SUPPORTING INFORMATION

5'-*C*-ETHYL-TETRAZOLYL-N^6-SUBSTITUTED ADENOSINE AND 2-CHLORO-ADENOSINE DERIVATIVES AS HIGHLY POTENT DUAL ACTING A_1 ADENOSINE RECEPTOR AGONISTS AND A_3 ADENOSINE RECEPTOR ANTAGONISTS

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Abbreviations

A_1AR, A_1 adenosine receptor; $A_{2A}AR$, A_{2A} adenosine receptor; $A_{2B}AR$, A_{2B} adenosine receptor; A_3AR, A_3 adenosine receptor; cAMP, cyclic adenosine-5'-monophosphate; CCPA, 2-chloro-N^6-cyclopentyl-adenosine; 5'Cl5'd-(±)-ENBA, 5'-chloro-5'-deoxy-N^6-(±)-(endo-norborn-2-yl)-adenosine; CHO, Chinese hamster ovary; DBU, 1,8-diazabicyclo[5.4.0]undec-7-ene; GPCR, G protein-coupled receptor; HEMADO, 2-hexyn-1-yl-N^6-methyladenosine; IOP, intraocular pressure; L-DOPA, 3,4-diidrossi-L-fenilalanina; NECA, 5'-N-ethyl-carboxamidoadenosine; NOE, Nuclear Overhauser Effect, R-PIA, (R)-N^6-phenylisopropyladenosine; TEA, triethylamine, TM, transmembrane domain; TMSiOTf, trimethylsilyl trifluoromethanesulfonate.

Experimental Section

Chemistry

All reagents and solvents were purchased from Sigma-Aldrich Chemical Co and were analytical grade. Thin layer chromatography (TLC) was run on silica gel 60 F254 plates; silica gel 60 (70–230 and 230-400 mesh, Merck) for column chromatography was used. Compounds **1-13** were characterized by ^{1}H-NMR, LC-MS and elemental analyses, and their purities (>96%) were quantified by HPLC. ^{1}H NMR spectra were determined at 400 MHz with a Varian Mercury AS400 instrument. The chemical shift values are expressed in δ values (ppm), and coupling constants (*J*) are in Hertz; TMS was used as an internal standard. The presence of all exchangeable protons was confirmed by the addition of D_2O. Mass spectra were recorded on an HP 1100 series instrument. All measurements were performed in the positive ion mode using atmospheric pressure electrospray ionization (API-ESI). Analytical HPLC measurements were run on an Agilent 1100 Series equipped with a diode array detector (DAD). The column was a Gemini-NX 5µm C-18 100Å 250 x 4.6 mm, the mobile phase was a mixture of water/methanol (95:5) at a flow rate of 1 mL/min. Area % purity was detected at 210nm or 245nm. The purity of the tested compounds was >98% based on the HPLC analysis. The ratio of β/α isomers was determined by HPLC-MS as follows. Column: Gemini-NX 5µm C-18 100Å 250 x 4.6 mm; solvent A: 97.5% water, 2.5% MeCN, 0.05% TFA; solvent B: 60% water, 40%MeCN, 0.05% TFA; gradient: 0-100% B over 30 min at a flow rate of 1.5 ml/min. Elemental analyses (C, H, and N) were determined on an EA 1108 CHNS-O (Fisons Instruments) analyzer and are within 0.4% of theoretical values.

(2*R*,3*R*,4*R*,5*R*)-2-(6-chloro-9*H*-purin-9-yl)-5-(2-ethyl-2*H*-tetrazol-5-yl)tetrahydrofuran-3,4-diyl diacetate (17)
To a stirred mixture of **14** (830 mg, 2.51 mmol), 6-chloropurine (**15**) (2.75 mmol), and DBU (7.50 mmol) in anhydrous acetonitrile (10 mL) at 0 °C was added TMSiOTf (10 mmol). The mixture was stirred at room temperature for 21 h and then heated to reflux for 3.5 h. The reaction was cooled, quenched with H_2O (70 mL), and extracted with AcOEt (3 x 40 mL).
The combined organic layers were dried, concentrated, and purified by flash chromatography on a silica gel column eluting with hexane-AcOEt (70:30) to afford compound **17** as a white foamy solid (63% yield). The final product was analyzed by HPLC-MS. The ratio of *β* and α anomers was 99:1.
^{1}H NMR (DMSO-d_6): δ 1.52 (t, *J* = 7.3 Hz, 3H, CH_2CH_3), 2.09 (s, 3H, $OCOCH_3$), 2.16 (s, 3H, $OCOCH_3$), 4.71 (q, *J* = 7.3 Hz, 2H, CH_2CH_3), 5.65 (d, *J* = 4.3 Hz, 1H, H-5'), 6.08 (t, *J* = 4.7 Hz, 1H, H-4'), 6.24 (t, *J* = 5.3 Hz, 1H, H-3'), 6.54 (d, *J* = 5.1 Hz, 1H, H-2'), 8.75 (s, 1 H, H-2), 8.90 (s, 1 H, H-8). ^{13}C NMR (DMSO-d_6): 14.43, 20.33, 20.63, 49.05, 73.81, 73.94, 76.82, 85.44, 131.11, 144.32, 152.06, 153.21, 153.33, 162.94, 169.27, 169.39 ppm. MS (API-ESI): *m/z* 437.10 $[M + H]^+$. Anal. calcd. for ($C_{16}H_{17}N_8O_5Cl$) C, 44.00; H, 3.92; N, 25.65; Found: C, 44.02; H, 3.91; N, 25.63.

(2*R*,3*R*,4*S*,5*R*)-2-(6-amino-9*H*-purin-9-yl)-5-(2-ethyl-2*H*-tetrazol-5-yl)tetrahydrofuran-3,4-diol (1). A mixture of compound (2*R*,3*R*,4*R*,5*R*)-2-(6-chloro-9*H*-purin-9-yl)-5-(2-ethyl-2*H*-tetrazol-5-yl)tetrahydrofuran-3,4-diyl diacetate (**17**) (1.0 mmol) and isopropanolic ammonia (25 mL) was heated at 60° for 3 h. Evaporation of the solvent to dryness gave a residue which was purified by flash column chromatography ($CHCl_3$-MeOH, 90:10) to give compound **1** (83% yield) as a white solid. ^{1}H NMR (DMSO-d_6): δ 1.53 (t, *J* = 7.3 Hz, 3H, CH_2CH_3), 4.60 (q, *J* = 4.7 Hz, 1H, H-4'), 4.70 (q, *J* = 7.3 Hz, 2H, CH_2CH_3), 4.80 (q, *J* = 5.1 Hz, 1H, H-3'), 5.18 (d, *J* = 4.3 Hz, 1H, H-5'), 5.75 (d, *J* = 5.5 Hz, 1H, OH), 5.83 (d, *J* = 5.6 Hz, 1H, OH), 6.10 (d, *J* = 4.7 Hz, 1H, H-2'), 7.30 (s, 2H, NH_2), 8.10 (s, 1 H, H-2), 8.40 (s, 1 H, H-8). ^{13}C NMR (DMSO-d_6): 14.12, 48.21, 73.53, 73.81, 76.88, 87.64, 118.86, 138.91, 149.55, 152.81, 156.06, 164.19 ppm. MS (API-ESI): *m/z* 334.13 $[M + H]^+$. Anal. calcd. for ($C_{12}H_{15}N_9O_3$) C, 43.24; H, 4.54; N, 37.82; Found: C, 43.26; H, 4.52; N, 37.83.

(2*R*,3*R*,4*S*,5*R*)-2-(6-amino-2-chloro-9*H*-purin-9-yl)-5-(2-ethyl-2*H*-tetrazol-5-yl)tetrahydrofuran-3,4-diol (2). Reaction of **18** [1] with isopropanolic ammonia for 4 h followed by flash chromatography on a silica gel column ($CHCl_3$-MeOH, 95:5) gave **2** as a white solid (80% yield). ^{1}H NMR (DMSO- d_6): δ 1.53 (t, *J* = 7.5 Hz, 3H, CH_2CH_3), 4.55 (q, *J* = 4.7 Hz, 1H, H-4'), 4.70 (q, *J* = 7.0 Hz, 2H, CH_2CH_3), 4.78 (q, *J* = 5.5 Hz, 1H, H-3'), 5.21 (d, *J* = 3.8 Hz, 1H, H-5'), 5.80 (d, *J* = 5.1 Hz, 1H, OH), 5.83 (d, *J* = 5.9 Hz, 1H, OH), 6.03 (d, *J* = 5.1 Hz, 1H, H-2'), 7.83 (brs, 2H, NH_2), 8.40 (s, 1 H, H-8). ^{13}C NMR (DMSO-d_6): 14.25, 48.41, 73.69,

73.97, 77.40, 87.79, 118.17, 139.65, 150.75, 153.44, 156.99, 164.31 ppm. MS (API-ESI): *m/z* 368.75 [M + H]$^+$. Anal. calcd. for ($C_{12}H_{14}ClN_9O_3$) C, 39.19; H, 3.84; N, 34.28; Found: C, 39.22; H, 3.86; N, 34.25.

General procedure for the amination of 17 and 18 into compounds 3-13.
To a stirred solution of (2*R*,3*R*,4*R*,5*R*)-2-(6-chloro-9*H*-purin-9-yl)-5-(2-ethyl-2*H*-tetrazol-5-yl)tetrahydrofuran-3,4-diyl diacetate (**17**) (1.0 mmol) or (2*R*,3*R*,4*R*,5*R*)-2-(2,6-dichloro-9*H*-purin-9-yl)-5-(2-ethyl-2*H*-tetrazol-5-yl)tetrahydrofuran-3,4-diyl diacetate (**18**) (1.0 mmol) in absolute ethanol (20 mL) and TEA (3 mmol) only in the case of compounds **7-10** and **13**, the appropriate amine (1.6 mmol) was added. The reaction mixture was refluxed for the time reported below and concentrated in vacuo. The residue was dissolved in methanolic ammonia (10 mL) and stirred at room temperature overnight. The solution was evaporated to dryness and the residue was purified by chromatography on a silica gel column.

(2*R*,3*S*,4*R*,5*R*)-2-(2-ethyl-2*H*-tetrazol-5-yl)-5-(6-(methylamino)-9*H*-purin-9-yl)tetrahydrofuran-3,4-diol (3). Reaction of **17** with methylamine for 1 h at room temperature followed by chromatography on a silica gel column ($CHCl_3$-MeOH, 95:5) gave **3** as a white solid (86% yield). ^{1}H NMR (DMSO-d_6): δ 1.47 (t, *J* = 7.3 Hz, 3H, CH_2CH_3), 2.91 (brs, 3H, CH_3), 4.54-4.59 (m, 1H, H-4'), 4.72 (q, *J* = 7.3 Hz, 2H, CH_2CH_3), 4.81 (q, *J* = 5.3 Hz, 1H, H-3'), 5.18 (d, *J* = 4.3 Hz, 1H, H-5'), 5.75 (d, *J* = 5.5 Hz, 1H, OH), 5.83 (d, *J* = 5.6 Hz, 1H, OH), 6.10 (d, *J* = 4.7 Hz, 1H, H-2'), 7.78 (brs, 1H, NH), 8.11 (s, 1H, H-2), 8.38 (s, 1H, H-8). ^{13}C NMR (DMSO-d_6): 14.21, 28.32, 48.38, 73.55, 73.79, 77.49, 87.84, 118.31, 138.92, 151.05, 153.74, 156.78, 164.26 ppm. MS (API-ESI): *m/z* 334.13 [M + H]$^+$. Anal. calcd. for ($C_{13}H_{17}N_9O_3$) C, 44.95; H, 4.93; N, 36.29; Found : C, 44.92; H, 4.89; N, 36.31.

(2*R*,3*R*,4*S*,5*R*)-2-(2-chloro-6-methylamino-9*H*-purin-9-yl)-5-(2-ethyl-2*H*-tetrazol-5-yl)tetrahydrofuran-3,4-diol (4). Reaction of **18** with methylamine for 1 h at room temperature followed by chromatography on a silica gel column ($CHCl_3$-MeOH, 95:5) gave **3** as a white solid (75% yield). ^{1}H NMR (DMSO-d_6): δ 1.52 (t, *J* = 7.5 Hz, 3H, CH_2CH_3), 2.92 (d, *J* = 4.7 Hz, 3H, CH_3), 4.51-4.59 (m, 1H, H-4'), 4.73 (q, *J* = 7.3 Hz, 2H, CH_2CH_3), 4.73-4.81 (m, 1H, H-3'), 5.20 (d, *J* = 4.3 Hz, 1H, H-5'), 5.81 (d, *J* = 5.6 Hz, 1H, OH), 5.89 (d, *J* = 6.0 Hz, 1H, OH), 6.03 (d, *J* = 5.6 Hz, 1H, H-2'), 8.32 (brs, 1H, NH), 8.41 (s, 1H, H-8). ^{13}C NMR (DMSO-d_6): 14.26, 28.21, 48.27, 73.52, 73.69, 77.51, 87.62, 118.06, 139.22, 151.95, 153.62, 156.43, 164.12 ppm. MS (API-ESI): *m/z* 382.11 [M + H]$^+$. Anal. calcd. for ($C_{13}H_{16}ClN_9O_3$) C, 40.90; H, 4.22; N, 33.02; Found: C, 40.88; H, 4.25; N, 33.04.

(2*R*,3*R*,4*S*,5*R*)-2-(6-cyclopentylamino-9*H*-purin-9-yl)-5-(2-ethyl-2*H*-tetrazol-5-yl) tetrahydrofuran-3,4-diol (5). Reaction of **17** with cyclopentylamine at reflux for 2 h followed by deprotection and chromatography on a silica gel column ($CHCl_3$-MeOH, 97:3) gave **5** as a white solid (65% yield). ^{1}H NMR (DMSO-d_6): δ 1.49 (t, *J* = 7.4 Hz, 3H, CH_2CH_3), 1.51-1.58 (m, 4H, cyclopentyl), 1.62-1.68 (m, 2H, cyclopentyl), 1.83-1.88 (m, 2H, cyclopentyl), 4.43-4.51 (m, 1H, NHC*H*), 4.54-4.60 (m, 1H, H-4'), 4.68 (q, *J* = 7.3 Hz, 2H, CH_2CH_3), 4.76-4.81 (m, 1H, H-3'), 5.27 (d, *J* = 4.7 Hz, 1H, H-5'), 5.74 (d, *J* = 5.5 Hz, 1H, OH), 5.81 (d, *J* = 6.0 Hz, 1H, OH), 6.11 (d, *J* = 4.7 Hz, 1H, H-2'), 7.74 (brs, 1H, NH), 8.18 (s, 1H, H-2), 8.37 (s, 1 H, H-8). ^{13}C NMR (DMSO-d_6): 14.22, 24.71(x2), 34.05 (x2), 48.23, 53.2, 73.12, 74.09, 77.23, 87.48, 117.16, 139.11, 151.86, 153.71, 156.43, 164.08 ppm. MS (API-ESI): *m/z* 402.19 [M + H]$^+$. Anal. calcd. for ($C_{17}H_{23}N_9O_3$) C, 50.86; H, 5.78; N, 31.40; Found: C, 50.84; H, 5.75; N, 31.43.

(2*R*,3*R*,4*S*,5*R*)-2-(2-chloro-6-cyclopentylamino-9*H*-purin-9-yl)-5-(2-ethyl-2*H*-tetrazol-5-yl)tetrahydro furan-3,4-diol (6). Reaction of **18** with cyclopentylamine at reflux for 2 h followed by deprotection and chromatography on a silica gel column ($CHCl_3$-MeOH, 97:3) gave **5** as a white solid (73% yield). ^{1}H NMR (DMSO-d_6): δ 1.43 (t, *J* = 7.4 Hz, 3H, CH_2CH_3), 1.49-1.57 (m, 4H, cyclopentyl), 1.61-1.66 (m, 2H, cyclopentyl), 1.81-1.94 (m, 2H, cyclopentyl), 4.35-4.42 (m, 1H, NHC*H*), 4.52-4.58 (m, 1H, H-4'), 4.72 (q, *J* = 7.5 Hz, 2H, CH_2CH_3), 4.77 (q, *J* = 5.1 Hz, 1H, H-3'), 5.21 (d, *J* = 4.3 Hz, 1H, H-5'), 5.78 (d, *J* = 5.6 Hz, 1H, OH), 5.83 (d, *J* = 5.6 Hz, 1H, OH), 6.04 (d, *J* = 5.1 Hz, 1H, H-2'), 8.33 (brs, 1H, NH), 8.39 (s, 1H, H-8). ^{13}C NMR (DMSO-d_6): 14.21, 23.99 (x2), 34.12 (x2), 48.19, 52.97, 73.06, 74.22, 77.12, 87.42, 118.88, 139.86, 150.97, 153.78, 156.06, 164.18 ppm. MS (API-ESI): *m/z* 436.15 [M + H]$^+$. Anal. calcd. for ($C_{17}H_{22}ClN_9O_3$) C, 46.85; H, 5.09; N, 28.92; Found: C, 46.87; H, 5.06; N, 28.94.

(2*R*,3*R*,4*S*,5*R*)-2-(6-(((1*R*,4*S*)-bicyclo[2.2.1]heptan-2-yl)amino)-9*H*-purin-9-yl)-5-(2-ethyl-2*H*-tetrazol-5-yl)tetrahydrofuran-3,4-diol (7). Reaction of **17** with (±)-*endo*-2-norbornylamine hydrochloride (3 mmol) and TEA (5.8 mmol) for 5 h followed by deprotection gave **7**, which was purified by chromatography on a silica gel column ($CHCl_3$-MeOH, 98:2) as a white solid (93% yield).^{1}H NMR (DMSO-d_6): δ 1.20-1.29 (m, 3H, norbornyl), 1.37-1.46 (m, 3H, norbornyl), 1.51 (t, *J* = 7.4 Hz, 3H, CH_2CH_3), 1.53-1.62 (m, 1H, norbornyl), 1.83-1.91 (m, 1H, norbornyl), 2.16 (s, 1H, norbornyl), 2.52 (s, 1H, norbornyl), 4.31-4.39 (m, 1H, NHC*H*), 4.58-4.64 (m, 1H, H-4'), 4.70 (q, *J* = 7.26 Hz, 2H, CH_2CH_3), 4.76-4.84 (m, 1H, H-3'), 5.18 (d, *J* = 4.7 Hz, 1H, H-5'), 5.74 (d, *J* = 5.6 Hz, 1H, OH), 5.81 (d, *J* = 6.0 Hz, 1H, OH), 6.11 (d, *J* = 4.7 Hz, 1H, H-2'), 7.81 (brs, 1H, NH), 8.20 (s, 1 H, H-2), 8.41 (s, 1H, H-8). ^{13}C NMR (DMSO-d_6): 14.23, 21.19, 29.09, 34.36, 36.35, 37.81, 38.88, 40.13, 51.89, 73.61, 76.72, 79.18, 87.71, 118.79, 138.64, 148.79, 152.69, 154.68, 164.27 ppm. MS (API-ESI): *m/z* 427.21 $[M + H]^+$. Anal. calcd. for ($C_{19}H_{25}N_9O_3$) C, 53.39; H, 5.90; N, 29.49; Found: C, 53.41; H, 5.88; N, 29.47.

(2*R*,3*R*,4*S*,5*R*)-2-(6-(((1*R*,4*S*)-bicyclo[2.2.1]heptan-2-yl)amino)-2-chloro-9*H*-purin-9-yl)-5-(2-ethyl-2*H*-tetrazol-5-yl)tetrahydrofuran-3,4-diol (8). Reaction of **18** with (±)-*endo*-2-norbornylamine hydrochloride (3 mmol) and TEA (5.8 mmol) for 4 h followed by deprotection gave **8**, which was purified by chromatography on a silica gel column ($CHCl_3$-MeOH, 98:2) as a white solid (74% yield).^{1}H NMR (DMSO-d_6): δ 1.22-1.28 (m, 3H, norbornyl), 1.36-1.48 (m, 3H, norbornyl), 1.53 (t, *J* = 7.4 Hz, 3H, CH_2CH_3), 1.55-1.63 (m, 1H, norbornyl), 1.82-1.89 (m, 1H, norbornyl), 2.18 (s, 1H, norbornyl), 2.54 (s, 1H, norbornyl), 4.19-4.29 (m, 1H, NHC*H*), 4.51-4.57 (m, 1H, H-4'), 4.68 (q, *J* = 6.2 Hz, 2H, CH_2CH_3), 4.73-4.78 (m, 1H, H-3'), 5.19 (d, *J* = 4.3 Hz, 1H, H-5'), 5.78 (d, *J* = 5.4 Hz, 1H, OH), 5.82 (d, *J* = 5.6 Hz, 1H, OH), 6.05 (d, *J* = 4.7 Hz, 1H, H-2'), 8.37 (brs, 1H, NH), 8.42 (s, 1H, H-8). ^{13}C NMR (DMSO-d_6): 14.18, 21.31, 29.12, 34.43, 36.39, 37.65, 38.56, 40.33, 52.09, 73.48, 76.61, 79.33, 87.82, 118.71, 138.46, 148.47, 153.46, 154.68, 164.32 ppm. MS (API-ESI): *m/z* 462.17 $[M + H]^+$. Anal. calcd. for ($C_{19}H_{24}ClN_9O_3$) C, 49.41; H, 5.24; N, 27.29; Found: C, 49.43; H, 5.22; N, 27.32.

(2*R*,3*S*,4*R*,5*R*)-2-(2-ethyl-2*H*-tetrazol-5-yl)-5-(6-((tetrahydrofuran-3-yl)amino)-9*H*-purin-9-yl)tetra hydrofuran-3,4-diol (9). Reaction of **17** with (*R*)-(+)-3-aminotetrahydrofuran toluene-4-sulfonate (1.6 mmol) and TEA (4.8 mmol) for 7 h followed by deprotection gave **9**, which was purified by chromatography on a silica gel column ($CHCl_3$-MeOH, 97:3) as a white solid (90% yield).^{1}H NMR (DMSO-d_6): δ 1.51 (t, *J* = 7.38 Hz, 3H, CH_2CH_3), 1.93–2.23 (2m, 2H, tetrahydrofuranyl), 3.56–3.93 (3m, 4H, tetrahydrofuranyl), 4.61 (q, *J* = 4.9 Hz, 1H, H-4'), 4.68-4.72 (m, 1H, NHC*H*), 4.74 (q, *J* = 7.3 Hz, 2H, CH_2CH_3), 4.81 (q, *J* = 5.1 Hz, 1H, H-3'), 5.18 (d, *J* = 4.3 Hz, 1H, H-5'), 5.75 (d, *J* = 5.6 Hz, 1H, OH), 5.81 (d, *J* = 6.0 Hz, 1H, OH), 6.12 (d, *J* = 5.1 Hz, 1H, H-2'), 7.98 (brs, 1H, NH), 8.22 (s, 1H, H-2), 8.42 (s, 1H, H-8). ^{13}C NMR (DMSO-d_6): 14.21, 31.92, 48.27, 50.94, 61.65, 66.54, 72.08, 73.84, 77.87, 87.81, 119.01, 138.89, 148.72, 152.16, 154.37, 164.21 ppm. MS (API-ESI): *m/z* 404.17 $[M + H]^+$. Anal. calcd. for ($C_{16}H_{21}N_9O_3$) C, 47.64; H, 5.25; N, 31.25; Found: C, 47.62; H, 5.27; N, 31.27.

(2*R*,3*R*,4*S*,5*R*)-2-(2-chloro-6-((tetrahydrofuran-3-yl)amino)-9*H*-purin-9-yl)-5-(2-ethyl-2*H*-tetrazol-5-yl)tetrahydrofuran-3,4-diol (10).
Reaction of **18** with (*R*)-(+)-3-aminotetrahydrofuran toluene-4-sulfonate (1.6 mmol) and TEA (4.8 mmol) for 8h followed by deprotection gave **10**, which was purified by chromatography on a silica gel column ($CHCl_3$-MeOH, 95:5) as a white solid (69% yield). ^{1}H NMR (DMSO-d_6): δ 1.49 (t, *J* = 7.4 Hz, 3H, CH_2CH_3), 1.91–2.22 (2m, 2H, tetrahydrofuranyl), 3.57–3.91 (3m, 4H, tetrahydrofuranyl), 4.52-4.58 (m, 1H, H-4'), 4.58-4.62 (m, 1H, NHC*H*), 4.71 (q, *J* = 7.5 Hz, 2H, CH_2CH_3), 4.78 (q, *J* = 5.1 Hz, 1H, H-3'), 5.21 (d, *J* = 4.3 Hz, 1H, H-5'), 5.82 (d, *J* = 5.6 Hz, 1H, OH), 5.88 (d, *J* = 6.0 Hz, 1H, OH), 6.05 (d, *J* = 5.1 Hz, 1H, H-2'), 8.43 (s, 1H, H-8), 8.58 (brs, 1H, NH). ^{13}C NMR (DMSO-d_6): 14.09, 31.59, 48.24, 50.97, 53.21, 66.61, 71.91, 73.53, 77.22, 87.69, 118.47, 139.44, 149.87, 153.22, 154.69, 164.12 ppm. MS (API-ESI): *m/z* 438.13 $[M + H]^+$. Anal. calcd. for ($C_{16}H_{20}ClN_9O_3$) C, 43.89; H, 4.60; N, 28.79; Found: C, 43.86; H, 4.82; N, 28.81.

(2*R*,3*R*,4*S*,5*R*)-2-(6-((4-chloro-2-fluorophenyl)amino)-9*H*-purin-9-yl)-5-(2-ethyl-2*H*-tetrazol-5-yl)tetrahydrofuran-3,4-diol (11). Reaction of **17** with 4-chloro-2-fluoro-aniline (1.6 mmol) for 6 h followed by deprotection gave **11**, which was purified by chromatography on a silica gel column ($CHCl_3$-MeOH, 98:2) as a white solid (66% yield).^{1}H NMR (DMSO-d_6): δ 1.48 (t, *J* = 7.4 Hz, 3H, CH_2CH_3), 4.62 (q, *J* = 4.7 Hz, 1H, H-4'), 4.73 (q, *J* = 7.3 Hz, 2H, CH_2CH_3), 4.82 (q, *J* = 5.1 Hz, 1H, H-3'), 5.22 (d, *J* = 4.7 Hz, 1H, H-5'), 5.78 (d, *J* = 5.6 Hz, 1H, OH), 5.83 (d, *J* = 6.0 Hz, 1H, OH), 6.18 (d, *J* = 5.1 Hz, 1H, H-2'), 7.26-7.29 (m,

1H, arom.), 7.48 (dd, *J* = 2.1, 10.3 Hz, 1H, arom.), 7.61 (t, *J* = 8.5 Hz, 1H, arom.), 8.25 (s, 1H, H-2), 8.54 (s, 1H, H-8), 9.65 (brs, 1H, NH). ^{13}C NMR (DMSO-d_6): 14.12, 48.21, 73.38, 73.82, 77.03, 87.88, 116.54, 119.83, 124.44, 125.68, 128.45, 129.52, 140.34, 150.06, 152.35, 155.08, 157.58, 164.14 ppm. MS (API-ESI): *m/z* 462.11 [M + H]$^+$. Anal. calcd. for ($C_{18}H_{17}ClN_9O_3$) C, 46.86; H, 3.71; N, 27.30; Found: C, 46.85; H, 3.73; N, 27.32.

(2*R*,3*R*,4*S*,5*R*)-2-(2-chloro-6-((4-chloro-2-fluorophenyl)amino)-9*H*-purin-9-yl)-5-(2-ethyl-2*H*-tetrazol-5-yl)tetrahydrofuran-3,4-diol (12). Reaction of **18** with 4-chloro-2-fluoro-aniline (1.6 mmol) for 9 h followed by deprotection gave **12**, which was purified by chromatography on a silica gel column ($CHCl_3$-MeOH, 98:2) as a white solid (61% yield). ^{1}H NMR (DMSO-d_6): δ 1.47 (t, *J* = 7.4 Hz, 3H, CH_2CH_3), 4.58 (q, *J* = 4.7 Hz, 1H, H-4'), 4.68 (q, *J* = 7.3 Hz, 2H, CH_2CH_3), 4.79 (q, *J* = 5.1 Hz, 1H, H-3'), 5.21 (d, *J* = 4.3 Hz, 1H, H-5'), 5.81 (d, *J* = 5.6 Hz, 1H, OH), 5.87 (d, *J* = 6.0 Hz, 1H, OH), 6.08 (d, *J* = 5.1 Hz, 1H, H-2'), 7.27-7.32 (m, 1H, arom.), 7.51-7.55 (m, 2H, arom.), 8.52 (s, 1H, H-8), 10.22 (brs, 1H, NH). ^{13}C NMR (DMSO-d_6): 14.18, 48.31, 73.66, 74.08, 77.33, 87.91, 116.23, 119.62, 124.21, 125.92, 128.76, 129.33, 141.02, 150.32, 152.21, 155.51, 157.73, 164.22 ppm. MS (API-ESI): *m/z* 496.07 [M + H]$^+$. Anal. calcd. for ($C_{18}H_{16}Cl_2FN_9O_3$) C, 43.56; H, 3.25; N, 25.40; Found: C, 43.54; H, 3.28; N, 25.41.

(2*R*,3*R*,4*S*,5*R*)-2-(2-chloro-6-((3-iodobenzyl)amino)-9*H*-purin-9-yl)-5-(2-ethyl-2*H*-tetrazol-5-yl) tetrahydrofuran-3,4-diol (13). Reaction of **18** with 3-iodobenzylamine hydrochloride (1.1 mmol) and TEA (3.1 mmol) for 9 h followed by deprotection gave **13**, which was purified by chromatography on a silica gel column ($CHCl_3$-MeOH, 98:2) as a white solid (61% yield). ^{1}H NMR (DMSO-d_6): δ 1.52 (t, *J* = 7.4 Hz, 3H, CH_2CH_3), 4.48-4.54 (m, 3H, H-4', CH_2Ph), 4.72 (q, *J* = 7.3 Hz, 2H, CH_2CH_3), 4.75 (q, *J* = 5.1 Hz, 1H, H-3'), 5.21 (d, *J* = 3.8 Hz, 1H, H-5'), 5.81 (d, *J* = 5.6 Hz, 1H, OH), 5.84 (d, *J* = 6.0 Hz, 1H, OH), 6.05 (d, *J* = 5.1 Hz, 1H, H-2'), 7.12 (t, *J* = 7.7 Hz, 1H, arom.), 7.32 (d, *J* = 7.3Hz, 1H, arom.), 7.58 (d, *J* = 7.7 Hz, 1H, arom.), 7.72 (s, 1H, arom.), 8.42 (s, 1H, H-8), 8.92 (t, *J* = 6.0 Hz, 1H, NH). ^{13}C NMR (DMSO-d_6): 14.22, 42.24, 48.35, 73.57, 74.18, 77.21, 87.78, 94.71, 119.72, 126.59, 130.47, 135.32, 135.68, 140.17, 142.88, 149.13, 152.54, 154.31, 164.14 ppm. MS (API-ESI): *m/z* 584.03 [M + H]$^+$. Anal. calcd. for ($C_{19}H_{19}ClN_9O_3$) C, 39.09; H, 3.28; N, 21.59; Found: C, 39.10; H, 3.31; N, 21.57.

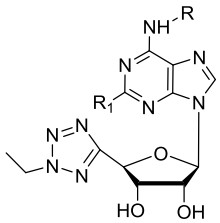
Membrane preparation

Membranes for radioligand binding were prepared as described earlier [2]. In brief, after homogenization of CHO cells stably transfected with the human adenosine receptor subtypes membranes were prepared in a two-step procedure. A first low-speed centrifugation (1,000 x g) was used to remove cell fragments and nuclei and was followed by a high-speed centrifugation (100,000 x g) of the supernatant in order to sediment a crude membrane fraction. The resulting membrane pellets were resuspended in the buffer used for the respective binding experiments, frozen in liquid nitrogen and stored in aliquots at 80°C. Adenylyl cyclase activity was measured in a membrane fraction obtained in a simplified procedure with only one high-speed centrifugation of the homogenate. The resulting crude membrane pellet was resuspended in 50 mM Tris/HCl, pH 7.4 and used immediately for the cyclase assay.

Radioligand binding

In competition experiments the following radioligands were used: 1 nM [^{3}H]CCPA for A_1 receptors, 10 nM [^{3}H]NECA for A_{2A} receptors, 1 nM [^{3}H]HEMADO for A_3 adenosine receptors [2, 3]. Nonspecific binding of [^{3}H]CCPA was determined in the presence of 1 mM theophylline, while nonspecific binding of [^{3}H]NECA and [^{3}H]HEMADO was estimated in the presence of 100 µM R-PIA. Dissociation constants (K_i-values) were calculated from radioligand competition experiments utilizing the program SCTFIT [4].
Due to the lack of a useful high-affinity radioligand for A_{2B} adenosine receptors, stimulation of adenylyl cyclase activity was measured to determine agonist potency (EC_{50}-values) [2]. If only partial agonistic activity was observed, efficacy was compared to 100 µM NECA as a full agonist. All values are given as geometric means with 95% confidence intervals ($n \geq 3$).

Table S1. Selectivity ratios for binding affinities



1-13

			Selectivity		
compd	R	R_1	A_{2A}/A_1	A_{2A}/A_3	A_3/A_1
1	H	H	9	2	4
2	H	Cl	21	12	2
3	CH_3	H	**220**	**1,630**	0.13
4	CH_3	Cl	**390**	**7,800**	0.05
5	cyclopentyl	H	**130**	12	11
6	cyclopentyl	Cl	**200**	16	13
7	(±)-*endo*-2-norbornyl	H	**140**	7	20
8	(±)-*endo*-2-norbornyl	Cl	**230**	10	22
9	tetrahydrofuranyl	H	**450**	**36**	13
10	tetrahydrofuranyl	Cl	**820**	**90**	9
11	2-fluoro-4-chloro-phenyl	H	**180**	**30**	6
12	2-fluoro-4-chloro-phenyl	Cl	**130**	**47**	3
13	2-iodo-benzyl	Cl	**45**	**180**	0.25

Values in bold mark compounds with selectivities ≥ 30 for both A_1 and A_3 vs A_{2A}. A_1 vs A_3 selectivity is ≤ 22 for all compounds.

Computational Chemistry

Molecular modeling and graphics manipulations were performed using the molecular operating environment (MOE) [5] and UCSF-CHIMERA software packages [6], running on a E4 Computer Engineering E1080 workstation provided of a Intel Core i7-930 Quad-Core processor. GOLD 5.2 [7] was used for all docking calculations. Figures were generated using Pymol 1.0 [8].

Residue Indexing

The convention used for the amino acid identifiers, according to the approach of Ballesteros and Weinstein, [9] facilitates comparison of aligned residues within the family of Group A GPCRs. To the most conserved residue in a given TM (TMX, where X is the TM number) is assigned the number X.50, and residues within a given TM are then indexed relative to the 50 position.

Three-Dimensional Structures of hA_1AR and hA_3AR

As, to date, no crystallographic information about the hA_1AR and hA_3AR is available, previously reported molecular models [10], built using the alignment and the homology modeling tools implemented in the program MOE, [11] were used in this study. The hA_1AR model was built using as template the crystal structure of the human $A_{2A}AR$ cocrystallized with the agonist UK-432097 (PDB ID: 3QAK), [12], while the $hA_{3A}R$ model was built using as template the crystal structure of the human $A_{2A}AR$ cocrystallized with the antagonist 6-(2,6-dimethylpyridin-4-yl)-5-phenyl-1,2,4-triazin-3-amine (T4G) (PDB ID: 3UZC) [13].

Docking simulations of 5'-*C*-tetrazole derivatives in the hA_1AR and hA_3AR models

Ligand structures were built using the MOE builder tool, as part of the MOE suite [11] and were subjected to a MMFF94x energy minimization until the rms conjugate gradient was < 0.05 kcal mol^{-1} $Å^{-1}$. The flexible docking of the ligands in the $hA_{1A}R$ and hA_3AR models was performed by means of the GOLD software, which uses a genetic algorithm for determining the docking modes of ligands and proteins. The coordinates of four key residues in the binding pocket of both hA_1AR and hA_3AR models, that is F171 (hA_1AR) or F168 (hA_3AR), $N^{6.55}$, $W^{6.48}$ and $H^{7.43}$, were chosen as active-site origin. The active-site radius was set equal to 13 Å. The mobility of $H^{7.43}$, $S^{7.42}$, $N^{2.50}$, $W^{6.48}$ and $L^{3.32}$ side chains was set up using the flexible side chains option in the GOLD front end, which incorporates the Lovell rotamer library [14]. Each GA run used the default parameters of 100 000 genetic operations on an initial population of 100 members divided into five subpopulations, with weights for crossover, mutation, and migration being set to 95, 95, and 10, respectively. GOLD allows a user-definable number of GA runs per ligand, each of which starts from a different orientation. For these experiments, the number of GA runs was set to 200 without the option of early termination, and scoring of the docked poses was performed with the original ChemPLP scoring function followed by rescoring with ChemScore [15]. The final receptor–ligand complex for each ligand was chosen interactively by selecting the highest scoring pose that was consistent with experimentally-derived information about the binding mode of the ligand.

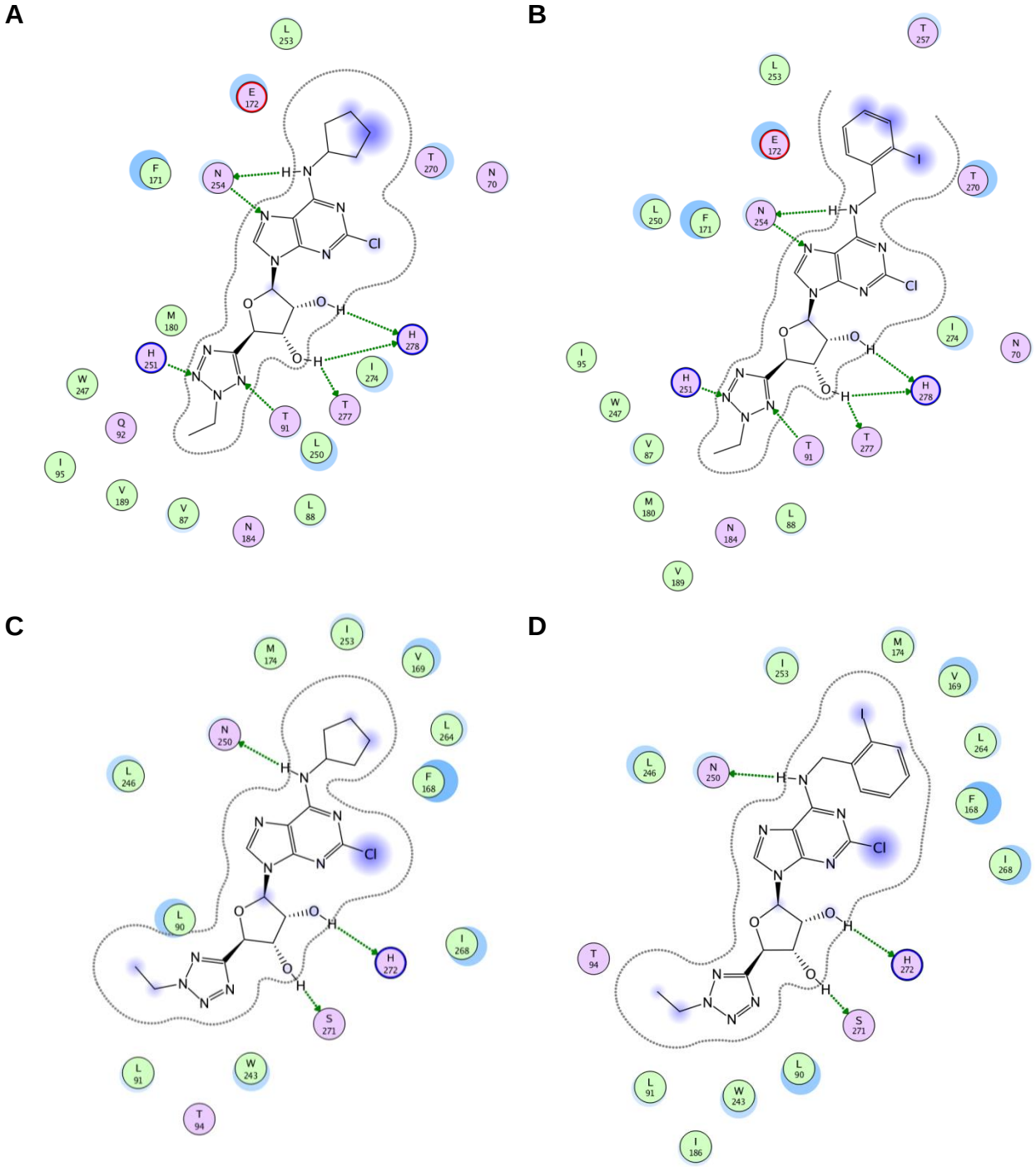


Figure S1. Ligand–receptor interaction diagram of compounds **6** and **13** docked into the hA_1AR (A and B) and hA_3AR (C and D) models (MOE 2013.08, Chemical Computing Group, Inc.). In these MOE interaction diagrams, green spheres = "greasy" residues; spheres with red outline = acidic residues; spheres with blue outline = basic residues; spheres with black outline = polar residues; blue background spheres = receptor exposure to solvent; blue spheres on ligand atoms = ligand exposure to solvent; green dotted lines = side chain donors/acceptors; gray dotted line = proximity contour.

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