v1.21 (Quantum Chemistry Program Exchange program 506). Final computations were performed on a Tektronix CAChe worksystem running Tektronix proprietary software[47] including their implementations of ZINDO v2.2 and MOPAC v5.10.[48] CAChe molecular mechanics uses Allinger's MM2 force field[49] as augmented by Tektronix. With both AMPAC and MOPAC, the AM1 Hamiltonian[50] was used.

The molecular mechanics of 1, 2, and 3 were investigated on the CAChe system[51] by minimizing the total molecular energy according to the molecular mechanics expression

$$E_{total} = E_{bonding} + E_{\theta} + E_{\phi} + E_{improp} + E_{elec} + E_{vdW} + E_{hb}$$

where $E_{bonding}$ describes bond lengths, E_{θ} describes bond angles, E_{ϕ} describes dihedral angles, E_{improp} describes improper torsions, E_{elec} describes electrostatic potential, E_{vdW} describes the van der Waals interactions, and E_{hb} describes hydrogen bonding. Further, molecular mechanics calculates energies relative to a hypothetical "perfect" geometry, rather than an absolute energy, and uses a temperature of 0 K. The structures cited here are those obtained from molecular mechanics, and the structure of LSD is in good agreement with the reported X-ray results.[27]

Calculations were performed on the free bases as well as on the N(6)-protonated cations, in vacuo, and no attempt was made to consider the effect of solvation. In addition, conformations where the N(6)-methyl was equatorial were of lower energy than those in which the methyl was axial. Therefore, only the equatorial conformations were used in the analyses.

As stated above, the molecular flexibility of 1, 2, and 3, is described primarily by the torsion angles τ_1, τ_2, and τ_3. All three dihedral angles were investigated on the CAChe system by performing geometry searches, or grid scans, with minimization at each step. In these searches, the molecular mechanics settings were as stated above. The angle τ_1 was searched over the ranges -70° to 0° and +70° to 0°, in 5-deg increments. This angle was scanned from both directions in order to better control the inversion of N(6) and to maintain the equatorial position of the N(6)-methyl. Similarly, τ_2 and τ_3 were searched over the range -180° to +180°, in 15-deg increments.

Acknowledgment. This work was supported by USPHS Grant DA-02189 from the National Institute on Drug Abuse. The University of Nebraska is gratefully acknowledged for support while Dr. Pfaff was on leave at Purdue University. (+)-Lysergic acid monohydrate was obtained from the National Institute on Drug Abuse. We also acknowledge valuable synthetic efforts by Stewart Frescas.

Registry No. 2, 137765-82-3; 2·maleate, 137765-83-4; 3, 137765-84-5; (+)-lysergic acid, 82-58-6; (*R*)-(-)-2-butylamine, 13250-12-9; (*S*)-(+)-2-butylamine, 513-49-5.

(47) CAChe Version 2.5, Tektronix, Inc., 1990.
(48) MOPAC v5.10 settings: optimization by the AM1 Hamiltonian; singlet multiplicity; CI level = default; maximum SCF cycles = 200; BFGS converger, XYZ coordinates. ZINDO v2.2 settings: energy only; singlet multiplicity; maximum SCF cycles = 200; INDO/1; SCF type = RHF.
(49) Allinger, N. L. Conformational analysis. 120. MM2. A Hydrocarbon Force Field Utilizing V_1 and V_2 Torsional Terms. *J. Am. Chem. Soc.* **1977**, *99*, 8127-8134.
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(51) CAChe Molecular Mechanics v2.5 settings: optimization by the block-diagonal Newton-Raphson method; relaxation factor = 1.00; energy value tolerance = 0.001 kcal/mol. Included terms: bond stretch, bond angle, dihedral angle, improper torsion, van der Waals, electrostatic, and hydrogen bonding.

The Three Binding Domain Model of Adenosine Receptors: Molecular Modeling Aspects

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Using molecular modeling, adenosine receptor ligands were fitted together to maximize correlations between the three most important factors controlling binding to the receptor, namely steric, hydrophobic, and electrostatic complimentarity. Structure-activity relationships can be explained by three binding domains on the receptors. These are hydrophobic, aromatic, and ribose binding domains. We propose that the N^6, C2, and C8 hydrophobic binding domains are not discreet but occupy the same region of the receptor.

Adenosine (1) is a naturally occurring endogenous nucleoside which has generated much interest due to its biological activity. Much of this activity is mediated via membrane-bound extracellular receptors which bind adenosine, its analogues, alkylxanthines, and various miscellaneous heterocycles.[1-3] Pharmacologically distinct receptors which inhibit (A_1) or stimulate (A_2) adenylate cyclase activity have been identified on the basis of differing structure-activity profiles. Interpretations of structure-activity relationships, based on the structure of (*R*)-(phenylisopropyl)adenosine and other N^6-substituted analogues, have resulted in details of the N^6 binding domain. The four atoms of the alkylamine moiety of (*R*)-(phenylisopropyl)adenosine lie in a Y-shaped groove, there is a site to fit the phenyl ring and in close proximity, capacious binding domains called S1 and S1-A based on evidence for little stereoselectivity and the ability to bind three carbons in this area.[4] There is a potential hydrogen-bond acceptor next to this N^6-subregion.[4] Novel N^6-bicyclo[2.2.1]alkyladenosines with unusually high potency allowed the receptor-excluded volume to be probed in considerable detail.[5] The sophistication of the model was increased using computer molecular modeling.[6] A

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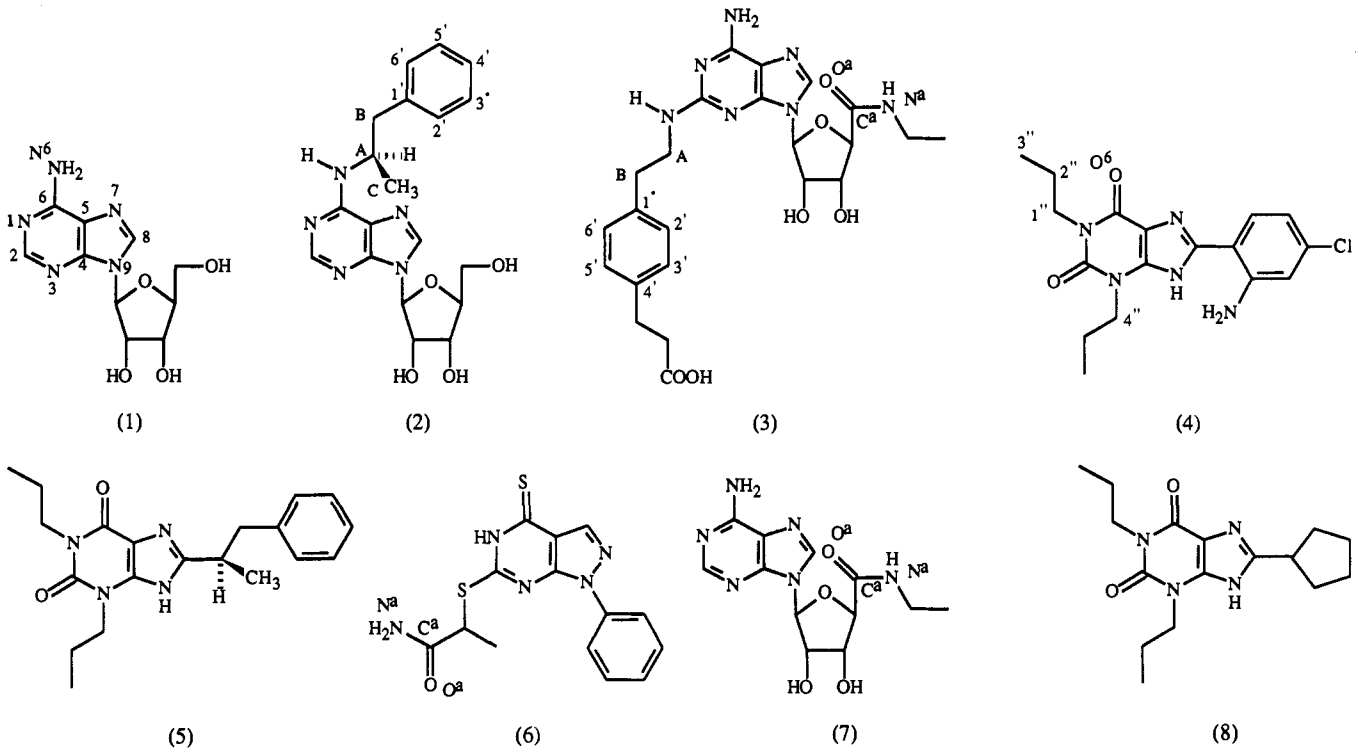
0022-2623/92/1835-0211$03.00/0

Chart I[a]



[a] For ease of illustration, the heterocycle atoms of all compounds were designated as in (1). Exocyclic atoms are designated as indicated. These designations are used to show charge correlations in Tables I and II.

possible position of the N^6 substituent relative to the purine ring was determined, and two new subregions, C (cycloalkyl) and B (bulk), were also distinguished. The C subregion accommodates 1 or 2 carbons of cyclopentyladenosine or cyclohexyladenosine, respectively. The B subregion is enclosed by the S1, S1-A, and aryl subregions and is occupied by the bulky norbornyl-substituted compounds, and as these compounds are potent at the A_1 receptor, this area must be available for binding in the A_1 receptor.

We have postulated that adenosine receptor ligands are recognized by only three primary binding domains.[7] This model proposes that the hithertofore N^6, C2, and C8 binding domains are not discrete, rather they are in the same region of the receptor. As a result of this hypothesis, the remaining important binding domains are recognized as a central aromatic and a ribose binding domain.[7] We have provided evidence for a conserved 6-membered ring, fitting the aromatic binding domain, with the synthesis of 4-(*n*-butylthio)-6-(phenylamino)-2-propionamidylthiopyrimidine.[8] It will be shown that adenosine agonists/antagonists arranged relative to each other according to this hypothesis maintain similar steric and hydrophobic interactions with the receptor so that lipophilic regions of the receptor and ligand correspond and parts of the receptor and ligand with opposite electrostatic potentials are in close proximity. In addition this arrangement maintains close proximity between common functional groups such as hydrogen-bond donors and acceptors allowing potential bonding sites to be maintained. In this study, more detail on the fitting of agonists/antagonists such as adenosine (**1**), (*R*)-(phenylisopropyl)adenosine (**2**), 5′-*N*-ethyl-2-[[4-(2-carboxyethyl)phenethyl]amino]adenosine-5′-uronamide (**3**),[9] 1,3-dipropyl-8-(2-amino-4-chlorophenyl)-xanthine (**4**),[10] 1,3-dipropyl-8(*R*)-(phenylisopropyl)xanthine (**5**),[11] α-((4-thioxo-1-phenylpyrazolo[3,4-*d*]pyrimidin-6-yl)thio)propionamide (**6**),[12] and *N*-ethyladenosine-5′-uronamide (**7**) has been introduced (see Chart I).

Procedure

Studies were performed on a VAX 11/750 computer and a Macintosh II terminal with CHEM-X[13] (Jan 90) molecular modeling software. Crystal structures of adenosine and caffeine were retrieved from the Cambridge Structural Database[14] and used or modified with standard bond an-

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(13) CHEM-X, developed and distributed by Chemical Design Limited, Oxford, England.
(14) CSD: Cambridge Crystallographic Data Centre, Lensfield Road, Cambridge, U.K.

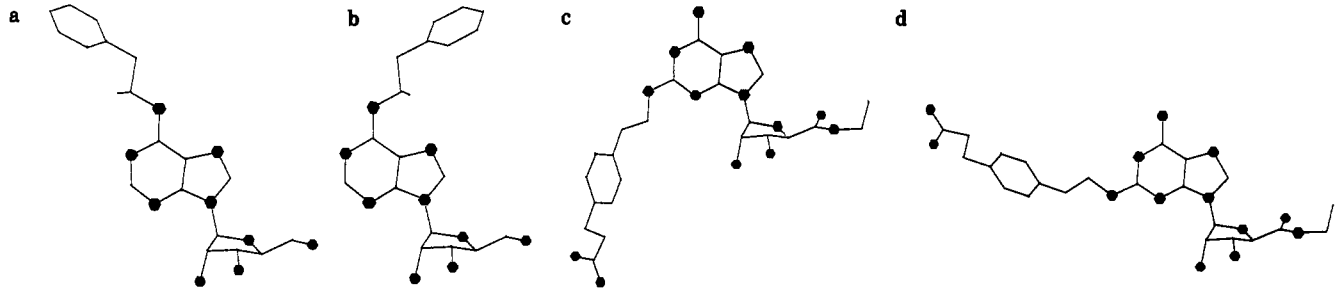

Figure 1. (a) The global minimum energy conformation of (*R*)-(phenylisopropyl)adenosine (**2**) with allowance for the double-bond nature of the C6–N^6 bond. This conformation is representative of a population of conformations within 10 kcal of the global minimum that exists where the N1–C6–N^6–C_A torsion angle is 0°. (b) The local minimum energy conformation of (*R*)-(phenylisopropyl)adenosine with allowance for the double-bond nature of the C6–N^6 bond. This conformation is 4.96 kcal above the global minimum energy and is representative of a population of conformations that exists where the N1–C6–N^6–C_A torsion angle is 180°. (c) The global minimum energy conformation of 5′-*N*-ethyl-2-[[4-(2-carboxyethyl)phenethyl]amino]adenosine-5′-uronamide (**3**) with allowance for the double-bond nature of the C2–N^2 bond. This conformation is representative of a population of conformations within 10 kcal of the global minimum that exists where the N1–C2–N^2–C_A torsion angle is 180°. (d) The local minimum energy conformation of 5′-*N*-ethyl-2-[[4-(2-carboxyethyl)phenethyl]amino]adenosine-5′-uronamide with allowance for the double-bond nature of the C2–N^2 bond. This conformation is 3.70 kcal above the global minimum energy and is representative of a population of conformations that exists where the N1–C2–N^2–C_A torsion angle is 0°.

gles and lengths. Global minima were determined by performing a systematic conformational search about rotatable bonds at increments of 30° using van der Waals energy calculation. Where heterocycles or substituents were drawn on-screen, molecular mechanics optimization was employed to optimize bond angles and lengths. Charges were calculated using the standard MNDO parameters and Pulay's method of convergence within the semiempirical molecular orbital MOPAC program.[15] Compounds were fitted together to minimize root-mean-square distances between pairs of selected atoms.

Results and Discussion

Previous definition of the N^6 binding domains[4-6] was achieved with all analogues oriented with heterocycles and ribose superimposed followed by consideration of the N^6 groups. The binding orientation of N^6-substituted adenosine analogues relative to one another was studied as a prelude to consideration of the relative relationships of this group of analogues to other analogues. The C6–N^6 bond of adenosine was noted from the X-ray data to have partial double bond character.[16] We have noted that there is hindered rotation about the corresponding exocyclic C–N bond of a triazolopyrimidine as evidenced by X-ray data and proton NMR.[17] The exocyclic C–N bond had significant double bond character (1.343-Å bond length) and the torsion angle about this bond was +0.9 (4)° by X-ray crystallographic analysis while the ratio of the two rotamers in solution was established as 2.7:1 from integration of the ^1H NMR spectrum.[17] Hindered rotation about this bond in adenosine and all N^6-substituted adenosine analogues fixes the dihedral angle about this bond at either 0 or 180° with energy barriers to rotation. Conformational analysis was performed on flexible bonds distal to this bond. Thus two populations of conformations within 10 kcal of the global minimum were generated. The minima from both populations of (*R*)-(phenylisopropyl)adenosine (**2**) are shown in Figure 1, parts a and b. The previous studies did not consider the short C6-N^6 bond length which results in hindered rotation about this bond. At this stage the two orientations of the hydrophobic groups were equally convincing since all N^6-substituted compounds had conformations in both populations within 10 kcal of the global minimum. The orientation of the C6–N^6 bond in the case of the global minima for all N^6-substituted compounds was always as illustrated in Figure 1a.

In order to find biologically relevant conformations for 5′-*N*-ethyl-2-[[4-(2-carboxyethyl)phenethyl]amino]-adenosine-5′-uronamide (**3**) a similar procedure to the N^6-substituted compounds was applied. The C2–N^2 bond was assumed to be fixed for the same reasons as above. Once again, two populations of conformations were obtained within 10 kcal of the global minimum. The local minima of the two populations are shown in Figure 1, parts c and d. Various options of fitting (*R*)-(phenylisopropyl)adenosine (**2**) and 5′-*N*-ethyl-2-[[4-(2-carboxyethyl)phenethyl]amino]adenosine-5′-uronamide (**3**) were examined. The superimposition of purines and ribose moieties as in Figure 2a leads to a model which suggests an N^6 binding domain and a C2 binding domain. The study of N^6,C2-disubstituted compounds have shown in most cases that these bis-substitutions decrease activity.[18] Two reasons have been proposed. Firstly that there is direct steric interference between the two side chains due to partial overlap of C2 and N^6 aryl binding pockets. Alternatively, there may an allosteric change in the receptor on binding one of the side chains, thereby closing the binding domain of the second chain. These explanations require the purine and ribose moieties of N^6-substituted analogues and C2-substituted analogues superimposed as in Figure 2a. An alternative to this view is the possibility of a single hydrophobic binding domain. Thus the two compounds were fitted to superimpose the hydrophobic groups. With two orientations for the hydrophobic group possible for (*R*)-(phenylisopropyl)adenosine and 5′-*N*-ethyl-2-[[4-(2-carboxyethyl)phenethyl]amino]-adenosine-5′-uronamide, there are four possible ways of aligning the two compounds; Figure 2, parts b–e, indicate these possibilities. Of the four possibilities, fits 2d and 2e place the ribose of each molecule in different positions and hence there is not good steric correlation. On this basis fits 2d and 2e were rejected. Figure 2, parts b and c, allow

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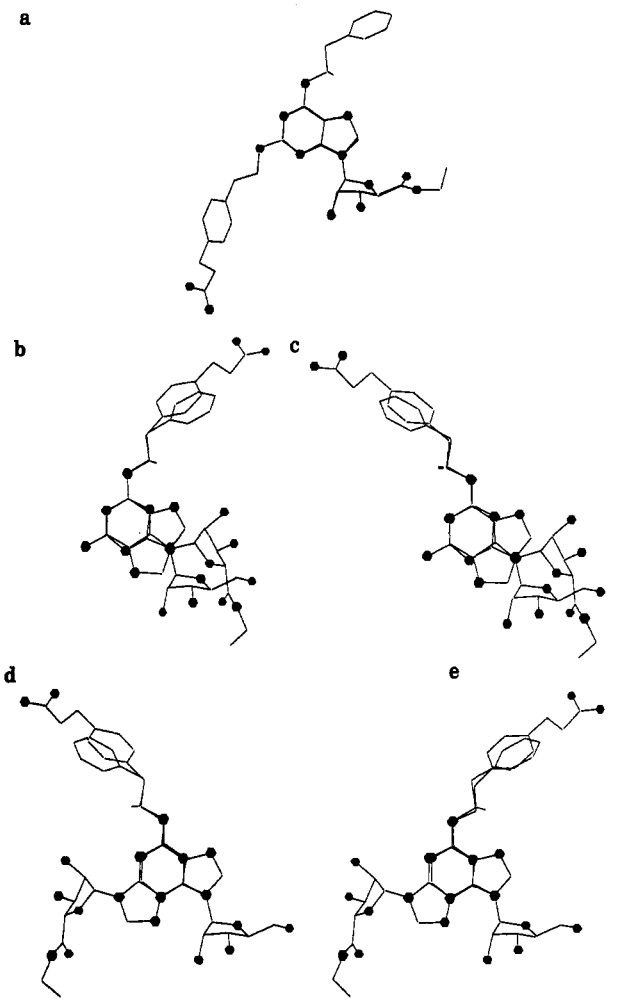

Figure 2. (a) Superimposition of (*R*)-phenylisopropyladenosine (**2**) and 5′-*N*-ethyl-2-[[4-(2-carboxyethyl)phenethyl]amino]-adenosine-5′-uronamide (**3**) in the standard manner which assumes separate C2 and N^6 binding domains. (b–e) Four methods of aligning (*R*)-(phenylisopropyl)adenosine relative to 5′-*N*-ethyl-2-[[4-(2-carboxyethyl)phenethyl]amino]adenosine-5′-uronamide. The pairs b and c and d and e align with heterocycles in the same relative orientation. The pair d and e were considered unlikely due to the distance between ribose moieties.

the ribose moieties to be in the same region, and these orientations allow the exocyclic amines bearing the hydrophobic groups to be in identical positions, thus allowing potential hydrogen-bonding. The charges of the corresponding atoms of (*R*)-(phenylisopropyl)adenosine and 5′-*N*-ethyl-2-[[4-(2-carboxyethyl)phenethyl]amino]-adenosine-5′-uronamide are shown in Table I. Note that the orientation of the heterocycle in both part b and part c of Figure 2 is the same, and therefore corresponding atoms are identical. We postulate based on parts b and c of Figure 2 that there is only one hydrophobic binding domain in both A_1 and A_2 receptors. This would explain the lack of additivity of bis-substituted compounds as one of the hydrophobic groups could bind in the pocket but the other would be placed in an unfavorable position. Fits 2b and 2c were equally convincing at this stage, and it was not until antagonists were introduced that this was resolved.

The hypothesis of a single hydrophobic binding domain requires the C8 phenyl ring of 1,3-dipropyl-8-(2-amino-4-chlorophenyl)xanthine (**4**) to superimpose with the N^6 hydrophobic group of (*R*)-(phenylisopropyl)adenosine (**2**). This initial orientation of the alkylxanthines with the C8 group superimposed on the N^6 hydrophobic substituent of N^6-substituted adenosines is supported by good hydrophobic correlations, by the stereoselectivity of the two 1,3-dipropyl-8-(phenylisopropyl)xanthine enantiomers,[11] and also by the A_1 selectivity of 8-cyclopenty-1,3-dipropylxanthine (**8**).[19] According to the fit, the cyclopentyl group of 8-cyclopentyl-1,3-dipropylxanthine would be positioned in close proximity to the area binding the cyclopentyl ring of cyclopentyladenosine, a compound with A_1 selectivity induced by the cyclopentyl ring. The two orientations of (*R*)-(phenylisopropyl)adenosine were superimposed on 1,3-dipropyl-8-(2-amino-4-chlorophenyl)-xanthine to maximize steric, hydrophobic, and electrostatic correlations. Four possible ways of orientating these two molecules were found. These are illustrated in Figure 3, parts a–d. A hydrophobic propyl group of 1,3-dipropyl-8-(2-amino-4-chlorophenyl)xanthine in both part b and part d of Figure 3 impinges on the ribose region. Sterically this may be allowed but may be unfavorable due to the different lipophilic nature of propyl and ribose. In addition parts b and d of Figure 3 display poor charge correlations between corresponding atoms. Hydrophobic interactions in parts a and c of figure 3 are similar. These fits allow potentially important hydrogen-bond donors in N^6 of (*R*)-(phenylisopropyl)adenosine and N9 of 1,3-dipropyl-8-(2-amino-4-chlorophenyl)xanthine to be in close proximity. The exocyclic amine of the N^6-substituted adenosines is an important hydrogen-bond donor since replacing this proton with a methyl reduces activity. The importance of a hydrogen-bond donor in the N9-position of alkylxanthines is borne out by the greater affinity of theophylline over caffeine, a structure–activity relationship which closely mimics the structure–activity relationship of the N^6-position of the adenosines.[4] Parts a and c of Figure 3 display good charge correlations when aligned against (*R*)-(phenylisopropyl)adenosine as evidenced by Table II so that, on the basis of electrostatic correlations, the orientations indicated in Figure 3, parts a and c, are most likely. Since all similar atom-type pairs are in close proximity in Figure 3a, in particular N1, C5 of (*R*)-(phenylisopropyl)adenosine align with N3, C5 of 1,3-dipropyl-8-(2-amino-4-chlorophenyl)xanthine, respectively, this orientation was favored over that indicated in Figure 3c where N1, C5 of (*R*)-(phenylisopropyl)adenosine align with C5, N3 of 1,3-dipropyl-8-(2-amino-4-chlorophenyl)-xanthine, respectively.

Similar fits for 1,3-dipropyl-8(*R*)-(phenylisopropyl)-xanthine (**5**) against (*R*)-(phenylisopropyl)adenosine (**2**) are shown in parts a and b of Figure 4. The hydrophobic groups of both compounds in Figure 4a,b were fitted together to minimize van der Waals energy, giving conformations of (*R*)-(phenylisopropyl)adenosine and 1,3-dipropyl-8(*R*)-(phenylisopropyl)xanthine which in Figure 4a were only 1.7 and 1.5 kcal above the global minima, respectively, and in Figure 4b were 0.0 and 3.57 kcal above the global minima, respectively. The methyl groups of both compounds approach to distance of 1 Å apart in Figure 4a and 0.5 Å in 4b. However, the phenyl rings of the compounds are more closely aligned in Figure 4a, being at an angle of only 7.2° and centroids 0.5 Å apart. In Figure 4b these rings are at an angle of 62.5° and the centroids are 1.2 Å apart. Further support for Figure 3a is provided by Figure 4, parts a and b. There is a much better correlation between hydrophobic groups in Figure

(19) Bruns, R. F.; Fergus, J. H.; Badger, E. W.; Santay, L. A.; Hartman, J. D.; Hays, S. J.; Huang, C. C. Binding of the A_1-Selective Adenosine Antagonist 8-Cyclopentyl-1,3-dipropylxanthine to Rat Brain Membrane. *Naunyn-Schmeidenbergs Arch Pharmacol.* **1987**, *335*, 59–63.

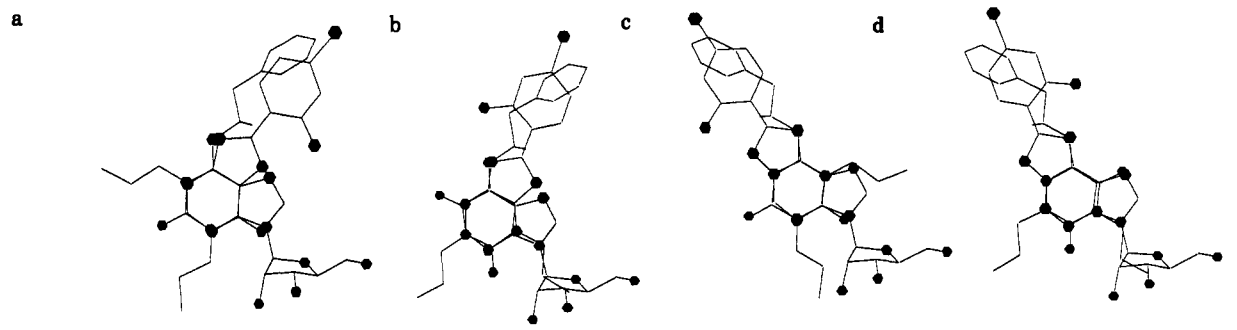

Figure 3. (a–d) The four possible superimpositions of 1,3-dipropyl-8-(2-amino-4-chlorophenyl)xanthine (4) and (*R*)-(phenylisopropyl)adenosine (2). Note that in a and c the N9 proton on 1,3-dipropyl-8-(2-amino-4-chlorophenyl)xanthine approaches the N^6 proton of (*R*)-(phenylisopropyl)adenosine, two important hydrogen-bond sites with similar structure–activity profiles.

Table I[a]

1		2		3		4		5		6		7	
atom	charge	atom	charge	atom	charge	atom	charge	atom	charge	atom	charge	atom	charge
N1	−0.38	N1	−0.38	N1	−0.38	N3	−0.30	N3	−0.30	C5	−0.23	N1	−0.39
C2	0.22	C2	0.22	C6	0.31	C2	0.48	C2	0.47	C6	0.25	C2	0.23
N3	−0.31	N3	−0.32	C5	−0.22	N1	−0.37	N1	−0.39	N1	−0.23	N3	−0.35
C4	0.13	C4	0.13	C4	0.18	C6	0.42	C6	0.42	C2	0.16	C4	0.14
C5	−0.21	C5	−0.20	N3	−0.35	C5	−0.15	C5	−0.17	N3	−0.26	C5	−0.19
C6	0.34	C6	0.34	C2	0.32	C4	0.12	C4	0.14	C4	0.11	C6	0.34
N7	−0.20	N7	−0.20	–		N7	−0.19	N7	−0.11	–		N7	−0.20
C8	0.13	C8	0.13	–		–		–		–		C8	0.09
N9	−0.28	N9	−0.28	N9	−0.31	O^6	−0.32	O^6	−0.32	S^2	0.07	N9	−0.23
N^6	−0.30	N^6	−0.31	N2	−0.31	N9	−0.19	N9	−0.20	N9	0.01	N^6	−0.33
–		–		N^6	−0.25	O^2	−0.39	O^2	−0.38	S^6	−0.38	–	
–		–		–		C1″	0.18	C1″	0.17	–		–	
–		–		–		C2″	−0.03	C2″	−0.04	–		–	
–		–		–		C3″	0.03	C3″	0.03	–		–	
–		C_A	0.11	C_A	0.16	–		C_A	0.01	–		–	
–		C_B	0.03	C_B	0.03	–		C_B	0.05	–		–	
–		C_C	0.02	–		–		C_C	0.04	–		–	
–		C1′	−0.10	C1′	−0.08	–		C1′	−0.11	–		–	
–		C2′	−0.03	C2′	−0.04	–		C2′	−0.03	–		–	
–		C3′	−0.06	C3′	−0.04	–		C3′	−0.06	–		–	
–		C4′	−0.05	C4′	−0.09	–		C4′	−0.06	–		–	
–		C5′	−0.06	C5′	−0.04	–		C5′	−0.06	–		–	
–		C6′	−0.04	C6′	−0.04	–		C6′	−0.04	–		–	
–		–		C^a	0.36	–		–		C^a	0.23	C^a	0.36
–		–		O^a	−0.36	–		–		O^a	−0.27	O^a	−0.36
–		–		N^a	−0.41	–		–		N^a	−0.37	N^a	−0.42

[a] Point charge correlations of a number of adenosine agonists/antagonists aligned as in Figures 2b, 3a, 4a, and 5a. Reading across the row shows the number of corresponding atoms from the designated fit together with their point charges calculated from MOPAC.

Table II

2		4 (Figure 3a)		4 (Figure 3c)	
atom	charge	atom	charge	atom	charge
N1	−0.38	N3	−0.30	C5	−0.15
C2	0.22	C2	0.48	C6	0.42
N3	−0.32	N1	−0.37	N1	−0.37
C4	0.13	C6	0.42	C2	0.48
C5	−0.20	C5	−0.15	N3	−0.30
C6	0.34	C4	0.12	C4	0.12
N7	−0.20	N7	−0.19	C4″	0.17
C8	0.13	–		–	
N9	−0.28	O^6	−0.32	O^2	−0.39
N^6	−0.31	N9	−0.19	N9	−0.19

[a] Point charge correlations of (*R*)-(phenylisopropyl)adenosine (2) and 1,3-dipropyl-8-(2-amino-4-chlorophenyl)xanthine (4) aligned as in parts a and c of Figure 3. Reading across the row shows the number of corresponding atoms from the designated fit together with their point charges calculated from MOPAC.

4a which is the same orientation as 3a, than in Figure 4b, which is the same orientation as 3c. In 3c and 4b a carbon of a propyl side chain correlates with N7 of (*R*)-(phenylisopropyl)adenosine. This orientation would lead to an unfavorable interaction between the receptor and the

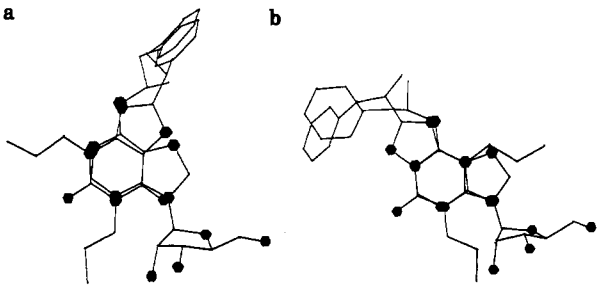

Figure 4. (a–b) Two orientations of (*R*)-(phenylisopropyl)-adenosine (2) and 1,3-dipropyl-8(*R*)-(phenylisopropyl)xanthine (5) maximizing electrostatic, hydrophobic, and hydrogen-bond correlations.

propyl side chain. It then follows that the most likely fit for (*R*)-(phenylisopropyl)adenosine and 5′-*N*-ethyl-2-[[4-(2-carboxyethyl)phenethyl]amino]adenosine-5′-uronamide is Figure 2b.

Using molecular modeling to superimpose (*R*)-(phenylisopropyl)adenosine and 1,3-dipropyl-8(*R*)-(phenylisopropyl)xanthine based on a different orientation of the heterocyclic rings, a group has recently concluded that the

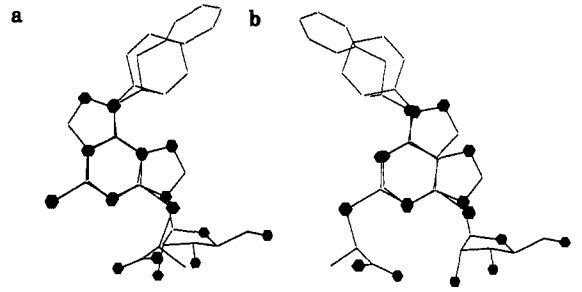

Figure 5. (a-b) The superimpositions of α-((4-thioxo-1-phenylpyrazolo[3,4-*d*]pyrimidin-6-yl)thio)propionamide (6) against the two orientations of (*R*)-(phenylisopropyl)adenosine (2).

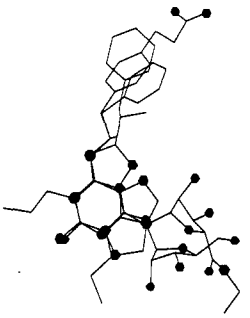

Figure 6. Superimpositions of (*R*)-(phenylisopropyl)adenosine (2), 5′-*N*-ethyl-2-[[4-(2-carboxyethyl)phenethyl]amino]-adenosine-5′-uronamide (3), and 1,3-dipropyl-8(*R*)-(phenylisopropyl)xanthine (5), illustrating the common hydrophobic binding domain occupied by the N^6, C2, and C8 hydrophobic groups and the remaining important binding domains of the central aromatic ring and the ribose domain which varies between the A1 and A2 receptors.

phenylisopropyl recognition unit of each molecule occupy the same space,[11] consistent with our proposal for one hydrophobic binding site. This fit is different to that proposed for theophylline and adenosine[20] where the 6:5 rings of the heterocycle were superimposed, and the fit provided good steric and electrostatic correlations. Since no aryl group was present, however, lipophilic factors were not taken into account.

α-((4-Thioxo-1-phenylpyrazolo[3,4-*d*]pyrimidin-6-yl)-thio)propionamide (6) was aligned with the two possible conformations of (*R*)-(phenylisopropyl)adenosine (2) to maximize lipophilic factors. These fits are shown in parts a and b of Figure 5. Figure 5a gives the highest electrostatic (Table I), steric, and lipophilic correlations between the molecules. A similar orientation against *N*-ethyladenosine-5′-uronamide (7) places the amide functionalities of *N*-ethyladenosine-5′-uronamide and α-((4-thioxo-1-phenylpyrazolo[3,4-*d*]pyrimidin-6-yl)thio)propionamide in close proximity. This provides further evidence for the hydrophobic group of (*R*)-(phenylisopropyl)adenosine being over the five-membered ring. We postulate that all active compounds in the classes represented will align in a similar manner to the sample compounds.

In conclusion, we present a model that rationalizes the existing N^6, C2, and C8 binding domains by demonstrating that they are the one region of the receptor (Figure 6). The model takes into account steric, hydrophobic, and electrostatic properties that may contribute to receptor binding potency. The model may be of value in development of novel structures with selective agonistic and antagonistic activity. Recent independent confirmation that the N^6 and C8 binding domains are common[11] provides support for our model. Our model is more expansive including the role of the ribose binding domain and accommodates the C2-substituted analogues.

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Inactivation of Calpain by Peptidyl Fluoromethyl Ketones

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The syntheses of Z-Leu-Leu-Tyr-CH_2F (1) and Z-Tyr-Ala-CH_2F (3) are described. The ability of Z-Leu-Leu-Tyr-CH_2F (1) and Z-Leu-Tyr-CH_2F (2) to inactivate in vitro calcium-activated proteinase from chicken gizzard are compared. Like the analogous diazomethyl ketones 4 and 5, these inhibitors were also found to inactivate cathepsin L in common with other inhibitors under current investigation. However, other specific inactivators for cathepsin L are available, for example, the fluoromethyl ketone 3 and diazomethyl ketone 6 of Z-Tyr-Ala-OH, which have no effect on the calcium-activated proteinase and therefore provide control inhibitors for observations made with Z-Leu-Leu-Tyr-CH_2F (1).

Introduction

Calpain, the calcium-activated neutral proteinase(s) of the cytoplasm, is a cysteinyl proteinase of considerable interest in a number of physiologically important roles such as signal transduction across membranes[1,2] possibly reflected in cytoskeletal alterations. A major area for this selective proteolysis role may be in the central nervous

*Abbreviations used: Z, benzyloxycarbonyl; AMC, 4-methyl-7-coumarinylamine; SDS, sodium dodecyl sulfate; Suc, succinyl.

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