5′-Substituted Adenosine Analogs as New High-Affinity Partial Agonists for the Adenosine A_1 Receptor

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5′-(Alkylthio)-, 5′-(methylseleno)-, and 5′-(alkylamino)-substituted analogues of N^6-cyclopentyladenosine (CPA) were synthesized in 30−50% overall yields. The affinities of these compounds for the adenosine A_1 and A_{2A} receptors were determined in rat brain membranes. The 5′-substituted CPA analogues proved selective for the adenosine A_1 receptors, displaying affinities in the nanomolar range. The compounds were also evaluated for their ability to stimulate [^{35}S]GTPγS binding, also in rat brain membranes. The K_i values in receptor binding studies corresponded well to the EC_{50} values thus obtained. Intrinsic activities of the compounds were tested in vitro by determining the GTP shift in receptor binding studies as well as the maximal binding of [^{35}S]GTPγS. It appeared that the 5′-thio and 5′-seleno derivatives in particular behaved as partial agonists.

Introduction

Extracellular adenosine has significant physiological activity. Through its interaction with adenosine receptors, the compound mediates a large variety of effects, e.g. on the cardiovascular, immune, and central nervous systems. Four subclasses of adenosine receptors have been identified: A_1, A_{2A}, A_{2B}, and A_3 receptors. Ligands for the adenosine receptors have a broad therapeutic potential because of the wide organ and tissue distribution of adenosine receptor subtypes.[1−3] Agonists for the adenosine receptors could, for example, be useful as sedatives, as platelet aggregation inhibitors, and in the diagnosis of diseases of coronary arteries.[4] However, severe cardiovascular side effects can be expected, caused by the strong hypotensive effects of the adenosine agonists.[5−7] These side effects are major drawbacks in the therapeutic use of adenosine receptor agonists. Partial agonists could have less pronounced cardiovascular effects and may act more selectively.[8−15] Another advantage of partial agonists would be that they probably induce less receptor downregulation and desensitization.

Previously we have synthesized partial agonists for the adenosine A_1 receptors by substituting the 8-position of adenosine agonists,[11,14,16] or by removing the 2′- or 3′-hydroxyl groups from the ribose ring (Figure 1).[12,13] For both types of partial agonists, the loss in affinity by the modification had to be compensated for by N^6-substitution.

Replacing the 5′-hydroxyl group with substituents such as an (alkylcarbonyl)amino group has led to potent agonists for the adenosine receptors, e.g. the nonselective NECA (5′-[*N*-[(ethylcarbonyl)amino]adenosine),[17,18] CGS 21680 (2-[[4-(2-carboxyethyl)phenethyl]amino]-5′-[*N*-(ethylcarbonyl)amino]adenosine), selective for the adenosine A_{2A} receptor,[19] and the adenosine A_3-selective ligand IB-MECA (N^6-(3-iodobenzyl)-5′-[(methylcarbonyl)amino]adenosine.[20] However, results obtained for 5′-methylthio substituents are somewhat ambiguous. Daly and Padgett claimed that 5′-(methylthio)adenosine and 2-fluoro-5′-(methylthio)adenosine are full agonists for the adenosine A_1 receptor and partial agonists on A_{2A} receptors,[21] whereas Taylor et al. described 5′-(methylthio)-*R*-PIA (5′-(methylthio)-N^6-*R*-1-phenyl-2-propyladenosine) as a partial agonist.[22] These data suggests that 5′-substituents could also affect the intrinsic activity of adenosine analogues, although the 5′-hydroxyl group itself, as present in the full agonist N^6-cyclopentyladenosine (CPA), does not appear essential for full intrinsic activity.[12,13]

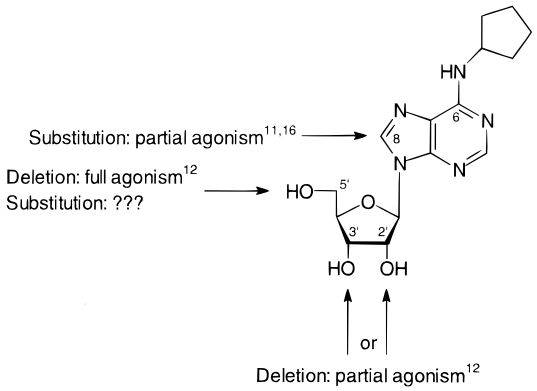

Figure 1. The N^6-cyclopentyl-substituted adenosine analogue CPA and the sites of substitution that can cause partial agonism.

In this paper the synthesis and testing are described of a series of CPA analogues modified at the 5′-position (Figure 1). Two in vitro systems were used to determine the intrinsic activities of the compounds at the adenosine A_1 receptor. The GTP (guanosine 5′-triphosphate) shift, i.e. the difference in affinity for the adenosine A_1

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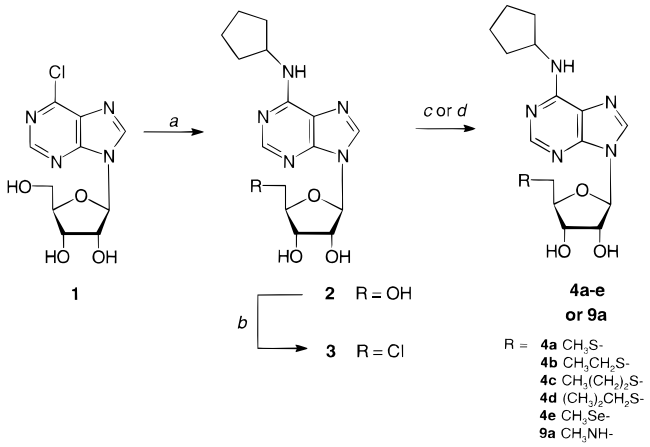
Scheme 1[a]



[a] (*a*) Cyclopentylamine/triethylamine/ethanol, 80 °C; (*b*) thionyl chloride/HMPA; (*c*) alkanethiol or alkaneselenol sodium salt/2 N NaOH, 80 °C; (*d*) methylamine, 2.5 bar.

receptor in rat brain in the presence and absence of GTP, and the maximal induction of [^{35}S]GTPγS (guanosine 5′-(γ-thio)triphosphate) binding to G proteins in the same membrane preparation by these compounds were determined.

Results and Discussion

Chemistry. The 5′-(alkylthio)-substituted analogues of CPA were synthesized in three steps, starting with 6-chloropurine riboside (**1**, Scheme 1).[23] CPA (**2**) was obtained by reacting **1** with cyclopentylamine in ethanol to substitute the chlorine atom. Subsequently, the 5′-hydroxyl group of CPA was replaced by a chlorine atom as leaving group, in a reaction with thionyl chloride in hexamethylphosphoramide (HMPA). A reaction between 5′-chloro-CPA (**3**) and the required alkanethiol or -selenol sodium salts in 2 N NaOH yielded the final products. The overall yields of compounds **4a**−**e** were between 30% and 50%. Thus, Scheme 1 describes a feasible route for the synthesis of 5′-alkylthio and 5′-alkylseleno analogues of N^6-substituted adenosines.

The main route for the synthesis of the 5′-alkylamino analogues of CPA is visualized in Scheme 2, as attempts to prepare analogue **9c** via Scheme 1 failed. To obtain a 5′-group that is selectively reactive with respect to the other two (2′- and 3′-) hydroxyl groups, the 5′-position of CPA (**2**) was selectively protected with a *tert*-butyldimethylsilyl group (**5**) to benzoylate the 2′- and 3′-hydroxyl groups (**6**). Deprotecting the 5′-position of this compound (**7**) and subsequent reaction with tosyl chloride yielded a CPA analogue with a good leaving group at the 5′-position (**8**). A reaction between **8** and alkylamines gave the three 5′-alkylamino analogues of CPA (**9a**−**c**). These compounds were synthesized in six steps with an overall yield of approximately 30%.

Attempts to prepare **4a** by a direct reaction of the 5-hydroxyl group with dimethyl disulfide and tributylphosphine, as was carried out with adenosine[24] and *R*-PIA,[21] were unsuccessful. The 5′-methylthio group could also be introduced following the route of Scheme 2 and substituting the tosyl moiety of **8**,[25] yielding 15% of **4a**. However, the high reactivity of the tosyl derivative caused ring closure by a reaction between the ribose 5′-position and N3 in the adenine ring.[26] Therefore replacement of the tosyl moiety by the less reactive iodine and subsequent substitution by a methylthio group without isolation of the intermediate was tried. This route yielded 10% of **4a**.

5′-(Methylamino)-CPA (**9a**) could also be synthesized in three steps via 5′-chloro-CPA (**3**) in a yield of 40%.[28] It was necessary to use pure methylamine to replace the 5′-chloro substituent of **3**, because trials with a 40% solution of methylamine in water failed. The facile preparation of the starting material and the occurrence of many side products in Scheme 2 made this latter route preferable, although the reaction of the chlorinated intermediate **3** with methylamine was much slower than that of the tosylated CPA analogue **8**.[29]

Pharmacology. (A) Affinity. The compounds were tested in radioligand binding studies to determine their affinities for the rat adenosine A_1 and A_{2A} receptors. The K_i values of the 5′-substituted CPA analogues for the adenosine A_1 receptor were in the nanomolar to micromolar range (Table 1). The 5′-(alkylthio)-substituted CPA analogues (**4a**−**d**) had affinities that were independent of chain length. Replacing the sulfur atom by a larger seleno atom did not affect the affinity; there was no difference in the K_i value of 5′-(methylthio)- (**4a**) and 5′-(methylseleno)-CPA (**4e**). The 5′-alkylamino analogues (**9a**−**c**) had lower affinities than those with 5′-alkylthio substituents (**4a**−**c**), and the affinities of alkylamino-substituted compounds appeared to decrease with increasing chain length. This lower affinity for the adenosine A_1 receptor might be due to protonation of the amino groups at physiological pH. Apparently, the receptor does not easily accommodate a positive charge in the 5′-region.

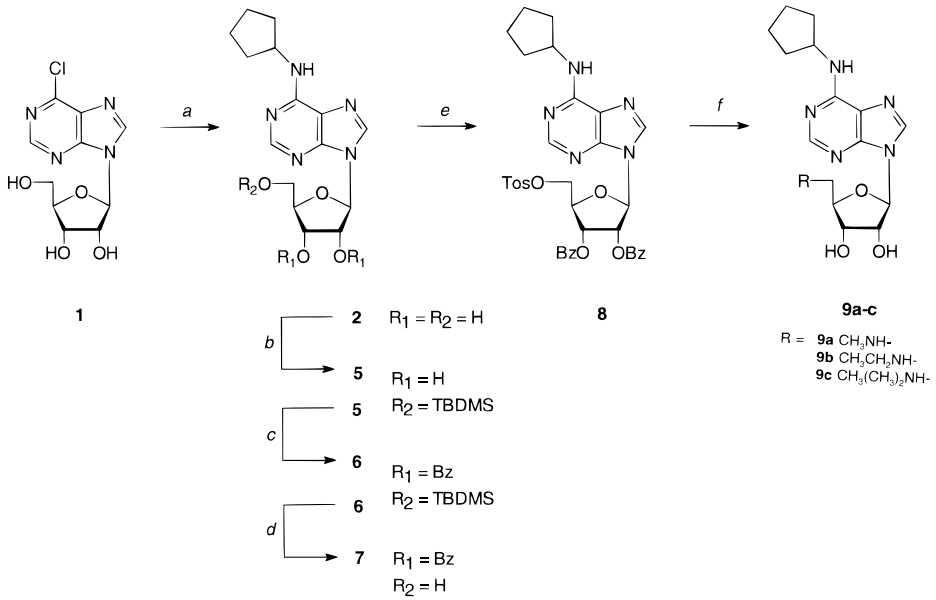
The 5′-substituted CPA analogues had only micromolar K_i values for the adenosine A_{2A} receptor. The 5′-substituted CPA analogues are therefore selective for the adenosine A_1 vs the A_{2A} receptor, probably due to the presence of the N^6-cyclopentyl substituent.

In addition, potencies of the various compounds on the adenosine A_1 receptor were determined in a [^{35}S]-GTPγS binding assay on rat brain tissue. The EC_{50} values of the CPA analogues were also in the nanomolar to micromolar range (Table 2). The potencies of the 5′-alkylthio analogues increased 3-fold with increasing chain length from methyl (**4a**) to *n*-propyl (**4c**). The EC_{50} values of the 5′-(alkylamino)-substituted compounds (**9a** and **9c**) increased 10-fold over the same range. The EC_{50} value of 5′-(methylseleno)-CPA (**4e**) was slightly higher than that of 5′-(methylthio)-CPA (**4a**).

All in all, there is a good correlation between the K_i values from binding experiments on the adenosine A_1 receptor (both in the presence and absence of GTP) and the EC_{50} values for [^{35}S]GTPγS binding ($r^2 = 0.98$ and $r^2 = 0.98$, respectively).

(B) Intrinsic Activity. The full agonist CPA (**2**) had a GTP shift of 6 (Table 1).[12] The GTP shifts of 5′-chloro-CPA (**3**) and 5′-(methylamino)-CPA (**9a**) equal that of CPA, which means that these compounds behave as full agonists in binding studies. All other compounds had GTP shifts that were lower than that of the full agonist CPA, but higher than unity, the value generally found for an antagonist. This indicates that these compounds could be partial agonists for the adenosine A_1 receptor.

Scheme 2[a]



[a] (*a*) Cyclopentylamine/triethylamine/ethanol, 80 °C; (*b*) TBDMS-Cl/pyridine; (*c*) benzoic anhydride/*N*-methylimidazole/pyridine; (*d*) tetrabutylammoniumfluoride/dioxane; (*e*) tosyl chloride/4-DMAP/CH_2Cl_2; (*f*) alkylamine, 2.5 bar.

Table 1. Affinities (apparent K_i Values in the Absence and Presence of GTP) and GTP Shifts of 5′-Substituted CPA Analogues ($n = 3$ ± SEM, or $n = 2$, Both Values Given)

no.	5′-R	K_i A_1[a] − GTP (nM)	K_i A_1[a] + GTP (nM)	GTP shift	K_i A_{2A}[b] (μM)
2	OH	5.90 (*5.78−6.02*)	35.2 (*29.7−40.7*)	6.0 (*5.1−6.8*)	0.58 ± 0.12
3	Cl	9.5 ± 3.2	61.5 (*34−89*)	5.6 (*4.4−6.8*)	8.75 ± 1.04
4a	SCH_3	59 ± 22	199 (*119−279*)	2.9 (*2.5−3.3*)	13.5 ± 1.7
4b	SCH_2CH_3	45 ± 8	130 (*102−158*)	2.9 (*2.9−3.0*)	19.0 ± 12.1
4c	$S(CH_2)_2CH_3$	76 ± 27	222 (*171−272*)	2.9 (*2.6−3.3*)	18.8 ± 6.8
4d	$SCH(CH_3)_2$	41 ± 9	149 ± 9	3.7 (*3.1−4.5*)	10.3 ± 2.8
4e	$SeCH_3$	76 ± 9	166 (*155−177*)	2.1 (*2.1−2.2*)	11.3 ± 2.6
9a	$NHCH_3$	440 ± 190	2600 ± 140	6.9 ± 3.9	45.5 (*41−50*)
9b	$NHCH_2CH_3$	2410 ± 1440	8560 ± 3460	4.2 ± 1.6	42.5 (*26−59*)
9c	$NH(CH_2)_2CH_3$	4640 ± 1990	14300 ± 3200	3.9 ± 3.0	14.5 (*12−17*)

[a] Displacement of [^{3}H]DPCPX from rat cortical membranes. [b] Displacement of [^{3}H]CGS 21680 from rat striatal membranes.

Table 2. EC_{50} values (95% Confidence Intervals in Parentheses) of 5′-Substituted CPA Analogues and Their Maximum Level of G Protein Activation (±SEM) As Determined by [^{35}S]GTPγS Binding to Rat Brain Membranes[a] ($n = 3-9$)

no.	5′-R	EC_{50} (nM)	intrinsic activity (%)
2	OH	14.3 (*14.1−14.4*)	100
3	Cl	26.0 (*24.4−27.6*)	88.0 ± 2.1
4a	SCH_3	99.0 (*70.2−139*)	40.2 ± 0.5
4b	SCH_2CH_3	127 (*124−131*)	65.6 ± 1.3
4c	$S(CH_2)_2CH_3$	384 (*365−403*)	84.1 ± 1.8
4d	$SCH(CH_3)_2$	189 (*156−229*)	85.3 ± 2.7
4e	$SeCH_3$	225 (*187−271*)	50.3 ± 0.4
9a	$NHCH_3$	1330 (*1230−1440*)	96.4 ± 0.6
9b	$NHCH_2CH_3$	−[b]	−[b]
9c	$NH(CH_2)_2CH_3$	13900 (*11000−17700*)	73.0 ± 3.6

[a] Binding of 0.3 nM [^{35}S]GTPγS to rat brain membranes. [b] −, not determined.

All compounds except 5′-(methylamino)-CPA (**9a**) are partial agonists in comparison to the [^{35}S]GTPγS binding induced by CPA (Table 2), and there is some correlation between the GTP shifts of the compounds and their maximal [^{35}S]GTPγS binding ($r^2 = 0.596$). Even the maximal effect of 5′-chloro-CPA (**3**) is only 88% of that of CPA (**2**). Steric hindrance cannot be an explanation for the submaximal activities of the 5′-substituted analogues, because the intrinsic activity of the alkylthio-substituted CPA analogues decreases with decreasing chain length. The two isosteres 5′-(*n*-propylthio)-CPA (**4c**) and 5′-(isopropylthio)-CPA (**4d**) have equal intrinsic activities, although these compounds differ 2-fold in their EC_{50} values. The maximal effect of 5′-(methylseleno)-CPA (**4e**, 50%) is slightly higher than that of 5′-(methylthio)-CPA (**4a**, 40%). Furthermore, the intrinsic activities of the 5′-(alkylamino)-substituted analogues **9a** and **9c** are higher than those of the corresponding alkylthio analogues **4a** and **4c**. The partial agonism must therefore be caused by more indirect effects. It should be mentioned here that in the current experimental setup it is impossible to study intrinsic activity on adenosine A_{2A} receptors. The lack of a suitable, commercially available, radiolabeled antagonist precludes the determination of GTP shifts. Second, the GTPγS assay does not work for G_s-coupled receptors such as the adenosine A_{2A} receptor.

We compared the present results with earlier ones obtained with other partial agonists for the adenosine A_1 receptor to try to find an explanation for the decreases in intrinsic activity by 5′-substitution. Previously we have synthesized 2′-, 3′-, and 5′-deoxy analogues of CPA.[12] The first two compounds were partial agonists for the adenosine A_1 receptor,[12,13] indicating that the 2′- and 3′-hydroxyl groups are essential for

affinity and intrinsic activity. However, removal of the 5′-hydroxyl group affected only the affinity and still yielded a full agonist for the adenosine A_1 receptor.[12,13] The 5′-hydroxyl group itself, therefore, appears not important for full intrinsic activity. The reduced intrinsic activity of the 5′-substituted CPA analogues in this study is therefore not due to the absence of a hydroxyl group at the 5′-position, but probably to more subtle changes such as the influence of the 5′-substituent on, for example, puckering of the ribose sugar.[11,14,16] Changes in puckering influence the orientation and position of the 2′- and 3′-hydroxy groups in the ligand binding site of the receptor.

Conclusions

5′-(Alkylthio)-, 5′-(alkylseleno)-, and 5′-(alkylamino)-substituted analogues of CPA were obtained in 30−50% overall yields. These compounds proved selective for the rat adenosine A_1 over the adenosine A_{2A} receptors. A strong correlation was found between the adenosine A_1 affinities in receptor binding studies and EC_{50} values for [^{35}S]GTPγS binding. GTP shifts and maximal effects in [^{35}S]GTPγS binding, as measures for intrinsic activity, demonstrated that most of the 5′-substituted CPA analogues behave as partial agonists in rat brain membranes. It can therefore be concluded that although the 5′-hydroxyl group itself is not essential for intrinsic activity, 5′-substitution with certain substituents can lead to partial agonists. In this way we obtained novel partial agonists with varying extents of intrinsic activity for the adenosine A_1 receptor.

Experimental Section

GTP and cyclopentylamine were obtained from Aldrich (Brussels, Belgium). Bovine serum albumin (BSA) was purchased from Sigma (St. Louis, MO). Adenosine deaminase (ADA) and GDP (guanosine 5′-diphosphate) were obtained from Boehringer Mannheim (Mannheim, Germany).

[^{3}H]DPCPX (1,3-dipropyl-8-cyclopentylxanthine, specific activity 108 Ci/mmol) and [^{3}H]CGS 21680 (specific activity 38.3 Ci/mmol) were purchased from NEN, Du Pont Nemours ('s-Hertogenbosch, The Netherlands). [^{35}S]GTPγS (1000−1500 Ci/mmol) was purchased from New England Nuclear (Bad Homburg, Germany). BCA (bicinchoninic acid) and BCA protein assay reagent were obtained from Pierce Chemical Co. (Rockford, IL). All other chemicals were from standard commercial sources and of analytical grade.

Synthesis. Thin-layer chromatography (TLC) was carried out using silica F_{254} preformed layers 0.1 mm thick on a plastic backing (Schleicher and Schüll DC Fertigfolien F1500 LS254). Spots were visualized either under UV light (254 or 356 nm) or by spraying with sulfuric acid/methanol (1:4) or molybdate reagent (H_2O/concentrated H_2SO_4/$(NH_4)_6Mo_7O_{24}\cdot 4H_2O$/$(NH_4)_2Ce(SO_4)_4\cdot 2H_2O$, 90/10/2.5/1, v/v/w/w) and charring at 140 °C for a few minutes. Preparative column chromatography was performed on silica gel (230−400 mesh ASTM), suspended in CH_2Cl_2. Melting points were determined in a Büchi capillary melting point apparatus and are uncorrected.

^{1}H NMR spectra of the intermediates and ^{13}C NMR spectra were recorded on a JEOL JNM-FX 200 (200 MHz) spectrometer at ambient temperature; the ^{1}H NMR spectra of the end products were determined with a Brucker WM 300 spectrometer (300 MHz), and the peaks were assigned by selective decoupling. Chemical shifts for ^{1}H and ^{13}C NMR are given in ppm (δ) relative to tetramethylsilane (TMS) as internal standard. All signals assigned to amino and hydroxyl groups were exchangeable with CD_3OD.

Mass spectra were recorded with a Finnigan MAT TSQ 70 or SSQ 710 or a MAT 900, the first two using an electrospray interface (EI), the latter with a direct insertion probe (DIP). EI and atmosferic pressure ionization (API) were used as ionization methods.

Elemental analysis was done at the Department of Microanalysis, Groningen University, The Netherlands.

N^6-Cyclopentyladenosine (CPA) (**2**)[30−32] and *5′*-chloro-5′-deoxy-CPA (**3**)[23] were synthesized according to literature procedures.

General Procedure for the Synthesis of 5′-(alkylthio)-, and 5′-(alkylseleno)-5′-deoxy Analogues of CPA (4a−e).[32] Compound **3** (100 mg, 0.28 mmol) was dissolved in 2.5 mL of 2 N NaOH. To this solution a 10-fold excess of alkanethiol sodium salt dissolved in 1 mL of water was added, and the mixture was stirred at 80 °C for 1 h and then neutralized with acetic acid. The precipitate was filtered off, while the mother liquors were concentrated and purified by column chromatography (eluent: CH_2Cl_2 → 4% MeOH/CH_2Cl_2).

5′-(Methylthio)-5′-deoxy-CPA (4a): yield 50 mg (0.137 mmol), 49%; mass EI *m*/*z* 366 (MH^+); T_m 65−70 °C (lit.[33] 50−60 °C); R_f (10% MeOH/CH_2Cl_2) 0.35; ^{1}H NMR ($CDCl_3$) δ 8.27 (s, 1H, H8), 7.98 (s, 1H, H2), 6.5 (bs, 1H, OH2′ or OH3′), 6.01 (d, J = 6.74 Hz, 1H, N^{6}H), 5.95 (d, $J_{1',2'}$ = 5.26 Hz, 1H, H1′), 4.67−4.64 (m, 2H, H2′/*H*CN^6), 4.45−4.40 (m, 2H, H3′/H4′), 4.1 (bs, 1H, OH2′ or OH3′), 2.87−2.83 (m, 2H, H5′/H5″), 2.17 (s, 3H, H_3CS), 2.14−2.07 (m, 2H, H2$_{\alpha-\text{cyclopentyl}}$/H5$_{\alpha-\text{cyclopentyl}}$), 1.80−1.50 (m, 6H, H2$_{\beta-\text{cyclopentyl}}$/H5$_{\beta-\text{cyclopentyl}}$/H3$_{\text{cyclopentyl}}$/H4$_{\text{cyclopentyl}}$). Anal. ($C_{16}H_{23}N_5O_3S$) C, H, N.

5′-(Ethylthio)-5′-deoxy-CPA (4b): yield 44 mg (0.116 mmol), 41%; mass EI *m*/*z* 380 (MH^+); T_m 48−53 °C; R_f (10% MeOH/CH_2Cl_2) 0.35; ^{1}H NMR ($CDCl_3$) δ 8.27 (s, 1H, H8), 8.00 (s, 1H, H2), 6.11 (d, J = 6.34 Hz, 1H, N^{6}H), 5.96 (d, $J_{1',2'}$ = 5.14 Hz, 1H, H1′), 4.57 (pseudo-t, 2H, H2′/*H*CN^6), 4.43−4.36 (m, 2H, H3′/H4′), 2.93−2.81 (dd, $J_{5',5''}$ = 14.0 Hz, $J_{4',5'}$ = 5.57 Hz, 2H, H5′/H5″), 2.60 (q, J_{CH_2,CH_3} = 7.42 Hz, 2H, H_2CS), 2.16−2.06 (m, 2H, H2$_{\alpha-\text{cyclopentyl}}$/H5$_{\alpha-\text{cyclopentyl}}$), 1.79−1.50 (m, 6H, H2$_{\beta-\text{cyclopentyl}}$/H5$_{\beta-\text{cyclopentyl}}$/H3$_{\text{cyclopentyl}}$/H4$_{\text{cyclopentyl}}$), 1.25 (t, J_{CH_2,CH_3} = 7.39 Hz, 3H, H_3CH$_2$CS). Anal. ($C_{17}H_{25}N_5O_3S$) C, H, N.

5′-(Propylthio)-5′-deoxy-CPA (4c): yield 50 mg (0.127 mmol), 45%; mass EI *m*/*z* 394 (MH^+); T_m 53−57 °C; R_f (10% MeOH/CH_2Cl_2) 0.4; ^{1}H NMR ($CDCl_3$) δ 8.26 (s, 1H, H8), 8.00 (s, 1H, H2), 6.18 (d, J = 7.59 Hz, 1H, N^{6}H), 5.99 (d, $J_{1',2'}$ = 5.01 Hz, 1H, H1′), 4.62 (pseudo-t, 2H, H2′/*H*CN^6), 4.39−4.36 (m, 2H, H3′/H4′), 2.88−2.85 (m, 2H, H5′/H5″), 2.61−2.51 (m, 2H, H_2CS), 2.12−2.08 (m, 2H, H2$_{\alpha-\text{cyclopentyl}}$/H5$_{\alpha-\text{cyclopentyl}}$), 1.76−1.52 (m, 8H, H2$_{\beta-\text{cyclopentyl}}$/H5$_{\beta-\text{cyclopentyl}}$/H3$_{\text{cyclopentyl}}$/H4$_{\text{cyclopentyl}}$/H$_3$C*H*$_2CH_2$CS), 0.95 (t, 3H, H_3C(H$_2$C)$_2$S). Anal. ($C_{18}H_{27}N_5O_3S$) C, H, N.

5′-(Isopropylthio)-5′-deoxy-CPA (4d): yield 40 mg (0.102 mmol), 36%; mass EI *m*/*z* 394 (MH^+); T_m 52−58 °C; R_f (10% MeOH/CH_2Cl_2) 0.4; ^{1}H NMR ($CDCl_3$) δ 8.30 (s, 1H, H8), 7.96 (s, 1H, H2), 6.5 (bs, 1H, OH), 5.90 (d + bs, $J_{1',2'}$ = 5.61 Hz, 2H, H1′/N^{6}H), 4.58−4.53 (m, 2H, H2′/*H*CN^6), 4.47−4.38 (m, 2H, H3′/H4′), 3.00−2.94 (m, 2H, H5′/H5″), 2.86−2.81 (m, 1H, HCS), 2.18−2.10 (m, 2H, H2$_{\alpha-\text{cyclopentyl}}$/H5$_{\alpha-\text{cyclopentyl}}$), 1.80−1.53 (m, 6H, H2$_{\beta-\text{cyclopentyl}}$/H5$_{\beta-\text{cyclopentyl}}$/H3$_{\text{cyclopentyl}}$/H4$_{\text{cyclopentyl}}$), 1.29−1.25 (m, 7H, (H$_3$C)$_2$HCS). Anal. ($C_{18}H_{27}N_5O_3S$) C, H, N.

5′-(Methylselenol)-5′deoxy-CPA (4e): yield 60 mg (0.145 mmol), 52%; mass API *m*/*z* 414 (MH^+); T_m 66−72 °C. R_f (10% MeOH/CH_2Cl_2), 0.35; ^{1}H NMR ($CDCl_3$) δ 8.22 (s, 1H, H8), 7.89 (s, 1H, H2), 5.87 (d + bs, $J_{1',2'}$ = 5.56, 2H, H1′/N^{6}H), 4.55−4.44 (m, 2H, H2′/*H*CN^6), 4.42−4.29 (m, 2H, H3′/H4′), 2.79−2.73 (q, $J_{4',5'/5''}$ = 6.41 Hz, 2H, H5′/H5″), 2.10−1.97 (m + s, 5H, H2$_{\alpha-\text{cyclopentyl}}$/H5$_{\alpha-\text{cyclopentyl}}$/$H_3$CSe), 1.75−1.46 (m, 6H, H2$_{\beta-\text{cyclopentyl}}$/H5$_{\beta-\text{cyclopentyl}}$/H3$_{\text{cyclopentyl}}$/H4$_{\text{cyclopentyl}}$). Anal. ($C_{16}H_{23}N_5O_3Se$) C, H, N.

5′-(Methylamino)-5′-deoxy-CPA (9a).[28] Compound **3** (50 mg, 0.142 mmol) was dissolved in dry and pure methylamine and stirred at room temperature under pressure (2.5 bar). After 3 days the methylamine was allowed to evaporate at room temperature, and the residue was purified by column chromatography (eluent: CH_2Cl_2 → 20% MeOH/CH_2Cl_2) to yield 20 mg of starting material and 25 mg of **9a**: yield 25 mg (0.072 mmol), 51%; mass EI *m*/*z* 349 (MH^+); T_m decomposition 200 °C; R_f (15% MeOH/CH_2Cl_2) 0.15; ^{1}H NMR (DMSO-d_6) δ

8.33 (s, 1H, H8), 8.22 (s, 1H, H2), 7.79−7.75 (m, 1H, N^6H), 7.46 (d, $J_{o,m}$ = 8.01 Hz, 2H, $H_{o-tosyl}$), 7.09 (d, 2H, $J_{o,m}$ = 8.04 Hz, $H_{m-tosyl}$), 5.93 (d, $J_{1',2'}$ = 5.69 Hz, 1H, H1′), 5.62 (d, 1H, OH), 5.47 (d, 1H, OH), 4.70 (q, $J_{1',2'}$ = 5.48, 1H, H2′), 4.50 (bs, 1H, *H*CN^6), 4.19−4.12 (m, 2H, H3′/H4′), 2.50 (s, 3H, H_3CNH), 2.27 (s, 3H, H_3C_{tosyl}), 1.95−1.89 (m, 2H, $H2_{\alpha-cyclopentyl}$/$H5_{\alpha-cyclopentyl}$), 1.71−1.52 (m, 6H, $H2_{\beta-cyclopentyl}$/$H5_{\beta-cyclopentyl}$/$H3_{cyclopentyl}$/$H4_{cyclopentyl}$). Anal. ($C_{16}H_{24}N_6O_3 \cdot C_7H_8O_3S$) C, H, N.

5′-*O*−(*tert*-Butyldimethylsilyl)-CPA (5).[34] Compound **2** (740 mg, 2.21 mmol) was coevaporated twice and dissolved in 4.5 mL of dry pyridine. To this solution was added 2.43 mmol (0.35 g) of TBDMS-Cl (*tert*-butyldimethylsilyl chloride), and the mixture was stirred at room temperature. After 2 h the reaction was quenched with MeOH and the mixture was evaporated to dryness. The residue was purified by column chromatography (eluent: CH_2Cl_2 → 4% MeOH/CH_2Cl_2) to yield **5**: yield 790 mg (1.76 mmol), 80%; mass: EI *m*/*z* 450 (MH^+); R_f (4% MeOH/CH_2Cl_2) 0.45; ^{1}H NMR ($CDCl_3$) δ 8.23 (s, 1H, H8), 8.07 (s, 1H, H2), 6.06 (d, $J_{6,1''}$ = 8.15 Hz, 1H, N^6H), 6.03 (d, $J_{1',2'}$ = 4.96 Hz, 1H, H1′), 4.50 (bs, 1H, CH−N^6H), 4.51−4.47 (m, 1H, H2′), 4.42−4.40 (m, 1H, H3′), 4.40−4.30 (m, 1H, H4′), 3.92−3.78 (m, 2H, H5′/H5″), 2.13−2.07 (m, 2H, $H2_{\alpha-cyclopentyl}$/$H5_{\alpha-cyclopentyl}$), 1.77−1.51 (m, 6H, $H2_{\beta-cyclopentyl}$/$H5_{\beta-cyclopentyl}$/$H3_{cyclopentyl}$/$H4_{cyclopentyl}$), 0.80 (s, 9H, $(CH_3)_3$), 0.03 (s, 3H, H_3C−Si), 0.003 (s, H_3C−Si).

2′,3′-Di-*O*-benzoyl-5′-O-(*tert*-butyldimethylsilyl)-CPA (6). Compound **5** (790 mg, 1.76 mmol) was coevaporated twice and dissolved in 3.5 mL of dry pyridine. Benzoic anhydride (12 g, 5.30 mmol) and *N*-methylimidazole (0.44 mL, 5.30 mmol) were added to this solution, and the mixture was stirred at room temperature for 90 min, then quenched with MeOH, and concentrated to dryness. The residue was dissolved in CH_2Cl_2 (20 mL) and washed with a 10% solution of $NaHCO_3$ (5 mL) and with water (5 mL). The organic layer was dried over magnesium sulfate, filtered, and concentrated to dryness. The product was purified by column chromatography (eluent: CH_2Cl_2 → 2% MeOH/CH_2Cl_2), and **6** was obtained: yield 970 mg (1.48 mmol), 84%; mass EI *m*/*z* 658 (MH^+); R_f (4% MeOH/CH_2Cl_2) 0.67; ^{1}H NMR ($CDCl_3$) δ 8.40 (s, 1H, H8), 8.28 (s, 1H, H2), 8.13−7.90 (m, 5H, H_{arom}), 7.61−7.30 (m, 8H, H_{arom}), 6.65 (d, $J_{1',2'}$ = 7.00 Hz, 2H, H1′/N^6H), 6.04 (dd, $J_{1',2'}$ = 6.95 Hz, $J_{2',3'}$ = 5.46 Hz, 1H, H2′), 5.90 (dd, $J_{2',3'}$ = 5.39 Hz, $J_{3',4'}$ = 2.21 Hz, 1H, H3′), 4.68−4.62 (m, 1H, *H*CN^6), 4.55−4.53 (m, 1H, H4′), 4.06−4.05 (m, 2H, H5′/H5″), 2.17−2.06 (m, 2H, $H2_{\alpha-cyclopentyl}$/$H5_{\alpha-cyclopentyl}$), 1.82−1.60 (m, 6H, $H2_{\beta-cyclopentyl}$/$H5_{\beta\ cyclopentyl}$)/$H3_{cyclopentyl}$/$H4_{cyclopentyl}$), 1.01 (s, 9H, $(H_3C)_3$), 0.23 (s, 6H, $(H_3C)_2$-Si).

2′,3′-Di-*O*-benzoyl-CPA (7). Compound **6** (970 mg, 1.475 mmol) was coevaporated twice and dissolved in 3.5 mL of dry dioxane. Then, 3.5 mL of a 1 M solution of tetrabutylammonium fluoride in dioxane (1.25 equiv) was added, and the mixture was stirred for 30 min. The reaction was quenched with 1 mL of water, and the solvents were evaporated. The residue was dissolved in 15 mL of CH_2Cl_2 and washed with a 10% solution of $NaHCO_3$ (15 mL) and water (15 mL). The organic layer was dried over $MgSO_4$, filtered, and concentrated. Purification with column chromatography (eluent: CH_2Cl_2 → 3% MeOH/CH_2Cl_2) yielded **7**: yield 650 mg (1.20 mmol), 81%; mass EI *m*/*z* 544 (MH^+); R_f (4% MeOH/CH_2Cl_2) 0.55; ^{1}H NMR ($CDCl_3$) δ 8.40 (s, 1H, H8), 8.12 (s, 1H, H2), 8.10−7.20 (m, 10 H, H_{arom}), 7.10 (d, 1H, N^6H), 6.43 (dd, $J_{1',2'}$ = 7.72 Hz, $J_{2',3'}$ = 5.39 Hz, 1H, H2′), 6.24 (d, $J_{1',2'}$ = 7.71 Hz, H1′), 6.07 (d, $J_{2',3'}$ = 5.30 Hz, 1H, H3′), 5.88 (bs, 1H, OH5′), 4.61 (bs, 2H, H4′/*H*CN^6), 4.12−3.99 (m, 2H, H5′/H5″), 2.18−2.08 (m, 2H, $H2_{\alpha-cyclopentyl}$/$H5_{\alpha-cyclopentyl}$), 1.79−1.53 (m, 6H, $H2_{\beta-cyclopentyl}$/$H5_{\beta-cyclopentyl}$/$H3_{cyclopentyl}$/$H4_{cyclopentyl}$).

5′-Tosyl-2′,3′-di-*O*-benzoyl-CPA (8).[35] Compound **7** (650 mg, 1.197 mmol) was coevaporated twice with dry dioxane and dissolved in 6 mL of dry CH_2Cl_2. A solution of 0.6 g of 4-DMAP (4-(dimethylamino)pyridine, 4.36 mmol) and 0.86 g of tosyl chloride (3.90 mmol) in 7.5 mL of dry CH_2Cl_2 was added, and the mixture was stirred overnight at room temperature. The product was concentrated and dissolved in 30 mL of CH_2Cl_2, washed with 5% $NaHCO_3$ aqueous solution (3 × 10 mL) and water (3 × 10 mL), dried over $MgSO_4$, filtered, and concentrated. The crude material was purified by column chromatography (eluent: 50% hexane/ether → 3% MeOH/ether) to obtain **8**: yield 700 mg (1.00 mmol), 85%; mass EI *m*/*z* 698 (MH^+); T_m 95−100 °C; R_f (4% MeOH/CH_2Cl_2) 0.74; ^{1}H NMR ($CDCl_3$) δ 8.30 (s, 1H, H8), 8.00 (s, 1H, H2), 8.00−7.10 (m, 14H, H_{arom}), 6.39 (d, $J_{1',2'}$ = 5.35 Hz, 1H, H1′), 6.12 (pseudo-t, $J_{1',2';2',3'}$ = 5.56 Hz, 1H, H2′), 5.99 (dd, $J_{2',3'}$ = 5.58 Hz, $J_{3',4'}$ = 4.61 Hz, 1H, H3′), 5.82 (bp, 1H, N^6H), 4.66−4.62 (m, 4H, H4′/H5′/H5″/*H*CN^6), 2.38 (s, 3H, H_3C_{tosyl}), 2.17−2.09 (m, 2H, $H2_{\alpha-cyclopentyl}$/$H5_{\alpha-cyclopentyl}$), 1.80−1.43 (m, 6H, $H2_{\beta-cyclopentyl}$/$H5_{\beta-cyclopentyl}$/$H3_{cyclopentyl}$/$H4_{cyclopentyl}$).

5′-(Alkylamino)-5′-deoxy-CPA (9).[29] Compound **8** (130 mg, 0.186 mmol) was dissolved in 5 mL of pure and dry alkylamine and stirred overnight at room temperatur under pressure (2.5 bar). The day after, the alkylamine was allowed to evaporate at room temperature, and the residue was purified by column chromatography (eluent: CH_2Cl_2 → 20% MeOH/CH_2Cl_2). Three different products were isolated and analyzed; the less polar one (15 mg) appeared to be the debenzoylated starting material, the second—a very small amount—was probably the 4′,5′-didehydro-CPA as inferred from its ^{1}H NMR spectrum, and the most polar was the desired product obtained as a tosylate salt.

5′-(Methylamino)-5′-deoxyadenosine (9a): yield 46 mg (130 mmol), 70%; Analysis as before.

5′-(Ethylamino)-5′-deoxy-CPA (9b): yield 50 mg (0.140 mmol), 75%; mass EI *m*/*z* 363 (MH^+); T_m decomposition 230 °C; R_f (15% MeOH/CH_2Cl_2) 0.25; ^{1}H NMR (DMSO-d_6) δ 8.34 (s, 1H, H8), 8.21 (s, 1H, H2), 7.75 (d, J = 7.42 Hz, 1H, N^6H), 7.46 (d, $J_{o,m}$ = 7.99 Hz, 2H, $H_{o-tosyl}$), 7.09 (d, $J_{o,m}$ = 7.97 Hz, 2H, $H_{m-tosyl}$), 5.94 (d, $J_{1',2'}$ = 5.45 Hz, 1H, H1′), 5.62 (d, $J_{H,OH}$ = 5.75 Hz, 1H, OH2′ or OH3′), 5.47 (d, $J_{H,OH}$ = 4.48 Hz, 1H, OH2′ or OH3′), 4.71 (q, $J_{1',2'}$ = 5.44 Hz, 1H, H2′), 4.50 (bs, 1H, *H*CN^6), 4.23−4.13 (m, 2H, H3′/H4′), 2.94 (q, J_{CH_2,CH_3} = 7.20 Hz, 1H, H_2CNH), 2.27 (s, 3H, H_3C_{tosyl}), 1.94−1.89 (m, 2H, $H2_{\alpha-cyclopentyl}$/$H5_{\alpha-cyclopentyl}$), 1.71−1.51 (m, 6H, $H2_{\beta-cyclopentyl}$/$H5_{\beta-cyclopentyl}$/$H3_{cyclopentyl}$/$H4_{cyclopentyl}$), 1.22−1.10 (m, 2H, H_3CH_2-CNH). Anal. ($C_{17}H_{26}N_6O_3 \cdot C_7H_8O_3S$) C, H, N.

5′-(Propylamino)-5′-deoxy-CPA (9c): yield 56 mg (0.149 mmol), 80%; mass API *m*/*z* 377 (MH^+); T_m decomposition 240 °C; R_f (15% MeOH/CH_2Cl_2) 0.3; ^{1}H NMR (DMSO-d_6) δ 8.33 (s, 1H, H8), 8.18 (s, 1H, H2), 7.67 (d, J = 6.9 Hz, 1H, N^6H), 7.46 (d, $J_{o,m}$ = 8.06 Hz, 2H, $H_{o-tosyl}$), 7.09 (d, $J_{o,m}$ = 8.04 Hz, 2H, $H_{m-tosyl}$), 5.85 (d, $J_{1',2'}$ = 5.79 Hz, 1H, H1′), 5.40 (d, $J_{H,OH}$ = 6.01 Hz, 1H, OH), 5.18−5.17 (m, 1H, OH), 4.70 (q, $J_{1',2'}$ = 5.62, 1H, H2′), 4.50 (bs, 1H, *H*CN^6), 4.15−4.14 (m, 1H, H3′), 4.03−4.01 (m, 1H, H4′), 2.87 (bs, 2H, H_2CNH), 2.26 (s, 3H, H_3C_{tosyl}), 1.94−1.88 (m, 4H, $H2_{\alpha-cyclopentyl}$/$H5_{\alpha-cyclopentyl}$), 1.71−1.52 (m, 8H, $H2_{\beta-cyclopentyl}$/$H5_{\beta-cyclopentyl}$/$H3_{cyclopentyl}$/$H4_{cyclopentyl}$/H_2CH_2-CNH), 0.93−0.75 (m, 4H, $H_3CH_2CH_2$CNH). Anal. ($C_{18}H_{28}N_6O_3 \cdot C_7H_8O_3S$) C, H, N.

Receptor Binding Studies. The adenosine A_1 binding assay was carried out on membranes of rat brain cortex. Membranes were prepared according to the method of Lohse et al.,[36] except that the membranes were incubated with 2 units/mL ADA at 37 °C before storage, as described by Pirovano et al.[9] Protein concentrations were measured with the BCA method.[37]

The adenosine A_1 binding assays were performed with 0.4 nM [^{3}H]DPCPX as the radioligand (K_d 0.28 nM[9]). The assays were performed as originally described by Lohse *et al.*[38] Nonspecific binding was determined in the presence of 10 μM *R*-PIA. The adenosine A_1 displacement studies were carried out both in the absence and in the presence of 1 mM GTP.

The adenosine A_{2A} assay was carried out on rat striatal membranes. Striata were dissected from rat brain tissue according to the method described by Glowinsky and Iversen,[39] and striatal membranes were prepared according to Bruns et al.,[17] except that the membranes were incubated with 2 units/mL ADA at 37 °C before storage.[9] The binding of 5.6 nM [^{3}H]-CGS 21680 (K_d 14.5 nM[12]) to the A_{2A} receptors of rat striatal membranes was determined as originally described by Johansson et al.,[40] but CPA was used in a 50 μM concentration

to determine the nonspecific binding of [^{3}H]CGS 21680, and 75 μg of striatal protein was used in the tests.

K_i values are means of three independent experiments performed in duplicate, unless indicated otherwise.

GTPγS Binding to Rat Brain Membranes. These experiments were carried out as described previously.[41,42] Nonspecific binding of [^{35}S]GTPγS to rat brain membranes was determined in the presence of 10 μM GTPγS. The EC_{50} values are shown as the geometrical mean values with 95% confidence limits, derived from three to nine independent experiments. Intrinsic activities are expressed as relative to CPA.

Data Analysis. Apparent K_i values were computed from the displacement curves by nonlinear regression of the competition curves with Prism (Graph Pad, San Diego, CA). GTP shifts were determined by dividing the apparent K_i value of the ligand in the presence of GTP by the apparent K_i value in the absence of GTP. EC_{50} values for stimulation of [^{35}S]GTPγS binding were calculated with Sigma Plot.

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Supporting Information Available: ^{13}C NMR spectral data for all compounds (3 pages). Ordering information is given on any current masthead page.

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