Structure−Activity Relationships of 2-Chloro-N^6-substituted-4′-thioadenosine-5′-uronamides as Highly Potent and Selective Agonists at the Human A_3 Adenosine Receptor

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We have established structure−activity relationships of novel 4′-thionucleoside analogues as the A_3 adenosine receptor (AR) agonists. Binding affinity, selectivity toward other AR subtypes, and efficacy in inhibition of adenylate cyclase were studied. From this study, 2-chloro-N^6-methyl-4′-thioadenosine-5′-methyluronamide (**36a**) emerged as the most potent and selective agonist at the human A_3 AR. We have also revealed that, similar to 4′-oxoadenosine analogues, at least one hydrogen on the 5′-uronamide moiety was necessary for high-affinity binding at the human A_3 AR, presumably to allow this group to donate a H bond within the binding site. Furthermore, bulky substituents on the 5′-uronamide reduced binding affinity, but in some cases large 5′-uronamide substituents, such as substituted benzyl and 2-phenylethyl groups, maintained moderate affinity with reduced efficacy, leading to A_3 AR partial agonists or antagonists. In several cases for which the corresponding 4′-oxonucleosides have been studied, the 4′-thionucleosides showed higher binding affinity to the A_3 AR.

Introduction

Adenosine is an endogenous signaling molecule regulating many physiological functions through four specific subtypes (A_1, A_{2A}, A_{2B}, and A_3) of adenosine receptors (ARs), at least one of which is expressed on the surface of most cells.[1] Among these four subtypes, the A_3 subtype is the most recently identified and is known to be involved in many diseases, such as cardiac[2] and cerebral ischemia,[3] cancer,[4] asthma,[5] glaucoma,[6] and inflammation.[7,8] Thus, the A_3 AR is regarded as a good therapeutic target for the development of clinically useful agents.

A number of nucleoside and nonnucleoside derivatives have been synthesized and tested for binding affinities at the human A_1, A_{2A}, A_{2B}, and A_3 ARs.[9−11] Among them, **1** (IB-MECA)[11] showed potent binding affinity to the human A_3 AR (K_i = 1.0 nM) with 51- and 2900-fold selectivity for the human A_1 and A_{2A} ARs, respectively, with binding affinity at the human A_{2B} AR >10 μM (Chart 1). The systematic structure−activity relationship study of compound **1** led to **2** (Cl-IB-MECA),[12] which showed increased selectivity in comparison to **1**, while exhibiting similar binding affinity (K_i = 1.4 nM) to the human A_3 AR. Compound **1** was found to show potent in vivo antitumor activity[4] and is now undergoing phase II clinical trials, and compound **2** is being used extensively as a pharmacological tool for studying A_3 AR.[13]

On the other hand, a cyclopropyl-fused carbocyclic nucleoside **3** (MRS 3558),[14] adopting a fixed conformation of the pseudoribose ring, exhibited higher binding affinity (K_i = 0.29 nM) than compounds **1** and **2** at the human A_3 AR. Compound **3** showed better selectivity for the A_1 AR and similar binding affinity to the A_{2A} AR, compared to **1**, while the opposite trend was observed in the case of **2**. This study indicated that the binding site of the human A_3 AR preferred the North sugar ring puckering.

Recently, on the basis of a bioisosteric rationale and the high binding affinity and selectivity of compounds **1**−**3**, we have reported the 2-chloro-N^6-substituted-4′-thioadenosine-5′-methyluronamides as highly potent and selective A_3 AR agonists, among which **4** (LJ-530) showed extremely high binding affinity (K_i = 0.28 nM) at the human A_3 AR with high selectivity to the human A_1 and human A_{2A} ARs.[15] Compound **5** (LJ-529) has also exhibited very high binding affinity (K_i = 0.38 nM) at the human A_3 AR but less selectivity than **4** to the human A_1 and A_{2A} ARs.

Thus, on the basis of high binding affinity and selectivity of 4′-thionucleosides **4** and **5**,[15] we carried out an extensive structure−activity relationship study of the N^6-position and/or methyluronamide of **4** and **5** to search for better A_3 AR agonists (Figure 1). We found that electronic as well as steric effects play an important role in binding affinity and selectivity to the ARs. In this paper, we report a full account of 4′-thioadenosine derivatives as highly potent and selective A_3 AR agonists.

Results and Discussion

Chemistry. For an extensive structure−activity relationship study of 4′-thionucleosides, we first synthesized the glycosyl donors 4-thiosugars **14** or **15** on a preparative scale, which was achieved, starting from D-gulonic γ-lactone (**6**) as shown in Scheme 1.[15] Compound **6** was converted to the diacetonide **7** by treating with acetone and anhydrous copper(II) sulfate under acidic conditions. Treatment of **7** with LAH in ether followed by treating the resulting diol **8** with mesyl chloride afforded the dimesylate **9** in excellent yield. Cyclization to the 4-thiofuranose **10** was achieved by heating **9** with anhydrous sodium sulfide in DMF. Treatment of **10** with 30% acetic acid at room

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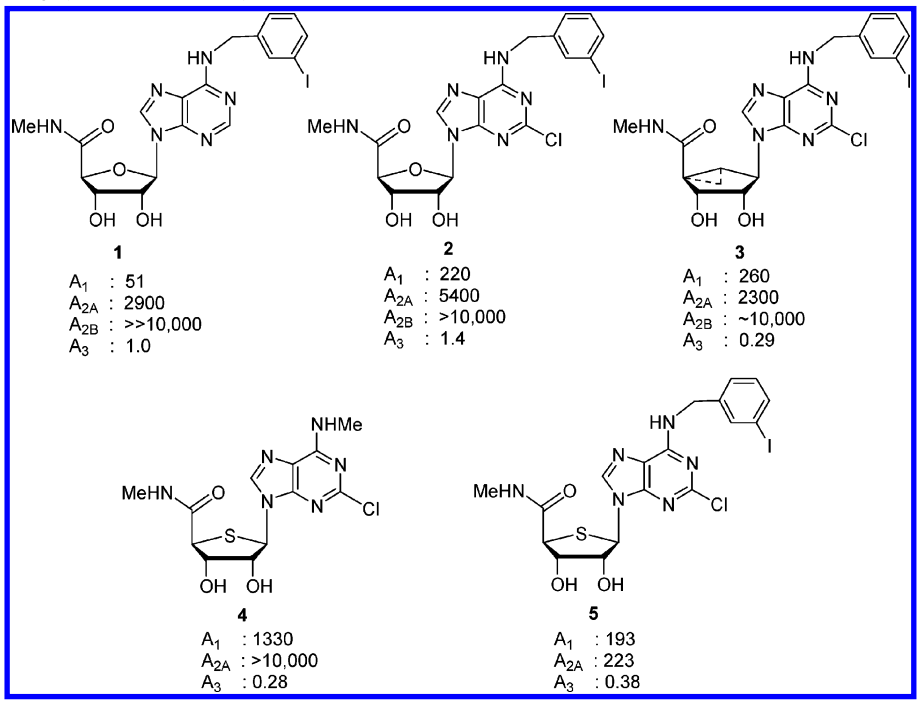
Chart 1. Binding Affinity (A_1, A_2, or A_3 AR) or Functional Activation of the Adenosine Derivatives at Human Adenosine Receptors[a]



[a] K_i (nM) values were determined (radioligand) in membrances from mammalian cells expressing hA_1 AR ([^{3}H]R-PIA), hA_2 AR ([^{3}H]CGS21680), or hA_3 AR ([^{125}I-AB-MECA). EC_{50} values at the hA_{2B} AR in a cAMP assay were determined in stably transfected CHO cells.[11,15,20,21]

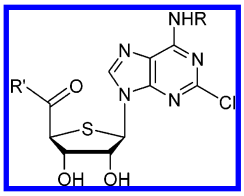


Figure 1. Structure of the desired 4′-thionucleosides (R = alkyl or arylalkyl, R′ = aminoalkyl or aminoarylalkyl).

temperature for 2 h gave **11** in 90% yield, on the basis of the recovered starting material.

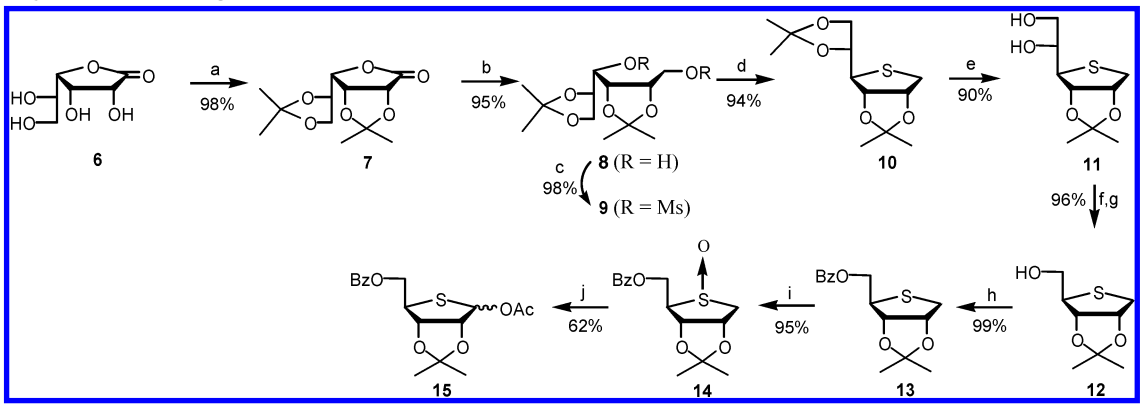
Oxidative cleavage of diol **11** with lead tetraacetate gave an aldehyde, which was reduced with sodium borohydride to give **12**. Protection of **12** with a benzoyl group afforded **13**. Treatment of **13** with mCPBA in methylene chloride gave the sulfoxide **14**, which served as a glycosyl donor for the condensation with nucleobases. The sulfoxide **14** was converted to another glycosyl donor **15** by refluxing with acetic anhydride at 100 °C.

The glycosyl donor **14** was directly condensed with 2,6-dichloropurine in the presence of TMSOTf and triethylamine in a solution of acetonitrile and 1,2-dichloroethane to give the β-anomer **16** (54%) with trace amounts of the α-anomer, while condensation of another glycosyl donor **15** with silylated 2,6-dichloropurine produced the same β-anomer **16** (60%) and its α-anomer in a 9:1 ratio,[15] but the two-step yield from sulfoxide **14** was only 37% (Scheme 2). Anomeric configurations were easily assigned by ^{1}H NOE experiments between 1′-H and 4′-H. To examine the effects of N^6-substituents and/or 5′-uronamides in binding to the binding site of the A_3 AR, compound **16** was treated with various amines such as ammonia, methylamine, cyclopropylamine, cyclopentylamine, 3-iodobenzylamine, and 2-methylbenzylamine to afford various N^6-substituted purine nucleosides **17**−**22**. For the synthesis of the various uronamides, N^6-substituted nucleosides **17**−**22** were first converted to the corresponding acids **29**−**34**. Treatment of **17**−**22** with 80% aqueous acetic acid followed by treating the resulting diols with TBSOTf yielded disilyl ethers, which were debenzoylated with sodium methoxide to give the 4′-hydroxymethyl analogues **23**−**28**, respectively. Oxidation of **23**−**28** with PDC in DMF gave the acid derivatives **29**−**34**, respectively.

Various N^6-substituted acid derivatives **29**−**34** were converted to the various uronamides **35**−**40g**, as shown in Scheme 3. In the previous paper,[15] acids were converted to the corresponding 5′-methyluronamides upon treatment with potassium carbonate and dimethyl sulfate in acetone followed by reacting the resulting esters with 40% methylamine, but an improved method was now employed for the synthesis of the uronamides. Direct coupling of the N^6-methyladenosine derivative **30** with various amines such as methylamine, dimethylamine, cyclopropylamine, cyclopropylmethylamine, isoamylamine, morpholine, 4-benzylpiperidine, 4-(4-fluorophenyl)piperazine, 3-fluorobenzylamine, 2-(3-fluorophenyl)ethylamine, and 3,3-diphenylpropylamine using EDC and HOBt afforded various 2-chloro-N^6-methyladenosine-5′-uronamides **36a**−**k** after desilylation. Similarly, N^6-cyclopropyl-, cyclopentyl-, 3-iodobenzyl-, and 2-methylbenzyladenosine derivatives **31**−**34** were also converted to the various uronamides **37a**−**e**, **38a,b**, **39a**−**m**, and **40a**−**g**, respectively.

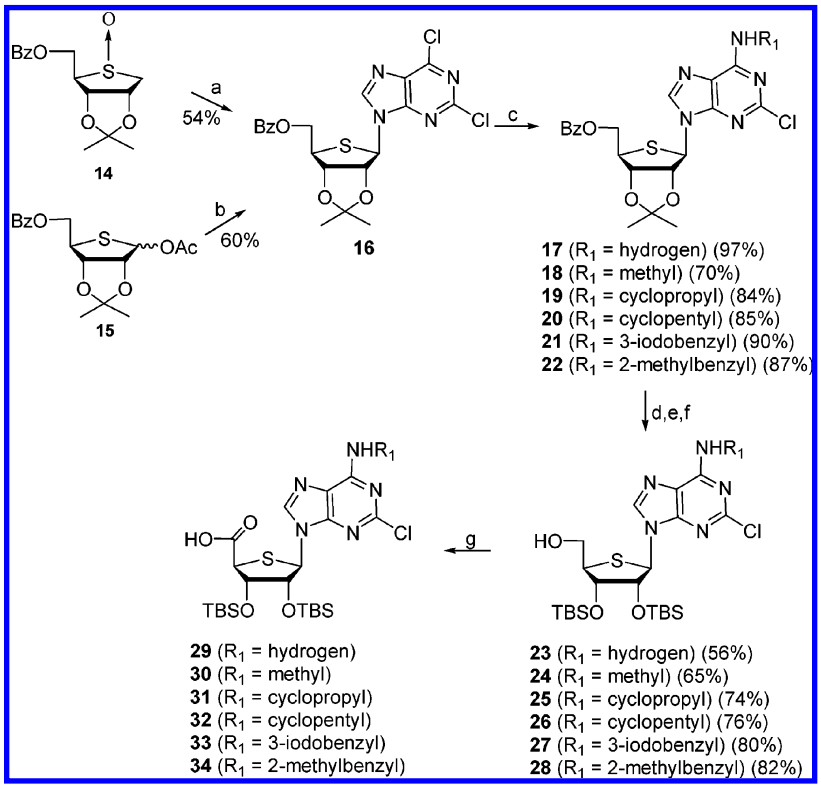
Biological Activity. All AR experiments were performed using adherent CHO (Chinese hamster ovary) cells stably transfected with cDNA encoding the human ARs.[11] Binding at the human A_3 AR in this study was carried out using [^{125}I]I-AB-MECA [N^6-(4-amino-3-iodobenzyl)adenosine-5′-*N*-ethylcarboxamidoadenosine] as a radioligand. In cases of weak binding, the percent inhibition of radioligand binding to the human A_3 AR was determined at 10 μM. Furthermore, the percent activation of the human A_3 AR (inhibition of adenylate cyclase in comparison to the full agonist **2**) was determined at 10 μM. Binding at human A_1 [using [^{3}H]R-PIA, N^6-(2-phenylisopropyl)adenosine, or [^{3}H]NECA, 5′-*N*-ethylcarboxamidoadenosine] or A_{2A} AR [using [^{3}H]CGS21680, (2-[*p*-(2-carboxyethyl)phenylethylamino]-5′-*N*-ethylcarboxamidoadenosine] was carried out as described in the Experimental Section.

Scheme 1. Synthesis of Thiosugar Intermediates[a]



[a] Reagents: (a) CH_3COCH_3, H_2SO_4, $CuSO_4$, rt; (b) $LiAlH_4$, ether; (c) MsCl, Et_3N, CH_2Cl_2; (d) Na_2S, DMF, heat; (e) 30% AcOH; (f) $Pb(OAc)_4$, EtOAc; (g) $NaBH_4$, MeOH; (h) BzCl, pyridine; (i) *m*-CPBA, CH_2Cl_2; (j) Ac_2O.

Scheme 2. Synthesis of 4′-Thionucleoside 5′-Carboxylic Acid Intermediates[a]

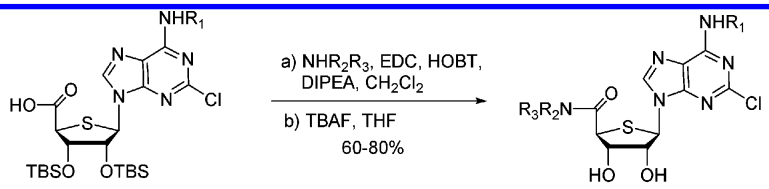


[a] Reagents and conditions: (a) 2,6-dichloropurine, TMSOTf, Et_3N, $CH_3CN-ClCH_2CH_2Cl$, rt to 83 °C; (b) silylated 2,6-dichloropurine, TMSOTf, $ClCH_2CH_2Cl$, rt to 80 °C; (c) R_1NH_2, Et_3N, EtOH, rt; (d) 80% AcOH, 70 °C; (e) TBSOTf, pyridine, 50 °C; (f) NaOMe, MeOH; (g) PDC, DMF.

As shown in Table 1, a variety of N^6-alkyl, cycloalkyl, and arylalkyl substituents on 4′-thioadenosine-5′-uronamide derivatives have produced high affinity at the human A_3 AR subtype, among which **36a** (R = CH_3, R′ = $NHCH_3$)[15] showed the most potent binding affinity (K_i = 0.28 ± 0.09 nM) at the A_3 AR. Compound **36a** was selective for the human A_3 AR versus the human A_1 AR by 4800-fold and showed extremely low binding affinity at the human A_{2A} AR (20% inhibition at 10 μM). It was also selective for the human A_3 AR versus the rat A_1 and rat A_{2A} ARs by 700- and 23 000-fold, respectively.[15] However, we have not determined the binding affinity of **36a** at rat A_3 AR, since it is expected to be weak due to the presence of the N^6-methyl group. The 4′-thionucleosides **36a** and its N^6-(3-iodobenzyl) analogue **39a** showed higher binding affinity to the human A_3 AR and higher selectivity in comparison to the human A_1 and A_{2A} ARs than the corresponding 4′-oxonucleosides **1** and **2**. However, due to the presence of the small N^6-alkyl group, the selectivity of the N^6-methyl derivative **36a** is not expected to be general across species.

All compounds (**36b,f**−**h**, **37d,e**, **39g**−**i**, **40c,f,g**) with dialkyl substitution at the 5′-uronamides showed reduced binding affinity at all the subtypes of ARs, indicating that the amide hydrogen, important for hydrogen bonding in the binding site of ARs, contributes to the observed affinity. Molecular modeling of adenosine receptors based on the template of the high-resolution structure of rhodopsin has already predicted that such a H-bond occurs between the exocyclic NH and the side chain of a conserved Asn residue in the sixth transmembrane helical domain of several ARs.[11a,17] In the case of monoalkyl 5′-uronamide derivatives, bulky substituents generally reduced binding affinity at the human A_3 AR, regardless of the substituent present at the N^6 position. A variety of N^6-alkyl, cycloalkyl, and arylalkyl substituted 4′-thio-5′-uronamide adenosine derivatives have produced moderate to high affinity at

Scheme 3. Synthesis of 4′-Thionucleoside 5′-Uronamide Derivatives[a]



29 (R_1 = hydrogen)
30 (R_1 = methyl)
31 (R_1 = cyclopropyl)
32 (R_1 = cyclopentyl)
33 (R_1 = 3-iodobenzyl)
34 (R_1 = 2-methylbenzyl)

35
36a-k
37a-e
38a-b
39a-m
40a-g

Compound No	R_1	R_2	R_3	Compound No	R_1	R_2	R_3
35	H	H	methyl	39b	3-iodobenzyl	H	ethyl
36a	methyl	H	methyl	39c	3-iodobenzyl	H	isoamyl
36b	methyl	CH_3	methyl	39d	3-iodobenzyl	H	cyclopropyl
36c	methyl	H	cyclopropyl	39e	3-iodobenzyl	H	cyclopropylmethyl
36d	methyl	H	cyclopropylmethyl	39f	3-iodobenzyl	H	cyclobutyl
36e	methyl	H	isoamyl	39g	3-iodobenzyl		4-benzylpiperidine
36f	methyl		morpholine	39h	3-iodobenzyl		4-(4-F-ph)piperazine
36g	methyl		4-benzylpiperidine	39i	3-iodobenzyl		morpholine
36h	methyl		4-(4-F-ph)piperazine	39j	3-iodobenzyl	H	3-Cl-benzyl
36i	methyl	H	3-F-benzyl	39k	3-iodobenzyl	H	3-CF_3-benzyl
36j	methyl	H	2-(3-F-phenyl)ethyl	39l	3-iodobenzyl	H	2-phenylethyl
36k	methyl	H	3,3-diphenylpropyl	39m	3-iodobenzyl	H	3,3-diphenypropyl
37a	cyclopropyl	H	methyl	40a	2-methylbenzyl	H	methyl
37b	cyclopropyl	H	ethyl	40b	2-methylbenzyl	H	ethyl
37c	cyclopropyl	H	cyclopropyl	40c	2-methylbenzyl	CH_3	methyl
37d	cyclopropyl		4-benzylpiperidine	40d	2-methylbenzyl	H	cyclopropyl
37e	cyclopropyl		morpholine	40e	2-methylbenzyl	H	cyclopropylmethyl
38a	cyclopentyl	H	methyl	40f	2-methylbenzyl		morpholine
38b	cyclopentyl	H	ethyl	40g	2-methylbenzyl		4-benzylpiperidine
39a	3-iodobenzyl	H	methyl				

[a] Reagents: (a) NHR_2R_3, EDC, HOBT, DIPEA, CH_2Cl_2; (b) TBAF, THF.

the human A_3 AR subtype. Among all the synthesized 5′-uronamide derivatives, 5′-methyluronamide derivatives exhibited the most potent binding affinity at the human A_3 AR, in which the binding affinity of the N^6 substituted compounds was in the following order: CH_3 > 3-iodobenzyl > H > 2-methylbenzyl > cyclopropyl > cyclopentyl. 5′-Uronamide substituents consisting of C1−C5 alkyl and cycloalkyl groups were capable of maintaining namomolar affinity and in some cases selectivity for the human A_3 AR in comparison to A_1 and A_{2A} ARs. This conclusion applied to N^6-methyl, cyclopropyl, and 3-iodobenzyl derivatives, but not to all N^6-substitution. While the 5′-*N*-cyclopropyl and cyclopropylmethyl uronamide derivatives displayed K_i values of 1−3 nM at the human A_3 AR in the above N^6 series, the corresponding N^6-(2-methylbenzyl) analogues were at least an order of magnitude less potent in binding. Thus, the effects of substitution at N^6 and 5′-positions were interdependent. To further illustrate this point, 5′-morpholineamides were ∼10-fold less potent in other N^6-series than was the N^6-(3-iodobenzyl) derivative **39i**. A 5′-(4-benzylpiperidine) derivative **40g** was considerably weaker in A_3 AR binding in the N^6-(2-methylbenzyl) series than in other N^6-series. A 5′-*N*-(3-methylbutyl) uronamide derivative **36e** was also highly potent and selective in binding to the A_3 AR. 5′-Uronamide substituents consisting of substituted benzyl and 2-phenylethyl groups generally resulted in intermediate affinity at the A_3 AR.

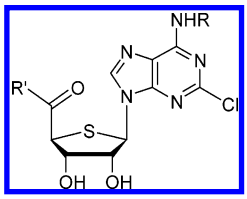
Compounds **35**, **36a,d**, **38a**, **39a,b,f**, and **40b,e** were full agonists in an assay of human A_3 receptor-mediated inhibition of cyclic AMP in transfected CHO cells.[16] However, in some cases large 5′-uronamide substituents reduced efficacy, leading to A_3 AR partial agonists or antagonists. For example, a 5′-*N*-(3-fluorobenzyl) uronamide derivative **36i** was a potent and selective partial agonist of the A_3 AR. The corresponding 5′-*N*-(2-(3-fluorophenyl)ethyl) uronamide derivative **36j**, although 5-fold less potent in binding, appeared to display less intrinsic efficacy toward the A_3 AR. The corresponding 5′-*N*-(3,3-diphenylpropyl) uronamide derivative **36k** displayed only moderate binding affinity and appeared to be a putative antagonist at the A_3 AR due to its ability to bind but not activate the receptor. In the series of N^6-(3-iodobenzyl) derivatives, **39c** and **39l** appeared to be either low efficacy agonists or antagonists. The 5′-*N*-(3-methylbutyl) uronamide **39c** was also selective in binding to the A_3 AR (K_i = 42 nM).

The reduction of intrinsic efficacy has been demonstrated previously for a variety of sterically bulky groups at N^6- and C2-positions[11] and for smaller substituents, such as thioethers, at the 5′-position.[10] These results are novel in that the range of 5′-substitutents found to maintain moderate binding affinity at the human the A_3 AR has been greatly expanded, leading to an initial structure−efficacy relationship.

Conclusion

We have established structure−activity relationships of novel 4′-thio analogues of compound **2**. An improved synthetic route to this series involved the direct condensation of the sulfoxide **14** with 2,6-dichloropurine, which was efficiently synthesized on a preparative scale from D-gulonic γ-lactone (**5**). This route was superior to the previous route based on condensation using 4-thioribosyl acetate **15** as an intermediate. From this study, 2-chloro-N^6-methyladenosine 5′-methyluronamide (**36a**) emerged as the most potent and selective agonist at the human A_3 AR. We have also revealed that, similar to 4′-oxoadenosine analogues, at least one hydrogen on the 5′-uronamide moiety is necessary for nanomolar binding affinity at the human A_3 AR, presumably to allow this group to donate a H bond within the

Table 1. Potency of 2-Chloro-4′-thioadenosine-5′-uronamide Derivatives at Human A_1, A_{2A}, and A_3 AR and Maximal Agonist Effects at Human A_3 AR Expressed in CHO Cells[a]



			K_i (nM) or % inhibition at 10 μM		hA$_3$ AR	
compd	R	R′	hA$_1$ AR	hA$_{2A}$ AR	K_i (nM)	% activation[b] at 10 μM
35	H	$NHCH_3$	89.2 ± 11.7	158 ± 29	0.40 ± 0.06	100 ± 5
36a	CH_3	$NHCH_3$	1330 ± 240	20%	0.28 ± 0.09	119 ± 12
36b	CH_3	$N(CH_3)_2$	10%	12%	1500 ± 1300	8.3 ± 5.9
36c	CH_3	cyclopropyl-NH	47.8 ± 5.7	2770 ± 580	2.82 ± 1.03	98 ± 8
36d	CH_3	cyclopropylmethyl-NH	638 ± 35	44%	1.10 ± 0.03	109 ± 9
36e	CH_3	isoamyl-NH	4070 ± 1220	14%	1.63 ± 0.17	92 ± 4
36f	CH_3	morpholine	4%	24%	3870 ± 580	38 ± 6
36g	CH_3	4-Benzyl -piperidine	10%	6%	3500 ± 340	3.1 ± 1.3
36h	CH_3	4-(4-F-Ph)-piperazine	28%	34%	2700 ± 880	2.9 ± 4.0
36i	CH_3	3-F-benzyl-NH	67%	41%	17.4 ± 3.8	57 ± 2
36j	CH_3	2-(3-F-phenyl)ethyl-NH	15%	30%	85.6 ± 35.6	11 ± 5
36k	CH_3	3,3-diphenyl-propyl-NH	20%	15%	415 ± 16	0.1 ± 2.8
37a	cyclopropyl	$NHCH_3$	37.3 ± 2.5	4890 ± 380	2.24 ± 1.21	99 ± 3
37b	cyclopropyl	$NHCH_2CH_3$	3.2 ± 0.3	604 ± 110	0.67 ± 0.07	97 ± 3
37c	cyclopropyl	cyclopropyl-NH	4.6 ± 0.3	325 ± 13	5.56 ± 1.77	85 ± 4
37d	cyclopropyl	4-benzylpiperidine	32%	33%	4020 ± 740	0
37e	cyclopropyl	morpholine	25%	11%	4440 ± 160	83 ± 5
38a	cyclopentyl	$NHCH_3$	13.9 ± 1.8	921 ± 76	4.27 ± 0.33	105 ± 2
38b	cyclopentyl	$NHCH_2CH_3$	2.0 ± 2	149 ± 29	2.83 ± 0.63	95 ± 3
39a	3-iodobenzyl	$NHCH_3$	193 ± 46	223 ± 36	0.38 ± 0.07	114 ± 9
39b	3-iodobenzyl	$NHCH_2CH_3$	144 ± 55	292 ± 120	0.89 ± 0.18	100 ± 6
39c	3-iodobenzyl	isoamyl-NH	31%	31%	41.9 ± 11.3	12 ± 6
39d	3-iodobenzyl	cyclopropyl-NH	92.0 ± 10.9	326 ± 23	2.96 ± 1.03	87 ± 4
39e	3-iodobenzyl	cyclopropylmethyl-NH	1720 ± 690	2220 ± 420	3.64 ± 0.60	96 ± 9
39f	3-iodobenzyl	cyclobutyl-NH	126 ± 2	549 ± 78	18.2 ± 13.4	100 ± 3
39g	3-iodobenzyl	4-benzylpiperidine	38%	17%	878 ± 285	74 ± 19
39h	3-iodobenzyl	4-(4-F-Ph)-piperazine	24%	12%	1440 ± 830	96 ± 10
39i	3-iodobenzyl	morpholine	18%	4%	510 ± 69	94 ± 6
39j	3-iodobenzyl	3-Cl-benzyl-NH	1710 ± 340	28%	308 ± 148	33 ± 9
39k	3-iodobenzyl	3-(trifluoromethyl)benzyl-NH	0%	11%	354 ± 18	31 ± 11
39l	3-iodobenzyl	2-phenylethyl-NH	20%	11%	433 ± 141	7.1 ± 2.7
39m	3-iodobenzyl	3,3-diphenylpropyl-NH	5%	3%	343 ± 48	31 ± 14
40a	2-methylbenzyl	$NHCH_3$	507 ± 66	1320 ± 270	2.18 ± 0.46	99 ± 6
40b	2-methylbenzyl	$NHCH_2CH_3$	75.8 ± 2.5	429 ± 97	2.50 ± 1.10	112 ± 9
40c	2-methylbenzyl	$N(CH_3)_2$	0%	22.5%	632 ± 70	9.1 ± 0.4
40d	2-methylbenzyl	cyclopropyl-NH	76.7 ± 9.3	202 ± 51	27.8 ± 3.8	91 ± 3
40e	2-methylbenzyl	cyclopropylmethyl-NH	1980 ± 310	3890 ± 630	29.7 ± 11.3	111 ± 11
40f	2-methylbenzyl	morpholine	4%	17%	7670 ± 800	61 ± 4
40g	2-methylbenzyl	4-benzylpiperidine	26%	15%	49200 ± 17500	31 ± 4

[a] All AR experiments were performed using adherent CHO cells stably transfected with cDNA encoding the human or rat ARs. Percent activation of the human A_3 AR was determined at 10 μM. Binding was carried out as described in the Experimental Section using as radioligand [^{3}H]R-PIA or [^{3}H]NECA at the human A_1 AR and [^{3}H]CGS 21680 at the human A_{2A} AR. Values from the present study are expressed as mean ± SEM, $n = 3-5$. [b] Percent activity (inhibition of foskolin-stimulated cyclic AMP formation) at 10 μM, relative to 10 μM Cl-IB-MECA (A_3).

binding site. Furthermore, bulky substituents on the 5′-uronamide reduced binding affinity, although in some cases large 5′-uronamide substituents, such as substituted benzyl and 2-phenylethyl groups, maintained moderate affinity with reduced efficacy, leading to A_3 AR partial agonists or antagonists. In several cases for which the corresponding 4′-oxonucleosides have been studied, the 4′-thionucleosides showed higher binding affinity to the A_3 AR. These findings may facilitate the identification of the modes of binding of nucleoside derivatives at ARs.

Experimental Section

General. Melting points are uncorrected. ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra were measured in $CDCl_3$ or CD_3OD and chemical shifts are reported in parts per million (δ) downfield from tetramethylsilane as internal standard. Column chromatography was performed using silica gel 60 (230−400 mesh). Anhydrous solvents were purified by the standard procedures.

Synthesis. Methanesulfonic Acid (*S*)-((4*R*)-2,2-Dimethyl-[1,3]-dioxolan-4-yl)((4*S*,5*S*)-5-methanesulfonyloxymethyl-2,2-dimethyl-[1,3]dioxolan-4-yl)methyl Ester (9). To a stirred slurry of **6** (20.2 g, 113.5 mmol) and anhydrous $CuSO_4$ (27.0 g, 169.8 mmol) in dry acetone (650 mL) was added concentrated H_2SO_4 (1.7 mL) and the mixture was stirred at room temperature for 24 h. The pH of the solution was adjusted to 7 with $Ca(OH)_2$, and the resulting slurry was filtered and evaporated to afford **7** (28.7 g, 98%) as a light-yellow solid, which was used in the next step without further purification. To a stirred solution of **7** (25.1 g, 97.2 mmol) in anhydrous THF (300 mL) was cautiously added LAH (7.2 g, 190.4 mmol) in several portions at 0 °C and the reaction mixture was stirred at room temperature for 5 h. The reaction mixture was quenched with ice, dried over anhydrous $MgSO_4$, filtered through a Celite pad, and evaporated. The resulting residue was purified

by silica gel column chromatography (hexane/ethyl acetate = 1:1) to give **8** (24.1 g, 95%) as a white semisolid: ^{1}H NMR ($CDCl_3$) δ 1.29 (s, 3 H, CH_3), 1.29 (s, 3 H, CH_3), 1.36 (s, 3 H, CH_3), 1.43 (s, 3 H, CH_3), 3.26 (s, 1 H, OH), 3.28 (s, 1 H, OH), 3.36−3.79 (m, 4 H, HOC*H*$_2$, OC*H*$_2$), 3.96−4.03 (m, 2 H, HOCH$_2$C*H*(OR)R′, OC*H*RR′), 4.12−4.22 (m, 2 H). Anal. ($C_{12}H_{22}O_6$) C, H.

To a solution of **8** (4.1 g, 15.7 mmol) in dry pyridine (20 mL) was added methanesulfonyl chloride (6.1 mL, 47.1 mmol) at 0 °C and the mixture was stirred at the same temperature for 4 h. The reaction mixture was quenched with ice and the reaction mixture was partitioned between EtOAc and H_2O. The organic layer was washed with aqueous $NaHCO_3$ solution and brine, dried over anhydrous $MgSO_4$, and evaporated. The residue was purified by silica gel column chromatography (hexane/ethyl acetate = 2:1) to give **9** (6.4 g, 98%) as a colorless syrup: ^{1}H NMR ($CDCl_3$) δ 1.37 (s, 3 H, CH_3), 1.39 (s, 3 H, CH_3), 1.46 (s, 3 H, CH_3), 1.52 (s, 3 H, CH_3) 3.10 (s, 3 H, SO_2CH_3), 3.18 (s, 3 H, SO_2CH_3), 3.92−4.08 (dd, 1 H, *J* = 7.1, 11.7 Hz, ROC*H*H), 4.08−4.20 (m, 2 H, ROCH*HC*H(OR′)), 4.39−4.49 (m, 4 H, MsOC*H*$_2$C*H*(OR)C*H*(OR′)), 4.81−4.87 (dd, 1 H, *J* = 4.8, 6.6 Hz, MsOC*H*RR′). Anal. ($C_{14}H_{26}O_{10}S_2$) C, H, S.

(3a*S*,4*R*,6a*R*)-4-((4*R*)-2,2-Dimethyl-[1,3]dioxolan-4-yl)-2,2-dimethyltetrahydrothieno[3,4-*d*][1,3]dioxole (10). To a stirred solution of **9** (10.1 g, 24.2 mmol) in DMF (260 mL) was added sodium sulfide nonahydrate (6.97 g, 29.0 mmol) and the mixture was heated at 100 °C for 3 h. After being cooled to room temperature, the mixture was diluted with ether and washed with H_2O (three times) and brine. The organic layer was dried over anhydrous $MgSO_4$, filtered, and evaporated. The resulting residue was purified by silica gel column chromatography (hexane/ethyl acetate = 5:1) to give **10** (5.9 g, 94%) as a syrup: ^{1}H NMR ($CDCl_3$) δ 1.33 (s, 3 H, CH_3), 1.34 (s, 3 H, CH_3), 1.44 (s, 3 H, CH_3), 1.52 (s, 3 H, CH_3), 2.88 (d, 1 H, *J* = 12.7 Hz, 6-C*H*H), 3.09 (td, 1 H, *J* = 2.2, 13.1 Hz, 6-CH*H*), 3.24 (d, 1 H, *J* = 8.8 Hz, 4-H), 3.76 (dd, 1 H, *J* = 5.7, 8.6 Hz, 5′-C*H*H), 3.98 (td, 1 H, *J* = 6.0, 9.0 Hz, 4′-H). 4.15 (dd, 1 H, *J* = 6.3, 8.5 Hz, 5′-CH*H*), 4.93 (d, 2 H, *J* = 1.9 Hz, 3a-H, 6a-H); ^{13}C NMR ($CDCl_3$) δ 23.8, 24.6, 25.6, 26.1, 36.9, 56.5, 68.3, 75.6, 82.3, 84.5; FAB-MS *m*/*z* 260 (M^+). Anal. ($C_{12}H_{20}O_4S$) C, H, S.

(1*R*)-1-((3a*S*,4*R*,6a*R*)-2,2-Dimethyltetrahydrothieno[3,4-*d*]-[1,3]dioxol-4-yl)ethane-1,2-diol (11). A solution of **10** (5.4 g, 20.1 mmol) in 30% aqueous AcOH (150 mL) was stirred at room temperature for 4 h and the reaction mixture was evaporated. The resulting residue was purified by silica gel column chromatography (hexane/ethyl acetate = 1:1) to give **11** (1.89 g, 43%) and recovered starting material **9** (2.82 g): ^{1}H NMR ($CDCl_3$) δ 1.34 (s, 3 H, CH_3), 1.53 (s, 3 H, CH_3), 2.90 (dd, 1 H, *J* = 2.2, 12.9 Hz, 6′-C*H*H), 3.09 (dd, 1 H, *J* = 4.8, 12.9 Hz, 6′-CH*H*), 3.27 (dd, 1 H, *J* = 1.9, 7.8 Hz, 4′-H), 3.49 (br s, 2 H, 2′ × OH), 3.58−3.72 (m, 2 H, HOC*H*HC*H*(OH)), 3.79 (dd, 1 H, *J* = 2.9, 11.0 Hz, HOCH*H*), 4.93 (m, 2 H, 3a-H, 6a-H). Anal. ($C_9H_{16}O_4S$) C, H, S.

(3a*S*,4*R*,6a*R*)-(2,2-Dimethyltetrahydrothieno[3,4-*d*][1,3]dioxol-4-yl)methanol (12). To a stirred solution of **11** (2.5 g, 11.2 mmol) in ethyl acetate (50 mL) was added $Pb(OAc)_4$ (5.4 g, 12.3 mmol) at 0 °C and the reaction mixture was stirred for 10 min, at which time TLC indicated the absence of starting material. The reaction mixture was filtered, the filtrate was diluted with EtOAc, and the organic layer was washed with saturated aqueous $NaHCO_3$ solution, dried over anhydrous $MgSO_4$, and evaporated. The residue was purified by silica gel column chromatography (hexane/ethyl acetate = 5:1) to give aldehyde (2.1 g, 98%) as a syrup: ^{1}H NMR ($CDCl_3$) δ 1.33 (s, 3 H, CH_3), 1.52 (s, 3 H, CH_3), 2.61 (dd, 1 H, *J* = 3.9, 13.2 Hz, 6-C*H*H), 2.87 (dd, 1 H, *J* = 4.1, 12.7 Hz, 6-CH*H*), 3.93 (s, 1 H, 4-H), 4.92 (t, 1 H, *J* = 4.1 Hz, 6a-H), 5.10 (d, 1 H, *J* = 5.4 Hz, 3a-H), 9.43 (s, 1 H, CHO).

To a stirred solution of aldehyde (5.6 g, 30.0 mmol) in MeOH (70 mL) was carefully added sodium borohydride (1.3 g, 33.6 mmol) in several portions at 0 °C, and the reaction mixture was stirred for 30 min at the same temperature and neutralized with glacial AcOH. After the removal of the solvent, the mixture was partitioned between EtOAc (150 mL) and brine (100 mL). The organic layer was dried over anhydrous $MgSO_4$, filtered, and evaporated. The resulting residue was purified by silica gel column chromatography (hexane/ethyl acetate = 2:1) to give **12** (5.5 g, 98%) as a syrup: ^{1}H NMR ($CDCl_3$) δ 1.33 (s, 3 H, CH_3), 1.53 (s, 3 H, CH_3), 2.41 (br s, 1 H, OH), 2.89 (dd, 1 H, *J* = 1.5, 12.9 Hz, 6-C*H*H), 3.09 (dd, 1 H, *J* = 4.9, 12.7 Hz, 6-CH*H*), 3.44 (td, 1 H, *J* = 1.0, 6.6 Hz, 4-H), 3.59 (d, 2 H, *J* = 5.4 Hz, HOC*H*$_2$), 4.71 (dd, 1 H, *J* = 1.2, 5.6 Hz, 6a-H), 4.91 (td, 1 H, *J* = 1.5, 5.3 Hz, 3a-H); FAB-MS *m*/*z* 190 (M^+). Anal. ($C_8H_{14}O_3S$) C, H, S.

Benzoic Acid (3a*S*,4*R*,6a*R*)-2,2-Dimethyltetrahydrothieno[3,4-*d*][1,3]dioxol-4-ylmethyl Ester (13). To a solution of **12** (2.1 g, 11.1 mmol) in pyridine (20 mL) was added benzoyl chloride (1.9 g, 13.4 mmol) at 0 °C and the reaction mixture was stirred at room temperature for 6 h, quenched with methanol, and evaporated. The residue was dissolved in ether (50 mL) and the insoluble pyridinium salt was filtered and washed with ether. The combined organic layer was dried over anhydrous $MgSO_4$, filtered, and evaporated. The resulting residue was purified by silica gel column chromatography (hexane/ethyl acetate = 4:1) to afford **13** (3.2 g, 99%) as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 1.26 (s, 3 H, CH_3), 1.47 (s, 3 H, CH_3), 2.87 (dd, 1 H, *J* = 1.5, 13.2 Hz, 6-C*H*H), 3.11 (dd, 1 H, *J* = 4.9, 13.2 Hz, 6-CH*H*), 3.57 (m, 1 H, 4-H), 4.25 (dd, 1 H, *J* = 8.0, 11.4 Hz, BzOC*H*H), 4.35 (dd, 1 H, *J* = 5.8, 11.4 Hz, BzOCH*H*), 4.72 (dd, 1 H, *J* = 1.2, 5.6 Hz, 6a-H), 4.91 (td, 1 H, *J* = 1.2, 4.4 Hz, 3a-H), 7.35−7.99 (m, 5 H, Ph); FAB-MS *m*/*z* 294 (M^+). Anal. ($C_{15}H_{18}O_4S$) C, H, S.

Benzoic Acid (3a*S*,4*R*,6*R*,6a*R*)- and (3a*S*,4*R*,6*S*,6a*R*)-6-Acetoxy-2,2-dimethyltetrahydrothieno[3,4-*d*][1,3]dioxol-4-ylmethyl Ester (15). To a stirred solution of **13** (1.4 g, 4.6 mmol) in CH_2Cl_2 (30 mL) was added a solution of *m*-CPBA (1.0 g, 4.6 mmol, 80%) in CH_2Cl_2 (15 mL) dropwise at −78 °C and the mixture was stirred at the same temperature for 45 min. The reaction mixture was quenched with aqueous saturated $NaHCO_3$ solution and extracted with CH_2Cl_2, and the organic layer was washed with brine, dried over anhydrous $MgSO_4$, filtered, and evaporated. The residue was purified by silica gel column chromatography (hexane/ethyl acetate = 2:1) to give **14** (1.4 g, 95%) as a white solid: ^{1}H NMR ($CDCl_3$) of one isomer δ 1.33 (s, 3 H, CH_3), 1.49 (s, 3 H, CH_3), 3.23 (dd, 1 H, *J* = 4.1, 14.4 Hz, 6-C*H*H), 3.38 (dd, 1 H, *J* = 6.3, 14.4 Hz, 6-CH*H*), 3.47 (m, 1 H, 4-H), 4.73 (dd, 1 H, *J* = 9.0, 11.9, Hz, BzOC*H*H), 4.89 (dd, 1 H, *J* = 4.9, 11.9 Hz, BzOCH*H*), 5.02 (t, 1 H, *J* = 6.1 Hz, 3a-H), 5.24 (m, 1 H, 6a-H), 7.41−8.03 (m, 5 H, Ph).

A solution of **14** (3.5 g, 11.3 mmol) in Ac_2O (90 mL) was heated at 100 °C for 6 h and evaporated. The residue was partitioned between ethyl acetate and H_2O. The organic layer was washed with saturated aqueous $NaHCO_3$ solution and brine, dried over anhydrous $MgSO_4$, filtered, and evaporated. The residue was purified by silica gel column chromatography (hexane/ethyl acetate = 3:1) to give **15** (2.5 g, 62%) as a syrup: ^{1}H NMR ($CDCl_3$) of one isomer δ 1.30 (s, 3 H, CH_3), 1.50 (s, 3 H, CH_3), 2.03 (s, 3 H, $COCH_3$), 3.77 (dd, 1 H, *J* = 5.8, 9.5 Hz, 4-H), 4.37 (dd, 1 H, *J* = 9.8, 11.4 Hz, BzOC*H*H), 4.23 (dd, 1 H, *J* = 6.1, 11.4 Hz, BzOCH*H*), 4.94 (d, 1 H, *J* = 5.6 Hz, 3a-H), 4.98 (d, 1 H, *J* = 5.6 Hz, 6a-H), 6.06 (s, 1 H, 6-H), 7.42−8.06 (m, 5 H, Ph); FAB-MS *m*/*z* 293 (M^+ − OAc). Anal. ($C_{17}H_{20}O_6S$) C,H, S.

Benzoic Acid (3a*S*,4*R*,6*R*,6a*R*)-6-(2,6-Dichloropurin-9-yl)-2,2-dimethyltetrahydrothieno[3,4-*d*][1,3]dioxol-4-ylmethyl Ester (16) and Its α-Isomer. To a suspension of 2,6-dichloropurine (9.93 g, 64.2 mmol) in a mixture of dry CH_3CN (100 mL) and 1,2-dichloroethane (50 mL) were added triethylamine (9.0 mL, 64.2 mmol) and TMSOTf (23.2 mL, 128.5 mmol), and the mixture was stirred at room temperature until the mixture was clear. The resulting solution was added to a solution of **14** (10.0 g, 32.1 mmol) in dry 1,2-dichloroethane (50 mL) dropwise. An additional amount of triethylamine (9.0 mL, 64.2 mmol) in dry 1,2-dichloroethane (25 mL) was added to the reaction mixture to initiate the Pummerer reaction. After being stirred at room temperature for 5 min, the reaction mixture was heated at 83 °C for 24 h. The reaction was quenched with ice and the reaction mixture was partitioned between EtOAc and H_2O. The separated organic layer was washed with

saturated $NaHCO_3$ solution (three times) and brine. The organic layer was dried over anhydrous $MgSO_4$ and evaporated. The residue was purified by a silica gel column chromatography (CH_2Cl_2/EtOAc = 20:1−10:1) to give **16** (8.34 g, 54%) as a white foam and its α-anomer (trace).

β-anomer **16**: UV (MeOH) λ_{max} 269 nm (pH 7); ^{1}H NMR ($CDCl_3$) δ 1.39 (s, 3 H, CH_3), 1.64 (s, 3 H, CH_3), 4.11 (td, 1 H, J = 2.7, 6.8 Hz, 4-H), 4.57 (dd, 1 H, J = 6.6, 11.4 Hz, BzOCH*H*), 4.75 (dd, 1 H, J = 7.1, 11.4 Hz, BzOC*H*H), 5.19 (dd, 1 H, J = 2.7, 5.4 Hz, 3a-H), 5.40 (dd, 1 H, J = 1.9, 5.4 Hz, 6a-H), 6.10 (d, 1 H, J = 1.9 Hz, 6-H), 7.37−7.97 (m, 5 H, Ph), 8.38 (s, 1 H, H-8); FAB-MS m/z 482 ($M^+ + 1$). Anal. ($C_{20}H_{18}Cl_2N_4O_4S$) C, H, N, S.

α-anomer: UV (MeOH) λ_{max} 269 nm (pH 7); ^{1}H NMR ($CDCl_3$) δ 1.37 (s, 3 H, CH_3), 1.64 (s, 3 H, CH_3), 4.08 (m, 1 H, 4-H), 4.51 (dd, 1 H, J = 6.3, 11.7 Hz, BzOC*H*H), 4.62 (dd, 1 H, J = 7.1, 11.7 Hz, BzOCH*H*), 5.01 (m, 1 H, 3a-H), 5.11 (m, 1 H, 6a-H), 6.50 (d, 1 H, J = 2.2 Hz, 6-H), 7.37−7.99 (m, 5 H, Ph), 8.87 (s, 1 H, H-8); FAB-MS m/z 482 ($M^+ + 1$). Anal. ($C_{20}H_{18}Cl_2N_4O_4S$) C, H, N, S.

Benzoic Acid (3a*S*,4*R*,6*R*,6a*R*)-6-(6-Amino-2-chloropurin-9-yl)-2,2-dimethyltetrahydrothieno[3,4-*d*][1,3]dioxol-4-ylmethyl Ester (17). A solution of **16** (454 mg, 0.94 mmol) in saturated ethanolic ammonia (25 mL) was heated at 50 °C in a sealed tube for 24 h. The reaction mixture was evaporated to dryness under reduced pressure. The resulting residue was purified by silica gel column chromatography (hexane/ethyl acetate = 1:1) to afford **17** (148 mg, 32%) and debenzoylated compound (235 mg, 65%). A solution of debenzoylated compound (235 mg) in pyridine (10 mL) was treated with benzoyl chloride (0.12 mL, 0.758 mmol) at room temperature for 12 h. The reaction mixture was partitioned between EtOAc and water and the organic layer was dried, filtered, and evaporated. The resulting residue was purified by silica gel column chromatography (hexane/ethyl acetate = 2:1) to afford compound **17** (240 mg, 80%): UV (MeOH) λ_{max} 264 nm (pH 7); ^{1}H NMR ($CDCl_3$) δ 1.37 (s, 3 H, CH_3), 1.63 (s, 3 H, CH_3), 4.01 (td, 1 H, J = 2.7, 7.3 Hz, 4-H), 4.57 (dd, 1 H, J = 6.8, 11.5 Hz, BzOC*H*H), 4.77 (dd, 1 H, J = 7.6, 11.5 Hz, BzOCH*H*), 5.23 (dd, 1 H, J = 2.7, 5.6 Hz, 3a-H), 5.38 (dd, 1 H, J = 1.9, 5.6 Hz, 6a-H), 6.02 (d, 1 H, J = 1.9 Hz, 6-H), 6.29 (br s, 2 H, NH_2), 7.40−8.02 (m, 6 H, Ph, H-8); FAB-MS m/z 463 ($M^+ + 1$). Anal. ($C_{20}H_{20}ClN_5O_4S$) C, H, N, S.

General Procedure A for the Synthesis of 18−22. To a solution of **16** in anhydrous EtOH (20 mL/mmol) were added triethylamine (3.0 equiv) and the appropriate amine (1.2 equiv). After being stirred at room temperature for 24 h, the reaction mixture was evaporated. The residue was purified by silica gel column chromatography (hexane/ethyl acetate = 1:1) to give various N^6-substituted nucleosides **18−22**. Spectral data for compounds **18−22** are provided in the Supporting Information.

General Procedure B for the Synthesis of 23−28. A solution of N^6-substituted nucleosides **(17−22)** in 80% aqueous AcOH solution (30 mL) was stirred at 70 °C for 12 h. The solvent was removed under reduced pressure and the mixture was neutralized with methanolic ammonia. After evaporation, the residue was purified by silica gel column chromatography (CH_2Cl_2/MeOH = 15:1) to give diol as a white foam.

To a stirred solution of diol in dry pyridine (20 mL) was added a solution of TBDMSOTf (5.0 equiv) dropwise and the reaction mixture was stirred at 50 °C for 5 h. The mixture was partitioned between CH_2Cl_2 and H_2O, and the organic layer was washed with water, aqueous $NaHCO_3$ solution, water, and brine; dried over anhydrous $MgSO_4$; and evaporated. The crude disilyl ether was used in the next step without further purification.

To a stirred solution of disilyl ether in anhydrous methanol (30 mL) was added sodium methoxide (1.5 equiv) and the mixture was stirred at room temperature for 4 h. After being neutralized with glacial acetic acid, the mixture was evaporated. The resulting residue was purified by silica gel column chromatography (hexane/ethyl acetate = 2:1) to give 4′-hydroxymethyl analogues **23−28**. Spectral data for compounds **23−28** are provided in the Supporting Information.

General Procedure C for the Synthesis of 29−34. To a stirred solution of 4′-hydroxymethyl analogues **(23−28)** in dry DMF (10 mL) was added pyridinium dichromate (10.0 equiv) and the reaction mixture was stirred at room temperature for 20 h. After being poured into water (50 mL per mmol), the reaction mixture was stirred at room temperature for 1 h. The precipitate was filtered, and the filter cake was washed with excess water and dried under high vacuum to give a brownish solid, which was used in the next step without further purification.

2-Chloropurin-9-yl)-3,4-dihydroxytetrahydrothiophene-2-carboxylic Acid Methyl Amide (35). Compound **23** (150 mg, 0.27 mmol) was oxidized with pyridinium dichromate (2.6 g, 6.91 mmol) to give the crude **29**, which was methylated with dimethyl sulfate (1 mL, 10.57 mmol) and K_2CO_3 (100 mg, 0.72 mmol) in acetone (6 mL) at room temperature for 2 h to give methyl ester (200 mg) after the usual workup. A stirred solution of crude methyl ester (200 mg) was treated with methylamine (20 mL, 40.0 mmol, 2 N THF solution) at room temperature for 24 h in a sealed tube. The volatiles were removed under reduced pressure, and the resulting residue was purified by silica gel column chromatography (hexane/ethyl acetate = 1:2) to give silyl amide (80 mg, 51%) as a white foam: UV (MeOH) λ_{max} 264 nm (pH 7); ^{1}H NMR ($CDCl_3$) δ 0.01 (m, 12 H, 4′ × Si−CH_3), 0.79 (s, 9 H, C(CH_3)$_3$), 0.85 (s, 9 H, C(CH_3)$_3$), 2.67 (d, 3 H, J = 4.1 Hz, N−CH_3), 3.78 (d, 1 H, J = 4.8 Hz, 2-H), 4.35 (m, 1 H, 3-H), 4.57 (m, 1 H, 4-H), 5.70 (d, 1 H, J = 5.6 Hz, 5-H), 7.72 (br s, 2 H, exchangeable with D_2O, NH_2), 7.72 (br s, 1 H, exchangeable with D_2O, NH), 8.10 (s, 1 H, H-8).

To a stirred solution of silyl amide (140 mg, 0.24 mmol) in THF (5 mL) was added TBAF (0.25 mL, 0.25 mmol, 1 M THF solution) and the reaction mixture was stirred at room temperature for 1 h. The solvent was evaporated and the resulting residue was purified by silica gel column chromatography (CH_2Cl_2/MeOH = 10:1) to give 3**5** (58 mg, 69%) as a white solid: mp 233−235 °C; $[\alpha]^{26}_D$ −20.2 (*c* 0.1, MeOH); UV (MeOH) λ_{max} 264 nm (pH 7); ^{1}H NMR (DMSO-d_6) δ 2.70 (d, 3 H, J = 4.1 Hz, N−CH_3), 3.82 (d, 1 H, J = 4.6 Hz, 2-H), 4.36 (dd, 1 H, J = 4.6, 8.7 Hz, 3-H), 4.53 (m, 1 H, 4-H), 5.60 (d, 1 H, J = 5.4 Hz, exchangeable with D_2O, OH), 5.78 (d, 1 H, J = 5.1 Hz, exchangeable with D_2O, OH), 5.82 (d, 1 H, J = 5.5 Hz, 5-H), 7.87 (br s, 2 H, exchangeable with D_2O, NH_2), 8.33 (br q, 1 H, exchangeable with D_2O, NH), 8.55 (s, 1 H, H-8); ^{13}C NMR (DMSO-d_6) δ 51.8, 57.5, 62.5, 75.4, 78.1, 118.4, 140.1, 150.5, 153.0, 156.7, 170.7; FAB-MS m/z 345 (M^+). Anal. ($C_{11}H_{13}ClN_6O_3S$) C, H, N, S.

General Procedure D for the Synthesis of 36a−40g. To a solution of acid derivatives **(30−34)**, EDC (1.5 equiv), HOBt (1.5 equiv), and appropriate amine (1.5 equiv) in CH_2Cl_2 (20 mL) was added DIPEA (3.0 equiv), and the mixture was stirred at room temperature for 12 h. The reaction mixture was evaporated and the residue was purified by a silica gel column chromatography (hexane/EtOAc = 10:1−5:1) to give silyl amides as a white foam.

To a stirred solution of silyl amides in THF (5 mL) was added TBAF (2.5 equiv) and the reaction mixture was stirred at room temperature for 1 h. The solvent was evaporated and the resulting residue was purified by silica gel column chromatography (CH_2Cl_2/MeOH = 10:1) to give **36a−40g**. Spectral data for compounds **36a−40g** are provided in the Supporting Information.

Pharmacological Methods. [^{125}I]-N^6-(4-amino-3-iodobenzyl)-adenosine-5′-*N*-methyluronamide (I-AB-MECA; 2000 Ci/mmol) and [^{3}H]cyclic AMP (40 Ci/mmol) were from Amersham Pharmacia Biotech (Buckinghamshire, UK).

Cell Culture and Membrane Preparation. CHO (Chinese hamster ovary) cells expressing the recombinant human A_3 AR were cultured in DMEM supplemented with 10% fetal bovine serum, 100 units/mL penicillin, 100 μg/mL streptomycin, 2 μmol/mL glutamine, and 800 μg/mL geneticin. CHO cells expressing rat A_3 ARs were cultured in DMEM and F12 (1:1). Cells were harvested by trypsinization. After homogenization and suspension, cells were centrifuged at 500*g* for 10 min, and the pellet was resuspended in 50 mM Tris·HCl buffer (pH 8.0) containing 10 mM $MgCl_2$, 1 mM EDTA, and 0.1 mg/mL CHAPS. The suspension was homogenized with an electric homogenizer for 10 s and was then recentrifuged

at 20 000 *g* for 20 min at 4 °C. The resultant pellets were resuspended in buffer in the presence of 3 units/mL adenosine deaminase, and the suspensions were stored at −80 °C until the binding experiments. The protein concentration was measured using the Bradford assay.[18]

Binding Assays to the Human A_1 and A_{2A} Receptors. For binding to human A_1 receptors, [^{3}H]R-PIA (2 nM) was incubated with membranes (40 μg/tube) from CHO cells stably expressing human A_1 receptors at 25 °C for 60 min in 50 mM Tris·HCl buffer (pH 7.4; $MgCl_2$, 10 mM) in a total assay volume of 200 μL. Nonspecific binding was determined using 10 μM of CPA. For human A_{2A} receptor binding, membranes (20 μg/tube) from HEK-293 cells stably expressing human A_{2A} receptors were incubated with 15 nM [^{3}H]CGS21680 at 25 °C for 60 min in 200 μL of 50 mM Tris·HCl, pH 7.4, containing 10 mM $MgCl_2$. NECA (10 μM) was used to define nonspecific binding. Reaction was terminated by filtration with GF/B filters.

Binding Assay to the Human A_3 Receptors Using [^{125}I]-4-Amino-3-iodobenzyladenosine-5′-*N*-methyluronamide ([^{125}I]I-AB-MECA). For competitive binding assay, each tube contained 50 μL of membrane suspension (20 μg of protein), 25 μL of [^{125}I]I-AB-MECA (1.0 nM), and 25 μL of increasing concentrations of the test ligands in Tris·HCl buffer (50 mM, pH 7.4) containing 10 mM $MgCl_2$, 1 mM EDTA. Nonspecific binding was determined using 10 μM of Cl-IB-MECA in the buffer. The mixtures were incubated at 25 °C for 60 min. Binding reactions were terminated by filtration through Whatman GF/B filters under reduced pressure using a MT-24 cell harvester (Brandell, Gaithersburgh, MD). Filters were washed three times with 9 mL of ice-cold buffer. Radioactivity was determined in a Beckman 5500B γ-counter.

Cyclic AMP Accumulation Assay. Intracellular cyclic AMP levels were measured with a competitive protein binding method.[19] CHO cells expressing recombinant human and rat A_3 ARs were harvested by trypsinization. After centrifugation and resuspension in medium, cells were planted in 24-well plates in 1.0 mL medium. After 24 h, the medium was removed, and cells were washed three times with 1 mL of DMEM, containing 50 mM HEPES, pH 7.4. Cells were then treated with agonists and/or test compounds in the presence of rolipram (10 μM) and adenosine deaminase (3 units/mL). After 45 min forskolin (10 μM) was added to the medium, and incubation was continued for an additional 15 min. The reaction was terminated by removing the supernatant, and cells were lysed upon the addition of 200 μL of 0.1 M ice-cold HCl. The cell lysate was resuspended and stored at −20 °C. For determination of cyclic AMP production, protein kinase A (PKA) was incubated with [^{3}H]cyclic AMP (2 nM) in K_2HPO_4/EDTA buffer (K_2HPO_4, 150 mM; EDTA, 10 mM), 20 μL of the cell lysate, and 30 μL of 0.1 M HCl or 50 μL of cyclic AMP solution (0−16 pmol/200 μL for standard curve). Bound radioactivity was separated by rapid filtration through Whatman GF/C filters and washed once with cold buffer. Bound radioactivity was measured by liquid scintillation spectrometry.

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Supporting Information Available: Spectral data for compounds **18**−**28** and **36**−**40** and results from elemental analyses data. This material is available free of charge via the Internet at http://pubs.acs.org.

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