# *N*-Cycloalkyl Derivatives of Adenosine and 1-Deazaadenosine as Agonists and Partial Agonists of the $A_1$ Adenosine Receptor

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A number of cycloalkyl substituents (from C-3 to C-8) have been introduced on the 6-amino group of adenosine, 1-deazaadenosine, and 2′-deoxyadenosine, bearing or not a chlorine atom at the 2-position, to evaluate the influence on the $A_1$ and $A_{2A}$ affinity of steric hindrance and lipophilicity. Furthermore, the guanosine 5′-triphosphate (GTP) shift and the maximal induction of guanosine 5′-($\gamma$-thio)triphosphate ([$^{35}$S]GTP$\gamma$S) binding to G proteins in rat brain membranes were used to determine the intrinsic activity of these nucleosides at the $A_1$ adenosine receptor. All compounds of the ribose-bearing series proved to be full agonists, the 1-deaza derivatives showing affinities for the $A_1$ receptor about 10-fold lower than the corresponding adenosines. On the other hand, all the 2′-deoxyribose derivatives bind to the $A_1$ receptor with affinities in the high nanomolar range, with the 2-chloro substituted compounds showing slightly higher affinities than the 2-unsubstituted counterparts. In terms of the potencies, the most potent compounds proved to be those bearing four- and five-membered rings. Both GTP shifts and [$^{35}$S]-GTP$\gamma$S experiments showed that most of the 2′-deoxyadenosine derivatives are partial agonists. The 2′-deoxyadenosine derivatives which were identified as partial agonists consistently detected fewer $A_1$ receptors in the high-affinity state than full agonists. However, it is worthwhile noting that there was not a simple linear relationship between receptor occupancy and activation. These results indicate that a critical density of $A_1$ adenosine receptors in the high-affinity state is required for G protein activation.

## Introduction

A variety of studies have demonstrated, on the basis of biochemical and pharmacological experiments, that most adenosine actions are mediated by at least four extracellular receptors: $A_1$, $A_{2A}$, $A_{2B}$, and $A_3$. Furthermore, adenosine is ubiquitous in mammalian cells, and it is structurally and metabolically related to bioactive nucleotides, methylating agents, and various coenzymes.[1,2]

The fact that adenosine and adenosine-related molecules are involved in the regulation of many aspects of cellular metabolism makes drugs with a purinergic mechanism an expanding therapeutic target.[1,3] However, to fully evaluate these prospects, subtype selective agonists and antagonists with high affinity and potency are required.[2−5] Moreover, differences of adenosine receptor distribution and activation upon various cells and tissue types may also be exploited to achieve selective responses by drugs acting on adenosine receptor sybtypes.[6]

On the other hand, the major drawbacks to the potential therapeutic use of adenosine agonists are to date the strong hypotensive and cardiac depressant effects.

Partial agonists, compounds whose intrinsic activity is less than that of a full agonist,[7,8] could have several advantages compared to full agonists including less pronounced cardiovascular effects and more selective actions. In addition, partial agonists may induce less receptor downregulation and desensitization.

In recent years, various modifications of purine and ribose moiety of *N*-cyclopentyladenosine (CPA) have been studied in order to investigate partial agonists.[9−16] It appeared from the results of these studies that partial agonists for adenosine $A_1$ receptors have been obtained by substituting the 8-position of CPA,[9,12−15] by removing the 2′- or 3′-hydroxyl group from the ribose ring,[10,11] and by replacing the 5′-hydroxyl group with methylseleno- or methylthio-group.[16]

We have previously examined the effects of several deaza analogues of adenosine on rat brain membranes and on human platelets in order to investigate the role of the purine nitrogens in the binding to adenosine receptors.[17] It was found that in this series 1-deazaadenosine displayed the highest affinity and potency for $A_1$ adenosine receptor subtype. Moreover, 3-deazaadenosine behaved as a very weak ligand for this subtype and 7-deaza- and 1,3-dideazaadenosine proved to be inactive. Furthermore, 1-deazaadenosine derivatives has been shown to inhibit adenosine deaminase (ADA)[18,19] and platelet aggregation induced by ADP.[20]

On this basis, a series of 1-deaza analogues of *N*-[*R*-(−)-1-methyl-2-phenethyl]adenosine (R-PIA), *N*-cyclo-

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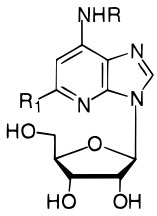
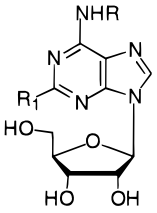
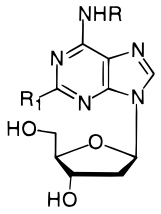


| | R | $R_1$ | | R | $R_1$ |
|---|---|---|---|---|---|
| **2a** | $cC_3H_5$ | Cl | **3a** | $cC_3H_5$ | H |
| **2b** | $cC_4H_7$ | Cl | **3b** | $cC_4H_7$ | H |
| **2c** | $cC_5H_9$ | Cl | **3c** | $cC_5H_9$ | H |
| **2d** | $cC_6H_{11}$ | Cl | **3d** | $cC_6H_{11}$ | H |
| **2e** | $cC_7H_{13}$ | Cl | **3e** | $cC_7H_{13}$ | H |
| **2f** | $cC_8H_{15}$ | Cl | **3f** | $cC_8H_{15}$ | H |



| | R | $R_1$ | | R | $R_1$ |
|---|---|---|---|---|---|
| **5a** | $cC_3H_5$ | Cl | **6a** | $cC_3H_5$ | H |
| **5b** | $cC_4H_7$ | Cl | **6b** | $cC_4H_7$ | H |
| **5c** | $cC_5H_9$ | Cl | **6c** | $cC_5H_9$ | H |
| **5d** | $cC_6H_{11}$ | Cl | **6d** | $cC_6H_{11}$ | H |
| **5e** | $cC_7H_{13}$ | Cl | **6e** | $cC_7H_{13}$ | H |
| **5f** | $cC_8H_{15}$ | Cl | **6f** | $cC_8H_{15}$ | H |



| | R | $R_1$ | | R | $R_1$ |
|---|---|---|---|---|---|
| **10a** | $cC_3H_5$ | Cl | **11a** | $cC_3H_5$ | H |
| **10b** | $cC_4H_7$ | Cl | **11b** | $cC_4H_7$ | H |
| **10c** | $cC_5H_9$ | Cl | **11c** | $cC_5H_9$ | H |
| **10d** | $cC_6H_{11}$ | Cl | **11d** | $cC_6H_{11}$ | H |
| **10e** | $cC_7H_{13}$ | Cl | **11e** | $cC_7H_{13}$ | H |
| **10f** | $cC_8H_{15}$ | Cl | **11f** | $cC_8H_{15}$ | H |

**Figure 1.** Structures of all the nucleosides evaluated in this paper.

hexyladenosine (CHA), and CPA were synthesized and evaluated in radioligand binding studies for their affinity at $A_1$ and $A_{2A}$ receptors.[20] The N-substituted 1-deazaadenosines largely retained the $A_1$ binding affinity of their adenosine counterparts, but lost some of their $A_2$ affinity, resulting in $A_1$ vs $A_2$ selective compounds.

The recent discoveries that adenosine derivatives can behave as partial agonists for the adenosine $A_1$ receptors prompted us to investigate the effect on $A_1$ and $A_{2A}$ binding affinity of the isosteric substitution of 1-nitrogen by a −CH− in 6-cycloalkyladenosine derivatives.

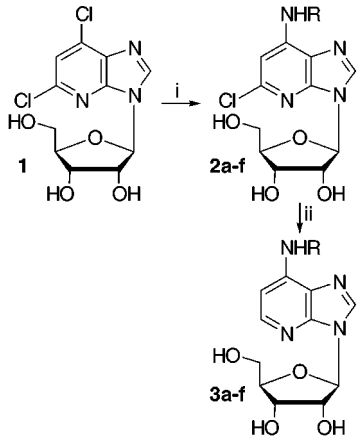
In this paper, the synthesis of a series of 1-deazaadenosine analogues with the 6-amino group bearing cycloalkyl substituents (from C-3 to C-8) and with or without a chlorine atom at the 2-position is reported, starting from 2,6-dichloro-1-deazapurine riboside (5-chloro-7-nitro-*β*-D-ribofuranosyl-3*H*-imidazo[4,5-*b*]pyridine, **1**).[17]

The same cycloalkyl rings have been introduced in the 6-position of adenosine and 2-chloroadenosine, starting from 2,6-dichloro-9-(2,3,5-*O*-triacetyl-*β*-D-ribofuranosyl)purine (**4**),[21] to evaluate the influence on the $A_1$ and $A_{2A}$ affinity of steric hindrance and lipophilicity.

Finally, a number of 2′-deoxyadenosines, bearing the same series of cycloalkylamino substituents in 6-position, have been prepared starting from 2,6-dichloro-9-(2-deoxy-3,5-di-*O*-*p*-toluoyl-*β*-D-*erythro*-pentofuranosyl)-9*H*-purine (**8**)[23] to assess the modulation of affinity and potency by the size of the cycloalkyl ring and by the presence of a chlorine atom on C-2, when these modifications are combined with removing the 2′ hydroxyl group.

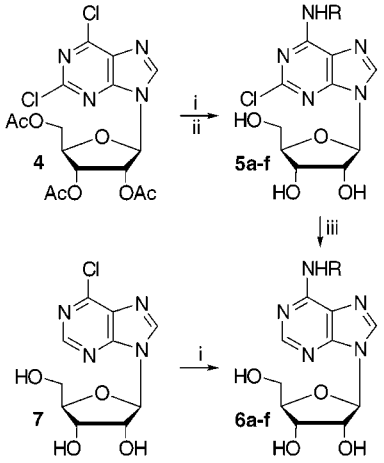
All these cycloalkylamino derivatives (Figure 1) were tested in vitro for their affinity for the adenosine $A_1$ and $A_{2A}$ receptors, and for their GTP shift, i.e., the difference in affinity for the adenosine $A_1$ receptor in rat brain in the presence and absence of GTP. Furthermore, the intrinsic activity of the nucleosides at the adenosine $A_1$ receptor was evaluated by measuring the maximal induction of [$^{35}$S]GTP$\gamma$S binding to G proteins in rat brain membrane preparations.

**Scheme 1.** Synthesis of *N*-Cycloalkyl-1-deazaadenosine Derivatives[a]



[a] R = see Figure 1. Reagents and conditions: (i) R-$NH_2$, EtOH, Δ; (ii) EtOH, Pd/C, $H_2$.

**Scheme 2.** Synthesis of *N*-Cycloalkyladenosine Derivatives[a]



[a] R = see Figure 1. Reagents and conditions: (i) R-$NH_2$, EtOH; (ii) $NH_3$/MeOH; (iii) MeOH, Pd/C, $H_2$.

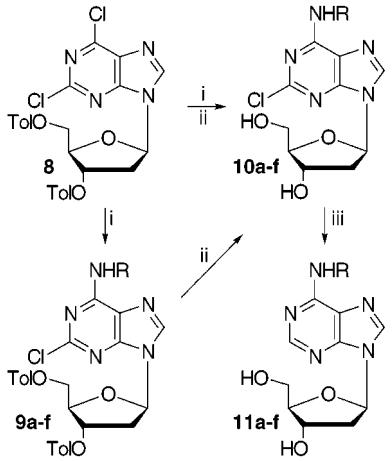
## Results and Discussion

**Chemistry.** The ribose derivatives **3a**−**f** and **6a**−**f** were synthesized, according to Schemes 1 and 2, starting from 2,6-dichloro-1-deazapurine and 2,6-dichloropurine nucleosides, respectively.

Reaction of 5,7-dichloro-3-β-D-ribofuranosyl-3*H*-imidazo[4,5-*b*]pyridine (**1**)[17] with the appropriate cycloalkylamine, in a steel bomb at 120 °C, gave the $N^7$-substituted-(5-chloro-3-β-D-ribofuranosyl)-3*H*-imidazo-[4,5-*b*]pyridines **2a**−**f**. Catalytic hydrogenolysis of the chlorinated compounds with 10% Pd/C in ethanol and 2 N NaOH afforded the corresponding derivatives **3a**−**f** (Scheme 1).

Treatment of 2,6-dichloro-9-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)purine (**4**)[21] with the appropriate cycloalkylamine, at room temperature for 4 h, gave a mixture of mono-, di-, and triacetylated 2-chloro-N-substituted derivatives; to accelerate the deacetylation process and to avoid side reactions, the crude material was treated with methanolic ammonia at room temperature to obtain the desired nucleosides **5a**−**f**. Catalytic hydrogenolysis of the chlorine atom in the 2-position with 10% Pd/C in ethanol and 2 N NaOH afforded the corresponding compounds **6a**−**f**. Alternatively, the synthesis of nucleosides **6a**−**f** can be achieved by reacting the commercially available 6-chloropurine riboside (**7**) with the appropriate cycloalkylamine in EtOH at reflux for 5 h[22] (Scheme 2).

**Scheme 3.** Synthesis of *N*-Cycloalkyl-2′-deoxyadenosine Derivatives[a]



[a] R = see Figure 1. Reagents and conditions: (i) R-$NH_2$, EtOH; (ii) $NH_3$/MeOH; (iii) MeOH, $H_2$, Pd/C.

The series of 2′-deoxyadenosine derivatives was obtained starting from 2,6-dichloro-9-(2-deoxy-3,5-di-*O*-*p*-toluoyl-β-D-*erythro*-pentofuranosyl)-9*H*-purine (**8**).[23] Treatment with the appropriate amine at room temperature for 4 h gave the fully protected 2′-deoxy-2-chloro-*N*-cycloalkyladenosines **9a**−**f**, which were isolated and characterized. Methanolic ammonia was added to the residue in order to achieve removing of toluoyl groups at room temperature to give **10a**−**f** in high yields. Finally, the dechlorinated nucleosides **11a**−**f** were obtained, as usual, by catalytic hydrogenolysis with 10% Pd/C in ethanol and 2 N NaOH. Compounds **11c** and **11d** were already obtained in very low yield by a less convenient route starting from 2′-deoxy-3′,5′-di-*O*-acetyl-6-iodopurine-9-β-D-riboside.[11]

**Pharmacology. Ribose Derivatives.** The compounds described in Schemes 1 and 2 were tested first in radioligand binding experiments to assess their affinities for the rat adenosine $A_1$ and $A_{2A}$ receptors, and the results are reported in Tables 3 and 4.

$A_1$ receptor affinities were determined on rat cortical membranes using the antagonist [$^3$H]DPCPX as radioligand. Affinities for the $A_{2A}$ receptor were established on rat striatal membranes using the agonist [$^3$H]CGS 21680.

The new 1-deazapurine nucleosides exhibited an appreciable $A_1$ affinity, the most active compound being the 2-chloro-*N*-cyclobutyl-1-deazaadenosine (**2b**, $K_i$ = 41 nM) (Table 3). The 2-chloro derivatives are, in general, slightly more active than the corresponding dechlorinated compounds, with the highest affinity shown by molecules bearing four- and five-membered rings. All these nucleosides exhibited a moderate $A_1$ vs $A_{2A}$ selectivity, the highest shown by molecules bearing cycloalkyl substituents with a low number of carbons.

In comparison with the 1-deaza analogues, the purine nucleosides exhibited higher affinity and selectivity at $A_1$ receptors, the most active compounds being the *N*-cyclobutyl- and *N*-cyclopentyladenosine derivatives **5b**, **6b**, **5c**, and **6c** (Table 4).

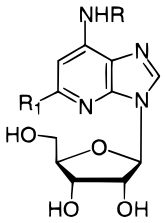
**Table 1.** Preparation of Compounds Reported in Schemes 1−3

| compd | R | time (h) | chromatography solvent | yield (%) | mp (°C) |
|---|---|---|---|---|---|
| **2a** | $cC_3H_5$ | 16 | $CHCl_3-CH_3OH$ (87:13) | 45 | 164−167 |
| **2b** | $cC_4H_7$ | 16 | $CH_3Cl-cC_6H_{12}-CH_3OH$ (70:20:10) | 47 | 209−211 |
| **2c** | $cC_5H_9$ | 24 | $CHCl_3-CH_3OH$ (97:3) | 35 | 168−170 |
| **2d** | $cC_6H_{11}$ | 24 | $CHCl_3-CH_3OH-NH_3$ (85:14:1) | 48 | 188−190 |
| **2e** | $cC_7H_{13}$ | 24 | $CHCl_3-cC_6H_{12}-CH_3OH$ (70:20:10) | 27 | 150 (dec) |
| **2f** | $cC_8H_{15}$ | 24 | $CHCl_3-cC_6H_{12}-CH_3OH$ (70:20:10) | 22 | 165−169 |
| **5a** | $cC_3H_5$ | 24 | $CHCl_3-CH_3CN-CH_3OH$ (82:9:9) | 70 | 169−171 dec lit.[24] 113−116 |
| **5b** | $cC_4H_7$ | 24 | $CHCl_3-CH_3CN-CH_3OH$ (80:10:10) | 91 | 181−183 |
| **5c** | $cC_5H_9$ | 24 | $CHCl_3-CH_3CN-CH_3OH$ (84:8:8) | 80 | 190−196 |
| **5d** | $cC_6H_{11}$ | 24 | $CHCl_3-CH_3CN-CH_3OH$ (84:8:8) | 92 | 108−111 |
| **5e** | $cC_7H_{13}$ | 48 | $CHCl_3-CH_3CN-CH_3OH$ (86:7:7) | 74 | 181−186 |
| **5f** | $cC_8H_{15}$ | 48 | $CHCl_3-CH_3CN-CH_3OH$ (86:7:7) | 80 | 189−192 |
| **10a** | $cC_3H_5$ | 72 | $CHCl_3-CH_3CN-CH_3OH$ (90:5:5) | 94 | 157−158 |
| **10b** | $cC_4H_7$ | 72 | $CHCl_3-CH_3CN-CH_3OH$ (90:5:5) | 86 | 140−142 |
| **10c** | $cC_5H_9$ | 76 | $CHCl_3-CH_3CN-cC_6H_{12}$ (80:10:10) | 88 | 125−128 |
| **10d** | $cC_6H_{11}$ | 76 | $CHCl_3-CH_3CN-CH_3OH$ (90:5:5) | 78 | 150−152 |
| **10e** | $cC_7H_{13}$ | 92 | $CHCl_3-CH_3CN-CH_3OH$ (90:5:5) | 90 | 178−180 |
| **10f** | $cC_8H_{15}$ | 92 | $CHCl_3-CH_3CN-CH_3OH$ (90:5:5) | 85 | 176−178 |

**Table 2.** Preparation of Compounds in Schemes 1−3

| compd | R | time (h) | chromatography solvent | yield (%) | mp (°C) |
|---|---|---|---|---|---|
| **3a** | $cC_3H_5$ | 16 | $CHCl_3-C_6H_6-CH_3CN-CH_3OH$ (60:20:10:10)[a] | 69 | 102−104 |
| **3b** | $cC_4H_7$ | 16 | $CHCl_3-C_6H_6-CH_3CN-CH_3OH$ (62:20:10:8)[a] | 62 | 150−157 |
| **3c** | $cC_5H_9$ | 5 | AcOEt−$CH_3OH$ (95:5) | 70 | 94−96 |
| **3d** | $cC_6H_{11}$ | 5 | AcOEt−$CH_3OH$ (95:5) | 67 | 134−137 |
| **3e** | $cC_7H_{13}$ | 24 | $CHCl_3-C_6H_6-CH_3CN-CH_3OH$ (60:20:10:10)[a] | 46 | 155−160 |
| **3f** | $cC_8H_{15}$ | 24 | $CHCl_3-C_6H_{14}-CH_3OH$ (78:20:2)[a] | 47 | 172−165 |
| **6a** | $cC_3H_5$ | 10 | $CHCl_3-CH_3CN-CH_3OH$ (80:10:10) | 40 | 171−174 lit.[22b] 184−187 |
| **6b** | $cC_4H_7$ | 10 | $CHCl_3-CH_3CN-CH_3OH$ (76:12:12) | 78 | 130−132 lit.[22c] 121−123 |
| **6c** | $cC_5H_9$ | 16 | $CHCl_3-CH_3CN-CH_3OH$ (84:8:8) | 70 | 120−122 lit.[22a] 77−81 |
| **6d** | $cC_6H_{11}$ | 16 | $CHCl_3-CH_3CN-CH_3OH$ (84:8:8) | 68 | 186−188 lit.[22a] 187−188 |
| **6e** | $cC_7H_{13}$ | 24 | $CHCl_3-CH_3CN-CH_3OH$ (83:8.5:8.5) | 60 | 234−245 lit.[22a] 93−97 |
| **6f** | $cC_8H_{15}$ | 24 | $CHCl_3-CH_3CN-CH_3OH$ (84:8:8) | 50 | 238−246 lit.[22a] 167−168 |
| **11a** | $cC_3H_5$ | 8 | $CHCl_3-cC_6H_{12}-CH_3CN-CH_3OH$ (76:10:7:7) | 80 | 155−157 |
| **11b** | $cC_4H_7$ | 8 | $CHCl_3-cC_6H_{12}-CH_3CN-CH_3OH$ (76:10:7:7) | 72 | 163−165 |
| **11c** | $cC_5H_9$ | 8 | $CHCl_3-cC_6H_{12}-CH_3CN-CH_3OH$ (76:10:7:7) | 60 | 158−160 lit.[11] 151 |
| **11d** | $cC_6H_{11}$ | 8 | $CHCl_3-CH_3CN-CH_3OH$ (86:7:7) | 82 | 158−160 lit.[11] 158−159 |
| **11e** | $cC_7H_{13}$ | 14 | $CHCl_3-cC_6H_{12}-CH_3CN-CH_3OH$ (80:10:5:5) | 80 | 178−180 |
| **11f** | $cC_8H_{15}$ | 14 | $CHCl_3-CH_3CN-CH_3OH$ (86:7:7) | 82 | 85−89 |

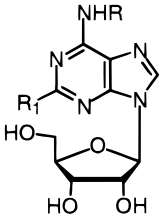
**Table 3.** $A_1$ and $A_{2A}$ Adenosine Receptor Affinities of 2,N-Substituted 1-Deazaadenosine Derivatives



| compd | R | $R_1$ | $K_i$ $A_1$ (nM)[a] | $K_i$ $A_{2A}$ (nM)[b] | $A_1$ selectivity ($K_i$ $A_{2A}$/$K_i$ $A_1$) |
|---|---|---|---|---|---|
| **2a** | $cC_3H_5$ | Cl | 151 ± 55 | 9640 ± 2260 | 64 |
| **3a** | $cC_3H_5$ | H | 326 ± 91 | 16400 ± 3480 | 50 |
| **2b** | $cC_4H_7$ | Cl | 41.0 ± 19 | 3110 ± 1510 | 76 |
| **3b** | $cC_4H_7$ | H | 109 ± 42 | 6550 ± 1740 | 59 |
| **2c** | $cC_5H_9$ | Cl | 49.0 ± 10 | 2840 ± 340 | 58 |
| **3c** | $cC_5H_9$ | H | 100 ± 12 | 10100 ± 3820 | 101 |
| **2d** | $cC_6H_{11}$ | Cl | 268 ± 146 | 5210 ± 1590 | 19 |
| **3d** | $cC_6H_{11}$ | H | 169 ± 49 | 5120 ± 1570 | 30 |
| **2e** | $cC_7H_{13}$ | Cl | 247 ± 133 | 4870 ± 940 | 19 |
| **3e** | $cC_7H_{13}$ | H | 364 ± 92 | 9200 ± 2030 | 25 |
| **2f** | $cC_8H_{15}$ | Cl | 595 ± 32 | 20000 ± 8750 | 34 |
| **3f** | $cC_8H_{15}$ | H | 539 ± 119 | 13700 ± 2430 | 25 |
| CPA | | | 5.07 ± 1.90 | 3370 ± 2660 | 665 |

[a] Displacement of [$^3$H]DPCPX from rat cortical membranes expressed as $K_i$ (nM). [b] Displacement of [$^3$H]CGS 21680 from rat striatal membranes expressed as $K_i$ (nM).

**Table 4.** $A_1$ and $A_{2A}$ Adenosine Receptor Affinities of 2,N-Substituted Adenosine Derivatives



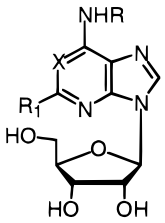
| compd | R | $R_1$ | $K_i$ $A_1$ (nM)[a] | $K_i$ $A_{2A}$ (nM)[b] | $A_1$ selectivity ($K_i$ $A_{2A}$/$K_i$ $A_1$) |
|---|---|---|---|---|---|
| **5a** | $cC_3H_5$ | Cl | 12.6 ± 5.16 | 2060 ± 1150 | 163 |
| **6a** | $cC_3H_5$ | H | 26.7 ± 4.74 | 510 ± 490 | 19 |
| **5b** | $cC_4H_7$ | Cl | 5.30 ± 3.38 | 460 ± 20 | 87 |
| **6b** | $cC_4H_7$ | H | 3.92 ± 2.79 | 420 ± 110 | 107 |
| **5c** (CCPA) | $cC_5H_9$ | Cl | 7.43 ± 4.56 | 640 ± 220 | 86 |
| **6c** (CPA) | $cC_5H_9$ | H | 5.07 ± 1.90 | 3370 ± 2660 | 665 |
| **5d** | $cC_6H_{11}$ | Cl | 31.7 ± 3.1 | 590 ± 130 | 19 |
| **6d** (CHA) | $cC_6H_{11}$ | H | 21.0 ± 11.6 | 600 ± 130 | 29 |
| **5e** | $cC_7H_{13}$ | Cl | 37.9 ± 3.1 | 1100 ± 160 | 29 |
| **6e** | $cC_7H_{13}$ | H | 19.6 ± 2.3 | 3150 ± 950 | 161 |
| **5f** | $cC_8H_{15}$ | Cl | 122 ± 71 | 4230 ± 930 | 35 |
| **6f** | $cC_8H_{15}$ | H | 53.5 ± 4.23 | 4180 ± 360 | 78 |

[a] Displacement of [$^3$H]DPCPX from rat cortical membranes expressed as $K_i$ (nM). [b] Displacement of [$^3$H]CGS 21680 from rat striatal membranes expressed as $K_i$ (nM).

In contrast to the 1-deaza analogues, the non-chlorinated compounds show, in general, higher affinity than the corresponding 2-chloro derivatives. At $A_{2A}$ receptors, the new nucleosides proved to be weak agonists, and the presence of the chlorine atom does not seem to significantly affect the affinity.

Some compounds of the 1-deaza derivative series (**2a**, **3a**, **2c**, **3c**, **2e**, and **3e**) and the corresponding purine analogues (**5a**, **6a**, **5c**, **6c**, **5e**, and **6e**) were evaluated for their relative intrinsic activity (RIA). The level of maximum stimulation of [$^{35}$S]GTP$\gamma$S binding induced by the full agonist 2-chloro-*N*-cyclopentyladenosine (CCPA, **5c**) was taken as 100% of the effect. The efficacy of all the tested compounds was almost identical (ranging from 90 to 100%), regardless of their affinities (Table 5). Therefore, these agonists were all considered to be full agonists.

**Table 5.** Intrinsic Activity of Selected 2,N-Substituted 1-Deazaadenosine and Adenosine Derivatives



| compd | R | $R_1$ | X | [$^{35}$S]GTP$\gamma$S[a] $EC_{50}$ | RIA[b] |
|---|---|---|---|---|---|
| **2a** | $cC_3H_5$ | Cl | CH | 704 (538−922) | 88.0 ± 2.1 |
| **3a** | $cC_3H_5$ | H | CH | 1530 (1280−1820) | 91.8 ± 2.3 |
| **2c** | $cC_5H_9$ | Cl | CH | 408 (289−575) | 87.8 ± 2.8 |
| **3c** | $cC_5H_9$ | H | CH | 475 (400−563) | 96.0 ± 1.2 |
| **2e** | $cC_7H_{13}$ | Cl | CH | 6580 (5270−8230) | 98.7 ± 1.0 |
| **3e** | $cC_7H_{13}$ | H | CH | 6360 (3840−10500) | 94.9 ± 2.1 |
| **5a** | $cC_3H_5$ | Cl | N | 57.3 (55.5−59.2) | 100.2 ± 3.3 |
| **6a** | $cC_3H_5$ | H | N | 709 (564−891) | 103.3 ± 1.3 |
| **5c** | $cC_5H_9$ | Cl | N | 30.0 (25.7−35.1) | 100 |
| **6c** | $cC_5H_9$ | H | N | 13.3 (10.1−17.5) | 101.6 ± 2.7 |
| **5e** | $cC_7H_{13}$ | Cl | N | 431 (371−502) | 100.8 ± 1.8 |
| **6e** | $cC_7H_{13}$ | H | N | 86.4 (69.4−108) | 101.5 ± 3.7 |

[a] Binding of [$^{35}$S]GTP$\gamma$S to rat brain membranes expressed as $EC_{50}$ (nM). [b] RIA = relative intrinsic activity (%).

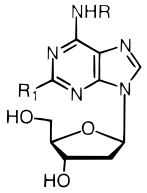
These data confirmed that the nitrogen atom at the 1-position of the purine ring is important, but not critical, for adenosine receptor affinity, whereas intrinsic activity is only marginally affected. The different size of cycloalkyl substituents at the 6-position of purine and 1-deazapurine systems modulates the affinity, with the highest affinity in both series shown by molecules bearing four- and five-membered rings (**2b**, $K_i$ $A_1$ = 40 nM, **2c**, $K_i$ $A_1$ = 50 nM and **6b**, $K_i$ $A_1$ = 3.92 nM, **6c**, $K_i$ $A_1$ = 5.07 nM, respectively; Tables 3 and 4).

**Deoxyribose Derivatives.** The 2′-deoxyadenosine derivatives were evaluated for their $A_1$ receptor affinity on rat cortical membranes, both in the presence and absence of GTP, using the antagonist [$^3$H]DPCPX as radioligand (Table 6). The $K_i$ values of this series of nucleosides for the $A_1$ adenosine receptor were all in the high nanomolar range, with the exception of 25 $\mu$M for compound **11a**.

$K_H$ and $K_L$ values for binding to the high- and low-affinity state of the receptor and the percentage of receptors in the high-affinity state (%$R_H$) were also calculated and reported in Table 6.

In addition, the potencies of the same nucleosides to stimulate G protein activation via the $A_1$ adenosine

**Table 6.** $A_1$ Adenosine Receptor Binding and G Protein Activation by 2,N-Substituted 2′-Deoxyadenosine Derivatives




| | | | [$^3$H]DPCPX without GTP[a] | | | | | [$^3$H]DPCPX + 1 mM GTP[a] | | [$^{35}$S]GTP$\gamma$S[b] | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| compd | R | $R_1$ | $K_i$ $A_1$ (nM) | $K_H$ | $K_L$ | $K_L$:$K_H$ | %$R_H$ | $K_i$ | SHIFT | $EC_{50}$ | RIA[c] |
| CCPA | $cC_5H_9$ | Cl | 0.50 (0.37−0.68) | 0.065 (0.039−0.11) | 11.2 (9.13−13.7) | 183 ± 28 | 58.6 ± 0.9 | 46.4 (43.5−49.5) | 96.8 ± 11.2 | 32.6 (24.6−43.2) | 100 |
| CPA | $cC_5H_9$ | H | 0.43 (0.34−0.54) | 0.052 (0.038−0.070) | 10.1 (7.88−12.9) | 201 ± 23 | 58.8 ± 1.2 | 44.7 (40.9−48.8) | 107 ± 10.8 | 16.0 (14.6−17.5) | 99.9 ± 1.6 |
| **10a** | $cC_3H_5$ | Cl | 724 (315−1667) | 115 (61.0−215) | 6030 (4870−7450) | 55.1 ± 11.6 | 44.2 ± 5.2 | 14300 (11300−18000) | 21.6 ± 6.0 | 6180 (5310−7200) | 20.8 ± 0.9 |
| **11a** | $cC_3H_5$ | H | 25200 (9060−70000) | | | | 0 | 134000 (106000−170000) | 6.1 ± 1.9 | | 0 |
| **10b** | $cC_4H_7$ | Cl | 245 (217−276) | 33.3 (25.2−44.0) | 1920 (1600−2320) | 58.0 ± 4.0 | 48.1 ± 2.0 | 3600 (3260−3970) | 14.9 ± 1.5 | 3710 (1330−10400) | 19.5 ± 0.4 |
| **11b** | $cC_4H_7$ | H | 524 (464−591) | 121 (85.6−170) | 8300 (6560−10500) | 69.1 ± 4.1 | 50.6 ± 1.7 | 23600 (23000−24100) | 45.1 ± 2.2 | 5110 (3950−6610) | 33.8 ± 2.4 |
| **10c** | $cC_5H_9$ | Cl | 154 (140−170) | 18.5 (17.5−19.7) | 800 (724−883) | 43.2 ± 0.9 | 44.0 ± 0.4 | 1320 (1160−1500) | 8.6 ± 0.2 | 1560 (1150−2120) | 8.3 ± 1.0 |
| **11c** | $cC_5H_9$ | H | 212 (190−236) | 39.3 (38.4−40.1) | 3520 (3270−3800) | 90.4 ± 4.3 | 52.6 ± 0.7 | 10200 (8610−12000) | 48.8 ± 6.2 | 5760 (4670−7100) | 42.1 ± 1.5 |
| **10d** | $cC_6H_{11}$ | Cl | 497 (425−580) | 61.7 (50.3−75.6) | 1590 (1440−1750) | 25.9 ± 1.9 | 35.7 ± 1.3 | 2560 (2320−2830) | 5.2 ± 0.5 | | 0 |
| **11d** | $cC_6H_{11}$ | H | 412 (363−468) | 92.5 (80.5−106) | 7330 (5970−8990) | 79.9 ± 7.0 | 51.9 ± 0.1 | 16700 (14200−19600) | 41.0 ± 4.8 | 9640 (5880−15800) | 40.1 ± 2.9 |
| **10e** | $cC_7H_{13}$ | Cl | 369 (315−435) | 30.7 (26.1−36.1) | 752 (623−906) | 24.6 ± 1.2 | 25.4 ± 2.0 | 1090 (1010−1180) | 3.0 ± 0.1 | | 0 |
| **11e** | $cC_7H_{13}$ | H | 769 (565−1050) | 124 (95.8−162) | 7410 (5740−9580) | 59.6 ± 1.1 | 45.1 ± 2.0 | 20400 (19400−21300) | 27.3 ± 4.6 | 13700 (10500−17700) | 24.9 ± 1.7 |
| **10f** | $cC_8H_{15}$ | Cl | 659 (494−880) | 23.6 (14.1−39.5) | 1020 (975−1060) | 45.9 ± 11.5 | 14.5 ± 3.0 | 1410 (1270−1550) | 2.2 ± 0.3 | | 0 |
| **11f** | $cC_8H_{15}$ | H | 586 (489−702) | 84.9 (75.6−95;4) | 4190 (3510−4990) | 50.4 ± 7.8 | 45.9 ± 3.0 | 9310 (8670−10000) | 16.0 ± 1.4 | | 0 |

[a] Displacement of [$^3$H]DPCPX from rat cortical membranes expressed as $K_i$, $K_H$ (affinity for the high-affinity state), or $K_L$ (affinity for the low-affinity state) (all given in nM). %$R_H$ denotes the percentage of receptors in the high-affinity state. [b] Binding of [$^{35}$S]GTP$\gamma$S to rat brain membranes expressed as $EC_{50}$ (nM). [c] RIA = relative intrinsic activity with reference to CCPA, which was set as 100%.

receptor were determined in [$^{35}$S]GTP$\gamma$S binding assay on rat brain membranes. In the right side of Table 6, the $EC_{50}$ values and the relative intrinsic activities (RIA) with respect to CCPA of the 2′-deoxyadenosine derivatives are reported. CCPA and CPA are used as controls. None of the tested compounds proved to be full agonists, the RIA values ranging from 0 to 42%.

Concerning structure−activity relationships, these results showed that there is not a relevant effect of the N-substituent ring size on affinity, although in both series the *N*-cyclopentyl derivatives **10c** and **11c** showed the highest affinity (Table 6).

Moreover, the 2-chloro substituted compounds showed in general slightly higher affinities, in comparison with the unsubstituted counterparts, in binding both to the high- and the low-affinity state of the $A_1$ receptors. It is worthwhile noting that this profile is similar to that showed by the 1-deazaadenosine derivatives (**2a**−**f** and **3a**−**f**) and opposite to that shown by the corresponding adenosine analogues (**5a**−**f** and **6a**−**f**).

However, even though the affinity values of the 2′-deoxyadenosine derivatives (modified on the sugar) are comparable to those of the 1-deazaadenosine analogues (modified on the purine moiety), these modifications of adenosine structure have a very different effect on the potencies of the corresponding compounds in G protein activation. In fact, the lack of a hydroxyl group in the 2′-position led to partial agonists at $A_1$ adenosine receptor (Table 6, right column), whereas the isosteric substitution of 1-nitrogen by a −CH− affected only the ligand potencies, but not intrinsic activities (Table 5).

Regarding the $EC_{50}$ values from [$^{35}$S]GTP$\gamma$S binding experiments, it is evident that in 2-chloro-2′-deoxyadenosine derivatives the potency increased with the size of the N-substituent (from cyclopropyl **10a** to cyclopentyl **10c**), whereas it showed the reverse tendency for the non-chlorinated nucleosides, with an optimum in both series for cC4 and cC5.

Regarding the intrinsic activities (Figure 2a), the presence of a chlorine atom on C-2 was favorable only in the case of the *N*-cyclopropyl substituted derivative (**10a**, RIA = 20.8% vs **11a**, RIA = 0%), all the other 2-chloro derivatives being less efficacious than the corresponding 2-unsubstituted analogues. The highest intrinsic activities are displayed by 2-dechlorinated compounds bearing a cyclopentyl or cyclohexyl ring on the 6-amino group (**11c**, RIA = 42.1% and **11d**, RIA = 40.1%). The GTP shift values showed exactly the same trend (Figure 2b). Both the GTP shifts and [$^{35}$S]GTP$\gamma$S binding experiments are equally indicative of full or partial agonism at $A_1$ adenosine receptors. We identified a highly significant linear correlation between GTP shifts obtained from receptor binding experiments and intrinsic activities of CPA, CCPA, and the 2′-deoxy-

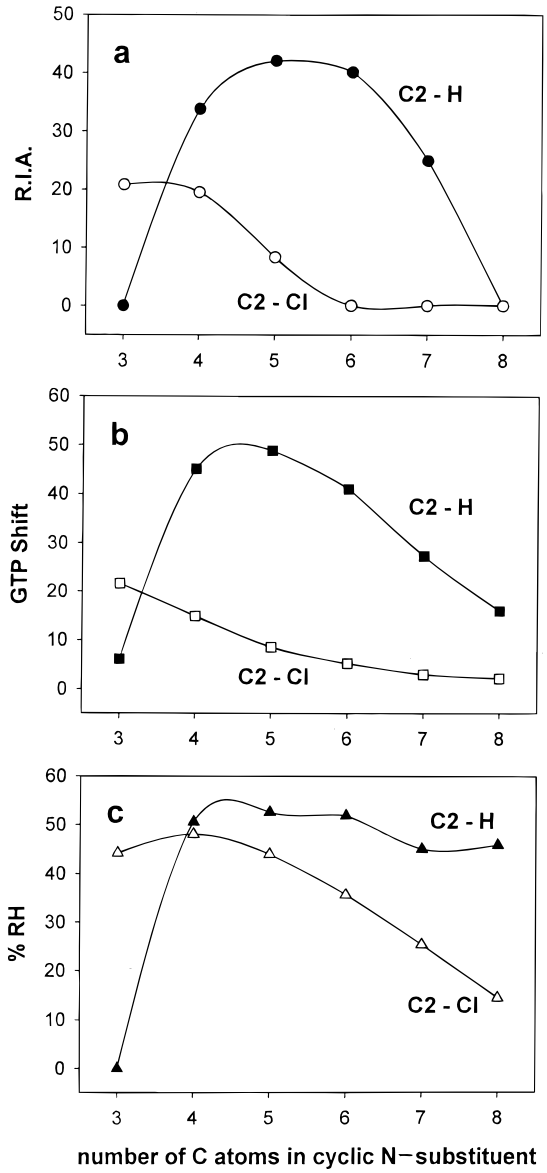



**Figure 2.** Structure−activity relationships for 2′-deoxyribose derivatives of adenosine. Panels a−c show the influence of C atom number in the cyclic N-substituent on relative intrinsic activity (RIA, determined by stimulation of [$^{35}$S]GTP$\gamma$S binding; a), on GTP shifts in displacement of [$^{3}$H]DPCPX binding to rat brain membranes in the absence or presence of 1 mM GTP, respectively (b), and on detection of receptors in the high-affinity state in the absence of GTP (%$R_H$; c).

adenosine derivatives from G protein activation studies ($r = 0.9872$, $P < 0.001$). As a further estimate of intrinsic activity, the ratios between $K_L$ and $K_H$ values for binding to the low- and high-affinity state of the receptor were calculated (Table 6), based on the assumption that agonists, but not antagonists, differentiate between G protein-coupled receptors in the high-affinity state and uncoupled receptors in the low-affinity state. In fact, we found a higly significant linear correlation between $K_L$:$K_H$ ratios in receptor binding and relative intrinsic activities determined in [$^{35}$S]GTP$\gamma$S binding experiments ($r = 0.9796$, $P < 0.001$).

The agonist intrinsic activity appears not directly related to how many of the receptors are "seen" by the ligand in the high-affinity state (Figure 2c). In fact, the non-chlorinated compounds **11b**−**f** show a high percentage of receptor binding to the high-affinity state, but they do not stimulate [$^{35}$S]GTP$\gamma$S binding.

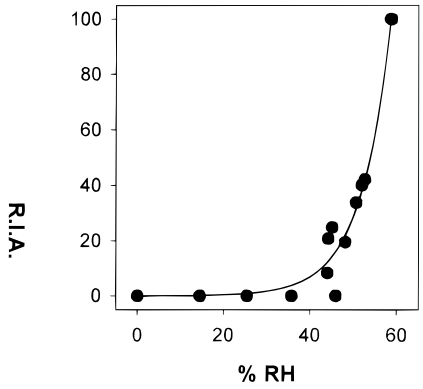



**Figure 3.** Relationship between binding to $A_1$ adenosine receptors in the high-affinity state and relative intrinsic activity. The detection of $A_1$ receptors by 2′-deoxyribose adenosine derivatives, CPA, and CCPA was determined from inhibition studies of [$^{3}$H]DPCPX binding in rat brain membranes to high-affinity sites in the absence of GTP (%$R_H$). Relative intrinsic activities (RIA) were measured as the maximum stimulation of [$^{35}$S]GTP$\gamma$S binding in rat brain membranes by ligands in relation to the full agonist CCPA.

In Figure 3 is reported the relationship between intrinsic activity and the percentage of receptors detected in the high-affinity state. The fact that this relationship led to an exponential curve means that with 40% of receptor binding in the high-affinity state a compound is almost inactive in G protein activation, as assessed by stimulation of [$^{35}$S]GTP$\gamma$S binding. With 50% binding to high-affinity state receptors, the intrinsic activity is 50%, whereas with 60% binding to the high-affinity state the intrinsic activity is 100%. These results argue against a simple 1:1 relationship between receptor occupancy and activation, and they indicate that below a critical density of receptors in the high-affinity state no stimulation can be achieved.

Further experiments were carried out to investigate whether the 2′-deoxyadenosine derivatives acted at the same $A_1$ receptor site as CCPA and whether they also exhibit antagonistic characteristics, as must be expected for partial agonists. Therefore, [$^{35}$S]GTP$\gamma$S binding was determined using a 100 $\mu$M concentration of nucleosides in the absence or presence of a maximally stimulating concentration of CCPA (1 $\mu$M). As shown in Figure 4, most of the compounds induced stimulation of [$^{35}$S]-GTP$\gamma$S binding somewhat above basal levels. In the presence of CCPA, some of the 2′-deoxyribose derivatives showed clear inhibitory effects, confirming the partial agonist behavior. In particular, the *N*-cyclopentyl derivatives **10c** and **11c**, with an RIA in stimulation of about 8% and 42%, respectively, caused a reduction of CCPA-induced [$^{35}$S]GTP$\gamma$S binding of about 50% and 15%, respectively. Therefore, even compounds which show very low or no stimulatory intrinsic activity are not inactive at $A_1$ receptors. They inhibit agonist-induced G protein activation, in good agreement with their antagonist-like receptor binding mode, which is characterized by low GTP shifts, low $K_L$:$K_H$ ratios, and with some precaution, detection of lower percentages of receptors in the high-affinity state.

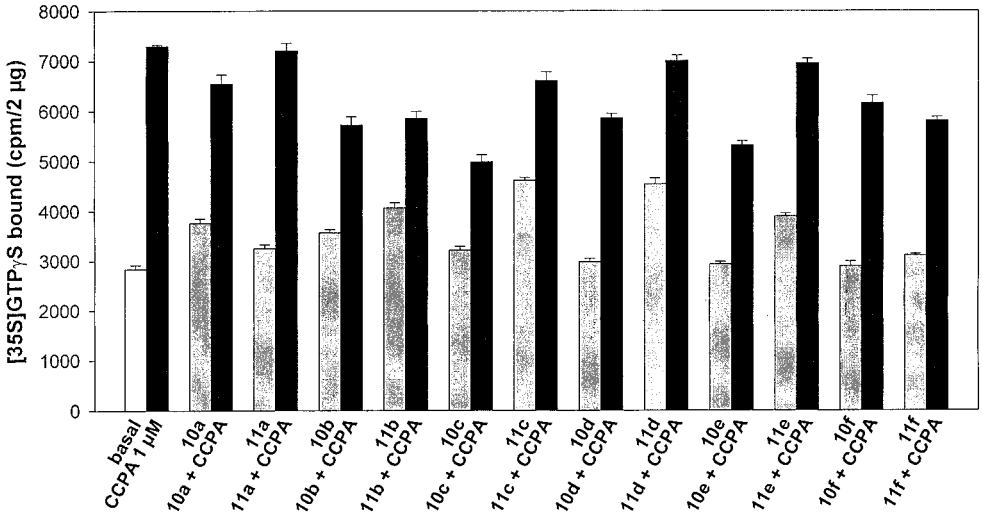



**Figure 4.** Stimulation of basal [$^{35}$S]GTP$\gamma$S binding and inhibition of CCPA-stimulated [$^{35}$S]GTP$\gamma$S binding by 2′-deoxyribose adenosine derivatives. [$^{35}$S]GTP$\gamma$S binding to rat brain membranes (2 $\mu$g) was measured in the absence (open column and gray columns) or presence (black columns) of 1 $\mu$M CCPA. 2′-Deoxyribose derivatives (100 $\mu$M concentration) stimulate basal [$^{35}$S]-GTP$\gamma$S binding (gray columns) and inhibit the stimulatory effect of CCPA (black columns).

## Conclusion

Three series of adenosine analogues, all bearing cycloalkyl substituents on the 6-amino group, have been synthesized and evaluated to determine their affinities and intrinsic activities at $A_1$ adenosine receptor. All compounds of the ribose-bearing series proved to be full agonists, the 1-deaza derivatives showing affinities for the $A_1$ receptor about 10-fold lower than the corresponding adenosine analogues. On the other hand, all the 2′-deoxyribose derivatives bind to the $A_1$ receptor with affinities in the high nanomolar range. Both GTP shifts and [$^{35}$S]GTP$\gamma$S experiments showed that most of the 2′-deoxyadenosine derivatives are partial agonists and they detected fewer $A_1$ receptors in the high-affinity state than full agonists.

Hence, it has been proved that modifications of purine moiety and of sugar in the adenosine structure have very different effects on the potencies of the corresponding compounds in G protein activation. In fact, the lack of a hydroxyl group at the 2′-position led to partial agonists at $A_1$ adenosine receptor, whereas the isosteric substitution of N-1 nitrogen by a carbon affected only the ligand potencies, but not intrinsic activities.

Finally, it has been clearly demonstrated that there was not a simple linear relationship between receptor occupancy and activation and that below a critical density of $A_1$ adenosine receptors in the high-affinity state no activation of G protein can be achieved.

## Experimental Section

**Chemistry.** Melting points were determined with a Büchi apparatus and are uncorrected. $^1$H NMR spectra were obtained with a Varian VX 300 MHz spectrometer. Thin-layer chromatography (TLC) was carried out on precoated TLC plates with silica gel 60 F-254 (Merck) and preparative TLC on precoated Whatman 60A TLC plates. For column chromatography, silica gel 60 (Merck) was used. Microanalytical results are within $\pm$0.4% of theoretical values.

**Preparation of 7-Cycloalkylamino-5-chloro-3-(*β*-D-ribofuranosyl)-3*H*-imidazo[4,5-*b*]pyridines (2a−f).** A mixture of 0.43 g (1.34 mmol) of 5,7-dichloro-3-(*β*-D-ribofuranosyl)-3*H*-imidazo[4,5-*b*]pyridine (**1**)[17] and 10 mL of the appropriate amine was heated in a steel bomb at the temperature and for the time listed in Table 1. The reaction mixture was evaporated, and the residue was chromatographed on a silica gel column eluting with the suitable mixture of solvents (Table 1) to give **2a**−**f** as chromatographically pure solids. Compounds **2c** and **2d** were already described elsewhere.[20]

**2a:** $^1$H NMR (Me$_2$SO-$d_6$) $\delta$ 0.60 (m, 2H, H cyclopropyl), 0.80 (m, 2H, H cyclopropyl), 2.61 (m, 1H, H-1 cyclopropyl), 3.62 (m, 2H, CH$_2$-5′), 3.95 (m, 1H, H-4′), 4.14 (m, 1H, H-3′), 4.57 (m, 1H, H-2′), 5.88 (d, 1H, $J$ = 6.1 Hz, H-1′), 6.60 (s, 1H, H-6), 7.68 (bs, 1H, NH), 8.36 (s, 1H, H-2). Anal. ($C_{14}H_{17}ClN_4O_4$) C, H, N.

**2b:** $^1$H NMR (Me$_2$SO-$d_6$) $\delta$ 1.75, 2.08, and 2.35 (m, 2H each, H cyclobutyl), 3.62 (m, 2H, CH$_2$-5′), 3.94 (m, 1H, H-4′), 4.15 (m, 1H, H-3′), 4.36 (bs, 1H, H-1 cyclobutyl), 4.58 (m, 1H, H-2′), 5.88 (d, 1H, $J$ = 6.1 Hz, H-1′), 6.34 (s, 1H, H-6), 7.52 (bs, 1H, NH), 8.37 (s, 1H, H-2). Anal. ($C_{15}H_{19}ClN_4O_4$) C, H, N.

**2e:** $^1$H NMR (Me$_2$SO-$d_6$) $\delta$ 1.50−1.70 (m, 10H, H cycloheptyl), 1.93 (m, 2H, cycloheptyl), 3.60 (m, 2H, CH$_2$-5′), 3.95 (m, 1H, H-4′), 4.14 (m, 2H, H-3′ and H-1 cycloheptyl), 4.57 (m, 1H, H-2′), 5.87 (d, 1H, $J$ = 6.0 Hz, H-1′), 6.37 (s, 1H, H-6), 6.99 (d, 1H, $J$ = 8.8 Hz, NH), 8.34 (s, 1H, H-2). Anal. ($C_{18}H_{25}ClN_4O_4$) C, H, N.

**2f:** $^1$H NMR (Me$_2$SO-$d_6$) $\delta$ 1.51−1.84 (m, 14H, H cyclooctyl), 3.60 (m, 2H, CH$_2$-5′), 3.95 (m, 1H, H-4′), 4.13 (m, 2H, H-3′ and H-1 cyclooctyl), 4.57 (m, 1H, H-2′), 5.86 (d, 1H, $J$ = 6.0 Hz, H-1′), 6.37 (s, 1H, H-6), 6.99 (d, 1H, $J$ = 8.7 Hz, NH), 8.34 (s, 1H, H-2). Anal. ($C_{19}H_{27}ClN_4O_4$) C, H, N.

**Preparation of 7-Cycloalkylamino-(*β*-D-ribofuranosyl)-3*H*-imidazo-[4,5-*b*]pyridines (3a−f).** To a solution of **2a**−**f** (0.5 mmol) in 40 mL of ethanol and 1 mL of 2 N NaOH was added 0.050 g of 10% Pd/C, and the mixture was shaken with hydrogen at the pressure and for the time listed in Table 2. The catalyst was removed, and the filtrate was concentrated to dryness. The residue was chromatographed on a silica gel column eluting with the suitable mixture of solvents to give **3a**−**f** (Table 2) as chromatographically pure solids. Compounds **3c** and **3d** were already described elsewhere.[20]

**3a:** $^1$H NMR (Me$_2$SO-$d_6$) $\delta$ 0.57 (m, 2H, H cyclopropyl), 0.78 (m, 2H, H cyclopropyl), 2.59 (m, 1H, H-1 cyclopropyl), 3.62 (m, 2H, CH$_2$-5′), 4.00 (m, 1H, H-4′), 4.15 (m, 1H, H-3′), 4.71 (m, 1H, H-2′), 5.91 (d, 1H, $J$ = 6.4 Hz, H-1′), 6.64 (d, 1H, $J_{6,5}$ = 5.7 Hz, H-6), 7.27 (bs, 1H, NH), 7.95 (d, 1H, $J_{5,6}$ = 5.7 Hz, H-5), 8.29 (s, 1H, H-2). Anal. ($C_{14}H_{18}N_4O_4$) C, H, N.

**3b:** $^1$H NMR (Me$_2$SO-$d_6$) $\delta$ 1.71, 2.06, and 2.34 (m, 2H each, H cyclobutyl), 3.61 (m, 2H, CH$_2$-5′), 3.99 (m, 1H, H-4′), 4.14 (m, 1H, H-3′), 4.37 (bs, 1H, H-1 cyclobutyl), 4.70 (m, 1H, H-2′), 5.90 (d, 1H, $J$ = 6.6 Hz, H-1′), 6.33 (d, 1H, $J_{6,5}$ = 5.7 Hz, H-6), 7.11 (d, 1H, $J$ = 7.0 Hz, NH), 7.95 (d, 1H, $J_{5,6}$ = 5.7 Hz, H-5), 8.29 (s, 1H, H-2). Anal. ($C_{15}H_{20}N_4O_4$) C, H, N.

**3e:** $^1$H NMR ($Me_2SO$-$d_6$) $\delta$ 1.51−1.71 (m, 10H, H cycloheptyl), 1.93 (m, 2H, cycloheptyl), 3.60 (m, 2H, $CH_2$-5′), 4.00 (m, 2H, H-4′ and H-1 cycloheptyl), 4.15 (m, 1H, H-3′), 4.71 (m, 1H, H-2′), 5.89 (d, 1H, $J$ = 6.6 Hz, H-1′), 6.36 (d, 1H, $J_{6,5}$ = 5.7 Hz, H-6), 6.56 (d, 1H, $J$ = 8.6 Hz, NH), 7.84 (d, 1H, $J_{5,6}$ = 5.7 Hz, H-5), 8.27 (s, 1H, H-2). Anal. ($C_{18}H_{26}N_4O_4$) C, H, N.

**3f:** $^1$H NMR ($Me_2SO$-$d_6$) $\delta$ 1.53−1.84 (m, 14H, H cyclooctyl), 3.60 (m, 2H, $CH_2$-5′), 3.92 (m, 1H, H-4′), 4.12 (m, 2H, H-3′ and H-1 cyclooctyl), 4.71 (m, 1H, H-2′), 5.89 (d, 1H, $J$ = 6.6 Hz, H-1′), 6.36 (d, 1H, $J_{6,5}$ = 5.7 Hz, H-6), 6.56 (d, 1H, $J$ = 8.6 Hz, NH), 7.84 (d, 1H, $J_{5,6}$ = 5.7 Hz, H-5), 8.27 (s, 1H, H-2). Anal. ($C_{19}H_{28}N_4O_4$) C, H, N.

**Preparation of 2-Chloro-6-cycloalkylamino-9-(β-D-ribofuranosyl)-9*H*-purines (2-Chloro-*N*-cycloalkyladenosines 5a−f).** A mixture of 0.3 g (0.67 mmol) of 2,6-dichloro-9-(2,3,5-tri-*O*-acetyl-β-D-ribofuranosyl)-9*H*-purine (**4**)[21] and 2 mL of the appropriate amine was stirred at room temperature for 4 h. The exceeding amine was evaporated in vacuo, methanol saturated at 0 °C with ammonia was added to the residue, and the mixture was stirred at room temperature for the time reported in Table 1. The reaction mixture was evaporated, and the residue was chromatographed on a silica gel column eluting with the suitable mixture of solvents (Table 1) to give **5a**−**f** as chromatographically pure solids. Compounds **5a**[24] and **5c**[25] were already described elsewhere.

**5b:** $^1$H NMR ($Me_2SO$-$d_6$) $\delta$ 1.71 (m, 2H, H cyclobutyl), 2.01−2.38 (m, 4H, H cyclobutyl), 3.61 (m, 2H, $CH_2$-5′), 3.95 (m, 1H, H-4′), 4.13 (m, 1H, H-3′), 4.51 (m, 1H, H-2′), 4.61 (m, 1H, H-1 cyclobutyl), 5.83 (d, 1H, $J$ = 5.9 Hz, H-1′), 8.41 (s, 1H, H-8), 8.65 (m, 1H, NH). Anal. ($C_{14}H_{18}ClN_5O_4$) C, H, N.

**5d:** $^1$H NMR ($Me_2SO$-$d_6$) $\delta$ 1.00−2.00 (m, 10H, H cyclohexyl), 3.63 (m, 2H, $CH_2$-5′), 3.97 (m, 1H, H-4′), 4.15 (m, 2H, H-3′ and H-1 cyclohexyl), 4.52 (m, 1H, H-2′), 5.82 (d, 1H, $J$ = 5.5 Hz, H-1′), 8.15 (d, 1H, $J$ = 8.0 Hz, NH), 8.40 (s, 1H, H-8). Anal. ($C_{16}H_{22}ClN_5O_4$) C, H, N.

**5e:** $^1$H NMR ($Me_2SO$-$d_6$) $\delta$ 1.35−1.78 (m, 10H, H cycloheptyl), 1.90 (m, 2H, cycloheptyl), 3.61 (m, 2H, $CH_2$-5′), 3.95 (m, 1H, H-4′), 4.15 (m, 2H, H-3′ and H-1 cycloheptyl), 4.52 (m, 1H, H-2′), 5.96 (d, 1H, $J$ = 5.8 Hz, H-1′), 8.29 (d, 1H, $J$ = 8.1 Hz, NH), 8.40 (s, 1H, H-8). Anal. ($C_{17}H_{24}ClN_5O_4$) C, H, N.

**5f:** $^1$H NMR ($Me_2SO$-$d_6$) $\delta$ 1.41−1.88 (m, 14H, H cyclooctyl), 3.60 (m, 2H, $CH_2$-5′), 3.95 (m, 1H, H-4′), 4.13 (m, 2H, H-3′), 4.26 (m, 1H, H-1 cyclooctyl), 4.52 (m, 1H, H-2′), 5.83 (d, 1H, $J$ = 6.1 Hz, H-1′), 8.29 (d, 1H, $J$ = 8.1 Hz, NH), 8.39 (s, 1H, H-8). Anal. ($C_{18}H_{26}ClN_5O_4$) C, H, N.

**Preparation of 6-Cycloalkylamino-9-(β-D-ribofuranosyl)-9*H*-purines (*N*-Cycloalkyladenosines 6a−f).** To a solution of **5a**−**f** (0.5 mmol) in 40 mL of ethanol and 1.5 mL of 2 N NaOH was added 0.050 g of 10% Pd/C, and the mixture was shaken with hydrogen at 45 psi for the time reported in Table 2. The catalyst was removed, and the filtrate was concentrated to dryness. The residue was chromatographed on a silica gel column eluting with the suitable mixture of solvents to give **6a**−**f** (Table 2) as chromatographically pure solids. Compounds **6a**−**f** were already synthesized starting from 6-chloropurine riboside (**7**).[22] However, they were not characterized by $^1$H NMR spectra. Compounds **6c** and **6d** are also commercially available.

**6a:** $^1$H NMR ($Me_2SO$-$d_6$) $\delta$ 0.64 (m, 2H, H cyclopropyl), 0.74 (m, 2H, H cyclopropyl), 3.07 (m, 1H, H-1 cyclopropyl), 3.60 (m, 2H, $CH_2$-5′), 3.97 (m, 1H, H-4′), 4.15 (m, 1H, H-3′), 4.61 (m, 1H, H-2′), 5.9 (d, 1H, $J$ = 6.1 Hz, H-1′), 8.03 (bs, 1H, NH), 8.25 (s, 1H, H-2), 8.37 (s, 1H, H-8). Anal. ($C_{13}H_{17}N_5O_4$) C, H, N.

**6b:** $^1$H NMR ($Me_2SO$-$d_6$) $\delta$ 1.67 (m, 2H, H cyclobutyl), 2.01−2.35 (m, 4H, H cyclobutyl), 3.59 (m, 2H, $CH_2$-5′), 3.97 (m, 1H, H-4′), 4.15 (m, 1H, H-3′), 4.61 (m, 1H, H-2′), 4.73 (m, 1H, H-1 cyclobutyl), 5.89 (d, 1H, $J$ = 5.9 Hz, H-1′), 8.18 (m, 2H, H-2 and NH), 8.38 (s, 1H, H-8). Anal. ($C_{14}H_{19}N_5O_4$) C, H, N.

**6e:** $^1$H NMR ($Me_2SO$-$d_6$) $\delta$ 1.35−1.98 (m, 12H, H cycloheptyl), 3.60 (m, 2H, $CH_2$-5′), 3.97 (m, 1H, H-4′), 4.14 (m, 2H, H-3′), 4.29 (m, 1H, H-1 cycloheptyl), 4.61 (m, 1H, H-2′), 5.88 (d, 1H, $J$ = 5.9 Hz, H-1′), 7.69 (d, 1H, $J$ = 7.9 Hz, NH), 8.19 (s, 1H, H-2), 8.35 (s, 1H, H-8). Anal. ($C_{17}H_{25}N_5O_4$) C, H, N.

**6f:** $^1$H NMR ($Me_2SO$-$d_6$) $\delta$ 1.40−1.90 (m, 14H, H cyclooctyl), 3.60 (m, 2H, $CH_2$-5′), 3.97 (m, 1H, H-4′), 4.15 (m, 2H, H-3′), 4.48 (m, 1H, H-1 cyclooctyl), 4.62 (m, 1H, H-2′), 5.89 (d, 1H, $J$ = 6.2 Hz, H-1′), 7.72 (d, 1H, $J$ = 7.6 Hz, NH), 8.20 (s, 1H, H-2), 8.36 (s, 1H, H-8). Anal. ($C_{18}H_{27}N_5O_4$) C, H, N.

**Preparation of 2-Chloro-6-cycloalkylamino-9-(2-deoxy-3,5-di-*O*-*p*-toluoyl-β-D-*erythro*-pentofuranosyl)-9*H*-purines (9a−f).** A mixture of 0.35 g (0.65 mmol) of 2,6-dichloro-9-(2-deoxy-3,5-di-*O*-*p*-toluoyl-β-D-*erythro*-pentofuranosyl)-9*H*-purine (**8**)[23] and 3 mL of the appropriate amine was stirred at room temperature for 4 h. The exceeding amine was evaporated in vacuo, and the residue was used for the next step without further purification.

Analytical samples of **9a**−**f** were obtained by preparative TLC eluting with $cC_6H_{12}$−EtOAc (65:35).

**9a:** $^1$H NMR ($Me_2SO$-$d_6$) $\delta$ 0.65 (m, 2H, H cyclopropyl), 0.76 (m, 2H, H cyclopropyl), 2.38 (s, 3H, $CH_3$), 2.42 (s, 3H, $CH_3$), 2.81 (m, 1H, H-2′), 3.00 (m, 1H, H-1 cyclopropyl), 3.20 (m, 1H, H-2″), 4.58 (m, 3H, $CH_2$-5′ and H-4′), 5.77 (m, 1H, H-3′), 6.47 (t, 1H, $J$ = 6.5 Hz, H-1′), 7.34 (m, 4H, H−Ph), 7.84 (d, 2H, $J$ = 8.1 Hz, H−Ph), 7.97 (d, 2H, $J$ = 8.1 Hz, H−Ph), 8.39 (s, 1H, H-8), 8.52 (bs, 1H, NH). Anal. ($C_{29}H_{28}ClN_5O_5$) C, H, N.

**9b:** $^1$H NMR ($Me_2SO$-$d_6$) $\delta$ 1.71 (m, 2H, H cyclobutyl), 1.97−2.33 (m, 4H, H cyclobutyl), 2.40 (s, 3H, $CH_3$), 2.52 (s, 3H, $CH_3$), 2.81 (m, 1H, H-2′), 3.24 (m, 1H, H-2″), 4.58 (m, 2H, $CH_2$-5′), 4.86 (m, 2H, H-4′ and H-1 cyclobutyl), 5.80 (m, 1H, H-3′), 6.46 (t, 1H, $J$ = 7.2 Hz, H-1′), 7.34 (m, 4H, H−Ph), 7.90 (m, 4H, H−Ph), 8.40 (s, 1H, H-8), 8.69 (m, 1H, NH). Anal. ($C_{30}H_{30}ClN_5O_5$) C, H, N.

**9c:** $^1$H NMR ($Me_2SO$-$d_6$) $\delta$ 1.47−1.91 (m, 6H, H cyclopentyl), 1.96 (m, 2H, H cyclopentyl), 2.38 (s, 3H, $CH_3$), 2.42 (s, 3H, $CH_3$), 2.79 (m, 1H, H-2′), 3.22 (m, 1H, H-2″), 4.37−4.68 (m, 4H, $CH_2$-5′, H-4′, and H-1 cyclopentyl), 5.78 (m, 1H, H-3′), 6.47 (t, 1H, $J$ = 6.6 Hz, H-1′), 7.35 (m, 4H, H−Ph), 7.84 (d, 2H, $J$ = 8.1 Hz, H−Ph), 7.97 (d, 2H, $J$ = 8.1 Hz, H−Ph), 8.38 (bs, 2H, H-8 and NH). Anal. ($C_{31}H_{32}ClN_5O_5$) C, H, N.

**9d:** $^1$H NMR ($Me_2SO$-$d_6$) $\delta$ 1.47−1.91 (m, 8H, H cyclohexyl), 1.96 (m, 2H, H cyclohexyl), 2.38 (s, 3H, $CH_3$), 2.42 (s, 3H, $CH_3$), 2.80 (m, 1H, H-2′), 3.20 (m, 1H, H-2″), 4.00 (m, 1H, H-1 cyclohexyl), 4.58 (m, 3H, $CH_2$-5′ and H-4′,), 5.80 (m, 1H, H-3′), 6.47 (t, 1H, $J$ = 6.3 Hz, H-1′), 7.35 (m, 4H, H−Ph), 7.84 (d, 2H, $J$ = 8.2 Hz, H−Ph), 7.97 (d, 2H, $J$ = 8.1 Hz, H−Ph), 8.25 (d, 1H, $J$ = 8.8 Hz, NH), 8.40 (s, 1H, H-8). Anal. ($C_{32}H_{34}ClN_5O_5$) C, H, N.

**9e:** $^1$H NMR ($Me_2SO$-$d_6$) $\delta$ 1.41−1.78 (m, 10H, H cycloheptyl), 1.90 (m, 2H, H cycloheptyl), 2.38 (s, 3H, $CH_3$), 2.42 (s, 3H, $CH_3$), 2.81 (m, 1H, H-2′), 3.21 (m, 1H, H-2″), 4.18 (m, 1H, H-1 cycloheptyl), 4.59 (m, 3H, $CH_2$-5′ and H-4′), 5.79 (m, 1H, H-3′), 6.47 (t, 1H, $J$ = 7.0 Hz, H-1′), 7.35 (m, 4H, H−Ph), 7.84 (d, 2H, $J$ = 8.2 Hz, H−Ph), 7.97 (d, 2H, $J$ = 8.1 Hz, H−Ph), 8.29 (d, 1H, $J$ = 8.7 Hz, NH), 8.40 (s, 1H, H-8). Anal. ($C_{33}H_{36}ClN_5O_5$) C, H, N.

**9f:** $^1$H NMR ($Me_2SO$-$d_6$) $\delta$ 1.55 (m, 12H, H cyclooctyl), 1.77 (m, 2H, H cyclooctyl), 2.38 (s, 3H, $CH_3$), 2.42 (s, 3H, $CH_3$), 2.81 (m, 1H, H-2′), 3.21 (m, 1H, H-2″), 4.25 (m, 1H, H-1 cyclooctyl), 4.59 (m, 3H, $CH_2$-5′ and H-4′), 5.79 (m, 1H, H-3′), 6.47 (t, 1H, $J$ = 7.1 Hz, H-1′), 7.34 (m, 4H, H−Ph), 7.83 (d, 2H, $J$ = 8.2 Hz, H−Ph), 7.97 (d, 2H, $J$ = 8.1 Hz, H−Ph), 8.30 (d, 1H, $J$ = 8.6 Hz, NH), 8.42 (s, 1H, H-8). Anal. ($C_{34}H_{38}ClN_5O_5$) C, H, N.

**Preparation of 2-Chloro-6-cycloalkylamino-9-(2-deoxy-β-D-*erythro*-pentofuranosyl)-9*H*-purines (2′-Deoxy-2-chloro-*N*-cycloalkyl Adenosines 10a−f).** To the crude mixture of **9a**−**f** 20 mL of methanol saturated at 0 °C with ammonia was added, and the reaction was allowed to stand at room temperature for the time reported in Table 1. After evaporation under vacuum, the residue was chromatographed on a silica gel column eluting with the suitable mixture of solvents (Table 1) to give **10a**−**f** as chromatographically pure solids. Compound **10d** was already described elsewhere.[26]

**10a:** $^1$H NMR ($Me_2SO$-$d_6$) $\delta$ 0.66 (m, 2H, cyclopropyl), 0.75 (m, 2H, H cyclopropyl), 2.30 (m, 1H, H-2′), 2.64 (m, 1H, H-2″), 2.99 (m, 1H, H-1 cyclopropyl), 3.57 (m, 2H, $CH_2$-5′), 3.82 (m,

1H, H-4′), 4.40 (m, 1H, H-3′), 6.28 (t, 1H, $J$ = 6.8 Hz, H-1′), 8.37 (s, 1H, H-8), 8.47 (bs, 1H, NH). Anal. ($C_{13}H_{16}ClN_5O_3$) C, H, N.

**10b:** $^1$H NMR ($Me_2SO$-$d_6$) $\delta$ 1.71 (m, 2H, H cyclobutyl), 2.00−2.40 (m, 5H, H cyclobutyl and H-2′), 2.63 (m, 1H, H-2″), 3.58 (m, 2H, $CH_2$-5′), 3.89 (m, 1H, H-4′), 4.40 (m, 1H, H-3′), 4.60 (m, 1H, H-1 cyclobutyl), 6.26 (t, 1H, $J$ = 6.9 Hz, H-1′), 8.39 (s, 1H, H-8), 8.61 (m, 1H, NH). Anal. ($C_{14}H_{18}ClN_5O_3$) C, H, N.

**10c:** $^1$H NMR ($Me_2SO$-$d_6$) $\delta$ 1.48−1.79 (m, 6H, H cyclopentyl), 1.94 (m, 2H, H cyclopentyl), 2.29 (m, 1H, H-2′), 2.64 (m, 1H, H-2″), 3.57 (m, 2H, $CH_2$-5′), 3.87 (m, 1H, H-4′), 4.40 (m, 1H, H-3′ and H-1 cyclopentyl), 6.28 (t, 1H, $J$ = 6.8 Hz, H-1′), 8.31 (s, 1H, NH), 8.37 (s, 1H, H-8). Anal. ($C_{15}H_{20}ClN_5O_3$) C, H, N.

**10e:** $^1$H NMR ($Me_2SO$-$d_6$) $\delta$ 1.43−1.78 (m, 10H, H cycloheptyl), 1.87 (m, 2H, H cycloheptyl), 2.28 (m, 1H, H-2′), 2.63 (m, 1H, H-2″), 3.56 (m, 2H, $CH_2$-5′), 3.86 (m, 1H, H-4′), 4.17 (m, 1H, H-1 cycloheptyl), 4.38 (m, 1H, H-3′), 6.26 (t, 1H, $J$ = 7.3 Hz, H-1′), 8.24 (d, 1H, $J$ = 8.4 Hz, NH), 8.36 (s, 1H, H-8). Anal. ($C_{17}H_{24}ClN_5O_3$) C, H, N.

**10f:** $^1$H NMR ($Me_2SO$-$d_6$) $\delta$ 1.40−1.90 (m, 14H, H cyclooctyl), 2.30 (m, 1H, H-2′), 2.63 (m, 1H, H-2″), 3.57 (m, 2H, $CH_2$-5′), 3.87 (m, 1H, H-4′), 4.25 (m, 1H, H-1 cyclooctyl), 4.39 (m, 1H, H-3′), 6.27 (t, 1H, $J$ = 6.8 Hz, H-1′), 7.34 (m, 4H, H−Ph), 7.83 (d, 2H, H−Ph), 7.97 (d, 2H, H−Ph), 8.25 (d, 1H, $J$ = 8.4 Hz, NH), 8.36 (s, 1H, H-8). Anal. ($C_{18}H_{26}ClN_5O_3$) C, H, N.

**Preparation of 6-Cycloalkylamino-9-(2-deoxy-*β*-D-*erythro*-pentofuranosyl)-9*H*-purines (2′-Deoxy-*N*-cycloalkyladenosines 11a−f).** To a solution of **10a**−**f** (0.5 mmol) in 40 mL of ethanol and 1 mL of 2 N NaOH was added 0.1 g of 10% Pd/C, and the mixture was shaken with hydrogen at 45 psi and for the time listed in Table 2. The catalyst was removed, and the filtrate was concentrated to dryness. The residue was chromatographed on a silica gel column eluting with the suitable mixture of solvents to give **11a**−**f** (Table 2) as chromatographically pure solids. Compounds **11c** and **11d** were already described elsewhere.[11]

**11a:** $^1$H NMR ($Me_2SO$-$d_6$) $\delta$ 0.64 (m, 2H, cyclopropyl), 0.74 (m, 2H, H cyclopropyl), 2.29 (m, 1H, H-2′), 2.74 (m, 1H, H-2″), 3.08 (m, 1H, H-1 cyclopropyl), 3.57 (m, 2H, $CH_2$-5′), 3.90 (m, 1H, H-4′), 4.43 (m, 1H, H-3′), 6.37 (t, 1H, $J$ = 6.2 Hz, H-1′), 7.97 (d, 1H, $J$ = 4.1 Hz, NH), 8.25 (s, 1H, H-2), 8.35 (s, 1H, H-8). Anal. ($C_{13}H_{17}N_5O_3$) C, H, N.

**11b:** $^1$H NMR ($Me_2SO$-$d_6$) $\delta$ 1.80 (m, 2H, H cyclobutyl), 2.04−2.35 (m, 5H, H cyclobutyl and H-2′), 2.69 (m, 1H, H-2″), 3.59 (m, 2H, $CH_2$-5′), 3.90 (m, 1H, H-4′), 4.42 (m, 1H, H-3′), 4.73 (m, 1H, H-1 cyclobutyl), 6.36 (t, 1H, $J$ = 6.6 Hz, H-1′), 8.08 (d, 1H, $J$ = 1.9 Hz, NH), 8.19 (s, 1H, H-2), 8.36 (s, 1H, H-8). Anal. ($C_{14}H_{19}N_5O_3$) C, H, N.

**11e:** $^1$H NMR ($Me_2SO$-$d_6$) $\delta$ 1.25−1.75 (m, 10H, H cycloheptyl), 1.89 (m, 2H, H cycloheptyl), 2.28 (m, 1H, H-2′), 2.73 (m, 1H, H-2″), 3.57 (m, 2H, $CH_2$-5′), 3.89 (m, 1H, H-4′), 4.31 (m, 1H, H-1 cycloheptyl), 4.41 (m, 1H, H-3′), 6.35 (t, 1H, $J$ = 6.2 Hz, H-1′), 7.66 (d, 1H, $J$ = 8.5 Hz, NH), 8.20 (s, 1H, H-2), 8.34 (s, 1H, H-8). Anal. ($C_{17}H_{25}N_5O_3$) C, H, N.

**11f:** $^1$H NMR ($Me_2SO$-$d_6$) $\delta$ 1.40−1.85 (m, 14H, H cyclooctyl), 2.28 (m, 1H, H-2′), 2.75 (m, 1H, H-2″), 3.60 (m, 2H, $CH_2$-5′), 3.89 (m, 1H, H-4′), 4.41 (m, 1H, H-3′ and H-1 cyclooctyl), 6.36 (t, 1H, $J$ = 6.0 Hz, H-1′), 7.62 (d, 1H, $J$ = 8.5 Hz, NH), 8.10 (s, 1H, H-2), 8.33 (s, 1H, H-8). Anal. ($C_{18}H_{27}N_5O_3$) C, H, N.

**Biological Assays. Adenosine Receptor Subtype Selectivity.** $A_1$ selectivity of derivatives was assessed essentially as described.[16] Membranes from rat brain cortex were prepared as described by Lohse et al.,[27] but incubated with adenosine deaminase before storage, as described by Pirovano et al.[28] Inhibition of [$^3$H]DPCPX binding to $A_1$ adenosine receptors in rat cortical membranes was performed according to Lohse et al.[29] For determination of affinity to $A_{2A}$ adenosine receptors, membranes from rat striatum were used. Membranes from rat striata were prepared as described by Bruns et al.[30] and pretreated with adenosine deaminase before storage. The method of binding of the $A_{2A}$-selective agonist [$^3$H]CGS 21680 followed the protocol of Jarvis et al.[31] $K_i$ values are the means of three independent experiments.

**Characterization of Binding of Adenosine Derivatives to High- and Low-Affinity States of the $A_1$ Adenosine Receptor.** Rat brain membranes were prepared as described.[32] [$^3$H]DPCPX binding (0.2 nM) to rat brain membranes was performed in 50 mM Tris-HCl buffer pH 7.4 in a total volume of 500 $\mu$L containing 40 $\mu$g of membrane protein, 0.5 units/mL adenosine deaminase, 0.02% 3-([3-cholamidopropyl]-dimethylammonio)-1-propanesulfonate (CHAPS), and increasing concentrations of unlabeled CPA, CCPA, or 2′-deoxyadenosine derivatives (**10a**−**f**, **11a**−**f**). The assays were performed both in the absence and presence of 1 mM GTP. Incubations were carried out for 3 h at 25 °C and were terminated by rapid filtration through Whatman GF/B glass fiber filters. Tubes and filters were washed twice with 4 mL of 50 mM Tris-HCl buffer pH 7.4, containing 0.02% CHAPS. All results are from three to seven independent experiments.

**[$^{35}$S]GTP$\gamma$S Binding to Rat Brain Membranes.** Stimulation of [$^{35}$S]GTP$\gamma$S binding to rat brain membranes by adenosine $A_1$ receptor activation was carried out as described previously.[8,15] The intrinsic activities of compounds under study are given as relative intrinsic activities (RIA) with reference to CCPA as a standard full agonist with its intrinsic activity set as 100%. Potencies and intrinsic activities of agonists are from 3 to 12 independent experiments for each compound.

**Data Analysis.** Apparent $K_i$ values for $A_1$ or $A_{2A}$ receptor binding were calculated from displacement curves of [$^3$H]-DPCPX and [$^3$H]CGS 21680 by nonlinear regression with Prism (Graph Pad, San Diego, CA). In a more detailed study of binding of 2′-deoxyadenosine derivatives to high- and low-affinity states of the $A_1$ receptor, competition curves were analyzed with SCTFIT.[33] All competition curves were analyzed according to one- and two-state models. $K_i$ values are affinities calculated assuming only a single affinity state. $K_H$ values are the affinities for the high-affinity state, $K_L$ values are the affinities for the low-affinity state of the $A_1$ adenosine receptor calculated from nonlinear curve fitting according to a two-state model. Curves were fitted to a one-state model only if fitting to a two-state model did not improve the fit significantly ($P$ < 0.05). $K_i$, $K_H$, and $K_L$ values are geometric means and are reported with 95% confidence limits from three to seven independent experiments for each compound. Percentages of $A_1$ receptors in the high-affinity state (%$R_H$) are given as the arithmetic means with standard errors (SEM). GTP shifts were calculated from the ratios of $K_i$ values in the presence and in the absence of GTP and are arithmetic means ± SEM. $EC_{50}$ values for stimulation of [$^{35}$S]GTP$\gamma$S binding were calculated with Sigma Plot and are reported as geometric means with 95% confidence limits. Relative intrinsic activities are arithmetic means ± SEM.

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**Supporting Information Available:** Elemental analyses of new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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