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Stereoselective Synthesis of Truncated 3'-Aminoadenosine Derivatives and their Binding Affinity at the A₃ Adenosine Receptor

Mun Ju Choi¹, Goirish Chandra¹, Hyuk Woo Lee¹, Xiyan Hou¹, Won Jun Choi^{1,2}, Khai Phan³, Kenneth A. Jacobson³, and Lak Shin Jeong^{1,*}

¹Laboratory of Medicinal Chemistry, College of Pharmacy and Department of Bioinspired Science, Ewha Womans University, Seoul 120-750, Korea

²College of Pharmacy, Dongguk University, Kyungki-do, 410-774, Korea

³Molecular Recognition Section, Laboratory of Bioorganic Chemistry, National Institute of Diabetes, and Digestive and Kidney Disease, National Institutes of Health, Bethesda, Maryland 20892, USA

Abstract

The stereoselective synthesis of truncated 3'-aminocarbanucleosides **4a–d** via a stereo- and regioselective conversion of a diol **9** to bromoacetate **11a** and their binding affinity towards the human A₃ adenosine receptor are described.

Introduction

Adenosine binds to four subtypes (A₁, A_{2A}, A_{2B}, and A₃) of adenosine receptors (ARs) located on cell membranes and regulates many physiological functions through modulation of cell signaling.¹ Second messengers such as inositol trisphosphate (IP₃), diacylglycerol (DAG), and cyclic AMP, which are essential for cell signaling, are controlled by ARs, and thus ARs have been promising targets for the treatment of several diseases associated with these signaling pathways.² For example, A₃ AR agonists show the therapeutic potentials against cancer,³ cerebral ischemia,⁴ and myocardial ischemia,⁵ while A₃ AR antagonists show anti-inflammatory,⁶ anti-asthma,⁷ and anti-glaucoma activities.⁸

The methanocarba nucleosides **1**,⁹ whose conformation is fixed to the Northern (*N*) conformation were reported to be potent and selective A₃ AR agonists, but their truncated analogues **2**¹⁰ were transformed to potent and species-independent A₃ AR antagonists, due to the absence of the 5'-uronamide, whose NH serves as a key hydrogen bonding donor essential for receptor activation. On the other hand, 3'-deoxy-3'-aminoadenosine derivatives **3**¹¹ were also discovered as potent and selective A₃ AR agonists. The 3'-amino group of **3** improved the selectivity to other subtypes as well as the aqueous solubility. Based on these findings, we designed compounds **4**, which combine the properties of truncated nucleosides and 3'-amino nucleosides to develop potent and selective A₃ AR antagonists. Introduction of the 3'-amino group was achieved by the regioselective opening of the acylinium ion with bromide, followed by treating the bromide with sodium azide. Herein, we report the stereo-

*To whom correspondence should be addressed: Lak Shin Jeong, Ph.D., Laboratory of Medicinal Chemistry, College of Pharmacy and Department of Bioinspired Science, Ewha Womans University, Seoul 120-750, Korea, Tel) 82-2-3277-3466, Fax) 82-2-3277-2851, lakjeong@ewha.ac.kr.

and regioselective synthesis of truncated 3'-aminonucleosides **4**, starting from D-ribose, and their binding affinity at the human A₃ AR.

Results and discussion

The synthetic strategy towards the desired nucleosides was to synthesize the glycosyl donor and then condense with purine bases.

Scheme 1 illustrates the synthesis of the key precursor **9** for the introduction of a 3'-amino group. D-Ribose was easily converted to cyclopentenone **5** according to our previously published procedure.¹² Treatment of cyclopentenone **5** with NaBH₄ in the presence of CeCl₃·7H₂O gave α-allylic alcohol **6** as a single diastereomer, resulting from the attack of the hydride to the less hindered convex face of the ketone.¹³ The stereoselective cyclopropanation of α-allylic alcohol **6** with diethyl zinc and methylene iodide afforded cyclopropyl-fused alcohol **7** as a single diastereomer, due to the directing effect of the allylic hydroxyl group.^{13,14} Benzoylation of **7** followed by hydrolysis of the isopropylidene group in benzoate **8** with 50% TFA yielded diol **9**, which is a key precursor for the manipulation to azidation.

Treatment of diol **9** with 2-acetoxyisobutyryl bromide¹⁵ gave bromoacetate **11a** as a single regioisomer (75%) without the formation of another regioisomer **11b** (Scheme 2). In this reaction, after diol **9** was first converted to acylinium ion **10**, the bromide attacked the C2 position of **10** in the pseudoaxial direction (route a), which was more highly favored than the same reaction in the pseudoequatorial direction (route b), giving desired **11a** as an exclusive regioisomer. Two regioisomers **11a** and **11b** were unambiguously distinguished by the diagnostic coupling constants typical of the boat conformation of the bicyclo[3.1.0]hexane system in ¹H NMR spectroscopy.¹⁶ In case of compound **11a**, the coupling constant of the J_{H4,H3} should be more than zero because of the *cis* relationship, while in case of **11b**, the corresponding coupling constant should be zero, because a H₃-C-C-H₄ dihedral angle with *trans* relationships are close to 90°. Thus, H-4 in **11a** should be split to a pseudo triplet or doublet of doublets, but H-4 in **11b** should appear as a doublet. ¹H NMR spectroscopy of **11a** indicated that H-4 showed a pseudo triplet at 5.95 ppm, confirming the structure of **11a**.

The desired **11a** was treated with NaN₃ in DMF at 100 °C to give the azido derivative **12a** (44%), but another stereoisomer **12b** (22%) was also obtained as a minor isomer (Scheme 3). As illustrated in Scheme 3, the S_N2 reaction of the bromide **11a** with sodium azide yielded the desired azido compound **12a** as a major isomer, but under heating condition, **11a** also underwent intramolecular S_N2 reaction, forming the intermediate **10**, which was attacked by sodium azide at the C2 position as explained in Scheme 2 to give **12b** as a minor isomer. The structures of **12a** and **12b** were also confirmed by the diagnostic coupling constants typical of the boat conformation of the bicyclo[3.1.0]hexane system in ¹H NMR spectroscopy.¹⁶ In case of **12a**, H-2 was split as a pseudo triplet at 4.12 ppm because of two dihedral angles (H₁-C-C-H₂ and H₃-C-C-H₄) with *cis* relationships, while in case of **12b**, H-2 was split as a singlet at xx ppm because of two dihedral angles (H₁-C-C-H₂ and H₃-C-C-H₄) with *trans* relationships.¹⁶

Removal of the protecting groups of **12a** with NaOMe afforded diol **13** (Scheme 4). Treatment of diol **13** with thionyl chloride followed by oxidation of resulting cyclic sulfite **14** with RuCl₃ and NaIO₄ gave cyclic sulfate **15** as glycosyl donor.¹⁷

The glycosyl donor **15** was transformed to the 3'-deoxy-3'-aminoadenosine derivatives **4a-d**, as illustrated in Scheme 5. Condensation of **15** with 2,6-dichloropurine and 6-chloropurine gave azido derivatives **16** and **17**, respectively after acid-catalyzed hydrolysis of the

sulfates.¹⁸ Treatment of **16** and **17** with 3-iodobenzylamine produced **18a** and **18c**, respectively. Treatment of **16** and **17** with methylamine produced **18c** and **18d**, respectively. Conversion of the 3'-azido group to the 3'-amino group was achieved by treating **18a-d** with Ph_3P and NH_4OH in $\text{THF-H}_2\text{O}$ to give **4a-d**, respectively.¹¹

Binding assays were carried out using standard radioligands and membrane preparations from Chinese hamster ovary (CHO) cells stably expressing the human (h) A_1 or A_3AR or human embryonic kidney cells (HEK-293) expressing the $\text{hA}_{2\text{A}}\text{AR}$.¹⁹ As shown in Table 1, all synthesized 3'-amino derivatives displayed much lower binding affinities at the human A_3AR than the reference A_3AR antagonist **2**. This result was unexpected because the 3'-amino group can serve as the same hydrogen bonding donor as the 3'-hydroxyl group and because some 3'-amino nucleosides are reported to be potent A_3AR agonists.¹¹ Only compound **4c** showed moderate binding affinity at the human A_3AR .

In summary, we have completed the stereoselective synthesis of truncated 3'-aminocarbanucleosides, which are fixed in a (*N*) conformation and their binding affinities at human ARs. The key step for the introduction of the 3'-amino group was to convert diol **9** to bromoacetate **12a** via acylinium ion **11a** in stereoselective and regioselective manners. It was also found that azidation of bromoacetate **12a** with sodium azide proceeded via an acylinium ion **11a** in addition to a direct $\text{S}_{\text{N}}2$ reaction.

Experimental Section

General Methods

^1H and ^{13}C NMR Spectra (CDCl_3 or CD_3OD) were recorded on 400 and 100 MHz NMR, respectively. The ^1H NMR data are reported as peak multiplicities: s for singlet, d for doublet, dd for doublet of doublets, t for triplet, q for quartet, br s for broad singlet and m for multiplet. Coupling constants are reported in hertz. The chemical shifts are reported as parts per million (δ) relative to the solvent peak. Column chromatography was performed on silica gel 60 (230-400 mesh). All the anhydrous solvents were distilled over CaH_2 , P_2O_5 or sodium/benzophenone prior to the reaction.

(3a*S*,4*S*,6*R*)-2,2-Dimethyl-4,6a-dihydro-3aHcyclopenta[1,3]dioxol-4-ol (**6**)

To a stirred solution of **5** (15.00 g, 81.42 mmol) in methanol (150 mL), sodium borohydride (3.21 g, 84.97 mmol) and cerium (III) chloride heptahydrate (36.39 g, 97.68 mmol) were added at 0 °C, and the reaction mixture was stirred at 0 °C for 30 min. The reaction mixture was partitioned between ethyl acetate and water, and the organic layer was dried (MgSO_4), filtered, and evaporated. The residue was purified by silica gel column chromatography (hexane:ethyl acetate= 4:1) to give **6** (12.72 g, 99%) as a colorless syrup: MS (FAB) m/z 157 $[\text{M}+\text{H}]^+$; $[\alpha]_{\text{D}}^{25} +15.4^\circ$ (c 2.36, CHCl_3); IR (neat) 3489, 2989, 1058 cm^{-1} ; ^1H NMR (CDCl_3) δ 5.89 (s, 2 H), 5.02 (d, J = 5.2 Hz, 1 H), 4.75 (t, J = 5.2 Hz, 1 H), 4.56 (dd, J = 5.2, 10.0 Hz, 1 H), 2.72 (d, J = 10.0 Hz, 1 H), 1.44 (s, 3 H), 1.40 (s, 3 H); ^{13}C NMR (CDCl_3) δ 136.63, 132.13, 112.55, 83.81, 77.37, 74.38, 27.85, 26.78. Anal. Calcd for $\text{C}_8\text{H}_{12}\text{O}_3$: C, 61.52; H, 7.74. Found: C, 61.52; H, 7.75.

3,3-Dimethyl-hexahydro-2,4-dioxo-cyclopropa[a]pentalen-5-ol (**7**)

To a stirred solution of **6** (5.00 g, 32.03 mmol) in methylene chloride (68 mL) cooled in ice bath, diethylzinc (64 mL, 64.07 mmol, 1.0 M solution in hexane) and diiodomethane (10.34 mL, 128.14 mmol) were added, and the reaction mixture was stirred at room temperature for 3 h. The mixture was quenched with cold aqueous ammonium chloride solution and extracted with methylene chloride. The organic layer was dried (MgSO_4), filtered, and evaporated. The residue was purified by silica gel column chromatography (hexane:ethyl

acetate= 4:1) to give **7** (3.20 g, 63%) as a colorless syrup: MS (ESI+) m/z 170.0944 [M]⁺; [α]_D^{27.1} 4.27° (*c* 3.72, CHCl₃); ¹H NMR (CDCl₃) δ 0.56-0.62 (m, 1 H), 0.93 (dd, *J* = 5.2, 13.6 Hz, 1 H), 1.25 (s, 1 H), 1.51 (s, 3 H), 1.57-1.63 (m, 1 H), 1.78-1.84 (m, 1 H), 2.43 (d, *J* = 8.4 Hz, 1 H, D₂O exchangeable), 4.43 (t, *J* = 6.8, 13.6 Hz, 1 H), 4.45-4.51 (m, 1 H), 4.84 (t, *J* = 6, 11.6 Hz, 1 H); ¹³C NMR (CDCl₃) δ 6.69, 21.41, 24.72, 26.20, 28.31, 71.55, 79.24, 81.09, 112.99.

Benzoic acid 3,3-dimethyl-hexahydro-2,4-dioxo-cyclopropa[a]pentalen-5-yl ester (**8**)

To a stirred solution of **7** (3.16 g, 19.94 mmol) in pyridine (20 mL), benzoyl chloride (6.94 mL, 59.83 mmol) was added, and the reaction mixture was stirred at room temperature for 3.5 h. The mixture was quenched with water and extracted with ethyl acetate. The organic layer was dried (MgSO₄), filtered, and evaporated. The residue was purified by silica gel column chromatography (hexane:ethyl acetate= 3:1) to give **8** (5.51 g, 99%) as a colorless syrup: MS (ESI+) m/z 274.1198 [M]⁺; [α]_D^{26.8} 4.27° (*c* 4.37, CHCl₃); ¹H NMR (CDCl₃) δ 0.72-0.78 (m, 1 H), 1.24 (s, 3 H), 1.30 (dd, *J* = 4.0, 13.6 Hz, 1 H), 1.47 (s, 3 H), 1.67-1.73 (m, 1 H), 1.89-1.96 (m, 1 H), 4.76 (t, *J* = 6.4, 13.6 Hz, 1 H), 4.95 (t, *J* = 6.0, 12.0 Hz, 1 H), 4.63 (t, *J* = 6.4, 12.4 Hz, 1 H), 7.43 (t, *J* = 7.4, 15.2 Hz, 1 H), 7.54 (t, *J* = 7.6, 14.8 Hz, 1 H), 8.09 (d, *J* = 11.6 Hz, 1 H); ¹³C NMR (CDCl₃) δ 7.46, 21.16, 24.69, 26.31, 74.33, 78.52, 80.87, 113.17, 128.43, 129.90, 130.39, 133.02, 166.47.

Benzoic acid 3,4-dihydroxy-bicyclo[3.1.0]hex-2-yl ester (**9**)

A solution of **8** (384.2 mg, 1.46 mmol) and 50% TFA (8 mL) was stirred at room temperature for overnight. The solvent was evaporated and further coevaporated with toluene to give colorless syrup. The syrup was purified by silica gel column chromatography (methylene chloride:methanol= 19:1) to give **9** (311.2 mg, 91%) as a white solid: mp 149.7-256.8 °C; MS (ESI+) m/z 234.0869 [M]⁺; [α]_D^{27.1} -0.07° (*c* 2.79, CHCl₃); ¹H NMR (CDCl₃) δ 0.53-0.59 (m, 1 H), 1.29 (dd, *J* = 5.2, 13.6 Hz, 1 H), 1.72-1.76 (m, 1 H), 1.78-1.84 (m, 1 H), 2.73 (s, 1 H, D₂O exchangeable), 2.75 (d, *J* = 3.2 Hz, 1 H, D₂O exchangeable), 2.20 (dd, *J* = 6.0, 16.8 Hz, 1 H), 4.46-4.51 (m, 1 H), 5.39 (t, *J* = 5.6, 10.8 Hz, 1 H), 7.43 (t, *J* = 8.6, 15.2 Hz, 1 H), 7.56 (t, *J* = 7.2, 14.8 Hz, 1 H), 8.07 (d, *J* = 8.0 Hz, 1 H); ¹³C NMR (CDCl₃) δ 5.62, 19.85, 22.62, 68.67, 71.85, 76.11, 128.64, 129.90, 130.15, 133.38, 166.67.

Benzoic acid 3-acetoxy-4-bromo-bicyclo[3.1.0]hex-2-yl ester (**11a**)

To a suspension of **9** (1.00 g, 4.27 mmol) in anhydrous acetonitrile (43 mL), a solution of 2-acetoxyisobutryl bromide (1.86 mL, 12.81 mmol) in anhydrous acetonitrile (10 mL) was added at 0 °C dropwise, and the reaction mixture was stirred at 0 °C for 3.5 h. Saturated sodium bicarbonate solution was added dropwise at 0 °C until the pH of solution became 8, and then the solution was neutralized with acetic acid to pH 5. The mixture was extracted with ethyl acetate, washed with sodium bicarbonate (x2), and brine. The organic layer was dried (MgSO₄), filtered, and evaporated. After evaporation, the residue was purified by silica gel column chromatography (hexane:ethyl acetate= 10:1) to give **11a** (1.08 g, 75%) as a colorless syrup: MS (ESI+) m/z 338.0184 [M]⁺; [α]_D^{27.1} 0.01° (*c* 15.1, CHCl₃); IR (KBr) 2101.88 cm⁻¹; ¹H NMR (CDCl₃) δ 0.72-0.78 (m, 1 H), 1.21-1.25 (m, 1 H), 1.92-1.97 (m, 1 H), 2.00 (s, 3 H), 2.11-2.16 (m, 1 H), 4.28 (s, 1 H), 5.60 (d, *J* = 6.8 Hz, 1 H), 5.95 (t, *J* = 5.6, 7.8 Hz, 1 H), 7.43 (t, *J* = 7.0, 14.2 Hz, 2 H), 7.55 (t, *J* = 6.6, 14.2 Hz, 1 H), 7.98 (d, *J* = 8.0 Hz, 2 H); ¹³C NMR (CDCl₃) δ 10.60, 20.87, 25.15, 51.93, 73.72, 76.55, 128.53, 129.67, 133.26, 165.90, 168.86, 128.53, 129.63, 129.96, 133.26.

Benzoic acid 3-acetoxy-4-azido-bicyclo[3.1.0]hex-2-yl ester (**12a**)

To a suspension of **11a** (235.4 mg, 0.69 mmol) in DMF (8 mL), NaN_3 (225.6 mg, 3.47 mmol) was added at room temperature, and the reaction mixture was stirred at 100 °C for 3 h. The solvent was evaporated and the residue was quenched with water, extracted with ether. The organic layer was dried (MgSO_4), filtered, and evaporated. After evaporation, the residue was purified by silica gel column chromatography (hexane:ethyl acetate= 9:1) to give **12a** (92.7 mg, 44%) as a colorless syrup: MS (ESI+) m/z 301.1057 $[\text{M}]^+$; $[\alpha]^{26.5}_{\text{D}}$ 0.03° (c 3.29, CHCl_3); IR (KBr) 2101.27 cm^{-1} ; ^1H NMR (CDCl_3) δ 0.79 (dd, J = 8.2, 21.0 Hz, 1 H), 1.51 (dd, J = 4.2, 14.0 Hz, 1 H), 1.82-1.85 (m, 1 H), 1.90-1.95 (m, 1 H), 2.08 (s, 3 H), 4.12 (t, J = 5.6, 11.2 Hz, 1 H), 5.48 (t, J = 6.8, 13.2 Hz, 1 H), 5.64 (t, J = 5.6, 11.0 Hz, 1 H), 7.44 (t, J = 7.4, 15.2 Hz, 2 H), 7.57 (t, J = 7.6, 15.2 Hz, 1 H), 8.00 (d, J = 7.6 Hz, 2 H); ^{13}C NMR (CDCl_3) δ 6.37, 18.73, 19.26, 20.74, 61.04, 69.46, 73.61, 128.61, 129.73, 129.92, 133.36, 165.87, 169.42.

4-Azido-bicyclo[3.1.0]hexane-2,3-diol (**13**)

To a stirred solution of **12a** (0.38 g, 1.26 mmol) in methanol (20 mL), sodium methoxide (0.2 g, 3.78 mmol) was added, and the reaction mixture was stirred at room temperature for 2 h. The solvent was evaporated and the residue was partitioned between ethyl acetate and water. The organic layer was dried (MgSO_4), filtered, and evaporated. The residue was purified by silica gel column chromatography (methylene chloride:methanol= 10:1) to give **13** (0.20 g, 99%) as a colorless syrup: $[\alpha]^{26.7}_{\text{D}}$ -0.01° (c 2.68, MeOH); IR (KBr) 2101.2 cm^{-1} ; ^1H NMR ($\text{DMSO}-d_6$) δ 0.37-0.42 (m, 1 H), 1.33 (dd, J = 4.2, 12.4 Hz, 1 H), 1.45-1.48 (m, 1 H), 1.55-1.60 (m, 1 H), 3.62 (t, J = 5.2, 10.4 Hz, 1 H), 3.75 (dd, J = 6.0, 16.4 Hz, 1 H), 4.15-4.19 (m, 1 H), 4.36 (d, J = 8.4, 1 H, D_2O exchangeable), 5.19 (d, J = 5.2 Hz, 1 H, D_2O exchangeable); ^{13}C NMR (MeOH- d_4) δ 6.47, 19.67, 21.65, 63.51, 71.48, 73.35.

5-Azido-hexahydro-2,4-dioxo-3-thia-cyclopropa[a]pentalene 3-oxide (**14**)

To a solution of **13** (55.2 mg, 0.36 mmol) in methylene chloride (3 mL), triethylamine (0.18 mL, 0.54 mmol) and thionyl chloride (0.04 mL, 0.54 mmol) were added at 0 °C, and the reaction mixture was stirred at 0 °C for 10 min. The reaction mixture was partitioned between methylene chloride and water. The organic layer was dried (MgSO_4), filtered, and evaporated. The residue was purified by silica gel column chromatography (hexane:ethyl acetate=2:1) to give a diastereomeric mixture of **14** (66.6 mg, 94%) as colorless syrup: $[\alpha]^{27.1}_{\text{D}}$ -2.33° (c 3.31, CHCl_3); IR (KBr) 2104.67 cm^{-1} ; ^1H NMR (CDCl_3) δ 0.96 (dd, J = 8.2, 22.8 Hz, 1 H), 1.00-1.04 (m, 1 H), 1.84-1.90 (m, 1 H), 1.96-2.03 (m, 1 H), 4.31 (t, J = 5.6, 11.6 Hz, 1 H), 5.24 (t, J = 6.6, 13.2 Hz, 1 H), 5.66 (t, J = 6.0, 12.0 Hz, 1 H); ^{13}C NMR (CDCl_3) δ 8.27, 20.40, 25.28, 60.40, 82.19, 87.45.

2-Azido-4-(2,6-dichloro-purin-9-yl)-bicyclo[3.1.0]hexan-3-ol (**16**)

To a solution of **14** (0.68 g, 3.40 mmol) in carbon tetrachloride, acetonitrile, and water (1:1:1.5, 17.5 mL), sodium metaperiodate (2.04 g, 6.5 mmol) and ruthenium chloride trihydrate (71.9 mg) were added, and the reaction mixture was stirred at room temperature for 10 min. The reaction mixture was partitioned between methylene chloride and water and the organic layer was dried (MgSO_4), filtered, and evaporated. The residue was purified by silica gel column chromatography (hexane:ethyl acetate=1:1) to give **15** as a colorless syrup, which was immediately used for the next reaction.

A suspension of 2,6-dichloropurine (0.96 g, 5.1 mmol), sodium hydride (0.2 g, 5.1 mmol, 60% dispersion in mineral oil), and 18-crown-6 (1.35 g, 5.1 mmol) in tetrahydrofuran (10 mL) was stirred at 80 °C for 3 h. To this solution, a solution of **15** in tetrahydrofuran (10 mL) was added, and the mixture was stirred at room temperature for 4 h. The mixture was

cooled down to 0 °C and 30% sulfuric acid was added carefully to make pH 2. The reaction mixture was stirred at room temperature overnight, then neutralized with sodium bicarbonate solution. The reaction mixture was partitioned between ethyl acetate and water. The organic layer was dried (MgSO₄), filtered and evaporated. The residue was purified by silica gel column chromatography (methylene chloride/methanol= 30/1) to give **16** (0.68 g, 61%) as a colorless syrup: MS (ESI+) *m/z* 325.0247 [M]⁺; [α]_D^{26.2} -0.77° (*c* 1.15, CHCl₃); UV (CHCl₃) λ_{max} 274.5 nm; IR (KBr) 2104.43 cm⁻¹; ¹H NMR (CDCl₃) δ 1.00-1.06 (m, 1 H), 1.41-1.44 (m, 1 H), 1.73-1.78 (m, 1 H), 2.18-2.24 (m, 1 H), 2.63 (s, 1 H, D₂O exchangeable), 4.20 (d, *J* = 3.2 Hz, 1 H), 4.68 (t, *J* = 5.6, 11.6 Hz, 1 H), 4.97 (s, 1 H), 8.17 (s, 1 H); ¹³C NMR (MeOH-*d*₄) δ 9.10, 19.11, 22.25, 63.89, 65.43, 78.59, 132.17, 147.42, 152.08, 153.82, 154.34.

2-Azido-4-(6-chloro-purin-9-yl)-bicyclo[3.1.0]hexan-3-ol (**17**)

Compound **15** (288.0 mg, 1.44 mmol) was condensed with 6-chloropurine to give compound **17** (228.1 mg, 54%) as a foam according to the similar procedure used for the preparation of **16**: MS (ESI+) *m/z* 291.0634 [M]⁺; [α]_D^{26.4} -1.65° (*c* 1.03, CHCl₃); UV (MeOH) λ_{max} 264.0 nm; IR (KBr) 2110.76 cm⁻¹; ¹H NMR (CDCl₃) δ 1.00-1.06 (m, 1 H), 1.43-1.46 (m, 1 H), 1.77-1.81 (m, 1 H), 2.17-2.23 (m, 1 H), 2.99 (t, *J* = 2.8, 5.6 Hz, 1 H, D₂O exchangeable), 4.23 (d, *J* = 6.6 Hz, 1 H), 4.69 (t, *J* = 5.6, 11.2 Hz, 1 H), 5.01 (s, 1 H), 8.20 (s, 1 H), 8.76 (s, 1 H); ¹³C NMR (CDCl₃) δ 8.95, 19.82, 21.87, 63.78, 64.05, 77.85, 132.34, 143.84, 151.41, 151.71, 152.25.

General Procedure for the Synthesis of **18a-d**

To a solution of **16** and **17** in EtOH (5 mL) was added appropriate amine (1.5 equiv) at room temperature, and the mixture was stirred at room temperature for a time period ranging from 20 min to 1 d, and evaporated. The residue was purified by a flash silica gel column chromatography (hexane:ethyl acetate= 1:1) to give **18a**, **18b** and **18c**, **18d**, respectively.

2-Azido-4-[2-chloro-6-(3-iodo-benzylamino)-purin-9-yl]-bicyclo[3.1.0]hexan-3-ol (**18a**)

Yield: 89%; white foam; MS (ESI+) *m/z* 522.0166 [M]⁺; [α]_D^{26.0} -0.72° (*c* 2.62, CHCl₃); UV (MeOH) λ_{max} 272.0 nm; IR (KBr) 2105.62 cm⁻¹; ¹H NMR (CDCl₃) δ 0.93-0.98 (m, 1 H), 1.66-1.70 (m, 1 H), 2.05-2.11 (m, 1 H), 4.18 (d, *J* = 6.8 Hz, 1 H), 4.68 (s, 1 H, D₂O exchangeable), 4.77 (s, 2 H), 4.84 (s, 1 H), 7.00 (s, 1 H, D₂O exchangeable), 7.04 (t, *J* = 8.0, 15.6 Hz, 1 H), 7.32 (d, *J* = 7.6 Hz, 1 H), 7.58 (d, *J* = 8.0 Hz, 1 H), 7.70 (t, *J* = 1.2, 2.8 Hz, 1 H), 7.72 (s, 1 H); ¹³C NMR (CDCl₃) δ 8.73, 14.38, 19.05, 21.42, 43.99, 60.60, 63.17, 77.50, 94.71, 118.91, 127.33, 130.58, 136.83, 138.50, 140.57, 149.90, 154.81, 155.17, 171.39.

2-Azido-4-(2-chloro-6-methylamino-purin-9-yl)-bicyclo[3.1.0]hexan-3-ol (**18b**)

Yield: 91%; white solid; mp 228.1-228.9 °C; MS (ESI+) *m/z* 320.0901 [M]⁺; [α]_D^{27.2} -0.03° (*c* 0.58, MeOH); UV (MeOH) λ_{max} 272.0 nm; IR (KBr) 2105.38 cm⁻¹; ¹H NMR (MeOH-*d*₄) δ 0.90-0.96 (m, 1 H), 1.55 (dd, *J* = 5.6, 13.6 Hz, 1 H), 1.72-1.77 (m, 1 H), 2.06-2.12 (m, 1 H), 3.09 (s, 3 H), 4.04 (d, *J* = 6.4 Hz, 1 H), 4.15 (t, *J* = 5.2, 10.8 Hz, 1 H), 4.79 (s, 1 H), 7.98 (s, 1 H); ¹³C NMR (MeOH-*d*₄) δ 8.66, 18.64, 21.68, 27.83, 63.10, 63.78, 77.88, 119.49, 139.15, 149.82, 155.30, 156.82.

2-Azido-4-[6-(3-iodo-benzylamino)-purin-9-yl]-bicyclo[3.1.0]hexan-3-ol (**18c**)

Yield: 76%; white foam; MS (ESI+) *m/z* 488.0567 [M]⁺; [α]_D^{27.0} -1.08° (*c* 1.98, CHCl₃); UV (CHCl₃) λ_{max} 267.5 nm; IR (KBr) 2104.26 cm⁻¹; ¹H NMR (CDCl₃) δ 0.98 (dd, *J* = 6.4, 22.0 Hz, 1 H), 1.54-1.59 (m, 1 H), 1.72-1.76 (m, 1 H), 2.07-2.13 (m, 1 H), 2.03 (s, 1 H, D₂O exchangeable), 4.16 (d, *J* = 5.6 Hz, 1 H), 4.32 (dd, *J* = 5.6, 16.4 Hz, 1 H), 4.83 (s, 2 H), 4.92 (s, 1 H), 6.65 (s, 1 H, D₂O exchangeable), 7.04 (t, *J* = 8.0, 15.6 Hz, 1 H), 7.32 (d, *J* = 7.6 Hz,

1 H), 7.59 (d, $J = 7.2$ Hz, 1 H), 7.71 (s, 1 H), 7.78 (s, 1 H), 8.39 (s, 1 H); ^{13}C NMR (CDCl_3) δ 8.80, 18.93, 21.18, 24.13, 29.90, 43.83, 62.99, 63.20, 94.80, 120.32, 127.04, 130.56, 136.64, 136.73, 138.14, 141.18, 153.36, 154.91.

2-Azido-4-(6-methylamino-purin-9-yl)-bicyclo[3.1.0]hexan-3-ol (18d)

Yield: 88%; white foam; MS (ESI+) m/z 286.1293 $[\text{M}]^+$; $[\alpha]^{27.1}_{\text{D}} -1.76^\circ$ (c 2.21, MeOH); UV (CHCl_3) λ_{max} 269.5 nm; IR (KBr) 2104.86 cm^{-1} ; ^1H NMR ($\text{MeOH}-d_4$) δ 0.90-0.96 (m, 1 H), 1.50 (dd, $J = 4.0, 13.2$ Hz, 1 H), 1.74-1.79 (m, 1 H), 2.06-2.12 (m, 1 H), 3.11 (s, 3 H), 4.10 (d, $J = 6.4$ Hz, 1 H), 4.22 (t, $J = 5.2, 10.8$ Hz, 1 H), 4.85 (s, 1 H), 8.14 (s, 1 H), 8.26 (s, 1 H); ^{13}C NMR ($\text{MeOH}-d_4$) δ 8.97, 19.25, 21.94, 27.86, 63.75, 64.23, 78.76, 120.94, 139.96, 153.83, 156.81.

General Procedure for the Synthesis of 4a-d

To a solution of **18a-d** in THF (1 mL), triphenylphosphine was added and the solution was cooled to 0 °C. After 30 min, ammonium hydroxide solution (0.1 mL) and water (0.02 mL) were added and the reaction mixture was stirred at room temperature overnight. The reaction mixture was concentrated and 1 *N*HCl was added to adjust to pH 1. The resultant solution was then partitioned between diethyl ether and water. The aqueous layer was further neutralized with sodium bicarbonate. After complete removal of water, the solid obtained was filtered, and washed with methylene chloride/methanol (6/1) solution. The residue was recrystallized using methylene chloride and methanol to give pure **4a-d** as a white solid.

2-Amino-4-[2-chloro-6-(3-iodo-benzylamino)-purin-9-yl]-bicyclo[3.1.0]hexan-3-ol (4a)

Yield: 86%; mp 255.8-262.2 °C; MS (ESI+) m/z 497.0344 $[\text{M}]^+$; $[\alpha]^{26.9}_{\text{D}} -0.75^\circ$ (c 0.32, AcOH); UV (MeOH) λ_{max} 272.0 nm; ^1H NMR ($\text{AcOH}-d_4$) δ 0.87-0.94 (m, 1 H), 1.24-1.31 (m, 1 H), 1.84-1.89 (m, 1 H), 2.17-2.21 (m, 1 H), 4.40 (d, $J = 6.4$ Hz, 1 H), 4.60 (t, $J = 5.2, 10.4$ Hz, 1 H), 4.75 (s, 2 H), 5.00 (s, 1 H), 7.07 (t, $J = 7.6, 15.6$ Hz, 1 H), 7.40 (d, $J = 7.6$ Hz, 1 H), 7.60 (d, $J = 8.0$ Hz, 1 H), 7.81 (s, 1 H), 8.22 (s, 1 H); ^{13}C NMR ($\text{AcOH}-d_4$) δ 8.04, 20.00, 21.89, 30.56, 44.21, 55.08, 64.82, 75.43, 94.79, 118.34, 128.17, 131.27, 137.41, 137.89, 140.71, 142.26, 150.45, 155.84, 156.04.

2-Amino-4-(2-chloro-6-methylamino-purin-9-yl)-bicyclo[3.1.0]hexan-3-ol (4b)

Yield: 85%; mp 249.2-259.6 °C; MS (ESI+) m/z 294.0996 $[\text{M}]^+$; $[\alpha]^{26.3}_{\text{D}} 0.05^\circ$ (c 3.74, AcOH); UV (MeOH) λ_{max} 272.0 nm; ^1H NMR ($\text{AcOH}-d_4$) δ 0.91 (dd, $J = 6.4, 22.4$ Hz, 1 H), 1.28-1.31 (m, 1 H), 1.85-1.89 (m, 1 H), 2.17-2.21 (m, 1 H), 3.08 (s, 3 H), 4.40 (d, $J = 6.8$ Hz, 1 H), 4.59 (t, $J = 5.6, 10.8$ Hz, 1 H), 4.99 (s, 1 H), 8.19 (s, 1 H); ^{13}C NMR ($\text{AcOH}-d_4$) δ 8.01, 21.88, 27.67, 55.04, 64.72, 75.41, 118.32, 139.92, 140.13, 149.78, 156.15, 156.54.

2-Amino-4-[6-(3-iodo-benzylamino)-purin-9-yl]-bicyclo[3.1.0]hexan-3-ol (4c)

Yield: 84%; mp 239.9-244.7 °C; MS (ESI+) m/z 462.0651 $[\text{M}]^+$; $[\alpha]^{26.3}_{\text{D}} -0.19^\circ$ (c 1.38, AcOH); UV (MeOH) λ_{max} 268.0 nm; ^1H NMR ($\text{AcOH}-d_4$) δ 0.94 (dd, $J = 6.4, 22.0$ Hz, 1 H), 1.37-1.38 (m, 1 H), 1.94 (m, 1 H), 2.21-2.25 (m, 1 H), 4.37 (d, $J = 6.8$ Hz, 1 H), 4.52 (t, $J = 6.0, 11.2$ Hz, 1 H), 4.82 (s, 2 H), 5.08 (s, 1 H), 7.06 (t, $J = 7.6, 15.6$ Hz, 1 H), 7.39 (d, $J = 7.2$ Hz, 1 H), 7.60 (d, $J = 8.0$ Hz, 1 H), 7.79 (s, 1 H), 8.35 (s, 1 H), 8.47 (s, 1 H); ^{13}C NMR ($\text{AcOH}-d_4$) δ 8.11, 20.00, 21.66, 44.27, 54.75, 64.35, 75.25, 94.80, 119.39, 127.70, 127.80, 131.26, 137.53, 140.21, 142.05, 148.44, 154.03, 155.17.

2-Amino-4-(6-methylamino-purin-9-yl)-bicyclo[3.1.0]hexan-3-ol (4d)

Yield: 86%; mp 238.7-242.4 °C; MS (ESI+) m/z 260.1388 $[\text{M}]^+$; $[\alpha]^{26.1}_{\text{D}} -0.22^\circ$ (c 1.43, MeOH); UV (MeOH) λ_{max} 267.0 nm; ^1H NMR ($\text{MeOH}-d_4$) δ 0.66-0.72 (m, 1 H), 1.24 (dd,

$J = 4.8, 12.8$ Hz, 1 H), 1.67-1.71 (m, 1 H), 1.89-1.96 (m, 1 H), 3.12 (s, 3 H), 3.74 (d, $J = 6.8$ Hz, 1 H), 3.79 (t, $J = 5.6, 10.4$ Hz, 1 H), 4.89 (s, 1 H), 8.18 (s, 1 H), 8.27 (s, 1 H); ^{13}C NMR (MeOH- d_4) δ 7.27, 19.85, 25.40, 54.97, 64.67, 77.10, 139.79, 153.79.

Pharmacology

Cell culture and membrane preparation

Chinese hamster ovary (CHO) cells stably expressing either the recombinant hA₁ or hA₃AR and human embryonic kidney (HEK) 293 cells stably expressing the human (h) A_{2A}AR were cultured in DMEM and F12 (1:1), supplemented with 10% fetal bovine serum, 100 units/mL penicillin, 100 $\mu\text{g/mL}$ streptomycin, and 2 $\mu\text{mol/mL}$ glutamine. In addition, 800 $\mu\text{g/mL}$ Geneticin and 500 $\mu\text{g/mL}$ hygromycin were added to the A_{2A} media and to the A₁ and A₃ media, respectively. After harvesting the cells, they were homogenized for 10 sec with an electric homogenizer, pipetted into 1-mL vials, and then stored at -80°C until binding experiments were conducted. The concentration of protein was determined using a BCA Protein Assay Kit from Pierce Biotechnology (Rockford, IL).¹⁹

Radioligand membrane binding studies

Radioligand binding assays at A₁, A_{2A}, and A₃ARs were performed according to the procedures described previously.²⁰⁻²² Each tube in the binding assay contained 100 μL of membrane suspension (20 μg of protein), 50 μL of a stock solution of agonist radioligand, and 50 μL of increasing concentrations of the test ligands in Tris-HCl buffer (50 mM, pH 7.5) containing 10 mM MgCl_2 . Nonspecific binding was determined using a final concentration of 10 μM NECA, a non-specific agonist, diluted with the buffer.

The mixtures were incubated at 25°C for 60 min. Binding reactions were terminated by filtration through Whatman GF/B filters under a reduced pressure using a MT-24 cell harvester (Brandell, Gaithersburg, MD). Filters were washed three times with 5 ml of 50 mM ice-cold Tris-HCl buffer (pH 7.5). The radioactive agonists [^3H]R-PIA and [^3H]CGS21680 were used for the A₁ and A_{2A}AR assays, respectively, while [^{125}I]AB-MECA was used for the A₃AR assay. All of the filters were washed 3 times with Tris-HCl, pH 7.5. Filters for A₁ and A_{2A}AR binding were placed in scintillation vials containing 5 ml of Hydrofluor scintillation buffer and counted using a PerkinElmer Tricarb 2810TR Liquid Scintillation Analyzer. Filters for A₃AR binding were counted using a PerkinElmer Cobra II γ -counter.

Data analysis

Binding and functional parameters were calculated using Prism 5.0 software (GraphPAD, San Diego, CA, USA). IC₅₀ values obtained from competition curves were converted to K_i values using the Cheng-Prusoff equation.²³ Data were expressed as mean $\pm$ standard error.

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References

- (a) Klotz K-N. Naunyn-Schmiedeberg's Arch Pharmacol. 2000; 362:382–391. [PubMed: 11111832]
(b) Baraldi PG, Cacciari B, Romagnoli R, Merighi S, Varani K, Borea PA, Spalluto G. Med Res Rev. 2000; 20:103–128. [PubMed: 10723024]
- 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. [PubMed: 12037688] (b) Lee EJ, Min HY, Chung HJ, Park EJ, Shin DH, Jeong LS, Lee SK. *Biochem Pharmacol*. 2005; 70:918. [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. (d) Kim SJ, Min HYH, Chung J, Park EJ, Hong JY, Kang YJ, Shin DH, Jeong LS, Lee SK. *Cancer Lett*. 2008; 264:309. [PubMed: 18321638]
4. von Lubitz DKJE, Ye W, McClellan J, Lin RC-S. *Ann N Y Acad Sci*. 1999; 890:93. [PubMed: 10668416]
5. Liang BT, Jacobson KA. *Proc Natl Acad Sci*. 1998; 95:6995. [PubMed: 9618527]
6. Sajjadi FG, Takabayashi K, Foster AC, Domingo RC, Firestein GS. *J Immunol*. 1996; 156:3435. [PubMed: 8617970]
7. Meade CJ, Mierau J, Leon I, Ensinger HA. *J Pharmacol Exp Ther*. 1996; 279:1148. [PubMed: 8968336]
8. Avila MY, Stone RA, Civan MM. *Invest Ophthalmol Vis Sci*. 2002; 43:3021.
9. Jacobson KA, Ji X-d, Li A-H, Melman N, Siddiqui MA, Shin K-J, Marquez VE, Ravi RG. *J Med Chem*. 2000; 43:2196–2203. [PubMed: 10841798]
10. Melman A, Wang B, Joshi BV, Gao Z-G, Castro S, Heller CL, Kim S-K, Jeong LS, Jacobson KA. *Bioorg Med Chem*. 2008; 16:8546–8556. [PubMed: 18752961]
11. DeNinno MP, Masamune H, Chenard LK, DiRico KJ, Eller C, Etienne JB, Tickner JE, Kennedy SP, Knight DR, Kong J, Oleynek JJ, Tracey WR, Hill RJ. *J Med Chem*. 2003; 46:353–355. [PubMed: 12540233]
12. (a) 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] (b) Moon HR, Choi WJ, Kim HO, Jeong LS. *Tetrahedron: Asymmetry*. 2002; 13:1189–1193.
13. (a) Luche J-L. *J Am Chem Soc*. 1978; 100:2226–2227. (b) Lee JA, Kim HO, Tosh DK, Moon HR, Kim SH, Jeong LS. *Org Lett*. 2006; 8:5081–5083. [PubMed: 17048848]
14. Altmann K-H, Kesselring R, Francotte E, Rihs G. *Tetrahedron Lett*. 1994; 35:2331–2334.
15. Zhang L, Cui Z, Zhang B. *Helv Chim Acta*. 2003; 86:703–710.
16. (a) Moon HR, Kim HO, Chun MW, Jeong LS. *J Org Chem*. 1999; 64:4733–4741. [PubMed: 11674546] (b) Marquez, VE. *Modified Nucleosides: in Biochemistry, Biotechnology and Medicine*. Herdewijn, P., editor. Wiley-VCH Verlag GmbH & Co. KGaA; Weinheim: 2008. p. 307–341.
17. (a) Lohray BB. *Synthesis*. 1992:1035–1052. (b) Lohray BB, Bhushan V. *Adv Heterocycl Chem*. 1997; 68:89–180. (c) Byun H-S, He L, Bittman R. *Tetrahedron*. 2000; 56:7051–7091.
18. (a) Bera S, Nair V. *Tetrahedron Lett*. 2001; 42:5813–5815. (b) Guenther S, Nair V. *Nucleosides Nucleotides Nucleic Acids*. 2004; 23:183–193. [PubMed: 15043146] (c) Hrebabecky H, Masojdkova M, Holy A. *Collect Czech Chem Commun*. 2005; 70:519–538.
19. Bradford MM. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem*. 1976; 72:248–254. [PubMed: 942051]
20. Klotz KN, Lohse MJ, Schwabe U, Cristalli G, Vittori S, Grifantini M. *Naunyn Schmiedebergs Arch Pharmacol*. 1989; 340:679–683. [PubMed: 2615857]
21. Jarvis MF, Schutz R, Hutchison AJ, Do E, Sills MA, Williams M. *J Pharmacol Exp Ther*. 1989; 251:888–893. [PubMed: 2600819]
22. Olah ME, Gallo-Rodriguez C, Jacobson KA, Stiles GL. *Mol Pharmacol*. 1994; 45:978–982. [PubMed: 8190112]
23. Cheng YC, Prusoff HR. *Biochem Pharmacol*. 1973; 22:3099. [PubMed: 4202581]

1 (X = CONHMe) (A₃ AR agonist)

2 (X = H) (A₃ AR antagonist)

R₁ = Cl or H

R₂ = alkyl or arylalkyl

3 (A₃ AR agonist)

R₁ = Cl or H

R₂ = alkyl or arylalkyl

Figure 1.

The rationale for the design of the target nucleosides

Scheme 1. Reagents and Conditions

a) NaBH_4 , $\text{CeCl}_3 \cdot 7\text{H}_2\text{O}$, $0\text{ }^\circ\text{C}$, 40 min; b) Et_2Zn , CH_2I_2 , $0\text{ }^\circ\text{C}$ to rt, 3 h; c) BzCl , pyridine, rt, 3.5 h; d) 50% TFA, rt, overnight.

Scheme 2.

Scheme 3.

Scheme 4. Reagents and Conditions

a) NaOMe, MeOH, rt, 1 h; b) $SOCl_2$, Et_3N , MC, 0 °C, 30 min; c) $RuCl_3 \cdot 3H_2O$, $NaIO_4$, CCl_4 /
 $CH_3CN/H_2O=1/1.5$, rt, 10 min.

Scheme 5. Reagents and Conditions

a) i. 2,6-dichloropurine or 6-chloropurine, NaH, rt, 4 h; ii. 35% H_2SO_4 , rt, overnight; b) 3-iodobenzylamine or methylamine, Et_3N , EtOH, rt, overnight; c) PPh_3 , NH_4OH , THF, rt, overnight.

Table 1

Binding affinities of known A₃AR antagonist **2** and 3'-amino derivatives **4a-d** at three subtypes of hARs.

Compound (X, R ₁ , R ₂)	Affinity (K _i , nM ± SEM, or % inhibition) ^a		
	hA ₁	hA _{2A}	hA ₃
2 (X = OH, R ₁ = Cl, R ₂ = 3-iodobenzyl)	3040 ± 610	1080 ± 310	1.44 ± 0.60
4a (X = NH ₂ , R ₁ = Cl, R ₂ = 3-iodobenzyl)	15 ± 8%	12% ± 5%	24.7 ± 0.6%
4b (X = NH ₂ , R ₁ = Cl, R ₂ = methyl)	8 ± 6%	5% ± 2%	42.3 ± 7.4%
4c (X = NH ₂ , R ₁ = H, R ₂ = 3-iodobenzyl)	10%	24 ± 3%	5460 ± 1280
4d (X = NH ₂ , R ₁ = H, R ₂ = methyl)	3 ± 2%	5 ± 2%	27.2 ± 5.3%

^a All binding experiments were performed using adherent mammalian cells stably transfected with cDNA encoding the appropriate hAR (A₁AR and A₃AR in CHO cells and A_{2A}AR in HEK-293 cells). Binding was carried out using 1 nM [³H]R-PIA, 10 nM [³H]CGS21680, or 0.5 nM [¹²⁵I]I-AB-MECA as radioligands for A₁, A_{2A}, and A₃ARs, respectively. Values are expressed as mean ± sem, n = 3-4 (outliers eliminated) and normalized against a non-specific binder, 5'-N-ethylcarboxamidoadenosine (NECA, 10 μM). Values expressed as a percentage refer to percent inhibition of specific radioligand binding at 10 μM, with nonspecific binding defined using 10 μM NECA.

^b Data from ref. 10.