A Model for the Antagonist Binding Site on the Adenosine A_1 Receptor, Based on Steric, Electrostatic, and Hydrophobic Properties

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With the aid of molecular modeling, both adenosine and adenosine A_1 receptor antagonists belonging to various chemical classes were compared with respect to their minimum-energy conformations and molecular electrostatic potentials, as computed by the semiempirical molecular orbital program MOPAC. Distinct steric and electrostatic similarities between adenosine and the prototypic adenosine antagonist theophylline are evident when both compounds are superimposed, with theophylline in a so-called flipped orientation. Similar patterns were found for all other A_1 antagonists investigated in this study. A model for the antagonist binding site on the adenosine A_1 receptor, based on steric, electrostatic, and hydrophobic properties contributing to potency, is proposed.

Many physiological effects of adenosine (1, Chart I) are mediated via membrane-bound receptors, among others in the nervous and the cardiovascular systems. Adenosine receptors are conventionally subdivided into A_1 and A_2, on the basis of different structure–activity relationships (SAR) profiles. At the A_1 receptor, N^6-[1(*R*)-phenyl-2-propyl]adenosine (*R*-PIA) is more potent than 5′-(*N*-ethylcarbamoyl)adenosine (NECA), and the *S* isomer of PIA is at least 10-fold less potent than *R*-PIA. At the A_2 receptor, there is little stereoselectivity and NECA is more potent than *R*-PIA.[1]

All adenosine receptor agonists reported so far are closely related to 1 itself. Only a few, slight modifications of the ribose moiety are allowed in order to retain agonist activity.[2] Substituents at N^6 or C2 may increase receptor affinity.[3]

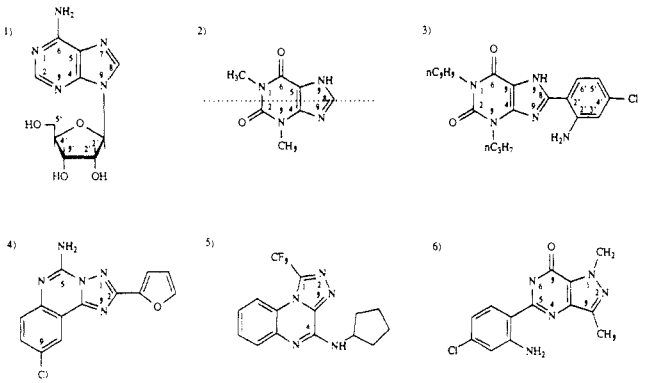
Adenosine receptor antagonists, on the other hand, belong to a wide variety of chemical classes. The xanthines, with caffeine and theophylline (2, Chart I) as best known representatives, were the first class of compounds to be identified as having adenosine antagonistic properties.[3] Adenosine receptor mediated effects of xanthines include central stimulant, cardiac stimulant, and diuretic actions.[4] The role of adenosine receptor antagonism in the antiasthmatic action of xanthines is less clear.[5]

Detailed structure–activity relationships studies of xanthines have revealed that substitution at N1, N3, and C8 can increase receptor affinity markedly. For example, 1,3-dipropyl-8-(2-amino-4-chloro)phenylxanthine (PACPX, 3, Chart I), has 1800-fold higher affinity at A_1 receptors from rat brain than its parent compound 2 (Table I).

In recent years, several non-xanthine classes of compounds with antagonistic properties at the adenosine receptor have been identified. These include pyrazolo[4,3-*d*]pyrimidines[6] and pyrazolo[4,3-*d*]pyrimidin-7-ones,[7] 9-methyladenines[8] and ribose-modified adenosine derivatives,[9] triazolo[4,3-*a*]quinoxalin-4-amines[10] and triazolo[1,5-*c*]quinazolines—most notably CGS 15943.[11] Also, barbiturates[12] and some antiepileptics like carbamazepine[13] have been reported to have moderate adenosine antagonistic properties. For some representative compounds, K_i values for inhibition of [^{3}H]cyclohexyladenosine binding to rat brain membranes—a measure for A_1 receptor affinity—are listed in Table I.

In general, the binding of a ligand to a receptor is governed by three important factors. First, the ligand should fit sterically to the receptor. Second, there should be

Chart I. Structures and Ring Numbering of Adenosine (1) and the Various Antagonists Theophylline[a] (2) PACPX (3), CGS 15943 (4), *N*-Cyclopentyl-1-(trifluoromethyl)[1,2,4]triazolo[4,3-*a*]-quinolin-4-amine (5), and 5-(2-Amino-4-chlorophenyl)-1,6-dihydro-1,3-dimethyl-7*H*-pyrazolo[4,3-*d*]pyrimidin-7-one (6)



[a] The dashed line indicates the long axis, as referred to in the text.

Table I. Affinities of Adenosine Receptor Antagonists Used in This Study

	A_1, K_i[a]
theophylline (2)	8.5 μM[b]
PACPX (3)	4.8 nM[c]
CGS 15943 (4)	21 nM[c]
N-cyclopentyl-1-(trifluoromethyl)[1,2,4]triazolo-[4,3-*a*]quinolin-4-amine (5)	7.3 nM[d]
5-(2-amino-4-chlorophenyl)-1,6-dihydro-1,3-dimethyl-7*H*-pyrazolo[4,3-*d*]pyrimidin-7-one (6)	310 nM[e]

[a] Inhibition of [^{3}H]CHA binding to rat brain membranes. [b] Reference 14. [c] Reference 11. [d] Reference 10. [e] Reference 7.

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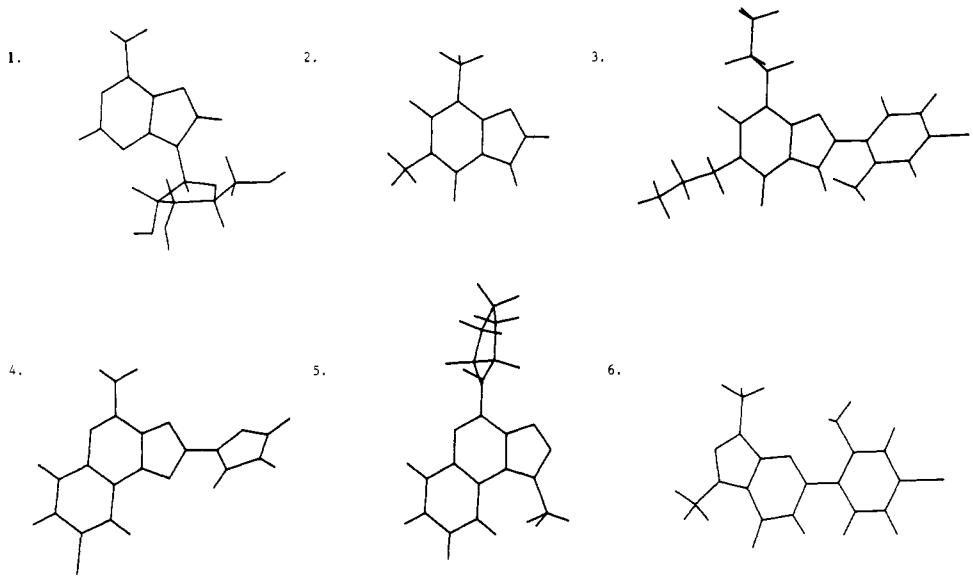

Figure 1. Minimum-energy conformations of compounds **1–6** as calculated by MOPAC. The plane of the heterocycle system is shown at an angle of 30°.

electrostatic complementarity: parts of the receptor and ligand with opposite electrostatic potentials (EP) should be in close proximity to each other. In the third place, lipophilic regions should match in order to have optimum hydrophobic interaction.

In the present study, **1** and a variety of adenosine antagonists were compared with respect to their minimum-energy conformation and molecular electrostatic potential (MEP), in order to gain more insight in the factors determining affinity for the A_1 receptor. The antagonists included in the study were **2**, **3**, CGS 15943 (**4**), *N*-cyclopentyl-1-(trifluoromethyl)[1,2,4]triazolo[4,3-*a*]quinolin-4-amine (**5**), and 5-(2-amino-4-chlorophenyl)-1,6-dihydro-1,3-dimethyl-7*H*-pyrazolo[4,3-*d*]pyrimidin-7-one (**6**). For structural formulas, see Chart I. It will be shown that similar electrostatic patterns can be found in all the structures investigated. Furthermore, there are distinct similarities in steric properties and the affinity-enhancing effects of hydrophobic substituents.

Computational Methods

Studies were performed with a VAX 11/785 computer and either a Visual 550 monochrome display or a Sigmex 6130 color display. Manipulations of structures and construction of the MEP's were carried out with the Chem-X (July 1988 update) molecular modeling software.[15] Minimum-energy conformations and charge distributions were calculated with the semiempirical molecular orbital MOPAC program,[16] using the standard MNDO parameters and Pulay's method of convergence. Crystal structures of **1** and **2** were retrieved from the Cambridge Structural Data Base[17] and were subsequently structurally optimized with MOPAC. Structures of other compounds were built on screen, starting from a xanthine framework and subsequently structurally optimized with MOPAC. All interatomic distances, bond angles, and dihedral angles were allowed to relax fully. If appropriate, a conformational search was performed for rotatable substituents in order to avoid the risk of local minima. This search was done in MOPAC, in 12 steps of 30°, with the AM1 Hamiltonian. This has recently been shown to account satisfactorily for rotational barriers.[18]

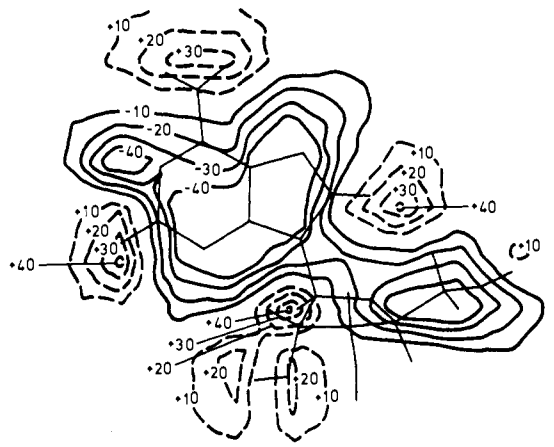

Figure 2. MEP of **1** in the plane of the 6:5-fused heterocyclic system. Contours are shown at 10, 20, 30, and 40 kcal/mol.

MEP plots were constructed in the plane of the 6:5-fused heterocyclic system common to all structures investigated. Charges were taken from the MOPAC minimizations. Subsequently, the MEP was computed by using the default Chem-X algorithm. The method Chem-X uses to compute the MEP common to two or more structures is outlined below. Structures are first superimposed and then an imaginary grid (20 × 20) is constructed in the plane of the 6:5-fused ring system. Initially, each grid point is assigned a value of zero. Subsequently, a certain significance value, for instance 5 kcal/mol, is read in. At each grid point, the electrostatic potential (EP) due to the first structure is computed. If the EP at a certain point is larger than +5 or smaller than −5 kcal/mol, the actual value of the EP is assigned to this grid point. All grid points with EP values between −5 and +5 kcal/mol are set to zero. Subsequently, the EP's at each point due to the second structure are computed. Whenever the EP at a certain

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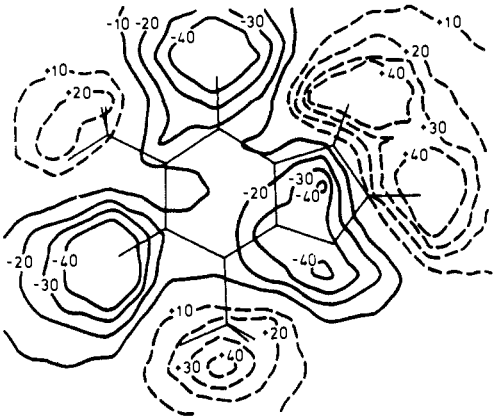

Figure 3. MEP of 2 (details as in Figure 2).

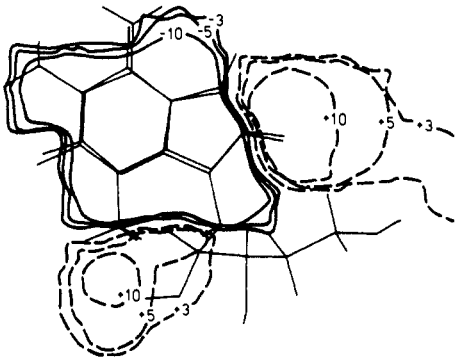

Figure 4. Fit and common MEP of 1 and 2 with all nitrogens coinciding.

point is larger than +5 or smaller than −5 kcal/mol due to both the first and the second structure, this grid point is assigned the value of the highest of the two EP's. Otherwise, a value of zero is assigned. The same is done for each following structure.

Results

The minimum-energy conformations for compounds 1–6, as calculated by MOPAC, are shown in Figure 1.

Fit of Adenosine and Theophylline. Charge distributions at various EP levels for the prototypic agonist 1 and the prototypic xanthine antagonist 2 are represented in Figures 2 and 3. Since xanthines competitively displace radiolabeled adenosine receptor agonists from the adenosine A_1 receptor, it is generally assumed that xanthine antagonists bind to the same region of the receptor as agonists do.[19] Both structures contain a purine ring, and therefore the most obvious way to superimpose them is with the atoms N1, N3, N7, and N9 of both ring systems coinciding. Figure 4 shows both this fit and the common EP at various potential levels. The xanthine ring of 2 overlaps almost completely with the adenine moiety of 1. Furthermore, there are three areas of common EP: the negative EP of the π-electrons of both ring systems, the positive EP of both H8 atoms and the positive EP of the methyl substituent of N3 in 2 and part of the ribose moiety of 1. In sharp contrast with these similarities, however, the positive EP's of H2 and the amino hydrogens of 1 coincide with the oppositely charged EP's of O^2 and O^6 in 2.

There is a second possibility to fit both structures, i.e. when 2 is turned 180° around the long axis (Figure 5). An

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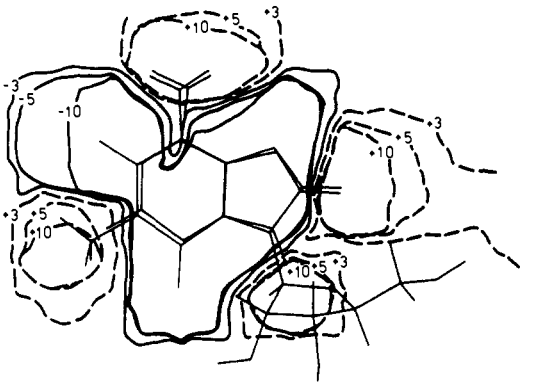

Figure 5. Fit and common MEP of 1 and 2 with the latter in a flipped orientation.

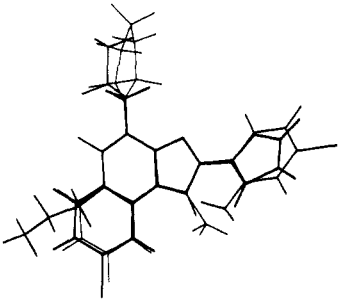

Figure 6. Fit of 3–5 shown at an angle of 30°.

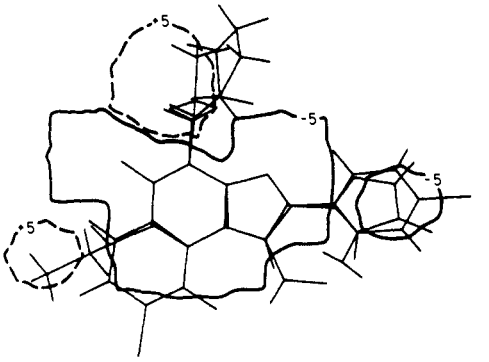

Figure 7. Common MEP of 3–5 in the plane of the heterocyclic system.

equally convincing steric fit is found, but in this case the electrostatic overlap is considerably larger. Not only the negative EP of both ring systems and the positive EP of H^8 overlap but also the two other positive areas in 1 coincide with those in 2. Additionally, there is a large overlap in the negative EP of the lone pairs of N1 and N3 in 1 and the carbonyl oxygens of 2.

Thus, when superimposed in this flipped orientation, *each single part* of 2 corresponds with a similarly charged part of 1. There is one large Y-shaped area of negative EP, spread out over the 6:5-fused ring system. This negative area is surrounded by four areas of positive EP, corresponding to the exocyclic amino group, H2, H8, and part of the ribose moiety of 1.

In Figures 4 and 5, contour levels are shown at 3, 5, and 10 kcal/mol. Essentially the same information is obtained in all three cases. For reasons of simplicity only one level—arbitrarily chosen at 5 kcal/mol— is shown in the succeeding MEP plots.

Fit of Potent Antagonists. Compounds 3–5 are all much more potent than 1 and 2, having affinities in the nanomolar range. Therefore, it would not be justified to compare their MEP with the MEP of either 1 or 2, so they are compared directly with each other. The fit we propose

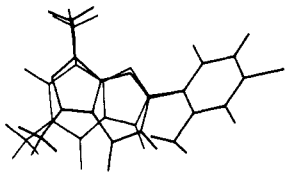

Figure 8. Fit of **2** and **6**, shown at an angle of 30°.

for them is represented in Figure 6; the common MEP is shown in Figure 7. Because of their close similarities in structure and MEP, **3** was placed in the same flipped orientation as **2**.

There are two possible tautomers of **4**, an amino and an imino form. MOPAC minimizations of both forms revealed that the amino tautomer is favored energetically by 14.5 kcal/mol. Therefore, we have used this canonical form in our modeling studies. Compound **4** is fitted on **3** with the 6:5-fused heterocycles coinciding. The furan ring of **4** overlaps with the C8 substituent of **3** and the benzene ring of **4** coincides partly with the N3 propyl substituent of **3**. When superimposed in this way, both steric and electronic overlap are considerable. It should be noted that the furan group of **4** is not shown in its minimum-energy conformation in Figures 6 and 7. According to our calculations, the angle between the planes of the furan ring and the heterocyclic system is 45° in the minimum-energy conformation of **4**. In order to orientate it in the same plane as the phenyl ring of **3**, it is necessary to rotate the furan ring slightly (torsion angle 33°), at a marginal cost of 0.3 kcal/mol (MOPAC energy). Of the various possibilities to fit **5**, optimum overlap with **3**—both sterically and electronically—is found if the tricyclic system is orientated in the same way as in **4**. Superimposition of **3**–**5** in the way described above results in the combined MEP of Figure 7. There are some distinct similarities with the combined MEP of **1** and **2**. Again, the Y-shaped area of negative EP is found. Furthermore, two areas of positive EP can be distinguished, similar to the two areas in the fit of **1** and **2**. They correspond with the exocyclic amino group and H2 of **1**. The other two areas of positive EP in the fit of **1** and **2** are not present. However, in the fit of **3**–**5** there is an additional area of negative EP, partly coinciding with the area of positive EP in the fit of **1** and **2** that corresponds with H8 of **1** and **2**.

Fit of Compound 6. Compound **6** has rather low affinity and therefore it would not be justified to compare it directly with much more potent compounds like **3**–**5**. Of the various ways to fit **6** on **2**, the one shown in Figure 8 is the only one that gives considerable steric and electrostatic overlap, which is shown in Figure 9. This is the fit that has been proposed by Hamilton et al.[7] In this fit, the 6-membered ring of one structure overlaps with the 5-membered ring of the other and vice versa. The conformation shown in Figure 8 is not the minimum-energy conformation shown in Figure 1. In order to orientate the 2-amino-4-chlorobenzene substituent of **6** in the same plane as the analogous substituent in **3**, it was rotated at a cost of 4 kcal/mol, as calculated by MOPAC. The angles between the plane of the heterocycle and the plane of the substituent are 41° for the fitted conformation and 178° for the minimum-energy conformation, respectively. Again, almost the same Y-shaped area of negative EP is found that was seen earlier in Figures 5 and 7. Furthermore, there are three common areas of positive EP that coincide with similar areas in Figure 5.

Discussion

The structures investigated in this study are exemplary for the range of A_1 antagonists reported so far. When they

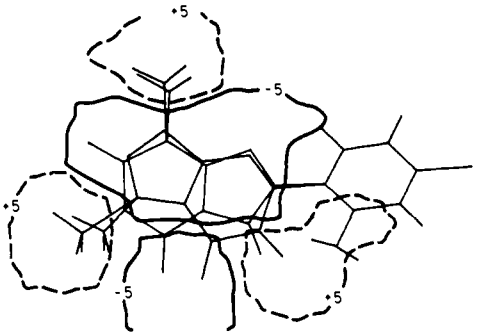

Figure 9. Common MEP of **2** and **6** in the plane of the heterocyclic system.

are compared with each other with respect to their various steric and electrostatic properties, some attributes seem to be generally valid, independent of the potency of these compounds. Furthermore, these very same attributes can be found in the agonist **1**, which is assumed to bind to the same region of the receptor. Thus, these properties may be of general importance for the ability to bind to the A_1 receptor. They will be discussed in terms of steric fit, electrostatic fit, and additional sites for hydrophobic interaction.

Steric Fit. The common property immediately becoming apparent on inspection of the structures used in this study is the 6:5-fused heterocyclic system. Therefore, the starting point for superimposition was the matching of these parts of the various structures.

There are two different ways to fit the adenine moiety of **1** and the xanthine structure of **2**. In both cases there is substantial steric overlap. However, there is considerably more overlap in EP when **1** and **2** are fitted with **2** in the flipped orientation (Figure 5) than in the situation where all nitrogen atoms of the 6:5-fused heterocyclic system coincide (Figure 4). If the assumption is right that adenosine and xanthines bind to the same region of the receptor, then this second possibility is surely the most obvious one. This is in agreement with the findings of Olsson et al., wo came to the same conclusion by comparing the dipole moments of adenine and **2**.[20] The fact that theophylline 7-ribonucleoside has affinity for the receptor,[20] whereas theophylline 9-ribonucleoside has not,[21] provides further support for this hypothesis.

It was assumed that **2** and **3** bind to the receptor in the same orientation (i.e. the 180°-reversed orientation) because of their close resemblances in structure and MEP. In the original publication,[11] **4** was depicted as the imino tautomer. Since MOPAC minimizations of the amino and the imino forms strongly favor the amino tautomer, we have used this canonical form in our modeling studies. In a recent paper by Francis et al.,[22] it is concluded on the basis of spectrometric evidence that the amino tautomer indeed is the preferred one. The same authors suggest two ways of fitting the amino tautomer of **4** on xanthine structures. In the first fit, the furan ring of **4** overlaps with the phenyl substituent of 8-phenylxanthine, and both 6:5-fused heterocyclic systems coincide. In the second fit,

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(21) Clanachan, A. S. *Can. J. Physiol. Pharmacol.* **1981**, *59*, 603–606.
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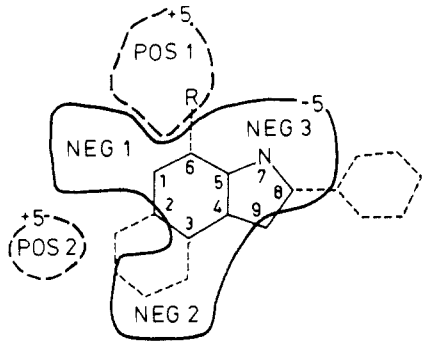

Figure 10. Common steric and electrostatic properties of the adenosine antagonists used in this study. Additional areas where substitution may enhance potency are indicated with dashed lines. Ring numbering is followed as in adenosine.

the benzene ring of 4 overlaps with the 2-amino-4-chlorophenyl substituent of **3**.

Although both fits show considerable steric overlap, electronic overlap with xanthine structures **2** or **3** is poor. Also with the agonist **1**, when fitted with the nitrogen atoms of the purine rings coinciding, there is little electronic overlap. A third possibility however, shown in Figures 6 and 7 and explained in the Results section, exhibits considerable overlap both sterically and electronically. The difference of this third fit with the first fit of Francis et al. is that again the xanthine is turned 180°. On the basis of the highly improved electrostatic overlap, we conclude that this is the most probable orientation for **4** to bind to the receptor. Our finding that the angle between the furan ring and the heterocyclic system of **4** is 45° is not in agreement with the results of Francis et al.,[22] who reported that the molecule is almost flat. The authors did not mention their method of energy minimization.

Compounds **4** and **5** have similar tricyclic systems, and when **5** is superimposed on **4** as shown in Figures 6 and 7, not only steric but also electrostatic overlap is optimal. In all fits described so far, the 6:5-fused heterocyclic systems are fully overlapping. This is not the case for the fit we propose for compound **6**. The 6-membered ring of **6** has to be fitted on the 5-membered ring of the xanthine and vice versa, in order to obtain a satisfactory electrostatic fit. In this way, also the 2-amino-4-chlorobenzene substituents of **3** and **6** can overlap, but an increase in intramolecular energy of 4 kcal/mol is needed for an optimum fit. It has been demonstrated by Hamilton et al., on the basis of highly correlated SAR at this site in xanthines and pyrazolo[4,3-*d*]pyrimidines, respectively, that these substituents most probably coincide.[7] Whether the relatively low potency of **6** is caused by the less than optimal steric overlap, the energy increase that is needed to fit both ring substituents, or yet another cause is a question that remains to be answered.

Electrostatic Fit. In Figures 5, 7, and 9 the common MEP of various combinations of structures are depicted. Independent of the affinity of antagonists—be it weak or potent—some similarities in charge distribution with the initial fit of the agonist **1** and the xanthine antagonist **2** are apparent. These are shown schematically in the model in Figure 10. Invariably, a large, Y-shaped area of negative EP is found, resulting from the aromatic system of the 6:5-fused heterocycle. The Y-shaped area extends out of the ring system at three points. For convenience, it has therefore been divided into three subsites, designated NEG 1, NEG 2, and NEG 3.

Apart from this common area of negative EP, there are also two common areas of positive EP, which are designated POS 1 and POS 2. The latter two are also seen in the fit of agonist **1** and antagonist **2** (Figure 5).

The negative EP of NEG 1 is caused in most cases by the lone pair of a nitrogen atom at position 1, with the exception of the xanthines, which have a carbonyl group in this place. The negative EP of NEG 2 may be caused either by an oxygen (xanthines, **6**), the lone pair of a nitrogen (**1**), or the π-electrons of a benzene ring (**4**, **5**). Thus, the origin of this negative EP does not appear to be critical.

For NEG 3, however, in all cases the negative EP is caused by the lone pair of a nitrogen atom. Probably, a nitrogen at position 7 is a prerequisite for affinity, quite unlike the various nitrogens at other positions. A likely explanation would be the involvement of this N7 as a hydrogen-bond acceptor (note: a nitrogen atom at position 7, as referred to in Figure 10, is meant; this N7 should correspond with N9 in xanthines, according to the proposed model). For NEG 1 and NEG 2, on the other hand, the negative value of the EP as such is more important than the atom or group that actually causes this negative EP. Thus, these areas might be important for the orientation of the molecule toward the receptor, rather than being involved in a more specific interaction, such as the formation of a hydrogen bond. Interestingly, a parallel can be seen with a series of deazaadenosines as A_1 receptor agonists. Whereas 1-deazaadenosine and derivatives, and to a lesser degree also 3-deazaadenosine, retain activity at the A_1 receptor, 7-deazaadenosine is completely inactive.[23,24] Again, a nitrogen atom at this place appears to be essential.

The positive EP at POS 1 originates from the H atoms and/or the substituents of the exocyclic amino function of **1**, **4**, or **5** or a ring substituent with positive EP in **2**, **3**, and **6**. The positive EP at POS 2 originates either from a substituent of a nitrogen at position 2 (xanthines, **6**), or—with the center of the positive charge slightly shifted to the left—from a hydrogen atom attached to the benzene ring of **4** and **5**.

Thus, for both POS 1 and POS 2, the origin of the positive EP does not seem to be critical, which suggests—analogous to subsites NEG 1 and NEG 2—a role for these areas in the orientation of the molecule toward the receptor.

In the fit of **1** and **2**, two other areas of positive EP are present. The first one is the region adjacent to N9 in **1** (or N7 in **2**). In fact, all antagonists, with the exception of **4**, also have a positive EP at this site. In the case of **4**, the area is neutral. Since compound **4** is a quite potent antagonist, a positive EP in this area is obviously not essential, but nevertheless it should not be ruled out that it might enhance receptor affinity.

The other area of positive EP is the region adjacent to C8. In contrast with compounds **1** and **2**, this area has a negative EP in the potent antagonists **3**–**5** and the less potent antagonist **6**. It might be argued that the higher affinity of the latter compounds is the result of a better electrostatic complementarity with the receptor in this area. On the other hand, the A_1 antagonist DPCPX (1,3-dipropyl-8-cyclopentylxanthine) has a positive EP in this area (data not shown) and yet it is highly potent (K_i = 0.46 nM for inhibition of [^{3}H]CHA binding to rat brain membranes[25]). Thus, the enhanced affinity of compounds

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with substituents in this region is more likely due to an increase in hydrophobic interactions with the receptor site.

Additional Sites for Hydrophobic Interaction. Three areas where lipophilic substitution may lead to enhanced affinity for the A_1 receptor can be discerned.

The first one coincides with POS 1. The influence of substitution at N^6 of 1 has been extensively studied (for an overview see ref 26). A_1 receptor affinity often increases markedly with a hydrophobic substituent at the N^6-position of agonists, and similar observations have been made with identically substituted derivatives of the adenosine antagonist 9-methyladenine. According to our model, this area corresponds with the substituent at N3 in xanthine derivatives. Indeed, increasing lipophilicity at this site in xanthines also leads to increasing receptor affinity.[27,28]

Only few data are available concerning substituents at the exocyclic amino group of 4,[22] but some analogies with N^6-substituted adenosine derivatives can be pointed out. Within the series of triazolo[1,5-*c*]quinolines, high affinity is retained with an isopropyl substituent, as is the case for the similarly N^6-substituted adenosine. The dimethylamino analogue of 4 has no affinity, which parallels the very low affinity of N^6-dimethyladenosine. Replacement of the amino group with oxygen also leads to a dramatic decrease in affinity, reminiscent of the inactivity of inosine.[28]

According to the proposed model, the exocyclic amino group of **5** also points toward this area. SARs for substitution at this position parallel the known SAR for N^6-substituted adenosines.[30] For instance, in a series of cycloalkyl substituents, a cyclopentyl group is optimal for A_1 receptor affinity and selectivity.[10] An exo-2-norbornanyl substituent also leads to a potent and A_1 selective compound,[10] as is the case for the analogously substituted adenosine.

The second area where lipophilic substitution may lead to enhanced receptor affinity is the area occupied by the propyl substituent at N1 of **3**. It corresponds partly with the POS 2 area. It has been demonstrated for xanthines that increasing lipophilicity at this site leads to increased affinity.[27,28] Furthermore, the benzene rings of the potent compounds 4 and **5** coincide with this area, indicating that this site is at least available for occupation and may well contribute to affinity.

The third important area is the region corresponding with a substituent at position 8. The furan ring of 4 is essential for high affinity. In both xanthines and pyrazolo[4,3-*d*]pyrimidines aromatic substituents at this position are known to increase receptor affinity considerably. In the pyrazolo[4,3-*d*]pyrimidine series, optimum affinity is reached with a 2-amino-4-chlorophenyl substituent. This substituent is also highly potent in the xanthine series.[7]

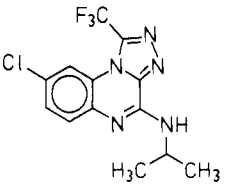

Figure 11. Structure of CP-66,278.

Finally, a factor which was not mentioned yet, but which may be important for high affinity, is the orientation of the substituent at position 8. For compound **3**, the rotational freedom of the C8-phenyl bond is limited. Upon rotation in steps of 30° (using MOPAC with the AM1 Hamiltonian), apart from the minimum-energy conformation indicated in Figure 1, a local minimum is detected at 240° (+2.8 kcal/mol), as well as a maximum at 180° (+21.5 kcal/mol). Thus, the phenyl substituent is forced out of the plane of the heterocycle, probably due to steric hindrance by the *o*-amino group. Since compound **3** is very potent, it is likely that the orientation of the ring substituent is not far from the optimum. For the much weaker compound **6**, however, an increase in intramolecular energy of 4 kcal/mol is needed to achieve maximum overlap between the 2-amino-4-chlorophenyl substituents of **3** and **6**. This may account for the relatively low potency of this compound. On the other hand, only a slight increase in intramolecular energy is needed to get maximum overlap between the furan ring of 4 and the 2-amino-4-chloro substituent of **3**, which is much more potent than **6**. Of course, it is very well possible that a ligand will sacrifice a few kilocalories/mole to adopt itself optimally to the receptor.

With the advent of new adenosine antagonists it will be possible to further extend and refine this model. One interesting new compound is CP-66,278, which is currently undergoing clinical trials as an antidepressant. It is a quite potent displacer of [^{3}H]CHA binding in rat brain membranes (IC_{50} = 24 nM). Its structure (Figure 11) resembles that of both **5** and **6**. When fitted in the same orientation as **5** and **6**, the charge distribution complies with the proposed model (data not shown). Again, substitution at the exocyclic amino group yields the same structure–activity profile as found for N^6-substituted adenosines.[31]

On the basis of this model, we have recently designed and synthesized a novel class of non-xanthine A_1 antagonists. Preliminary results indicate that some of the compounds in this series have A_1 affinities in the lower nanomolar range. Details will be published elsewhere. In conclusion, we present a model for the antagonist binding site of the adenosine A_1 receptor, which takes into account various steric, electrostatic, and hydrophobic properties that may contribute to potency as an A_1 antagonist. The model may be of value in the optimization of existing antagonists and in the development of novel structures with A_1-antagonistic activity.

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