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Conformational Search for the N^6-Substituted Adenosine Analogues and Related Adenosine A_1 Receptor Antagonists

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Abstract—The search for 3-D requirements for the adenosine A_1 receptor affinity is useful to aid in the design of more potent and/or novel ligands as pharmacological tools and therapeutics for the receptor. To emboss 3-D requirements for adenosine A_1 receptor affinity among adenosine receptor antagonists, adenosine and xanthine analogs, conformations for the N^6-substituted adenosine analogues and related adenosine A_1 receptor antagonists were thoroughly searched by semi-empirical quantum mechanics calculations. Newly established global minima for these compounds (C1′-N^6-C6-N1 torsion: 10°) are consistent with retrieved structures from the Cambridge Structural Database and previously published NMR data on the solution conformation of N^6-substituted adenosine analogues. However, these newly studied global minima for adenosine analogues are found to be different from those previously reported (C1′-N^6-C6-N1 torsion: ±75°). Copyright © 1996 Elsevier Science Ltd

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 which exploit the adenosine signal transmission pathway has been conducted. Most of the targets have been either adenosine receptor agonists or antagonists.[3] Two promising areas where A_1 antagonists may be therapeutic are cognitive deficit and acute renal failure (ARF).[4] Several A_1-selective compounds, such as KW-3902[5] are under clinical trial at this time for use as renal protectants, and these compounds may prove to be useful therapeutics.

The first generation of A_1-selective antagonists, although structurally diverse, originates mainly from adenine and xanthine bases. The search for the 3-D requirements for the A_1 receptor affinity based on these compounds is useful to aid in the design of more potent and/or novel ligands as pharmacological tools and therapeutics. Our efforts have been directed toward embossing 3-D requirements for A_1 receptor affinity among these adenosine and xanthine analogues.[6] However, many of the ligands were too flexible to define 3-D requirements for binding to the A_1 receptor. In this sense such a study needs a rigid molecule with high receptor affinity as a template. Among the adenosine and the xanthine analogues previously reported, we employed xanthine analogues as rigid templates for further analysis.[6] In addition, we also assessed the energy surface of the N^6-substituted adenosine analogues to test the validity of the conformation. We thoroughly studied global minima for N^6-substituted adenosine analogues by semi-empirical quantum mechanics calculations. These newly studied global minima for adenosine analogues are found to be different from those previously reported.[7] However, they are consistent with observed structures for adenosine analogues based on X-ray crystallography[8] and NMR.[9,10] Thus we obtained valid structures for further searching of the 3-D requirements for the A_1 receptor affinity among structurally diverse molecules, which enabled us to conclude that the global minima for adenosine analogues corresponded to the bio-active conformations.[6] Here we present the thorough conformational search for the N^6-substituted adenosine analogues by semi-empirical quantum mechanics calculations.

Procedure

The ligands used, shown in Figure 1, were the A_1-selective agonist, N^6-cyclopentyladenosine (**1**),[11] N^6-cyclohexyladenosine (**2**),[12] N^6-1-adamantyladenosine (**3**),[13] and (*R*)-N^6-1-phenyl-2-propyladenosine (R-PIA, **4**),[14] and the competitive antagonists, CP68247 (**5**),[15] (*R*)-7,8-dimethyl-2-phenyl-9-(1-phenylethyl)-7-deazaadenine (**6**),[16] APPP (**7**),[17] and N^4-cyclopentyl-2-phenyl-1*H*-imidazo[4,5-*c*]quinolin-4-amine (**8**).[18] All compounds were modified from X-ray data of the Cambridge Structural Database (CSD),[19] (**1, 2, 3, 4**) or built on screen using Quanta version 3.3.[20] Geometry were initially optimized using CHARMm[21] and the Newton–Raphson minimizer, followed by optimization with

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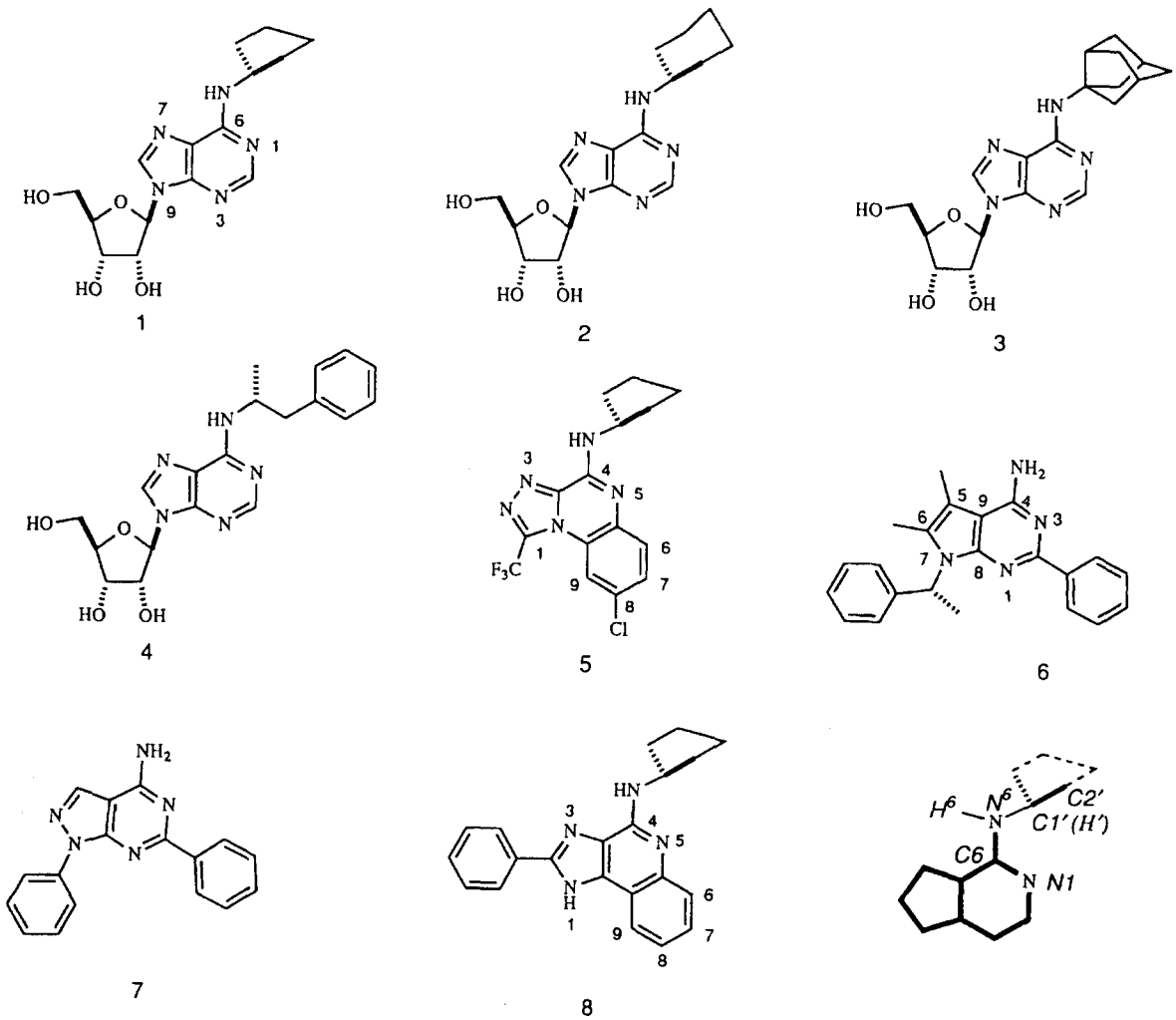

Figure 1. Ligands used in this study. Bold face diagram indicates the general numbering system.

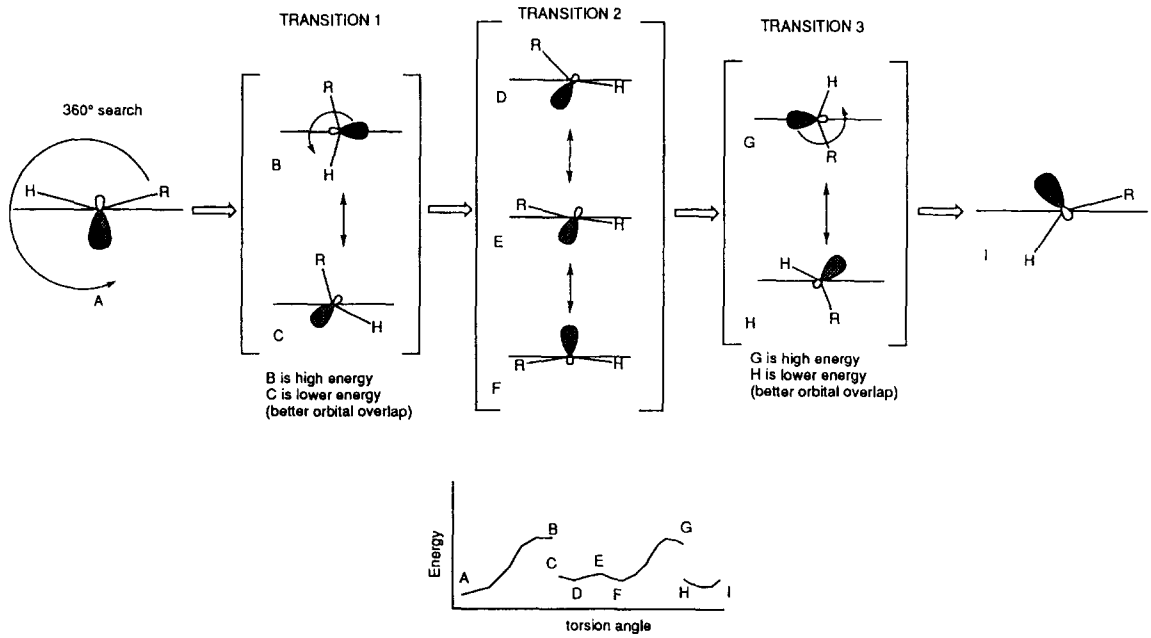

Figure 2. Explanation for energy surface seen in MOPAC path search about the N^6–C6 bond.

MOPAC6 using the AM1 Hamiltonian[22] and the keywords: Gradients, Precise, and EF.[23] 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

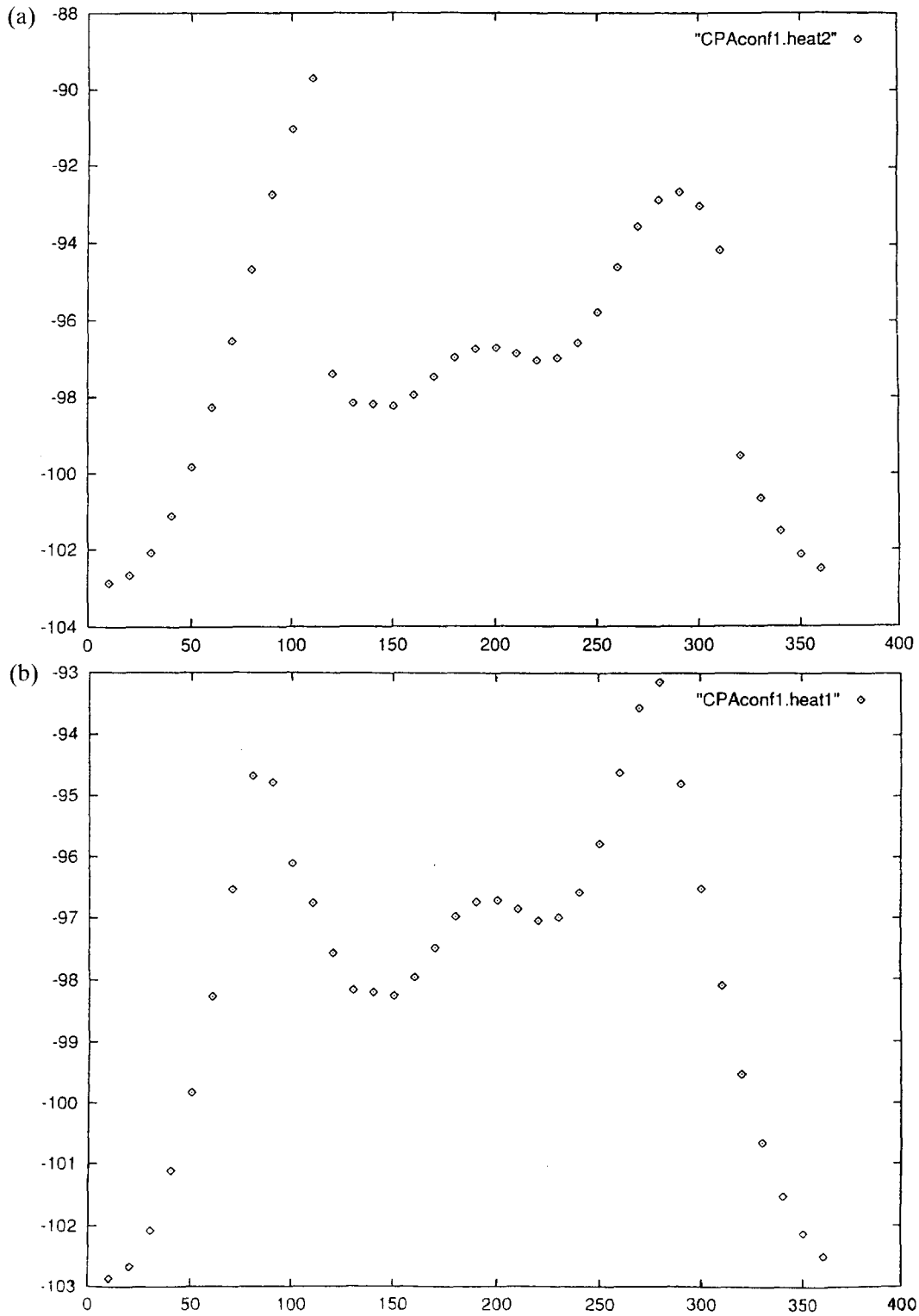

Figure 3. The sp^2–sp^3 nature of the exocyclic nitrogen leads to three transition states in a 360° search. The MOPAC optimizer is unable to provide a smooth transition through the B–C and G–H transition states, this gives rise to the disrupted energy surface shown in Figure 3a, using **1** as the example. By back-calculation from conformer C and conformer H, a realistic energy surface was obtained, shown in Figure 3b, again using **1** as the example.

Table 1. C1′-N^6-C6-N1 torsion angle and thermodynamic parameters calculated by AM1

Cpd	C1′(H)-N^6-C6-N1 torsion angle		EB kcal[a]	HF(GM) kcal[b]	HF(LM) kcal[c]	ΔH local minimum kcal[d]
	Global minimum	Local minimum				
1	13.5°	−162.7°	≈8	−102.9	−99.6	3.3
2	13.8°	−159.0°	≈6	−111.8	−108.5	3.3
3	7.4°	−98.4°	ND	−111.5	−105.6	5.9
4	12.5°	−157.8°	≈9	−70.5	−67.1	3.4
5	12.4°	−143.6°	≈9	−35.5	−31.7	3.8
6	−14.5°	—	≈5	122.4	—	—
7	12.2°	—	≈6	174.0	—	—
8	15.7°	−125.0°	≈6	108.3	111.8	3.5

[a]Energy barrier.
[b]Heat of formation (global minimum).
[c]Heat of formation (local minimum).
[d]Difference from the global minimum.

(30)), followed by precise optimization of appropriate conformers to establish exact energies/geometry for global and local minima.

Results and Discussion

The pendent phenyl moiety on the pyrimidine rings of **6** and **7**, and the pendent phenyl of the pyrazole ring of **7**, lie at an angle of 40±4° to the heterocycle.

Van Galen et al.[7] suggested that the biologically active conformation of adenosine analogues adopted a torsion about N1-C6-N^6-C1′ bond of ±75°, apparently the only energetically favorable angles that many adenosine analogues could adopt. This was emphasized by the restricted rotation about N^6-1-adamantyladenosine (**3**), with it adopting a global minimum N1-C6-N^6-C1′ torsion angle of 90°. In the present study, the energy surfaces were calculated by a conformational search about the N1-C6-N^6-C1′ or N1-C6-N^6-H1′ torsion, from 0 to 360° at 10° intervals with MOPAC6, for all compounds assessed (Figs 2 and 3). Appropriate conformers were chosen for precise optimization in order to give exact details about the global and local minima (Table 1). The global minimum energy conformations for these were found at approximately 10°, with a local minima at approximately −150° (with the exception of **3** and **8**). These torsion angles allow for maximum orbital overlap between the N^6-lone pair and the π-cloud of the heterocycle with minimum steric hindrance. This study showed a local minimum 5.9 kcal above the global minimum for **3** at the N1-C6-N^6-C1′ torsion angle of −98° which may correspond to the global minima at 90° in the previous study.[7,24] The energy surface for rotation about the exocyclic amine is complex (Fig. 2), due to the exocyclic nitrogen existing as a distorted pyramidal structure (sp^2–sp^3 hybrid). The energy surface (Fig. 3a) is therefore complicated by a transition state in which the nitrogen inverts to maximize overlap between the N^6-lone pair and the π-cloud of the heterocycle. The smooth energy surface seen in Figure 3b has been obtained by back calculation of conformers from points 'C' + 'H' in Figure 2. In our hands, conformations of N^6-substituted adenosine analogues with N1-C6-N^6-C1′ torsions of ±75° were ≈6 kcal/mol above global minimum energy (for compound **1** the value determined using MOPAC6, with full geometry optimization excluding the N1-C6-N^6-C1′ torsion, using the keywords AM1, Gradients, Precise, and EF was 5.818 kcal/mol above the global minimum, Fig. 3).

Table 2. C1′-N^6-C6-N1 torsion angle of purin-6-(*N*-substituted) amines in CSD

REFCODE	C1′-N^6-C6-N1 (°)
ADGLAL10	1.5
BADFOB10	−3.9
BARFUP	5.2
BERHAH	−9.0
BODMOW	−0.8
CATBII	−5.6
DEFPOT	−10.2
FIFMOW	−28.1
GOJWOR	7.7
KEXKON	−5.7, 2.7, 9.7, 17.1
PURTRE	−3.3
SACXEZ	1.4
VUGYOL	147.4

Other examples are DMADEN10, DUTZOH10, GEKWAN, KINTIN10, KPRCGM20, MADENC10, PCMTRE10, PCTRIB10, PMTADN10, PUCGLR10 and SUHJAG.

Examination of X-ray crystal structures, retrieved from the CSD,[8] of variously substituted purin-6-(*N*-substituted)amines revealed that in almost all cases, the lone pair of the exocyclic nitrogen lies at, or close to, 90° to the purine ring. The X-N^6-C6-N1 torsion angle was close to ±10° in all cases except for FIFMOW (−28.1°) and VUGYOL (147.4°) (Table 2). FIFMOW has N^6-benzoyl moiety and VUGYOL is a strained cyclophane like compound. Furthermore, examination of the C6–N^6 bond length revealed distances between 1.312 and 1.391 Å, values within the range for peptide bonds, indicating partial double-bond character for these bonds.[8] ^{1}H[9,10] and ^{15}N NMR[9] studies of adenosine compounds and a closely related triazolo[4,5-*d*]pyrimidine[10] indicated hindered rotation about the exocyclic

nitrogen bond at room temperature. In the case of N^6-substituted adenosine compounds, a hydrogen bond is formed between the N^6-H and N7 resulting in an upfield shift of the N7 signal.[25] The NMR evidence supports a solution state conformation similar to the solid state and gas phase conformations.

Conclusions

A new global minimum for the N^6-substituted adenosine analogues is presented (N1-C6-N^6-C1′ torsion 10°; C6-N^6-C1′-C2′ torsion 170–300°). The new conformation is supported by solid state evidence from the X-ray crystal structures of many similar compounds retrieved from the CSD and solution state NMR evidence from N^6-substituted adenosine analogues and related compounds. This is the first adenosine modeling study to report the sp^2–sp^3 hybrid nature of the N^6-nitrogen.

Acknowledgments

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