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Design, synthesis, and biological activity of N^6-substituted-4′-thioadenosines at the human A_3 adenosine receptor

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Abstract—A large series of N^6-substituted-4′-thioadenosines were synthesized starting from D-gulonic-γ-lactone, and structure–activity relationships were studied at the human A_3 and other subtypes of adenosine receptors (ARs). 2-Chloro-substituted and 2-H analogues were compared. 2-Chloro-N^6-methyl-4′-thioadenosine **19b** was a highly potent and selective agonist (K_i = 0.8 ± 0.1 nM in binding) at the A_3AR, and displayed the same relative efficacy in receptor activation as a known full agonist, Cl-IB-MECA. Most of N^6-substituted-4′-thioadenosines were less potent in binding than the corresponding N^6-substituted-adenosines or N^6-substituted-4′-thioadenosine-5′-uronamides. N^6-(3-Iodobenzyl) derivative **19g** was demonstrated to be an A_3AR-selective partial agonist displaying a K_i value of 3.2 nM.

1. Introduction

Extracellular adenosine acts as a signaling molecule by activating four subtypes (A_1, A_{2A}, A_{2B}, and A_3) of adenosine receptors (ARs).[1] The structure–activity relationships of adenosine derivatives acting at the four subtypes have been explored in detail. Several selective A_3AR agonists are currently in clinical trials for the treatment of cancer, pulmonary diseases, pain, and other diseases.[2]

Agonists acting at the A_3AR are associated with cardioprotective, cerebroprotective, and cytostatic properties.[3–7] The first selective A_3AR agonist to be reported was **1** (IB-MECA), which is currently in clinical trials for the treatment of colon carcinoma and rheumatoid arthritis (Chart 1).[8–10] Among the most selective agonists of this receptor are **2** (Cl-IB-MECA)[11] and its 4′-thio analogue **3** (LJ-529).[12] Adenosine-5′-uronamide derivatives **1**–**3** and other highly selective A_3AR agonists, such as CP-608039,[13] contain the potency- and efficacy-enhancing 5′-alkyluronamide moiety.

The exploration of N^6-substitution of adenine-9-riboside derivatives, for example, **4**–**7**, has provided new leads into the design of A_3AR-selective agonists.[14–16] Binding affinity and relative efficacy at the human A_3AR of a wide range of N^6-substituted adenosine derivatives were studied in intact Chinese hamster ovary (CHO) cells stably expressing A_3 and other ARs.[14] At the human A_3AR, small groups such as N^6-methyladenosine (**4**) enhanced affinity, although N^6-methyl analogues are nearly inactive at the rat A_3AR. N^6-Cyclopropylmethyladenosine (**5**) was equipotent to **4** as a full agonist at the human A_3AR but demonstrated significantly enhanced affinity at the A_1AR. The N^6-(3-iodobenzyl) derivatives, known to enhance the affinity at both the rat and human A_3AR and contained in compound **6** and 5′-uronamide derivatives **1**–**3**, also led to a reduction in efficacy in the

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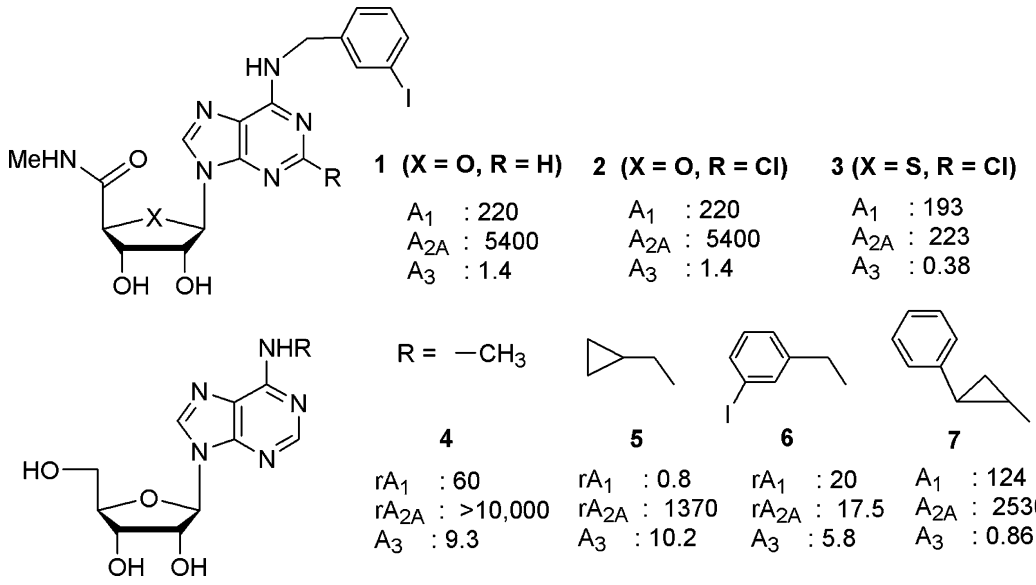


Chart 1. Binding affinity of the adenosine derivatives at human adenosine receptors.[†]

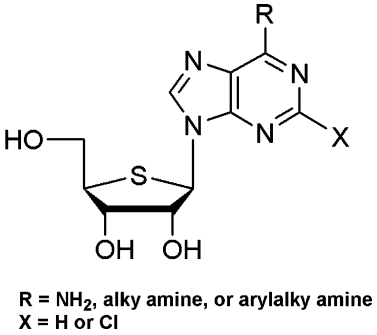


Figure 1. Structures of the target 4′-thionucleosides.

5′-OH series. Other bulky substitutions at the N^6-position of the adenine moiety, including other benzyl substitutions, 2,2-diphenylethyl, and various cycloalkyl groups, were found to decrease the maximum agonist efficacy at the A_3AR.[12,17] However, N^6-(*trans*-2-phenylcyclopropyl)adenosine (**7**) exhibited full agonism at the human A_3AR and greatly enhanced affinity and selectivity. A 2-chloro substituent was demonstrated to further decrease the A_3AR efficacy in combination with N^6-substitution.[18]

While the structure–activity relationships (SARs) have been well explored within the 4′-oxo series of 5′-OH derivatives with respect to both affinity and relative efficacy, the SAR within the 4′-thio series is not yet well developed. Although there is a bioisosteric rationale for the replacement of the ring oxygen with sulfur, there may be differences in the effects on potency, selectivity, and efficacy. Thus, in the search for improved A_3AR agonists or partial agonists, herein we describe the synthesis and in vitro biological characterization of a large series of 4′-thioadenosine derivatives (Fig. 1).

2. Results and discussion

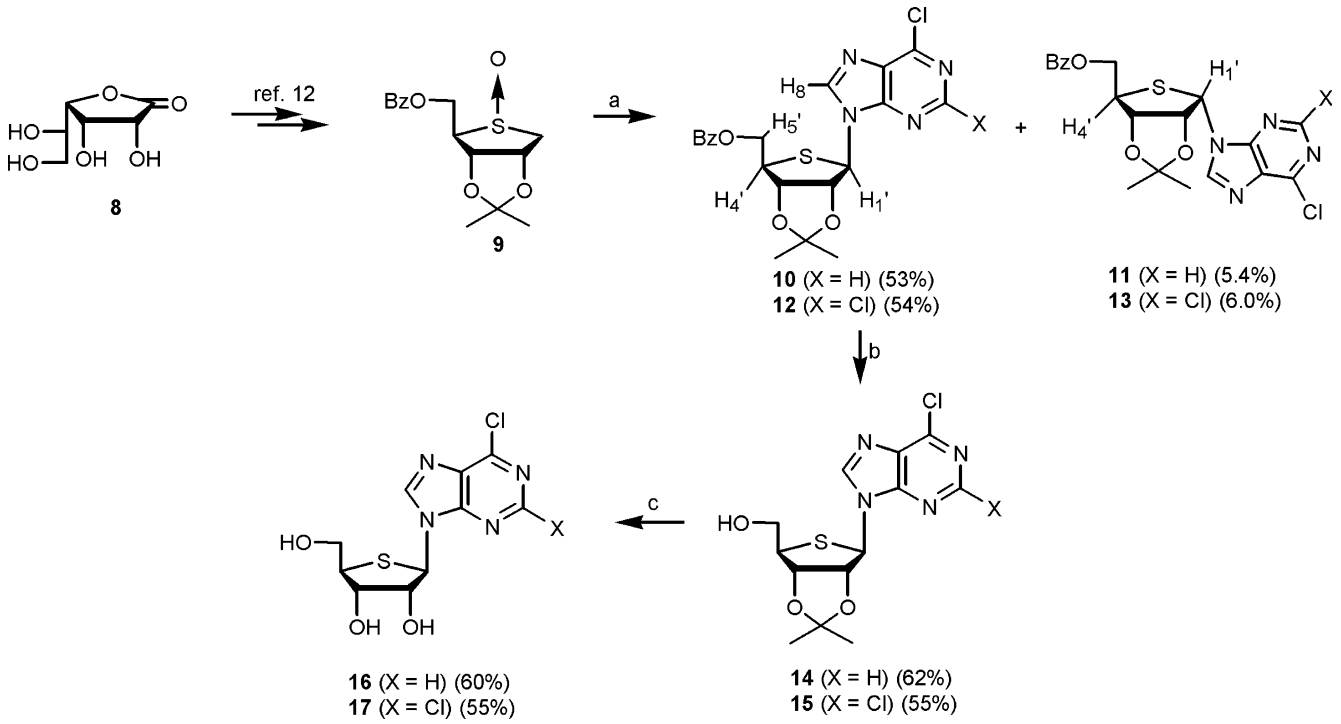
2.1. Chemistry

Synthesis of the desired 4′-thioadenosine derivatives started from D-gulonic-γ-lactone (**8**), as shown in Scheme 1.

D-Gulonic-γ-lactone (**8**) was converted to the glycosyl donor **9** according to the reported procedure[12] developed by our laboratory. Direct condensation of **9** with 6-chloropurine or 2,6-dichloropurine in the presence of TMSOTf and triethylamine in a solution of acetonitrile and 1,2-dichloroethane afforded the β-anomers **10** or **12** with minor formation of α-anomers **11** or **13**, respectively. The initially formed N^3-isomer was smoothly converted to the N^9-isomer upon heating to 80 °C, as reported by Chu and co-workers.[19] Anomeric configurations of **10** and **11** were readily assigned by ^{1}H NMR NOE experiments. Irradiation of 1′-H of compound **10** gave NOE on its 4′-H, indicating β-anomer, while no NOE was observed on the same experiment in the case of compound **11**, resulting in α-anomer. The same pattern was observed in the case of **12** and **13**. Removal of the benzoyl group of **10** and **12** was achieved using methylmagnesium iodide in THF to give **14** and **15**, respectively. Treatment with sodium methoxide or methanolic ammonia, typically used for the deprotection of a benzoyl group, resulted in the formation of 6-methoxy or 6-aminopurine derivative. Treatment of **14** and **15** with 80% aqueous acetic acid at 70 °C gave the triols **16** and **17**, respectively.

Substitution of N^6-positions of **16** and **17** with various primary and secondary amines for the synthesis of the final nucleosides **18a–v** and **19a–n**, respectively, is shown in Scheme 2. The amines used in this substitution were

[†] K_i values (nM) in binding to A_1 A_{2A}, and A_3 adenosine receptors (human, unless noted as rat, r).



Scheme 1. Reagents and conditions: (a) 6-Chloropurine or 2,6-dichloropurine, TMSOTf, ET_3N, CH_3CN-$ClCH_2CH_2Cl$, rt to 80 °C; (b) 3.0 M MeMgI in ether, THF, rt, 4 h; (c) 80% AcOH, 70 °C, 12 h.

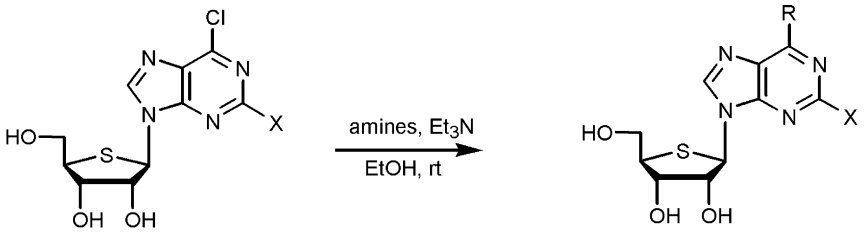
alkyl-, cycloalkyl-, arylalkyl-, and cyclic-amines. In total, 36 final nucleoside derivatives were prepared as shown in Scheme 2.

2.2. Biological activity

All AR experiments were performed using adherent Chinese hamster ovary (CHO) cells stably transfected with cDNA encoding the human ARs.[20] Binding at the human A_3AR 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_3AR was determined at 10 μM. Furthermore, the percent activation of the human A_3AR (inhibition of adenylate cyclase in comparison to the full agonist **2**) was determined at 10 μM. Binding at the human A_1AR (using [^{3}H]R-PIA, N^6-(2-phenylisopropyl)adenosine) or A_{2A}AR (using [^{3}H]CGS2 1680, (2-[*p*-(2-carboxyethyl)phenylethylamino]-5′-*N*-ethylcarboxamido-adenosine)) was carried out as described in Section 3.1.

As shown in Table 1, a variety of N^6-alkyl, cycloalkyl, and arylalkyl substituents in 4′-thioadenosine derivatives have produced nanomolar binding affinity at the human A_3AR subtype, among which **19b** (R = CH_3NH, X = Cl)[12] exhibited the highest binding affinity (K_i = 0.8 ± 0.1 nM) at this AR subtype. Compound **19b** was selective for the human A_3AR versus the human A_1AR by 790-fold and showed almost no measurable binding affinity at the human A_{2A}AR (4% inhibition at 10 μM). Within the 4′-thioadenosine series (X = H), compound **18h** showed the highest binding affinity (K_i = 1.9 ± 0.4 nM) at the human A_3AR with selectivity over the human A_1 and A_{2A}ARs by 24- and 300-fold, respectively. The primary amine-substituted N^6-alkyl or N^6-cycloalkyl-4′-thioadenosine derivatives (**18b**–**18f**) exhibited moderate binding affinity at the human A_3AR, while the derivatives (**18s**–**18v**) substituted with secondary amines, such as piperidine, piperazine, and morpholine, were totally devoid of activity at the human ARs, indicating that, similar to 4′-oxoadenosine derivatives, at least one hydrogen atom at the N^6-position is essential for hydrogen bonding in the binding site of the AR. In the 3-halogen-substituted benzylamine series **18g**–**18l**, the larger halogen atom, the higher binding affinity was observed. In the arylalkyl series, monophenyl-substituted alkylamines such as **18n**–**18p** showed high affinity at the human A_3AR, while diphenyl-substituted alkylamines such as **18q** and **18r** lost their binding at the human A_3AR possibly due to the steric repulsion at the binding site despite a favorable hydrophobic interaction. In comparison with the 4′-oxo series, **4**–**7**, the corresponding 4′-thio derivatives **18b**, **18f**, **18h**, and **18p** were found to exhibit lower binding affinity at the human A_3AR.

In the case of 2-chloro-4′-thioadenosine series (X = Cl), the order of compounds showing high binding affinity at the A_3AR is as follows: **19b** (R = CH_3NH, K_i = 0.8 ± 0.1) > **19m** (R = *trans*-2-phenyl-cyclopropyl-NH, K_i = 1.9 ± 0.4) > **19g** (R = 3-iodobenzyl-NH, K_i = 3.2 ± 0.9) > **19j** (R = phenethyl-NH, K_i = 4.40 ± 0.33) ~ **19k** (R =



16 (X = H)
17 (X = Cl)

18a-v (X = H)
19a-n (X = Cl)

Compound No	R	X	Compound No	R	X
18a	NH_2	H	18u	4-(4-fluorobenzyl)piperazine	H
18b	CH_3NH	H	18v	morpholine	H
18c	cyclopropyl-NH	H	19a	NH_2	Cl
18d	cyclobutyl-NH	H	19b	CH_3NH	Cl
18e	3-methylbutyl-NH	H	19c	cyclopentyl-NH	Cl
18f	cyclopropylmethyl-NH	H	19d	benzyl-NH	Cl
18g	benzyl-NH	H	19e	2-methylbenzyl-NH	Cl
18h	3-iodobenzyl-NH	H	19f	2-ethoxybenzyl-NH	Cl
18i	3-chlorobenzyl-NH	H	19g	3-iodobenzyl-NH	Cl
18j	3-methylbenzyl-NH	H	19h	alpha-naphthylmethyl-NH	Cl
18k	3-fluorobenzyl-NH	H	19i	fluoren-9-yl-methyl-NH	Cl
18l	3-(trifluoromethyl)benzyl-NH	H	19j	phenethyl-NH	Cl
18m	naphth-1-yl-methyl-NH	H	19k	3-fluorophenethyl-NH	Cl
18n	phenethyl-NH	H	19l	1,2-diphenylethyl	Cl
18o	3-fluorophenethyl-NH	H	19m	trans-2-phenyl-cyclopropyl-NH	Cl
18p	trans-2-phenylcyclopropyl-NH	H	19n	3,3-diphenylpropyl-NH	Cl
18q	1,2-diphenylethyl-NH	H			
18r	3,3-diphenylpropyl-NH	H			
18s	piperidine	H			
18t	4-benzylpiperidine	H			

Scheme 2.

3-fluoro-phenethyl-NH, $K_i = 4.7 \pm 1.6$) ~ **19a** (R = NH_2, $K_i = 4.9 \pm 1.3$). Compounds with bulky substituents such as **19h** (R = α-naphthylmethyl-NH), **19l** (R = 1,2-diphenylethyl-NH), and **19n** (R = 3,3-diphenylpropyl-NH) lost their binding to the A_3AR, while substituted-benzylamine analogues, such as **19d**–**19f** with the exception of **19g**, were found to show moderate binding affinity at the human A_3AR. Among compounds (**18h**, **18i**, **18n**, **18p**, **19a**, **19b**, **19g**, **19j**, **19k**, and **19m**) showing high binding affinity at the human A_3AR, compounds **18p** and **19m** substituted with N^6-(*trans*-2-phenylcyclopropyl)amino group and **19b** substituted with a N^6-methylamino group were found to be full agonists at the human A_3AR.

Other compounds exhibited partial agonism at the human A_3AR. For example, N^6-benzyl **19d** and N^6-(2-ethyloxybenzyl) **19f** derivatives were partial agonists with >100-fold selectivity for the A_3AR. Higher affinity (3.2 nM) was observed for the A_3AR-selective partial agonist, N^6-(3-iodobenzyl) derivative **19g**.

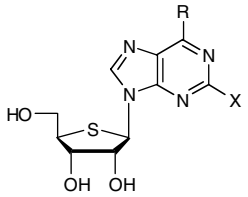
3. Conclusions

We have established structure–activity relationships of a large series of N^6-substituted-4′-thioadenosines at the human A_3AR. From this study, 2-chloro-N^6-methyl-4′-thioadenosine (**19b**) was discovered as the most potent and selective full agonist ($K_i = 0.8 \pm 0.1$ nM) at the human A_3AR. Most of N^6-substituted-4′-thioadenosines were less potent than the corresponding N^6-substituted-adenosines or N^6-substituted-4′-thioadenosine-5′-uronamides. It was also revealed that, similar to 4′-oxoadenosine derivatives, at least one hydrogen atom at the N^6-position is essential for hydrogen bonding in the binding site of the AR, and bulky substituents at the N^6-position reduced binding affinity. These findings may provide good insights into the identification of the binding interaction between nucleoside derivatives and ARs.

3.1. Experimental section

^{1}H NMR spectra ($CDCl_3$, CD_3OD, or DMSO-d_6) were recorded on Varian Unity Inova 400 MHz. Chemical shifts were reported in ppm units with TMS as the internal standard. ^{13}C NMR spectra ($CDCl_3$, CD_3OD, or DMSO-d_6) were recorded on Varian Unity Inova 100 MHz. Optical rotations were determined on Jasco III in methanol. UV spectra were recorded on U-3000 made by Histachi in methanol. Elementary analyses were measured on EA1110. The crude products were purified using a silica gel 60 (230–400 mesh, Merck).

Table 1. Potency of 4′-thioadenosine derivatives at human A_1, A_{2A}, and A_3ARs and maximal agonist effects at human A_3ARs expressed in CHO cells[a]



Compound	R	X	K_i (hA_1AR) nM[a] or % displ. at 10 μM	K_i ($hA_{2A}AR$) nM[a] or % displ. at 10 μM	K_i (hA_3AR) nM[a] or % displ. at 10 μM	% Activation (hA_3AR)[b] at 10 μM
18a	NH_2	H	5240 ± 740	5740 ± 980	445 ± 54	0
18b	CH_3–NH	H	359 ± 69	26%	10.3 ± 0.7	60 ± 11
18c	Cyclopropyl-NH	H	22.5 ± 0.3	40%	45.2 ± 5.8	78 ± 6
18d	Cyclobutyl-NH	H	15.6 ± 4.0	4950 ± 160	48.0 ± 4.9	91 ± 2
18e	3-Methyl-butyl-NH	H	0%	28%	65.3 ± 3.6	99 ± 4
18f	Cyclopropyl-methyl-NH	H	7%	23%	22.9 ± 0.8	96 ± 4
18g	Benzyl-NH	H	597 ± 8	2110 ± 740	155 ± 33	87 ± 7
18h	3-Iodo-benzyl-NH	H	45.9 ± 2.1	575 ± 75	1.9 ± 0.4	60 ± 4
18i	3-Chloro-benzyl-NH	H	91.8 ± 6.0	995 ± 206	6.7 ± 0.4	62 ± 3
18j	3-Methyl-benzyl-NH	H	179 ± 19	3610 ± 1110	13.9 ± 5.7	48 ± 9
18k	3-Fluoro-benzyl-NH	H	651 ± 110	13%	57.6 ± 12.9	63 ± 11
18l	3-(Trifluoro-methyl)-benzyl-NH	H	430 ± 37	42%	32.7 ± 6.7	29 ± 7
18m	Naphth-1-yl-methyl-NH	H	129 ± 23	206 ± 15	42.2 ± 13.0	72 ± 4
18n	2-Phenethyl-NH	H	82.6 ± 6.8	507 ± 31	5.6 ± 1.1	86 ± 5
18o	3-Fluoro-phenethyl-NH	H	83.4 ± 6.3	774 ± 86	11.3 ± 0.6	89 ± 9
18p	*trans*-2-Phenyl-cyclopropyl-NH	H	136 ± 13	2330 ± 700	6.6 ± 2.9	114 ± 8
18q	1,2-Diphenyl-ethyl-NH	H	504 ± 77	1500 ± 400	1080 ± 70	54 ± 3
18r	3,3-Diphenyl-propyl-NH	H	1950 ± 40	911 ± 209	1650 ± 150	0 ± 3
18s	Piperidine	H	25%	21%	21%	0
18t	4-Benzyl-piperidine	H	0%	5%	10%	0
18u	4-(4-Fluoro-benzyl)-piperazine	H	0%	26%	22%	0
18v	Morpholine	H	26%	19%	16%	0
19a	NH_2[c]	Cl	47%	2440 ± 310	4.9 ± 1.3	64 ± 18
19b	CH_3–NH	Cl	629 ± 168	4%	0.8 ± 0.1	96 ± 5
19c	Cyclopentyl-NH	Cl	32.0 ± 6.5	1410 ± 140	94.4 ± 29.2	68 ± 3
19d	Benzyl-NH	Cl	2280 ± 180	47%	18.2 ± 2.6	63 ± 4
19e	2-Methyl-benzyl-NH	Cl	853 ± 133	3280 ± 170	48.9 ± 16.6	62 ± 4
19f	2-Ethyloxy-benzyl-NH	Cl	2570 ± 630	4630 ± 510	17.2 ± 2.2	60 ± 2
19g	3-Iodo-benzyl-NH[c]	Cl	554 ± 64	1190 ± 290	3.2 ± 0.9	32 ± 7
19h	α-Naphthylmethyl-NH	Cl	581 ± 36	1340 ± 160	268 ± 185	45 ± 5
19i	Fluoren-9-yl-methyl-NH	Cl	474 ± 199	34%	50.4 ± 26.5	112 ± 0
19j	2-Phenethyl-NH	Cl	139 ± 71	1460 ± 20	4.40 ± 0.33	81 ± 9
19k	3-Fluoro-phenethyl-NH	Cl	294 ± 33	1650 ± 110	4.7 ± 1.6	71 ± 7
19l	1,2-Diphenylethyl	Cl	3320 ± 770	3910 ± 690	1300 ± 610	38 ± 5
19m	*trans*-2-Phenyl-cyclopropyl-NH	Cl	856 ± 155	1930 ± 320	1.9 ± 0.4	102 ± 4
19n	3,3-Diphenyl-propyl-NH	Cl	38%	3790 ± 320	720 ± 193	0

[a] All AR experiments were performed using adherent CHO cells stably transfected with cDNA encoding the human ARs. Percent activation of the human A_3AR was determined at 10 μM. Binding at human A_1 and A_{2A}ARs in this study was carried out as described in Methods using [^{3}H]R-PIA or [^{3}H]CGS 21680 as a radioligand. Values from the present study are expressed as means ± SEM, n = 3–5.

[b] Percent activity at 10 μM, relative to 10 μM Cl-IB-MECA (A_3).

[c] Binding affinity values from Gao et al.[20]

Reagents were purchased from Aldrich Chemical Company. All the anhydrous solvents were distilled over CaH_2 or P_2O_5 or Na/benzophenone prior to the reaction.

3.2. Chemical synthesis

3.2.1. General procedure for the condensation. To a suspension of 6-chloropurine (21.69 mmol) or 2,6-dichloropurine (21.69 mmol) in a solution of dry CH_3CN (20 mL) and 1,2-dichloroethane (10 mL) were added Et_3N (21.69 mmol) and TMSOTf (43.38 mmol), and the mixture was stirred at room temperature until the solution became clear. A solution of sulfoxide **28** (10.85 mmol) in dry 1,2-dichloroethane (10 mL) was added to the resulting solution in one shot at room temperature. An additional amount of Et_3N (21.69 mmol) was added to the reaction mixture to initiate the Pummerer reaction. The reaction mixture was stirred under reflux at 80 °C for 4 days, during which time the initially formed N-3 isomer was converted to N-9 isomer. The reaction mixture was partitioned between EtOAc and aqueous saturated $NaHCO_3$ solution, and the organic layer was washed with brine, dried ($MgSO_4$), filtered,

and evaporated. The residue was purified by flash silica gel column chromatography (hexane/EtOAc = 5:1) to give the condensed product.

3.3. 6-Chloro-9-[(5-*O*-benzoyl-2,3-*O*-isopropylidene)-4-thio-β-D-ribofuranosyl]purine (10)

Yield = 53%; white foam; FAB-MS *m*/*z* 447 (M^++1); UV (MeOH) λ_{max} 274 nm (pH 7); $[\alpha]_D^{25}$ 12.35 (*c* 1.00, MeOH); ^{1}H NMR ($CDCl_3$) δ 1.39 (s, 3H, CH_3), 1.66 (s, 3H, CH_3), 4.11 (td, 1H, *J* = 2.8, 6.4 Hz, 4-H), 4.53 (dd, 1H, *J* = 6.4, 12.0 Hz, 5-H_a), 4.74 (dd, 1H, *J* = 6.8, 12.0 Hz, 5-H_b), 5.15 (dd, 1H, *J* = 1.6, 5.4 Hz, 3-H), 5.45 (dd, 1H, *J* = 2.0, 5.6 Hz, 2-H), 6.07 (d, 1H, *J* = 2.0 Hz, 1-H), 7.35–7.91 (m, 5 H, Ph), 8.39 (s, 1H, H-8), 8.80 (s, 1H, H-2); ^{13}C NMR ($CDCl_3$) δ 25.20, 27.28, 54.09, 65.70, 68.00, 85.85, 89.23, 112.64, 128.39, 129.39, 129.40, 129.64, 133.27, 152.30, 153.32, 154.79, 166.01; Anal. Calcd for $C_{20}H_{19}ClN_4O_4S$: C, 53.75; H, 4.29; N, 12.54; S, 7.17. Found: C, 53.77; H, 4.69; N, 12.43; S, 7.55.

3.4. 2,6-Dichloro-9-[(5-*O*-Benzoyl-2,3-*O*-isopropylidene)-4-thio-β-D-ribofuranosyl]purine (12)

Yield = 54%; white foam; FAB-MS *m*/*z* 482 (M^++1); $[\alpha]_D^{25}$ −1.46 (*c* 1.03, MeOH); UV (MeOH) λ_{max} 269 nm (pH 7); ^{1}H NMR ($CDCl_3$) δ 1.39 (s, 3H, CH_3), 1.64 (s, 3H, CH_3), 4.11 (td, 1H, *J* = 2.7, 6.8 Hz, 4-H), 4.57 (dd, 1H, *J* = 6.6, 11.4 Hz, 5-H_a), 4.75 (dd, 1H, *J* = 7.1, 11.4 Hz, 5-H_b), 5.19 (dd, 1H, *J* = 2.7, 5.4 Hz, 3-H), 5.40 (dd, 1H, *J* = 1.9, 5.4 Hz, 2-H), 6.10 (d, 1H, *J* = 1.9 Hz, 1-H), 7.37–7.97 (m, 5 H, Ph), 8.38 (s, 1H, H-8); ^{13}C NMR (CD_3OD) δ 25.44, 27.54, 54.17, 65.59, 68.30, 85.84, 89.14, 113.34, 128.64, 129.35, 129.74, 133.65, 144.94, 152.39, 152.47, 153.32, 166.17. Anal. Calcd for $C_{20}H_{18}Cl_2N_4O_4S$: C, 49.90; H, 3.77; N, 11.64; S, 6.66. Found: C, 49.53; H, 3.42; N, 11.93; S, 6.36.

3.5. General procedure for the debenzoylation

To a stirred solution of **10** (11.73 mmol) or **12** (11.73 mmol) in THF (125 mL) was added methylmagnesium iodide (MeMgI) (33.8 mmol, 3.0 M solution in diethyl ether) at 0 °C and the mixture was stirred for 4 h at room temperature. The mixture was partitioned between EtOAc and water, dried, filtered and evaporated. The residue was purified by flash silica gel column chromatography (hexane/EtOAc = 1:1) to give the debenzoylated products, **14** or **15**, respectively.

3.6. 6-Chloro-9-[(2,3-*O*-isopropylidene)-4-thio-β-D-ribofuranosyl]purine (14)

Yield = 62%; white solid; UV (MeOH) λ_{max} 274 nm (pH 7); $[\alpha]_D^{25}$ −13.54 (*c* 1.00, MeOH); ^{1}H NMR ($CDCl_3$) δ 1.39 (s, 3H, CH_3), 1.68 (s, 3H, CH_3), 3.95 (m, 1H, 4-H), 4.13–4.16 (m, 2H, 5-H), 5.04 (dd, 1H, *J* = 4.6, 1.2 Hz, 3-H), 5.21 (dd, 1H, *J* = 4.2, 3.2 Hz, 2-H), 6.74 (d, 1H, *J* = 3.2 Hz, 1-H), 8.62 (s, 1H, H-8), 8.82 (s, 1 H, H-2); Anal. Calcd for $C_{13}H_{15}ClN_4O_3S$: C, 45.55; H, 4.41; N, 16.34; S, 9.35. Found: C, 44.98; H, 4.21; N, 16.12; S, 9.30.

3.7. 2,6-Dichloro-9-[(2,3-*O*-isopropylidene)-4-thio-β-D-ribofuranosyl]purine (15)

Yield = 65%; yellowish syrup; UV (MeOH) λ_{max} 274 nm (pH 7); $[\alpha]_D^{25}$ −5.81 (*c* 0.86, MeOH); ^{1}H NMR ($CDCl_3$) δ 1.36 (s, 3H, CH_3), 1.66 (s, 3H, CH_3), 3.93–3.97 (m, 1H, 4-H), 4.07–4.14 (m, 2H, 5-H), 5.01 (dd, 1H, *J* = 1.2, 4.8 Hz, 3-H), 5.20 (dd, 1 H, *J* = 3.2, 5.2 Hz, 2-H), 6.71 (d, 1H, *J* = 3.2 Hz, 1-H), 8.60 (s, 1H, H-8); Anal. Calcd for $C_{13}H_{14}Cl_2N_4O_3S$: C, 41.39; H, 3.74; N, 14.85; S, 8.50. Found: C, 41.33; H, 3.56; N, 14.45; S, 8.21.

3.8. General procedure for the removal of the isopropylidene group

A solution of **14** (0.19 mmol) or **15** (0.19 mmol) in 80% aqueous AcOH solution (6.36 mL) was stirred under reflux at 70 °C for 12 h. After removing the solvent under reduced pressure, the residue was purified by flash silica gel column chromatography (CH_2Cl_2/MeOH = 10:1) to give **16** or **17**, respectively.

3.9. 6-Chloro-9-(4-thio-β-D-ribofuranosyl)purine (16)

Yield = 60%; white solid; UV (MeOH) λ_{max} 270 nm (pH 7); $[\alpha]_D^{25}$ −8.43 (*c* 1.00, MeOH); ^{1}H NMR (CD_3OD) δ 3.53 (dd, 1H, *J* = 4.8, 9.6 Hz, 4-H), 3.84–3.90 (m, 2H, 5-H), 4.27 (t, 1H, *J* = 3.6 Hz, 3-H), 4.65 (dd, 1H, *J* = 3.6, 4.8 Hz, 2-H), 5.98 (d, 1H, *J* = 4.8 Hz, 1-H), 8.84 (s, 1H, H-8), 8.98 (s, 1H, H-2); Anal. Calcd for $C_{10}H_{11}ClN_4O_3S$: C, 39.67; H, 3.66; N, 11.71; S, 10.59. Found: C, 39.98; H, 3.23; N, 11.31; S, 10.33.

3.10. 2,6-Dichloro-9-(4-thio-β-D-ribofuranosyl)purine (17)

Yield = 55%; yellowish solid; UV (MeOH) λ_{max} 275 nm (pH 7); $[\alpha]_D^{25}$ −0.50 (*c* 1.00, MeOH); ^{1}H NMR (CD_3OD) δ 3.54 (dd, 1H, *J* = 5.2, 9.6 Hz, 4-H), 3.87–3.97 (m, 2H, 5-H), 4.33 (t, 1H, *J* = 3.6 Hz, 3-H), 4.71 (dd, 1H, *J* = 3.6, 5.2 Hz, 2-H), 6.02 (d, 1H, *J* = 5.2 Hz, 1-H), 8.97 (s, 1H, H-8); Anal. Calcd for $C_{10}H_{10}Cl_2N_4O_3S$: C, 35.62; H, 2.99; N, 16.62; S, 9.51. Found: C, 35.23; H, 3.32; N, 16.22; S, 9.50.

3.11. General procedure for the N^6-substitution reaction

To a stirred solution of 6-chloropurine or 2,6-dichloropurine derivative (0.298 mmol) and an appropriate amine·HCl or free amines (0.446 mmol) in EtOH (6 mL) was added Et_3N (0.894 mmol) and the solution was stirred overnight at room temperature. After removing the solvent under reduced pressure, the residue was purified by flash silica gel column chromatography (CH_2Cl_2/MeOH = 15:1) to give the N^6-substituted amine derivatives.

3.12. 6-Amino-9-(4-thio-β-D-ribofuranosyl)purine (18a)

Yield = 58%; white solid; mp 125.4–126.3 °C; FAB-MS *m*/*z* 284 (M^+); $[\alpha]_D^{25}$ −47.1 (*c* 0.05, MeOH); UV (MeOH) λ_{max} 260 nm (pH 7); ^{1}H NMR (DMSO-d_6) δ 3.32 (m, 1H, 4-H), 3.64 (m, 1H, 5-H_a), 3.79 (m, 1H, H_b-5), 4.20 (br dd, 1H, *J* = 3.7, 7.4 Hz, 3-H), 4.66 (m, 1H, 2-H),

5.21 (t, 1H, $J = 5.5$ Hz, exchangeable with D_2O, $HOCH_2$), 5.35 (d, 1H, $J = 4.6$ Hz, exchangeable with D_2O, O*H*), 5.60 (d, 1H, $J = 6.4$ Hz, exchangeable with D_2O, O*H*), 5.85 (d, 1H, $J = 6.4$ Hz, 1-H), 7.28 (br s, 2H, exchangeable with D_2O, NH_2), 8.14 (s, 1H, H-8), 8.45 (s, 1H, H-2); Anal. Calcd for $C_{10}H_{13}N_5O_3S$: C, 42.40; H, 4.63; N, 24.72; S, 11.32. Found: C, 42.56; H, 4.34; N, 24.44; S, 11.03.

3.13. 6-Methylamino-9-(4-thio-β-D-ribofuranosyl)purine (18b)

Yield = 64%; white solid; mp 178.0–180.0 °C; FAB-MS *m*/*z* 298 (M^+); $[\alpha]_D^{25}$ −56.6 (*c* 0.05, MeOH); UV (MeOH) λ_{max} 266 nm (pH 7); ^{1}H NMR (DMSO-d_6) δ 2.95 (d, 3H, $J = 3.6$ Hz, $NHCH_3$), 3.20 (m, 1H, 4-H), 3.63 (m, 1H, 5-H_a), 3.80 (m, 1H, 5-H_b), 4.20 (dd, 1H, $J = 3.7$, 7.4 Hz, 3-H), 4.67 (m, 1H, 2-H), 5.22 (t, 1H, $J = 5.5$ Hz, exchangeable with D_2O, $HOCH_2$), 5.35 (d, 1H, $J = 4.6$ Hz, exchangeable with D_2O, O*H*), 5.57 (d, 1H, $J = 5.6$ Hz, exchangeable with D_2O, O*H*), 5.86 (d, 1H, $J = 6.8$ Hz, 1-H), 7.76 (br s, 1H, exchangeable with D_2O, $NHCH_3$), 8.24 (s, 1H, H-8), 8.44 (s, 1H, H-2); Anal. Calcd for $C_{11}H_{15}N_5O_3S$: C, 44.44; H, 5.08; N, 23.55; S, 10.78. Found: C, 44.42; H, 4.87; N, 23.56; S, 10.88.

3.14. 6-Cyclopropylamino-9-(4-thio-β-D-ribofuranosyl)purine (18c)

Yield = 61%; white solid; mp 255.3–258.0 °C; FAB-MS *m*/*z* 324 (M^+); $[\alpha]_D^{25}$ −74.0 (*c* 0.05, MeOH); UV (MeOH) λ_{max} 270 nm (pH 7); ^{1}H NMR (DMSO-d_6) δ 0.61 (m, 2H, cyclopropyl), 0.72 (m, 2H, cyclopropyl), 3.04 (m, 1H, cyclopropyl), 3.34 (m, 1H, 4-H), 3.63 (m, 1H, 5-H_a), 3.79 (m, 1H, 5-H_b), 4.20 (br dd, 1H, $J = 3.2$, 6.4 Hz, 3-H), 4.67 (m, 1H, 2-H), 5.22 (t, 1H, $J = 5.5$ Hz, exchangeable with D_2O, $HOCH_2$), 5.35 (d, 1H, $J = 4.6$ Hz, exchangeable with D_2O, O*H*), 5.57 (d, 1H, $J = 6.2$ Hz, exchangeable with D_2O, O*H*), 5.86 (d, 1H, $J = 6.4$ Hz, 1-H), 7.95 (br s, 1H, exchangeable with D_2O, N*H*-cyclopropyl), 8.25 (s, 1H, H-8), 8.45 (s, 1H, H-2); Anal. Calcd for $C_{13}H_{17}N_5O_3S$: C, 48.29; H, 5.30; N, 21.66; S, 9.92. Found: C, 48.69; H, 5.67; N, 21.25; S, 9.73.

3.15. 6-Cyclobutylamino-9-(4-thio-β-D-ribofuranosyl)-purine (18d)

Yield = 54%; white solid; mp 181.3–183.1 °C; FAB-MS *m*/*z* 338 (M^+); $[\alpha]_D^{25}$ −63.2 (*c* 0.05, MeOH); UV (MeOH) λ_{max} 272 nm (pH 7); ^{1}H NMR (DMSO-d_6) δ 1.61 (m, 2H, cyclobutyl), 2.09–2.24 (m, 4H, cyclobutyl), 3.20 (m, 1H, cyclopropyl), 3.39 (m, 1H, 4-H), 3.62 (m, 1H, 5-H_a), 3.78 (m, 1H, 5-H_b), 4.19 (dd, 1H, $J = 2.4$, 4.8 Hz, 3-H), 4.65 (m, 1H, 2-H), 5.20 (t, 1H, $J = 5.8$ Hz, exchangeable with D_2O, $HOCH_2$), 5.33 (d, 1H, $J = 4.6$ Hz, exchangeable with D_2O, O*H*), 5.56 (d, 1H, $J = 5.8$ Hz, exchangeable with D_2O, O*H*), 5.86 (d, 1H, $J = 4.8$ Hz, 1-H), 8.08 (br s, 1H, exchangeable with D_2O, N*H*-cyclobutyl), 8.20 (s, 1H, H-8), 8.46 (s, 1H, H-2); Anal. Calcd for $C_{14}H_{19}N_5O_3S$: C, 49.84; H, 5.68; N, 20.76; S, 9.50. Found: C, 49.55; H, 5.99; N, 20.43; S, 9.41.

3.16. 6-(3-Methylbutyl)amino-9-(4-thio-β-D-ribofuranosyl)purine (18e)

Yield = 61%; white solid; mp 176.3–177.5 °C; FAB-MS *m*/*z* 354 (M^+); $[\alpha]_D^{25}$ −72.5 (*c* 0.05, MeOH); UV (MeOH) λ_{max} 267 nm (pH 7); ^{1}H NMR (DMSO-d_6) δ 0.89 (d, 6 H, $J = 3.6$ Hz, $CH(CH_3)_2$), 1.48 (m, 2H), 1.63 (m, 1H), 3.33 (t, 2H, $NHCH_2$), 3.39 (m, 1H, 4-H), 3.64 (m, 1H, 5-H_a), 3.81 (m, 1H, 5-H_b), 4.20 (dd, 1H, $J = 3.2$, 6.4 Hz, 3-H), 4.66 (m, 1H, 2-H), 5.20 (t, 1H, $J = 5.5$ Hz, exchangeable with D_2O, $HOCH_2$), 5.32 (d, 1H, $J = 4.6$ Hz, exchangeable with D_2O, O*H*), 5.55 (d, 1H, $J = 6.4$ Hz, exchangeable with D_2O, O*H*), 5.86 (d, 1H, $J = 6.4$ Hz, 1-H), 7.79 (br s, 1H, exchangeable with D_2O, N*H*-isoamyl), 8.21 (s, 1H, H-8), 8.43 (s, 1H, H-2); Anal. Calcd for $C_{15}H_{23}N_5O_3S$: C, 50.97; H, 6.56; N, 19.81; S, 9.07. Found: C, 51.28; H, 6.34; N, 19.41; S, 8.98.

3.17. 6-(Cyclopropylmethyl)amino-9-(4-thio-β-D-ribofuranosyl)purine (18f)

Yield = 72%; white solid; mp 214.5–216.4 °C; FAB-MS *m*/*z* 338 (M^+); $[\alpha]_D^{25}$ −26.3 (*c* 0.05, MeOH); UV (MeOH) λ_{max} 270 nm (pH 7); ^{1}H NMR (DMSO-d_6) δ 0.26 (m, 2H, cyclopropyl), 0.40 (m, 2H, cyclopropyl), 1.14 (m, 1H, cyclopropyl), 3.34 (t, 2H, $NHCH_2$), 3.38 (m, 1H, 4-H), 3.63 (m, 1H, 5-H_a), 3.79 (m, 1H, 5-H_b), 4.20 (dd, 1 H, $J = 3.6$, 7.2 Hz, 3-H), 4.67 (m, 1H, 2-H), 5.22 (t, 1H, $J = 5.5$ Hz, exchangeable with D_2O, $HOCH_2$), 5.34 (d, 1H, $J = 4.6$ Hz, exchangeable with D_2O, O*H*), 5.56 (d, 1H, $J = 6.4$ Hz, exchangeable with D_2O, O*H*), 5.86 (d, 1H, $J = 6.4$ Hz, 1-H), 7.90 (br s, 1H, exchangeable with D_2O, N*H*-cyclopropyl), 8.21 (s, 1H, H-8), 8.45 (s, 1H, H-2); Anal. Calcd for $C_{14}H_{19}N_5O_3S$: C, 49.84; H, 5.68; N, 20.76; S, 9.50. Found: C, 49.56; H, 5.86; N, 20.36; S, 9.13.

3.18. 6-Benzylamino-9-(4-thio-β-D-ribofuranosyl)purine (18g)

Yield = 63%; white solid; mp 168.8–170.7 °C; FAB-MS *m*/*z* 374 (M^+); $[\alpha]_D^{25}$ −58.4 (*c* 0.05, MeOH); UV (MeOH) λ_{max} 270 nm (pH 7); ^{1}H NMR (DMSO-d_6) δ 3.33 (m, 1H, 4-H), 3.63 (m, 1H, 5-H_a), 3.79 (m, 1H, 5-H_b), 4.21 (br, 1H, $J = 3.2$, 6.4 Hz, 3-H), 4.69 (br s, 3H, $PhCH_2$ and 2-H), 5.21 (t, 1H, $J = 5.6$ Hz, exchangeable with D_2O, $HOCH_2$), 5.34 (d, 1H, $J = 4.6$ Hz, exchangeable with D_2O, O*H*), 5.57 (d, 1H, $J = 5.8$ Hz, exchangeable with D_2O, O*H*), 5.87 (d, 1H, $J = 6.4$ Hz, 1-H), 7.27–7.33 (m, 5 H, Ph), 8.22 (s, 1H, H-8), 8.49 (br s, 1H, exchangeable with D_2O, N*H*) 8.50 (s, 1H, H-2); Anal. Calcd for $C_{17}H_{19}N_5O_3S$: C, 54.68; H, 5.13; N, 18.75; S, 8.59. Found: C, 54.69; H, 5.11; N, 18.35; S, 8.99.

3.19. 6-(3-Iodobenzyl)amino-9-(4-thio-β-D-ribofuranosyl)purine (18h)

Yield = 78%; white solid; mp 194.3–196.0 °C; FAB-MS *m*/*z* 500 (M^+); $[\alpha]_D^{25}$ −54.0 (*c* 0.05, MeOH); UV (MeOH) λ_{max} 268 nm (pH 7); ^{1}H NMR (DMSO-d_6) δ 3.33 (m, 1H, 4-H), 3.62 (m, 1H, 5-H_a), 3.80 (m, 1H, 5-H_b), 4.21 (dd, 1 H, $J = 3.2$, 6.4 Hz, 3-H), 4.69 (br m, 3H, $PhCH_2$

and 2-H), 5.21 (t, 1H, J = 5.5 Hz, exchangeable with D_2O, $HOCH_2$), 5.33 (d, 1H, J = 4.6 Hz, exchangeable with D_2O, OH), 5.55 (d, 1H, J = 6.3 Hz, exchangeable with D_2O, OH), 5.88 (d, 1H, J = 6.4 Hz, 1-H), 7.11 (t, 1H, J = 7.8 Hz, 5′-H), 7.37 (d, 1H, J = 7.6 Hz, 6′-H), 7.59 (d, 1H, J = 7.8 Hz, 4′-H), 7.72 (s, 1H, 2′-H), 8.22 (s, 1H, H-8), 8.45 (br s, 1H, exchangeable with D_2O, NH) 8.49 (s, 1H, H-2); Anal. Calcd for $C_{17}H_{18}IN_5O_3S$: C, 40.89; H, 3.63; N, 14.03; S, 6.42. Found: C, 40.78; H, 3.43; N, 14.01; S, 6.13.

3.20. 6-(3-Chlorobenzyl)amino-9-(4-thio-β-D-ribofuranosyl)purine (18i)

Yield = 70%; white solid; mp 183.5–185.0 °C; FAB-MS m/z 408 (M^+); $[\alpha]_D^{25}$ −67.6 (c 0.05, MeOH); UV (MeOH) λ_{max} 268 nm (pH 7); ^{1}H NMR (DMSO-d_6) δ 3.34 (m, 1H, 4-H), 3.63 (m, 1H, 5-H_a), 3.80 (m, 1H, 5-H_b), 4.21 (dd, 1 H, J = 3.6, 7.2 Hz, 3-H), 4.68 (br s, 3H, PhCH_2 and 2-H), 5.20 (t, 1H, J = 5.5 Hz, exchangeable with D_2O, $HOCH_2$), 5.33 (d, 1H, J = 4.6 Hz, exchangeable with D_2O, OH), 5.56 (d, 1H, J = 6.4 Hz, exchangeable with D_2O, OH), 5.86 (d, 1H, J = 6.4 Hz, 1-H), 7.26–7.30 (m, 3H, Ph), 7.38 (s, 1 H, 2′-H), 8.22 (s, 1H, H-8), 8.48 (br s, 1H, exchangeable with D_2O, NH), 8.50 (s, 1H, H-2); Anal. Calcd for $C_{17}H_{18}ClN_5O_3S$: C, 50.06; H, 4.45; N, 17.17; S, 7.86. Found: C, 49.98; H, 4.16; N, 16.99; S, 7.99.

3.21. 6-(3-Methylbenzyl)amino-9-(4-thio-β-D-ribofuranosyl)purine (18j)

Yield = 67%; white solid; mp 151.3–513.0 °C; FAB-MS m/z 388 (M^+); $[\alpha]_D^{25}$ −75.1 (c 0.05, MeOH); UV (MeOH) λ_{max} 267 nm (pH 7); ^{1}H NMR (DMSO-d_6) δ 2.26 (s, 3H, PhCH_3), 3.34 (m, 1H, 4-H), 3.63 (m, 1H, 5-H_a), 3.79 (m, 1H, 5-H_b), 4.20 (br dd, 1H, J = 3.7, 7.4 Hz, 3-H), 4.67 (br s, 3H, $PhCH_2$ and 2-H), 5.21 (t, 1H, J = 5.5 Hz, exchangeable with D_2O, $HOCH_2$), 5.33 (d, 1H, J = 4.6 Hz, exchangeable with D_2O, OH), 5.56 (d, 1H, J = 6.3 Hz, exchangeable with D_2O, OH), 5.87 (d, 1H, J = 6.4 Hz, 1-H), 7.01–7.19 (m, 4H, Ph), 8.21 (s, 1H, H-8), 8.37 (br s, 1H, exchangeable with D_2O, NH) 8.47 (s, 1H, H-2); Anal. Calcd for $C_{18}H_{21}N_5O_3S$: C, 55.80; H, 5.46; N, 18.07; S, 8.28. Found: C, 55.76; H, 5.67; N, 18.47; S, 8.23.

3.22. 6-(3-Fluorobenzyl)amino-9-(4-thio-β-D-ribofuranosyl)purine (18k)

Yield = 61%; white solid; mp 161.7–162.8 °C; FAB-MS m/z 392 (M^+); $[\alpha]_D^{25}$ −66.6 (c 0.05, MeOH); UV (MeOH) λ_{max} 271 nm (pH 7); ^{1}H NMR (DMSO-d_6) δ 3.34 (m, 1H, 4-H), 3.62 (m, 1H, 5-H_a), 3.79 (m, 1H, 5-H_b), 4.21 (br s, 1H, 3-H), 4.70 (br s, 3H, $PhCH_2$ and 2-H), 5.21 (t, 1H, J = 4.8 Hz, exchangeable with D_2O, $HOCH_2$), 5.34 (br, 1H, exchangeable with D_2O, OH), 5.55 (d, 1H, J = 6.3 Hz, exchangeable with D_2O, OH), 5.88 (d, 1H, J = 6.8 Hz, 1-H), 7.02–7.35 (m, 4H, Ph), 8.22 (s, 1H, H-8), 8.49 (br s, 1H, exchangeable with D_2O, NH) 8.50 (s, 1H, H-2); Anal. Calcd for $C_{17}H_{18}FN_5O_3S$: C, 52.17; H, 4.64; N, 17.89; S, 8.19. Found: C, 52.46; H, 4.99; N, 17.56; S, 7.98.

3.23. 6-(3-Trifluoromethylbenzyl)amino-9-(4-thio-β-D-ribofuranosyl)purine (18l)

Yield = 58%; white solid; mp 124.6–125.8 °C; FAB-MS m/z 442 (M^+); $[\alpha]_D^{25}$ −51.6 (c 0.05, MeOH); UV (MeOH) λ_{max} 266 nm (pH 7); ^{1}H NMR (DMSO-d_6) δ 3.34 (m, 1H, 4-H), 3.61 (m, 1H, 5-H_a), 3.79 (m, 1H, 5-H_b), 4.21 (dd, 1H, J = 3.4, 6.8 Hz, 3-H), 4.79 (br s, 3H, PhCH_2 and 2-H), 5.21 (t, 1H, J = 5.6 Hz, exchangeable with D_2O, $HOCH_2$), 5.34 (d, 1H, J = 4.6 Hz, exchangeable with D_2O, OH), 5.55 (d, 1H, J = 5.8 Hz, exchangeable with D_2O, OH), 5.87 (d, 1H, J = 6.4 Hz, 1-H), 7.52–7.66 (m, 3H, Ph), 7.72 (s, 1H, 2′-H), 8.22 (s, 1H, H-8), 8.50 (s, 1H, H-2), 8.51 (br s, 1H, exchangeable with D_2O, NH); Anal. Calcd for $C_{18}H_{18}F_3N_5O_3S$: C, 48.98; H, 4.11; N, 15.86; S, 7.26. Found: C, 49.01; H, 3.98; N, 15.44; S, 7.25.

3.24. 6-(1-Naphthylmethyl)amino-9-(4-thio-β-D-ribofuranosyl)purine (18m)

Yield = 71%; white solid; mp 167.8–169.0 °C; FAB-MS m/z 424 (M^+); $[\alpha]_D^{25}$ −48.5 (c 0.05, MeOH); UV (MeOH) λ_{max} 272 nm (pH 7); ^{1}H NMR (DMSO-d_6) δ 3.33 (m, 1H, 4-H), 3.60 (m, 1H, 5-H_a), 3.79 (m, 1H, 5-H_b), 4.21 (br dd, 1H, J = 3.7, 7.4 Hz, 3-H), 4.69 (m, 1H, 2-H), 5.18 (br s, 2H, naphthalenyl-CH_2), 5.21 (t, 1H, J = 5.5 Hz, exchangeable with D_2O, $HOCH_2$), 5.34 (br, 1H, exchangeable with D_2O, OH), 5.55 (br, 1H, exchangeable with D_2O, OH), 5.88 (d, 1H, J = 6.2 Hz, 1-H), 7.42–7.94 (m, 7 H, Ph), 8.22 (s, 1H, H-8), 8.47 (br s, 1H, exchangeable with D_2O, NH), 8.49 (s, 1H, H-2); Anal. Calcd for $C_{21}H_{21}N_5O_3S$: C, 59.56; H, 5.00; N, 16.54; S, 7.57. Found: C, 59.17; H, 5.40; N, 16.13; S, 7.17.

3.25. 6-Phenethylamino-9-(4-thio-β-D-ribofuranosyl)-purine (18n)

Yield = 66%; white solid; mp 150.3–152.4 °C; FAB-MS m/z 388 (M^+); $[\alpha]_D^{25}$ −65.5 (c 0.05, MeOH); UV (MeOH) λ_{max} 268 nm (pH 7); ^{1}H NMR (DMSO-d_6) δ 2.92 (t, 2H, J = 7.6 Hz, PhCH_2), 3.34 (m, 1H, 4-H), 3.64 (m, 1H, 5-H_a), 3.70 (br s, 2H, NH–CH_2), 3.79 (m, 1H, 5-H_b), 4.21 (dd, 1 H, J = 3.6, 6.2 Hz, 3-H), 4.66 (m, 1H, 2-H), 5.21 (t, 1H, J = 5.8 Hz, exchangeable with D_2O, $HOCH_2$), 5.34 (d, 1H, J = 5.2 Hz, exchangeable with D_2O, OH), 5.55 (d, 1H, J = 6.4 Hz, exchangeable with D_2O, OH), 5.87 (d, 1H, J = 6.4 Hz, 1-H), 7.18–7.31 (m, 5 H, Ph), 7.89 (br s, 1H, exchangeable with D_2O, NH), 8.25 (s, 1H, H-8), 8.45 (s, 1H, H-2); Anal. Calcd for $C_{18}H_{21}N_5O_3S$: C, 55.80; H, 5.46; N, 18.07; S, 8.28. Found: C, 55.94; H, 5.15; N, 17.99; S, 8.09.

3.26. 6-(3-Fluorophenethyl)amino-9-(4-thio-β-D-ribofuranosyl)purine (18o)

Yield = 63%; white solid; mp 186.3–188.0 °C; FAB-MS m/z 406 (M^+); $[\alpha]_D^{25}$ −45.8 (c 0.05, MeOH); UV (MeOH) λ_{max} 270 nm (pH 7); ^{1}H NMR (DMSO-d_6) δ 2.95 (t, 2H, J = 8.0 Hz, PhCH_2), 3.33 (m, 1H, 4-H), 3.61 (m, 1H, 5-H_a), 3.72 (br s, 2H, NH–CH_2), 3.79 (m, 1H, 5-H_b), 4.20 (dd, 1 H, J = 3.2, 6.4 Hz, 3-H), 4.66 (m, 1H, 2-H), 5.20

(t, 1H, J = 5.6 Hz, exchangeable with D_2O, $HOCH_2$), 5.34 (d, 1H, J = 4.6 Hz, exchangeable with D_2O, OH), 5.55 (d, 1H, J = 6.3 Hz, exchangeable with D_2O, OH), 5.87 (d, 1H, J = 6.4 Hz, 1-H), 6.99–7.34 (m, 4H, Ph), 7.93 (br s, 1H, exchangeable with D_2O, NH), 8.25 (s, 1H, H-8), 8.45 (s, 1H, H-2); Anal. Calcd for $C_{18}H_{20}FN_5O_3S$: C, 53.32; H, 4.97; N, 17.27; S, 7.91. Found: C, 52.98; H, 5.08; N, 16.99; S, 7.51.

3.27. 6-(*trans*-2-Phenyl-cyclopropyl)amino-9-(4-thio-β-D-ribofuranosyl)purine (18p)

Yield = 74%; white solid; mp 201.2–203.0 °C; FAB-MS m/z 400 (M^+); $[\alpha]_D^{25}$ −62.1 (c 0.05, MeOH); UV (MeOH) λ_{max} 272 nm (pH 7); ^{1}H NMR (DMSO-d_6) δ 1.23–1.44 (m, 2H, cyclopropyl CH_2), 2.10 (m, 1H, cyclopropyl CH–Ph), 3.20 (br s, 1H, NH-cyclopropyl CH) 3.35 (m, 1H, 4-H), 3.61 (m, 1H, 5-H_a), 3.79 (m, 1H, 5-H_b), 4.20 (br dd, 1H, J = 3.6, 7.2 Hz, 3-H), 4.66 (m, 1H, 2-H), 5.20 (t, 1H, J = 5.8 Hz, exchangeable with D_2O, $HOCH_2$), 5.32 (d, 1H, J = 4.6 Hz, exchangeable with D_2O, OH), 5.54 (d, 1H, J = 6.4 Hz, exchangeable with D_2O, OH), 5.88 (d, 1H, J = 6.4 Hz, 1-H), 7.16–7.30 (m, 5 H, Ph), 8.23 (br s, 1H, exchangeable with D_2O, NH), 8.26 (s, 1H, H-8), 8.47 (s, 1H, H-2); Anal. Calcd for $C_{19}H_{21}N_5O_3S$: C, 57.13; H, 5.30; N, 17.53; S, 8.03. Found: C, 57.45; H, 5.56; N, 17.14; S, 7.98.

3.28. 6-(1,2-Diphenylethyl)amino-9-(4-thio-β-D-ribofuranosyl)purine (18q)

Yield = 68%; white solid; mp 112.7–114.0 °C; FAB-MS m/z 464 (M^+); $[\alpha]_D^{25}$ −57.7 (c 0.05, MeOH); UV (MeOH) λ_{max} 271 nm (pH 7); ^{1}H NMR (DMSO-d_6) δ 3.05–3.10 (m, 2H, NHCHCH_2), 3.35 (dd, 1H, J = 4.8 Hz, 4-H), 3.60 (m, 1H, 5-H_a), 3.77 (m, 1H, 5-H_b), 4.18 (br dd, 1H, J = 3.6, 7.2 Hz, 3-H), 4.63 (m, 1H, 2-H), 5.23 (t, 1H, J = 5.5 Hz, exchangeable with D_2O, $HOCH_2$), 5.33 (d, 1H, J = 4.6 Hz, exchangeable with D_2O, OH), 5.60 (br s, 1H, NH–CH), 5.63 (d, 1H, J = 6.3 Hz, exchangeable with D_2O, OH), 5.80 (d, 1H, J = 6.4 Hz, 1-H), 7.10–7.52 (m, 10 H, 2× Ph), 8.11 (s, 1H, H-8), 8.46 (s, 1H, H-2), 8.47 (br s, 1H, exchangeable with D_2O, NH); Anal. Calcd for $C_{24}H_{25}N_5O_3S$: C, 62.19; H, 5.44; N, 15.11; S, 6.92. Found: C, 62.18; H, 5.57; N, 15.01; S, 6.88.

3.29. 6-(3,3-Diphenylpropyl)amino-9-(4-thio-β-D-ribofuranosyl)purine (18r)

Yield = 79%; white solid; mp 138.5–139.7 °C; FAB-MS m/z 478 (M^+); $[\alpha]_D^{25}$ −66.8 (c 0.05, MeOH); UV (MeOH) λ_{max} 269 nm (pH 7); ^{1}H NMR (DMSO-d_6) δ 2.35 (dd, 2H, J = 14.4 Hz, 7.2 Hz, NHCH$_2$CH_2), 3.31–3.38 (m, 3H, 4-H and NH–CH_2), 3.61 (m, 1H, 5-H_a), 3.79 (m, 1H, 5-H_b), 4.07 (t, 1 H, J = 8.6 Hz), 4.19 (dd, 1H, J = 3.6, 7.2 Hz, 3-H), 4.65 (m, 1H, 2-H), 5.20 (t, 1H, J = 5.6 Hz, exchangeable with D_2O, $HOCH_2$), 5.32 (d, 1H, J = 4.6 Hz, exchangeable with D_2O, OH), 5.52 (d, 1H, J = 6.3 Hz, exchangeable with D_2O, OH), 5.86 (d, 1H, J = 6.4 Hz, 1-H), 7.14–7.34 (m, 10 H, 2× Ph), 7.89 (br s, 1H, exchangeable with D_2O, NH), 8.17 (s, 1H, H-8), 8.44 (s, 1H, H-2); Anal. Calcd for $C_{25}H_{27}N_5O_3S$: C, 62.87; H, 5.70; N, 14.66; S, 6.71. Found: C, 62.56; H, 5.55; N, 14.26; S, 6.67.

3.30. 6-(Piperidin-1-yl)amino-9-(4-thio-β-D-ribofuranosyl)purine (18s)

Yield = 85%; white solid; mp 124.9–125.8 °C; FAB-MS m/z 352 (M^+); $[\alpha]_D^{25}$ −74.6 (c 0.05, MeOH); UV (MeOH) λ_{max} 279 nm (pH 7); ^{1}H NMR (DMSO-d_6) δ 1.57–1.68 (m, 6 H, piperidinyl), 3.38 (m, 1H, 4′-H), 3.61 (m, 1H, 5-H_a), 3.79 (m, 1H, 5-H_b), 4.07 (t, 1H, J = 8.6 Hz), 4.19 (br dd, 5 H, piperidyl-N(CH_2)$_2$ and 3-H), 4.63 (m, 1H, 2-H), 5.19 (t, 1H, J = 5.5 Hz, exchangeable with D_2O, $HOCH_2$), 5.31 (d, 1H, J = 4.6 Hz, exchangeable with D_2O, OH), 5.56 (d, 1H, J = 6.3 Hz, exchangeable with D_2O, OH), 5.88 (d, 1H, J = 6.4 Hz, 1-H), 8.22 (s, 1H, H-8), 8.47 (s, 1H, H-2); Anal. Calcd for $C_{15}H_{21}N_5O_3S$: C, 51.27; H, 6.02; N, 19.93; S, 9.12. Found: C, 51.56; H, 6.42; N, 19.67; S, 9.02.

3.31. 6-(4-Benzylpiperidin-1-yl)amino-9-(4-thio-β-D-ribofuranosyl)purine (18t)

Yield = 82%; white solid; mp 194.3–196.0 °C; FAB-MS m/z 442 (M^+); $[\alpha]_D^{25}$ −63.3 (c 0.05, MeOH); UV (MeOH) λ_{max} 280 nm (pH 7); ^{1}H NMR (DMSO-d_6) δ 1.13 (m, 2H, piperidinyl), 1.69 (m, 2H, piperidinyl), 1.88 (m, 1H, BnCH), 3.08 (br m, 4H, N(CH_2)$_2$), 3.38 (m, 1H, 4-H), 3.60 (m, 1 H, 5-H_a), 3.77 (m, 1H, 5-H_b), 4.18 (br dd, 1H, J = 3.7, 7.4 Hz, 3-H), 4.62 (m, 1H, 2-H), 5.19 (t, 1 H, J = 5.5 Hz, exchangeable with D_2O, $HOCH_2$), 5.31 (d, 1H, J = 4.6 Hz, exchangeable with D_2O, OH), 5.33 (br s, 2H, PhCH_2), 5.55 (d, 1H, J = 6.3 Hz, exchangeable with D_2O, OH), 5.88 (d, 1H, J = 6.4 Hz, 1-H), 7.17–7.30 (m, 5 H, Ph), 8.22 (s, 1H, H-8), 8.47 (s, 1H, H-2); Anal. Calcd for $C_{22}H_{27}N_5O_3S$: C, 59.84; H, 6.16; N, 15.86; S, 7.26. Found: C, 59.98; H, 6.02; N, 15.99; S, 7.05.

3.32. 6-[4-(4-Fluorobenzyl)-piperazin-1-yl]amino-9-(4-thio-β-D-ribofuranosyl)purine (18u)

Yield = 79%; white solid; mp 218.3–220.0 °C; FAB-MS m/z 460 (M^+); $[\alpha]_D^{25}$ −82.36 (c 0.05, MeOH); UV (MeOH) λ_{max} 282 nm (pH 7); ^{1}H NMR (DMSO-d_6) δ 3.28–3.35 (br m, 8 H, piperazinyl), 3.38 (m, 1H, 4-H), 3.61 (m, 1H, 5-H_a), 3.78 (m, 1H, 5-H_b), 4.19 (br dd, 1H, J = 3.6, 7.2 Hz, 3-H), 4.37 (br s, 2H, PhCH_2), 4.64 (m, 1H, 2-H), 5.20 (t, 1 H, J = 4.8 Hz, exchangeable with D_2O, $HOCH_2$), 5.33 (d, 1H, J = 4.6 Hz, exchangeable with D_2O, OH), 5.58 (d, 1H, J = 6.4 Hz, exchangeable with D_2O, OH), 5.90 (d, 1H, J = 6.4 Hz, 1-H), 7.03–7.10 (m, 5 H, Ph), 8.28 (s, 1H, H-8), 8.54 (s, 1H, H-2); Anal. Calcd for $C_{21}H_{25}FN_6O_3S$: C, 54.77; H, 5.47; N, 18.25; S, 6.96. Found: C, 54.39; H, 5.25; N, 18.56; S, 6.76.

3.33. 6-(Morpholin-1-yl)amino-9-(4-thio-β-D-ribofuranosyl)purine (18v)

Yield = 68%; white solid; mp 144.5–146.0 °C; FAB-MS m/z 354 (M^+); $[\alpha]_D^{25}$ −90.7 (c 0.05, MeOH); UV (MeOH)

λ_{max} 282 nm (pH 7); ^{1}H NMR (DMSO-d_6) δ 3.39 (m, 1H, 4-H), 3.60 (m, 1H, 5-H$_a$), 3.63 (br s, 4H, morpholinyl), 3.79 (m, 1H, 5-H$_b$), 4.19 (br, 5 H, morpholine-H and 3-H), 4.63 (m, 1 H, 2-H), 5.20 (t, 1H, J = 5.5 Hz, exchangeable with D_2O, $HOCH_2$), 5.34 (d, 1H, J = 4.6 Hz, exchangeable with D_2O, OH), 5.57 (d, 1H, J = 6.3 Hz, exchangeable with D_2O, OH), 5.89 (d, 1H, J = 6.4 Hz, 1-H), 8.27 (s, 1H, H-8), 8.53 (s, 1H, H-2); Anal. Calcd for $C_{14}H_{19}N_5O_4S$: C, 47.58; H, 5.42; N, 19.82; S, 9.07. Found: C, 47.78; H, 5.23; N, 19.98; S, 9.35.

3.34. 2-Chloro-6-amino-9-(4-thio-β-D-ribofuranosyl)-purine (19a)

Yield = 53%; white solid; mp 220.1–222.2 °C; FAB-MS m/z 318 (M^+); $[\alpha]_D^{25}$ −24.6 (c 0.07, MeOH); UV (MeOH) λ_{max} 264 nm (pH 7); ^{1}H NMR (DMSO-d_6) δ 3.30 (br m, 1H, 4-H), 3.62 (m, 1H, 5-H$_a$), 3.79 (m, 1H, 5-H$_b$), 4.20 (br dd, 1H, J = 3.7, 7.4 Hz, 3-H), 4.61 (m, 1H, 2-H), 5.16 (t, 1H, J = 5.5 Hz, exchangeable with D_2O, $HOCH_2$), 5.34 (d, 1H, J = 4.6 Hz, exchangeable with D_2O, OH), 5.58 (d, 1H, J = 6.3 Hz, exchangeable with D_2O, OH), 5.77 (d, 1H, J = 6.8 Hz, 1-H), 7.81 (br s, 2H, exchangeable with D_2O, NH_2), 8.48 (s, 1H, H-8); Anal. Calcd for $C_{10}H_{12}ClN_5O_3S$: C, 37.80; H, 3.81; N, 22.04; S, 10.09. Found: C, 37.92; H, 3.75; N, 22.24; S, 10.41.

3.35. 2-Chloro-6-methylamino-9-(4-thio-β-D-ribofuranosyl)purine (19b)

Yield = 52%; white solid; mp 239.3–241.3 °C; FAB-MS m/z 332 (M^+); $[\alpha]_D^{25}$ −30.6 (c 0.1, MeOH); UV (MeOH) λ_{max} 271 nm (pH 7); ^{1}H NMR (DMSO-d_6) δ 2.92 (d, 3H, J = 4.3 Hz, N-CH_3), 3.30 (m, 1H, 4-H), 3.62 (m, 1H, 5-H$_a$), 3.79 (m, 1H, 5-H$_b$), 4.20 (dd, 1H, J = 3.6, 7.9 Hz, 3-H), 4.61 (m, 1H, 2-H), 5.16 (t, 1H, J = 5.5 Hz, exchangeable with D_2O, $HOCH_2$), 5.34 (d, 1H, J = 4.6 Hz, exchangeable with D_2O, OH), 5.58 (d, 1H, J = 6.3 Hz, exchangeable with D_2O, OH), 5.77 (d, 1H, J = 6.8 Hz, 1-H), 8.26 (br q, 1H, exchangeable with D_2O, NH), 8.47 (s, 1H, H-8); Anal. Calcd for $C_{11}H_{14}ClN_5O_3S$: C, 39.82; H, 4.25; N, 21.11; S, 9.66. Found: C, 39.97; H, 4.42; N, 21.20; S, 9.77.

3.36. 2-Chloro-6-cyclopentylamino-9-(4-thio-β-D-ribofuranosyl)purine (19c)

Yield = 84%; white solid; mp 187.5–188.6 °C; FAB-MS m/z 386 (M^+); $[\alpha]_D^{25}$ −31.4 (c 0.05, MeOH); UV (MeOH) λ_{max} 266 nm (pH 7); ^{1}H NMR (DMSO-d_6) δ 1.34–1.75 (br m, 8 H, cyclopropyl), 3.30 (m, 1H, 4-H), 3.62 (m, 1H, 5-H$_a$), 3.76 (m, 1H, 5-H$_b$), 4.21 (br dd, 1H, J = 3.7, 6.8 Hz, 3-H), 4.44 (m, 1H, NH–CH), 4.63 (m, 1H, 2-H), 5.14 (t, 1H, J = 5.5 Hz, exchangeable with D_2O, $HOCH_2$), 5.33 (d, 1H,J = 4.6 Hz, exchangeable with D_2O, OH), 5.57 (d, 1H, J = 6.0 Hz, exchangeable with D_2O, OH), 5.78 (d, 1H, J = 6.4 Hz, 1-H), 8.20 (br s, 1H, exchangeable with D_2O, NH), 8.45 (s, 1H, H-8); Anal. Calcd for $C_{15}H_{20}ClN_5O_3S$: C, 46.69; H, 5.22; N, 18.15; S, 8.31. Found: C, 46.78; H, 5.45; N, 18.45; S, 7.99.

3.37. 6-Benzylamino-2-chloro-9-(4-thio-β-D-ribofuranosyl)purine (19d)

Yield = 75%; white solid; mp 135.0–140.0 °C; UV (MeOH) λ_{max} 274 nm; MS (FAB) m/z 408.0 (M^+); $[\alpha]_D^{25}$ −3.51 (c 0.97, MeOH); ^{1}H NMR (CD_3OD) δ 3.52 (dd, 1H, J = 4.8 Hz ,4-H), 3.79 (br t, 2H, NH–CH_2), 3.85–3.96 (m, 2H, 5-H), 4.29 (t, 1H, J = 4.4 Hz, 3.6 Hz, 3-H), 4.66 (dd, 1H, J = 3.6, 5.4 Hz, 2-H), 4.75 (br s, 2H, NHCH_2), 5.88 (d, 1H, J = 5.6 Hz, 1-H), 7.23-7.39 (m, 5 H, Ph), 8.40 (s, 1H, H-8); Anal. Calcd for $C_{17}H_{18}ClN_5O_3S$: C, 50.06, H, 4.45, N, 17.17, S, 7.86. Found: C, 50.23, H, 4.05, N, 17.35, S, 7.65.

3.38. 2-Chloro-6-(2-methylbenzyl)amino-9-(4-thio-β-D-ribofuranosyl)purine (19e)

Yield = 72%; white solid; mp 186.3–189.3 °C; UV (MeOH) λ_{max} 274 nm; MS (FAB) m/z 422.0 (M^+); $[\alpha]_D^{25}$ −3.78 (c 0.98, MeOH); ^{1}H NMR (CD_3OD) δ 2.37 (d, 3H, J = 10.8 Hz, 2-CH_3–Ph–), 3.52 (dd, 1H, J = 4.8, 5.2 Hz, 4-H), 3.83–3.95 (dd, 2H, J = 4.8, 11.6 Hz, 5-H), 4.29 (t, 1H, J = 3.6, 4.8 Hz, 3-H), 4.66 (dd, 1H, J = 3.6, 5.2 Hz, 2-H), 4.74 (br s, 2H, NH–CH_2), 5.87 (d, 1H, J = 5.2 Hz, 1-H), 7.13–7.32 (m, 5 H, Ph), 8.40 (s, 1H, H-8); Anal. Calcd for $C_{18}H_{20}ClN_5O_3S$: C, 51.24, H, 4.78, N, 16.60, S, 7.60. Found: C, 51.57, H, 5.04, N, 16.26, S, 7.29.

3.39. 2-Chloro-6-(2-ethoxybenzyl)amino-9-(4-thio-β-D-ribofuranosyl)purine (19f)

Yield = 86%; white solid; mp 147.9–150.4 °C; UV (MeOH) λ_{max} 276 nm; MS (FAB) m/z 452.1 (M^+); $[\alpha]_D^{25}$ −3.20 (c 1.03, MeOH); ^{1}H NMR (CD_3OD) δ 1.40 (t, 3H, J = 6.0, 7.2 Hz, OCH_2CH_3), 3.52 (dd, 1H, J = 4.4, 5.2 Hz, 4-H), 3.85–3.96 (m, 2H, 5-H), 4.10 (q, 2H, J = 6.4, 7.6 Hz, OCH_2CH_3), 4.29 (t, 1H, J = 3.6, 4.8 Hz, 3-H), 4.58 (br s, 1H, NH–CH_2), 4.65 (t, 1H, J = 4.0, 5.2 Hz, 2-H), 5.87 (d, 1H, J = 5.2 Hz, 1-H), 6.86–7.32 (m, 4H, Ph), 8.41 (s, 1H, H-8); Anal. Calcd for $C_{19}H_{22}ClN_5O_3S$: C, 50.50, H, 4.91, N, 15.50, S, 7.10. Found: C, 50.85, H, 4.96, N, 15.63, S, 6.90.

3.40. 2-Chloro-6-(3-iodobenzyl)amino-9-(4-thio-β-D-ribofuranosyl)purine (19g)

Yield = 53%; white solid; mp 112.5–114.3 °C; FAB-MS m/z 534 (M^+); $[\alpha]_D^{25}$ −28.3 (c 0.1, MeOH); UV (MeOH) λ_{max} 273 nm (pH 7); ^{1}H NMR (DMSO-d_6) δ 3.29 (m, 1H, 4-H), 3.63 (m, 1H, 5-H$_a$), 3.78 (m, 1H, 5-H$_b$), 4.20 (br d, 1H, J = 3.4 Hz, 3-H), 4.61 (m, 3H, 2-H and N-CH_2), 5.17 (t, 1H, J = 5.5 Hz, exchangeable with D_2O, $HOCH_2$), 5.34 (d, 1H, J = 4.7 Hz, exchangeable with D_2O, OH), 5.58 (d, 1H, J = 6.1 Hz, exchangeable with D_2O, OH), 5.78 (d, 1H, J = 6.8 Hz, 1-H), 7.13 (t, 1H, J = 7.7 Hz, 5′-H), 7.34 (d, 1H, J = 7.5 Hz, 6′-H), 7.59 (d, 1H, J = 7.8 Hz, 4′-H), 7.74 (s, 1H, 2′-H), 8.53 (s, 1H, H-8), 8.92 (br t, 1H, J = 5.8 Hz, exchangeable with D_2O, NH); Anal. Calcd for $C_{17}H_{17}ClIN_5O_3S$: C, 38.25; H, 3.21; N, 13.12; S, 6.01. Found: C, 38.47; H, 3.42; N, 13.42; S, 6.20.

3.41. 2-Chloro-6-(2-naphthylmethyl)amino-9-(4-thio-β-D-ribofuranosyl)purine (19h)

Yield = 86%; white solid; mp 145.4–150.9 °C; UV (MeOH) λ_{max} 280 nm; MS (FAB) *m/z* 458 (M^+); $[\alpha]_D^{25}$ −4.15 (*c* 1.06, MeOH); ^{1}H NMR (CD_3OD) δ 3.52 (dd, 1H, *J* = 4.8, 5.2 Hz, 4-H), 3.85–3.95 (m, 2H, 5-H), 4.28 (t, 1H, *J* = 4.0, 4.4 Hz, 3-H), 4.65 (dd, 1H, *J* = 3.6, 5.2 Hz, 2-H), 5.20 (br s, 2H, NH–C*H*$_2$), 5.86 (d, 1H, *J* = 5.2 Hz, 1-H), 7.41–8.14 (m, 7 H, naphthalenyl H), 8.42 (s, 1H, H-8); Anal. Calcd for $C_{21}H_{20}ClN_5O_3S$: C, 55.08, H, 4.40, N, 15.29, S, 7.00. Found: C, 55.03, H, 4.80, N, 15.53, S, 7.31.

3.42. 2-Chloro-6-(fluoren-9-yl)amino-9-(4-thio-β-D-ribofuranosyl)purine (19i)

Yield = 85%; white solid; mp 139.2–146.1 °C; UV (MeOH) λ_{max} 273 nm; MS (FAB) *m/z* 429.4 (M^+-H_2O-Cl); $[\alpha]_D^{25}$ 5.60 (*c* 1.00, MeOH); ^{1}H NMR (CD_3OD) δ 3.55 (dd, 1H, *J* = 4.8 Hz, 4-H), 3.86–3.97 (m, 2H, 5-H), 4.29 (t, 1H, *J* = 4.0 Hz, 3-H), 4.68 (t, 1H, *J* = 3.6, 5.2 Hz, 2-H), 5.90 (d, 1H, *J* = 5.6 Hz, 1-H), 6.60 (s, 1H, NH–C*H*), 7.27-7.79 (m, 8 H, fluorenyl H), 8.34 (s, 1H, H-8); Anal. Calcd for $C_{23}H_{20}ClN_5O_3S$: C, 57.32, H, 4.18, N, 14.53, S, 6.65. Found: C, 57.44, H, 4.53, N, 14.31, S, 6. 68.

3.43. 2-Chloro-6-phenethylamino-9-(4-thio-β-D-ribofuranosyl)purine (19j)

Yield = 85%; white solid; mp 131.2–135.3 °C; UV (MeOH) λ_{max} 273 nm; MS (FAB) *m/z* 422.1 (M^++Na); $[\alpha]_D^{25}$ −3.18 (*c* 1.07, MeOH); ^{1}H NMR (CD_3OD) δ 2.97 (t, 2H, *J* = 7.6 Hz, C*H*$_2$–Ph), 3.52 (dd, 1H,*J* = 4.8 Hz, 4-H), 3.79 (br t, 2H, NH–C*H*$_2$), 3.79-3.96 (m, 2H, 5-H), 4.30 (t, 1H, *J* = 4.4 Hz, 3-H), 4.65 (dd, 1H, *J* = 3.6, 5.2 Hz, 2-H), 5.87 (d, 1H, *J* = 5.6 Hz, 1-H), 7.16–7.27 (m, 5H, Ph), 8.39 (s, 1H, H-8); Anal. Calcd for $C_{18}H_{20}ClN_5O_3S$: C, 51.24, H, 4.78, N, 16.60, S, 7.60. Found: C, 51.30, H, 4.84, N, 16.47, S,7.84.

3.44. 2-Chloro-6-(3-fluorophenethyl)amino-9-(4-thio-β-D-ribofuranosyl)purine (19k)

Yield = 83%; white solid; mp 126.1–130.0 °C; UV (MeOH) λ_{max} 272 nm; MS (FAB) *m/z* 440 (M^+); $[\alpha]_D^{25}$ −3.86 (*c* 1.01, MeOH); ^{1}H NMR (CD_3OD) δ 2.99 (t, 2H, *J* = 7.6 Hz, C*H*$_2$–Ph), 3.52 (dd, 1H, *J* = 4.8, 5.2 Hz, 4-H), 3.81 (br t, 2H, NH–C*H*$_2$), 3.85–3.96 (m, 2H, 5-H), 4.29 (t, 1H, *J* = 4.4 Hz, 3-H), 4.65 (dd, 1H, *J* = 3.8, 5.4 Hz, 2-H), 5.87 (d, 1H, *J* = 5.2 Hz, 1-H), 6.89–7.30 (m, 4H, Ph), 8.39 (s, 1H, H-8); Anal. Calcd for $C_{18}H_{19}ClN_5O_3S$: C, 49.15, H, 4.35, N, 15.92, S, 7.29. Found: C, 49.18, H, 4.31, N, 15.53, S, 7.01.

3.45. 2-Chloro-6-(1,2-diphenylethyl)amino-9-(4-thio-β-D-ribofuranosyl)purine (19l)

Yield = 85%; white solid; mp 79.3–80.8 °C; UV (MeOH) λ_{max} 275 nm; MS (FAB) *m/z* 498 (M^+); $[\alpha]_D^{25}$ −2.06 (*c* 0.97, MeOH); ^{1}H NMR (CD_3OD) δ 3.16–3.28 (m, 2 H, NHCHC*H*$_2$), 3.51 (dd, 1H, *J* = 4.8 Hz, 4-H), 3.84–3.94 (m, 2H, 5-H), 4.26 (t, 1H, *J* = 4.8 Hz, 3-H), 4.61 (d, 1H, *J* = 4.0 Hz, 2-H), 5.60 (br s, 1H, NH–C*H*), 5.83 (d, 1H, *J* = 5.6 Hz, 1-H), 7.07–7.42 (m, 10 H, 2× Ph), 8.40 (s, 1H, H-8); Anal. Calcd for $C_{24}H_{24}ClN_5O_3S$: C, 57.88, H, 4.86, N, 14.06, S, 6.44. Found: C, 57.79, H, 4.46, N, 14.58, S, 6.45.

3.46. 2-Chloro-6-(*trans*-2-phenyl-cyclopropyl)amino-9-(4-thio-β-D-ribofuranosyl)purine (19m)

Yield = 79%; white solid; mp 181.8–183.8 °C; UV (MeOH) λ_{max} 275 nm; MS (FAB) *m/z* 434.1 (M^+); $[\alpha]_D^{25}$ −2.12 (*c* 0.99, MeOH); ^{1}H NMR (CD_3OD) δ 1.28–1.39 (m, 2H, cyclopropyl CH_2), 2.16–2.21 (m, 1H, cyclopropyl C*H*-Ph), 3.11 (br s, 1H, NH-cyclopropyl C*H*), 3.53 (dd, 1H, *J* = 4.8, 5.2 Hz, 4-H), 3.86–3.96 (m, 2H, 5-H), 4.30 (t, 1H, *J* = 4.4 Hz, 3-H), 4.66 (dd, 1H, *J* = 3.8, 5.4 Hz, 2-H), 5.89 (d, 1H, *J* = 5.6 Hz, 1-H), 7.28–7.29 (m, 5 H, Ph), 8.42 (s, 1H, H-8); Anal. Calcd for $C_{19}H_{20}ClN_5O_3S$: C, 52.59, H, 4.65, N, 16.14, S, 7.39. Found: C, 52.91, H, 4.65, N, 16.20, S, 7.63.

3.47. 2-Chloro-6-(3,3-diphenylpropyl)amino-9-(4-thio-β-D-ribofuranosyl)purine (19n)

Yield = 82%; white solid; mp 113.9–116.8 °C; UV (MeOH) λ_{max} 273 nm; MS (FAB) *m/z* 512 (M^+); $[\alpha]_D^{25}$ −2.57 (*c* 1.05, MeOH); ^{1}H NMR (CD_3OD) δ 2.43 (dd, 2H, *J* = 7.2, 14.4 Hz, $NHCH_2CH_2$), 3.48–3.54 (m, 3H, 4-H and NH–C*H*$_2$), 3.85–3.96 (dd, 2H, *J* = 4.8, 11.6 Hz, 5-H), 4.09 (pseudo t, 1H, *J* = 7.2, 8.4 Hz, CH_2CH_2CH), 4.29 (pseudo t, 1H, *J* = 3.6, 4.4 Hz, 3-H), 4.65 (pseudo t, 1H, *J* = 4.0, 4.8 Hz, 2-H), 5.86 (d, 1H, *J* = 5.2 Hz, 1-H), 7.12–7.31 (m, 10 H, 2× Ph), 8.38 (s, 1H, H-8); Anal. Calcd for $C_{25}H_{26}ClN_5O_3S$: C, 58.64, H, 5.12, N, 13.68, S, 6.26. Found: C, 58.38, H, 5.40, N, 13.46, S, 6.92.

3.48. Biological assays

3.48.1. Cell culture and membrane preparation. Chinese hamster ovary (CHO) cells expressing the recombinant human A_1, A_{2A}, or A_3AR were cultured in DMEM (Dulbecco's modified Eagle's medium) and F12 (1:1) supplemented with 10% fetal bovine serum, 100 U/mL penicillin, 100 μg/mL streptomycin, and 2 μmol/mL glutamine. 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 7.4) containing 10 mM $MgCl_2$. The suspension was homogenized with an electric homogenizer for 10 s and was then re-centrifuged at 20,000*g* for 20 min at 4 °C. The resultant pellets were resuspended in buffer containing 3 U/mL adenosine deaminase, and the suspension was stored at −80 °C until the binding experiments. The protein concentration was measured using the Bradford assay.[21]

3.48.2. Binding assays at the human A_1 and A_{2A}ARs. For binding to the human A_1AR, 50 μL of increasing concentrations of a test ligand and 50 μL of [^{3}H]R-PIA (2 nM) were incubated with membranes (40 μg/tube) from CHO cells stably expressing the human A_1AR 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 N^6-cyclopentyladenosine. For human A_{2A}AR binding, membranes (20 μg/tube) from HEK-293 cells stably expressing the human A_{2A}AR were incubated at 25 °C for 60 min with a final concentration of 15 nM [^{3}H]CGS21680 in a mixture containing 50 μL of increasing concentrations of a test ligand and 200 μL of 50 mM Tris–HCl, pH 7.4, containing 10 mM $MgCl_2$. *N*-5′-Ethyluronamidoadenosine (10 μM) was used to define nonspecific binding. The reaction was terminated by filtration with GF/B filters.

3.48.3. Binding assay at the human A_3AR. Each tube in the competitive binding assay contained 100 μl membrane suspension (20 μg protein), 50 μl [^{125}I]I-AB-MECA (1.0 nM), and 50 μl of increasing concentrations of the test ligands in Tris–HCl buffer (50 mM, pH 8.0) containing 10 mM $MgCl_2$, 1 mM EDTA. Nonspecific binding was determined using 10 μM of 5′-*N*-ethylcarboxamidoadenosine 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, USA). Filters were washed three times with 9 mL ice-cold buffer. Radioactivity was determined in a Beckman 5500B γ-counter.

3.49. Cyclic AMP accumulation assay

Intracellular cyclic AMP levels were measured with a competitive protein binding method.[22] CHO cells that expressed recombinant human A_3ARs were harvested by trypsinization. After centrifugation and resuspension in medium, cells were plated in 24-well plates in 0.5 mL medium. After 24 h, the medium was removed, and cells were washed three times with 1 mL DMEM, containing 50 mM *N*-(2-hydroxyethyl)piperazine-*N*′-(2-ethanesulfonic acid) (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 U/mL). After 45 min forskolin (10 μM) was added to the medium, and incubation was continued an additional 15 min. The reaction was terminated upon removal of 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.

3.50. Statistical analysis

Binding and functional parameters were calculated using Prism 5.0 software (GraphPAD, San Diego, CA, USA). IC_{50} values obtained from competition curves were converted to K_i values using the Cheng-Prusoff equation.[23] Data were expressed as means ± standard error.

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