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Theoretical Structure–Activity Studies of Adenosine A_1 Ligands: Requirements for Receptor Affinity

Michael J. Dooley,† Motomichi Kono* and Fumio Suzuki*

Pharmaceutical Research Laboratories, Kyowa Hakko Kogyo Co. Ltd, 1188 Shimotogari, Nagaizumi-cho, Sunto-gun, Shizuoka-ken 411, Japan

Abstract—The three-dimensional (3-D) requirements for A_1 adenosine receptor affinity have been studied based on hydrogen-bonding functionality correlation between a group of twelve A_1 adenosine receptor ligands representing ten structurally different classes of compounds. Electrostatic potential similarity indices and shape similarity indices strongly support the proposed receptor-bound orientations of the ligands. We conclude, in areas common to both agonist and antagonist binding at the A_1 receptor, that the ligands are recognized by a similar physicochemical 3-D environment. The finding of similar 3-D requirements for agonists and antagonists suggests a fairly static receptor structure in the region common to agonist and antagonist binding. The ribose moiety is remote from antagonist binding site. Such a 3-D environment rationalizes the binding of a number of potent novel antagonists including KW-3902, not previously reported in modeling studies.

Introduction

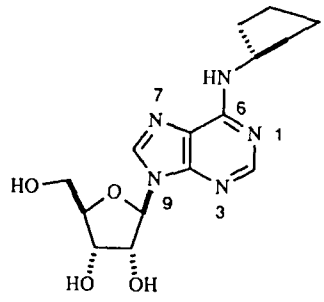
Adenosine is a neuromodulator with wide-ranging effects throughout the body.[1] The physiological responses to adenosine are elicited through interaction with four major subtypes of adenosine receptor, designated A_1, A_{2a}, A_{2b}, and A_3.[2] Because of the potent bioactivity of adenosine, intensive research to produce novel therapeutics that exploit the adenosine signal transmission pathway has been conducted. Most of the targets have been either adenosine receptor agonists or antagonists.[3] However, only adenosine is currently in clinical use.[4] Two promising areas where A_1 antagonists may be therapeutic are cognitive deficit and acute renal failure (ARF).[5] Several A_1-selective compounds, such as KW-3902 [6] are under clinical trial at this time for use as renal protectants, and these compounds may prove to be useful therapeutics.

Previous molecular modelling studies[7,8] of adenosine receptor ligands led to the independent development of two conceptually similar models for alkylxanthine binding to adenosine A_1 receptors. The underlying concepts of both models was that the C8 binding domain for the alkylxanthines and the N^6 binding domain of the adenosine agonists overlap. A third, conceptually different model[9] in which the C8 binding domain for the alkylxanthines and the N^6 binding domain of the adenosine agonists are separate, has also been developed. Novel compounds have been synthesized which support two models[7,9] for alkylxanthine binding. Therefore, the actual receptor-bound orientation of the alkylxanthine class of compounds has yet to be conclusively defined. Furthermore, a number of potent A_1 selective antagonists remain to be included to refine the 3-D requirements for binding. This report presents an inclusive 3-D requirements map for the A_1 adenosine receptor that defines the receptor-bound orientation of the alkylxanthines and other potent A_1 selective ligands. Our investigation supports the concept of a common N^6 and C8 binding domain for the N^6-substituted adenosine agonists and alkylxanthines and in particular supports the Peet model.[7] Our calculations indicated that the bio-active conformation about the C6–N^6 bond of the adenosine agonists presented a torsion for the N1–C6–N^6–Ca angle in the range of $\pm 10°$.[10] This value is very different from the accepted value in the literature.[11] This result is critical to the successful development of a refined 3-D map for the A_1 adenosine receptor affinity and receptor modeling projects, since the geometry of the docked ligands could drastically influence the choice of putative binding sites in the modeled receptor.

Procedure

The ligands used as the basis of this study, shown in Figure 1, were the A_1-selective agonist, cyclopentyladenosine (**1**)[12] and the competitive antagonists, CP68247 (**2**),[13] PACPX (**3**),[12] KW-3902 (**4**),[6] 8-cyclopentyl-1-propyl-3-(4-aminophenylethyl)xanthine (**5**),[14] 7,8-dihydro-8-ethyl-2-(3-noradamantyl)-4-propyl-1*H*-imidazo-[2,1-*i*]purin-5(4*H*)-one (**6**),[15] 1,3-dimethyl-5-(2-amino-4-chlorophenyl)pyrazolo[4,3-*d*]pyrimidin-7-one (**7**),[16] 1-benzyl-6-(4-methoxyphenyl)-3-propyl-1',2,3,4-tetrahydro-5*H*-imidazo[1',2':1,5]pyrazolo[3,4-*d*]pyrimidin-2,4-dione (**8**),[17] (*R*)-7,8-dimethyl-2-phenyl-9-(1-phenylethyl)-7-deazaadenine (**9**),[18] (*R*)-2-phenyl-9-(1-phenyl-

†Present address: Centre for Drug Design and Development, The University of Queensland, Brisbane Qld 4072, Australia.



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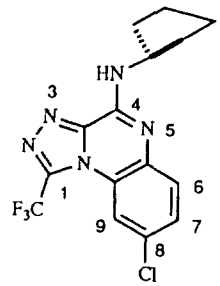

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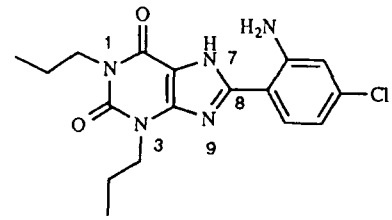

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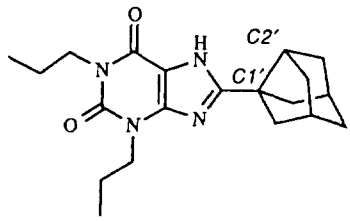

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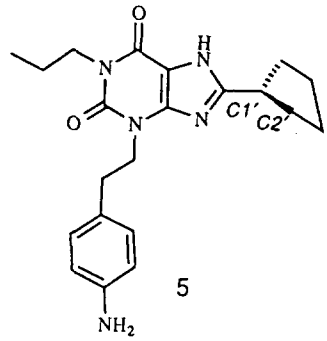

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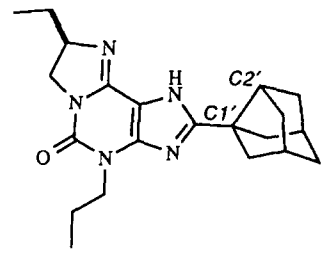

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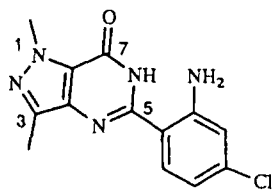

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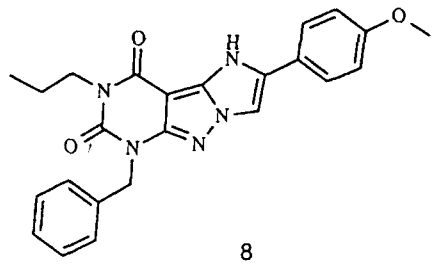

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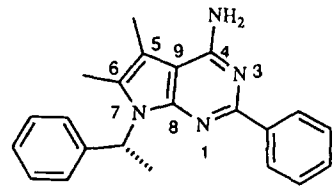

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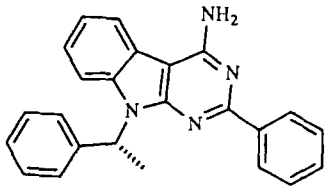

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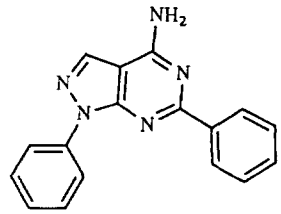

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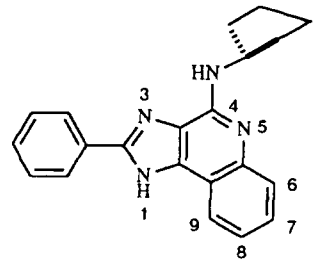

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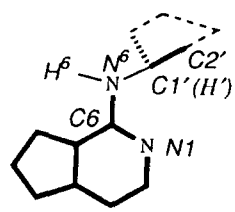

Figure 1. Ligands used in the development of the A_1 adenosine receptor pharmacophore. Bold faced diagram indicates general numbering system applied to **1**, **2**, **9**, **10**, **11**, **12**, for the pupose of clarity in the text.

ethyl)-9*H*-pyrimido[4,5-*b*]indol-4-amine (**10**),[18] APPP (**11**),[19] and N^4-cyclopentyl-2-phenyl-1*H*-imidazo[4,5-*c*]-quinolin-4-amine (**12**).[20] Compounds **2**, **6**, **8**, **9**, **10** and **12** were selected because they were clearly the most potent in their respective series. Compound **1** was selected because it is one of the most potent agonists and is the one most widely used for pharmacological studies. Three of the most potent xanthines, **3**, **4** and **5**, were chosen to represent three different substituents responsible for promoting affinity of xanthines. That is, **3** is a representative of *C*8-aromatic substituted xanthines, **4** is a representative of *C*8-cycloalkylxanthines and **5** is a representative of xanthines with large *N*3-hydrophobic groups. Although there are some structural differences in potent agonists, it is generally assumed that all agonists bind in only one orientation at the receptor. The use of more than one agonist in this study therefore accomplishes little. Although **7**'s series has only moderate potency, the SAR has been quantitatively correlated with the SAR of the alkylxanthines. Compound **7** was the particular compound chosen by Hamilton et al.[16] to illustrate the orientation relative to the xanthine PACPX (**3**), and in the study by Van Galen et al.[9] Therefore, **7** was chosen to maintain consistency with these previous papers. There are no other compounds in the series represented by **11** and therefore it is currently the most potent by default. The relevant biological data for all compounds is shown in Table 1. All compounds possessed K_i or IC_{50} values of less than 30 nM in rat or guinea pig tissue, except **7**. The type of compounds represented by **4**, **5** and **6** are generally more potent in rat tissue than in guinea pig tissue.[6]

All compounds shown in Figure 1 were either retrieved from domestic X-ray data[21] (**4**), or modified from domestic X-ray data or the Cambridge Structural Database (CSD), (**1**, **3**, **5**, **6**),[22] or built on screen using Quanta version 3.3[23] (remainder). Geometry were initially optimized using CHARMm[24] and the Newton–Raphson minimizer, followed by optimization with MOPAC6 using the AM1 Hamiltonian and the keywords: Gradients, Precise, and EF.[25] Minimum energy conformations were determined by conformational searches in MOPAC6 using either the reaction path search (keywords: Step=*m* (10), Points=*n* (36)) or the grid search (keywords: Step1=*m* (30), Step2=*n* (30)), followed by precise optimization of appropriate conformers to establish exact energies/geometry for global and local minima.

Table 1. A_1 receptor binding data for the compounds of this study

Cpd	A_1 K_i (nm) (IC_{50} where indicated)
1	0.59[11.a]
2	28.0[12.a]
3	2.5[11.a]
4	1.3[6.b]
5	5.6[13.b]
6	2.7[14.b]
7	310[15.a] (IC_{50})
8	4.7[16.a]
9	4.7[17.a]
10	2.6[17.a]
11	23.0[18.a]
12	10.0[19.a]

a: rat.
b: guinea pig.

The optimized structures were transferred to the modeling package, RECEPS version 2.0.[26] The AUTOFIT function of RECEPS[27] performs a combinatorial search for all possible correspondence of hydrogen-bonding functional groups between molecules. Flexible fit calculations of RECEPS were performed only between a compound with rigid hydrogen bond functionality (either **4** or **6**) and a flexible compound in order to establish the most likely bioactive conformations of the flexible compounds. From this initial test, it was established that optimal conformations were adequately represented by the global minima of all compounds except **3**, **5**, and **7**. These global minima were actually used in further (rigid) RECEPS calculations. The global minimum energy conformations of **3**, **5** and **7** were alternatively used in further RECEPS calculations, since hydrogen bonding functionality could not establish the optimal conformations for **3**, **5** and **7**. Thus every pairwise combination of the eleven antagonists and one agonist was tested within RECEPS, specifying all hydrogen-bonding functionality, to give a 12×12 matrix in which every compound was compared with every other. The best seven fits (see footnote a, Table 2) between each molecule were saved and examined. A search for those fits which produced a self consistent 12×12 matrix (i.e. any one compound compared with all others maintained a consistent superimposition) was conducted, since these indicated that all compounds could bind to a common set of hydrogen bond functionality on a putative receptor.

Electrostatic potential similarity indices and shape similarity indices between the ligands in the RECEPS-suggested orientations were calculated. Similar electrostatic character and shape might reflect a common 3-D requirements for the receptor affinity for all ligands. Before calculation of these indices, it was noted that pendent groups on the heterocycles which fell in the same region in the RECEPS fits, actually superimposed well in the global minimum energy conformations. Therefore, a time consuming flexible analysis became superfluous, only rigid analysis of compounds was performed. The molecular modeling software ASP[28] was used, in conjunction with the graphical interface PIMMS,[29] for the calculation and optimization of similarity indices. Shape and electrostatic potential Carbo[30] similarity indices were calculated for each fit. The Carbo shape index ranges from 0 up to 1, for perfect shape similarity, whilst the Carbo electrostatic potential index ranges from -1 for complete complimentarity through to 1 for complete similarity. Often the optimization of similarity indices led to a change in orientation which was visually judged to no longer support the self-consistent matrix. In these

cases, indicated by XXX in Table 2, only the unoptimized similarity indices could be used as a guide to the similarity of the molecules in the proposed orientation. Optimization of similarity indices was carried out using the 3-gaussian approximation option of ASP.

Results and Discussion

RECEPS and ASP calculations

Likely relative orientation of ligands were determined by correlating hydrogen-bonding functionality with putative hydrogen-bond sites on the receptor through the use of the RECEPS program.[26] Firstly, hydrogen-bond correlation is particularly useful for finding likely receptor bound orientations because they have 'tight' geometry criteria, i.e. there are narrow ranges for lengths and angles which are optimal for hydrogen-bond formation.[31,32] Secondly, although there is no definitive evidence, (i.e. X-ray or NMR evidence) there is ample evidence from SAR studies[33] that these bonds play an important role in the interaction between ligand and adenosine receptor. Interactions between ligand and adenosine receptor governed by hydrogen bonding was an important assumption in at least three previous adenosine receptor ligand modeling studies.[7,8,34] Finally, RECEPS has been shown, in systems where hydrogen bonds play an important role, to accurately predict the receptor bound orientations of the ligands (confirmed by observation of ligand–receptor X-ray data) even when all hydrogen bond functionality of the ligands do not form hydrogen bonds with the receptor.[27] Our single assumption is that the binding region for all ligands share the critical region for the receptor recognition. This is a valid assumption since the eleven antagonists used in this study are all competitive, i.e. they competitively displace radiolabelled adenosine receptor agonists from the A_1 adenosine receptor.[9,35] By organizing the RECEPS fits into matrices, it is possible to search for fits that produced a singular and specific orientation for each ligand (a self-consistent matrix).

The initial RECEPS comparison by flexible fit between **4/6** with rigid hydrogen bonding functionality and the flexible compounds suggested that the bioactive conformation of the exocyclic amino group of **1**, **2**, **9**, **10**, **11** and **12**, adopted a torsion angle about C1′–N^6–C6–N1 or H^6–N^6–C6–N1 in the region of 0°. The dummy atoms representing the putative receptor hydrogen bond functionality for compounds **4** and **6** are in the same plane as the heterocycle, therefore, the 0° torsion angle allows maximum correlation between these dummy atoms and the dummy atoms of the flexible compounds. RECEPS calculations comparing either **4** or **6** with **3**, **5** and **7**, could not indicate possible bioactive conformations, since the maximum correlation between dummy atoms was independent of the position of the flexible hydrogen bond functionality (the details of the bioactive conformations of *C8*-cycloalkylxanthines are described below in the the section 'Bioactive conformations of *C*8-cycloalkylxan-

Table 2. RECEPS and ASP similarity indices for compounds **1–12** in their proposed receptor bound orientation

Compounds		Indices				
Ref.	Test	RECEPS fit[a]	ASP electrostat[b]		ASP shape[b]	
			unopt[c]	optim[d]	unopt	optim
1	2	2	0.435	0.594	0.695	0.752
	3	3	0.396	0.474	0.653	0.746
	4	3	0.492	0.642	0.713	0.829
	5	3	0.385	0.460	0.680	0.747
	6	6	0.561	XXX	0.653	XXX
	7	2	0.567	XXX	0.688	0.767
	8	7	0.380	XXX	0.558	XXX
	9	3	0.542	0.618	0.547	0.669
	10	3	0.389	XXX	0.623	0.654
	11	3	0.557	XXX	0.540	0.652
	12	3	0.495	XXX	0.600	XXX
	12[e]	2	0.581	XXX	0.668	0.711
2	3	1	0.597	0.618	0.762	0.836
	4	1	0.523	0.628	0.724	0.846
	5	1	0.476	0.564	0.672	0.760
	6	2	0.461	0.537	0.716	0.832
	7	5	0.544	0.576	0.817	0.878
	8	5	0.485	XXX	0.594	XXX
	9	1	0.382	XXX	0.764	0.818
	10	1	0.256	XXX	0.733	0.795
	11	1	0.313	XXX	0.730	0.860
	12	1	0.559	0.574	0.851	0.859
	12[e]	6	0.469	0.574	0.787	0.841
3	4	1	0.684	0.784	0.758	0.843
	5	1	0.610	0.671	0.780	0.803
	6	1	0.570	0.650	0.703	0.805
	7	1	0.689	0.702	0.898	0.906
	8	1	0.434	0.576	0.558	0.746
	9	1	0.358	0.425	0.715	0.808
	10	1	0.352	0.459	0.750	0.796
	11	4	0.349	XXX	0.748	0.850
	12	2	0.462	0.534	0.649	XXX
	12[e]	1	0.633	0.659	0.829	0.875
4	5	1	0.696	0.713	0.771	0.826
	6	1	0.891	0.892	0.903	0.918
	7	1	0.617	0.694	0.787	0.867
	8	1	0.544	0.710	0.729	0.846
	9	1	0.539	0.549	0.754	0.824
	10	4	0.527	0.600	0.797	0.808
	11	4	0.539	XXX	0.768	0.831
	12	2	0.514	0.640	0.612	XXX
	12[e]	1	0.642	0,747	0.727	0.827
5	6	1	0.561	0.590	0.684	0.791
	7	1	0.477	0.520	0.738	0.765
	8	1	0.544	0.574	0.572	0.703
	9	1	0.320	XXX	0.638	0.801
	10	1	0.281	XXX	0.695	0.794
	11	4	0.398	XXX	0.720	0.804
	12	2	0.398	0.508	0.574	XXX
	12[e]	1	0.525	XXX	0.726	0.831
6	7	2	0.634	0.696	0.738	0.829
	8	1	0.604	0.673	0.678	0.785
	9	1	0.630	0.637	0.724	0.838
	10	1	0.608	0.696	0.754	0.814
	11	2	0.674	0.660	0.751	0.870
	12	3	0.598	0.684	0.676	XXX
	12[e]	1	0.683	0.769	0.726	0.829
7	8	4	0.394	0.512	0.578	0.748
	9	3	0.606	0.670	0.729	0.802
	10	3	0.562	XXX	0.806	0.762
	11	1	0.603	0.660	0.634	0.736
	12	4	0.692	0.516	0.721	0.777
	12[e]	1	0.628	0.682	0.823	0.826

Table 2. Continued

Compounds		Indices				
Ref.	Test	RECEPS fit[a]	ASP elecrostat[b]		ASP shape[b]	
			unopt[c]	optim[d]	unopt	optim
8	9	1	0.473	XXX	0.729	0.802
	10	1	0.428	0.583	0.673	0.762
	11	3	0.424	XXX	0.634	0.736
	12	4	0.447	0.516	0.501	XXX
	12[e]	3	0.481	XXX	0.558	0.766
9	10	1	0.924	0.941	0.931	0.980
	11	1	0.840	0.873	0.828	0.866
	12	4	0.607	0.684	0.676	0.720
	12[e]	5	0.588	0.677	0.723	0.821
10	11	1	0.818	0.870	0.811	0.888
	12	4	0.607	0.648	0.670	0.698
	12[e]	5	0.645	0.670	0.783	0.820
11	12	3	0.610	0.688	0.676	XXX
	12[e]	2	0.612	0.708	0.772	0.896

[a]The number indicates the rank of the RECEPS fit, the smaller number (1 being the best) representing a fit giving the most dummy atom correspondences and the least RMS deviation between them.
[b]ASP electrostat: Carbo electrostatic potential similarity indices, ASP shape: shape similarity indices.
[c]unopt: similarity indices for the initial RECEPS fit.
[d]optim: the optimized similarity indices. Where a value is given, optimization resulted in only a small movement of the molecules and the self-consistent matrix was preserved. Where XXX is indicated, the optimization resulted in a change in orientation disrupting the self-consistent matrix. In this case, the unoptimized index should be used as a guide to similarity.
[e]Upper fit, orientation of **12** superimposed on **1**. Lower fit, orientation of **12** superimposed on **3**.

thines'). The global minimum energy conformations of **3**, **5** and **7** were used in further RECEPS calculations. Conformational searches of **1**, **2**, **9**, **10**, **11**, and **12** using MOPAC revealed the global minimum energy conformations,[10] which were found to be similar to the conformations suggested by the initial RECEPS flexible fit calculations. These global minimum conformations including that of **8** were used as representatives of bioactive conformations in further (rigid) RECEPS calculations.

Searching each of the seven saved orientations for every pair-wise fit in the matrix led to the isolation of only one orientation for **1**–**11** and two possible orientations for **12**, which maintained a self-consistent matrix and therefore it was established that all the compounds were capable of binding to a common hydrogen bond network (the details of these fits are given below in the section 'Details on specific fits').

The fits which produced self-consistent matrices were transferred to ASP where the electrostatic potential and shape similarity indices were calculated. Observation of the RECEPS fits revealed that flexible pendent groups on the compounds, which fell in the same area, actually superimposed well in the global minimum conformation. The results of the RECEPS calculations and the ASP calculations are shown in Table 2. A global comparison of ligands was carried out since it is now recognized that very often there is electrostatic potential complimentarity not between individual moiety and a part of the receptor binding site but between the whole ligand and the receptor binding site.[36,37] All electrostatic potential similarity indices are positive, indicating overall similarity between all ligands in the fits, and strongly supporting the proposed receptor-bound orientations. In the proposed fits, the shape similarity indices between all ligands are consistently high, indicating a requirement for a similar shape allowance. Interestingly, in many cases, the suggested RECEPS fit correlates with maximum electrostatic potential and shape similarity between ligands. To further emphasize this point, examination of Table 2 shows that there is coincidence between appropriate hydrogen bonding correlation and maximum electrostatic potential and shape similarity for **1** (agonist) and **2**, **3**, **4**, **5** and **9** (antagonists). This is also the case for comparison between the antagonists, e.g. **2** and **3**–**7**, **12**; **3** and **4**–**10**; **4** and **5**–**10**, **11**; **5** and **6**–**8**; **6** and **7**–**11**, etc. In other cases there is coincidence between appropriate hydrogen bonding correlation and one of either maximum electrostatic potential or shape similarity, e.g. **1** (agonist) and **7**, **10**–**11** (antagonists), **2** and **9**–**12**, **3** and **11**, **12**, etc.

Bioactive conformations of *C*8-cycloalkylxanthines

The energy surfaces for rotation about the C8–C1′ bond of the alkylxanthines **3**, **4** and **5** are in agreement with a previous report.[38] The N7-C8-C1′-C2′ torsion angle of **3** is approximately 50°. In addition the 1,3-substituents of these compounds, and the corresponding substituents of **6** and **8**, reside above the same face of the heterocycle in the global minimum conformation and at approximately 90° to the heterocycle.

A possible bioactive conformation of the *C*8-cycloalkylxanthine regarding the N9–C8–C1′–C2′ torsion has been reported previously[38] to be 330°. They determined the bioactive conformations on the basis of the comparison of the energy surface between 7-methyl-8-cycloalkylxanthines and their 7-H analogues with higher receptor affinity. They completely regarded the difference of the receptor affinity between 7-methyl-8-cycloalkylxanthines and their 7-H analogues as intramolecular source. However there is strong evidence that the N7–H is an important hydrogen bond donor in A_1 receptor binding.[39] Thus an alternative explanation for the decrease in binding energy of the 7-methyl-alkylxanthine-receptor–ligand complex relative to the 7-*H*-alkylxanthine-receptor–ligand complex is a loss of a hydrogen bond between N7–H of xanthines and the A_1 adenosine receptor. The loss of a hydrogen bond would alone account for between 2 kcal/mol and 10 kcal/mol[40] loss in binding energy, not considering the potential for steric conflict.

A comparison of the N^6-substituted adenosine analogues, **1** and N^6-cyclohexyladenosine with the

*C*8-cycloalkylxanthines, **4**, **5**, 1,3-dipropyl-8-cyclopentylxanthine, and 1,3-dipropyl-8-cyclohexylxanthine and **2** and **12**, was used to establish possible bioactive conformations for the 8-cycloalkylxanthines. By aligning these compounds of the global energy minima in the orientations suggested by flexible fit of RECEPS hydrogen bonding functionality, it was noted that the cycloalkyl groups of all compounds fell in the same region and that the C1′ carbon of all compounds were no more than 0.8 Å apart. Furthermore, by adjusting the C6–N^6–C1′–C2′ and N9–C8–C1′–C2′ torsion angles the cycloalkyl groups of analogous compounds (e.g. **1** and **5** or 1,3-dipropyl-8-cyclopentylxanthine; N^6-cyclohexyladenosine and 1,3-dipropyl-8-cyclohexylxanthine) could be superimposed almost perfectly. The energy barrier to rotation about the C8–C1′ bond of the cycloalkylxanthines is very low (<2 kcal/mol), therefore these compounds alone could not be used to establish bioactive conformations. The energy barrier to rotation about the N^6–C1′ bond of the N^6-substituted adenosine analogues, **2**, and **12**, is quite high (≈7 kcal/mol) and torsions for C6–N^6–C1′–C2′ in the range of 170–300° are within 3 kcal/mol of the global minimum. This range corresponds to torsion angles about the N9–C8–C1′–C2′ bond of cycloalkylxanthines from 170 to 300° (within 2 kcal/mol of the global minimum). These torsion ranges include the global minima of the above compounds, (*R*)-N^6-1-phenyl-2-propyladenosine, N^6-1-adamantyladenosine, **2**, **4**, **5**, **6** and **12**. Throughout these torsion ranges, the N^6-cycloalkyl and *C*8-cycloalkyl groups are able to be superimposed very well. Figure 6 illustrates the fit of **1** (global minimum) with 1,3-dipropyl-8-cyclopentylxanthine, and N^6-cyclohexyladenosine (global minimum) with 1,3-dipropyl-8-cyclohexylxanthine (orthogonal pairs).

As the summary, first we determined the orientation and the N1–C6–N^6–C1′ torsion of N^6-cycloalkyladenosine by the flexible fit of RECEPS hydrogen bonding functionality using **4**/**6** as rigid templates. The obtained N1–C6–N^6–C1′ torsion of N^6-cycloalkyladenosine turned out to be very close to those of the global energy minima. Then on the contrary, the restricted C6–N^6–C1′–C2′ torsion of N^6-substituted adenosine analogues in their global minima helped to restrict the corresponding N9–C8–C1′–C2′ torsion of cycloalkylxanthines. These conformations have new alignment of the N^6- and 8-substituents in comparison with the bioactive conformations suggested previously.[11,38]

A further point which should be addressed within the context of bioactive conformation searching, is the sp^2–sp^3 nature of the N^6-nitrogen. Nitrogen 'flipping' gives rise to two populations of essentially equivalent conformers which are 'mirror-images' of each other (excluding the ribose moiety). In the examples shown in this study, the N^6-substituent is above the heterocycle face opposite to the ribose moiety, the other possibility is for the N^6-substituent to be above the same heterocycle face as the ribose moiety. The receptor is likely to have a preference, but these two populations are essentially energetically identical and it is impossible with current knowledge to define the correct orientation. It is possible to fit the alkylxanthines and indeed all compounds of this study in an identical manner, with regard the heterocycles and ribose position, with either orientation of nitrogen. The resultant bioactive conformations for each orientation of nitrogen will be essentially energetically equivalent 'mirror-images'. We chose to show only one population for clarity.

Details on specific fits

The only orientation of **2** that was maintained a self-consistent matrix, is shown in Figure 2. The fused 5:6 heterocycle of **2** superimposed on the heterocycle of **1**. This orientation gave good electrostatic potential similarity, and excellent shape similarity to the other molecules in this study. This orientation has been suggested previously,[13] based on support from SAR for substituents on the exocyclic nitrogen, which shows similarity to the SAR for adenosine analogues. The 1-trifluoromethyl-group of **2** projects into a position where the ribose C1′ of adenosine analogues would bind to the receptor. In the series of compounds represented by **2**, an ethyl or pentafluoroethyl group, and to a lesser extent, a methyl group is tolerated in the 1-position.

The alkylxanthines **3**–**5**, and the imidazopurine, **6**, were each found to bind in only one self-consistent orientation, those shown in Figure 2. The orientation of the xanthines is similar to that suggested by Peet,[7] with the *C*8-substituent entering the N^6-binding domain (hereafter designated the N^6–C8 binding domain), the N7–H lying close to the N^6–H, conserving a potential hydrogen-bonding site (hereafter designated the N^6–N7 acceptor), and O^6 occupying the same position as N7 of adenosine analogues (hereafter designated the N7–O^6 site). The *N*1-alkyl substituent entered the same position as the ribose group (hereafter designated the ribose-N1 binding domain), and superimposes on the 1-trifluoromethyl-group of **2**. This is reflected by consistent SAR for this position among the alkylxanthines and the compounds represented by **2**. From a steric point of view, the ribose binding domain can easily accommodate the *n*-propyl group. It is possible that the propyl group can access an area of the ribose-N1 site that has an appropriate lipophilic nature. The SAR of certain key xanthine targets offers highly convincing support for this orientation.[7,34] The *N*3-substituent of compounds **3**–**6** entered the region hereafter defined as the N3 binding domain. Generally compounds **3**–**6** showed good electrostatic potential similarity and excellent shape similarity with the other compounds, based on ASP calculations.

Compound **7** is not particularly potent. However from the SAR of this series of compounds, the orientation relative to alkylxanthines is almost certain, and was identified by Hamilton et al.[16] The fit against **1** is shown in Figure 2. The 5-phenyl substituent binds into the N^6–C8 binding domain of the receptor and is almost coplanar with the phenyl ring of **3**, the *N*1-, and

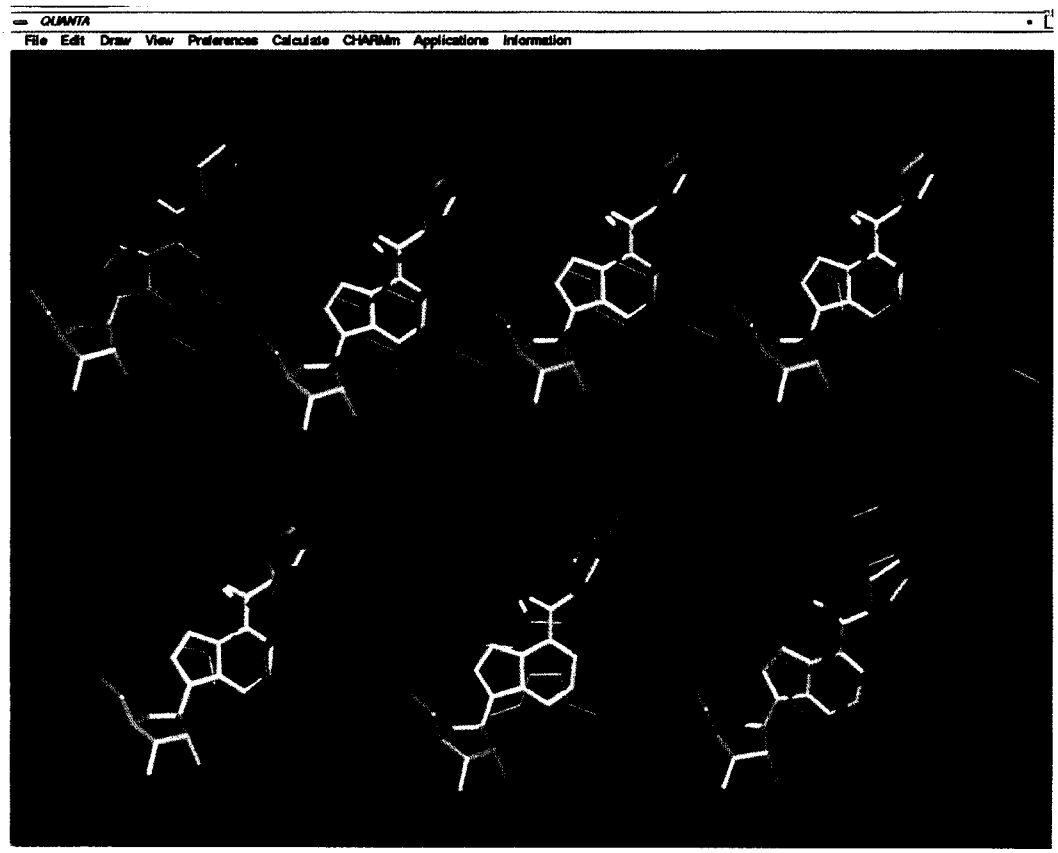

Figure 2. Likely receptor-bound orientations of compounds **2**–**8**, fitted against **1** for reference. Top row (left to right) **2**–**5**. Bottom row (left to right) **6**–**8**. Yellow atoms indicate important protons which can interact with a common site on the receptor.

*C*3-methyl groups bind into the same position as the N1 and N3 alkyl groups of the alkylxanthines, and O^7 binds into the N7–O^6 site. The N6–H is in the correct position to bind to the N^6–N7 acceptor.

In this orientation, **7** generally showed good electrostatic potential similarity and excellent shape similarity with the compounds of this study, despite its relatively weak potency. On the contrary in the orientations of **9**, **10** and **11** (described below), they showed relatively poor similarities, despite their fairly good potency. Although there is not experimental evidence to rationalize these apparent conflicts, some repulsive or attractive force caused by small subsidiary interaction might influence the whole molecular recognition regardless of the extent of their similarity coincidence as a whole to the recognition site of the receptor. Moreover, many other factors such as log P, desolvation energy of the ligand, and dipole moment of the ligand influence the activity of the ligand. Thus it might be expected that the similarity indices would not necessarily correlate with activity of the ligands.

The orientation of compound **8** essentially mimicked that of the alkylxanthines. The benzyl group projected into the N3 binding domain, the *n*-propyl group projected into the ribose-N1 binding domain and the *p*-methoxyphenyl substituent entered the N^6–C8 binding domain. The fit against **1** is shown in Figure 2. This orientation maintained a hydrogen-bond donor for the N^6–N7 acceptor. ASP calculations showed that in this orientation, **8** showed good similarity to all compounds apart from **1** and **2**.

Only one orientation was found to be likely for the three compounds **9**, **10** and **11**. The heterocyclic rings of **9**, **10** and **11** superimposed on the heterocyclic ring of **7**. The fits of **9** against **1**, **3** and **7** are shown in Figure 3. In this orientation, the exocyclic nitrogen of **9**, **10** and **11** bind near the N7 nitrogen of **1** and maintained the N^6–N7 acceptor on the receptor. The phenyl group pendent to the pyrimidine ring binds into the N^6–C8 binding domain and is almost coplanar with the phenyl rings of **3** and **7**. The bulky phenethyl substituents of **9** and **10** bind into the N3 binding domain close to the benzyl group of **8**. The *C*5-methyl substituent of **9** and the *C*5-methine of **10** bind into the ribose-N1 binding domain close to the N1 alkyl groups of **3**, **4** and **5**, the C3 methyl of **7**, and the trifluoromethyl group of **2**. At the N7–O^6 site, the receptor is also capable of binding an oxygen, for example, O^6 of the alkylxanthines and O^7 of **7**. This orientation may explain why compounds in the series represented by **9** and **10** tolerate an oxygen in place of the exocyclic nitrogen. These 'hypoxanthine' analogues, for example **13**[18] of Figure 4 would bind with the oxygen in the same position as O^6 of **3**, **4**, **5** and O^7 of **7**, i.e. the N7–O^6 site. Additionally, in this orientation, if the exocyclic nitrogen of **9** and **10** is replaced by oxygen, the compound becomes the N3–H tautomer and the

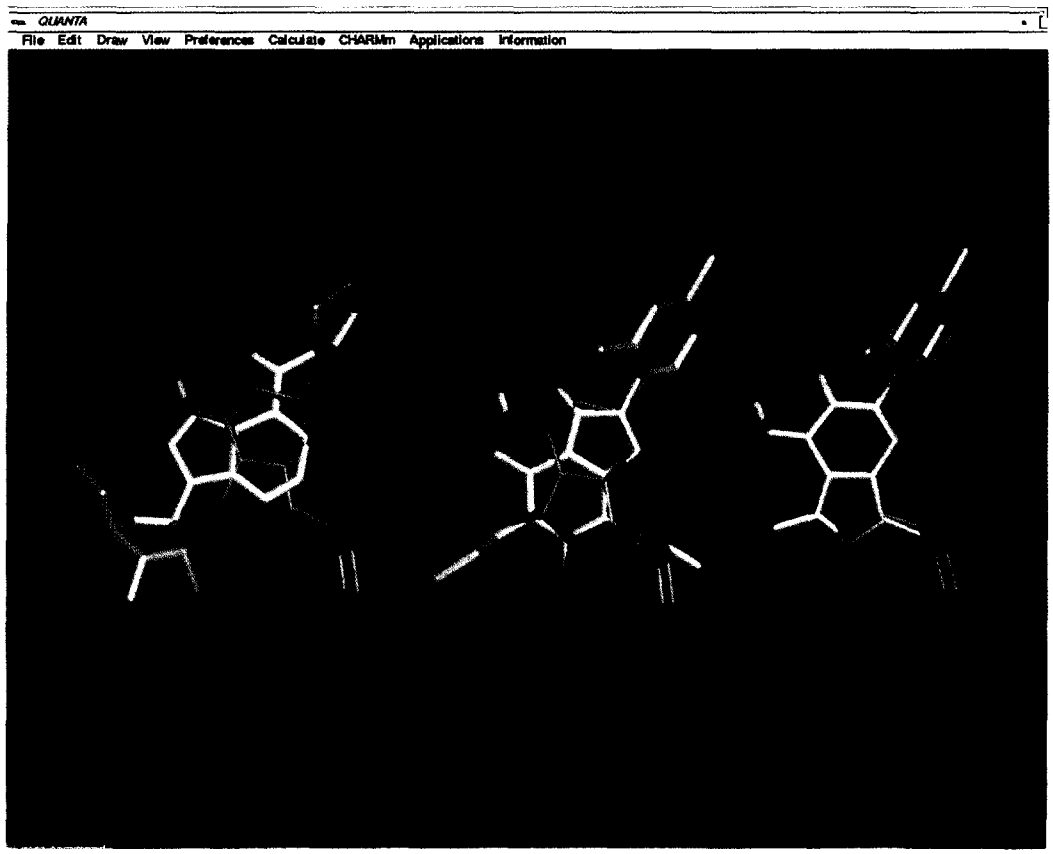

Figure 3. The orientation of **9** relative to **1** (left), **3** (middle) and **7** (right). Yellow atoms indicate important protons which can interact with a common site on the receptor.

potential to hydrogen-bond to the N^6–N7 acceptor is not lost. If one considers the binding mode of the A_2-selective compound **14**[41] at the A_1 receptor it is possible to see from Figure 4, that it may have the same binding mode as **7** due to their similarity (indeed **7** might be an A_2-selective compound, the biological information has never been published[2]). If Figure 4 is representative of the actual binding mode of the 'hypoxanthine' compound **13**, it may be reasonable to suggest that compounds belonging to this 'hypoxanthine' series should show increased A_2 affinity. Indeed this is the case.[18]

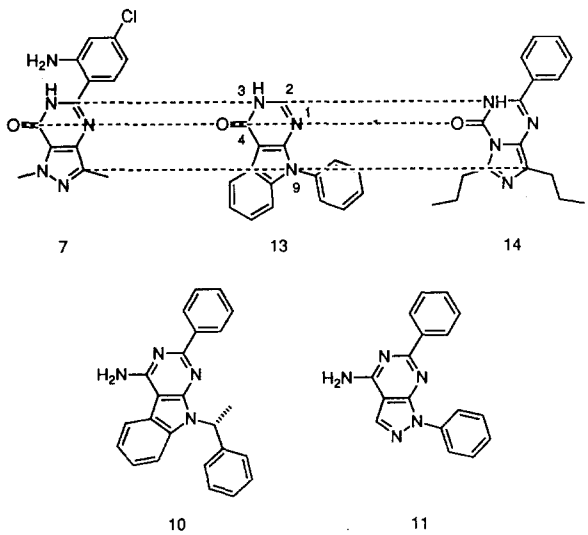

Figure 4. Proposed binding mode of **13** and **14** with **7**, **10** and **11** for comparison.

An orientation of compounds **9**, **10** and **11** that might seem intuitively correct is with the heterocycles superimposed on the heterocycle of **1**. However, many of the required fits were not revealed in the RECEPS calculation. Indeed in this orientation, the ASP calculations (not shown) revealed only poor to fair similarity with other compounds. The Van der Waals surfaces of **9** and **10** largely overlapped the cage for the N^6–N7 acceptor of the receptor, thus in this orientation, **9** and **10** would be likely to have an unfavorable steric interaction with the receptor.

Two orientations of **12** are possible. The first concurs with the proposal by Van Galen[20] and is shown in Figure 5, against **1**. In this orientation, the 5:6 fused heterocycle of **1** and **12** superimpose. The implication of this fit, given a common N^6–C8 binding domain, is that there is an area capable of binding an aryl substituent near the C8 binding site of adenosine analogues. The ASP calculations showed that this orientation gave fair similarity indices against **1**, but generally good similarity indices against the other compounds. This orientation is supported by similar SAR for substituents on the exocyclic nitrogen of **12**, **1** and **2**. In the second possible orientation, **12** bound in

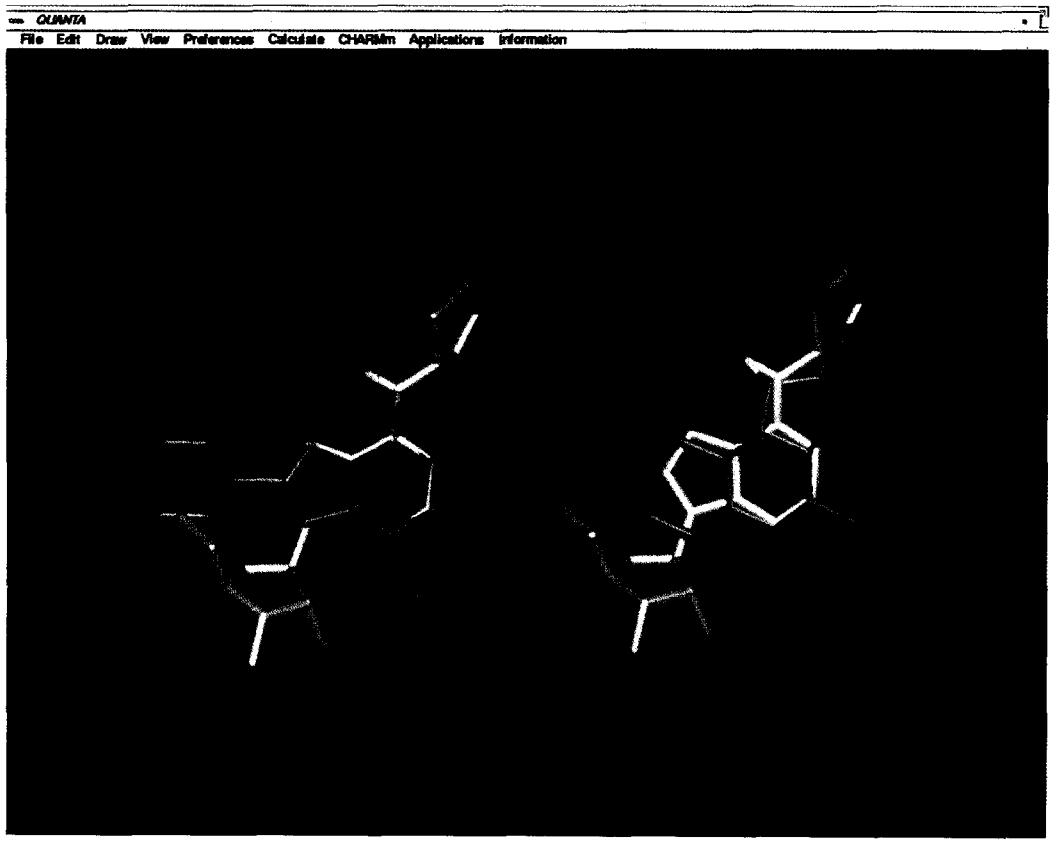

Figure 5. The two orientations of **12**, relative to **1**. Yellow atoms indicate important protons which can interact with a common site on the receptor.

a manner which superimposed the 5:6 fused heterocycle with the heterocycle of the alkylxanthines as shown in Figure 5. In this orientation, the 2-phenyl substituent bound into the N^6–C8 binding domain of the receptor, almost coplanar with the phenyl rings of **3**, **7**, **9**, **10**, and **11**, the N1–H was positioned to bind to the N^6–N7 acceptor and the N^4-substituent bound in the N3 binding domain. The electrostatic potential and shape similarities for this orientation were generally better than for the previous orientation. However, this orientation does not agree with the observed SAR for the N^4 position in this series of compounds. In particular, the stereochemical preference for the (*R*)- rather than the (*S*)-phenylisopropyl substituent at N^4, closely matches the same preference at the N^6–C8 binding domain.[20] On this basis the first orientation of **12** was concluded to be the most likely. However, to be thorough, the SAR for the C2 position of this series should be checked in more detail. For example, the (*R*)-*C*2 and (*S*)-*C*2-phenylisopropyl analogues of **12** could be synthesized to determine if there is any stereochemical requirement similar to the N^6-region of the adenosine analogues.

Generation of 3-D requirements for the A_1 receptor affinity

On the basis of the RECEPS orientations, 3-D requirements for the A_1 receptor affinity were developed and is illustrated in Figure 7.

Because of the strong evidence,[33,39] it is likely that both the N^6–H and N7–H protons act as hydrogen-bond donors to receptor sites. In this study we presented evidence that the N^6–H and N7–H protons bind to a common receptor site which we called the N^6–N7 acceptor. From the 3-D requirements for the receptor affinity illustrated in Figure 7, the N7 nitrogen of adenosine analogues and the O^6 oxygen of alkylxanthines, are in the correct position and orientation to be hydrogen-bond acceptors to the 'N^6–N7 acceptor'. Replacement of the N7 nitrogen by carbon,[42] and the O^6 oxygen by sulfur[43] (a poor hydrogen-bonder), drastically reduces the activity of adenosine analogues and alkylxanthines respectively, indicating a potential role in hydrogen bond formation with a receptor hydrogen-bond donor. Because of the close proximity of the N^6–N7 acceptor and this receptor donor site, it is likely that they are the same residue of the protein.

By considering the overlays of **1** and **4**, and **1** and **9**, it is possible to see that the hydrogen bond functionality at the N7–O6 site is an acceptor in the former case and a donor in the latter. A purely speculative rationalization is offered. If it is assumed that the receptor functionality responsible for hydrogen bonding at this position is a histidine residue (there is evidence for this[44,45]), then rotation and tautomerization of this residue might be used to explain the duality. It may be possible to check this hypothesis using the computer generated model of the A_1 adenosine receptor.[44]

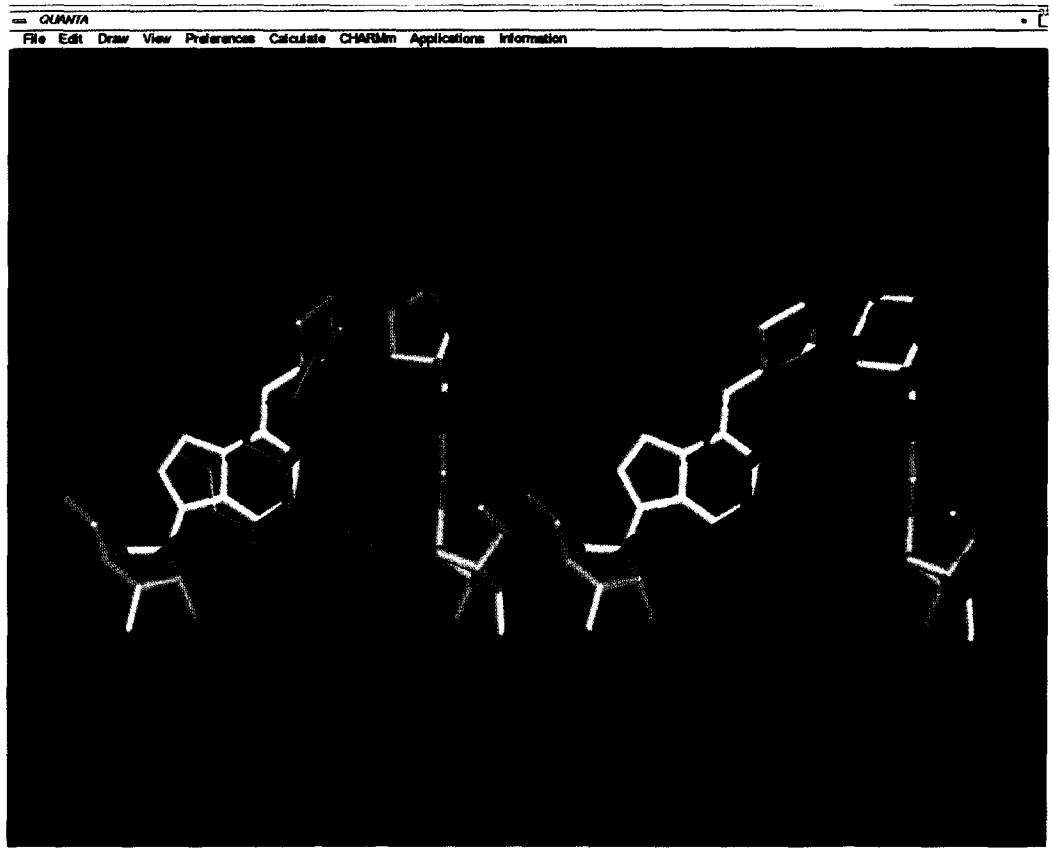

Figure 6. Orthogonal pairs of **12** and 1,3-dipropyl-8-cyclopentylxanthine (left pair) and N^6-cyclohexyladenosine and 1,3-dipropyl-8-cyclohexylxanthine (right pair) indicating the close alignment of cycloalkyl-groups.

Indeed these 3-D requirements for the receptor affinity and the computer generated adenosine receptor, model might be used 'interactively' to make further refinements to the A_1 receptor binding site.

The question of how it is possible for antagonists and agonists to be recognized by a similar pharmacophore arises. These 3-D requirements for the receptor affinity offer an explanation. Careful inspection of Figures 2

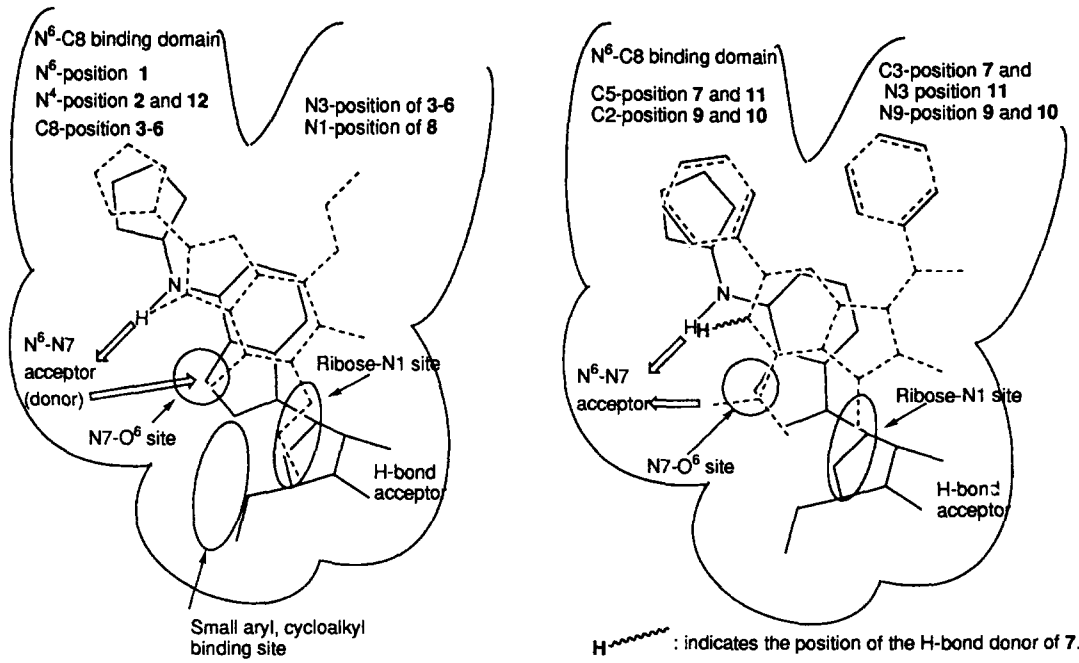

Figure 7. Pharmacophoric map of the A_1 receptor derived from the compounds used in this study. At left is shown the binding mode of compounds **1–6**, **8** and **12**, and at right, the binding mode of **7** and **9–11**.

and 7 reveals that in all fits between the agonist, **1** and the antagonists, there is no region of the antagonists which overlaps, to any significant degree, with the ribose, the necessary moiety for agonist activity. Therefore in this model it would be entirely possible for the agonist induced change in the receptor to have little influence upon the common regions for agonists and antagonists binding.

Conclusions

This report presents 3-D requirements for the A_1 adenosine receptor affinity based on maximizing hydrogen-bonding correlation between molecules, which is strongly supported by electrostatic potential and shape similarity indices. Suggested binding orientations are offered for a number of novel antagonists, not previously modeled, and from this extra detail, novel target compounds have been designed and are currently being synthesized.

The finding of similar 3-D requirents for the receptor affinity for agonists and antagonists suggests a fairly static receptor structure in the region common to agonist and antagonist binding. The ribose moiety is remote from antagonist binding and it is therefore likely to be a change localized in this area, and having little influence on the region binding the heterocycles, which elicits agonist activity.

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