Supporting Information:

Structure-Guided Design of A_3 Adenosine Receptor-Selective Nucleosides: Combination of 2-Arylethynyl and Bicyclo[3.1.0]hexane Substitutions

Dilip K. Tosh, Francesca Deflorian, Khai Phan, Zhan-Guo Gao, Tina C. Wan, Elizabeth Gizewski, John A. Auchampach, Kenneth A. Jacobson

Contents: page

Figures

Figure S1. Panel A: 2D depiction layout of the active sites of receptor-ligand complex. Spatial proximity and major interactions between the residues of A_3AR-ops and docked agonist **35** are indicated. Panel B: Docking pose of (N)-methanocarba adenosine derivative **35** in the binding site of the hybrid A_3AR-ops model. The nucleoside moiety of the ligand is anchored in the binding site by key H-bond interactions (highlighted with dotted lines) with the residues Thr94 (TM3), Asn250 (TM5), and Ser271 and His272 (TM7), while the C2 chain is located in a pocket among EL2, TM2, and TM7. The ligand is shown using the ball-and-stick representation, with carbon atoms colored in orange. The receptor residues are depicted in stick with grey carbons.

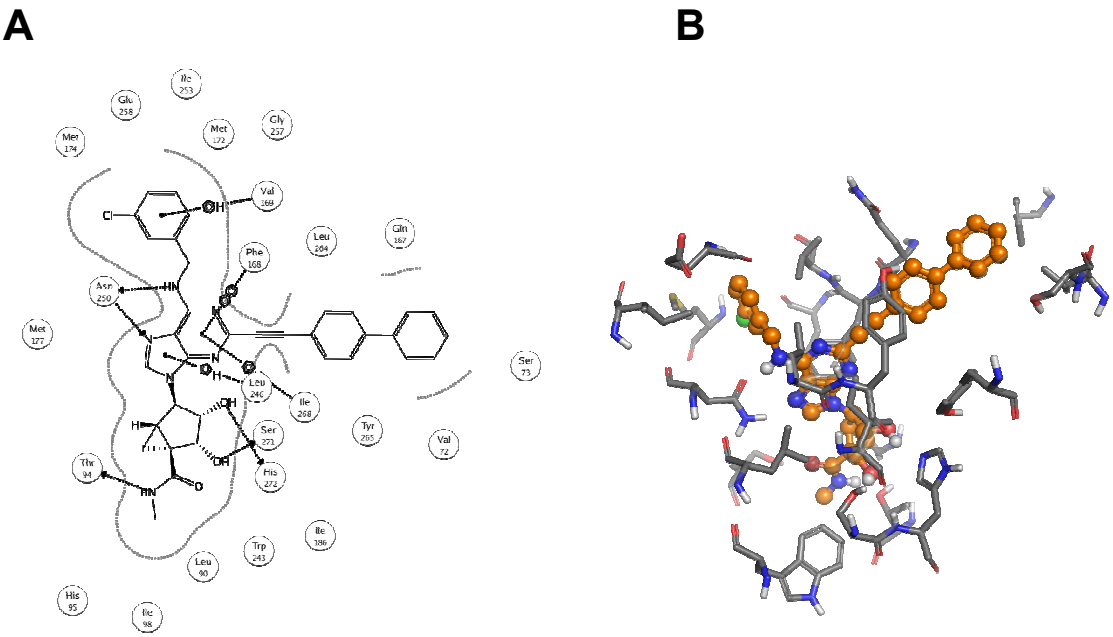


Figure S2. Docking poses of chlorophenylethynyl derivatives **14** (panel A) and **15** (panel B) in $A_{2A}AR$ structure.

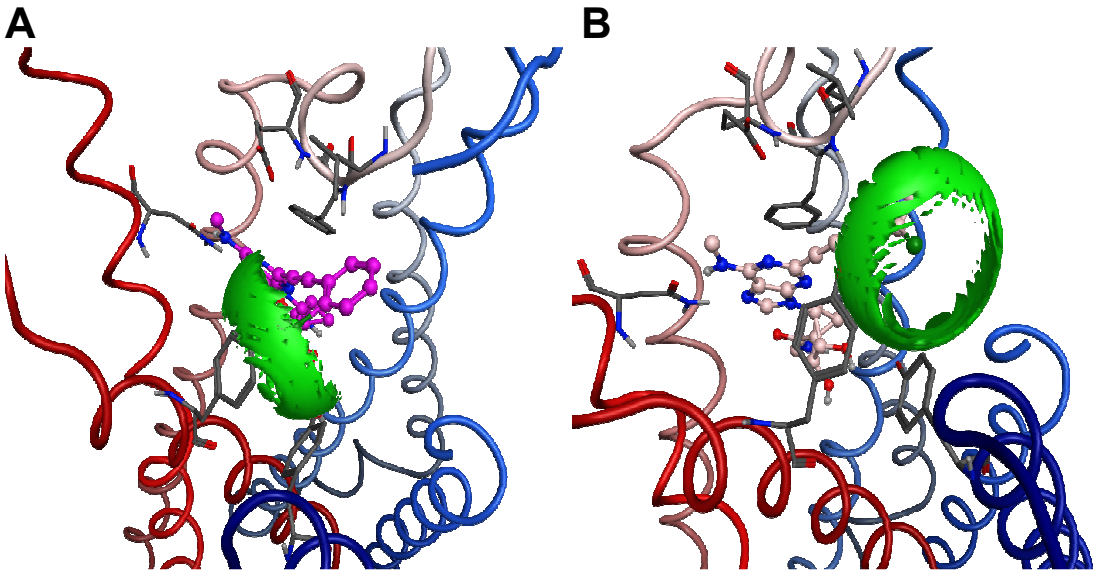


Figure S3. *o*-Cl **14** (A) and *m*-Cl **15** (B) N^6-methyl chlorophenylethynyl derivatives docked in the modified $hA_{2A}AR$ crystal structure. Compound **15** has enhanced affinity at this AR subtype.

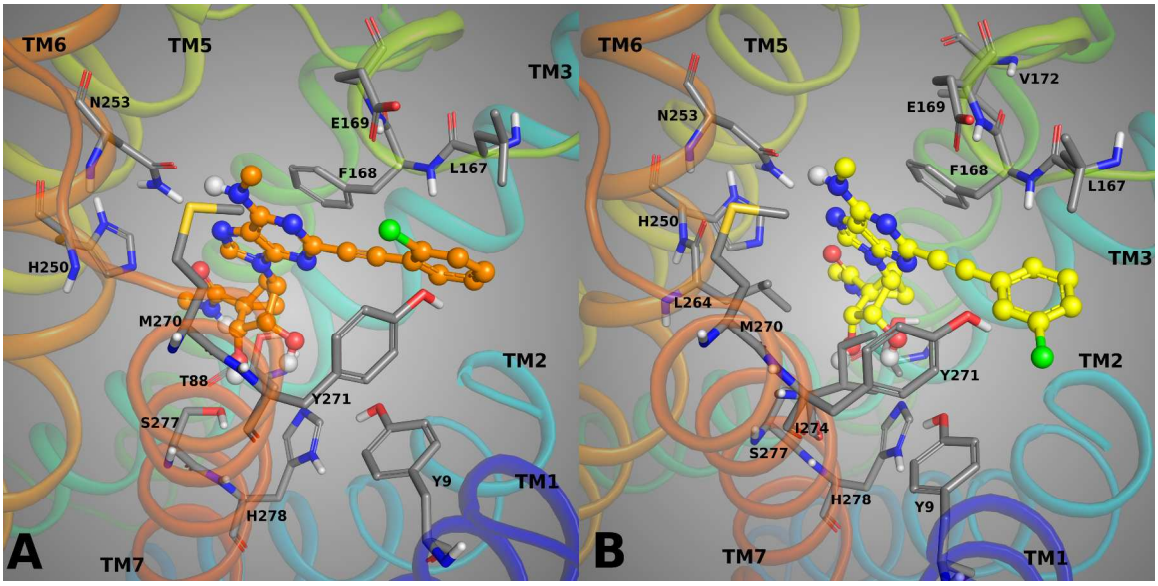


Figure S4. *o*-Cl **14** (A) and *m*-Cl **15** (B) N^6-methyl chlorophenylethynyl derivatives docked in the hA_3AR homology model (after movement of TM2). Compound **14** has enhanced affinity at this AR subtype.

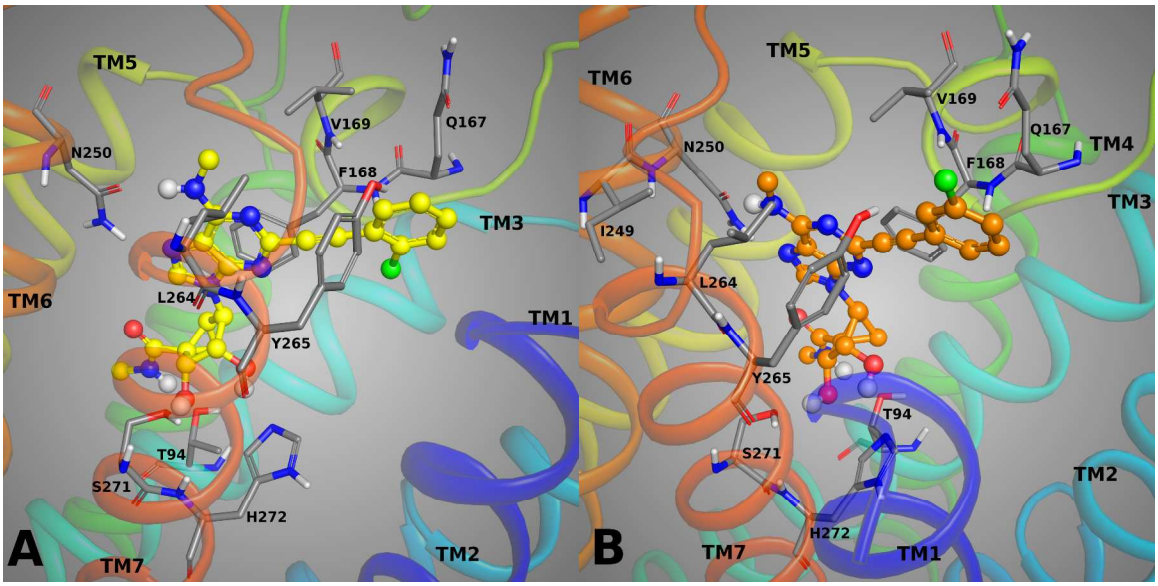
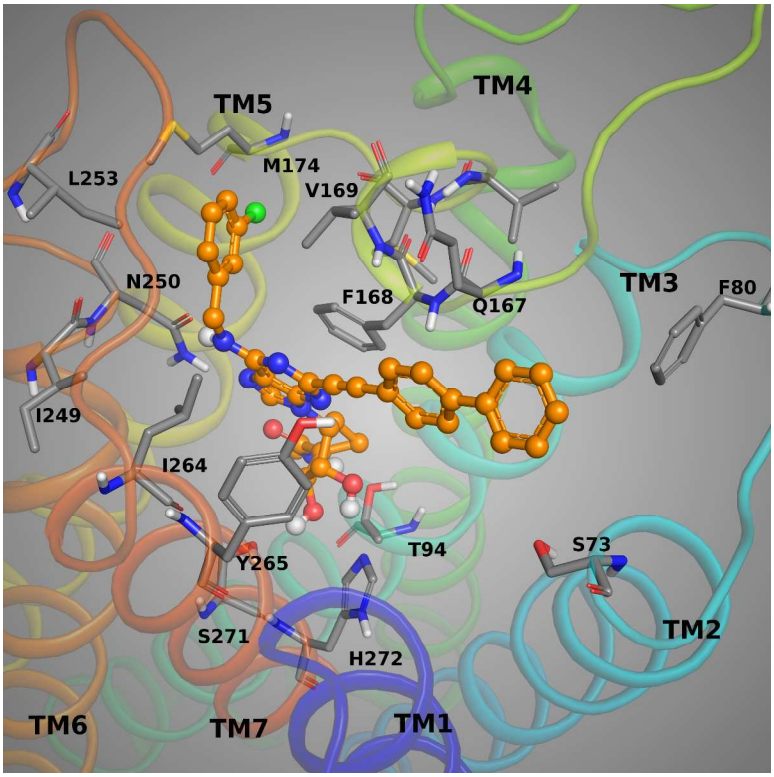


Figure S5. N^6-3-chlorobenzyl analogue **34** docked in the hA_3AR.



Modeling procedures

1) **Homology Modeling of A_3AR:** the crystal structure of the A_{2A}AR co-crystallized with the agonist UK-342,097, UK-A_{2A}AR, (PDB ID: 3QAK)[1] was used as structural template for the modeling of the agonist-bound A_3AR using MOE Homology Modeling[2] tool, as described in Tosh et al.[3] In this study, two more hybrid models of A_3AR were built using multiple template structures. The UK-A_{2A}AR structure was used as template for the entire A_3AR structure but the extracellular terminus of TM2 (residues from Val63 to Ser73 of A_3AR) and EL1 (residues from Leu74 to Tyr81 of A_3AR). The X-ray structure of the β_2 adrenergic receptor in complex with the Gs protein (PDB ID: 3SN6)[4] and the crystal structure of the opsin in its active conformation (PDB ID: 3DQB)[5] were used as templates to build the extracellular terminus of TM2. During the homology modeling of both the hybrid models of A_3AR, here called A_3AR-$\beta_2$adr and A_3AR-ops no structural template was used for the modeling of EL1. The alignment and homology modeling tools of MOE were used for the construction of the hybrid A_3AR models.
2) **Induced-fit docking of methanocarba derivatives in the A_3AR models:** the compounds structures were built in Maestro[6] and submitted to 1000 steps Polak-Ribiere conjugate gradient (PRCG) minimization until a gradient of 0.01 kJ/(mol Å^2). The molecular docking of the ligands was performed by means of the Induced Fit Docking (IFD) as in the Schrödinger package.[7] The docking site was defined with key residues in the binding pocket of the A_3AR model, namely Asn250, Ser271, His272, and Phe168, and a 26 Å x26 Å x26 Å box was centered on those residues. In the first stage of the IFD protocol, a docking of the ligands was performed in a rigid binding site with Glide 5.0 using the SP (standard precision) procedure using a van der Walls scaling of 0.5 for both receptor and ligand non polar atoms. For each methanocarba derivative, the top 20 docking conformations were retained and subjected to the receptor sampling by means of the Refinement module in Prime 2.0. The Prime side-chain sampling and energy minimization were performed on all the residues within a 5Å of the ligand in any of the 20 poses using the OPLS parameter and a surface Generalized Born implicit solvent model. The complexes within 30 kcal/mol of the minimum energy structure, ranked by Prime energy, were retained for the re-docking stage. In the final step, Glide with the XP (extra precision) scoring function and default parameters were used to re-dock all the ligands into their respective conformations produced by Prime. No constraints were used for all the docking calculations. The top scored model for each ligand was chosen as final binding conformation.
3) **Induced-fit docking of methanocarba derivatives in the A_{2A}AR structure:** three residues of the extracellular terminal of TM2 of the UK-A_{2A}AR structure (residues from Ile66 to Thr68) were removed before the IFD of agonists **14** and **15**, in order to avoid steric clash between the ligands and the residues in TM2 during the docking procedure. The same IFD parameters described above were applied for the docking of agonists **14** and **15**.

The graphical pictures were generated with the Pymol program.[8]

References (molecular modeling):

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3. Tosh, D.K., Phan, K., Deflorian, F., Wei, Q., Yoo, L.S., Gao, Z.G., Jacobson, K.A. Click Modification in the N(6) Region of A(3) Adenosine Receptor-Selective Carbocyclic Nucleosides for Dendrimeric Tethering that Preserves Pharmacophore Recognition. *Bioconjug. Chem.* **2012**, *23*, 232-247.
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6. Schrödinger, LLC, Portland, OR
7. Sherman, W., Day, T., Jacobson, M.P., Friesner, R.A., Farid, R. Novel Procedure for Modeling Ligand/Receptor Induced Fit Effects. *J. Med. Chem.*, **2006**, *49*, 534-553.
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Synthetic procedures for protected nucleosides **45 – 46**.

(1*S*,2*R*,3*S*,4*R*,5*S*)-(2,3-*O*-Isopropylidene)-*N*-methyl-4-(6-(methylamino)-2-(phenylethynyl)-9*H*-purin-9-yl)bicyclo[3.1.0]hexane-1-carboxamide (45a). $PdCl_2(PPh_3)_2$ (5.16 mg, 0.01 mmol), CuI (1.3 mg, 0.007 mmol), phenylacetylene (48 μL, 0.44 mmol) and triethylamine (0.1 mL, 0.73 mmol) was added to a solution of compound **43** (35.6 mg, 0.07 mmol) in anhydrous DMF (1 mL), and stirred at room temperature overnight. Solvent was evaporated under vacuum, and the residue was purified on flash silica gel column chromatography (CH_2Cl_2:MeOH = 40:1) to give compound **45a** (29 mg, 86%) as a syrup. ^{1}H NMR (CD_3OD) δ 8.13 (s, 1H), 7.73-7.70 (m, 2H), 7.47-7.44 (m, 3H), 5.81 (d, *J* = 7.2 Hz, 1H), 5.03 (s, 1H), 4.85 (d, *J* = 6.8 Hz, 1H), 3.15 (br s, 3H), 2.78 (s, 3H), 2.18-2.14 (m, 1H), 1.57-1.53 (m, 4H), 1.42 (t, *J* = 5.2 Hz, 1H), 1.31 (s, 3H). HRMS calculated for $C_{25}H_{27}N_6O_3$ $(M + H)^+$: 459.2145; found 459.2150.

(1*S*,2*R*,3*S*,4*R*,5*S*)-4-(2-((2-Fluorophenyl)ethynyl)-6-(methylamino)-9*H*-purin-9-yl)-2,3-*O*-isopropylidene-*N*-methylbicyclo[3.1.0]hexane-1-carboxamide (45b). Compound **45b** (79%) was prepared from compound **43** following the same method as used for compound **45a**. ^{1}H NMR (CD_3OD) δ 8.15 (s, 1H), 7.66-7.57 (m, 1H), 7.53-7.47 (m, 1H), 7.30-7.23 (m, 2H), 5.79 (d, *J* = 7.2 Hz, 1H), 5.04 (s, 1H), 3.15 (br s, 3H), 2.76 (s, 3H), 2.17-2.14 (m, 1H), 1.57-1.53 (m, 4H), 1.42 (t, *J* = 5.2 Hz, 1H), 1.31 (s, 3H). HRMS calculated for $C_{25}H_{26}FN_6O_3$ $(M + H)^+$: 477.2050; found 477.2040.

(1*S*,2*R*,3*S*,4*R*,5*S*)-4-(2-((3-Fluorophenyl)ethynyl)-6-(methylamino)-9*H*-purin-9-yl)-2,3-*O*-isopropylidene-*N*-methylbicyclo[3.1.0]hexane-1-carboxamide (45c). Compound **45c** (75%) was prepared from compound **43** following the same method as used for compound **45a**.^{1}H NMR (CD_3OD) δ 8.14 (s, 1H), 7.66-7.53 (m, 2H), 7.50-7.45 (m, 1H), 7.25-7.20 (m, 1H), 5.81 (d, *J* = 7.2 Hz, 1H), 5.03 (s, 1H), 3.15 (br s, 3H), 2.78 (s, 3H), 2.18-2.14 (m, 1H), 1.57-1.53 (m, 4H), 1.43 (t, *J* = 5.2 Hz, 1H), 1.31 (s, 3H). HRMS calculated for $C_{25}H_{26}FN_6O_3$ $(M + H)^+$: 477.2050; found 477.2052.

(1*S*,2*R*,3*S*,4*R*,5*S*)-4-(2-((4-Fluorophenyl)ethynyl)-6-(methylamino)-9*H*-purin-9-yl)-2,3-*O*-isopropylidene-*N*-methylbicyclo[3.1.0]hexane-1-carboxamide (45d). Compound **45d** (77%) was prepared from compound **43** following the same method as used for compound **45a**. ^{1}H NMR (CD_3OD) δ 8.14 (s, 1H), 7.78-7.72 (m, 2H), 7.23-7.19 (m, 2H), 5.80 (d, *J* = 6.8 Hz, 1H), 5.03 (s, 1H), 3.14 (br s, 3H), 2.77 (s, 3H), 2.17-2.14 (m, 1H), 1.56-1.43 (m, 4H), 1.43 (t, *J* = 5.6 Hz, 1H), 1.31 (s, 3H). HRMS calculated for $C_{25}H_{26}FN_6O_3$ $(M + H)^+$: 477.2050; found 477.2033.

(1*S*,2*R*,3*S*,4*R*,5*S*)-4-(2-((2-Chlorophenyl)ethynyl)-6-(methylamino)-9*H*-purin-9-yl)-2,3-*O*-isopropylidene-*N*-methylbicyclo[3.1.0]hexane-1-carboxamide (45e). Compound **45e** (81%) was prepared from compound **43** following the same method as used for compound **45a**. ^{1}H NMR (CD_3OD) δ 8.15 (s, 1H), 7.78 (d, *J* = 5.6 Hz, 1H), 7.57 (d, *J* = 6.8 Hz, 1H), 7.47-7.38 (m, 2H), 5.81 (d, *J* = 6.0 Hz, 1H), 5.04 (s, 1H), 4.91 (d, *J* = 7.2 Hz, 1H) 3.15 (br s, 3H), 2.74 (s, 3H), 2.17-2.13 (m, 1H), 1.56-1.52 (m, 4H), 1.41 (t, *J* = 5.2 Hz, 1H), 1.31 (s, 3H). HRMS calculated for $C_{25}H_{26}ClN_6O_3$ $(M + H)^+$: 493.1755; found 493.1749.

(1*S*,2*R*,3*S*,4*R*,5*S*)-4-(2-((3-Chlorophenyl)ethynyl)-6-(methylamino)-9*H*-purin-9-yl)-2,3-*O*-isopropylidene-*N*-methylbicyclo[3.1.0]hexane-1-carboxamide (45f). Compound **45f** (79%) was prepared from compound **43** following the same method as used for compound **45a**. ^{1}H NMR (CD_3OD) δ 8.15 (s, 1H), 7.75 (s, 1H), 7.67-7.63 (m, 1H), 7.50-7.42 (m, 2H), 5.81 (d, *J* = 6.8 Hz, 1H), 5.03 (s, 1H), 3.14 (br s, 3H), 2.78 (s, 3H), 2.18-2.15 (m, 1H), 1.57-

1.53 (m, 4H), 1.43 (t, *J* = 5.2 Hz, 1H), 1.31 (s, 3H). HRMS calculated for $C_{25}H_{26}ClN_6O_3$ (M + H)$^+$: 493.1755; found 493.1762.

(1*S*,2*R*,3*S*,4*R*,5*S*)-4-(2-((4-Chlorophenyl)ethynyl)-6-(methylamino)-9*H*-purin-9-yl)-2,3-*O*-isopropylidene-*N*-methylbicyclo[3.1.0]hexane-1-carboxamide (45g). Compound **45g** (82%) was prepared from compound **43** following the same method as used for compound **45a**. ^{1}H NMR (CD_3OD) δ 8.14 (s, 1H), 7.70 (d, *J* = 8.4 Hz, 2H), 7.47 (d, *J* = 8.4 Hz, 2H), 5.80 (d, *J* = 7.2 Hz, 1H), 5.03 (s, 1H), 3.14 (br s, 3H), 2.77 (s, 3H), 2.20-2.14 (m, 1H), 1.55-1.53 (m, 4H), 1.43 (t, *J* = 5.2 Hz, 1H), 1.31 (s, 3H). HRMS calculated for $C_{25}H_{26}ClN_6O_3$ (M + H)$^+$: 493.1755; found 493.1771.

(1*S*,2*R*,3*S*,4*R*,5*S*)-4-(2-((4-Bromophenyl)ethynyl)-6-(methylamino)-9*H*-purin-9-yl)-2,3-*O*-isopropylidene-*N*-methylbicyclo[3.1.0]hexane-1-carboxamide (45h). Compound **45h** (74%) was prepared from compound **43** following the same method as used for compound **45a**. ^{1}H NMR (CD_3OD) δ 8.13 (s, 1H), 7.67-7.61 (m, 2H), 7.57-7.55 (m, 2H), 5.79 (d, *J* = 7.2 Hz, 1H), 5.03 (s, 1H), 3.14 (br s, 3H), 2.78 (s, 3H), 2.21-2.14 (m, 1H), 1.54-1.53 (m, 4H), 1.43 (t, *J* = 5.2 Hz, 1H), 1.31 (s, 3H). HRMS calculated for $C_{25}H_{26}BrN_6O_3$ (M + H)$^+$: 537.1250; found 537.1234.

(1*S*,2*R*,3*S*,4*R*,5*S*)-4-(2-((3-Aminophenyl)ethynyl)-6-(methylamino)-9*H*-purin-9-yl)-2,3-*O*-isopropylidene-*N*-methylbicyclo[3.1.0]hexane-1-carboxamide (45i). Compound **45i** (71%) was prepared from compound **43** following the same method as used for compound **45a**.^{1}H NMR (CD_3OD) δ 8.12 (s, 1H), 7.16 (t, *J* = 8.0 Hz, 1H), 7.01 (t, *J* = 7.6 Hz, 2H), 6.80 (d, *J* = 6.4 Hz, 1H), 5.79 (d, *J* = 6.8 Hz, 1H), 5.03 (s, 1H), 3.15 (br s, 3H), 2.79 (s, 3H), 2.16-2.13 (m, 1H), 1.56-1.53 (m, 4H), 1.41 (t, *J* = 5.2 Hz, 1H), 1.31 (s, 3H). HRMS calculated for $C_{27}H_{33}N_7O_3$ (M + H)$^+$: 474.2254; found 474.2262.

(1*S*,2*R*,3*S*,4*R*,5*S*)-4-(2-((3,4-Difluorophenyl)ethynyl)-6-(methylamino)-9*H*-purin-9-yl)-2,3-*O*-isopropylidene-*N*-methylbicyclo[3.1.0]hexane-1-carboxamide (45j). Compound **45j** (81%) was prepared from compound **43** following the same method as used for compound **45a**. ^{1}H NMR (CD_3OD) δ 8.17 (s, 1H), 7.67-7.63 (m, 1H), 7.59-7.54 (m, 1H), 7.41-7.34 (m, 1H), 5.81 (d, *J* = 6.8 Hz, 1H), 5.03 (s, 1H), 3.14 (br s, 3H), 2.77 (s, 3H), 2.18-2.14 (m, 1H), 1.57-1.53 (m, 4H), 1.43 (t, *J* = 5.2 Hz, 1H), 1.31 (s, 3H). HRMS calculated for $C_{25}H_{25}F_2N_6O_3$ (M + H)$^+$: 495.1956; found 495.1945.

(1*S*,2*R*,3*S*,4*R*,5*S*)-4-(2-((3,5-Difluorophenyl)ethynyl)-6-(methylamino)-9*H*-purin-9-yl)-2,3-*O*-isopropylidene-*N*-methylbicyclo[3.1.0]hexane-1-carboxamide (45k). Compound **45k** (82%) was prepared from compound **43** following the same method as used for compound **45a**. ^{1}H NMR (CD_3OD) δ 8.15 (s, 1H), 7.68-7.55 (m, 1H), 7.38-7.35 (m, 1H), 7.14-7.09 (m, 1H), 5.81 (d, *J* = 7.2 Hz, 1H), 5.03 (s, 1H), 3.14 (br s, 3H), 2.77 (s, 3H), 2.18-2.15 (m, 1H), 1.56-1.53 (m, 4H), 1.44 (t, *J* = 5.2 Hz, 1H), 1.31 (s, 3H). HRMS calculated for $C_{25}H_{25}F_2N_6O_3$ (M + H)$^+$: 495.1956; found 495.1966.

(1*S*,2*R*,3*S*,4*R*,5*S*)-4-(2-((4-Ethylphenyl)ethynyl)-6-(methylamino)-9*H*-purin-9-yl)-2,3-*O*-isopropylidene-*N*-methylbicyclo[3.1.0]hexane-1-carboxamide (45l). Compound **45l** (78%) was prepared from compound **43** following the same method as used for compound **45a**. ^{1}H NMR (CD_3OD) δ 8.12 (s, 1H), 7.62 (d, *J* = 8.0 Hz, 2H), 7.30 (d, *J* = 8.0 Hz, 2H), 5.80 (d, *J* = 6.4 Hz, 1H), 5.03 (s, 1H), 3.15 (br s, 3H), 2.78 (s, 3H), 2.72 (q, *J* = 7.6 Hz, 2H), 2.17-2.13 (m, 1H), 1.57-1.53 (m, 4H), 1.42 (t, *J* = 5.2 Hz, 1H), 1.31-1.28 (m, 6H). HRMS calculated for $C_{27}H_{31}N_6O_3$ (M + H)$^+$: 487.2458; found 487.2451.

(1*S*,2*R*,3*S*,4*R*,5*S*)-4-(2-((4-*tert*-Butylphenyl)ethynyl)-6-(methylamino)-9*H*-purin-9-yl)-2,3-*O*-isopropylidene-*N*-methylbicyclo[3.1.0]hexane-1-carboxamide (45m). Compound

45m (74%) was prepared from compound **43** following the same method as used for compound **45a**. ^{1}H NMR (CD_3OD) δ 8.15 (s, 1H), 7.64 (d, *J* = 8.0 Hz, 2H), 7.50 (d, *J* = 8.0 Hz, 2H), 5.81 (d, *J* = 6.8 Hz, 1H), 5.04 (s, 1H), 3.15 (br s, 3H), 2.78 (s, 3H), 2.19-2.13 (m, 1H), 1.58-1.53 (m, 4H), 1.42 (t, *J* = 5.2 Hz, 1H), 1.37 (s, 9H), 1.33 (s, 3H). HRMS calculated for $C_{29}H_{35}N_6O_3$ $(M + H)^+$: 515.2771; found 515.2751.

(1*S*,2*R*,3*S*,4*R*,5*S*)-4-(2-((4-Acetylphenyl)ethynyl)-6-(methylamino)-9*H*-purin-9-yl)-2,3-*O*-isopropylidene-*N*-methylbicyclo[3.1.0]hexane-1-carboxamide (45n). Compound **45n** (82%) was prepared from compound **43** following the same method as used for compound **45a**. ^{1}H NMR (CD_3OD) δ 8.15 (s, 1H), 8.08 (d, *J* = 8.4 Hz, 2H), 7.84 (d, *J* = 8.4 Hz, 2H), 5.81 (d, *J* = 6.8 Hz, 1H), 5.04 (s, 1H), 3.14 (br s, 3H), 2.77 (s, 3H), 2.65 (s, 3H), 2.19-2.15 (m, 1H), 1.56-1.53 (m, 4H), 1.44 (t, *J* = 5.2 Hz, 1H), 1.31 (s, 3H). HRMS calculated for $C_{27}H_{29}N_6O_4$ $(M + H)^+$: 501.2250; found 501.2245.

(1*S*,2*R*,3*S*,4*R*,5*S*)-4-(2-(Biphenyl-4-ylethynyl)-6-(methylamino)-9*H*-purin-9-yl)-2,3-*O*-isopropylidene-*N*-methylbicyclo[3.1.0]hexane-1-carboxamide (45o). Compound **45o** (85%) was prepared from compound **43** following the same method as used for compound **45a**. ^{1}H NMR (CD_3OD) δ 8.13 (s, 1H), 7.79 (d, *J* = 8.4 Hz, 2H), 7.74-7.68 (m, 4H), 7.48 (t, *J* = 7.2 Hz, 2H), 7.41-7.37 (m, 1H), 5.82 (d, *J* = 6.8 Hz, 1H), 5.04 (s, 1H), 3.16 (br s, 3H), 2.80 (s, 3H), 2.18-2.15 (m, 1H), 1.57-1.54 (m, 4H), 1.43 (t, *J* = 5.2 Hz, 1H), 1.34 (s, 3H). HRMS calculated for $C_{31}H_{31}N_6O_4$ $(M + H)^+$: 535.2458; found 535.2477.

(1*S*,2*R*,3*S*,4*R*,5*S*)-4-(2-((Naphthalene-1-yl)ethynyl)-6-(methylamino)-9*H*-purin-9-yl)-2,3-*O*-isopropylidene-*N*-methylbicyclo[3.1.0]hexane-1-carboxamide (45p). Compound **45p** (76%) was prepared from compound **43** following the same method as used for compound **45a**. ^{1}H NMR (CD_3OD) δ 8.56 (d, *J* = 8.4 Hz, 1H), 8.15 (s, 1H), 8.01-7.96 (m, 2H), 7.75-7.51 (m, 4H), 5.85 (d, *J* = 7.2 Hz, 1H), 5.08 (s, 1H), 4.93 (d, *J* = 7.2 Hz, 1H) 3.15 (br s, 3H), 2.68 (s, 3H), 2.21-2.18 (m, 1H), 1.57-1.54 (m, 4H), 1.43 (t, *J* = 5.2 Hz, 1H), 1.34 (s, 3H). HRMS calculated for $C_{29}H_{28}N_6O_3Na$ $(M + Na)^+$: 531.2121; found 531.2114.

(1*S*,2*R*,3*S*,4*R*,5*S*)-4-(2-((Phenanthren-9-yl)ethynyl)-6-(methylamino)-9*H*-purin-9-yl)-2,3-*O*-isopropylidene-*N*-methylbicyclo[3.1.0]hexane-1-carboxamide (45q). Compound **45b** (71%) was prepared from compound **43** following the same method as used for compound **45a**. ^{1}H NMR (CD_3OD) δ 8.87-8.78 (m, 2H), 8.67-8.66 (m, 1H), 8.35 (s, 1H), 8.15 (s, 1H), 8.02-7.99 (m, 2H), 7.79-7.63 (m, 3H), 5.88 (d, *J* = 6.8 Hz, 1H), 5.09 (s, 1H), 4.95 (d, *J* = 7.2 Hz, 1H) 3.18 (br s, 3H), 2.69 (s, 3H), 2.25-2.18 (m, 1H), 1.58-1.54 (m, 4H), 1.44 (t, *J* = 5.2 Hz, 1H), 1.35 (s, 3H). HRMS calculated for $C_{33}H_{31}N_6O_3$ $(M + H)^+$: 559.2458; found 559.2462.

(1*S*,2*R*,3*S*,4*R*,5*S*)-(2,3-*O*-Isopropylidene)-*N*-methyl-4-(6-(methylamino)-2-(pyridin-2-ylethynyl)-9*H*-purin-9-yl)bicyclo[3.1.0]hexane-1-carboxamide (46). Compound **46** (78%) was prepared from compound **43** following the same method as used for compound **45a**.^{1}H NMR (CD_3OD) δ 8.65 (d, *J* = 4.4 Hz, 1H), 8.16 (s, 1H), 7.98 (t, *J* = 6.0 Hz, 1H), 7.85 (d, *J* = 8.0 Hz, 1H), 7.57-7.51 (m, 1H), 6.00 (d, *J* = 6.8 Hz, 1H), 5.01 (s, 1H), 4.95 (d, *J* = 7.2 Hz, 1H), 3.13 (br s, 3H), 2.73 (s, 3H), 2.11-2.07 (m, 1H), 1.59-1.56 (m, 4H), 1.41 (t, *J* = 5.2 Hz, 1H), 1.33 (s, 3H). HRMS calculated for $C_{24}H_{26}N_7O_3$ $(M + H)^+$: 460.2097; found 460.2079.

Synthetic procedures for arylalkynyl intermediate **51**.

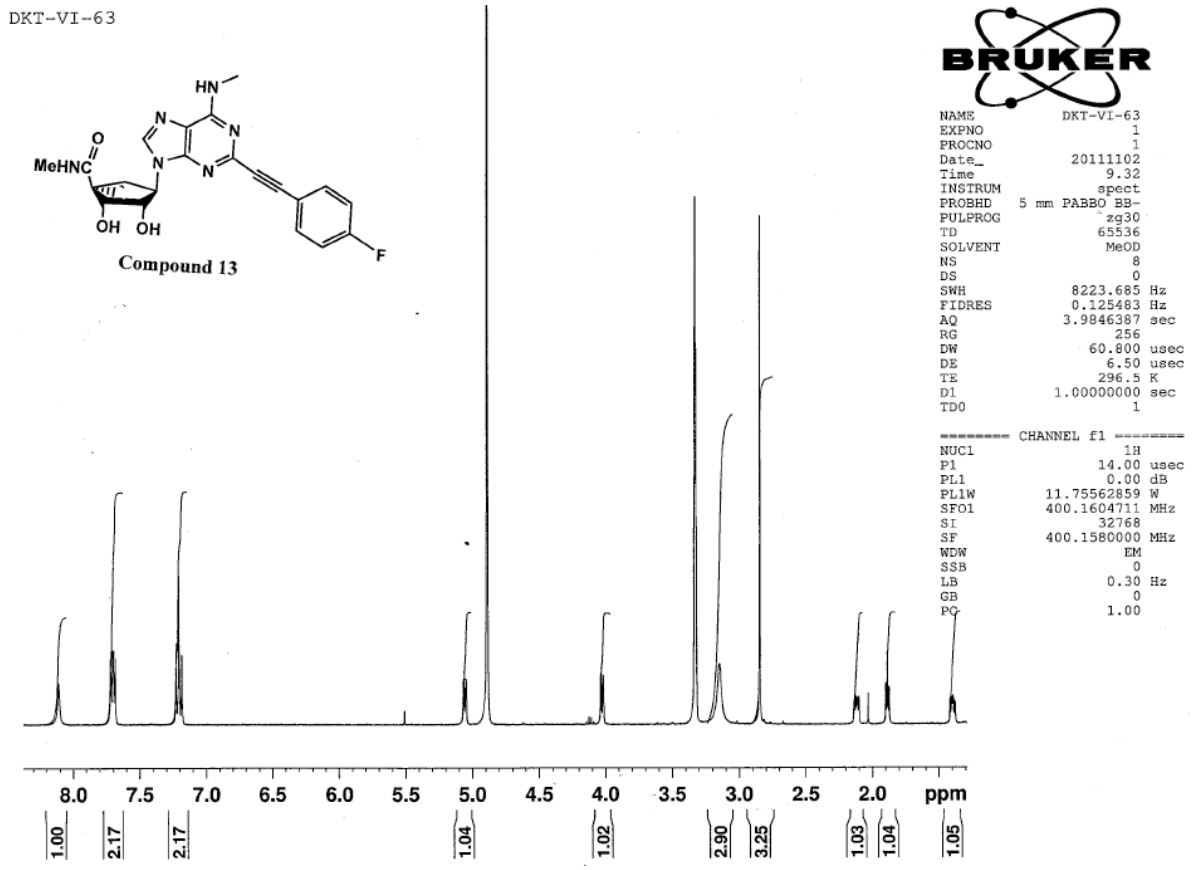
Trimethyl(pyren-4-ylethynyl)silane (50)

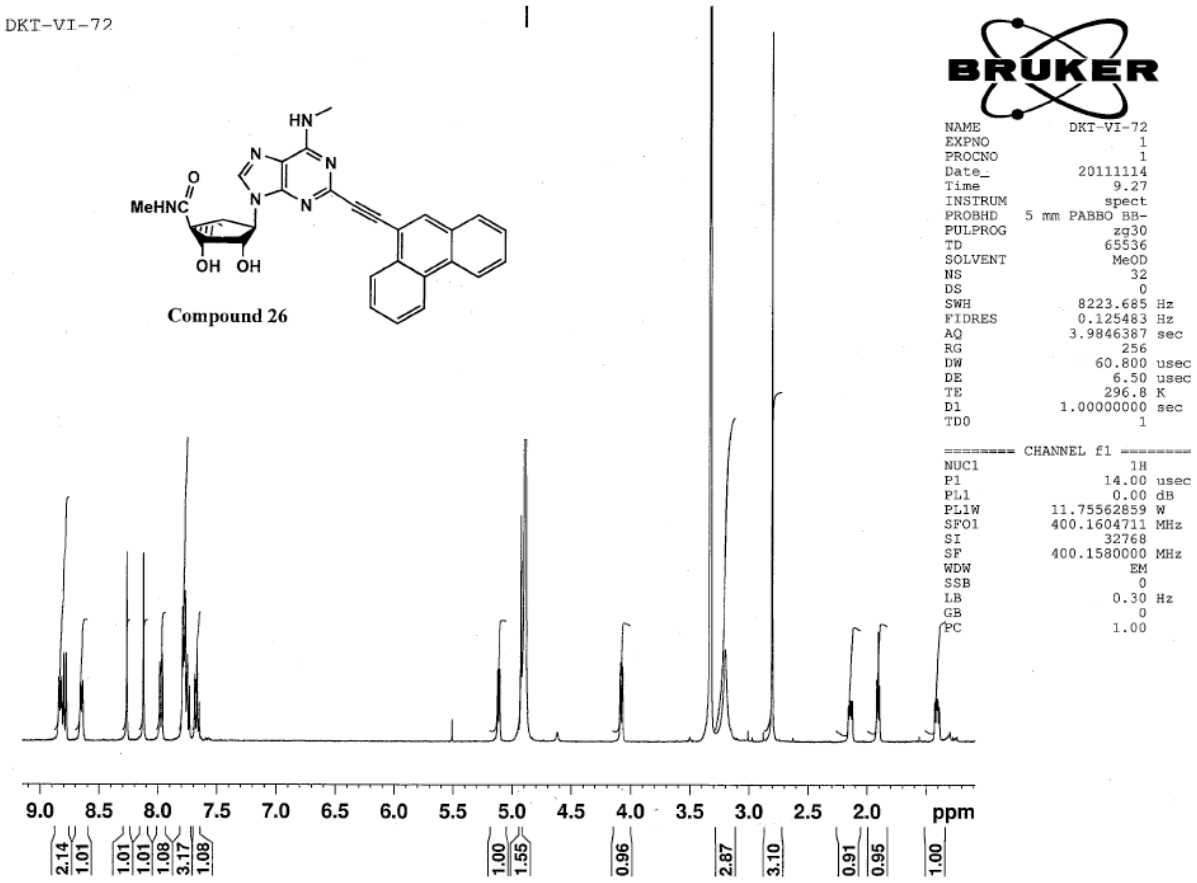
$PdCl_2(PPh_3)_2$ (60 mg, 0.08 mmol), CuI (8 mg, 0.04 mmol), trimethylsilyl acetylene (0.36 mL, 2.58 mmol) and triethylamine (0.6 mL, 4.3 mmol) was added to a solution of 4-bromo-pyrene (121 mg, 0.43 mmol) in anhydrous DMF (2.5 mL), and heated at 60 ^oC for overnight. Solvent was evaporated under vacuum, and the residue was purified on flash silica gel column chromatography by eluting with only hexane to compound **50** (78 mg, 62%) as a syrup. ^1H NMR (CD_3OD) δ 8.61 (dd, J_1 = 1.2, J_2 = 6.8 Hz, 1H), 8.33 (s, 1H), 8.27-8.25 (m, 1H), 8.23-8.19 (m, 2H), 8.11-8.08 (m, 3H), 8.02 (d, J = 3.6 Hz, 1H), 0.40 (s, 9H). HRMS calculated for $C_{21}H_{18}Si$ (M^+): 298.1178; found 298.1178.

4-Ethynylpyrene (51)

Tetrabutylammonium fluoride (0.17 mL, 1M solution in THF) was added to a solution of compound **50** (43 mg, 0.14 mmol) in dry THF (1.5 mL) and stirred at room temperature for 1h. Solvent was evaporated under vacuum and the residue was purified on flash silica gel column chromatography (hexane:ethylacetate = 70:1) to give 4-ethynylpyrene **51** (25 mg, 77%) as white powder. ^1H NMR (CD_3OD) δ 8.72 (d, J = 7.6 Hz, 1H), 8.41 (s, 1H), 8.24 (t, J = 8.0 Hz, 2H), 8.18 (d, J = 7.6 Hz, 1H), 8.13-8.05 (m, 3H), 8.03 (t, J = 7.6 Hz, 1H), 3.58 (s, 1H). HRMS calculated for $C_{18}H_{10}$ (M^+): 226.0783; found 226.0783.

Spectroscopic characterization of representative nucleoside derivatives 13, 26, 29, and 34.

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N
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O
OH OH
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PROCNO 1
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FIDRES 0.125483 Hz
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RG 256
DW 60.800 usec
DE 6.50 usec
TE 296.8 K
D1 1.00000000 sec
TD0 1
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PL1 0.00 dB
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SFO1 400.1604711 MHz
SI 32768
SF 400.1580000 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
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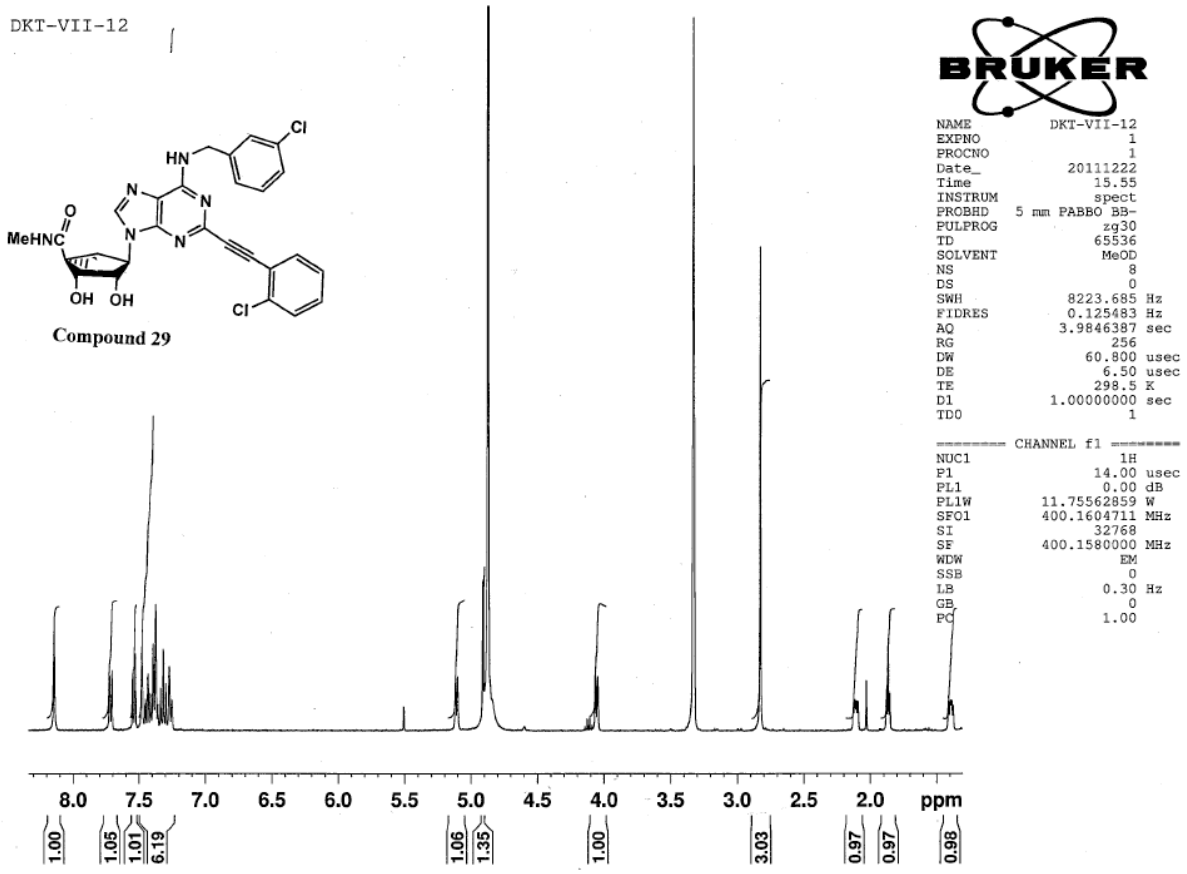
2.14 1.01 1.01 1.01 1.08 3.17 1.08 1.00 1.55 0.96 2.87 3.10 0.91 0.95 1.00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PROCNO 1
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DE 6.50 usec
TE 298.5 K
D1 1.00000000 sec
TD0 1
CHANNEL f1
NUC1 1H
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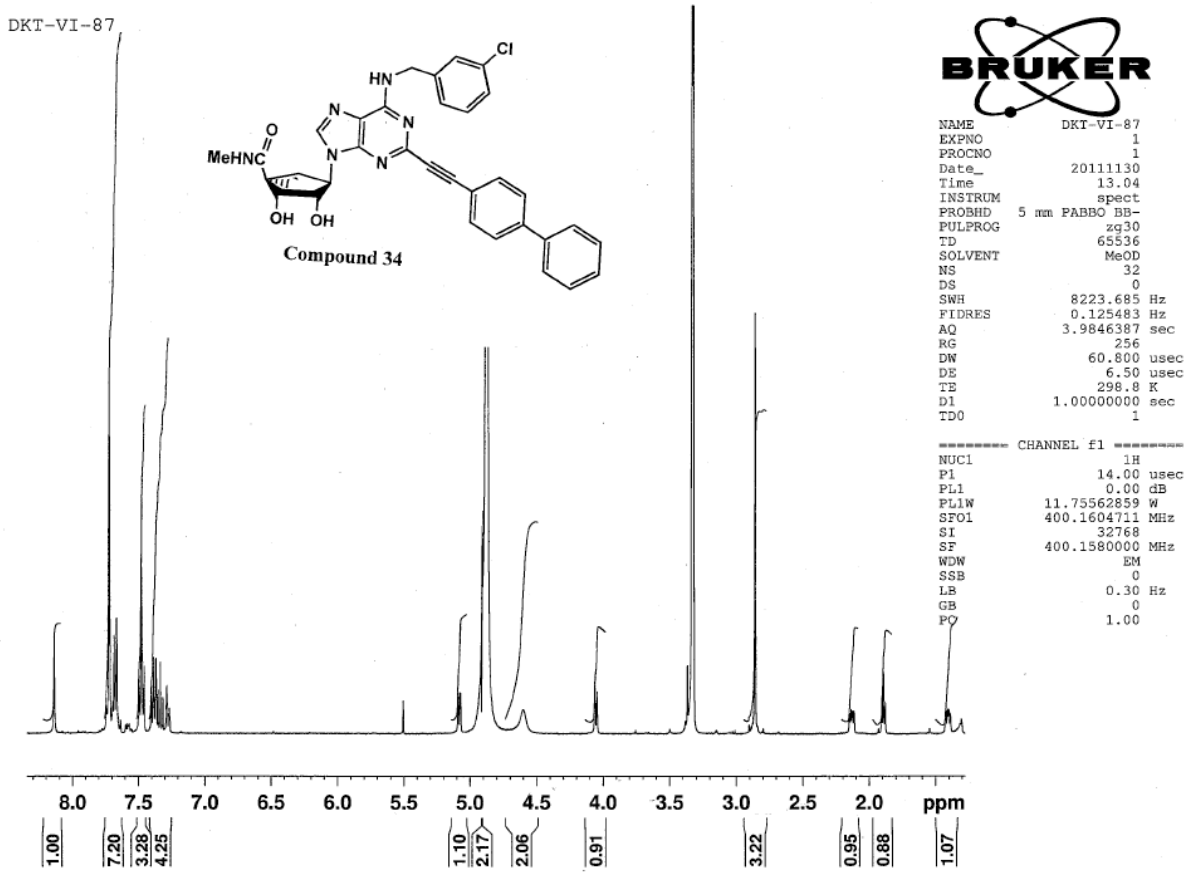
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 ppm
1.00 1.05 1.01 6.19 1.06 1.35 1.00 3.03 0.97 0.97 0.98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N
N
N
O
MeHNC
OH OH
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PULPROG zg30
TD 65536
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FIDRES 0.125483 Hz
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RG 256
DW 60.800 usec
DE 6.50 usec
TE 298.8 K
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TD0 1
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NUC1 1H
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SI 32768
SF 400.1580000 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 ppm
1.00 7.20 3.28 4.25 1.10 2.17 2.06 0.91 3.22 0.95 0.88 1.07

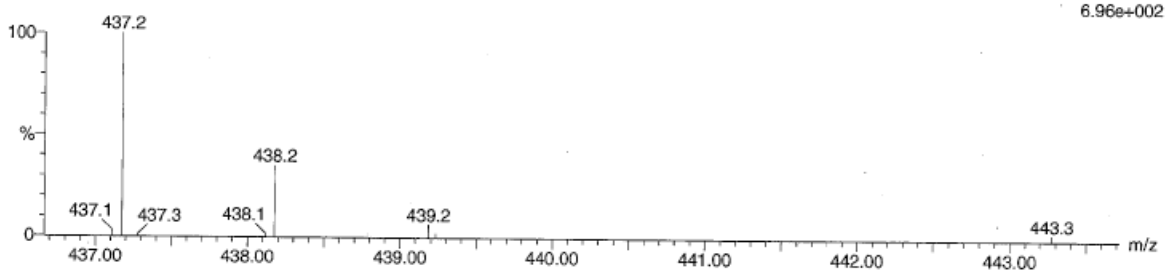
Single Mass Analysis
Tolerance = 9.0 mDa / DBE: min = -1.5, max = 65.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
245 formula(e) evaluated with 5 results within limits (up to 50 closest results for each mass)
Elements Used:
C: 0-500 H: 0-1000 N: 0-6 O: 0-6 F: 1-1

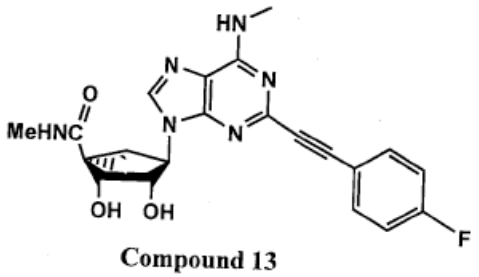
02-Nov-2011 10:47:12
TOF MS ES+

DKT-VI-63_436 101 (1.718) Cn (Cen,7, 50.00, Ar); Sm (SG, 2x3.00); Sb (12,10.00)

6.96e+002



Minimum:				-1.5		
Maximum:		9.0	10.0	65.0		
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Formula
437.1722	437.1737	-1.5	-3.4	14.5	7.1	C22 H22 N6 O3 F
	437.1706	1.6	3.7	22.5	0.4	C33 H22 F
	437.1764	-4.2	-9.6	13.5	3.0	C26 H26 O5 F
	437.1778	-5.6	-12.8	18.5	1.7	C27 H22 N4 O F
	437.1665	5.7	13.0	18.5	1.4	C28 H22 N2 O2 F



Compound 13

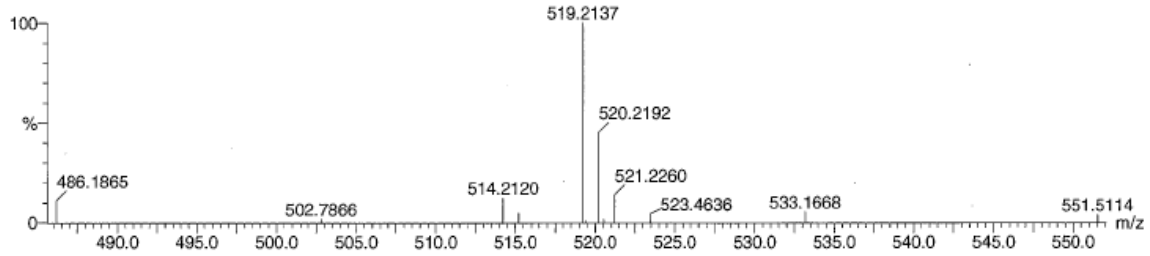
Single Mass Analysis
Tolerance = 9.0 mDa / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
104 formula(e) evaluated with 4 results within limits (up to 50 closest results for each mass)
Elements Used:
C: 0-500 H: 0-1000 N: 6-6 O: 0-21

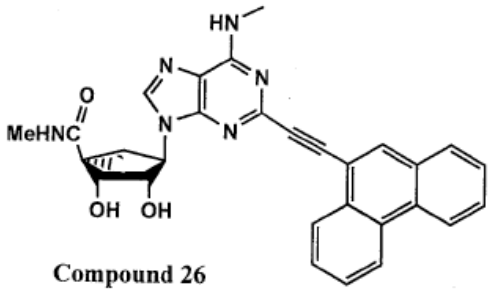
14-Nov-2011 10:22:08
TOF MS ES+

dkt-vi-72 125 (2.126) AM (Cen,9, 50.00, Ht,10000.0,0.00,1.00); Sm (SG, 2x3.00)

7.10e+001



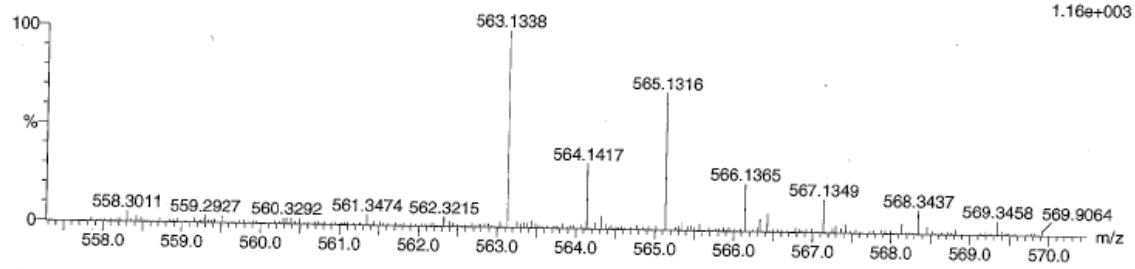
Minimum:				-1.5		
Maximum:		9.0	10.0	50.0		
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Formula
519.2137	519.2145	-0.8	-1.5	20.5	1.6	C30 H27 N6 O3
	519.2110	2.7	5.2	-1.5	8.0	C12 H35 N6 O16
	519.2203	-6.6	-12.7	11.5	3.4	C23 H31 N6 O8
	519.2051	8.6	16.6	7.5	4.9	C19 H31 N6 O11



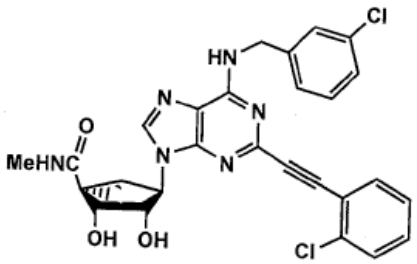
Compound 26

Single Mass Analysis
Tolerance = 12.0 mDa / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
110 formula(e) evaluated with 2 results within limits (up to 50 closest results for each mass)
Elements Used:
C: 0-500 H: 0-1000 N: 4-7 O: 2-6 35Cl: 2-2
22-Dec-2011 11:42:15
TOF MS ES+
SKT-VII-12_563r 132 (2.246) Cn (Cen,5, 50.00, Ar); Sm (SG, 2x3.00); Sb (12,10.00); Cm (110:148)



Minimum:				-1.5		
Maximum:		12.0	10.0	50.0		
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Formula
563.1338	563.1365	-2.7	-4.8	18.5	334.9	C28 H25 N6 O3 35Cl2
	563.1253	8.5	15.1	18.5	332.1	C29 H25 N4 O4 35Cl2



Compound 29

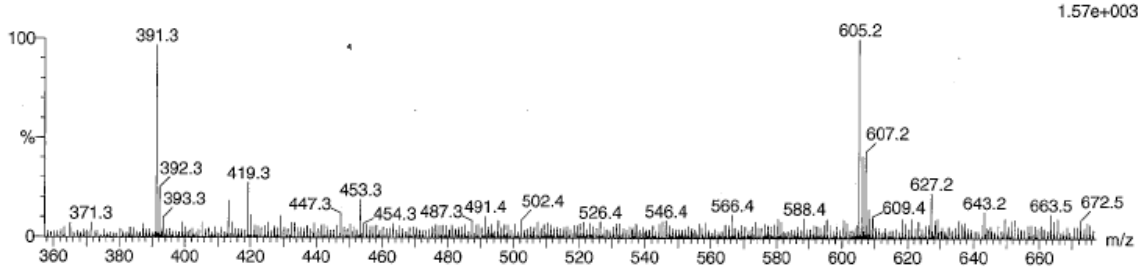
Single Mass Analysis
Tolerance = 5.0 mDa / DBE: min = -1.5, max = 50.0
Element prediction: Off
Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions
166 formula(e) evaluated with 2 results within limits (up to 50 closest results for each mass)
Elements Used:
C: 0-500 H: 0-1000 N: 4-8 O: 0-4 35Cl: 1-1

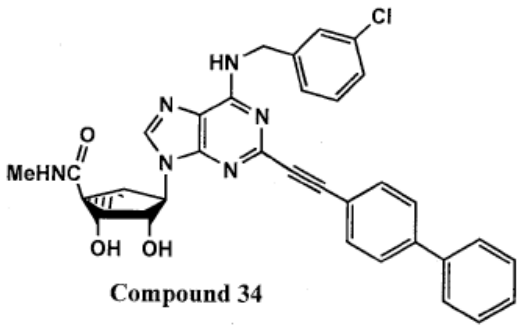
30-Nov-2011 13:19:51
TOF MS ES+

DKT-VI-87_605 167 (2.841) Cn (Cen,7, 50.00, Ar); Sm (SG, 2x3.00); Sb (12,10.00); Cm (163:177)

1.57e+003



Minimum:		5.0	10.0	-1.5		
Maximum:				50.0		
Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Formula
605.2083	605.2068	1.5	2.5	22.5	214.0	C34 H30 N6 O3 35Cl
	605.2108	-2.5	-4.1	26.5	200.8	C39 H30 N4 O 35Cl



Compound 34

Pharmacological assay procedures

Receptor binding and functional assays (see main text for references)

[^{3}H]R-*N*^{6}-Phenylisopropyladenosine (**52**, [^{3}H]R-PIA, 63 Ci/mmol), [^{125}I]*N*^{6}-(4-Amino-3-iodobenzyl)adenosine-5′-*N*-methyluronamide (**53**, [^{125}I]I-AB-MECA, 2200 Ci/mmol), and [^{3}H](2-[p-(2-carboxyethyl)phenyl-ethylamino]-5′-*N*-ethylcarboxamido-adenosine) (**54**, [^{3}H]CGS21680, 40.5 Ci/mmol) were purchased from Perkin–Elmer Life and Analytical Science (Boston, MA). Test compounds were prepared as 5 mM stock solutions in DMSO and stored frozen.

Cell Culture and Membrane Preparation - CHO cells stably expressing the recombinant hA_1, hA_3, and rA_3Rs, and HEK-293 cells stably expressing the $hA_{2A}AR$ were cultured in Dulbecco's modified Eagle medium (DMEM) and F12 (1:1) supplemented with 10% fetal bovine serum, 100 units/mL penicillin, 100 μg/mL streptomycin, and 2 μmol/mL glutamine. In addition, 800 μg/mL geneticin was added to the A_{2A} media, while 500 μg/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 ^oC. The suspension was homogenized with an electric homogenizer for 10 sec, pipetted 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, IL).[48]

Binding assays: Into each tube in the binding assay was added 50 μL of increasing concentrations of the test ligand in Tris-HCl buffer (50 mM, pH 7.5) containing 10 mM $MgCl_2$, 50 μL of the appropriate agonist radioligand, and finally 100 μL of membrane suspension. For the A_1AR (22 μg of protein/tube) the radioligand used was [^{3}H]**52** (final concentration of 3.5 nM). For the $A_{2A}AR$ (20 μg/tube) the radioligand used was [^{3}H]**53** (10 nM). For the A_3AR (21 μg/tube) the radioligand used was [^{125}I]**54** (0.34 nM). Nonspecific binding was determined using a final concentration of 10 μM adenosine-5'-*N*-ethylcarboxamide (NECA) **48** 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_3AR binding were counted using a Packard Cobra II γ-counter. The K_i values were determined using GraphPad Prism for all assays.

Similar competition binding assays were conducted using HEK 293 cell membranes expressing mARs using [^{125}I]**54** to label A_1 or A_3ARs and [^{3}H]**53** to label $A_{2A}ARs$.[49] IC_{50} values were converted to K_i values as described.[50] Nonspecific binding was determined in the presence of 200 μM NECA **48**.

cAMP accumulation assay: Intracellular cAMP levels were measured with a competitive protein binding method.[34] CHO cells that expressed the recombinant hA_3AR were harvested by trypsinization. After centrifugation and resuspended in medium, cells were planted in 96-well plates in 0.1 mL medium. After 24 h, the medium was removed and cells

were washed three times with 0.2 mL DMEM, containing 50 mM HEPES, pH 7.4. Cells were then treated with the agonist **48** or test compound in the presence of rolipram (10 μM) and adenosine deaminase (3 units/mL). After 30 min forskolin (10 μM) 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 100 μL of 0.1 M ice-cold HCl. The cell lysate was resuspended and stored at -20°C. For determination of cAMP production, 50 μL of the HCl solution was used in the Amersham cAMP Enzyme Immunoassay following the instructions provided with the kit. The results were interpreted using a SpectroMax M5 Microplate reader at 450 nm.