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NEW BASE-ALTERED ADENOSINE ANALOGUES: SYNTHESIS AND AFFINITY AT ADENOSINE A_1 and A_{2A} RECEPTORS

Seung B. Ha,[a] Neli Melman,[b] Kenneth A. Jacobson,[b] and Vasu Nair[a,*]

[a]*Department of Chemistry, The University of Iowa, Iowa City, Iowa 52242, U.S.A.*
[b]*Molecular Recognition Section, Laboratory of Bioorganic Chemistry, NIDDK, National Institutes of Health, Bethesda, MD 20892, U.S.A.*

Abstract: N^6-Substituted adenosine analogues containing cyclic hydrazines or chiral hydroxy (ar)alkyl groups, designed to interact with the S2 and S3 receptor subregions, have been synthesized and their binding to the adenosine A_1 and A_{2A} receptors have been investigated. Examples of both types of compounds were found to exhibit highly selective binding (K_i in low nM range) to the rat A_1 receptor. © 1997 Elsevier Science Ltd.

Introduction

The physiological functions of adenosine have been extensively studied in recent years. Adenosine exerts its biological effects via extracellular purinergic receptors, termed A_1, A_{2A}, A_{2B}, and A_3, which are distributed throughout a wide variety of tissues in mammalian systems.[1-3] Although adenosine has been approved for clinical use by the FDA for the treatment of supraventricular tachycardia, its therapeutic application is limited by its rapid metabolic inactivation and its nonselectivity for the receptor subtypes.[4] There has been considerable interest in the development of adenosine receptor agonists that mimic the pharmacological properties of adenosine but with greater metabolic stability and with higher receptor specificity.[5-7] Adenosine agonists with high A_1 or A_{2A} receptor selectivity are of potential interest as antihypertensives, antiarrhythmics, analgesics, antipsychotics, and anticonvulsants.[8,9] Several recent reports of highly potent and selective adenosine A_1 or A_{2A} receptor agonists have focused attention on strategic modifications at the N^6-, C^2-, and 5'-modified adenosines.[5-7,10]

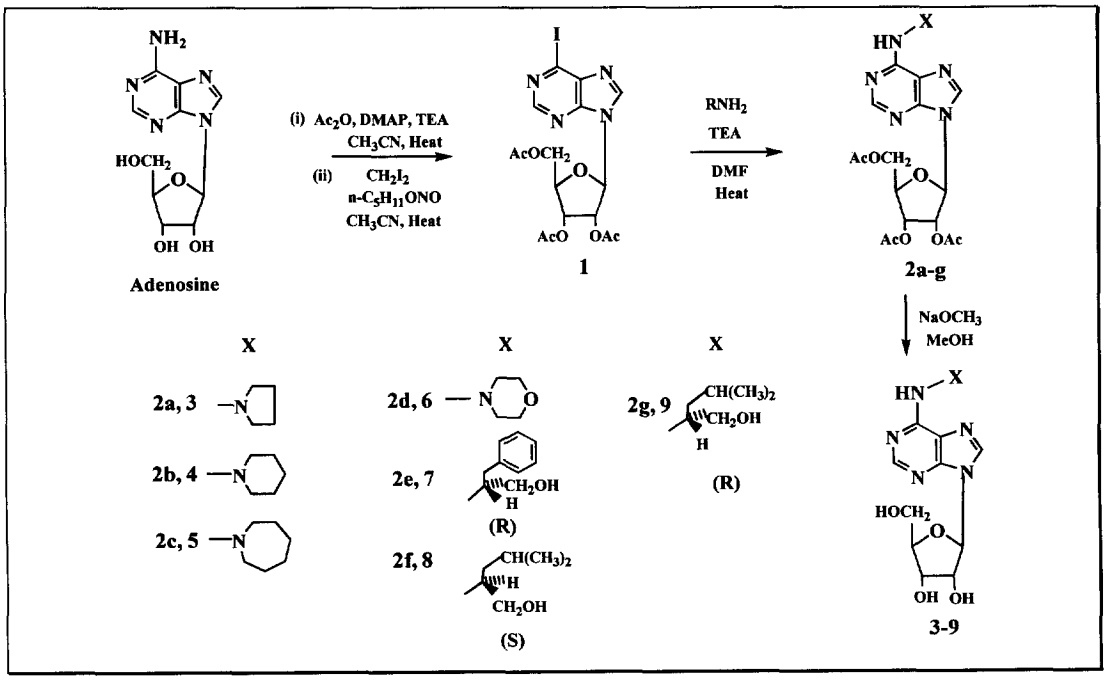
However, there have been very few adenosine analogues where a N-N bond exists at the purine 6-position. 6-Hydrazinopurine riboside[11] has receptor affinity in the micromolar range (K_i values for A_1 = 29.7 μM, A_2 = 7.3 μM) but 2-chloro-N^6-[4-(phenylthio)-1-piperidinyl] adenosine[12] exhibits strong A_1 receptor binding and A_1 to A_2 receptor selectivity (A_1 K_i = 0.9 nM, A_2 K_i = 470 nM, A_2/A_1 ratio = 522). The nitrogen isostere of CPA, N^6-(1-pyrrolidinyl)adenosine has been reported by us as a potent and selective A_1 agonist (K_i = 8.0 nM for A_1 and 2800 nM for A_2 and a selectivity ratio A_2/A_1 of 350).[10]

The model of the N^6-region of the A_1 receptor has been derived from the structure of (*R*)-PIA and is based on the assumption that each single part of the C6 substituent (N^6, C^1, C^2, C^3 and phenyl) positively contributes to the affinity.[13,14] Each of these parts corresponds to a receptor subregion, termed N^6, S1, S2, S3, and aryl. The chirality at the C^2 carbon, occupying the S2 subregion, produces a high degree of stereoselectivity

for *R*- vs. *S*- isomers. N^6-[(*S*)-1-Hydroxy-3-phenyl-2-propyl]adenosine, which has a hydroxyl group on the C^3 carbon corresponding to the S3 subregion, retains high affinity and selectivity for the A_1 receptor (A_1 K_i = 2.7 nM, A_2 K_i = 390 nM, and a selectivity ratio A_2/A_1 of 144).[13] This paper reports on the design, synthesis, and adenosine receptor binding studies of new N^6-substituted adenosine analogues containing cyclic hydrazines or chiral hydroxy (ar)alkyl groups.

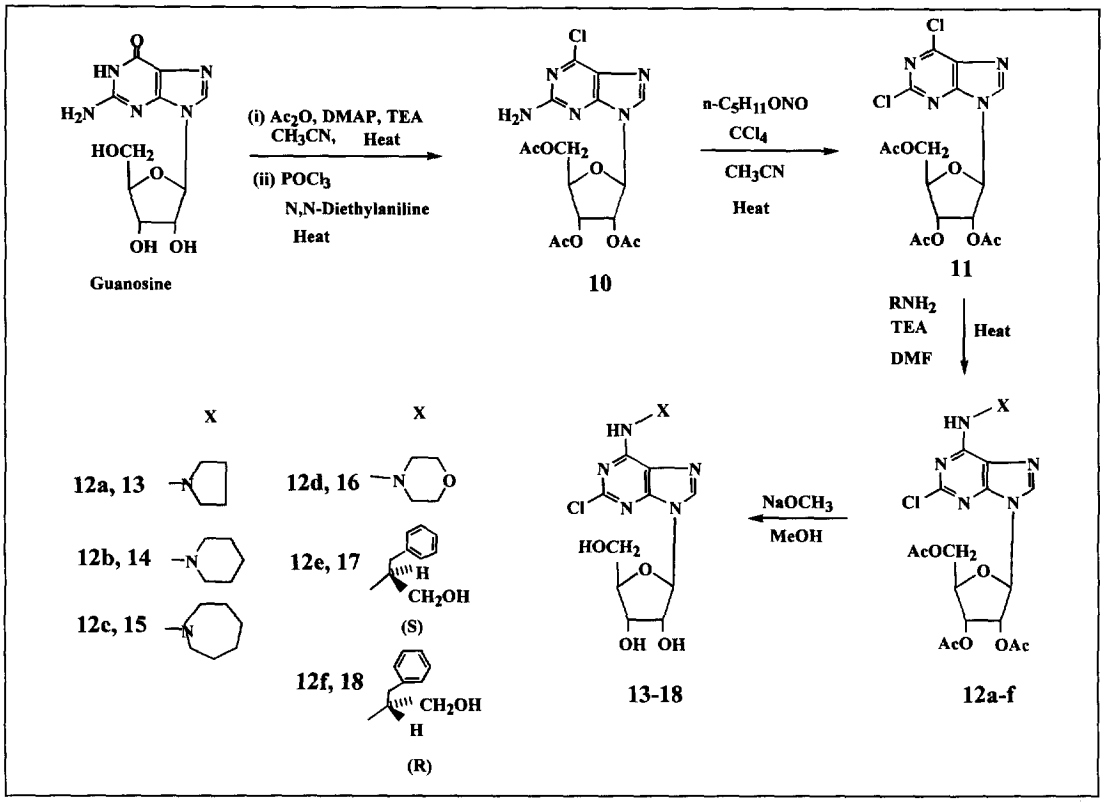
Chemistry

Adenosine was used as the starting material in the synthesis of N^6-substituted adenosine analogues (**3**–**9**). It was acetylated with acetic anhydride, 4-dimethylaminopyridine, triethylamine in acetonitrile at 60 °C (Scheme 1).[15] 2',3',5'-Tri-*O*-acetyladenosine was converted to the 6-iodo compound **1** by a thermally-induced radical deamination-halogenation reaction with *n*-pentyl nitrite and diiodomethane in acetonitrile at 60 °C.[16] The 6-iodo compound **1** was treated with cyclic hydrazines or chiral (ar)alkylamines in the presence of triethylamine in *N*,*N*-dimethylformamide or chloroform/ethanol at 60 °C to provide the N^6-substituted triacetates **2a**–**g**, which were subsequently deprotected with sodium methoxide in anhydrous methanol or anhydrous ammonia in absolute ethanol to afford target compounds **3**–**9**.



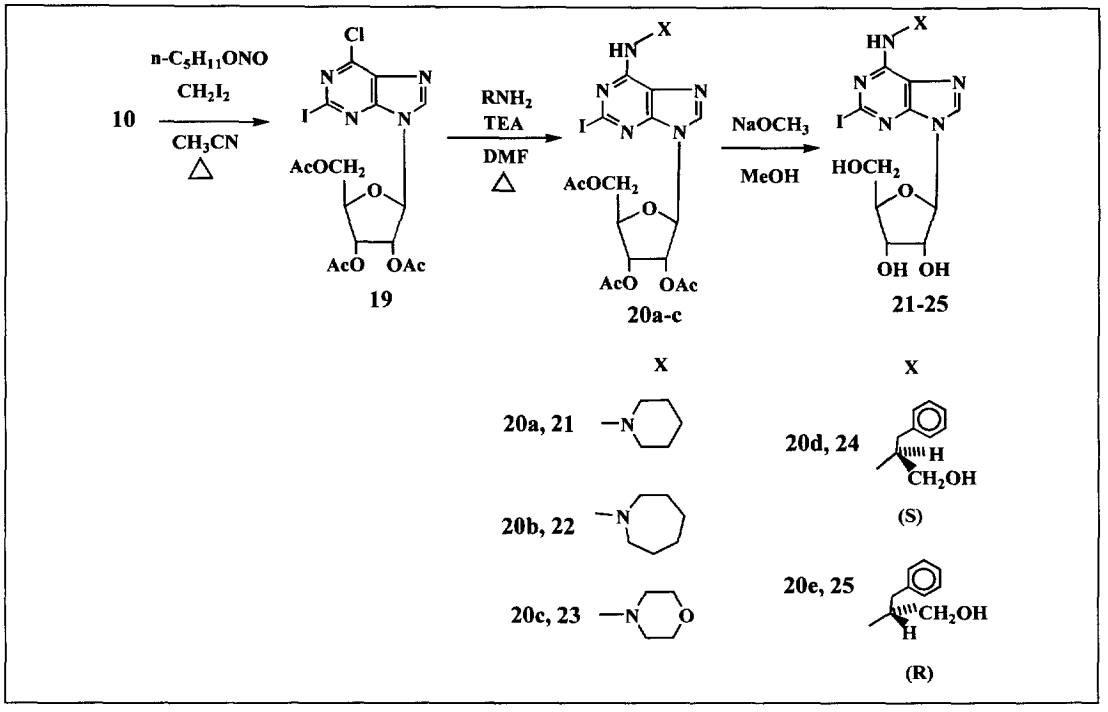
Scheme 1

Guanosine served as the starting material in the synthesis of 2-chloro-N^6-substituted adenosine analogues (**13–18**) (Scheme 2). It was acetylated by the same procedure that was used for adenosine but at



Scheme 2

ambient temperatures followed by reaction with phosphorus oxychloride and *N,N*-diethylaniline at 60 °C to give 2-amino-6-chloro compound **10** in 87% yield (Scheme 2).[17] The key intermediate, the 2,6-dichloro compound **11**, was prepared by the radical deamination-halogenation reaction of 2-amino-6-chloropurine riboside **10** in the presence of *n*-pentyl nitrite and excess carbon tetrachloride.[16] The 6-chloro group of **11** was selectively displaced by the cyclic hydrazines or chiral (ar)alkylamines in the presence of triethylamine in DMF or chloroform/ethanol at 60 °C by taking advantage of the greater nucleophilic lability of the 6-position compared to the 2-position. Subsequent deprotection with sodium methoxide in methanol or anhydrous ammonia in absolute ethanol afforded the target compounds **13–18**. 2-Iodo-N^6-substituted adenosine analogues (**21–25**) were prepared via the key intermediate, the 6-chloro-2-iodo compound **19**, by the same synthetic methodology used for the synthesis of the 2-chloro compounds (Scheme 3).



Scheme 3

Results and Discussion

The A_1 receptor affinity and the A_{2A}/A_1 selectivity of some chosen analogues were carried out with rat brain or striatal membranes using radioligand binding assays (Table 1). The high A1 affinity and selectivity of N^6-(1-pyrrolidinyl)adenosine (**3**), the corresponding N^6-(1-piperidinyl) adenosine (**4**) and its 2-chloro analogue **14**, and the 2-chloro N^6-(1-morpholino)adenosine (**16**) with K_i values for the A_1 receptor of 7.3, 3.6, 4.9, and 8 nM, respectively, and with A_{2A} values being in the μM range or higher are of interest. These results, when compared to the low affinity of 6-hydrazinopurine riboside (K_i for A_1 = 29.7 ± 6.6 μM, K_i for A_{2A} = 7.34 ± 1.12 μM),[11] suggest that the destabilizing effects of the polar hydrazino functionality can be offset by stabilizing interactions of larger N^6-substituents whose additional carbons interact with the distal hydrophobic N^6-subregion. The morpholino analogue **6** is >2000-fold less potent at A_1 receptors than the piperidinyl analogue, **4**. Thus, the distal ether functionality destabilizes the binding to the receptor. This destabilization can be overcome by adding a 2-chloro substituent as in **16**. The chiral compounds synthesized are expected to have interaction with a number of N^6-subregions (S1, S2, S3, and aryl) and can be used as probes to study spatial and stereochemical requirements, especially in the S2 receptor subregion. For example, while the (*S*) and (*R*) isomers of N^6-(1-hydroxy-4-methyl-2-pentyl)adenosines **8** and **9** showed low A_1 binding affinity (139 and 224

Table 1. Affinities of Selected Adenosine Analogues in Radioligand Binding Assays at A_1 and A_{2A} Receptors [a,b]

Compound No.	A_1	A_{2A}
3	8.0	2800[10]
4	7.30 ± 1.25	29% at 10^{-4} M
6	15,500 ± 1900	36 ±7 % at 10^{-4} M
8	139 ± 44	<10% at 10^{-5} M
9	224 ± 48	30,300 ± 14,800
14	3.55 ± 0.35	936 ± 237
16	4.89 ± 0.27	1900 ± 520
17	2.41 ± 0.46	492 ± 87
18	452 ± 65	10,400 ± 3600
21	71.0 ± 23.4	8630 ± 2220
23	113 ± 35	19,400 ± 7000
24	23.0 ± 8.0	1360 ± 310
25	989 ± 105	49 ± 2% at 10^{-4} M

[a]Displacement of specific [^{3}H](*R*)-PIA binding in rat brain membranes, expressed as K_i ± S.E.M. in nM (n = 3–6), or % of displacement at indicated conc. [b] Displacement of specific [^{3}H]CGS 21680 binding in rat striatal membranes, expressed as K_i ± S.E.M. in nM (n = 3–6), or % of displacement at indicated conc. Radioligand Binding Assays. For all binding experiments, adenosine deaminase was present (3 IU/mL) during the incubation with radioligand. [^{3}H]CGS 21680 binding to striatal A_{2A}-receptors in rat brain was carried out as described[18] using 20 μM 2-chloroadenosine to determine nonspecific binding. The binding of [^{3}H]R-PIA to rat cortical A_1-receptors was carried out as previously described.[19] For competition studies, IC_{50} values were determined using the Inplot computer program (Graphpad, San Diego, CA) and converted to apparent K_i values using K_D values and the Cheng–Prusoff equation.[20] K_D values in rat brain for [^{3}H]PIA and [^{3}H]CGS 21680 binding were 1.0 and 15 nM, respectively, at A_1 and A_{2A} receptors. Concentrations of [^{3}H]PIA and [^{3}H]CGS 21680 used in competition experiments were 1.0 and 5.0 nM, respectively.

nM, respectively) and poor A_{2A} affinity (mM range), dramatic differences are seen in the affinities of the (*S*) and (*R*) isomers of N^6-(1-hydroxy-3-phenyl-2-propyl)adenosines. For example, in the case of **17** and **18**, the compound with (*S*) chirality of the N^6-substituent is a factor of about 200 times more potent than the corresponding (*R*)-isomer at A_1 receptors. There is also much greater selectivity between A_1 and A_{2A} binding for the (*S*) compared to the (*R*) isomer. Related results were obtained for the 2-iodo compounds **24** and **25**. The 2-iodo analogues (**21**, **23**, **24**, **25**) were each less potent at both A_1 and A_{2A} receptors than the corresponding 2-chloro analogues (**14**, **16**, **17**, **18**). Curiously, for the piperidinyl analogues, a 2-iodo group (**21**) diminished potency at A_1 receptors versus the 2-H analogue (**4**), while for the morpholino analogues, a 2-iodo group (**23**) enhanced A_1 potency versus the 2-unsubstituted compound (**6**). At the A_{2A} receptors, introduction of a 2-halo

group resulted in enhanced potency compared to the 2-H case. The target compounds are stable with respect to deamination by mammalian adenosine deaminase. They are not expected to be substrates for cellular kinases.

In conclusion, N^6-substituted adenosine analogues containing cyclic hydrazines or chiral hydroxy (ar)alkyl groups, designed to interact with the S2 and S3 receptor subregions, have been synthesized. Affinity studies of selected analogues to the adenosine A_1 and A_{2A} receptors were carried out with rat brain or striatal membranes using radioligand binding assays. Both types of compounds investigated were found to exhibit highly selective binding to the A_1 receptor (A_1 K_i = low nM range, A_{2A} K_i = > μM range). For pairs of diastereoisomers, the (*S*)-isomer was significantly more potent than the (*R*)-isomer. Interestingly, the (*S*)-isomers of **8**, **17**, and **24** resemble more closely the structure of (*R*)-PIA (more active isomer) than (*S*)-PIA (less active isomer) in terms of alignment of the $-CH_3$ of PIA compared to the $-CH_2OH$ of **8**, **17** and **24**.

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