required as, in some cases, it was possible to match two sets of extrema that were close to each other (i.e., only focusing on a small region around each of the molecules), and to generate a very low rmsd value for a superposition with little steric overlap of the molecules. However, when a match was obtained using three or more extrema that were widely spaced around the inhibitor, then a sterically plausible overlay was automatically created. The reported overlay corresponds to the lowest rmsd with a sterically feasible overlay.

Results

cAMP in the assumed anti conformation has a complex electrostatic potential surface (see Fig. 2). The deepest negative electrostatic potential minimum occurs near the O6' of the phosphate group. Three other important potential minima are arranged around N1, N3 and N7 of the adenosine ring. The main regions of positive electrostatic potential occur with maxima near N6 of the purine ring and around O7' and O2' of the sugar ring. Despite the complexity of the electrostatic potential surface around cAMP, it was possible to match four extrema of each specific inhibitor very closely with those of cAMP, to produce a small rmsd (Tables 1 and 2). This overlay generally resulted in other pairs of extrema (with the same sign) coming within 3 Å of each other, though there were some potentially destabilising clashes for the poorer inhibitors.

Specific PDE III inhibitors

For the specific PDE III inhibitors, the deepest electrostatic potential minimum was generally found near the cyclic amide group. Anagrelide is one exception; here the deepest electrostatic minimum was found between the two chlorine atoms, in the molecular plane. All overlays of the specific PDE III inhibitors reported in Table 1 match the

deep cAMP $O6'_{min}$ (–207 kJ mol^{-1}), in agreement with a model of the cAMP binding site proposed by Moos et al. [11], which is based on the ability of the inhibitor cyclic amide function to occupy the cAMP 5'-phosphate region in the binding site. Three of the best inhibitors, anagrelide, SK&F95800 and dazonone, all match the $O1'_{min}$ (–74 kJ mol^{-1}) of cAMP. In each case the inhibitor compounds match the position of this cAMP minimum with a minimum from the π-electron density of the aromatic ring system which these molecules have in common. Such electrostatic minima are generally poorly represented by point charge models.

Anagrelide

This compound is the best inhibitor of the original sample studied by Davis et al. [7]. The four-point relative orientation proposed in Table 1, and shown in Fig. 3, produces many favourable corresponding pairs of extrema. While the electrostatic minima associated with Cl3 and Cl4 do not indicate possible hydrogen bonding groups, they are still regions of significant electrostatic potential which can be matched with the electrostatic potential of cAMP. The region of positive potential above and below the plane of the cyclic amide of anagrelide matches the large positive region on the electrostatic potential surface of cAMP (see Fig. 2). The activity of anagrelide and its analogue dazonone has been attributed by Venuti et al. [23] to the ability of the cyclic amide group to mimic the cAMP phosphate, and to the aromatic π-electron density of the inhibitors, which provide a further strong interaction in the absence of hydrogen bonding substituents at C3 and C4. For anagrelide, this interaction is identified by the overlay of the minimum Ar^{A}_{min} with $O1'_{min}$ of cAMP. Thus, the matching of electrostatic extrema has reproduced the overlay proposed on the grounds that structural elements of the specific PDE III inhibitors can mimic the anti conformation of cAMP.

Dazonone

This inhibitor is structurally similar to anagrelide, but the electrostatic potential surface differs in some regions. Dazonone has a single Cl atom, and the associated electrostatic minima are arranged symmetrically above and below the molecular plane, rather than in the plane as is the case in anagrelide. A region of negative electrostatic potential in anagrelide around the aromatic ring near Cl3 is replaced by a region of positive potential in dazonone, which results in the weak potential clash observed for this relative orientation (Table 1). The proposed relative orientation of dazonone is fairly similar to that of anagrelide, despite the fact that the explicitly matched extrema are somewhat different.

SK&F95800

This is the second best inhibitor in this sample and it overlays cAMP with a relatively good rmsd value. The first four moderate clashes listed in Table 1 all occur in one small region of the overlay. They result from the close SK&F95800 double maxima C10'-H_{max} and C5'-H_{max}, which lie near the negative potential of cAMP $N3_{min}$ and $O2'_{min}$ in this orientation. In addition, there are two strongly clashing regions around N7 and O2' in the cAMP electrostatic potential, which are approximately 2.0 Å from SK&F95800 electrostatic extrema in each case.

SK&F93741

The low-energy conformation, SK&F93741g, produces a good rmsd value, with four favourable overlay points separated by >1.0 Å. However, a single strong clash appears at the 3.0 Å limit. The best overlay of the alternative conformation (with the acetyl group rotated in a trans conformation, to mimic SK&F95800), SK&F93741ac, matches different points and gives a comparable rmsd value, with only two moderate clashes. SK&F95800 is a better inhibitor than SK&F93741, despite the fact that it lacks a methyl group which is known to increase inhibitory activity [15]. Hence, it is not necessary to assume that SK&F93741 adopts the higher energy conformation, analogous to SK&F95800, in order to have an electrostatically favourable binding orientation.

SK&F93505

This compound is structurally related to SK&F93741, differing only in the lack of an acetyl group at N7', but is a much poorer PDE III inhibitor. The relative orientation shown in Table 1 satisfies potential hydrogen bonding sites at the four chosen points. However, there are three strong and potentially destabilising clashes, all separated by more than 2.0 Å. Not all extrema will have corresponding complementary extrema at the receptor. These clashes may be important.

Milrinone

Milrinone is shown in Fig. 4, in its proposed relative binding orientation with the four matching extrema indicated. Milrinone primarily overlays with electrostatic maxima of cAMP, with no clashes of regions of opposite potential. Figure 5 shows the electrostatic potential surfaces of milrinone and cAMP in their proposed relative binding orientations. Similarity between the electrostatic potential surfaces can be seen once the molecules are correctly orientated. The electrostatic matching for this inhibitor is particularly good in the regions of positive electrostatic potential; the explicitly matched extrema are clustered around the sugar residue of cAMP. As a result, milrinone does not match at the cAMP N3 minima in this orientation, as proposed previously [7] for these 'short' inhibitors, but lies slightly in front of the cAMP purine ring plane, with the negative potential areas $N1_{min}$ and $N6^{A}_{min}$ coinciding. The relative orientation reported here is

in agreement with earlier findings that nonplanar conformations in certain molecules allow for more favourable matching with portions of the cAMP sugar regions, by 'introducing a favourable electrostatic potential in the vicinity of the 2'-hydroxyl to compensate for nonplanarity' [11]. We identify this favourable electrostatic overlap as the coincidence of the cAMP maximum O2'-H_{max} and the milrinone maximum C4-H_{max}. This accounts for the earlier difficulties of Davis et al. [7] in describing the binding of milrinone, as they neglected the potential maxima because of their crude method of calculation.

Amrinone

This is the worst overall inhibitor of the original set, where a reasonable match of electrostatic properties is still possible at some points, but at the expense of clashes elsewhere. The lowest rmsd is quite high, and these overlay points result from a string of electrostatic minima arranged symmetrically above and below the amrinone molecular plane. Closer examination shows that these minima are clustered around cAMP $N3_{min}$ (–132 kJ mol^{-1}) or $O2'_{min}$ (–105 kJ mol^{-1}), so they do not represent several potential ligand–binding site interaction points, but rather indicate similar electrostatic properties existing for both molecules across this region. There are several potential clashes, including two strong extrema of opposite signs within 2.0 Å of each other. Such potentially destabilising clashes feature in all plausible superpositions for amrinone, and so it would not be expected to bind to the same site as cAMP very strongly, which correlates with its low inhibitory strength.

Nonspecific PDE inhibitors

The nonspecific PDE inhibitors are expected to bind in the receptor region occupied by the purine moiety of cAMP, and indeed many of the nonspecific inhibitors are structurally similar to the purine ring system. The results for the nonspecific PDE inhibitors are listed in Table 2. All inhibitors can overlay at least three electrostatic extrema associated with the purine moiety of cAMP. Given the structural similarity of many nonspecific PDE inhibitors to the purine ring system, this might be supposed from a comparison of molecular structures. Matching extrema for adenine does, trivially, result in a direct overlay of the adenosine ring. However, a direct ring system overlay of the inhibitors onto cAMP does not necessarily produce the best agreement in electrostatic properties. This is particularly apparent in the case of theophylline. The best overlay reported in Table 2 corresponds approximately to the 'N^6-C8' model introduced by Peet et al. [24] and investigated by Van der Wenden et al. [5]. In this overlay the N1, N3 and N9 atoms of theophylline are superimposed onto N9, N3 and N1 of cAMP. We have closely approximated this model by matching the relevant electrostatic maxima and minima, rather than the atomic positions. All the nonspecific inhibitors studied here can overlay at least three extrema surrounding the purine ring of cAMP with few clashes, in general agreement with the results of Davis et al [7].

Conclusions

The use of accurate electrostatic models has demonstrated that there is far more correspondence between the electrostatic properties of the substrate and inhibitors of phosphodiesterase III than was noted in the earlier work, which used minima only. The observation by Davis et al. [7], that matching a couple of electrostatic minima provides a rationale for determining relative binding orientations has been extended to a new method of comparing electrostatic similarity, which could be applied to other systems where such effects are dominant. By optimising the overlay of three or four electrostatic extrema of the natural substrate and each inhibitor, it is possible to find an overlay which is a plausible relative binding orientation, being both sterically feasible and having a close correspondence between potential hydrogen bonding sites and other regions where strong electrostatic interactions with the binding site are possible. This success for such a structurally diverse range of inhibitors increases our confidence that the proposed overlays may correspond to the relative binding orientations within the enzyme. The complexity of the three-dimensional arrangement of the six maxima and eight minima around cAMP suggests that it is unlikely that a plausible overlay could be found for many molecules, and therefore that the method could be a useful screen in designing new inhibitors.

This approach of matching the potential extrema is a distillation of the more general belief that electrostatic potential fields play a vital role in molecular recognition processes. If the matched extrema are spread out around the inhibitor, then this approach will also ensure that there is reasonable steric overlay. We also observe that such overlays of known inhibitors usually have similar electrostatic properties in other regions, in that extrema not used in the match are generally located in regions of the correct sign. This suggests that the approach is physically reasonable and may be matching points where strong interaction with the binding site is present. Certainly the method is matching potential hydrogen bonding sites. Indeed, the common appearance of some cAMP extrema in many overlays, specifically $O6'_{min}$ and $O2'_{min}$, suggests that there are appropriate polar residues in this region in the binding site.

The uncertainty of how close an overlay of extrema is necessary for binding is less important than the uncertainty as to whether there is a suitable polar group in the vicinity of a potential extrema match or clash. Thus, like all methods of comparing ligands, it is unlikely to provide

a good quantitative correlation with even the binding energy of the ligand to the protein, let alone more remote experimental quantities such as inhibitor strengths. Nevertheless, there are qualitative correlations. The method is being automated to consider all possible sets of extrema for matching.

The use of DMA electrostatic models has made a considerable difference to the numbers of extrema that match. Davis et al. [7] matched only two minima for the specific inhibitors, since their semiempirical atomic charge calculations severely underestimated the magnitude of the maxima and did not use any minima above the aromatic rings. Thus, a reliable method of calculating the electrostatic properties which reflects the anisotropy of the atomic electron distributions is essential. This can be done by calculating the electrostatic properties directly from the wave function [6], but the DMA method is more practical for large systems and for systems with a high degree of conformational flexibility.

In applying this method to systems with more structural flexibility, it will be necessary to consider conformations other than the global minimum. It is possible to calculate the extrema in other conformations readily from a DMA, without performing another ab initio calculation, by neglecting changes in the atomic charge distribution which occur with changes in conformation. The errors in this approximation are unlikely to be significant for this type of similarity comparison [25]. However, for large molecules where many conformations or induced movement has to be taken into account, even a DMA ab initio-based method will be computationally too demanding. Thus, methods of representing the lone pair and π-electron density by additional point charges, without requiring the calculation of a wave function, are being developed [26]. The anisotropic distribution of electron density in atoms contributes to the exquisite sensitivity of electrostatic extrema to both molecular structure and conformation which helps explain the large variations in biological interactions.

We observe that it is possible to match the electrostatic extrema of a diverse range of inhibitors onto those of the natural substrate, producing a relative orientation of the molecules in which both can favourably interact with the same unknown binding site. Thus, the approach could be useful in designing new ligands in a range of systems where the natural substrate or a good lead compound is known. This will be established by the application of the approach to other systems, and further study of the intermolecular interactions within protein binding sites.

Acknowledgements

R.P.A. is grateful to the SERC for support in the form of a studentship. B.L. worked at UCL as an ERASMUS student. S.L.P. thanks the SERC for computer facilities provided under GR/G11668 and GR/J31865, and the Royal Society for a research grant for the graphics work.

References

1 Weinstein, H., Osman, R., Topiol, S. and Green, A.P., Ann. New York Acad. Sci., 367 (1981) 434.
2 Weinstein, H., Osman, R., Green, J.P. and Topiol, S., In Politzer, P. and Truhlar, D.G. (Eds.) Chemical Applications of Atomic and Molecular Electrostatic Potentials, Plenum Press, New York, NY, 1981, pp. 309–334.
3 Carbo, R., Leyda, L. and Arnau, M., Int. J. Quantum Chem., 17 (1980) 1185.
4 Hodgkin, E.E. and Richards, W.G., Int. J. Quantum Chem., Quantum Biol. Symp., 14 (1987) 105.
5 van der Wenden, E.M., Price, S.L., Apaya, R.P., IJzerman, A.P. and Soudijn, W., J. Comput.-Aided Mol. Design, 9 (1995) 44.
6 Sanz, F., Manaut, F., Rodríguez, J., Lozoya, E. and López-de-Briñas, E., J. Comput.-Aided Mol. Design, 7 (1993) 337.
7 Davis, A., Warrington, B.H. and Vinter, J.G., J. Comput.-Aided Mol. Design, 1 (1987) 97.
8 Stone, A.J. and Alderton, M., Mol. Phys., 56 (1985) 1047.
9 Buckingham, A.D. and Fowler, P.W., Can. J. Chem., 63 (1985) 2018.
10 Kato, Y., Inoue, A., Yamada, M., Tomioka, N. and Itai, A., J. Comput.-Aided Mol. Design, 6 (1992) 475.
11 Moos, W.H., Humblet, C.C., Sircar, I., Rithner, C., Weishaar, R.E., Bristol, J.A. and McPhail, A.T., J. Med. Chem., 30 (1987) 1963.
12 MacroModel, Version 3.1, Columbia University, New York, NY, 1990.
13 Weiner, S.J., Kollman, P.A., Nguyen, D.T. and Case, D.A., J. Comput. Chem., 7 (1986) 230.
14 Bristol, J.A., Sircar, I., Moos, W.H., Evans, D.B. and Weishaar, R.E., J. Med. Chem., 27 (1984) 1099.
15 Prout, K., Bannister, C., Burns, K., Chen, M., Warrington, B.H. and Vinter, J.G., Acta Crystallogr., B50 (1994) 71.
16 Robertson, D.W., Beedle, E.E., Swartzendruber, J.K., Jones, N.D., Elzey, T.K., Kauffmann, R.F., Wilson, H. and Hayes, J.S., J. Med. Chem., 29 (1986) 635.
17 CADPAC5: The Cambridge Analytical Derivatives Package, Issue 5.0, 1992, a suite of quantum chemistry programs developed by R.D. Amos with contributions from I.L. Alberts, J.S. Andrews, S.M. Colwell, N.C. Handy, D. Jayatilaka, P.J. Knowles, R. Kobayashi, N. Koga, K.E. Laidig, P.E. Malsen, C.W. Murray, J.E. Rice, J. Sanz, D. Simandiras, A.J. Stone and M.-D. Su.
18 Binkley, J.S., Pople, J.A. and Hehre, W.J., J. Am. Chem. Soc., 102 (1980) 939.
19 Price, S.L., Andrews, J.S., Murray, C.W. and Amos, R.D., J. Am. Chem. Soc., 114 (1992) 8268.
20 Stone, A.J., Chem. Phys. Lett., 83 (1981) 233.
21 Price, S.L. and Stone, A.J., J. Chem. Phys., 86 (1987) 2859.
22 Stone, A.J., ORIENT, Version 2, a program for calculating the electrostatic interactions between molecules, University of Cambridge, Cambridge, 1990.
23 Venuti, M.C., Stephenson, R.A., Alvarez, R., Bruno, J.J. and Strosberg, A.M., J. Med. Chem., 31 (1988) 2136.
24 Peet, N.P., Lentz, N.L., Meng, E.C., Dudley, M.W., Ogden, A.M.L., Demeter, D.A., Weintraub, H.J.R. and Bey, P., J. Med. Chem., 33 (1990) 3127.
25 Price, S.L. and Stone, A.J., J. Chem. Soc., Faraday Trans., 88 (1992) 1755.
26 Vinter, J.G., J. Comput.-Aided Mol. Design, 8 (1994) 653.