N^6,5′-Disubstituted Adenosine Derivatives as Partial Agonists for the Human Adenosine A_3 Receptor

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5′-(Alkylthio)-substituted analogues of N^6-benzyl- and N^6-(3-iodobenzyl)adenosine were synthesized in 37−61% overall yields. The affinities of these compounds for the adenosine A_1, A_{2A}, and A_3 receptors were determined using rat brain cortex, rat brain striata, and stably transfected human A_3 receptors in HEK 293 cells, respectively. The compounds proved to be selective for the adenosine A_3 receptor and displayed affinities in the nanomolar range. Compounds **8**, **10**, and **11** had the highest affinities for the A_3 receptor with K_i values ranging from 8.8 to 27.7 nM. In the N^6-benzyl series, compound **4** (LUF 5403), with a 5′-methylthio group, maintained a reasonable affinity and had the highest selectivity for the A_3 receptor. Compound **12** (LUF 5411), with an N^6-(3-iodobenzyl) group and a 5′-(*n*-propylthio) substituent, had the highest A_3 selectivity of all of the compounds and also displayed high affinity for this receptor (K_i = 44.3 nM). The compounds were also evaluated for their ability to stimulate [^{35}S]-GTPγ[S] binding in cell membranes expressing the human adenosine A_3 receptor. It appeared that the N^6,5′-disubstituted adenosine derivatives behaved as partial agonists. Compounds **2**, **4**, **8**, and **10** had the highest intrinsic activities. Additionally, when tested in a cAMP assay, these compounds also behaved as partial agonists.

Introduction

Adenosine is thought to mediate a wide variety of effects as a result of its activation of specific membrane-bound receptors called P_1-purinoceptors. The three subclasses of P_1-purinoceptors are A_1, A_2, and A_3, with A_2 further subdivided into A_{2A} and A_{2B}. All three classes are coupled to the enzyme adenylate cyclase. A_1 and A_3 adenosine receptors are linked to the inhibition of adenylate cyclase. Activated A_{2A} and A_{2B} receptors can stimulate adenylate cyclase. The ubiquitous human adenosine A_3 receptor, the target receptor in this study, has the highest expression in the lungs and the liver but is also found in the CNS, testes, heart, and immune system.[1,2] Therefore, the desired actions of A_3 receptor ligands may be accompanied by serious side effects. For example, chronic administration of adenosine A_3 receptor agonists has been found to be cardio- and cerebroprotective.[3−5] However, stimulation of the rat A_3 receptor in the lungs and immune system causes bronchoconstriction and the release of allergic mediators, respectively.[6] Potentially, various ways exist to circumvent these side effects, including the use of partial agonists.[7] Through partial agonists differences in receptor−effector coupling in various tissues may be exploited and selectivity of action in vivo may be achieved. Additionally, less receptor downregulation and desensitization is another probable advantage of partial agonists.

The present study explores the possibility of high-affinity partial agonists for the A_3 receptor. For this purpose, A_3 receptor selectivity needed to be achieved. Van Galen et al.[2] and Gallo-Rodriguez et al.[8] have reported that certain N^6-substituted adenosines are selective agonists for the adenosine A_3 receptor. Their study showed that a benzyl or a 3-iodobenzyl substituent at the N^6-position resulted in highly potent and selective ligands for the A_3 receptor. In later series of Mogensen et al.[9] and Baraldi et al.,[10] bulky and other purine 6-amino substituents have been explored further. This has led to the synthesis of *N*-carboxamido, *N*-alkoxy, and *N*-urea derivatives, all selective ligands for the adenosine A_3 receptor (Table 1).

In addition to a substituent for A_3 selectivity, a substituent for partial agonism had to be introduced. It is known that at the 5′-position *N*-alkylcarboxamide substituents are tolerated.[2,8] In their study, Mogensen et al.[9] have also introduced other 5′-substituents, for example, an 5′-isoxazole moiety. This substituent has been shown to act as a bioisostere for the 5′-alkylamides (Table 1). In a recent study from our laboratory on the adenosine A_1 receptor, Van der Wenden et al.[11] substituted the A_1 receptor-selective ligand N^6-cyclopentyladenosine (CPA) at the 5′-position with alkylthio and alkylseleno groups. These substituents are well-tolerated at the 5′-position. Additionally, these compounds behaved as partial agonists for the A_1 receptor.

In this paper, the synthesis and biological evaluation for a series of adenosine analogues substituted at the N^6-position with a benzyl or 3-iodobenzyl group for A_3 selectivity and at the 5′-position with alkylthio groups for potential partial agonism are described. The compounds were tested in radioligand binding assays for affinity. Their intrinsic activities were determined both in [^{35}S]GTPγ[S] (guanosine 5′-(γ-thio)triphosphate) binding assays[12,13] and in cAMP assays.

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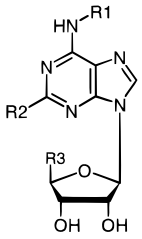
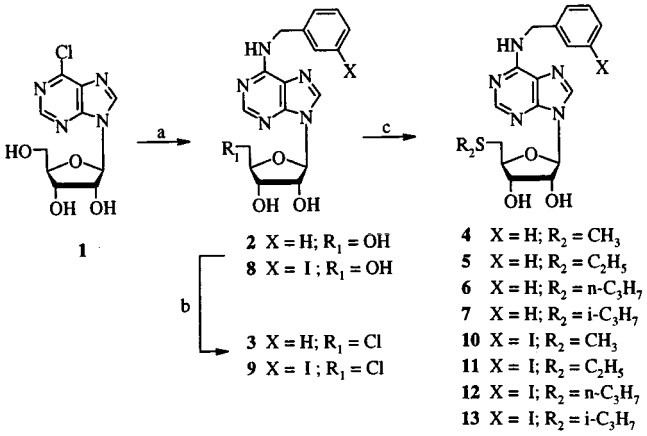
Table 1. Affinities of Some Selected Key A_3 Agonists Expressed as K_i Values (nM)[9,10]



R1	R2	R3	K_i A_3 (nM)	
$PhCH_2$	H	$CH_3NHCOCH_2$	41[a]	*N*-benzyl-MECA
3-I-$PhCH_2$	H	$CH_3NHCOCH_2$	1.1 ± 0.3[b]	IB-MECA
3-I-$PhCH_2$	Cl	$CH_3NHCOCH_2$	0.33 ± 0.08[b]	Cl-IB-MECA
3-I-$PhCH_2$	Cl	isoxazole	7.8[a]	
CH_3O	Cl	isoxazole	31[a]	
4-SO_2NH_2-PhNH	H	$CH_3CH_2NHCOCH_2$	9.73 ± 0.75[b]	

[a] Displacement of [^{125}I]AB-MECA from human A_3 receptors expressed in HEK 293 cells (no SEM given).[9] [b] Displacement of [^{125}I]AB-MECA from rat A_3 receptors expressed in CHO cells (n = 3−6, ±SEM).[10]

Scheme 1[a]



[a] (a) Absolute EtOH, Et_3N and benzylamine or *m*-iodobenzylamine hydrochloride; (b) (i) pyridine, CH_3CN, $SOCl_2$, (ii) MeOH, H_2O, concd ammonia; (c) RSH, 2 M NaOH, DMF; R = CH_3, C_2H_5, *n*-C_3H_7, or *i*-C_3H_7.

Chemistry

The synthetic route for the preparation of these 5′-(alkylthio)-derived N^6-substituted adenosines involved a three-step synthesis (Scheme 1). It was based on the introduction of a 5′-alkylthio substituent to the N^6-derivatized 5′-halogenide adenosine moiety.

The starting point for synthesis was from commercially available 6-chloropurine riboside (**1**). N^6-Benzyladenosine[14] (**2**; 70% yield) and N^6-(3-iodobenzyl)-adenosine[15] (**8**; 76% yield) were obtained by reacting **1** with benzylamine or 3-iodobenzylamine hydrochloride, respectively, in ethanol.[8,15] For the preparation of compounds **4**−**7** and **10**−**13**, a good leaving group at the 5′-position was needed. Several reactions were performed in order to replace the 5′-hydroxyl group of **2** and **8** for a chlorine. Treatment of **2** with freshly distilled thionyl chloride ($SOCl_2$) and hexamethylphosphoramide (HMPA)[16] yielded **3** at only 43%. Due to this low yield and the carcinogenic properties of HMPA, **2** was instead treated with $SOCl_2$ and 1,3-dimethyl-3,4,5,6-tetrahydro-2(1*H*)-pyrimidinone (DMPU); **3** was obtained in 75% yield. Alternatively, stirring **2** with $SOCl_2$ in pyridine and CH_3CN followed by treatment with MeOH, water, and concentrated ammonia facilitated the workup and yielded **3** at 86% and **9** at 99%.

Several attempts to convert **3** into the 5′-(alkylthio)-substituted adenosines by reaction with the appropriate thiol in either 2 M NaOH,[11] or NaH and dimethylformamide (DMF), or 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) and CH_3CN did not succeed. Solubility problems, as proved by the recovery of starting material after workup, caused these failures. Compounds **4**−**7** and **10**−**13** could be isolated in reasonable overall yields (37−61%) under slighty modified conditions, viz. mixing **3** with the appropriate thiol in aqueous NaOH with the addition of some DMF to improve the solubility of all reagents.

Biological Evaluation

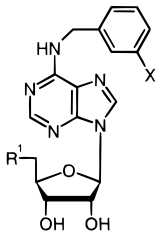
All compounds were tested in radioligand binding assays to determine their affinities for the A_1 receptor in rat brain cortex, the A_{2A} receptor in rat striatum, and the human A_3 receptor as expressed in HEK 293 cells (Table 2). For the A_1 receptor, the tritiated antagonist [^{3}H]-1,3-dipropyl-8-cyclopentylxanthine ([^{3}H]DPCPX) was used. Since radiolabeled antagonists were not commercially available for the A_{2A} and A_3 receptors when the binding studies were performed, [^{3}H]CGS21680, an A_{2A} agonist, and [^{125}I]AB-MECA, an A_3 agonist, were used. Displacement experiments were performed in the absence of GTP.

All compounds were also tested in functional assays for the A_3 receptor. The signal transduction by adenosine A_3 receptors was investigated by measuring the modulation of guanosine 5′-*O*-(3-[^{35}S]thio)triphosphate ([^{35}S]GTPγ[S]) binding to cell membranes when compounds **2**−**13** were bound to the receptor. This reflects the GDP−GTP exchange reaction stimulated by receptor agonists. Membranes from CHO cells stably transfected with human A_3 receptors were used, since no significant stimulation occurred with membranes prepared from the HEK 293 cells used in the binding studies. The second functional assay quantified the ability of a selection of these compounds to inhibit cAMP production in human A_3 receptors expressed in HEK 293 cells.

Results and Discussion

The synthetic route depicted in Scheme 1 yielded target compounds **2**−**13**. The introduction of the N^6-

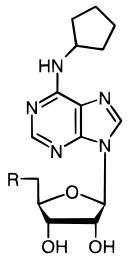
Table 2. Affinities at Adenosine A_1, A_{2A}, and A_3 Receptors Expressed as K_i Values (nM; $n = 3$, ±SEM, or $n = 2$, both values given) or Percentage Displacement at 10 μM



			K_i (nm) or % displacement at 10^{-5} M			
no.	X	R^1	A_1[a]	A_{2A}[b]	A_3[c]	A_1/A_3
2	H	OH	4450 ± 3020	733 ± 440	550 ± 54	8.1
3	H	Cl	47% ± 14%	641 ± 93	1860 ± 330	>5.4
4	H	SCH_3	31% ± 11%	25% ± 5%	128 ± 29	>78.1
5	H	SC_2H_5	29% ± 9%	38% ± 6%	159 ± 63	>62.9
6	H	S-*n*-C_3H_7	5875 (4920−6830)	7990 ± 3010	674 ± 122	8.7
7	H	S-*i*-C_3H_7	14% ± 5%	6% ± 5%	211 ± 50	>47.4
8	I	OH	78.8 (73.6−83.9)	340 ± 170	27.7 ± 7.8	2.8
9	I	Cl	270 ± 150	457 ± 225	188 ± 74	1.4
10	I	SCH_3	610 ± 210	1825 (2320−1330)	8.78 ± 3.74	69.5
11	I	SC_2H_5	720 ± 140	1081 ± 612	17.5 ± 4.6	41.1
12	I	S-*n*-C_3H_7	42% ± 26%	42% ± 23%	44.3 ± 16.9	>225.7
13	I	S-*i*-C_3H_7	35% ± 17%	45% ± 12%	46.3 ± 17.9	>216.0

[a] Displacement of [^{3}H]DPCPX from rat cortical membranes.[34] [b] Displacement of [^{3}H]CGS 21680 from rat striatal membranes.[35] [c] Displacement of [^{125}I]AB-MECA from the human A_3 receptor expressed in HEK 293 cells.[2,36]

Table 3. Affinities of N^6-Cyclopentyladenosine (CPA) Analogues at Adenosine A_1, A_{2A}, and A_3 Receptors Expressed as K_i Values (nM; $n = 3$, ±SEM, or $n = 2$, both values given)



		K_i (nm)			
no.	R	A_1[a,d]	A_{2A}[b,d]	A_3[c]	A_1/A_3
14	OH	5.90 (5.78−6.02)	580 ± 120	120 ± 15	0.05
15	Cl	9.5 ± 3.2	8750 ± 1040	1290 ± 380	0.007
16	SCH_3	59 ± 22	13540 ± 1730	74 ± 4.7	0.80
17	SC_2H_5	45 ± 8.0	19010 ± 12070	90 ± 13	0.50
18	S-*n*-C_3H_7	76 ± 27	18780 ± 6820	193 ± 21	0.39
19	S-*i*-C_3H_7	41 ± 9.0	10250 ± 2830	137 ± 15	0.30

[a] Displacement of [^{3}H]DPCPX from rat cortical membranes.[34] [b] Displacement of [^{3}H]CGS 21680 from rat striatal membranes.[35] [c] Displacement of [^{125}I]AB-MECA from the human A_3 receptor expressed in HEK 293 cells.[2,36] [d] Values from Van der Wenden et al.[11]

substituent, either a benzyl or a 3-iodobenzyl, proceeded according to literature methods.[8,15] Subsequently, the 5′-alkylthio groups were introduced. Direct reaction of the 5′-hydroxyl group with dimethyl disulfide and tributylphosphine had been proven to be unsuccessful for CPA analogues (**14**−**19**, Table 3).[11] Therefore, as mentioned, a good leaving group at the 5′-position was needed to introduce the alkylthio substituent. Hence, the 5′-hydroxyl group was replaced by a chlorine atom. The synthesis of 5′-halogenated ribonucleosides is generally performed by the introduction of *p*-toluenesulfonyl or methanesulfonyl into the 5′-position of a suitably blocked ribonucleoside and subsequent replacement of the sulfonyl group with an alkali halide to give the blocked 5′-halogeno-5′deoxy ribonucleoside.[18−20] However, the high reactivity of tosyl derivatives readily can cause ring closure when the ribose 5′- and 3-positions of the adenine ring react.[21] Direct conversions of nucleosides to 5′-chloro derivatives have been performed with thionyl chloride ($SOCl_2$) and DMF.[22] The advantage of this procedure is that the 2′- and 3′-hydroxyl groups do not need to be protected and the intermediate is more stable than the 5′-tosylate. However, reaction of cytidine with $SOCl_2$ and DMF has afforded a ring-closed product, viz. 2,2′-cyclocytidine, instead of 5′-chloro-5′-deoxycytidine.[23] Kikugawa et al.[24] and Borchardt et al.[16] have found that $SOCl_2$ mixed with hexamethylphosphoramide (HMPA) is a good reagent for selective halogenation of the 5′-position of ribonucleosides, without the undesired cyclization. Robins et al.[25] introduced a modification to this procedure. Instead of $SOCl_2$ and HMPA, the authors used a mixture of $SOCl_2$, acetonitrile, and pyridine with satisfactory results. Treatment of pyrimidine or purine nucleosides with $SOCl_2$/aceto-

nitrile/pyridine has been shown to result in the formation of the diastereomeric sulfite esters. Such esters readily hydrolyze in aqueous methanolic ammonia, thus providing a very convenient route to 5′-chloro-5′-deoxy nucleosides. Therefore, with this method the protection of the 2′- and 3′-hydroxyl groups was unnecessary and the 5′-chloro derivatives were obtained in good yield without prior isolation of the sulfite ester.

Table 2 displays radioligand binding data for all synthesized disubstituted final products. For reasons of comparison, data for 5′-substituted N^6-cyclopentyladenosine (CPA) derivatives are also listed (**14**−**19**, Table 3). The A_1 and A_{2A} receptor binding data of these 5′-substituted CPA compounds were previously reported,[11] and the adenosine A_3 receptor affinities were determined in the present study. The CPA derivatives had A_1 receptor affinities in the nanomolar range and were selective for this receptor. However, they also showed reasonable affinity for the A_3 receptor.

Replacement of the N^6-cyclopentyl substituent by an N^6-benzyl or an N^6-(3-iodobenzyl) group (**2**−**13**) increased the selectivity of these compounds for the A_3 receptor (Tables 1 and 2). The affinities of the compounds with an N^6-benzyl group (**2**−**7**) for the A_3 receptor were quite similar to those of the CPA analogues (**14**−**19**). The N^6-benzyl group induced a decrease in affinity for the rat A_1 receptor rather than for the human A_3 receptor and thus induced A_3 selectivity. The N^6-(3-iodobenzyl) substituent (**8**−**13**) did not decrease the affinity for the A_1 receptor as much as the N^6-benzyl group, but it increased the affinity for the A_3 receptor rather strongly. This led to high-affinity and selective compounds for the A_3 receptor. This result agrees with published data by Jacobson and co-workers.[2,4,15,26] It has been shown that the N^6 region of the A_3 receptor appeared to tolerate several substituents.[2] In a series of N^6-aralkyl derivatives of adenosine, an N^6-benzyl group was optimal for A_3 selectivity compared to other N^6-aryl substituents, in particular N^6-phenyl and N^6-phenethyl groups. Further substitution of this benzyl group at the meta-position with iodine caused even higher affinity for the A_3 receptor, as shown also with our compounds.[8,15] In the case of 5′-uronamide adenosine derivatives, replacement of the N^6-amino group by the N^6-benzylamino group decreased the affinity for the A_1 and A_{2A} receptors by 9−14-fold and increased the affinity for the A_3 receptor by 4−17-fold. Replacement with the N^6-(3-iodobenzyl)amino group showed similar affinities for the A_1 and A_{2A} receptors but demonstrated increased affinity for the A_3 receptor by 65−128-fold.[8]

The introduction of the 5′-alkylthio substituents also caused changes in affinity and selectivity. The primary goal in introducing the 5′-alkylthio groups was to induce partial agonism. Likewise, when these groups were introduced at the 5′-position of CPA, the resulting compounds behaved as partial agonists for the A_1 receptor.[11] However, the affinity for the A_1 receptor of these 5′-alkylthio-substituted CPA derivatives decreased, and the selectivity of the compounds changed. The unsubstituted analogue (CPA itself) and 5′-chloro CPA (**16**) still had the highest affinities for the A_1 receptor and moderate affinities for the A_3 receptor. However, the 5′-alkylthio derivatives of CPA no longer demonstrated high A_1 selectivity compared to the A_3 receptor subtype. In particular, the affinities of compounds **16** (5′-methylthio CPA) and **17** (5′-ethylthio CPA) were noteworthy. Their affinities for the A_1 receptor decreased, while the affinities for the A_3 receptor increased. These results were encouraging for the compounds selective for the A_3 receptor. Indeed, the introduction of 5′-alkylthio substituents to these compounds (**2**−**13**, Table 2) induced similar changes in affinity and selectivity as shown in the CPA analogues. The large decrease in affinity for the A_1 receptor in all cases and comparable or increased affinity for the A_3 receptor resulted in an increase in A_3 selectivity for all compounds. For example, within the N^6-benzyl series the A_1/A_3 selectivity of compound **4** (5′-methylthio) compared to compound **2** (5′-hydroxyl) increased by 10-fold, the A_{2A}/A_3 selectivity by 59-fold, and the A_3 affinity by 4-fold. A similar comparison within the N^6-(3-iodobenzyl) series showed that the replacement of the 5′-hydroxyl group (**8**) by an *n*-propylthio group (**12**) increased A_1/A_3 selectivity by 81-fold and A_{2A}/A_3 selectivity by 18-fold. However, the affinity for the A_3 receptor decreased somewhat (by 2-fold). In the literature changes in affinity and selectivity caused by 5′-substituents have mostly been described for 5′-uronamides.[26] The potency of these compounds at either the A_{2A} or A_3 receptor has been found to be enhanced when compared to their A_1 affinity. NECA has been reported to be among the most potent agonists at the A_3 receptor, although it is not A_3-selective.[8,27,28] Replacement of the 5′-hydroxyl group of N^6-benzyladenosine or N^6-(3-iodobenzyl)adenosine with 5′-*N*-alkyluronamide groups increased affinity for all three receptor subtypes with the best selectivity gain at the A_3 receptor, however.[2,15]

Concerning the intrinsic activities, all compounds (**2**−**13**) showed varying submaximal but higher than basal levels of [^{35}S]GTPγ[S] binding (Table 4). Thus, the compounds behaved as partial agonists in this assay. Compounds **2**, **4**, **8**, and **10** (either the hydroxyl or methylthio substituent at the 5′-position within both the N^6-benzyl and N^6-(3-iodobenzyl) series) had the highest intrinsic activities, whereas the other compounds were considerably less active. The latter two compounds, **8** and **10**, also had lower EC_{50} values for the A_3 receptor than NECA, the reference compound. The intrinsic activities of compounds **2**, **4**, **8**, and **10** (10 μM) were also determined in cyclic AMP assays. NECA showed a

Table 4. Agonistic Activity of N^6,5′-Disubstituted Adenosine Analogues Expressed as Percentage of [^{35}S]GTPγ[S] Binding to the Adenosine A_3 Receptors Stimulated by the Analogues at 10^{-4} M (maximal stimulation), Compared to the Maximal [^{35}S]GTPγ[S] Binding Caused by NECA (100%, $n = 3$)[a]

no.	max [^{35}S]GTPγ[S] bound (%)	EC_{50} (nM)
basal	0	
NECA	100	89 ± 23
2	81 ± 1	384 ± 123
3	13 ± 1	
4	52 ± 2	473 ± 103
5	19 ± 1	
6	7 ± 0	
7	22 ± 1	
8	56 ± 2	14 ± 10
9	8 ± 0	
10	41 ± 1	27 ± 18
11	21 ± 0	
12	10 ± 0	
13	16 ± 0	

[a] Some EC_{50} values ($n = 3$−5, ±SEM) are also given.

Table 5. Agonistic and Antagonistic Behavior of N^6,5′-Disubstituted Adenosine Analogues in a cAMP Assay[a]

no.	% inhibition	% inhibition in presence of NECA (10 μM)
NECA	100	
2	82 ± 15	94 (92−95)
4	60 ± 11	93 (88−98)
8	86 ± 9	91 (84−96)
10	64 ± 4	87 (77−96)

[a] Left column: percentage inhibition of forskolin-stimulated (10 μM) cAMP production, mediated by the human adenosine A_3 receptor stably transfected on CHO cells, caused by N^6,5′-disubstituted adenosine analogues (10 μM) indicating partial agonism. Right column: percentage inhibition of forskolin-stimulated cAMP production caused by N^6,5′-disubstituted adenosine analogues in combination with NECA (10 μM both) indicating partial antagonism, compared to the inhibition caused by NECA alone (10 μM) (n = 3, ±SEM, or n = 2, both values given).

maximal inhibition of the forskolin-stimulated (10 μM) cAMP production (100%), whereas **2**, **4**, **8**, and **10** inhibited this cAMP production to a lesser extent (Table 5) which is again evidence for their partial agonistic behavior.

All of these findings support and extend observations by Daly and Padgett,[29] Taylor et al.,[30] and Van der Wenden et al.[11] Taylor described the agonistic activity of 5′-deoxy-5′-(methylthio)-(*R*)-*N*-(1-methyl-2-phenylethyl)adenosine ((*R*)-PIA) in its effects on blood pressure and heart rate in rats, suggesting it to be a partial agonist for the adenosine A_1 receptor. More recently, Daly and Padgett have shown that 5′-deoxy-5′-(methylthio)adenosine was a weak agonist at both the A_1 and A_{2A} receptors. Using PC12 cell membranes with A_1 and A_{2A} receptors, the response caused by this compound was submaximal at the highest concentrations tested. In particular, the partial agonistic activity at the A_{2A} receptors of PC12 cell membranes was an unexpected finding in view of the competitive antagonistic activity of 5′-deoxy-5′-(methylthio)adenosine at the A_{2B} receptor of human fibroblast cells.[31] Furthermore, Van der Wenden et al. have shown that the replacement of the 5′-hydroxyl group of the A_1 receptor-selective ligand CPA with 5′-alkylthio and 5′-alkylseleno substituents indeed induced partial agonism for the A_1 receptor.[11] Van Galen et al. and Kim et al. have quantified the partially agonistic behavior of another class of compounds, viz. derivatives of 1,3-dibutylxanthine 7-riboside.[2,32] These compounds were found to be low-affinity partial agonists for the adenosine A_3 receptor. Finally, the partial agonistic properties of the N^6-monosubstituted adenosines (**2** and **8**) were noteworthy. With intact ribose moieties which are usually considered to be characteristic of full agonists for the A_3 receptor, these compounds were in fact partial agonists in the functional assay utilized herein.

The compounds (100 μM) were also evaluated for antagonistic effect, an important feature of partial agonists. All compounds showed partially antagonistic properties in the [^{35}S]GTPγ[S] binding assay in the precence of 1 μM NECA, by reducing the amount of [^{35}S]-GTPγ[S] binding caused by NECA to a varying extent (Figure 1). Again, compounds **2**, **4**, **8**, and **10** showed the highest intrinsic activities. When compounds **2**, **4**, **8**, and **10** (10 μM) were tested in the cyclic AMP assay in combination with NECA (10 μM), they showed partial antagonistic properties also. In all four cases the inhibition of cAMP production was less effective than with NECA alone, but always higher than tested alone with the partial agonists (Table 5).

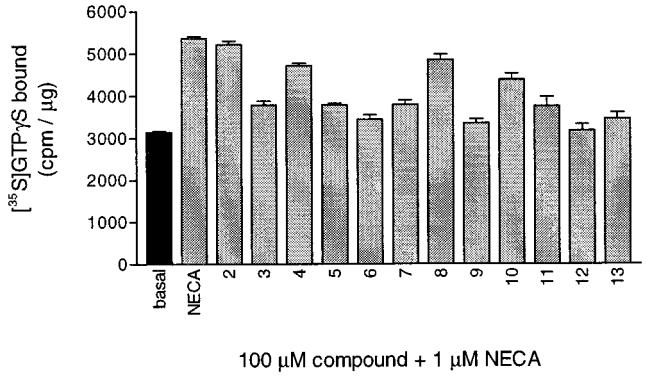


Figure 1. Inhibition of NECA-stimulated (1 μM) [^{35}S]GTPγ-[S] binding to human adenosine A_3 receptors stably transfected in CHO cells, by N^6,5′-disubstituted adenosine analogues (100 μM; n = 3, ±SEM).

Conclusions

N^6,5′-Disubstituted adenosine derivatives described in the present study are partial agonists for the human adenosine A_3 receptor. It appeared that both substituents affected A_3 receptor affinity, selectivity, and intrinsic activity in a 'titratable' way. The 5′-alkylthio substituents both induced partial agonism and increased A_3 receptor selectivity in all cases. The compounds also increased A_3 receptor affinity in some cases (**4**, **5**, **7**, **10**, and **11**). Surprisingly, when adenosine was substituted at the N^6-position only (**2** and **8**), thus preserving the ribose moiety, partial agonism was shown. The compounds will be useful tools in pharmacology by inducing selectivity of effects due to differences in receptor−effector coupling in various tissues in vitro and in vivo. Thus, these and future partial agonists for the A_3 receptor may have reduced acute side effects, while retaining the useful cardio- and cerebroprotection after chronic administration.[3−5]

Experimental Section

Chemicals and Solvents. 6-Chloropurine riboside (**1**) was obtained from Aldrich (Aldrich Chemie, Sigma-Aldrich Chemie BV, Zwijndrecht, The Netherlands). All other reagents were from standard commercial sources and of analytical grade. [^{3}H]-DPCPX (1,3-dipropyl-8-cyclopentylxanthine), [^{3}H]CGS 21680, and [^{125}I]AB-MECA were purchased from NEN (Hoofddorp, The Netherlands).

Chromatography. Thin-layer chromatography (TLC) was carried out using aluminum sheets (20 × 20 cm) with silica gel F_{254} from Merck. Spots were visualized under UV (254 nm). Preparative column chromatography was performed on silica gel (230−400 mesh ASTM).

Instruments and Analyses. Elemental analyses were performed for C, H, N (Department of Microanalysis, Groningen University, The Netherlands). ^{13}C NMR spectra were measured at 50.1 MHz with a JEOL JNM-FX 200 spectrometer equipped with a PG 200 computer operating in the Fourier transform mode. ^{1}H NMR spectra were measured at 200 MHz, using the above-mentioned spectrometer, or at 300 MHz, using a Bruker WM-300 spectrometer equipped with an ASPECT-2000 computer operating in the Fourier transform mode. Chemical shifts for ^{1}H and ^{13}C NMR are given in ppm (δ) relative to tetramethylsilane (TMS) as internal standard.

All high-resolution mass spectra were measured on a Finnigan MAT900 mass spectrometer equipped with a direct

insertion probe for EI experiments (70 eV with resolution 1000) or on a Finnigan MAT TSQ-70 spectrometer equipped with an electrospray interface for ESI experiments. Spectra were collected by constant infusion of the analyte dissolved in 80/20 methanol/H_2O. ESI is a soft ionization technique resulting in protonated, sodiated species in positive ionization mode and deprotonated species in negative ionization mode.

Resolution of the compounds was achieved by reverse-phase HPLC (Gilson HPLC system, 712 system controller software; Gilson Netherlands, Meyvis en Co BV, Bergen op Zoom, The Netherlands) using a 65% MeOH/31.5% H_2O/3.5% CH_3CN (v/v) mobile phase, an Alltima C18 5-μm (250 mm × 4.6 mm) or a nucleotide/nucleoside 7-μm (250 mm × 4.6 mm) column (Alltech Nederland BV, Breda, The Netherlands) at a flow rate of 0.7 mL/min. The peaks were defined by measurement of UV absorbance (254 nm). Retention times are given. Melting points (not corrected) were determined in a Büchi capillary melting point apparatus.

Syntheses. General Procedure for the Amination of 1 into Compounds 2 and 8.[15] To a solution of **1** (0.98 g, 3.42 mmol) in absolute ethanol (35 mL) were added the corresponding amine (6.84 mmol) and dry triethylamine (4.27 mmol, 0.60 mL), and the mixture was refluxed overnight at 80 °C. The solution was allowed to cool and was concentrated in vacuo. Water (50 mL) was added to the residue, and this aqueous layer was extracted with three portions of ethyl acetate (30 mL). The organic layer was dried with $MgSO_4$, filtered, and concentrated. The product was crystallized from methanol.

***N*^{6}-Benzyladenosine (2).** The reaction was carried out with benzylamine (1.10 mmol, 0.12 mL) at 80 °C overnight: yield 88.4 mg (0.25 mmol, 70%); mp 167−169 °C; R_f 0.29 (10% MeOH in CH_2Cl_2); ^{1}H NMR (DMSO-d_6) δ 8.43 (bs, 1H, NH), 8.37 (s, 1H, H-8), 7.30−7.17 (m, 5H, benzyl), 8.18 (s, 1H, H-2), 5.87 (d, J = 6.18 Hz, 1H, H-1′), 5.46−5.37 (m, 2H, 3′-OH, 5′-OH), 5.19−5.17 (m, 1H, 2′-OH), 4.75−4.69 (m, 2H, NHCH_2), 4.69−4.59 (m, 1H, H-2′), 4.15−4.11 (m, 1H, H-3′), 3.98−3.93 (m, 1H, H-4′), 3.74−3.41 (m, 2H, H-5′); HPLC, nucleotide/nucleoside column; retention time 5.55 min.

***N*^{6}-(3-Iodobenzyl)adenosine (8).** The reaction was carried out with 3-iodobenzylamine hydrochloride (1.50 g, 5.58 mmol), at 80 °C overnight: yield 1.05 g (2.17 mmol, 76%); mp 169−172 °C; R_f 0.43 (CH_2Cl_2:MeOH = 96:4); ^{1}H NMR (DMSO-d_6) δ 8.56−8.42 (bs, 1H, NH), 8.38 (s, 1H, H-8), 8.20 (s, 1H, H-2), 7.71 (s, 1H, NHCH$_2$CC*H*CI), 7.57 (pd, J = 7.9 Hz, 1H, CCHCIC*H*), 7.33 (pd, 1H, CC*H*CH), 7.09 (pt, J = 6.18 Hz, 1H, CCHC*H*), 5.89 (d, J = 6.18 Hz, 1H, H-1′), 5.45 (d, J = 6.18 Hz, 1H, 2′-OH), 5.39 (pt, 1H, 5′-OH), 5.19 (d, J = 4.46 Hz, 1H, 3′-OH), 4.78−4.54 (m, 2H, CH_2NH), 4.78−4.54 (m, 1H, H-2′), 4.21−4.10 (m, 1H, H-3′), 4.01−3.93 (m, 1H, H-4′), 3.75−3.45 (m, 2H, H-5′); HPLC, nucleotide/nucleoside column; retention time 4.35 min.

General Procedure for the Chlorination of Compounds 2 and 8 into 3 and 9, Respectively. To a stirred suspension of the starting material (0.22 mmol) in pyridine (0.44 mmol, 35.6 μL) and CH_3CN (0.66 mL) cooled in an ice bath was slowly added $SOCl_2$ (1.10 mmol, 81 μL). Stirring was continued at ∼5 °C for 3−4 h with subsequent warming to ambient temperature overnight. The resulting suspension was concentrated in vacuo; the 2′,3′-protected intermediate was not isolated. Then methanol (2 mL), H_2O (0.2 mL), and concentrated ammonia (0.11 mL) were added, stirring was continued for 30 min at ambient temperature, and the solution was concentrated. Water was added (15 mL), the solution was neutralized ($NaHCO_3$), and the aqueous layer was extracted with three portions of ethyl acetate (10 mL). The organic layer was dried with $MgSO_4$, filtered, and concentrated. The yellow powder thus obtained was stirred in MeOH for 30 min and filtered yielding the pure white product.

***N*^{6}-Benzyl-5′-chloro-5′-deoxyadenosine (3).** The reaction was carried out with *N*^{6}-benzyladenosine (**2**; 1.10 g, 3.03 mmol): yield 1.11 g (2.93 mmol, 97%); mp 200−202 °C; R_f 0.65 (10% MeOH in CH_2Cl_2); ^{1}H NMR (DMSO-d_6) δ 8.49−8.36 (m, 1H, NH), 8.36 (s, 1H, H-8), 8.21 (s, 1H, H-2), 7.35−7.21 (m, 5H, benzyl), 5.93 (d, J = 4.80 Hz, 1H, H-1′), 5.61 (d, J = 5.49 Hz, 1H, 2′-OH), 5.47 (dd, J = 4.81 Hz, J = 1.37 Hz, 1H, 3′-OH), 4.76−4.69 (m, 2H, NHCH_2), 4.76−4.69 (m, 1H, H-2′), 4.29−4.17 (m, 1H, H-3′), 4.17−4.02 (m, 1H, H-4′), 4.02−3.75 (m, 2H, H-5′); ^{13}C NMR (DMSO-d_6) δ 154.5 (C-6), 152.5 (C-2), 149.8 (C-4), 140.1 (NHCH$_2$*C*, benzyl), 139.9 (C-8), 128.1 (C*C*H, benzyl), 127.0 (CCH*C*H, benzyl), 126.5 (CCHCH*C*H, benzyl), 119.2 (C-5), 87.5 (C-1′), 83.6 (C-4′), 72.6 (C2′), 71.2 (C-3′), 44.7 (NH*C*H$_2$), 44.7 (C-5′); MS m/z 376 (M + H)$^+$; HPLC, nucleotide/nucleoside column; retention time 6.77 min. Anal. ($C_{17}H_{18}N_5O_3Cl$·0.1$CH_3CO_2C_2H_5$·0.4 H_2O) C, H, N.

5′-Chloro-5′-deoxy-*N*^{6}-(3-iodobenzyl)adenosine (9). The reaction was carried out with *N*^{6}-(3-iodobenzyl)adenosine (**8**; 1.33 g, 2.77 mmol): yield 1.38 g (2.75 mmol, 99%); mp 192−194 °C; ^{1}H NMR (DMSO-d_6) δ 8.46 (bs, 1H, NH), 8.38 (s, 1H, H-8), 8.22 (s, 1H, H-2), 7.71 (s, 1H, CC*H*CI), 7.57 (d, J = 7.56 Hz, 1H, CCHCIC*H*), 7.35 (d, J = 7.55 Hz, 1H, CC*H*CH), 7.09 (t, J = 7.55 Hz, 1H, CCHC*H*), 5.94 (d, J = 5.49, 1H, H-1′), 5.59 (d, J = 6.17 Hz, 1H, 2′-OH), 5.45 (d, J = 4.80 Hz, 1H, 3′-OH), 4.77−4.64 (m, 2H, NHCH_2), 4.77−4.64 (m, 1H, H-2′), 4.24−4.21 (m, 1H, H-3′), 4.10−4.07 (m, 1H, H-4′), 3.93−3.84 (m, 2H, H-5′); ^{13}C NMR (DMSO-d_6) δ 154.6 (C-6), 152.4 (C-2), 142.7 (C-4), 135.6 (CCHCI*C*H, benzyl), 135.2 (C*C*HCI), 132.4 (NHCH$_2$*C*), 130.3 (CCH*C*H), 126.5 (C*C*HCH), 119.3 (C-5), 94.4 (C-3′), 87.5 (C-1′), 83.6 (C-4′), 72.6 (C-2′), 71.1 (C-3′), 44.7 (NH*C*H$_2$), 44.7 (C-5′); MS m/z 502 (M + H)$^+$; HPLC, nucleotide/nucleoside column; retention time 10.79 min. Anal. ($C_{17}H_{17}N_5O_3ClI$) C, H, N.

General Procedure for the Introduction of the 5′-(Alkylthio) Substituent Starting from Compounds 3 and 9 into Compounds 4−7 and 10−13, Respectively. To a stirred solution of the corresponding alkylthiol (2.13 mmol) in NaOH (0.6 mL, 2 M) was added the 5′-chloro-5′-deoxy compound **3** or **9** (0.21 mmol). DMF was added dropwise until complete dissolution. The mixture was refluxed for 2 h, cooled, and acidified with acetic acid, and water was added (10 mL). The white precipitate formed was filtered and dried. The product was recrystallized from MeOH and ethyl acetate.

***N*^{6}-Benzyl-5′-deoxy-5′-(methylthio)adenosine (4).** The reaction was carried out with sodium thiomethoxide (149.0 mg, 2.13 mmol) and **3** (80.0 mg, 0.21 mmol): yield 61.0 mg (0.16 mmol, 74%); mp 210−211 °C; ^{1}H NMR (DMSO-d_6) δ 8.35 (s, 1H, H-8), 8.33 (bs, 1H, NH), 8.21 (s, 1H, H-2), 7.34−7.17 (m, 5H, benzyl), 5.90 (d, J = 5.65 Hz, 1H, H-1′), 5.44 (d, J = 5.94 Hz, 1H, 2′-OH), 5.26 (d, J = 5.02 Hz, 1H, 3′-OH), 4.74 (q, J = 5.42 Hz, 1H, H-2′), 4.74 (q, J = 5.42 Hz, 2H, NHCH_2), 4.15 (q, J = 4.18 Hz, 1H, H-3′), 4.03 (pq, J = 3.79 Hz, 1H, H-4′), 2.83 (dq, J = 15.11 Hz, J = 5.76 Hz, 2H, H-5′), 2.02 (s, 3H, CH_3); MS m/z 388.2 (M + H)$^+$; HPLC, nucleotide/nucleoside column; retention time 8.72 min. Anal. ($C_{18}H_{21}N_5O_3S$·0.5H_2O) C, H, N.

***N*^{6}-Benzyl-5′-deoxy-5′-(ethylthio)adenosine (5).** The reaction was carried out with ethanethiol (158 μL, 2.13 mmol) and **3** (80.0 mg, 0.21 mmol): yield 52.5 mg (0.13 mmol, 61%); mp 165−166 °C; R_f 0.53 (10% MeOH in CH_2Cl_2); ^{1}H NMR (DMSO-d_6) δ 8.34 (s, 1H, H-8), 8.28 (bs, 1H, NH), 8.21 (s, 1H, H-2), 7.34−7.17 (m, 5H, benzyl), 5.90 (d, J = 5.57 Hz, 1H, H-1′), 5.42 (d, J = 5.96 Hz, 1H, 2′-OH), 5.23 (d, J = 5.12 Hz, 1H, 3′-OH), 4.74 (pq, J = 5.56 Hz, J = 5.58 Hz, 1H, H-2′), 4.74 (pq, J = 5.56 Hz, J = 5.58 Hz, 2H, NHCH_2), 4.16 (q, J = 4.25 Hz, 1H, H-3′), 4.01 (dq, J = 3.81 Hz, 1H, H-4′), 2.85 (dq, J = 16.33 Hz, J = 10.58 Hz, 2H, H-5′), 2.51 (q, J = 7.38 Hz, 2H, SCH_2), 1.13 (t, J = 7.36 Hz, 3H, CH_3); MS m/z 402.2 (M + H)$^+$; HPLC, Alltima column; retention time 14.23 min. Anal. ($C_{19}H_{23}N_5O_3S$·0.7H_2O) C, H, N.

***N*^{6}-Benzyl-5′-deoxy-5′-(*n*-propylthio)adenosine (6).** The reaction was carried out with 1-propanethiol (193 μL, 2.13 mmol) and **3** (80.0 mg, 0.21 mmol): yield 76.6 mg (0.18 mmol, 87%); mp 159−160 °C; ^{1}H NMR (DMSO-d_6) δ 8.34 (s, 1H, H-8), 8.26 (bs, 1H, NH), 8.20 (s, 1H, H-2), 7.35−7.16 (m, 5H, benzyl), 5.89 (d, J = 5.58 Hz, 1H, H-1′), 4.73 (pt, J = 5.36 Hz, 1H, H-2′),), 4.73 (pt, J = 5.36 Hz, 2H, NHCH_2), 4.15 (t, J = 4.14 Hz, 1H, H-3′), 4.00 (pq, J = 6.59 Hz, 1H, H-4′), 2.86 (dq, J = 15.38 Hz, J = 5.62 Hz, 2H, H-5′), 2.47 (q, J = 6.50 Hz, 2H, SCH_2), 1.48 (q, J = 7.23 Hz, 2H, SCH_2CH_2), 0.85 (t, J = 7.31 Hz, 3H, CH_3); ^{13}C NMR (DMSO-d_6) δ 154.4 (C-6, C-4), 152.5

(C-2), 140.0 ($NHCH_2C$), 139.8 (C-8), 128.2 ($NHCH_2CCHCH$), 127.0 ($NHCH_2CCH$), 126.5 (CCHCHCH), 119.4 (C-5), 87.6 (C-1′), 84.1 (C-4′), 72.8 (C-2′), 72.7 (C-3′), 42.9 ($NHCH_2$), 34.1 (C-5′, SCH_2), 22.5 (SCH_2CH_2), 13.2 (CH_3); MS *m/z* 416 $(M + H)^+$; HPLC, Alltima column; retention time 21.43 min. Anal. ($C_{20}H_{25}N_5O_3S \cdot 0.5H_2O$) C, H, N.

N^6-Benzyl-5′-deoxy-5′-(isopropylthio)adenosine (7). The reaction was carried out with 2-propanethiol (198 μL, 2.13 mmol) and **3** (80.0 mg, 0.21 mmol): yield 70.5 mg (0.17 mmol, 80%); mp 115−116 °C; ^{1}H NMR (DMSO-d_6) δ 8.34 (s, 1H, H-8), 8.27 (bs, 1H, NH), 8.20 (s, 1H, H-2), 7.35−7.17 (m, 5H, benzyl), 5.89 (d, *J* = 5.54 Hz, 1H, H-1′), 5.41 (d, *J* = 5.95 Hz, 1H, 2′-OH), 5.22 (d, *J* = 5.08 Hz, 1H, 3′-OH), 4.74 (pq, *J* = 5.34 Hz, 1H, H-2′), 4.74 (pq, *J* = 5.34 Hz, 2H, $NHCH_2$), 4.16 (q, *J* = 4.28 Hz, 1H, H-3′), 4.00 (pq, *J* = 4.04 Hz, 1H, H-4′), 2.98−2.77 (m, 2H, H-5′), 2.98−2.77 (m, 1H, SCH), 1.18−1.14 (m, 6H, CH_3); ^{13}C NMR (DMSO-d_6) δ 154.4 (C-6, C-4), 152.5 (C-2), 140.0 ($NHCH_2C$), 128.1 (CCHCH), 127.0 (CCH), 126.5 (CCHCHCH), 120.0 (C-5), 87.4 (C-1′), 84.1 (C-4′), 72.6 (C-2′, C-3′), 42.9 ($NHCH_2$), 34.7 (SCH), 32.5 (C-5′), 23.3 (CH_3); MS *m/z* 416.3 $(M + H)^+$; HPLC, Alltima column; retention time 18.75 min. Anal. ($C_{20}H_{25}N_5O_3S \cdot 0.7H_2O$) C, H, N.

5′-Deoxy-N^6-(3-iodobenzyl)-5′-(methylthio)adenosine (10). The reaction was carried out with sodium thiomethoxide (149.0 mg, 2.13 mmol) and **9** (100.0 mg, 0.21 mmol): yield 69.1 mg (0.13 mmol, 63%); mp 145−146 °C; ^{1}H NMR (DMSO-d_6) δ 8.36 (s, 1H, H-8), 8.34 (s, 1H, NH), 8.22 (s, 1H, H-2), 7.73 (bs, 1H, CCHCI), 7.57 (d, *J* = 7.90 Hz, 1H, CCHCICH), 7.36 (d, *J* = 7.75 Hz, 1H, CCHCH), 7.10 (t, *J* = 7.74 Hz, 1H, CCHCH), 5.91 (d, *J* = 5.57 Hz, 1H, H-1′), 5.47 (bs, 1H, 2′-OH), 5.39 (bs, 1H, 3′-OH), 4.74 (pt, *J* = 5.11 Hz, 1H, H-2′), 4.74 (pt, *J* = 5.11 Hz, 2H, $NHCH_2$), 4.17 (t, *J* = 4.12 Hz, 1H, H-3′), 4.04 (q, *J* = 3.98 Hz, 1H, H-4′), 2.84 (dq, *J* = 14.53 Hz, *J* = 5.71 Hz, 2H, H-5′), 2.06 (s, 3H, CH_3); MS *m/z* 514 $(M + H)^+$; HPLC, nucleotide/nucleoside column; retention time 11.16 min. Anal. ($C_{18}H_{20}N_5O_3SI \cdot 0.6H_2O$) C, H, N.

5′-Deoxy-5′-(ethylthio)-N^6-(3-iodobenzyl)adenosine (11). The reaction was carried out with ethanethiol (158 μL, 2.13 mmol) and **9** (100.0 mg, 0.21 mmol): yield 90.5 mg (0.17 mmol, 80%); mp 154−156 °C; ^{1}H NMR (DMSO-d_6) δ 8.35 (s, 1H, H-8), 8.34 (bs, 1H, NH), 8.21 (s, 1H, H-2), 7.72 (bs, 1H, CCHCI), 7.56 (d, *J* = 7.82 Hz, 1H, CCHCICH), 7.35 (d, *J* = 7.62 Hz, 1H, CCHCH), 7.09 (t, *J* = 7.75 Hz, 1H, CCHCH), 5.90 (d, *J* = 5.53 Hz, 1H, H-1′), 5.42 (d, *J* = 5.89 Hz, 1H, 2′-OH), 5.23 (d, *J* = 5.03 Hz, 1H, 3′-OH), 4.77−4.72 (m, 1H, H-2′), 4.77−4.72 (m, 2H, $NHCH_2$), 4.16 (q, *J* = 4.16 Hz, 1H, H-3′), 4.01 (pq, *J* = 4.04 Hz, 1H, H-4′), 2.83 (dq, *J* = 16.74 Hz, *J* = 9.91 Hz, 2H, H-5′), 2.50 (q, 2H, SCH_2), 1.12 (t, *J* = 7.36 Hz, 3H, CH_3); MS *m/z* 528.2 $(M + H)^+$; HPLC, Alltima column; retention time 30.75 min. Anal. ($C_{19}H_{22}N_5O_3SI$) C, H, N.

5′-Deoxy-N^6-(3-iodobenzyl)-5′-(*n*-propylthio)adenosine (12). The reaction was carried out with 1-propanethiol (193 μL, 2.13 mmol) and **9** (100.0 mg, 0.21 mmol): yield 79.7 mg (0.15 mmol, 70%); mp 133−135 °C; ^{1}H NMR (DMSO-d_6) δ 8.36 (s, 1H, H-8), 8.33 (bs, 1H, NH), 8.21 (s, 1H, H-2), 7.72 (bs, 1H, CCHCI), 7.56 (d, *J* = 7.83 Hz, 1H, CCHCICH), 7.35 (d, *J* = 7.27 Hz, 1H, CCHCH), 7.09 (t, *J* = 7.66 Hz, 1H, CCHCH), 5.90 (d, *J* = 5.49 Hz, 1H, H-1′), 5.42 (d, *J* = 5.73 Hz, 1H, 2′-OH), 5.22 (d, *J* = 4.75 Hz, 1H, 3′-OH), 4.79−4.69 (m, 1H, H-2′), 4.79−4.69 (m, 2H, $NHCH_2$), 4.16 (q, *J* = 4.68 Hz, 1H, H-3′), 4.01 (pq, *J* = 4.08 Hz, 1H, H-4′), 2.84 (dq, *J* = 13.99 Hz, *J* = 5.70 Hz, 2H, H-5′), 2.48−2.43 (m, 2H, SCH_2), 1.48 (q, *J* = 7.25 Hz, 2H, SCH_2CH_2), 0.84 (t, *J* = 7.29 Hz, 3H, CH_3); ^{13}C NMR (DMSO-d_6) δ 154.2 (C-6, C-4), 152.5 (C-2), 142.8 ($NHCH_2C$), 140.0 (C-8), 135.3 (CCHCI), 130.4 (CCHCH), 126.6 (CCHCH), 119.5 (C-5), 94.7 (CI), 87.5 (C-1′), 84.2 (C-4′), 72.6 (C-2′, C-3′), 42.1 ($NHCH_2$), 33.9 (C-5′, SCH_2), 22.5 (SCH_2CH_2), 13.2 (CH_3); MS *m/z* 542.1 $(M + H)^+$; HPLC, Alltima column; retention time 20.65 min. Anal. ($C_{20}H_{24}N_5O_3SI$) C, H, N.

5′-Deoxy-N^6-(3-iodobenzyl)-5′-(isopropylthio)adenosine (13). The reaction was carried out with 2-propanethiol (198 μL, 2.13 mmol) and **9** (100.0 mg, 0.21 mmol): yield 80.8 mg (0.15 mmol, 70%); mp 140−141 °C; ^{1}H NMR (DMSO-d_6) δ 8.36 (s, 1H, H-8), 8.32 (bs, 1H, NH), 8.21 (s, 1H, H-2), 7.72 (bs, 1H, CCHCI), 7.56 (d, *J* = 7.66 Hz, 1H, CCHCICH), 7.35 (d, *J* = 7.87 Hz, 1H, CCHCH), 7.09 (t, *J* = 7.68 Hz, 1H, CCHCH), 5.89 (d, *J* = 5.39 Hz, 1H, H-1′), 5.46−5.37 (m, 1H, 2′-OH), 5.27−5.18 (m, 1H, 3′-OH), 4.79−4.62 (m, 1H, H-2′), 4.79−4.62 (m, 2H, $NHCH_2$), 4.20−4.13 (m, 1H, H-3′), 4.00 (pq, *J* = 4.39 Hz, 1H, H-4′), 2.97−2.77 (m, 2H, H-5′), 2.97−2.77 (m, 1H, SCH), 1.16 (t, *J* = 6.54 Hz, 6H, CH_3); MS *m/z* 542.1 $(M + H)^+$; HPLC, Alltima column; retention time 19.60 min. Anal. ($C_{20}H_{24}N_5O_3SI$) C, H, N.

Radioligand Binding Studies. Measurements with [^{3}H]-DPCPX in the absence of GTP were performed according to a protocol published previously.[34] Adenosine A_{2A} receptor affinities were determined according to Jarvis et al.[35] Adenosine A_3 receptor affinities were determined essentially as described.[2,36] Briefly, assays were performed in 50/10/1 buffer (50 mM Tris/10 mM $MgCl_2$/1 mM ethylenediaminetetraacetic acid (EDTA) and 0.01% 3-([3-cholamidopropyl]dimethylammonio)-1-propanesulfonate (CHAPS)) in glass tubes and contained 50 μL of a HEK 293 cell membrane suspension (10−30 μg), 25 μL of [^{125}I]AB-MECA (final concentration 0.15 nM), and 25 μL of ligand. Incubations were carried out for 1 h at 37 °C and were terminated by rapid filtration over Whatman GF/B filters, using a Brandell cell harvester (Brandell, Gaithersburg, MD). Tubes were washed three times with 3 mL of buffer. Radioactivity was determined in a Beckman 5500B γ-counter. Nonspecific binding was determined in the presence of 10^{-5} M (*R*)-PIA.

GTPγS Binding. The extent of stimulation of [^{35}S]GTPγ-[S] binding was determined by the method of Lorenzen et al.[12,13] with some minor modifications. In the final incubation conditions, which allowed the characterization of compounds **2**−**13** as potential partial agonists of the A_3 receptor, the GDP concentration was reduced to 1 μM and NaCl was omitted. Incubations were performed at 25 °C for 90 min with 1 μg of membrane protein (human A_3 receptor expressed in CHO cells). NECA was used as a full agonist in this assay, and its stimulation of [^{35}S]GTPγ[S] binding to the G protein was set to 100% (Table 4).

cAMP Assay. CHO cell cultures were grown overnight as a monolayer in 24-well tissue culture plates (400 μL/well; 2×10^5 cells/well). Cyclic AMP generation was performed in Dulbecco's modified Eagles medium (DMEM)/*N*-(2-hydroxyethyl)piperazine-*N*′-2-ethansulfonic acid (HEPES) buffer (0.60 g of HEPES/50 mL of DMEM, pH 7.4). To each well, washed three times with DMEM/HEPES buffer (250 μL) and 200 μL of DMEM/HEPES buffer was added 50 μL of adenosine deaminase (final concentration 10 IU/mL). After the mixture was incubated for 30 min at 37 °C, 50 μL of a mixture of rolipram and cilostamide (final concentration each 50 μM), 50 μL of forskolin (final concentration 10 μM), and 50 μL of agonist (final concentration 10 μM; NECA or compounds **2**, **4**, **8**, or **10**) were added. After 1 h, the reaction was terminated by removing the medium and adding 200 μL of 0.1 M HCl. Wells were stored at −20 °C until assay. When the compounds were tested as partial antagonists, to each well were added 150 μL of DMEM/HEPES buffer, 50 μL of adenosine deaminase (final concentration 10 IU/mL), and 50 μL of one of the compounds (final concentration 10 μM each). After incubation for 30 min at 37 °C, 50 μL of a mixture of rolipram and cilostamide (final concentration 50 μM each), 50 μL of forskolin (final concentration 10 μM), and 50 μL of NECA (final concentration 10 μM) were added.

The amounts of cyclic AMP were determined after a protocol with cAMP binding protein[37] with the following minor modifications. As a buffer was used 150 mM K_2HPO_4/10 mM EDTA/0.2% bovine serum albumine (BSA) at pH 7.5. Samples (10 μL + 40 μL of 0.1 M HCl) were incubated for at least 2.5 h at 0 °C before filtration over Whatman GF/B filters. Filters were additionally rinsed with 2 × 2 mL of Tris HCl buffer (pH 7.4, 4 °C). Filters were counted in Packard Emulsifier Safe scintillation fluid (3.5 mL) after 2 h of extraction.

Data Analysis. Apparent K_i values were computed from the displacement curves by nonlinear regression of the com-

petition curves with Prism (Graph Pad, San Diego, CA). EC_{50} values for stimulation of [^{35}S]GTPγ[S] binding were calculated with SigmaPlot.

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