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Design, synthesis, and binding of homologated truncated 4′-thioadenosine derivatives at the human A_3 adenosine receptors

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ABSTRACT

We synthesized homologated truncated 4′-thioadenosine analogues **3** in which a methylene (CH_2) group was inserted in place of the glycosidic bond of a potent and selective A_3 adenosine receptor antagonist **2**. The analogues were designed to induce maximum binding interaction in the binding site of the A_3 adenosine receptor. However, all homologated nucleosides were devoid of binding affinity at all subtypes of adenosine receptors, indicating that free rotation through the single bond allowed the compound to adopt an indefinite number of conformations, disrupting the favorable binding interaction essential for receptor recognition.

1. Introduction

Adenosine, an endogenous modulator controls many physiological functions through binding to the adenosine receptors (ARs) which consist of four subtypes, A_1, A_{2A}, A_{2B}, and A_3.[1] Binding of adenosine to the ARs changes the levels of the second messengers such as diacylglycerol (DAG) and cyclic 3′,5′-monophosphate (cAMP) for signal transduction.[1] Therefore, adenosine receptors are regarded as promising therapeutic targets for the treatment of many types of diseases related to these signal transduction pathways.[2]

Among four subtypes of ARs, A_3 AR is most recently identified receptor and many efforts have been made to modulate A_3 AR for the treatment of several diseases such as cancer, cardiac ischemia, asthma, glaucoma, and inflammation.[3] 4′-Thionucloesides were recently discovered to be good templates for the design of A_3 AR ligands.[4,5] Compound **1**[4,5] was found to be a very potent A_3 AR agonist (K_i = 0.38 nM) and showed very potent in vivo antitumor activity in several types of tumor xenograft models (Fig. 1). A mechanistic study revealed that compound **1** induced cell-cycle arrest by lowering levels of c-myc and cyclin D1 in a concentration- and time-dependent manner at lower concentrations and induced apoptosis through PARP cleavage at higher concentrations.[6] Compound **1** also inhibited the Wnt signaling pathway at a 10 nM concentration.[6]

A molecular modeling study indicated that the NH of the methylamide of **1** serves as a hydrogen bonding donor required for the receptor activation.[7] On the basis of these findings, it was hypothesized that the truncated analogue **2** derived from compound **1** in which a hydrogen bonding donor NH, essential for the A_3AR agonist activity, was removed, could function as an A_3 AR antagonist. Compound **2** turned out to be a potent and selective antagonist at the human (K_i = 4.16 nM) as well as rat (K_i = 3.89 nM) A_3 AR.[8]

Introduction of a C–C single bond in a structure increases the free rotation. The increase of the free rotation allows the molecule to adopt many conformations, making it possible to induce maximum binding interaction in the binding site of the receptor. On the basis of this hypothesis, we designed and synthesized the homologated structure **3** in which a methylene (CH_2) group was inserted in place of the glycosidic bond of a potent and selective

Abbreviations: AR, adenosine receptor; CCPA, 2-chloro-N^6-cyclopentyladenosine; CHO, Chinese hamster ovary; Cl-IB-MECA, 2-chloro-N^6-(3-iodobenzyl)-5′-*N*-methylcarbamoyladenosine; [^{125}I]I-AB-MECA, [^{125}I]N^6-(4-aminobenzyl)-5′-*N*-methylcarboxamidoadenosine; NECA, 5′-*N*-ethylcarboxamidoadenosine; CPA, N^6-cyclopentyladenosine; CGS 21680, 2-[4-(2-carboxyethyl)phenethylamino]-5′-*N*-ethylcarboxamido-adenosine; PARP, poly (ADP-ribose) polymerase.

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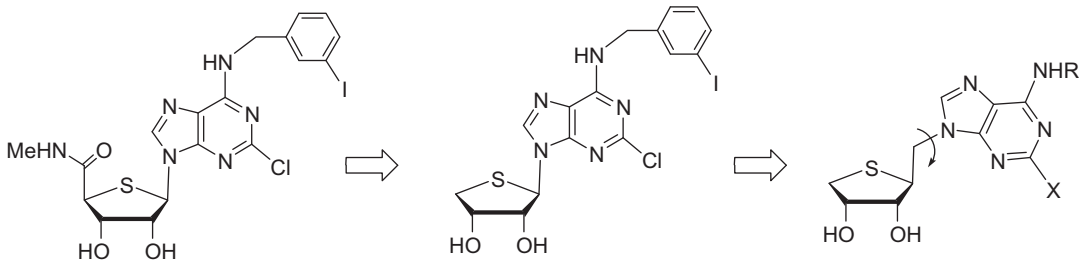


Figure 1. The rationale for the design of the target nucleoside 3.

A_3 AR antagonist **2** with the expectation of maximum binding interaction in the binding site of the receptor, as potential A_3 AR antagonists. Herein, we report the synthesis of the homologated truncated 4′-thioadenosine analogues **3** from D-mannose and the results of binding assays at the human ARs.

2. Results and discussion

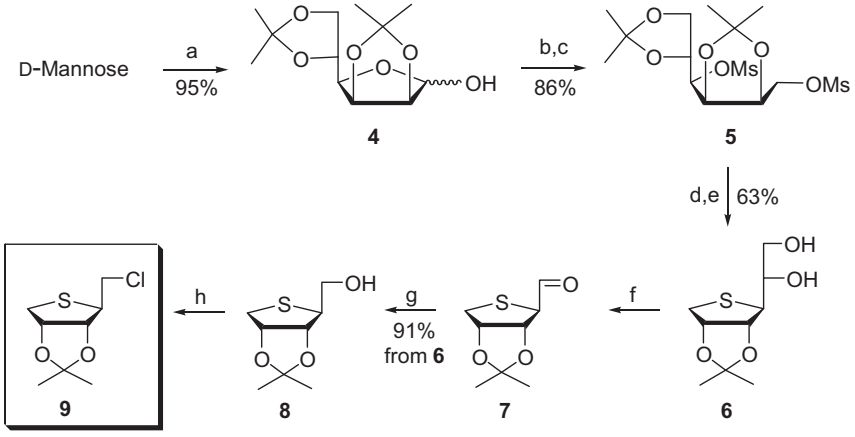
Our synthetic strategy was to synthesize the homologated glycosyl donor and then to condense it with a purine base. The homologated glycosyl donor **7** was first synthesized from D-mannose as shown in Scheme 1. D-Mannose was converted to the diol **4** according to our previously published procedure.[8] D-Mannose was converted to the diacetonide **4** by treating with 2,2-dimethoxypropane and acetone under acidic conditions. Reduction of **4** with sodium borohydride followed by mesylation of the resulting diol afforded the dimesylate **5**. Treatment of **5** with anhydrous sodium sulfide in DMF at 80 °C yielded the thiosugar **6**, which was hydrolyzed with 60% acetic acid to give diol **6**. Oxidative cleavage of the diol **6** with 1.2 equiv of $Pb(OAc)_4$ produced the aldehyde **7**, but use of excess $Pb(OAc)_4$ gave the anomeric acetate, resulting from the oxidative decarboxylation.[8] Reduction of the aldehyde **7** with $NaBH_4$ afforded the primary alcohol **8**. Treatment of **8** with phosphorous oxychloride yielded the glycosyl donor **9**, which was directly used for the condensation with purine bases.

The final nucleosides **3a–d** and **3e–h** were synthesized from direct S_N2 displacement of the chloride **9** with 6-chloropurine and 2,6-dichloropurine anions, as shown in Scheme 2. Condensation of **9** with 6-chloropurine and 2,6-dichloropurine in the presence of NaH in DMF afforded the desired N_9-isomers, 6-chloropurine derivative **10** and the 2,6-dichloropurine derivative **11**, respectively without concomitant formation of the corresponding N_7-isomers. The structural assignment of N_9-isomer and N_7-isomer was accomplished by the comparison of UV and ^{13}C NMR data reported in literatures.[8,9] Removal of the isopropylidene group of **10** using 2 N HCl gave the 2-H analogue **12**. Treatment of the 6-chloropurine derivative **12** with 3-halobenzyl amines yielded the N^6-(3-halobenzyl)amine derivatives **3a–d**. Similarly, the 2,6-dichloropurine derivative **11** was converted to the 2-Cl analogues **3e–h** via compound **13**.

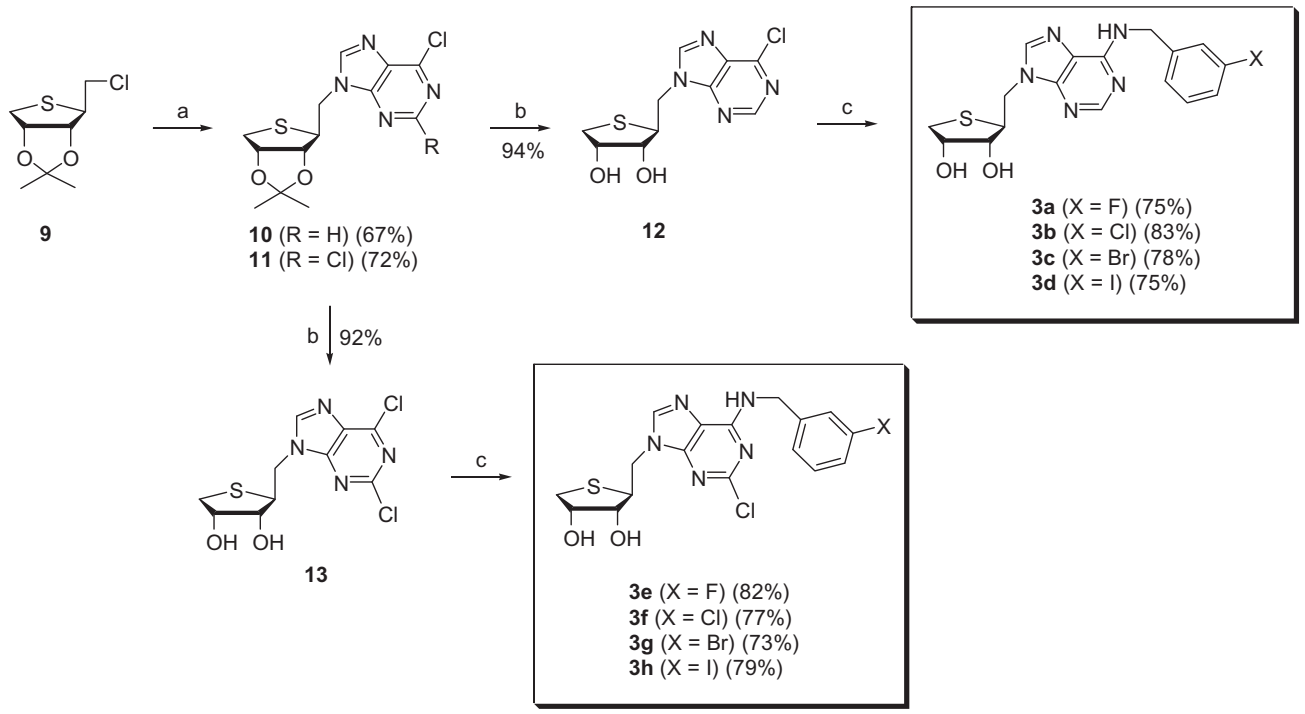
All AR binding experiments were performed using adherent mammalian CHO (Chinese hamster ovary) cells stably transfected with cDNA encoding the appropriate human ARs.[10] Binding was carried out using 1 nM [^{3}H]CCPA, 10 nM [^{3}H]CGS 21680, or 0.5 nM [^{125}I]I-AB-MECA as radioligands for A_1, A_{2A}, and A_3 ARs, respectively. Values are expressed as mean ± sem, n = 3–5 (outliers eliminated). If a percentage is given, it represents the percent inhibition at a fixed concentration of 10 μM. As shown in Table 1, all homologated compounds synthesized in this study bound weakly for determination of affinities at three subtypes of ARs. This result indicated that the homologation of the rotatable methylene (CH_2) between 1′ and N^9 positions disrupted favorable binding interactions at the ARs.

Since A_{2A} is the only AR subtype whose X-ray crystal structure is available (PDB code: 3EML),[11] compounds **2** and **3h** were docked into the A_{2A} AR structure, considering the flexibility of the binding site residues (Fig. 2).

The docking results of compounds **2** and **3h** were significantly different as shown in Figure 2. Compound **2** (K_i = 341 nM for hA_{2A}) showed a binding mode that was consistent with previous docking.[7] Its N^6-amino group formed an H-bond with Glu169, and the 2′- and 3′-OH groups were involved in H-bonding with Ser277 and His278. In addition, the adenine ring made the π–π stacking with Phe168 (Fig. 2A).

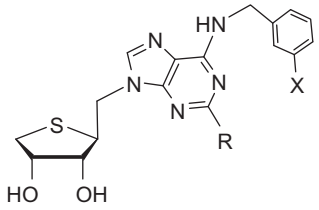


Scheme 1. Reagents and conditions: (a) 2,2-dimethoxypropane, camphosulfonic acid, acetone, rt; (b) $NaBH_4$, EtOH, rt; (c) MsCl, Et_3N, CH_2Cl_2, rt; (d) Na_2S, DMF, 80 °C; (e) 60% AcOH, rt; (f) $Pb(OAc)_4$, EtOAc, 0 °C; (g) $NaBH_4$, EtOH, 0 °C; (h) $POCl_3$, CH_3CN, rt.



Scheme 2. Reagents and conditions: (a) NaH, 6-chloropurine or 2,6-dichloropurine, DMF, rt; (b) 2 N HCl, THF, rt; (c) 3-halobenzylamine, Et_3N, EtOH.

Table 1
Inhibition of radioligand binding by known A_3AR antagonist **2** and homologated 4′-thioadenosine derivatives **3a–d** and **3e–h** at three subtypes of ARs[a]



Compounds	K_i (hA_1AR) nM or % displ. at 10 μM	K_i ($hA_{2A}AR$) nM or % displ. at 10 μM	K_i (hA_3AR) nM or % displ. at 10 μM
3a (R = H, X = F)	0.3 ± 0.3%	14.0 ± 5.6%	8.7 ± 6.0%
3b (R = H, X = Cl)	3.3 ± 3.3%	13.0 ± 8.2%	8.9 ± 1.8%
3c (R = H, X = Br)	6.6 ± 3.8%	25.8 ± 6.3%	6.1 ± 3.2%
3d (R = H, X = I)	9.6 ± 4.7%	18.2 ± 7.1%	13.9 ± 5.5%
3e (R = Cl, X = F)	7.9 ± 4.0%	12.0 ± 12.0%	17.6 ± 4.6%
3f (R = Cl, X = Cl)	3.2 ± 1.9%	11.0 ± 6.5%	13.6 ± 2.9%
3g (R = Cl, X = Br)	23.6 ± 3.4%	2.0 ± 1.2%	21.0 ± 4.7%
3h (R = Cl, X = I)	16.4 ± 4.6%	18.4 ± 4.3%	10.0 ± 5.0%
2	2490 ± 940	341 ± 75	4.16 ± 0.50

[a] All AR experiments were performed using adherent mammalian cells stably transfected with cDNA encoding the human ARs (CHO cells for A_1AR and A_3AR, HEK-293 cells for A_{2A}AR). Percent inhibition of binding at the human A_3AR was determined at 10 μM. Binding was carried out using radioligand [^{3}H]R-PIA at the human A_1AR, [^{3}H]CGS 21680 at the human A_{2A}AR, or [^{125}I]I-AB-MECA at the human A_3AR. Values from the present study are expressed as mean ± sem, n = 3–5.

In contrast with compound **2**, compound **3h** showed various binding modes, losing important interactions (Fig. 2B). It may retain or lose the H-bonding interaction with Glu169 and/or the π–π stacking with Phe168. Furthermore, it is more likely to lose H-bonding of the OH groups in the thio-ribose with Ser277/His278. This might be due to the rotatable extra methylene group (between the thio-ribose and the adenine ring), which might explain why compound **3h** lost its binding affinity at the A_{2A} AR. Structural and conformational factors in the hydrophilic ribose binding region are known to be critical for nucleoside recognition in the ARs.[7]

3. Conclusions

In this study, the synthesis of homologated truncated 4′-thioadenosine derivatives from D-mannose and assay of their binding affinities at the ARs were accomplished. The CH_2 homologation inserted in place of the glycosyl bond was introduced to allow max-

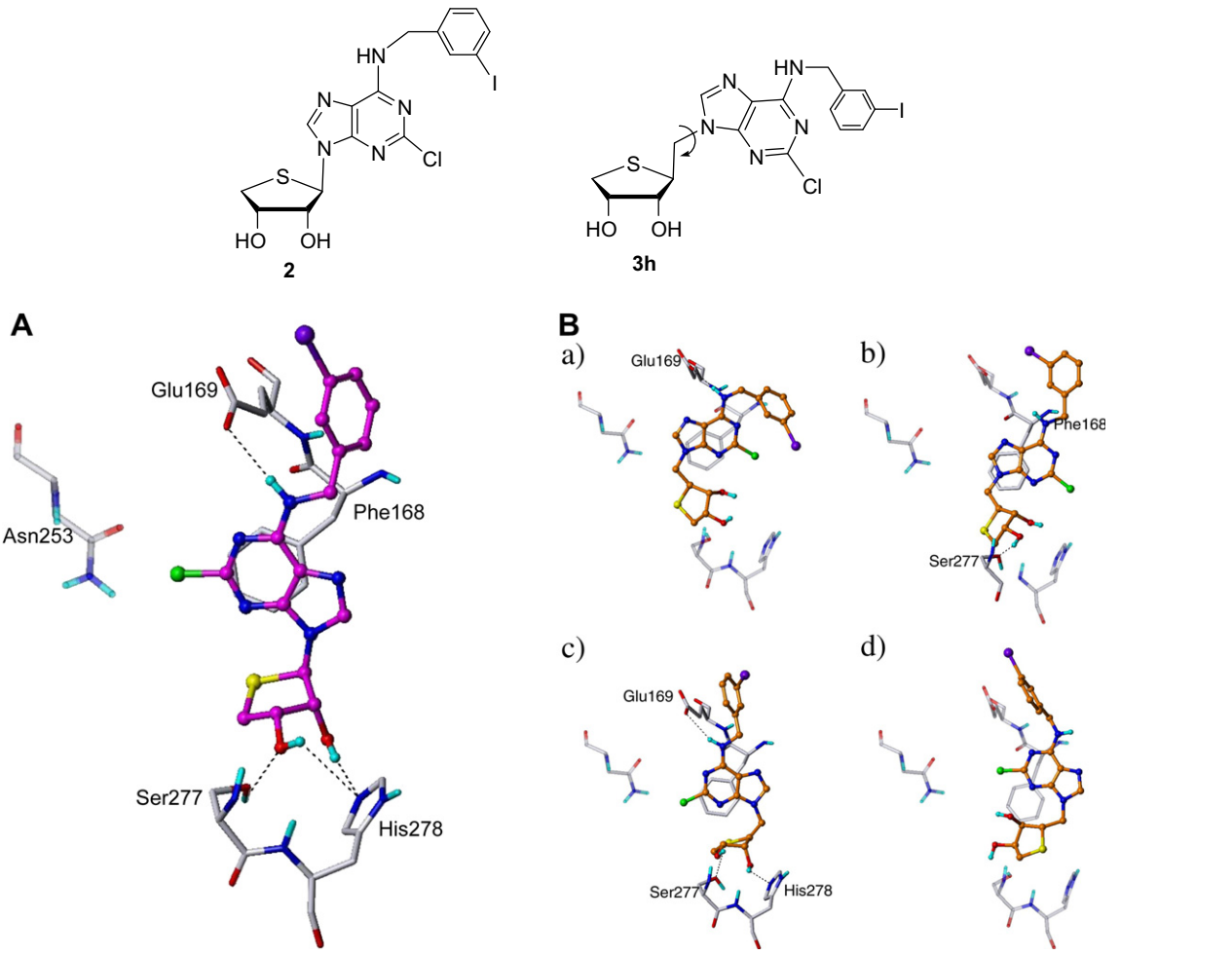


Figure 2. Predicted binding modes of compounds **2** and **3h** in the A_{2A} AR crystal structure. (A) Compound **2** (in magenta) interacts with the binding site residues. (B) Compound **3h** (in orange) shows various binding modes. The key residues are displayed in capped-stick with carbon atoms in white. Hydrogen bonds are drawn in black dashed lines and non-polar hydrogens are undisplayed for clarity.

imum binding interaction through the single bond rotation, but the rotatable extra methylene group resulted in losing important interactions as illustrated in various predicted binding modes.

4. Experimental section

4.1. General methods

^{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. Elemental analyses were measured on EA1110. The crude products were purified using a silica gel 60 (230–400 mesh, Merck). 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.

4.2. Chemical synthesis

4.2.1. (2,2-Dimethyl-tetrahydro-thieno[3,4-*d*][1,3]dioxol-4-yl)-methanol (6)

To a stirred solution of **6**[8] (20 g, 90.8 mmol) in ethyl acetate (500 mL) was added $Pb(OAc)_4$ (48.3 g, 109 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 and the filtrate was diluted with EtOAc. The organic layer was washed with saturated aqueous $NaHCO_3$ solution, dried over anhydrous $MgSO_4$, and evaporated to give the aldehyde **7**, which was used for next step without further purification. To a stirred solution of aldehyde **7** (5.6 g, 30.0 mmol) in EtOH (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/EtOAc = 2:1) to give **8** (5.2 g, 91%) as a colorless syrup: ^{1}H NMR ($CDCl_3$) δ 1.27 (s, 3H, CH_3), 1.47 (s, 3H, CH_3), 2.68 (br s, 1H, OH), 2.84 (dd, 1H, J = 2.0, 12.6 Hz, C*H*H), 3.06 (dd, 1H, J = 5.2, 13.0 Hz, CH*H*), 3.38 (m, 1H, CH), 3.56 (dd, 2H, J = 2.8, 8.8 Hz, HOCH_2), 4.66 (dd, 1H, J = 1.6, 5.6 Hz, OCH), 4.86 (td, 1H, J = 1.6, 4.8 Hz, OCH); ^{13}C NMR ($CDCl_3$) δ 24.75, 26.66, 37.42, 56.62, 63.32, 83.70, 85.94, 111.21; FAB-MS m/z 191 ($M+H^+$).

4.2.2. 4-Chloromethyl-2,2-dimethyl-tetrahydro-thieno[3,4-*d*][1,3]dioxole (9)

To a stirred solution of **8** (8.5 g, 40.7 mmol) in anhydrous acetonitrile (100 mL) was added $POCl_3$ (4.47 mL, 48.9 mmol) at 0 °C and the reaction mixture was stirred for 30 min at room temperature. The reaction mixture was dried in vacuo and co-evaporated with

toluene twice to give **9**, which was directly used for next step without further purification: ^{1}H NMR ($CDCl_3$) δ 1.27 (s, 3H, CH_3), 1.46 (s, 3H, CH_3), 2.88 (m, 2H, CH_2), 3.08 (dd, 1H, *J* = 4.8, 13.2 Hz, CH), 3.44 (m, 1H, C*H*HCl), 3.65 (dd, 1H, *J* = 4.0, 8.2 Hz, CH*H*Cl), 4.81 (dd, 1H, *J* = 0.8, 5.6 Hz, OCH), 4.89 (m, 1H, OCH); ^{13}C NMR ($CDCl_3$) δ 24.73, 26.54, 37.87, 45.72, 55.32, 83.58, 86.18, 111.31.

4.2.3. General procedure for the condensation

To a solution of 6-chloropurine (21.69 mmol) and 2,6-dichloropurine (21.69 mmol) in a solution of anhydrous DMF (50 mL) were added NaH (21.69 mmol) and the mixture was stirred at room temperature until the solution became clear. A solution of **9** (18.07 mmol) in anhydrous DMF (10 mL) was added to the resulting solution at room temperature and stirred overnight at room temperature. The reaction mixture was diluted with EtOAc (100 mL) and washed with water several times, dried with anhydrous $MgSO_4$ and evaporated. The residue was purified by flash silica gel column chromatography (hexane/EtOAc = 2:1) to give the condensed products **10** and **11**, respectively.

4.2.3.1. 6-Chloro-9-(2,2-dimethyl-tetrahydro-thieno[3,4-*d*][1,3] dioxol-4-ylmethyl)-9*H*-purine (10). Yield = 67%; white foam; UV (MeOH) λ_{max} 265 nm (pH 7); $[\alpha]_D^{25}$ +128.0° (*c* 0.25, MeOH); FAB-MS *m/z* 327 ($M+H^+$); ^{1}H NMR ($CDCl_3$) δ 1.19 (s, 3H, CH_3), 1.38 (s, 3H, CH_3), 2.88 (m, 2H, CH_2), 3.72 (td, 1H, *J* = 1.2, 2.4 Hz, CH), 4.26 (dd, 1H, *J* = 8.0, 16.0 Hz, CH*H*N), 4.59 (dd, 1H, *J* = 6.8, 14.0 Hz, C*H*HN), 4.66 (dd, 1H, *J* = 2.0, 5.0 Hz, OCH), 4.79 (m, 1H, OCH), 8.28 (s, 1H, H-8), 8.67 (s, 1H, H-2); ^{13}C NMR ($CDCl_3$) δ 24.70, 26.49, 36.99, 46.07, 54.13, 83.20, 86.02, 111.88, 131.18, 145.53, 151.07, 151.84, 152.16; Anal. Calcd for $C_{13}H_{15}ClN_4O_2S$: C, 47.78; H, 4.63; N, 17.14; S, 9.81. Found: C, 47.82; H, 4.65; N, 17.20; S, 9.85.

4.2.3.2. 2,6-Dichloro-9-(2,2-dimethyl-tetrahydro-thieno[3,4-*d*][1,3]dioxol-4-ylmethyl)-9*H*-purine (11). Yield = 72%; white foam; UV (MeOH) λ_{max} 275 nm (pH 7); $[\alpha]_D^{25}$ +173.3° (*c* 0.30, MeOH); FAB-MS *m/z* 361 (M^+); ^{1}H NMR ($CDCl_3$) δ 1.27 (s, 3H, CH_3), 1.47 (s, 3H, CH_3), 2.98 (m, 2H, CH_2), 3.74 (td, 1H, *J* = 2.0, 6.2 Hz, CH), 4.21 (dd, 1H, *J* = 8.8, 14.2 Hz, CH*H*N), 4.47 (dd, 1H, *J* = 6.8, 14.2 Hz, C*H*HN), 4.69 (dd, 1H, *J* = 2.0, 5.6 Hz, OCH), 4.89 (m, 1H, OCH), 8.23 (s, 1H, H-8); ^{13}C NMR ($CDCl_3$) δ 24.82, 26.62, 37.04, 46.18, 54.13, 83.32, 86.22, 112.20, 146.13, 145.53, 152.14, 153.32, 153.37; Anal. Calcd for $C_{13}H_{14}Cl_2N_4O_2S$: C, 43.22; H, 3.91; N, 15.51; S, 8.88. Found: C, 43.29; H, 3.96; N, 15.63; S, 8.82.

4.2.4. General procedure for the removal of the isopropylidene group

To a stirred solution of **10** (12.4 mmol) and **11** (12.4 mmol) in THF (50 mL) was added 2 N HCl at room temperature and the mixture was stirred for 24 h at same temperature. The mixture was neutralized with NH_4OH and evaporated. The residue was purified by flash silica gel column chromatography (CH_2Cl_2/EtOAc/MeOH = 10:10:1) to give the products, **12** and **13**, respectively.

4.2.4.1. 2-(6-Chloro-purin-9-ylmethyl)-tetrahydro-thiophene-3,4-diol (12). Yield = 94%; white foam; UV (MeOH) λ_{max} 265 nm (pH 7); FAB-MS *m/z* 287 ($M+H^+$); $[\alpha]_D^{25}$ +53.1° (*c* 0.65, MeOH); ^{1}H NMR (DMSO-d_6) δ 2.62 (dd, 1H, *J* = 4.0, 10.0 Hz, C*H*H), 2.90 (dd, 1H, *J* = 4.0, 10.0 Hz, CH*H*), 3.70 (m, 1H, CH), 3.80 (br s, 1H, C*H*HN), 4.12 (br s, 1H, CH*H*N), 4.40 (dd, 1H, *J* = 7.2, 13.6 Hz, C*H*OH), 4.60 (dd, 1H, *J* = 7.2, 13.6 Hz, C*H*OH), 5.03 (br s, 2H, OH), 8.70 (s, 1H, H-8), 8.78 (s, 1H, H-2); ^{13}C NMR (DMSO-d_6) δ 33.04, 47.31, 48.00, 73.54, 77.40, 130.68, 147.60, 148.95, 151.51, 151.96.

4.2.4.2. 2-(2,6-Dichloro-purin-9-ylmethyl)-tetrahydro-thiophene-3,4-diol (13). Yield = 92%; white foam; UV (MeOH) λ_{max} 275 nm (pH 7); FAB-MS *m/z* 321 (M^+); $[\alpha]_D^{25}$ +64.3° (*c* 0.60, MeOH); ^{1}H NMR (DMSO-d_6) δ 2.61 (dd, 1H, *J* = 4.0, 10.8 Hz, C*H*H), 2.92 (dd, 1H, *J* = 4.4, 10.8 Hz, CH*H*), 3.67(m, 1H, C*H*HN), 3.80 (dd, 1H, *J* = 3.2, 6.6 Hz, CH*H*N), 4.11 (m, 1H, C*H*OH), 4.37 (dd, 1H, *J* = 7.2, 14.0 Hz, C*H*OH), 4.55 (dd, 1H, *J* = 6.8, 14.2 Hz, C*H*OH), 5.04 (br s, 2H, OH), 8.72 (s, 1H, H-8); ^{13}C NMR (DMSO-d_6) δ 33.19, 47.52, 73.58, 77.74, 130.40, 147.75, 148.65, 149.57, 153.53, 153.75.

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

To a stirred solution of 6-chloropurine derivative **12** (0.45 mmol) or 2,6-dichloropurine derivative **13** (0.45 mmol) and an appropriate amine hydrochloride salts or free amines (0.90 mmol) in EtOH (10 mL) was added Et_3N (1.35 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/EtOAc/MeOH = 10:10:1) to give the N^6-substituted amine derivatives **3a**–**h**.

4.2.5.1. N^6-(3-Fluorobenzylamino)-9-ylmethyl-(4-thio-β-D-ribofuranosyl)adenine (3a). Yield = 75%; white solid; mp 117–120 °C; UV (MeOH) λ_{max} 274 nm (pH 7); $[\alpha]_D^{25}$ +53.3° (*c* 0.30, MeOH); ^{1}H NMR (DMSO-d_6) δ 2.61 (dd, 1H, *J* = 5.2, 10.8 Hz, C*H*H), 2.88 (dd, 1H, *J* = 5.2, 10.4 Hz, CH*H*), 3.68 (m, 1H, CH), 3.78 (m, 1H, NHC*H*H), 4.14 (m, 1H, NHCH*H*), 4.19 (dd, 1H, *J* = 7.6, 14.0 Hz, C*H*HN), 4.47 (dd, 1H, *J* = 6.0, 14.2 Hz, CH*H*N), 4.66 (br s, 2H, C*H*OH × 2), 5.02 (d, 1H, *J* = 4.8 Hz, OH), 5.09 (d, 1H, *J* = 5.6 Hz, OH), 7.10 (t, 1H, *J* = 8.0 Hz, NBn-*H*), 7.36 (d, 1H, *J* = 7.6 Hz, NBn-*H*), 7.58 (d, 1H, *J* = 7.6 Hz, NBn-*H*), 7.73 (s, 1H, NBn-*H*), 8.16 (s, 1H, H-8), 8.21 (s, 1H, H-2), 8.36 (br s, 1H, N*H*Bn). ^{13}C NMR (DMSO-d_6) δ 32.82, 42.26, 46.81, 48.77, 73.56, 76.88, 94.70, 118.94, 126.69, 130.45, 135.32, 135.76, 141.04, 142.94, 148.94, 152.34, 154.17; FAB-MS *m/z* 376 ($M+H^+$); Anal. Calcd for $C_{17}H_{18}FN_5O_2S$: C, 54.39; H, 4.83; N, 18.65; S, 8.54. Found: C, 54.32; H, 4.96; N, 18.77; S, 8.62.

4.2.5.2. N^6-(3-Chlorobenzylamino)-9-ylmethyl-(4-thio-β-D-ribofuranosyl)adenine (3b). Yield = 83%; white solid; mp 143–147 °C; UV (MeOH) λ_{max} 275 nm (pH 7); $[\alpha]_D^{25}$ +71.8° (*c* 0.32, MeOH); ^{1}H NMR (DMSO-d_6) δ 2.61 (dd, 1H, *J* = 5.2, 10.4 Hz, C*H*H), 2.87 (dd, 1H, *J* = 5.2, 10.4 Hz, CH*H*), 3.68 (m, 1H, CH), 3.78 (m, 1H, NHCH*H*), 4.16 (m, 1H, NHCH*H*), 4.19 (dd, 1H, *J* = 7.6, 14.0 Hz, C*H*HN), 4.46 (dd, 1H, *J* = 8.0, 14.0 Hz, CH*H*N), 4.69 (br s, 2H, C*H*OH × 2), 5.01 (d, 1H, *J* = 4.8 Hz, OH), 5.08 (d, 1H, *J* = 5.6 Hz, OH), 7.25 (t, 1H, *J* = 8.0 Hz, NBn-*H*), 7.35 (d, 1H, *J* = 7.6 Hz, NBn-*H*), 7.39 (d, 1H, *J* = 7.6 Hz, NBn-*H*), 7.54 (s, 1H, NBn-*H*), 8.16 (s, 1H, H-8), 8.22 (s, 1H, H-2), 8.38 (br s, 1H, N*H*Bn); ^{13}C NMR (DMSO-d_6) δ 32.81, 42.40, 46.81, 48.76, 73.56, 76.83, 118.93, 121.52, 126.28, 129.46, 129.86, 130.42, 141.06, 143.09, 148.97, 152.34, 154.18; FAB-MS *m/z* 392 ($M+H^+$); Anal. Calcd for $C_{17}H_{18}ClN_5O_2S$: C, 52.10; H, 4.63; N, 17.87; S, 8.18. Found: C, 52.33; H, 4.64; N, 17.90; S, 8.16.

4.2.5.3. N^6-(3-Bromobenzylamino)-9-ylmethyl-(4-thio-β-D-ribofuranosyl)adenine (3c). Yield = 78%; white solid; mp 134–139 °C; UV (MeOH) λ_{max} 270 nm (pH 7); $[\alpha]_D^{25}$ +72.0° (*c* 0.25, MeOH); ^{1}H NMR (DMSO-d_6) δ 2.62 (dd, 1H, *J* = 5.2, 10.6 Hz, C*H*H), 2.87 (dd, 1H, *J* = 4.8, 10.2 Hz, CH*H*), 3.67 (m, 1H, CH), 3.78 (m, 1H, NHCH*H*), 4.14 (m, 1H, NHC*H*H), 4.19 (dd, 1H, *J* = 7.6, 14.2 Hz, C*H*HN), 4.47 (dd, 1H, *J* = 6.4, 14.0 Hz, CH*H*N), 4.70 (br s, 2H, C*H*OH × 2), 5.02 (d, 1H, *J* = 5.2 Hz, OH), 5.08 (d, 1H, *J* = 5.6 Hz, OH), 7.31 (m, 3H, NBn-*H*), 7.39 (s, 1H, NBn-*H*), 8.16 (s, 1H, H-8), 8.21 (s, 1H, H-2), 8.37 (br s, 1H, N*H*Bn). ^{13}C NMR (DMSO-d_6) δ 32.18, 42.45, 46.81, 48.77, 73.56, 76.84, 118.96, 125.87, 126.56, 126.95, 130.10, 132.86, 141.06, 142.83, 148.99, 152.34, 154.21; FAB-MS *m/z* 436 (M^+); Anal. Calcd for $C_{17}H_{18}BrN_5O_2S$: C, 46.80;

H, 4.16; N, 16.05; S, 7.35. Found: C, 46.89; H, 4.21; N, 16.07; S, 7.41.

4.2.5.4. N^6-(3-Iodobenzylamino)-9-ylmethyl-(4-thio-β-D-ribofuranosyl)adenine (3d). Yield = 75%; white solid; mp 172–175 °C; UV (MeOH) λ_{max} 272 nm (pH 7); $[\alpha]_D^{25}$ +68.5° (*c* 0.35, MeOH); ^{1}H NMR (DMSO-d_6) δ 2.61 (dd, 1H, *J* = 5.2, 10.4 Hz, C*H*H), 2.87 (dd, 1H, *J* = 5.2, 10.6 Hz, CH*H*), 3.67 (m, 1H, CH), 3.78 (m, 1H, NHCH*H*), 4.14 (m, 1H, NHC*H*H), 4.19 (dd, 1H, *J* = 7.6, 18.0 Hz, C*H*HN), 4.46 (dd, 1H, *J* = 6.4, 14.0 Hz, CH*H*N), 4.71 (br s, 2H, C*H*OH × 2), 5.02 (d, 1H, *J* = 4.8 Hz, OH), 5.08 (d, 1H, *J* = 5.6 Hz, OH), 7.00–7.19 (m, 3H, NBn-*H*), 7.32 (m, 1H, NBn-*H*), 8.16 (s, 1H, H-8), 8.21 (s, 1H, H-2), 8.36 (br s, 1H, N*H*Bn); ^{13}C NMR (DMSO-d_6) δ 32.81, 42.51, 46.80, 48.76, 73.56, 76.83, 118.94, 123.15, 130.08, 130.16, 141.03, 143.28, 148.98, 152.34, 154.25, 160.95, 163.37; FAB-MS *m/z* 484 (M+H$^+$); Anal. Calcd for $C_{17}H_{18}IN_5O_2S$: C, 42.25; H, 3.75; N, 14.49; S, 6.63. Found: C, 42.34; H, 3.78; N, 14.51; S, 6.65.

4.2.5.5. 2-Chloro-N^6-(3-fluorobenzylamino)-9-ylmethyl-(4-thio-β-D-ribofuranosyl)adenine (3e). Yield = 82%; white solid; mp 164–167 °C; UV (MeOH) λ_{max} 275 nm (pH 7); $[\alpha]_D^{25}$ +48.0° (*c* 0.50, MeOH); ^{1}H NMR (DMSO-d_6) δ 2.61 (dd, 1H, *J* = 4.8, 10.8 Hz, C*H*H), 2.89 (dd, 1H, *J* = 4.8, 10.4 Hz, CH*H*), 3.65 (m, 1H, CH), 3.78 (m, 1H, NHCH*H*), 4.16 (m, 2H, NHC*H*H, C*H*HN), 4.43 (dd, 1H, *J* = 6.4, 14.0 Hz, CH*H*N), 4.60 (br d, 2H, *J* = 5.2 Hz, C*H*OH × 2), 5.03 (d, 1H, *J* = 4.8 Hz, OH), 5.09 (d, 1H, *J* = 6.0 Hz, OH), 7.12 (t, 1H, *J* = 7.6 Hz, NBn-*H*), 7.36 (d, 1H, *J* = 7.6 Hz, NBn-*H*), 7.59 (d, 1H, *J* = 8.0 Hz, NBn-*H*), 7.74 (s, 1H, NBn-*H*), 8.17 (s, 1H, H-8), 8.83 (br s, 1H, N*H*Bn); ^{13}C NMR (DMSO-d_6) δ 32.93, 42.54, 47.06, 48.36, 73.58, 77.14, 94.73, 118.06, 126.87, 130.52, 135.56, 136.08, 141.67, 141.94, 149.95, 152.95, 154.70; FAB-MS *m/z* 410 (M+H$^+$); Anal. Calcd for $C_{17}H_{17}ClFN_5O_2S$: C, 49.82; H, 4.18; N, 17.09; S, 7.82. Found: C, 49.94; H, 4.25; N, 17.12; S, 7.85.

4.2.5.6. 2-Chloro-N^6-(3-chlorobenzylamino)-9-ylmethyl-(4-thio-β-D-ribofuranosyl)adenine (3f). Yield = 77%; white solid; mp 154–158 °C; UV (MeOH) λ_{max} 275 nm (pH 7); $[\alpha]_D^{25}$ +77.5° (*c* 0.40, MeOH); ^{1}H NMR (DMSO-d_6) δ 2.62 (dd, 1H, *J* = 4.8, 10.6 Hz, C*H*H), 2.90 (dd, 1H, *J* = 4.8, 10.8 Hz, CH*H*), 3.65 (m, 1H, CH), 3.78 (m, 1H, NHCH*H*), 4.11–4.20 (m, 2H, NHC*H*H, C*H*HN), 4.44 (dd, 1H, *J* = 6.0, 14.0 Hz, CH*H*N), 4.63 (br d, 2H, *J* = 6.5 Hz, C*H*OH × 2), 5.04 (d, 1H, *J* = 4.8 Hz, OH), 5.09 (d, 1H, *J* = 5.6 Hz, OH), 7.27 (t, 1H, *J* = 7.6 Hz, NBn-*H*), 7.35 (d, 1H, *J* = 7.6 Hz, NBn-*H*), 7.42 (d, 1H, *J* = 7.6 Hz, NBn-*H*), 7.55 (s, 1H, NBn-*H*), 8.17 (s, 1H, H-8), 8.84 (br s, 1H, N*H*Bn); ^{13}C NMR (DMSO-d_6) δ 32.93, 42.65, 47.06, 48.36, 73.59, 77.15, 118.07, 121.56, 126.45, 129.71, 130.14, 130.51, 141.69, 142.09, 149.97, 152.95, 154.72; FAB-MS *m/z* 426 (M$^+$); Anal. Calcd for $C_{17}H_{17}Cl_2N_5O_2S$: C, 47.89; H, 4.02; N, 16.43; S, 7.52. Found: C, 47.92; H, 4.05; N, 16.50; S, 7.55.

4.2.5.7. N^6-(3-Bromobenzylamino)-2-chloro-9-ylmethyl-(4-thio-β-D-ribofuranosyl)adenine (3g). Yield = 73%; white solid; mp 165–169 °C; UV (MeOH) λ_{max} 274 nm (pH 7); $[\alpha]_D^{25}$ +70.0° (*c* 0.50, MeOH); ^{1}H NMR (DMSO-d_6) δ 2.62 (dd, 1H, *J* = 4.4, 10.6 Hz, C*H*H), 2.89 (dd, 1H, *J* = 5.2, 10.6 Hz, CH*H*), 3.66 (m, 1H, CH), 3.79 (m, 1H, NHCH*H*), 4.13–4.21 (m, 2H, NHC*H*H, C*H*HN), 4.44 (dd, 1H, *J* = 6.4, 13.8 Hz, CH*H*N), 4.65 (br d, 2H, *J* = 5.2 Hz, C*H*OH × 2), 5.04 (d, 1H, *J* = 4.8 Hz, OH), 5.10 (d, 1H, *J* = 5.6 Hz, OH), 7.02–7.30 (m, 4H, NBn-*H*), 8.17 (s, 1H, H-8), 8.84 (br s, 1H, N*H*Bn); ^{13}C NMR (DMSO-d_6) δ 32.95, 42.76, 47.08, 48.38, 73.62, 77.18, 118.01, 123.32, 130.21, 130.29, 141.67, 142.32, 149.98, 152.99, 154.81, 160.96, 163.37; FAB-MS *m/z* 472 (M+H$^+$); Anal. Calcd for $C_{17}H_{17}BrClN_5O_2S$: C, 43.37; H, 3.64; N, 14.88; S, 6.81. Found: C, 43.40; H, 3.70; N, 14.90; S, 6.85.

4.2.5.8. 2-Chloro-N^6-(3-iodobenzylamino)-9-ylmethyl-(4-thio-β-D-ribofuranosyl)adenine (3h). Yield = 79%; white solid; mp 174–180 °C; UV (MeOH) λ_{max} 274 nm (pH 7); $[\alpha]_D^{25}$ +136.4° (*c* 0.33, MeOH); ^{1}H NMR (DMSO-d_6) δ 2.62 (dd, 1H, *J* = 4.8, 10.6 Hz, C*H*H), 2.89 (dd, 1H, *J* = 4.8, 10.4 Hz, CH*H*), 3.65 (m, 1H, CH), 3.78 (m, 1H, NHCH*H*), 4.13–4.20 (m, 2H, NHC*H*H, C*H*HN), 4.44 (dd, 1H, *J* = 6.0, 14.0 Hz, CH*H*N), 4.64 (br d, 2H, *J* = 4.8 Hz, C*H*OH × 2), 5.04 (d, 1H, *J* = 4.8 Hz, OH), 5.09 (d, 1H, *J* = 5.6 Hz, OH), 7.27–7.40 (m, 4H, NBn-*H*), 8.17 (s, 1H, H-8), 8.84 (br s, 1H, N*H*Bn); ^{13}C NMR (DMSO-d_6) δ 32.94, 42.71, 47.07, 48.36, 73.60, 77.17, 118.07, 126.04, 126.81, 127.22, 130.19, 132.93, 141.69, 141.84, 149.98, 152.96, 154.75; FAB-MS *m/z* 518 (M+H$^+$); Anal. Calcd for $C_{17}H_{17}ClIN_5O_2S$: C, 39.43; H, 3.31; N, 13.53; S, 6.19. Found: C, 39.52; H, 3.35; N, 13.58; S, 6.22.

4.3. Molecular modeling study

Ligand structures were generated with Concord and energy minimized using MMFF94s force field and MMFF94 charge until the rms of Powell gradient was 0.05 kcal mol^{-1} A^{-1} in SYBYL 8.1.1 (Tripos Inc., St. Louis, MO, USA). The X-ray crystal structure of human A_{2A} AR (PDB code: 3EML) was prepared using Biopolymer Structure Preparation Tool in SYBYL. The docking study was performed using GOLD v.4.1.2 (Cambridge Crystallographic Data Centre, Cambridge, UK), which employs a genetic algorithm (GA) and allows for full ligand flexibility and partial protein flexibility. The binding site was defined as the 9 Å around the co-crystallized ligand (ZM-241385). The side chains of the eight residues (i.e., Thr88, Phe168, Glu169, Trp246, Leu249, Asn253, Ser277, and His278) in the crystal ligand binding site were set to be flexible with 'crystal mode'. GoldScore scoring function was used and other parameters were set as suggested by the GOLD authors except the number of GA runs as 30. All computation calculations were undertaken on Intel® Xeon™ Quad-core workstation with Linux Cent OS release 4.6.

4.4. Binding assay

Human A_1 and A_{2A} adenosine receptors: For binding to human A_1 receptors, [^{3}H]PIA (1 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}ARs were incubated with 15 nM [^{3}H]CGS21680 at 25 °C for 60 min in 200 μL 50 mM Tris–HCl, pH 7.4, containing 10 mM $MgCl_2$. Reaction was terminated by filtration with GF/B filters.

Human A_3 adenosine receptor: For competitive binding assay, each tube contained 100 μL suspension of membranes (20 μg protein) from CHO cells stably expressing the human A_3AR, 50 μL of [^{125}I]I-AB-MECA (0.5 nM), and 50 μL of increasing concentrations of the nucleoside derivative 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, USA). Filters were washed three times with 9 mL ice-cold buffer. Radioactivity was determined in a Beckman 5500B γ-counter.

For binding at all three subtypes, K_i values are expressed as mean ± sem, *n* = 3–5 (outliers eliminated), and normalized against a non-specific binder, 5′-*N*-ethylcarboxamidoadenosine (NECA, 10 μM).[9] Alternately, for weak binding a percent inhibition of specific radioligand binding at 10 μM, relative to inhibition by 10 μM NECA assigned as 100%, is given.

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