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Structure-Activity Relationships of Truncated Adenosine Derivatives as Highly Potent and Selective Human A₃ Adenosine Receptor Antagonists

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Abstract

On the basis of potent and selective binding affinity of truncated 4'-thioadenosine derivatives at the human A₃ adenosine receptor (AR), their bioisosteric 4'-oxo derivatives were designed and synthesized from commercially available 2,3-*O*-isopropylidene- β -erythrono lactone. The derivatives tested in AR binding assays were substituted at the C2 and N⁶ positions. All synthesized nucleosides exhibited potent and selective binding affinity at the human A₃ AR. They were less potent than the corresponding 4'-thio analogues, but showed higher selectivity to other subtypes. The 2-Cl series generally were better than the 2-H series in view of binding affinity and selectivity. Among compounds tested, compound **5d** (X = Cl, R = 3-bromobenzyl) showed the highest binding affinity ($K_i = 13.0 \pm 6.9$ nM) at the hA₃ AR with high selectivity (at least 1000-fold) in comparison to other AR subtypes. Like the corresponding truncated 4'-thio series, compound **5d** antagonized the action of an agonist to inhibit forskolin-stimulated adenylate cyclase in hA₃ AR-expressing CHO cells. Although the 4'-oxo series were less potent than the 4'-thio series, this class of human A₃ AR antagonists is also regarded as another good template for the design of A₃ AR antagonists and for further drug development.

Keywords

A₃ Adenosine Receptor; Antagonists; Truncated Adenosine; Structure-Activity Relationships

Introduction

Adenosine, an endogenous chemical messenger binds to four subtypes (A₁, A_{2A}, A_{2B}, and A₃) of adenosine receptors (ARs) and regulates many physiological functions in the cell.¹

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A₃ AR activation stimulates phospholipase C (PLC), increasing the levels of inositol triphosphate (IP₃) and diacylglycerol (DAG) and inhibits adenylate cyclase, decreasing the level of cAMP. Since IP₃, DAG, and cAMP serve as second messengers important for cell signaling, A₃ AR has been promising targets for the treatment of several diseases associated with cell signaling.² Thus, A₃ AR agonists have the therapeutic potentials against cancer,³ cerebral ischemia,⁴ and myocardial ischemia,⁵ while A₃ AR antagonists can be developed as anti-inflammatory,⁶ anti-asthma,⁷ and anti-glaucoma agents⁸.

A number of purine and non-purine compounds have been developed as potent and selective A₃ AR ligands.^{9,10} Most of adenosine derivatives were found to be A₃ AR agonists, among which compound **1** (Cl-IB-MECA)¹¹ showed high binding affinity (K_i for hA₃ = 1.4 nM) at the human (h) A₃ AR with high selectivities to other subtypes (Chart 1) and is under clinical trials as an anticancer agent. On the basis of a bioisosteric rationale, we designed and synthesized the 4'-thio analogue **2**¹² of Cl-IB-MECA, which exhibited better binding affinity (K_i for hA₃ = 0.38 nM) than compound **1**.

On the other hand, nonpurine heterocyclic compounds¹³ have been reported to be better templates than purine derivatives in developing potent and selective hA₃ AR antagonists. However, these A₃ AR antagonists were weak or ineffective in binding to the rat A₃ AR, indicating that they were not suitable for efficacy evaluation in small animal models and thus as drug candidates.¹⁴ Thus, searching for potent and selective species-independent A₃ AR antagonists has been an immediate goal. Because a few nucleoside derivatives have been reported to be species-independent A₃ AR antagonists¹⁴, we began to search for novel potent and selective A₃ AR antagonists, derived from nucleoside templates.

As a result, the truncated 4'-thio analogue **3**¹⁵ removing a hydrogen bonding donor ability of the 5'-uronamide required for the activation of the A₃ AR in compound **2** was found to be one of the best A₃ AR antagonists which bound selectively to the A₃ AR in a species-independent manner (K_i = 4.16 nM at the hA₃ AR and K_i = 3.9 nM at the rat A₃ AR) (Chart 1). Thus, it is of interest to design bioisosteric analogues of compound **3**, such as the truncated 4'-oxo derivatives **4** and **5**, and to compare their binding affinities at the hA₃ AR. In this article, we report the synthesis of truncated adenosine derivatives **4** and **5** as potent and selective hA₃ AR antagonists.

Results and discussion

Chemistry

Our synthetic strategy to the final nucleosides was first to synthesize the glycosyl donor and then condense with purine bases. Synthesis of the glycosyl donor **7**¹⁶ from commercially available 2,3-*O*-isopropylidene- β -erythrone is shown in Scheme 1.

Reduction of **5** with DIBAL in toluene gave the lactol **6**¹⁶, which was treated with acetic anhydride in pyridine to give the glycosyl donor **7**.

The glycosyl donor **7** was then condensed with silylated 6-chloropurine and 2,6-dichloropurine to give 6-chloro derivative **8** and 2,6-dichloropurine derivative **9**, respectively (Scheme 2). During condensation reaction, the initially formed N-3 isomer was smoothly converted to the N-9 isomer on refluxing. Removals of the isopropylidene groups in **8** and **9** under acidic conditions afforded **10** and **11**, respectively.

Treatment of **10** with methylamine and 3-halobenzyl amines yielded the *N*⁶-methylamino derivative **4a** and the *N*⁶-(3-halobenzyl)amino derivatives **4b-e**. Similarly, compound **11**

was converted to the 2-chloro-*N*⁶-methylamino derivative **5a** and the 2-chloro-*N*⁶-(3-halobenzyl)amino derivatives **5b-e**.

Biological activity

Binding assays were carried out using adherent mammalian cells stably transfected with cDNA encoding the appropriate hARs (A₁ and A₃ in CHO cells and A_{2A} in HEK-293 cells) and standard radioligands (1 nM [³H]CCPA for A₁ AR, 10 nM [³H]CGS21680 for A_{2A} AR, and 0.5 nM [¹²⁵I]I-AB-MECA for A₃ AR).^{17,18} Table 1 shows that all synthesized compounds exhibited potent and selective binding affinity at the hA₃ AR, although they were less potent than the corresponding 4'-thio analogues. In general, the 2-Cl series showed higher binding affinity to the hA₃ AR and better selectivity to other subtypes (A₁ and A_{2A}) than the 2-H series. The similar trend was observed in the corresponding 4'-thio analogues. Within the 2-Cl series, compound **5d** (R = 3-bromobenzyl) showed the highest binding affinity (*K*_i = 13 nM) at the hA₃ AR with high selectivity to other subtypes, A₁ AR and A_{2A} AR. The magnitude of the binding affinity in the 3-halobenzyl series is decreased in the following order: Br-Bn > I-Bn > Cl-Bn > F-Bn, indicating that the proper size of halogen is essential for optimal hydrophobic interaction at the hA₃ AR. The same trend was observed in the 2-H series.

One representative truncated 4'-oxo analogue was chosen for testing in a functional assay consisting of 10 μM forskolin-stimulated cyclic AMP production in CHO cells stably expressing the hA₃ AR. Compound **5d** was shown to be an antagonist of the hA₃ AR in this assay and did not activate the hA₃ AR. Figure 1 shows that the concentration response curve for inhibition of adenylate cyclase by the full agonist Cl-IB-MECA **1** was right shifted by 300 nM **5d**. The EC₅₀ value of **1** shifted from 1.14 to 11.9 nM in the presence of the antagonist. Functional efficacy of these truncated derivatives at the A₃ AR in rat and other species remains to be determined.

Conclusions

On the basis of a bioisosteric rationale, novel truncated adenosine derivatives were designed and synthesized as potent and selective hA₃ AR antagonists. All the synthesized nucleosides showed high binding affinity at the hA₃ AR with high selectivity to the hA₁ and hA_{2A} ARs. Among compounds tested, compound **5d** (X = Cl, R = 3-bromobenzyl) showed the highest binding affinity (*K*_i = 13.0 ± 6.9 nM) at the hA₃ AR with high selectivity (at least 1000-fold) in comparison to other AR subtypes. Like the corresponding truncated 4'-thio analogue series, compound **5d** was shown to be an antagonist of the hA₃ AR in an assay of adenylate cyclase. Although the truncated 4'-oxo series showed lower binding affinity at the hA₃ AR than the corresponding 4'-thio series, this class of potent hA₃ AR antagonists is also regarded as another good template for the design of A₃ AR antagonists and for further drug development.

Experimental Section

General methods

Melting points are uncorrected. ¹H NMR (400 MHz) and ¹³C NMR (100 MHz) spectra were measured in CDCl₃, CD₃OD or DMSO-*d*₆ 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

(3a*R*,4*R*,6a*R*)-2,2-Dimethyltetrahydrofuro[3,4-*d*][1,3]dioxol-4-ol (**6**)

To a stirred suspension of 2,3-*O*-isopropylidene- β -erythrone (8.0 g, 50.58 mmol) in toluene (150 mL) was added DIBAL (61.1 mL, 61.1 mmol, 1 M solution in toluene) at -78 °C and the reaction mixture was stirred at the same temperature for 30 min and then quenched by the slow addition of methanol. The colloidal suspension was filtered on a Celite pad and washed with EtOAc. The combined organic layers were dried over MgSO₄, filtered, and evaporated. The residue was purified by flash silica gel column chromatography (hexane:EtOAc = 3:1) to give **6** (7.20 g, 89%) as a syrup, whose spectral data were identical with those of authentic sample¹⁶.

(3a*R*,4*R*,6a*R*)-2,2-Dimethyltetrahydrofuro[3,4-*d*][1,3]dioxol-4-yl-acetate (**7**)

To a stirred solution of **6**¹⁶ (6.2 g, 38.7 mmol) in pyridine (30 mL) was added acetic anhydride (4.39 mL, 46.44 mmol) at 0 °C and the reaction mixture was stirred for 3 h at rt and evaporated. The residue was dissolved in EtOAc and washed with water. The organic layer was dried over MgSO₄ and evaporated. The residue was purified by flash silica gel column chromatography (hexane:EtOAc = 8:1) to give **7** (6.4 g, 82%) as a syrup: ¹H NMR (CDCl₃) δ 6.16 (s, 1 H), 4.86 (dd, 1 H, *J* = 3.6, 6.0 Hz), 4.66 (d, 1 H, *J* = 6.0 Hz), 4.12 (d, 1 H, *J* = 6.4 Hz), 3.99 (dd, 1 H, *J* = 3.6, 10.8 Hz), 2.05 (s, 3 H), 1.48 (s, 3 H), 1.33 (s, 3 H).

6-Chloro-9-((3a*R*,4*R*,6a*S*)-2,2-dimethyltetrahydrothieno[3,4-*d*][1,3]dioxol-4-yl)-9*H*-purine (**8**)

6-Chloropurine (2.29 g, 14.84 mmol), ammonium sulfate (98 mg, 0.742 mmol), and HMDS (25 mL) were refluxed overnight under inert and dry condition. The solution was evaporated under high vacuum. The resulting solid was re-dissolved in 1,2-dichloroethane (30 mL) and cooled in ice. To this mixture, a solution of **7** (1.5 g, 7.42 mmol) in 1,2-dichloroethane (10 mL) and TMSOTf (2.69 mL, 14.84 mmol) were successively added and the mixture was stirred at 0 °C for 30 min, at rt for 1 h, and then heated at 80 °C for 2 h. The mixture was cooled, diluted with CH₂Cl₂ and washed with saturated NaHCO₃ solution. The organic layer was dried over MgSO₄ and evaporated. The yellowish syrup was purified by a flash silica gel column chromatography (CH₂Cl₂:MeOH = 50:1) to give **8** (1.5 g, 68%) as a white solid: mp 118.5-119.2 °C; UV (MeOH) λ_{max} 264.0 nm; ¹H NMR (CD₃OD) δ 8.73 (s, 1 H), 8.62 (s, 1 H), 6.27 (s, 1 H), 5.52 (d, 1 H, *J* = 5.6 Hz), 5.31 (dd, 1 H, *J* = 3.6, 6.0 Hz), 4.27 (dd, 1 H, *J* = 3.6, 10.4 Hz), 4.19 (d, 1 H, *J* = 10.4 Hz), 1.54 (s, 3 H), 1.38 (s, 3 H); ¹³C NMR (CD₃OD) δ 153.2, 152.8, 151.7, 147.9, 132.9, 114.3, 93.1, 85.9, 82.8, 77.0, 26.8, 25.1; [α]_D²⁵ -45.04 (*c* 0.333, DMSO); FAB-MS *m/z* 297 [M+H]⁺. Anal. Calcd for C₁₂H₁₃ClN₄O₃: C, 48.58; H, 4.42; N, 18.88. Found: C, 49.26; H, 4.61; N, 18.70.

2,6-Dichloro-9-((3a*R*,6*R*,6a*R*)-2,2-dimethyltetrahydrofuro[3,4-*d*][1,3]dioxol-6-yl)-9*H*-purine (**9**)

Compound **7** (702 mg, 3.47 mmol) was condensed with 2,6-dichloropurine to give compound **9** (793 mg, 69%) as a foam according to the similar procedure used for the preparation of **8**: UV (MeOH) λ_{max} 276.5 nm; ¹H NMR (CDCl₃) δ 8.15 (s, 1 H), 6.07 (s, 1 H), 5.41 (d, 1 H, *J* = 6.0 Hz), 5.29-5.26 (m, 1 H), 4.31-4.25 (m, 2 H), 1.57 (s, 3 H), 1.41 (s, 3 H); [α]_D²⁵ -21.00 (*c* 0.10, DMSO); FAB-MS *m/z* 331 [M+H]⁺. Anal. Calcd for C₁₂H₁₂Cl₂N₄O₃: C, 43.52; H, 3.65; N, 16.92. Found: C, 43.08; H, 3.61; N, 16.70.

(2*R*,3*R*,4*S*)-2-(6-Chloro-9*H*-purin-9-yl)-tetrahydrothiophene-3,4-diol (**10**)

To a solution of **8** (300 mg, 1.01 mmol) in THF (5 mL) was added 2 *N*hydrochloric acid (2.5 mL) and the mixture was stirred at rt overnight. The mixture was neutralized with 1 *N* NaOH solution, and then carefully evaporated. The mixture was purified by a flash silica gel

column chromatography (CH₂Cl₂:MeOH = 20:1) to give **10** (165 mg, 64%) as a white solid: mp 165.5-166.7 °C; UV (MeOH) $\lambda_{\max}$ 264.0 nm; $\lambda_{\max}$ ¹H NMR (DMSO-*d*₆) δ 8.95 (s, 1 H), 8.82 (s, 1 H), 6.01 (d, 1 H, *J* = 6.4 Hz), 5.54 (d, 1 H, *J* = 6.4 Hz), 5.29 (d, 1 H, *J* = 4 Hz), 4.83 (ddd, 1 H, *J* = 4.8, 6.4, 10.8 Hz), 4.41 (dd, 1 H, *J* = 3.2, 9.2 Hz), 4.33-4.29 (m, 1 H), 3.86 (dd, 1 H, *J* = 1.6, 9.2 Hz); ¹³C NMR (DMSO-*d*₆) δ 151.8, 151.7, 149.4, 146.5, 131.5, 88.4, 74.6, 74.1, 70.3; [α]^{25.6}_D -95.71 (*c* 0.14, DMSO); FAB-MS *m/z* 257 [M+H]⁺. Anal. Calcd for C₉H₉ClN₄O₃: C, 42.12; H, 3.53; N, 21.83. Found: C, 42.17; H, 3.83; N, 20.43.

(2*R*,3*R*,4*R*)-2-(2,6-Dichloro-9*H*-purin-9-yl)tetrahydrofuran-3,4-diol (11)

Compound **9** (683 mg, 2.06 mmol) was converted to **11** (344 mg, 57%) as a white solid according to the similar procedure used for the preparation of **10**: mp 122.7-123.4 °C; UV (MeOH) $\lambda_{\max}$ 276.5 nm; ¹H NMR (DMSO-*d*₆) δ 8.98 (s, 1 H), 5.96 (d, 1 H, *J* = 6.4 Hz), 5.57 (d, 1 H, *J* = 6.0 Hz), 5.32 (d, 1 H, *J* = 4.0 Hz), 4.74-4.69 (m, 1 H), 4.41 (dd, 1 H, *J* = 3.6, 9.2 Hz), 4.32-4.29 (m, 1 H), 3.87 (dd, 1 H, *J* = 2.0, 9.6 Hz); ¹³C NMR (DMSO-*d*₆) δ 153.2, 151.2, 150.04, 147.1, 131.2, 88.4, 74.8, 74.1, 70.1; [α]²⁵_D -68.09 (*c* 0.14, DMSO); FAB-MS *m/z* 291 [M+H]⁺. Anal. Calcd for C₉H₈Cl₂N₄O₃: C, 37.13; H, 2.77; N, 19.25. Found: C, 37.23; H, 3.11; N, 19.45.

General procedure for the synthesis of 4a-e and 5a-e

To a solution of **10** and **11** in EtOH (5 mL) was added appropriate amines (1.5 equiv) at room temperature and the mixture was stirred at room temperature for 1 d to 3 d and evaporated. The residue was purified by a flash silica gel column chromatography (CH₂Cl₂:MeOH = 20:1) to give **4a-e** and **5a-e**, respectively.

(2*R*,3*R*,4*R*)-Tetrahydro-2-(6-(methylamino)-9*H*-purin-9-yl)-furan-3,4-diol (4a)

73% yield; white solid; mp 208.5-209.0 °C; UV (MeOH) $\lambda_{\max}$ 265.0 nm; ¹H NMR (DMSO-*d*₆) δ 8.33 (s, 1 H), 8.23 (s, 1 H), 7.73 (brs, 1 H), 5.85 (d, 1 H, *J* = 6.4 Hz), 5.43 (d, 1 H, *J* = 6.4 Hz), 5.19 (d, 1 H, *J* = 4.0 Hz), 4.87-4.82 (m, 1 H), 3.34 (dd, 1 H, *J* = 4.0, 9.6 Hz), 4.27-4.25 (m, 1 H), 3.79 (dd, 1 H, *J* = 1.2, 9.2 Hz), 2.95 (brs, 3 H); ¹³C NMR (DMSO-*d*₆) δ 155.0, 152.6, 148.5, 139.9, 119.9, 87.7, 74.2, 73.6, 70.3, 26.9; [α]^{25.8}_D -115.45 (*c* 0.11, DMSO); FAB-MS *m/z* 252 [M+H]⁺. Anal. Calcd for C₁₀H₁₃N₅O₃: C, 47.81; H, 5.22; N, 27.87. Found: C, 47.50; H, 5.55; N, 27.66.

(2*R*,3*R*,4*R*)-2-(6-(3-Fluorobenzylamino)-9*H*-purin-9-yl)tetrahydrofuran-3,4-diol (4b)

80% yield; white solid; mp 196.3-197.0 °C; UV (MeOH) $\lambda_{\max}$ 268.0 nm; ¹H NMR (DMSO-*d*₆) δ 8.42 (brs, 1 H), 8.39 (s, 1 H), 8.21 (s, 1 H), 7.36-7.30 (m, 1 H), 7.18-7.11 (m, 2 H), 7.03 (dt, 1 H, *J* = 2.0, 8.4 Hz), 5.86 (d, 1 H, *J* = 6.4 Hz), 5.43 (d, 1 H, *J* = 6.4 Hz), 5.18 (d, 1 H, *J* = 4.0 Hz), 4.88-4.83 (m, 1 H), 4.71 (brs, 2 H), 4.34 (dd, 1 H, *J* = 3.6, 9.2 Hz), 4.28-4.25 (m, 1 H), 3.79 (dd, 1 H, *J* = 1.6, 9.2 Hz); ¹³C NMR (DMSO-*d*₆) δ 163.4, 160.9, 154.3, 152.5, 148.9, 143.2, 140.3, 130.13 (d, *J* = 31.6 Hz), 123.1 (d, *J* = 8.0 Hz), 119.8, 113.5 (dd, *J* = 82.4, 143.6 Hz), 87.8, 74.2, 73.7, 70.3, 42.5; [α]^{25.7}_D -96.52 (*c* 0.115, DMSO); FAB-MS *m/z* 346 [M+H]⁺. Anal. Calcd for C₁₆H₁₆FN₅O₃: C, 55.65; H, 4.67; N, 20.28. Found: C, 55.29; H, 4.75; N, 19.93.

(2*R*,3*R*,4*R*)-2-(6-(3-Chlorobenzylamino)-9*H*-purin-9-yl)-tetrahydrofuran-3,4-diol (4c)

83% yield; white solid; mp 187.0-187.7 °C; UV (MeOH) $\lambda_{\max}$ 267.0 nm; ¹H NMR (DMSO-*d*₆) δ 8.43 (brs, 1 H), 8.39 (s, 1 H), 8.22 (s, 1 H), 7.38 (s, 1 H), 7.34-7.24 (m, 3 H), 5.85 (d, 1 H, *J* = 6.4 Hz), 5.44 (d, 1 H, *J* = 6.8 Hz), 5.20 (d, 1 H, *J* = 3.6 Hz), 4.85 (dd, 1 H, *J* = 6.4, 10.8 Hz), 4.70 (s, 2 H), 4.35 (dd, 1 H, *J* = 3.6, 9.2 Hz), 4.28-4.25 (m, 1 H), 3.80 (dd, 1 H, *J* = 1.6, 9.2 Hz); ¹³C NMR (DMSO-*d*₆) δ 154.3, 152.5, 148.9, 142.8, 140.4, 132.9, 130.2, 126.9, 126.6, 125.8, 119.8, 87.8, 74.3, 73.7, 70.4, 42.4; [α]^{25.8}_D -92.92 (*c* 0.099, DMSO);

FAB-MS m/z 362 $[M+H]^+$. Anal. Calcd for $C_{16}H_{16}ClN_5O_3$: C, 53.12; H, 4.46; N, 19.36. Found: C, 53.12; H, 4.57; N, 18.96.

(2R,3R,4R)-2-(6-(3-Bromobenzylamino)-9H-purin-9-yl)-tetrahydrofuran-3,4-diol (4d)

86% yield; white solid; mp 174.0-174.9 °C; UV (MeOH) λ_{max} 267.0 nm; 1H NMR (DMSO- d_6) δ 8.44 (brs, 1 H), 8.39 (s, 1 H), 8.21 (s, 1 H), 7.52 (s, 1 H), 7.42 (dt, 1 H, J = 2.0, 8.8 Hz), 7.36 (d, 1 H, J = 8.0 Hz), 7.25 (d, 1 H, J = 8.0 Hz), 5.86 (d, 1 H, J = 6.4 Hz), 5.43 (d, 1 H, J = 6.4 Hz), 5.19 (d, 1 H, J = 3.6 Hz), 4.88-4.82 (m, 1 H), 4.69 (brs, 2 H), 4.35 (dd, 1 H, J = 3.6, 9.2 Hz), 4.28-4.25 (m, 1 H), 3.80 (dd, 1 H, J = 1.6, 9.6 Hz); ^{13}C NMR (DMSO- d_6) δ 154.3, 152.5, 148.9, 143.0, 140.4, 130.4, 129.8, 129.5, 126.2, 121.5, 119.8, 87.8, 74.2, 73.7, 70.3, 42.3; $[\alpha]^{23.3}_D$ -88.00 (c 0.15, DMSO); FAB-MS m/z 406 $[M]^+$. Anal. Calcd for $C_{16}H_{16}BrN_5O_3$: C, 47.31; H, 3.97; N, 17.24. Found: C, 47.30; H, 3.94; N, 17.47.

(2R,3R,4R)-2-(6-(3-Iodobenzylamino)-9H-purin-9-yl)-tetrahydrofuran-3,4-diol (4e)

77% yield; white solid; mp 202.8-203.5 °C; UV (MeOH) λ_{max} 267.0 nm; 1H NMR (DMSO- d_6) δ 8.42 (brs, 1 H), 8.39 (s, 1 H), 8.21 (s, 1 H), 7.71 (s, 1 H), 7.57 (dt, 1 H, J = 2.8, 8.0 Hz), 7.35 (d, 1 H, J = 7.6 Hz), 7.10 (d, 1 H, J = 7.6 Hz), 5.86 (d, 1 H, J = 6.4 Hz), 5.44 (d, 1 H, J = 6.4 Hz), 5.19 (d, 1 H, J = 3.6 Hz), 4.88-4.83 (m, 1 H), 4.66 (brs, 2 H), 3.35 (dd, 1 H, J = 3.6, 9.2 Hz), 4.27-4.25 (m, 1 H), 3.80 (dd, 1 H, J = 1.6, 9.6 Hz); ^{13}C NMR (DMSO- d_6) δ 154.3, 152.5, 148.9, 142.9, 140.37, 135.7, 135.3, 130.5, 126.6, 119.8, 94.7, 87.8, 74.2, 73.7, 70.3, 42.2; $[\alpha]^{25.6}_D$ -67.74 (c 0.124, DMSO); FAB-MS m/z 454 $[M+H]^+$. Anal. Calcd for $C_{16}H_{16}IN_5O_3$: C, 42.40; H, 3.56; N, 15.45. Found: C, 42.44; H, 3.59; N, 15.22.

(2R,3R,4R)-2-(2-Chloro-6-(methylamino)-9H-purin-9-yl)-furan-3,4-diol (5a)

85% yield; white solid; mp 247 °C (dec); UV (MeOH) λ_{max} 269.0 nm; 1H NMR (DMSO- d_6) δ 8.38 (s, 1 H), 8.25 (q, 1 H, J = 4.4 Hz), 5.79 (d, 1 H, J = 6.8 Hz), 5.48 (d, 1 H, J = 6.4 Hz), 5.22 (d, 1 H, J = 4.0 Hz), 4.73-4.69 (m, 1 H), 4.30 (dd, 1 H, J = 3.6, 9.2 Hz), 4.25-4.23 (m, 1 H), 3.79 (dd, 1 H, J = 1.6, 9.2 Hz), 2.92 (d, 3 H, J = 4.4 Hz); ^{13}C NMR (DMSO- d_6) δ 155.5, 153.3, 149.4, 140.2, 118.8, 87.4, 74.4, 73.6, 70.2, 27.1; $[\alpha]^{23.5}_D$ -40.77 (c 0.13, DMSO); FAB-MS m/z 286 $[M+H]^+$. Anal. Calcd for $C_{10}H_{12}ClN_5O_3$: C, 42.04; H, 4.23; N, 24.51. Found: C, 42.38; H, 4.14; N, 24.27.

(2R,3R,4R)-2-(2-Chloro-6-(3-fluorobenzylamino)-9H-purin-9-yl)-tetrahydrofuran-3,4-diol (5b)

83% yield; white solid; mp 187.0-187.9 °C; UV (MeOH) λ_{max} 271.0 nm; 1H NMR (DMSO- d_6) δ 8.92 (t, 1 H, J = 6.0 Hz), 8.43 (s, 1 H), 7.39-7.33 (m, 1 H), 7.18-7.13 (m, 2 H), 7.06 (dt, 1 H, J = 2.0, 8.4 Hz), 5.81 (d, 1 H, J = 6.8 Hz), 5.47 (d, 1 H, J = 6.0 Hz), 5.22 (d, 1 H, J = 4.0 Hz), 4.74-4.64 (m, 3 H), 4.41 (dd, 1 H, J = 3.6, 9.2 Hz), 4.25 (brs, 1 H), 3.80 (dd, 1 H, J = 1.2, 9.6 Hz); ^{13}C NMR (DMSO- d_6) δ 163.4, 163.9, 154.9, 153.1, 149.9, 142.2 (d, J = 26.8 Hz), 140.7, 130.3 (d, J = 34.8 Hz), 123.2, 118.8, 113.8 (dd, J = 82.4, 122.0 Hz), 87.5, 74.4, 73.7, 70.2, 42.7; $[\alpha]^{23.2}_D$ -73.40 (c 0.094, DMSO); FAB-MS m/z 380 $[M+H]^+$. Anal. Calcd for $C_{16}H_{15}ClFN_5O_3$: C, 50.60; H, 3.98; N, 18.44. Found: C, 50.30; H, 4.01; N, 18.04.

(2R,3R,4R)-2-(2-Chloro-6-(3-chlorobenzylamino)-9H-purin-9-yl)-tetrahydrofuran-3,4-diol (5c)

76% yield; white solid; mp 196.4-197.0 °C; UV (MeOH) λ_{max} 271.5 nm; 1H NMR (DMSO- d_6) δ 8.92 (t, 1 H, J = 6.0 Hz), 8.43 (s, 1 H), 7.39 (s, 1 H), 7.37-7.28 (m, 3 H), 5.80 (d, 1 H, J = 6.8 Hz), 5.47 (d, 1 H, J = 6.4 Hz), 5.22 (d, 1 H, J = 4.0 Hz), 4.73-4.64 (m, 3 H), 4.32 (dd, 1 H, J = 3.6, 9.2 Hz), 4.24 (brs, 1 H), 3.79 (dd, 1 H, J = 1.6, 9.2 Hz); ^{13}C NMR (DMSO- d_6) δ 154.8, 153.1, 149.9, 141.8, 140.7, 132.9, 130.2, 127.1, 126.8, 125.9, 118.7, 87.5, 74.4, 73.7, 70.2, 42.7; $[\alpha]^{22.9}_D$ -78.19 (c 0.133, DMSO); FAB-MS m/z 396 $[M]^+$.

Anal. Calcd for $C_{16}H_{15}Cl_2N_5O_3$: C, 48.50; H, 3.82; N, 17.68. Found: C, 48.56; H, 3.89; N, 17.12.

(2*R*,3*R*,4*R*)-2-(2-Chloro-6-(3-bromobenzylamino)-9*H*-purin-9-yl)-tetrahydrofuran-3,4-diol (5d)

81% yield; white solid; mp 181.5–181.7 °C; UV (MeOH) $\lambda_{\max}$ 274.5 nm; 1H NMR (DMSO- d_6) δ 8.92 (t, 1 H, J = 6.0 Hz), 8.43 (s, 1 H), 7.55 (s, 1 H), 7.44 (d, 1 H, J = 8.0 Hz), 7.35–7.33 (m, 1 H), 7.30–7.26 (m, 1 H), 5.81 (d, 1 H, J = 6.4 Hz), 5.47 (d, 1 H, J = 6.4 Hz), 5.22 (d, 1 H, J = 4.0 Hz), 4.69–4.66 (m, 1 H), 4.62 (s, 2 H), 4.32 (dd, 1 H, J = 3.6, 9.2 Hz), 4.25 (brs, 1 H), 3.80 (dd, 1 H, J = 1.6, 9.2 Hz); ^{13}C NMR (DMSO- d_6) δ 154.9, 153.2, 149.9, 142.1, 140.7, 130.6, 130.1, 129.8, 126.4, 121.6, 118.8, 87.5, 74.4, 73.7, 70.2, 42.6; $[\alpha]^{25}_D$ -62.75 (c 0.10, DMSO); FAB-MS m/z 440 $[M+H]^+$. Anal. Calcd for $C_{16}H_{15}ClBrN_5O_3$: C, 43.61; H, 3.43; N, 15.89. Found: C, 43.92; H, 3.44; N, 16.05.

(2*R*,3*R*,4*R*)-2-(2-Chloro-6-(3-iodobenzylamino)-9*H*-purin-9-yl)-tetrahydrofuran-3,4-diol (5e)

78% yield; white solid; mp 195.5–195.8 °C; UV (MeOH) $\lambda_{\max}$ 274.0 nm; 1H NMR (DMSO- d_6) δ 8.91 (t, 1 H, J = 6.4 Hz), 8.44 (s, 1 H), 7.75 (s, 1 H), 7.61 (d, 1 H, J = 8.0 Hz), 7.36 (d, 1 H, J = 7.6 Hz), 7.13 (t, 1 H, J = 4.0 Hz), 5.81 (d, 1 H, J = 6.8 Hz), 5.47 (d, 1 H, J = 6.8 Hz), 5.23 (d, 1 H, J = 4.0 Hz), 4.72 (dd, 1 H, J = 6.4, 10.8 Hz), 4.61 (d, 1 H, J = 6.0 Hz), 4.34 (dd, 1 H, J = 3.6, 9.2 Hz), 3.81 (dd, 1 H, J = 1.2, 9.2 Hz); ^{13}C NMR (DMSO- d_6) δ 154.8, 153.2, 149.9, 141.9, 140.7, 136.0, 135.6, 130.6, 126.8, 118.8, 94.8, 87.5, 74.4, 73.7, 70.2, 42.5; $[\alpha]^{25}_D$ -68.07 (c 0.12, DMSO); FAB-MS m/z 488 $[M+H]^+$. Anal. Calcd for $C_{16}H_{15}ClIN_5O_3$: C, 39.41; H, 3.10; N, 14.36. Found: C, 39.66; H, 3.08; N, 14.53.

Pharmacological Methods

$[^{125}I]N^6$ -(4-Amino-3-iodobenzyl)adenosine-5'-*N*-methyluronamide (I-AB-MECA; 2200 Ci/mmol), $[^3H]$ cyclic AMP (40 Ci/mmol), and $[^3H]$ CGS21680 (2-[p-(2-carboxyethyl)phenylethylamino]-5'-*N*-ethylcarboxamido-adenosine) (40.5 Ci/mmol) were purchased from Perkin–Elmer Life and Analytical Science (Boston, MA). $[^3H]$ CCPA (2-chloro- N^6 -cyclopentyladenosine) (42.6 Ci/mmol) was a custom synthesis product (Perkin Elmer). Test compounds were prepared as 5 mM stock solutions in DMSO and stored frozen.

Cell Culture and Membrane Preparation

CHO (Chinese hamster ovary) cells stably expressing the recombinant human A_1 and A_3 , and HEK-293 (Human Embryonic Kidney) cells stably expressing human A_{2A} were cultured in Dulbecco's modified Eagle medium (DMEM) and F12 (1:1) supplemented with 10% fetal bovine serum, 100 units/mL penicillin, 100 μ g/mL streptomycin, and 2 μ mol/mL glutamine. In addition, 800 μ g/mL geneticin was added to the A_{2A} media, while 800 μ g/mL hygromycin was added to the A_1 and A_3 media. After harvesting, cells were homogenized and suspended in PBS. Cells were then centrifuged at 240 g for 5 min, and the pellet was resuspended in 50 mM Tris-HCl buffer (pH 7.5) containing 10 mM $MgCl_2$. The suspension was homogenized and was then ultra-centrifuged at 14,330 g for 30 min at 4 °C. The resultant pellets were resuspended in Tris buffer, incubated with adenosine deaminase (3 units/mL) for 30 min at 37 °C. The suspension was homogenized with an electric homogenizer for 10 sec, pipet into 1 mL vials and then stored at -80 °C until the binding experiments. The protein concentration was measured using the BCA Protein Assay Kit from Pierce Biotechnology, Inc. (Rockford, 1000 mL).¹⁹

Binding assays

Into each tube in the binding assay was added 50 μ L of increasing concentrations of the test ligand in Tris-HCl buffer (50 mM, pH 7.5) containing 10 mM $MgCl_2$, 50 μ L of the appropriate agonist radioligand, and finally 100 μ L of membrane suspension. For A_1 receptors (22 μ g of protein/tube) the radioligand used was [3H]CCPA (final concentration of 1.0 nM). For A_{2A} receptors (20 μ g/tube) the radioligand used was [3H]CGS21680 (10 nM). For A_3 receptors (21 μ g/tube) the radioligand used was ^{125}I -AB-MECA (0.34 nM). Nonspecific binding was determined using a final concentration of 10 μ M 5'-*N*-ethylcarboxamidoadenosine (NECA) diluted with the buffer. The mixtures were incubated at 25 °C for 60 min in a shaking water bath. Binding reactions were terminated by filtration through Brandel GF/B filters under a reduced pressure using a M-24 cell harvester (Brandel, Gaithersburg, MD). Filters were washed three times with 3 mL of 50 mM ice-cold Tris-HCl buffer (pH 7.5). Filters for A_1 and A_{2A} AR binding were placed in scintillation vials containing 5 mL of Hydrofluor scintillation buffer and counted using a Perkin Elmer Liquid Scintillation Analyzer (Tri-Carb 2810TR). Filters for A_3 AR binding were counted using a Packard Cobra II γ -counter. The K_i values were determined using GraphPad Prism for all assays.

Cyclic AMP accumulation assay

Intracellular cyclic AMP levels were measured with a competitive protein binding method.²⁰ CHO cells that expressed the recombinant hA_3AR were harvested by trypsinization. After centrifugation and resuspended 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 DMEM, containing 50 mM HEPES, pH 7.4. Cells were then treated with the agonist NECA and/or test compound (e.g. **5d**) in the presence of rolipram (10 μ M) and adenosine deaminase (3 units/mL). After 30 min forskolin (10 μ M) 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 μ L of 0.1 M ice-cold HCl. The cell lysate was resuspended and stored at -20 °C. For determination of cyclic AMP production, a cyclic AMP kit (Sigma) was used.

Statistical analysis

Binding and functional parameters were calculated using Prism 4.0 software (GraphPAD, San Diego, CA, USA). IC_{50} values obtained from competition curves were converted to K_i values using the Cheng-Prusoff equation.²¹ Data were expressed as mean $\pm$ standard error.

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References

1. Olah ME, Stiles GL. Pharmacol. Ther. 2000; 85:55–75. [PubMed: 10722120]
2. Jacobson KA, Gao Z-G. Nature Rev. Drug Disc. 2006; 5:247–264.
3. a Fishman P, Madi L, Bar-Yehuda S, Barer F, Del Valle L, Khalili K. Oncogene. 2002; 21:4060–4064. [PubMed: 12037688] b Lee EJ, Min HY, Chung HJ, Park EJ, Shin DH, Jeong LS, Lee SK. Biochem. Pharmacol. 2005; 70:918–924. [PubMed: 16051194] c Chung H, Jung J-Y, Cho S-D, Hong K-A, Kim H-J, Shin D-H, Kim H, Kim HO, Lee HW, Jeong LS, Gong K. Mol. Cancer Ther. 2006; 5:685–692. [PubMed: 16546983] d Kim SJ, Min HYH, Chung J, Park EJ, Hong JY, Kang YJ, Shin DH, Jeong LS, Lee SK. Cancer Lett. 2008; 264:309–315. [PubMed: 18321638]

4. von Lubitz DKJE, Ye W, McClellan J, Lin RC-S. *Ann. N Y Acad. Sci.* 1999; 890:93–106. [PubMed: 10668416]
5. Liang BT, Jacobson KA. *Proc. Natl. Acad. Sci.* 1998; 95:6995–6999. [PubMed: 9618527]
6. Sajjadi FG, Takabayashi K, Foster AC, Domingo RC, Firestein GS. *J. Immunol.* 1996; 156:3435–3442. [PubMed: 8617970]
7. Meade CJ, Mierau J, Leon I, Ensinger HA. *J. Pharmacol. Exp. Ther.* 1996; 279:1148–1156. [PubMed: 8968336]
8. Avila MY, Stone RA, Civan MM. *Invest. Ophthalmol. Visual Sci.* 2002; 43:3021–3026.
9. Klotz K-N. *Naunyn-Schmiedeberg's Arch. Pharmacol.* 2000; 362:382–391. [PubMed: 11111832]
10. Baraldi PG, Cacciari B, Romagnoli R, Merighi S, Varani K, Borea PA, Spalluto G. *Med. Res. Rev.* 2000; 20:103–128. [PubMed: 10723024]
11. Kim HO, Ji X.-d. Siddiqi SM, Olah ME, Stiles GL, Jacobson KA. *J. Med. Chem.* 1994; 37:3614–3621. [PubMed: 7932588]
12. a Jeong LS, Jin DZ, Kim HO, Shin DH, Moon HR, Gunaga P, Chun MW, Kim Y-C, Melman N, Gao Z-G, Jacobson KA. *J. Med. Chem.* 2003; 46:3775–3777. [PubMed: 12930138] b Jeong LS, Lee HW, Jacobson KA, Kim HO, Shin DH, Lee JA, Gao Z-G, Lu C, Duong HT, Gunaga P, Lee SK, Jin DZ, Chun MW, Moon HR. *J. Med. Chem.* 2006; 49:273–281. [PubMed: 16392812] c Jeong LS, Lee HW, Kim HO, Jung JY, Gao Z-G, Duong HT, Rao S, Jacobson KA, Shin DH, Lee JA, Gunaga P, Lee SK, Jin DZ, Chun MW. *Bioorg. Med. Chem.* 2006; 14:4718–4730. [PubMed: 16603368]
13. Baraldi PG, Tabrizi MA, Romagnoli R, Fruttarolo F, Merighi S, Varani K, Gessi S, Borea PA. *Curr. Med. Chem.* 2005; 12:1319–1329. [PubMed: 15974999]
14. a Gao ZG, Blaustein J, Gross AS, Melman N, Jacobson KA. *Biochem. Pharmacol.* 2003; 65:1675–1684. [PubMed: 12754103] b Yang H, Avila MY, Peterson-Yantorno K, Coca-Prados M, Stone RA, Jacobson KA, Civan MM. *Current Eye Res.* 2005; 30:747–754.
15. Jeong LS, Choe SA, Gunaga P, Kim HO, Lee HW, Lee SK, Tosh DK, Patel A, Palaniappan KK, Gao Z-G, Jacobson KA, Moon HR. *J. Med. Chem.* 2007; 50:3159–3162. [PubMed: 17555308]
16. Choi WJ, Park JG, Yoo SJ, Kim HO, Moon HR, Chun MW, Jung YH, Jeong LS. *J. Org. Chem.* 2001; 66:6490–6494. [PubMed: 11559205]
17. a Klotz K-N, Hessling J, Hegler J, Owman C, Kull B, Fredholm BB, Lohse MJ. *Naunyn-Schmiedeberg's Arch. Pharmacol.* 1998; 357:1–9. [PubMed: 9459566] b Gao ZG, Mamedova L, Chen P, Jacobson KA. *Biochem. Pharmacol.* 2004; 68:1985–1993. [PubMed: 15476669]
18. Kim YC, Ji X.-d. Melman N, Linden J, Jacobson KA. *J. Med. Chem.* 2000; 43:1165–1172. [PubMed: 10737749]
19. Walsh MK, Wang X, Weimer BC. *J. Biochem. Biophys. Methods.* 2001; 47:221–231. [PubMed: 11245893]
20. Nordstedt C, Fredholm BB. *Anal. Biochem.* 1990; 189:231–234. [PubMed: 2177960]
21. Cheng Y-C, Prusoff WH. *Biochem. Pharmacol.* 1973; 22:3099–3108. [PubMed: 4202581]

Chart 1^a

^aBinding affinity (A₁ AR, A_{2A} AR, or A₃ AR) at human adenosine receptors

Chart 1.

Reagents and conditions: a) DIBAL, toluene, -78 °C, 30 min; b) Ac₂O, pyridine, rt, 3 h.

Scheme 1.

Reagents and conditions: a) silylated 6-chloropurine or 2,6-dichloropurine, TMSOTf, 1,2-dichloroethane, rt to 80 °C; b) 2 N HCl, THF, rt, overnight; c) RNH₂, Et₃N, EtOH, rt, 1-3 days

Scheme 2.

Figure 1.
Right-shift of the concentration-response curves of the full agonist Cl-IB-MECA **1** by 300 nM of antagonist **5d**.

Table 1

Binding affinities of known A₃ AR agonists, **1** and **2** and antagonist **3**, and truncated 4'-adenosine derivatives **4a-e** and **5a-e** at three subtypes of hARs.

Compound	Affinity, K_i , nM $\pm$ SEM (or % inhibition) ^a		
	hA ₁	hA _{2A}	hA ₃
1 (Cl-IB-MECA)	220	5400	1.4
2 (thio-Cl-IB-MECA)	193	223	0.38
3 (X = Cl, Y = S, R = 3-iodobenzyl)	2490	341	4.16
4a (X = H, Y = O, R = methyl)	<10%	25 $\pm$ 5%	70.1 $\pm$ 18.3
4b (X = H, Y = O, R = 3-fluorobenzyl)	16 $\pm$ 2%	6560 $\pm$ 1560	537 $\pm$ 58
4c (X = H, Y = O, R = 3-chlorobenzyl)	35 $\pm$ 7%	3410 $\pm$ 400	76.1 $\pm$ 3.9
4d (X = H, Y = O, R = 3-bromobenzyl)	3300 $\pm$ 1480	1400 $\pm$ 120	59.0 $\pm$ 0.2
4e (X = H, Y = O, R = 3-iodobenzyl)	2080 $\pm$ 1160	997 $\pm$ 125	90.2 $\pm$ 39.8
5a (X = Cl, Y = O, R = methyl)	<10%	15 $\pm$ 5%	37.5 $\pm$ 5.0
5b (X = Cl, Y = O, R = 3-fluorobenzyl)	10%	42 $\pm$ 4%	284 $\pm$ 37
5c (X = Cl, Y = O, R = 3-chlorobenzyl)	36%	3630 $\pm$ 1080	75.0 $\pm$ 11.7
5d (X = Cl, Y = O, R = 3-bromobenzyl)	<10%	30%	13.0 $\pm$ 6.9
5e (X = Cl, Y = O, R = 3-iodobenzyl)	<10%	43%	42.9 $\pm$ 8.9

^aAll binding experiments were performed using adherent mammalian cells stably transfected with cDNA encoding the appropriate hAR (A₁ AR and A₃ AR in CHO cells and A_{2A} AR in HEK-293 cells). Binding was carried out using 1 nM [³H]CCPA, 10 nM [³H]CGS21680, or 0.5 nM [¹²⁵I]-AB-MECA as radioligands for A₁, A_{2A}, and A₃ ARs, respectively. Values are expressed as mean $\pm$ sem, n = 3-4 (outliers eliminated), and normalized against a non-specific binder, 5'-N-ethylcarboxamidoadenosine (NECA, 10 μ M). If a percentage is given, it represents the percent inhibition at a fixed concentration of 10 μ M.