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N⁶-Substituted-5'-N-Methylcarbamoyl-4'-selenoadenosines as Potent and Selective A₃ Adenosine Receptor Agonists with Unusual Sugar Puckering and Nucleobase Orientation

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Abstract

Potent and selective A₃ adenosine receptor (AR) agonists were identified by replacement of 4'-oxo- or 4'-thionucleosides with bioisosteric selenium. Unlike previous agonists, 4'-seleno analogues preferred a glycosidic *syn* conformation and a South sugar pucker, as shown in the X-ray crystal structure of 5'-N-methylcarbamoyl derivative **3p**. Among compounds tested, N⁶-3-iodobenzyl analogue **3d** was found to be the most potent A₃AR full agonist (K_i = 0.57 nM), which was 800- and 1900-fold selective for A₁ and A_{2A}ARs, respectively. In the N⁶-cycloalkyl series, 2-Cl analogues generally exhibited better hA₃AR affinity than 2-H analogues, while 2-H > 2-Cl in the N⁶-3-halobenzyl series. The N⁷-isomers **3t** and **3u** were much weaker in binding than corresponding N⁹-isomers, but compound **3t** lacked A₃AR activation, appearing to be a weak antagonist. 2-Cl-N⁶-3-iodobenzyl analogue **3p** inhibited chemoattractant-induced migration of

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Supporting Information: The Supporting Information is available free of charge via the Internet at <http://pubs.acs.org>. Elemental analyses data, X-ray crystal packing structure of **3p**, ¹H NMR experiments of **3p** in the solution state, ¹H and ¹³C NMR copies of all final compounds **3a-u** (PDF), and hA₃AR homology model (PDB file). Molecular formula strings (CSV)

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microglia/monocytes without inducing cell death at 50 μ M. This suggests the potential for the development of 4'-selenonucleoside A₃AR agonists as novel anti-stroke agents.

Graphical abstract

Structure-activity relationships of 4'-selenonucleosides as potent and selective human A₃ adenosine receptor agonists and their effects on chemotaxis of inflammatory cells are described.

Keywords

A₃ adenosine receptor agonist; 4'-selenonucleoside; bioisostere; sugar puckering; structure-activity relationship

Introduction

Adenosine receptors (ARs), consisting of four subtypes (A₁, A_{2A}, A_{2B}, and A₃), have continued to be promising targets for the developments of new therapeutic agents.^{1,2} The pharmacological actions of ARs are initiated upon binding of extracellular adenosine to each receptor subtype, to modulate the levels of second messengers such as adenosine 3',5'-cyclic phosphate (cAMP), diacylglycerol (DAG), and inositol trisphosphate (IP₃), in turn affecting cellular signal transduction.³ Thus, altering an imbalance in activation of ARs can correct abnormal cell signaling that occurs in certain pathological states.⁴ Therefore, agonists or antagonists that can modulate the AR activity can be developed as novel chemotherapeutic agents.

A₃AR is the most recently identified receptor among the ARs, yet numerous A₃AR agonists and antagonists have already been developed as potential therapeutic agents for the treatments of many diseases such as cancer, cardiac ischemia, cerebral ischemia, renal ischemia, glaucoma, and rheumatoid arthritis.⁵ Among these, 2-chloro-*N*-(3-iodobenzyl)-5'-*N*-methylcarbamoyladenosine (**1**, Cl-IB-MECA, X = O, R¹ = 3-iodobenzyl, R² = Cl, K_i = 1.4 nM for human (h) A₃AR)⁶ which belong to the 4'-oxonucleosides, is regarded as a first generation A₃AR agonist (Figure 1). Compound **1** is in a phase II clinical trial for hepatocellular carcinoma and is projected to enter a clinical trial for nonalcoholic steatohepatitis.⁷ On the basis of the structure of **1**, its bioisosteric 4'-thio analogue **2** (thio-Cl-IB-MECA, X = S, R¹ = 3-iodobenzyl, R² = Cl, K_i = 0.38 nM for A₃AR)⁸ was discovered as the second generation A₃AR agonist. Compound **2** exhibited potent biological activities in models of cancer⁹ and cerebral ischemia¹⁰ with novel mechanisms of action.

On the other hand, it has long been known that the sugar conformation of nucleoside derivatives exists in dynamic equilibrium between North and South conformations, whose energy barrier in the 4'-oxo series is less than roughly 4 Kcal/mol.¹¹ The nucleobase of nucleoside derivatives also exists in either an *anti* or *syn* orientation, which is affected by the substitution of the nucleobase. It has been reported that compound **1** prefers the North (³T₂) sugar puckering to the South (²T₃) sugar puckering in binding to the A₃AR, which was confirmed by high affinity A₃AR agonist (*N*)-methanocarba derivative (*K*_i = 1.5 nM for A₃AR) that was fixed in the North (³T₂) sugar conformation,¹² while South (²T₃)-fixed analogues exhibited much lower binding affinity at the A₃AR than corresponding North analogues.¹³ Compound **2** was also reported to show the same sugar puckering as **1**.⁸

Another structural requirement that is essential for binding to A₃AR is the orientation of purine base. Nucleoside analogues with a *syn* orientation exhibited much lower binding affinity to adenosine receptors, indicating that A₃AR agonists should adopt the North (³T₂) sugar puckering with an *anti* nucleobase orientation.^{13,14}

We have recently reported the syntheses and unusual sugar conformations of 4'-selenonucleosides based on the bioisosteric concept of substituting native oxygen with selenium.¹⁵ From these studies, 4'-selenonucleosides showed an opposite South conformation in comparison to the 4'-oxonucleosides adopting a North conformation, probably due to steric effects induced by a bulky selenium atom.¹⁶ Thus, it was interesting to design the 4'-selenonucleoside derivatives **3** and to compare their conformation with that of **1** with respect to A₃AR agonist activity. It was also interesting to compare the orientation of the purine base of **3** with that of **1**, with the previous assumption that an A₃AR agonist attained an *anti* orientation when bound to the receptor. In addition to the desired *N*⁹-derivatives **3**, we also synthesized the corresponding *N*⁷-analogues for comparing binding affinities at the A₃AR. Herein, we report the synthesis, binding affinity, and conformational study of 4'-selenonucleoside derivatives **3** as potent and selective A₃AR agonists with unusual sugar puckering and nucleobase orientation.

Results and Discussion

Chemistry

For the synthesis of the final compound **3**, the key precursors **6a** and **6b** were first synthesized from D-ribose as shown in Scheme 1.

Using a reported procedure¹⁷ developed by our laboratory, D-ribose was converted to the 4-selenosugar **4**, which was converted to the glycosyl donor **5**¹⁷ by treatment with *m*CPBA followed by reaction with acetic anhydride at 100 °C. Condensation of **5** with silylated 6-chloropurine and 2,6-dichloropurine in the presence of TMSOTf as a Lewis acid catalyst afforded the β-*N*⁹-anomers **6a** (49%) and **7a** (20%) along with the β-*N*⁷-anomer **6b** (15%) and **7b** (29%), respectively.¹⁷ The β-*N*⁷-anomers **6b** and **7b** were smoothly converted to the β-*N*⁹-anomers **6a** and **7a** by heating with TMSOTf in toluene, respectively.¹⁷ The anomeric β-configurations of **6a** and **7a** were readily confirmed by NOE experiments between 1'-H and 4'-H, while the *N*⁹- and *N*⁷-regioisomers were confirmed by the X-ray crystal structures.¹⁷

The N^9 -precursors **6a** and **7a** were converted to the key intermediates **12a** and **12b**, respectively, as depicted in Scheme 2. The TBDPS protecting groups in **6a** and **7a** were first changed to the benzoyl (Bz) group for the selective conversion to the 5'- N -methyluronamide, but its removal with base such as NaOMe, methanolic ammonia, NaBH₄, or MeMgCl resulted in N^6 -substitution along with debenzoylation. Thus, a *p*-nitrobenzoyl (PNB) group was finally selected because it can be removed under milder basic conditions than the Bz group. Compounds **6a** and **7a** were treated with HF-Et₃N in THF to give the 5'-hydroxy compounds **8a** and **8b**, which were protected with a PNB group to afford **9a** and **9b**, respectively. The 2',3'-acetonide protecting groups of **9a** and **9b** were also changed to the TBS groups because the 2',3'-acetonide was problematic on removal under acidic conditions at the final stage. Thus, treatment of **9a** and **9b** with 50% aqueous TFA yielded diols **10a** and **10b**, which were again protected with TBS groups to afford **11a** and **11b**, respectively. The PNB groups of **11a** and **11b** were smoothly removed by treating with 1 *N* NaOH in 1,4-dioxane to give the 5'-hydroxyl derivatives **12a** and **12b**, respectively, which are ready for the transformation to the 5'- N -methyluronamide.

For the conversion of the primary alcohol into the carboxylic acid, compounds **12a** and **12b** were treated with PDC in DMF, used in the preparations of the corresponding 4'-oxo- and 4'-thionucleosides, but this treatment resulted in extensive decomposition and very low yields. Thus, we decided to synthesize the acid in two steps via the aldehyde. Albright-Goldman oxidation¹⁸ of **12a** and **12b** with DMSO/Ac₂O at 100 ° for 1 h produced the aldehydes **13a** and **13b**, respectively in good yields. Further oxidation of **13a** and **13b** using Tollens' reagents (Ag(NH₃)₂(OH)),¹⁹ prepared by treating AgNO₃, NaOH, and NH₄OH, afforded the corresponding acids, which without purification, were treated with HATU and methylamine hydrochloride in the presence of *N,N*-diisopropylethylamine (DIPEA) to afford the 5'-uronamides **14a** and **14b**, respectively in good yields. Removals of the TBS groups of **14a** and **14b** with TBAF or HF-Et₃N yielded **15a** and **15b**, respectively. Compounds **15a** and **15b** were treated with various amines such as alkylamines and arylalkylamines to afford the final 4'-selenonucleoside derivatives **3a-s**.

The corresponding N^7 -isomers **3t** and **3u** were also synthesized as shown in Scheme 4 and binding affinities at the ARs compared. Compounds **6b** and **7b** were treated with 3-iodobenzylamine in EtOH to give **16a** and **16b**, respectively. Removal of the TBDPS groups in **16a** and **16b** with TBAF yielded **17a** and **17b**, which were benzoylated and successively treated with 88% HCO₂H to afford diols **19a** and **19b**, respectively. Protection of **19a** and **19b** with TBS groups yielded **20a** and **20b**, which were treated with NaOMe to give the primary alcohols **21a** and **21b**, respectively. Oxidation of **21a** and **21b** with PDC in DMF, followed by the treatment of the crude acid with CH₃NH₂-HCl in the presence of HOBt and EDC•HCl yielded the 5'-uronamides **22a** and **22b**, respectively in reasonable yields. It is interesting to note that the N^7 -isomer was better tolerated under the PDC oxidation conditions than the N^9 -isomer. Removal of the TBS groups of **22a** and **22b** with TBAF afforded the final nucleosides **3t** and **3u**, respectively.

Conformational analysis

The conformation of a representative synthesized 4'-seleno compound was analyzed using ^1H NMR and X-ray crystallography. The X-ray crystal structure of **3p** was obtained as illustrated in Figure 2.²⁰

Surprisingly, compound **3p** exhibited a 2'-exo/3'-endo (South) conformation, which is opposite to that of the corresponding 4'-oxo- or 4'-thionucleoside, probably due to the steric effects induced by bulky selenium atom instead of gauche effects.¹⁶ Furthermore, **3p** exhibited a *syn* orientation of the purine base, which is not common to the corresponding 4'-oxo- or 4'-thionucleoside. The three parameters that are essential for the description of the nucleoside conformation were measured from the X-ray structure of **3p**. The torsion angle χ (C5-N9-C1'-Se4'), which describes the geometry of the glycosyl bond between the purine base and the sugar ring was 61.92°, indicating a *syn* conformation. Another torsion angle γ (C3'-C4'-C5'-N6') describing the rotation about the exocyclic C4'-C5' bond was -83.77°, indicating that it adopts a -*sc* orientation. The last parameter is the pseudorotational phase angle P, indicating which atoms are displaced out of the plane and the direction of the displacement. The P value was 0.030, which is the characteristic of C3'-endo South conformation ($0 < P < 18^\circ$).

The reason why compound **3p** assumes a *syn* conformation despite the possible steric repulsion of the purine base with the sugar ring might be explained by the following factors: (1) Intramolecular hydrogen bonding between N3 of purine base and the amide NH of N6'.²¹ (2) The intermolecular π - π stacking interaction between purine ring and other aromatic rings on crystal stacking (Figure 1S). (3) The 'South' sugar puckering.

^1H NMR experiments (Figure 2S) were also performed to confirm the sugar puckering and base orientation of **3p** in the solution state. The population (64.5%) of South conformation was preferred over that of North conformation at 25 °C, showing the P and ν_{max} (maximum torsion angle) values were 190.93° and 47.45, respectively. ^1H - ^1H NOESY spectrum of **3p** confirmed the *syn* conformation of the purine base. In summary, compound **3p** preferred the South conformation and the *syn* orientation in the solid as well as in the solution state.

However, correlation of biological activity with a crystal structure or ^1H NMR data must be carefully interpreted because conformational preferences of a molecule are greatly influenced by its environments such as the solid state (X-ray crystal structure), solution (NMR experiments), gas state (theoretical calculations), or binding to a protein.²² The *syn* crystal structure conformation of a nucleoside obtained in the solid state is just one of the many possible ones, due to the flexibility of the glycosidic bond. It has been also reported that the potent nonselective AR agonist adenosine-5'-N-ethyluronamide (NECA, **23**) prefers a *syn* conformation as determined in NMR experiments, demonstrating that a *syn* conformation in solution was permissive to potent biological activity.²³ However, when bound to the receptor, adenosine adopted an *anti* conformation, which was confirmed by conformationally restricted analogues^{12,13} and X-ray crystallography²⁴ of agonist-bound A_{2A}AR complexes. The conformational state of a nucleoside can be also indicated by the chemical shift of the 2'-H in ^1H NMR.²⁵ The chemical shifts of 2'-H of the *syn* conformation nucleosides are approximately 5.2 ppm, while those of the *anti* conformation

are around 4.2 ppm. Adenosine shows an intermediate value of 4.62, suggesting that it rapidly changes its conformation with a slight preference for the *anti* conformation. For **3p**, the chemical shift of 2'-H was found to be close to that of adenosine (4.56 and 4.62, respectively), suggesting that it adopts both conformations in solution, similar to adenosine, although it showed the *syn* conformation in the solid state.

Binding affinity

The binding affinities of all final compounds at the ARs were measured using standard radioligands and membrane preparations.²⁶ The hA₁AR or hA₃AR was expressed in Chinese hamster ovary (CHO) cells, and the hA_{2A}AR was expressed in human embryonic kidney (HEK)-293 cells. [¹²⁵I]*N*⁶-(3-Iodo-4-aminobenzyl)-5'-*N*-methylcarboxamidoadenosine (I-AB-MECA) was used to measure the binding affinity at the hA₃AR, while [³H](-)-*N*⁶-2-phenylisopropyl adenosine (R-PIA) and [³H]2-[*p*-(2-carboxyethyl)phenylethylamino]-5'-*N*-ethylcarboxamidoadenosine (CGS21680) were used for the hA₁AR and hA_{2A}AR, respectively. The percent inhibition of radioligand binding to the hA₁AR and hA_{2A}AR at 10 μM was used in cases of weak binding. Nonspecific binding was defined using 10 μM of **23**.

As shown in Table 1, all final *N*⁶-4'-selenonucleosides **3a-s** produced nanomolar binding affinity with *K_i* values in the range of 0.5 ~ 7 nM at the hA₃AR subtype with high selectivity to other subtypes hA₁ and hA_{2A}ARs, among which **3d** exhibited the best binding affinity (*K_i* = 0.57 nM) at the hA₃AR. Compound **3d** (*K_i* = 0.57 nM) showed about 3 times higher binding affinity at the hA₃AR subtype than the corresponding 4'-oxonucleoside **1a** (*K_i* = 1.8 nM), while exhibiting about 2 times lower binding affinity than the corresponding 4'-thionucleoside **2a** (*K_i* = 0.25 nM). This result is unusual in that these nucleosides **3a-s** with a possible South conformation and *syn* orientation could also act as potent and selective A₃AR agonists, because the North conformation and *anti* orientation have been regarded as the key structural requirements for A₃AR agonists. In the *N*⁶-cycloalkyl series, 2-Cl analogues **3q-s** exhibited better binding affinity at the hA₃AR than 2-H analogues **3i-k** and the binding affinity at the hA₃AR decreased in the following order: cyclobutyl > cyclopropyl > cyclopentyl. In the *N*⁶-3-halobenzyl series, 2-H analogues **3a-d** generally exhibited higher binding affinity at the hA₃AR than 2-Cl analogues **3m-p**. The binding affinity at the hA₃AR in the 2-H series decreased in the following order: 3-I derivative **3d** > 3-Br derivative **3c** > 3-F derivative **3a** > 3-Cl derivative **3b**, while in the 2-Cl series, 3-Cl derivative **3n** ≈ 3-Br derivative **3o** > 3-F derivative **3m** > 3-I derivative **3p**. As expected, the corresponding *N*⁷-isomers **3t** and **3u** showed much weaker binding affinity at all three subtypes maybe due to poor hydrophobic interaction of 3-iodobenzyl group in the binding site of hA₃AR. From the SAR analysis, it was found that the 4'-selenonucleosides had similar requirements for the A₃AR affinity to those of the corresponding 4'-oxo- or 4'-thionucleosides.^{8,12} For example, a bulky hydrophobic substituent at the *N*⁶ position was compatible with high affinity, but with better selectivity to other subtypes, hA₁ and hA_{2A}ARs. The wide range of steric bulk tolerated at the *N*⁶ substituent was indicated by the *N*⁶-methyl **3l** (*K_i* = 1.49 nM) and naphthyl **3g** (*K_i* = 1.76 nM) analogues producing similarly high A₃AR affinity. It should also be mentioned that despite the high binding affinity at the hA₃AR of the 4'-selenonucleosides they adopted the unusual inactive conformations such as South sugar puckering as well as a

syn base orientation because of a steric effect induced by the bulky selenium atom. This result demonstrates that they may be converted to the active North and *anti* conformation in the binding site of the receptor, overwhelming the steric effect caused by the selenium atom.

At the hA₁ and hA_{2A}ARs, moderate affinity was observed for many Se analogues, with the most potent at the hA₁ being *N*⁶-cycloalkyl derivative **3j** (*K_i* = 38 nM) and **3k** (*K_i* = 25 nM). In the *N*⁶-cycloalkyl series, the presence of a 2-Cl reduced the hA₁AR affinity by ~2-fold, while the hA_{2A}AR affinity nearly identical. The most potent affinities at the hA_{2A}AR (~200 nM) were observed for **3g** and **3n**, which do not share C2 and *N*⁶ substituent.

In a cAMP functional assay²⁶ at the hA₃AR expressed in CHO cells, the most potent compound **3d** behaved as a potent full agonist, similar to **1a,b** and **2a,b**. In a full concentration-response curve compound **3d** displayed an EC₅₀ value of 2.7 ± 2.2 nM in stimulation of cAMP formation via the hA₃AR (Figure 3).

Compound **3d** displayed a maximal hA₃AR-mediated inhibition of cAMP formation of 91 ± 16% (n = 3, 10 μM), relative to the full agonist **23** (= 100%) and similarly other compounds proved to be full agonists of the hA₃AR. Interestingly, the *N*⁷-isomer **3t** induced 0.2% of activation seen with full agonist **23**, indicating that it was an A₃AR antagonist of μM affinity.

Molecular modeling study

2-Chloro-*N*⁶-(3-iodobenzyl) analogue **3p** exhibited high hA₃AR binding affinity (*K_i* = 4.2 nM), and its X-ray crystal structure showed extraordinary 2'-endo/3'-exo (South) puckering and *syn* conformation via intra-molecular H-bonding between the side-chain amide and the adenine ring (Figure 4C). To investigate how **3p** binds with hA₃AR, we first built the homology model of hA₃AR using an agonist-bound hA_{2A}AR X-ray crystal structure (PDB id: 3QAK)²⁴ as the template, which was used to perform the induced-fit docking study. As shown in Figures 4A and 4B, **3p** bound very well at the orthosteric agonist binding site of hA₃AR. The Se-containing sugar ring maintained its 2'-endo/3'-exo (South) puckering and displayed H-bonds with Ser271 and His272. The side chain amide, involved in *syn* conformation of the glycosidic bond in the X-ray crystal structure of **3p**, exhibited H-bonding interaction with Thr94 which was suggested as one of the key residues for agonist activity.²⁹ The adenine ring exhibited π-π interactions with Phe168 and H-bonding with Asn250. In addition, the C2-chlorine showed hydrophobic interactions with Val72, Phe168, and Ile268. The iodobenzene ring displayed hydrophobic interactions with Val169, Ile253, Leu264, Tyr265, and Ile268.

The docked conformation was superimposed on the X-ray crystal structure of **3p** as shown in Figure 4D. Compound **3p** preserved its ring puckering as a South form. However, it transformed into an *anti* conformation by losing the original intra-molecular H-bonding. It appeared that the side chain amide and the adenine ring dramatically moved away to obtain more favorable interactions with binding site residues in hA₃AR.

Thus, the *syn* conformation in solution or in the pure crystal state of the potent 4'-selenonucleoside **3p** appears to be modified when bound to the A₃AR in order to maintain

conserved H-bonding and hydrophobic contacts of the ribose and adenine moieties with the receptor

Biological activity

We have recently reported that A₃AR agonist **2b** reduced brain ischemic injury and inhibited inflammatory cell migration in rats with higher potency than **1b**.¹⁰ In order to compare the inhibition by **2b** and **3d** of inflammatory cell migration, we examined the effects of these agonists on chemoattractant-induced migration of microglia/monocytes in a chemotaxis chamber (Figure 5). The chemotaxis assay revealed that compound **3d** inhibited monocyte chemotactic protein 1 (MCP-1, 100 ng/mL)-induced migration of microglial cells with higher potency than **2b**. Furthermore, **3d** at concentrations up to 50 μM exhibited much less cytotoxicity than **2b** in PrestoBlue cell viability test. These results demonstrate that 4'-selenonucleosides may serve as better candidates than the corresponding 4'-thionucleosides as protective agents in cerebral ischemia.

We have also examined the cytotoxic effects of the representative A₃AR agonists, **3a-d** in MRC-9 (lung normal cells).³⁰ All compounds did not show any cytotoxicity up to 20 μM.

Conclusion

We have discovered novel 4'-selenonucleosides **3a-s** as potent and selective hA₃AR agonists, whose binding affinity at the hA₃AR was almost comparable to that of **1** or **2**. They were found to adopt an unusual South sugar puckering and *syn* nucleobase orientation, which were confirmed by X-ray crystallography and ¹H NMR analyses. These findings are surprising in that known hA₃AR agonists prefer the North conformation and *anti* nucleobase orientation. Compound **3d** exhibited more potent inhibition of MCP-1 induced microglial chemotaxis than **1b** or **2b**, indicating that 4'-selenonucleosides have a great potential to be developed as novel anti-stroke agents. It is believed that this study opens up a new field in designing new A₃AR ligands.

Experimental Section

General Methods

¹H and ¹³C NMR spectra (CDCl₃, CD₃OD or DMSO-*d*₆) were recorded on Bruker AV 400 (400/100 MHz), Bruker AMX 500 (500/125 MHz), or Jeol JNM-ECA600 (600/150 MHz) instrument. Chemical shifts are reported as parts per million (δ) relative to the solvent peak. Coupling constants (*J*) are reported in hertz (Hz). Mass spectra were recorded on a Thermo LCQ XP instrument. Optical rotations were determined on Jasco III in appropriate solvent. UV spectra were recorded on U-3000 made by Hitachi in methanol or water. Infrared spectra were recorded on FT-IR (FTS-135) made by Bio-Rad. Melting points were determined on a Buchan B-540 instrument and are uncorrected. Elemental analyses (C, H, and N) were used to determine the purity of all synthesized compounds, and the results were within ± 0.4% of the calculated values, confirming 95% purity. Reactions were checked with TLC (Merck precoated 60F254 plates). Flash column chromatography was performed on silica gel 60 (230-400 mesh, Merck). Unless otherwise noted, materials were obtained from commercial

suppliers and were used without purification. All solvents were purified and dried by standard techniques just before use. THF and Et₂O were freshly distilled from sodium and benzophenone. Methylene chloride, toluene, and benzene were purified by refluxing with CaH₂. Hexanes and ethylacetate were purified by simple distillation.

General procedure for the synthesis of 3a-s

To a stirred solution of **15a-b** (1 equiv) in ethanol were added amine (3 equiv) and triethylamine (6 equiv), and the reaction mixture was stirred at 95 °C for 15 - 30 h. All volatiles was evaporated, and the residue was purified by silica gel column chromatography (dichloromethane : methanol = 100 : 1 - 10 : 1) to give **3a-s** as a white solid.

(2S,3S,4R,5R)-5-(6-((3-Fluorobenzyl)amino)-9H-purin-9-yl)-3,4-dihydroxy-N-methyltetrahydroselenophene-2-carboxamide (3a)—White solid; Yield: 85%; mp 123-130 °C; $[\alpha]_D^{20}$ -19.35 (*c* 0.34, CH₂Cl₂+CH₃OH); UV (CH₂Cl₂) $\lambda_{\max}$ 269.0 nm; ¹H NMR (400 MHz, CD₃OD) δ 8.54 (s, 1 H), 8.29 (s, 1 H), 7.35-7.30 (m, 1 H), 7.20 (d, 1 H, *J* = 7.6 Hz), 7.11 (d, 1 H, *J* = 10.0 Hz), 6.97 (td, 1 H, *J* = 2.6, 8.2 Hz), 6.21 (d, 1 H, *J* = 6.0 Hz), 4.87 (dd, 1 H, *J* = 5.4, 7.8 Hz), 4.58 (dd, 1 H, *J* = 3.4, 4.2 Hz), 4.10 (d, 1 H, *J* = 4.4 Hz), 2.82 (s, 3 H); ¹³C NMR (100 MHz, CD₃OD) δ 174.4, 153.9, 142.1, 131.3, 131.2, 124.24, 124.21, 115.2, 115.0, 114.9, 114.7, 81.6, 77.8, 56.9, 47.2, 26.9; HRMS (FAB) found 467.0754 [calcd for C₁₈H₁₉FN₆O₃Se (M+H)⁺ 467.0746]; Anal. (C₁₈H₁₉FN₆O₃Se) C, H, N.

(2S,3S,4R,5R)-5-(6-((3-Chlorobenzyl)amino)-9H-purin-9-yl)-3,4-dihydroxy-N-methyltetrahydroselenophene-2-carboxamide (3b)—White solid; Yield: 99%; mp 219-227 °C; $[\alpha]_D^{20}$ -18.24 (*c* 0.27, CH₂Cl₂+CH₃OH); UV (CH₂Cl₂) $\lambda_{\max}$ 269.0 nm; ¹H NMR (400 MHz, CD₃OD+ CDCl₃) δ 8.54 (s, 1 H), 8.29 (s, 1 H), 7.43-7.23 (m, 4 H), 6.21 (d, 1 H, *J* = 6.0 Hz), 4.89-4.88 (m, 1 H), 4.85 (bs, 1 H), 4.58 (q, 1 H, *J* = 3.4, 4.2 Hz), 4.10 (d, 1 H, *J* = 4.0 Hz), 4.06 (s, 1 H), 2.82 (s, 3 H); ¹³C NMR (100 MHz, CD₃OD+ CDCl₃) δ 173.1, 155.3, 153.3, 141.5, 135.5, 134.9, 130.4, 129.5, 128.0, 127.9, 126.2, 120.4, 80.8, 78.2, 77.2, 56.4, 47.2, 26.9; HRMS (FAB) found 483.0456 [calcd for C₁₈H₁₉ClN₆O₃Se (M+H)⁺ 483.0451]; Anal. (C₁₈H₁₉ClN₆O₃Se) C, H, N.

(2S,3S,4R,5R)-5-(6-((3-Bromobenzyl)amino)-9H-purin-9-yl)-3,4-dihydroxy-N-methyltetrahydroselenophene-2-carboxamide (3c)—White solid; Yield: 79%; mp 252 ° (decomposed); $[\alpha]_D^{20}$ -15.12 (*c* 0.17, CH₂Cl₂+CH₃OH); UV (CH₂Cl₂) $\lambda_{\max}$ 269.0 nm; ¹H NMR (400 MHz, CD₃OD) δ 8.54 (s, 1 H), 8.29 (s, 1 H), 7.56 (s, 1 H), 7.41-7.35 (m, 2 H), 7.24 (t, 1 H, *J* = 7.6 Hz), 6.21 (d, 1 H, *J* = 5.6 Hz), 4.89-4.85 (m, 1 H), 4.58 (t, 1 H, *J* = 3.8 Hz), 4.10 (d, 1 H, *J* = 4.0 Hz), 2.82 (s, 3 H); ¹³C NMR (100 MHz, CD₃OD+CDCl₃) δ 173.0, 155.1, 153.0, 141.6, 132.9, 132.4, 131.3, 130.9, 130.7, 128.0, 126.6, 123.0, 80.8, 78.2, 77.1, 56.4, 47.2, 26.9; HRMS (FAB) found 526.9947 [calcd for C₁₈H₁₉BrN₆O₃Se (M+H)⁺ 526.9945]; Anal. (C₁₈H₁₉BrN₆O₃Se) C, H, N.

(2S,3S,4R,5R)-3,4-Dihydroxy-5-(6-((3-iodobenzyl)amino)-9H-purin-9-yl)-N-methyltetrahydroselenophene-2-carboxamide (3d)—White solid; Yield: 80%; mp 180-185 °C; $[\alpha]_D^{20}$ 157.50 (*c* 0.10, CH₂Cl₂+CH₃OH); UV (CH₂Cl₂) $\lambda_{\max}$ 269.0 nm; ¹H NMR (400 MHz, DMSO-*d*₆) δ 8.51 (bs, 1 H), 8.45 (s, 1 H), 8.24 (s, 1 H), 7.72 (s, 1 H), 7.58

(d, 1 H, $J = 8.0$ Hz), 7.36 (d, 1 H, $J = 8.0$ Hz), 7.11 (t, 1 H, $J = 7.8$ Hz), 6.02 (d, 1 H, $J = 6.0$ Hz), 5.72 (d, 1 H, $J = 6.0$ Hz), 5.50 (d, 1 H, $J = 5.2$ Hz), 4.78 (td, 1 H, $J = 3.0, 6.0$ Hz), 4.66 (bs, 1 H), 4.44 (q, 1 H, $J = 4.6, 7.8$ Hz), 3.95 (d, 1 H, $J = 4.0$ Hz), 2.64 (d, 3 H, $J = 4.4$ Hz); ^{13}C NMR (100 MHz, DMSO- d_6) δ 171.6, 154.3, 152.6, 142.9, 140.0, 135.8, 135.5, 130.6, 126.7, 94.8, 79.5, 75.7, 55.2, 45.3, 26.1; HRMS (FAB) found 574.9800 [calcd for $\text{C}_{18}\text{H}_{19}\text{N}_6\text{O}_3\text{Se}$ ($\text{M}+\text{H}$) $^+$ 574.9807]; Anal. ($\text{C}_{18}\text{H}_{19}\text{N}_6\text{O}_3\text{Se}$) C, H, N.

(2S,3S,4R,5R)-3,4-Dihydroxy-5-(6-((2-methoxybenzyl)amino)-9H-purin-9-yl)-N-methyltetrahydroselenophene-2-carboxamide (3e)—White solid; Yield: 74%; mp 103–108 °C; $[\alpha]_{\text{D}}^{20}$ 14.67 (c 0.20, CH_3OH); UV (CH_2Cl_2) λ_{max} 272.0 nm; ^1H NMR (400 MHz, CD_3OD) δ 8.53 (s, 1 H), 8.28 (s, 1 H), 7.30–7.23 (m, 2 H), 6.99 (d, 1 H, $J = 7.6$ Hz), 6.88 (dd, 1 H, $J = 6.8, 7.2$ Hz), 6.20 (d, 1 H, $J = 6.0$ Hz), 4.58 (dd, 1 H, $J = 3.4, 4.2$ Hz), 4.10 (d, 1 H, $J = 4.4$ Hz), 3.89 (s, 3 H), 2.81 (s, 3 H); ^{13}C NMR (300 MHz, CD_3OD) δ 175.0, 159.7, 156.8, 154.6, 142.6, 132.8, 132.4, 130.5, 130.4, 122.6, 122.1, 112.6, 112.1, 82.3, 78.6, 57.7, 56.7, 47.9, 27.7; HRMS (FAB) found 479.0945 [calcd for $\text{C}_{19}\text{H}_{22}\text{N}_6\text{O}_4\text{Se}$ ($\text{M}+\text{H}$) $^+$ 479.0946]; Anal. ($\text{C}_{19}\text{H}_{22}\text{N}_6\text{O}_4\text{Se}$) C, H, N.

(2S,3S,4R,5R)-5-(6-((2-Chlorobenzyl)amino)-9H-purin-9-yl)-3,4-dihydroxy-N-methyltetrahydroselenophene-2-carboxamide (3f)—White solid; Yield: 84%; mp 154–160 °C; $[\alpha]_{\text{D}}^{20}$ -35.26 (c 0.58, CH_3OH); UV (CH_2Cl_2) λ_{max} 268.5 nm; ^1H NMR (400 MHz, CD_3OD) δ 8.55 (s, 1 H), 8.29 (s, 1 H), 7.44–7.41 (m, 2 H), 7.28–7.23 (m, 2 H), 6.21 (d, 1 H, $J = 6.0$ Hz), 4.92 (bs, 1 H), 4.88 (dd, 2 H, $J = 3.2, 6.0$ Hz), 4.58 (dd, 1 H, $J = 3.4, 4.2$ Hz), 4.10 (d, 1 H, $J = 4.4$ Hz); ^{13}C NMR (100 MHz, CD_3OD) δ 174.4, 153.9, 142.1, 134.4, 130.5, 130.1, 129.8, 128.1, 81.6, 77.8, 56.9, 47.2, 26.9; HRMS (FAB) found 483.0458 [calcd for $\text{C}_{18}\text{H}_{19}\text{ClN}_6\text{O}_3\text{Se}$ ($\text{M}+\text{H}$) $^+$ 483.0451]; Anal. ($\text{C}_{18}\text{H}_{19}\text{ClN}_6\text{O}_3\text{Se}$) C, H, N.

(2S,3S,4R,5R)-3,4-Dihydroxy-N-methyl-5-(6-((naphthalen-1-ylmethyl)amino)-9H-purin-9-yl)tetrahydroselenophene-2-carboxamide (3g)—White solid; Yield: 79%; mp 233 ° (decomposed); $[\alpha]_{\text{D}}^{20}$ -22.18 (c 0.28, $\text{CH}_2\text{Cl}_2+\text{CH}_3\text{OH}$); UV (CH_2Cl_2) λ_{max} 273.0 nm; ^1H NMR (400 MHz, CD_3OD) δ 8.51 (s, 1 H), 8.35 (s, 1 H), 7.92–7.89 (m, 1 H), 7.83 (d, 1 H, $J = 8.4$ Hz), 7.56–7.48 (m, 2 H), 7.44 (t, 1 H, $J = 7.8$ Hz), 6.22 (d, 1 H, $J = 5.6$ Hz), 5.29 (bs, 2 H), 4.58 (dd, 1 H, $J = 3.2, 4.0$ Hz), 4.10 (d, 1 H, $J = 4.0$ Hz); ^{13}C NMR (100 MHz, CD_3OD) δ 173.9, 153.8, 141.8, 135.1, 132.6, 130.0, 129.6, 129.2, 128.2, 127.4, 127.3, 126.3, 124.2, 123.2, 81.4, 77.6, 56.7, 47.1, 27.0; HRMS (FAB) found 499.0992 [calcd for $\text{C}_{22}\text{H}_{22}\text{N}_6\text{O}_3\text{Se}$ ($\text{M}+\text{H}$) $^+$ 499.0997]; Anal. ($\text{C}_{21}\text{H}_{20}\text{N}_6\text{O}_3\text{Se}$) C, H, N.

(2S,3S,4R,5R)-3,4-Dihydroxy-N-methyl-5-(6-(((1R,2R)-2-phenylcyclopropyl)amino)-9H-purin-9-yl)tetrahydroselenophene-2-carboxamide (3h)—White solid; Yield: 78%; mp 125–129 °C; $[\alpha]_{\text{D}}^{20}$ 34.53 (c 0.35, CH_3OH); UV (CH_2Cl_2) λ_{max} 274.0 nm; ^1H NMR (400 MHz, CD_3OD) δ 8.53 (s, 1 H), 8.32 (s, 1 H), 7.30–7.15 (m, 6 H), 6.21 (d, 1 H, $J = 6.0$ Hz), 4.59–4.57 (m, 2 H), 4.10 (d, 1 H, $J = 4.4$ Hz), 2.82 (s, 3 H), 2.23–2.16 (m, 1 H), 1.40–1.29 (m, 2 H); ^{13}C NMR (100 MHz, CD_3OD) δ 173.6, 156.4, 153.4, 141.8, 141.6, 129.4, 129.1, 127.0, 126.8, 81.2, 78.7, 77.4,

56.6, 47.1, 34.0, 27.0, 25.7; HRMS (FAB) found 475.0998 [calcd for C₂₀H₂₂N₆O₃Se (M+H)⁺ 475.0997]; Anal. (C₂₀H₂₂N₆O₃Se) C, H, N.

(2S,3S,4R,5R)-5-(6-(Cyclopropylamino)-9H-purin-9-yl)-3,4-dihydroxy-N-methyltetrahydroselenophene-2-carboxamide (3i)—White solid; Yield: 98%; mp 165-170 °C; [α]_D²⁰ -29.83 (c 0.58, CH₃OH); UV (CH₂Cl₂) λ_{max} 270.5 nm; ¹H NMR (400 MHz, CD₃OD) δ 8.53 (s, 1 H), 8.32 (s, 1 H), 6.21 (d, 1 H, *J* = 6.0 Hz), 4.58 (dd, 1 H, *J* = 3.4, 4.2 Hz), 4.10 (d, 1 H, *J* = 4.4 Hz), 2.81 (s, 3 H), 0.92-0.87 (m, 2 H), 0.67-0.63 (m, 2 H); ¹³C NMR (100 MHz, CD₃OD) δ 174.4, 157.2, 153.7, 142.1, 81.7, 77.8, 56.9, 47.1, 26.9, 7.6; HRMS (FAB) found 399.0685 [calcd for C₁₄H₁₈N₆O₃Se (M+H)⁺ 399.0684]; Anal. (C₁₄H₁₈N₆O₃Se) C, H, N.

(2S,3S,4R,5R)-5-(6-(Cyclobutylamino)-9H-purin-9-yl)-3,4-dihydroxy-N-methyltetrahydroselenophene-2-carboxamide (3j)—White solid; Yield: 79%; mp 153-158 °C; [α]_D²⁰ -38.92 (c 0.65, CH₃OH); UV (CH₂Cl₂) λ_{max} 271.0 nm; ¹H NMR (400 MHz, CD₃OD) δ 8.53 (s, 1 H), 8.25 (s, 1 H), 6.19 (d, 1 H, *J* = 6.0 Hz), 4.57 (dd, 1 H, *J* = 3.4, 4.2 Hz), 4.10 (d, 1 H, *J* = 4.4 Hz), 2.81 (s, 3 H), 2.49-2.41 (m, 2 H), 2.12-2.04 (m, 2 H), 1.86-1.79 (m, 2 H); ¹³C NMR (100 MHz, CD₃OD) δ 174.4, 153.9, 141.9, 81.6, 77.8, 56.9, 47.1, 32.0, 26.9, 15.9; HRMS (FAB) found 413.0841 [calcd for C₁₅H₂₀N₆O₃Se (M+H)⁺ 413.0840]; Anal. (C₁₅H₂₀N₆O₃Se) C, H, N.

(2S,3S,4R,5R)-5-(6-(Cyclopentylamino)-9H-purin-9-yl)-3,4-dihydroxy-N-methyltetrahydroselenophene-2-carboxamide (3k)—White solid; Yield: 94%; mp 135 ° (decomposed); [α]_D²⁰ -110.77 (c 0.13, CH₃OH); UV (CH₂Cl₂) λ_{max} 271.0 nm; ¹H NMR (400 MHz, CD₃OD+CDCl₃) δ 8.50 (s, 1 H), 8.26 (s, 1 H), 6.17 (d, 1 H, *J* = 5.6 Hz), 4.83 (dd, 1 H, *J* = 3.2, 6.0 Hz), 4.58 (dd, 1 H, *J* = 3.2, 4.4 Hz), 4.10 (d, 1 H, *J* = 4.8 Hz), 2.82 (s, 3 H), 2.16-2.05 (m, 2 H), 1.83-1.79 (m, 2 H), 1.73-1.59 (m, 4 H); ¹³C NMR (100 MHz, CD₃OD+CDCl₃) δ 174.0, 155.5, 153.7, 149.7, 141.5, 120.6, 81.5, 81.4, 77.6, 56.8, 56.7, 53.5, 47.1, 33.9, 27.0, 26.9, 24.6; HRMS (FAB) found 427.0993 [calcd for C₁₆H₂₂N₆O₃Se (M+H)⁺ 427.0997]; Anal. (C₁₆H₂₂N₆O₃Se) C, H, N.

(2S,3S,4R,5R)-3,4-Dihydroxy-N-methyl-5-(6-(methylamino)-9H-purin-9-yl)tetrahydroselenophene-2-carboxamide (3l)—White solid; Yield: 92%; mp 173-178 °C; [α]_D²⁰ -29.57 (c 0.47, CH₃OH); UV (CH₂Cl₂) λ_{max} 267.5 nm; ¹H NMR (400 MHz, CD₃OD) δ 8.51 (s, 1 H), 8.28 (s, 1 H), 6.20 (d, 1 H, *J* = 6.0 Hz), 4.58 (dd, 1 H, *J* = 3.6, 4.0 Hz), 4.10 (d, 1 H, *J* = 4.4 Hz), 3.12 (bs, 3 H), 2.82 (s, 3 H); ¹³C NMR (100 MHz, CD₃OD) δ 174.4, 153.9, 141.8, 81.6, 77.8, 56.9, 47.1, 26.9; HRMS (FAB) found 373.0527 [calcd for C₁₂H₁₆N₆O₃Se (M+H)⁺ 373.0527]; Anal. (C₁₂H₁₆N₆O₃Se) C, H, N.

(2S,3S,4R,5R)-5-(2-Chloro-6-((3-fluorobenzyl)amino)-9H-purin-9-yl)-3,4-dihydroxy-N-methyltetrahydroselenophene-2-carboxamide (3m)—White solid; Yield: 85%; mp 139-144 °C; [α]_D²⁰ -15.0 (c 0.34, CH₃OH); UV (CH₂Cl₂) λ_{max} 273.0 nm; ¹H NMR (500 MHz, CD₃OD + CDCl₃) δ 8.41 (s, 1 H), 7.28 (dd, *J* = 7.6, 13.9 Hz, 1 H), 7.15 (d, *J* = 7.5 Hz, 1 H), 7.07 (d, *J* = 9.6 Hz, 1 H), 6.93 (t, *J* = 7.6 Hz, 1 H), 6.06 (d, *J* = 5.8 Hz, 1 H), 4.75 (s, 3 H), 4.54 (t, *J* = 3.5 Hz, 1 H), 4.07 (d, *J* = 4.4 Hz, 1 H), 2.84 (s, 3 H); ¹³C NMR (75 MHz, CD₃OD+CDCl₃) δ 174.3, 166.3, 163.0, 156.8, 155.9, 151.7, 142.9, 131.7

(d, $J = 8.3$ Hz), 125.0, 120.3, 116.0 (d, $J = 22.6$ Hz), 115.5 (d, $J = 21.3$ Hz), 81.9, 78.2, 57.5, 48.0, 45.2, 27.9; HRMS (ESI) found 501.0349 [calcd for $C_{18}H_{19}ClFN_6O_3Se$ (M + H)⁺ 501.0356]; Anal. ($C_{18}H_{18}ClFN_6O_3Se$) C, H, N.

(2S,3S,4R,5R)-5-(2-Chloro-6-((3-chlorobenzyl)amino)-9H-purin-9-yl)-3,4-dihydroxy-N-methyltetrahydroselenophene-2-carboxamide (3n)—White solid; Yield: 76%; mp 166-170 °C; $[\alpha]_D^{20}$ -14.6 (c 1.00, CH₃OH); UV (CH₂Cl₂) λ_{max} 275.0 nm; ¹H NMR (500 MHz, CDCl₃) δ 8.14 (s, 1 H), 7.32 (s, 1 H), 7.21 (s, 3 H), 5.92 (d, $J = 6.0$ Hz, 1 H), 4.71 (s, 2 H), 4.68-4.67 (m, 1 H), 4.46 (t, $J = 3.8$ Hz, 1 H), 4.05-4.04 (m, 1 H), 3.32 (s, 1 H), 2.88 (s, 3 H); ¹³C NMR (75 MHz, CD₃OD+CDCl₃) δ 174.4, 143.0, 142.5, 135.9, 131.5, 129.4, 129.2, 129.0, 127.7, 82.0, 79.7, 78.3, 57.5, 48.0, 45.3, 41.4, 31.3, 27.9; HRMS (ESI) found 517.0049 [calcd for $C_{18}H_{19}Cl_2N_6O_3Se$ (M+H)⁺ 517.0061]; Anal. ($C_{18}H_{18}Cl_2N_6O_3Se$) C, H, N.

(2S,3S,4R,5R)-5-(6-((3-Bromobenzyl)amino)-2-chloro-9H-purin-9-yl)-3,4-dihydroxy-N-methyltetrahydroselenophene-2-carboxamide (3o)—White solid; Yield: 81%; mp 165-170 °C; $[\alpha]_D^{20}$ -36.5 (c 0.33, CH₃OH); UV (CH₂Cl₂) λ_{max} 276.0 nm; ¹H NMR (400 MHz, CDCl₃ + CD₃OD) δ 8.16 (s, 1 H), 7.45 (s, 1 H), 7.32 (d, $J = 7.8$ Hz, 1 H), 7.22 (d, $J = 6.9$ Hz, 1 H), 7.12 (t, $J = 7.8$ Hz, 1 H), 5.91 (d, $J = 5.8$ Hz, 1 H), 4.67 (s, 2 H), 4.65-4.63 (m, 1 H), 4.43 (t, $J = 4.7$ Hz, 1 H), 2.84 (s, 3 H); ¹³C NMR (150 MHz, CD₃OD) δ 175.0, 145.7, 144.3, 143.3, 132.5, 132.17, 132.15, 132.8, 129.6, 128.4, 124.2, 101.9, 62.1, 58.3, 57.7, 48.0, 45.3, 27.9; HRMS (ESI) found 560.9463 [calcd for $C_{18}H_{19}BrClN_6O_3Se$ (M+H)⁺ 560.9556]; Anal. ($C_{18}H_{18}BrN_6O_3Se$) C, H, N.

(2S,3S,4R,5R)-5-(2-Chloro-6-((3-iodobenzyl)amino)-9H-purin-9-yl)-3,4-dihydroxy-N-methyltetrahydroselenophene-2-carboxamide (3p)—White solid; Yield: 87%; mp 154-158 °C; $[\alpha]_D^{20}$ -19.5 (c 0.48, CH₃OH); UV (CH₂Cl₂) λ_{max} 273.0 nm; ¹H NMR (500 MHz, CD₃OD) δ 8.48 (s, 1 H), 7.77 (s, 1 H), 7.60 (d, $J = 7.8$ Hz, 1 H), 7.39 (d, $J = 7.7$ Hz, 1 H), 7.09 (t, $J = 7.8$ Hz, 1 H), 6.10 (d, $J = 7.5$ Hz, 1 H), 4.79 (dd, $J = 3.3, 5.8$ Hz, 1 H), 4.75 (broad s, 1 H), 4.56 (t, $J = 3.9$ Hz, 1 H), 4.09 (d, $J = 4.5$ Hz, 1 H), 2.82 (s, 3 H); ¹³C NMR (125 MHz, CD₃OD) δ 175.0, 157.2, 156.3, 152.4, 143.4, 143.3, 138.7, 138.2, 132.2, 129.0, 120.7, 95.7, 82.3, 78.6, 57.8, 48.0, 45.2, 27.8; HRMS (ESI) found 608.9481 [calcd for $C_{18}H_{19}ClIN_6O_3Se$ (M+H)⁺ 608.9417]; Anal. ($C_{18}H_{18}ClIN_6O_3Se$) C, H, N.

(2S,3S,4R,5R)-5-(2-Chloro-6-(cyclopropylamino)-9H-purin-9-yl)-3,4-dihydroxy-N-methyltetrahydroselenophene-2-carboxamide (3q)—White solid; Yield: 86%; mp 130-135 °C; $[\alpha]_D^{20}$ -27.3 (c 1.98, CH₃OH); UV (CH₂Cl₂) λ_{max} 275.0 nm; ¹H NMR (400 MHz, CD₃OD) δ 8.47 (s, 1 H), 6.11 (d, $J = 5.8$ Hz, 1 H), 4.79 (dd, $J = 3.3, 5.8$ Hz, 1 H, partially merged with H₂O), 4.56 (t, $J = 3.8$ Hz, 1 H), 4.09 (d, $J = 4.5$ Hz, 1 H), 2.82 (s, 3 H), 0.89-0.85 (m, 2 H), 0.65-0.62 (m, 2 H); ¹³C NMR (75 MHz, CD₃OD) δ 175.0, 158.7, 156.2, 143.1, 142.8, 120.7, 82.3, 80.6, 78.6, 76.3, 66.9, 57.7, 48.0, 27.8, 27.3; HRMS (ESI) found 433.0268 [calcd for $C_{14}H_{18}ClN_6O_3Se$ (M+H)⁺ 433.0294]; Anal. ($C_{14}H_{17}ClN_6O_3Se$) C, H, N.

(2S,3S,4R,5R)-5-(2-Chloro-6-(cyclobutylamino)-9H-purin-9-yl)-3,4-dihydroxy-N-methyltetrahydroselenophene-2-carboxamide (3r)—White solid; Yield: 82%; mp 152–157 °C; $[\alpha]_D^{20}$ -20.5 (*c* 1.66, CH₃OH); UV (CH₂Cl₂) $\lambda_{\max}$ 277.0 nm; ¹H NMR (500 MHz, DMSO-*d*₆) δ 8.68 (d, *J* = 7.6 Hz, 1 H), 8.44 (s, 1 H), 8.16 (d, *J* = 4.0 Hz, 1 H), 5.92 (d, *J* = 6.1 Hz, 1 H), 5.74 (d, *J* = 5.5 Hz, 1 H), 5.51 (d, *J* = 4.9 Hz, 1 H), 4.71 (s, 1 H), 4.62–4.58 (m, 1 H), 4.41–4.40 (m, 1 H), 3.95 (d, *J* = 4.0 Hz, 1 H), 2.64 (d, *J* = 4.5 Hz, 3 H), 2.30–2.22 (m, 2 H), 2.13–2.05 (m, 2 H), 1.70–1.63 (m, 2 H); ¹³C NMR (100 MHz, DMSO-*d*₆) δ 181.5, 163.9, 163.2, 160.2, 150.1, 128.0, 89.7, 85.5, 65.0, 55.1, 40.8, 39.98, 39.93, 36.0, 24.6; HRMS (ESI) found 447.0443 [calcd for C₁₅H₂₀ClN₆O₃Se (M+H)⁺ 447.0451]; Anal. (C₁₅H₁₉ClN₆O₃Se) C, H, N.

(2S,3S,4R,5R)-5-(2-Chloro-6-(cyclopentylamino)-9H-purin-9-yl)-3,4-dihydroxy-N-methyltetrahydroselenophene-2-carboxamide (3s)—White solid; Yield: 79%; mp 148–152 °C; $[\alpha]_D^{20}$ -21.7 (*c* 0.18, CH₃OH); UV (CH₂Cl₂) $\lambda_{\max}$ 277.0 nm; ¹H NMR (500 MHz, CDCl₃) δ 8.24 (s, 1 H), 8.10 (s, 1 H), 6.29 (s, 1 H), 6.11 (d, *J* = 4.3 Hz, 1 H), 5.88 (s, 1 H), 5.02 (s, 1 H), 4.76 (s, 1 H), 4.42 (d, *J* = 4.7 Hz, 1 H), 4.17 (s, 1 H), 2.88 (d, *J* = 3.7 Hz, 3 H), 2.37 (s, 1 H), 2.02 (s, 2 H), 1.66 (s, 2 H), 1.59 (s, 2 H), 1.43 (s, 2 H); ¹³C NMR (75 MHz, CD₃OD) δ 190.4, 175.0, 157.0, 142.8, 82.3, 78.5, 57.8, 54.4, 47.9, 34.5, 27.8, 25.5; HRMS (ESI) found 461.0607 [calcd for C₁₆H₂₂ClN₆O₃Se (M+H)⁺ 461.0607]; Anal. (C₁₆H₂₁ClN₆O₃Se) C, H, N.

(2S,3S,4R,5R)-3,4-Dihydroxy-5-(6-((3-iodobenzyl)amino)-7H-purin-7-yl)-N-methyl tetrahydroselenophene-2-carboxamide (3t)—To a solution of **22a** (480 mg, 0.6 mmol) in THF (5 mL) was added tetra-*n*-butylammonium fluoride (0.39 mL, 1.32 mmol) and the reaction mixture was stirred at room temperature for 1 h. The reaction mixture was evaporated and the residue was purified by silica gel chromatography (dichloromethane : methanol = 10 : 1) to give **3t** (300 mg, 87%) as a white solid: $[\alpha]_D^{20}$ 22.00 (*c* 0.10, CH₃OH); UV (CH₃OH) $\lambda_{\max}$ 283.5 nm; ¹H NMR (400 MHz, CD₃OD) δ 8.46 (s, 1 H), 8.31 (s, 1 H), 7.82 (s, 1 H), 7.55 (d, *J* = 7.2 Hz, 1 H), 7.44 (d, *J* = 7.6 Hz, 1 H), 7.06 (t, *J* = 7.6 Hz, 1 H), 6.39 (d, *J* = 9.2 Hz, 1 H), 5.12 (d, *J* = 15.6 Hz, 1 H), 5.03 (dd, *J* = 3.6, 9.2 Hz, 1 H), 4.73 (d, *J* = 15.2 Hz, 1 H), 4.36 (dd, *J* = 1.0, 3.4 Hz, 1 H), 4.06 (d, *J* = 1.2 Hz, 1 H), 2.68 (s, 3 H); ¹³C NMR (75 MHz, CDCl₃+CD₃OD) δ 174.2, 160.9, 154.0, 152.1, 148.0, 143.4, 137.7, 137.2, 131.4, 128.0, 113.1, 95.3, 82.9, 76.4, 60.8, 47.7, 45.3, 36.1, 27.5; HRMS (ESI) found 574.9803 [calcd for C₁₈H₁₉I N₆O₃Se (M+H)⁺ 574.9807]; Anal. (C₁₈H₁₉IN₆O₃Se) C, H, N.

(2S,3S,4R,5R)-5-(2-Chloro-6-((3-iodobenzyl)amino)-7H-purin-7-yl)-3,4-dihydroxy-N-methyltetrahydroselenophene-2-carboxamide (3u)—Compound **22b** (254 mg, 0.30 mmol) was converted to **3u** (160 mg, 87%) as a white solid, using similar procedure used in the preparation of **3t**: $[\alpha]_D^{20}$ 40.00 (*c* 0.10, CH₃OH); UV (MeOH) $\lambda_{\max}$ 280.5 nm; ¹H NMR (400 MHz, CD₃OD) δ 8.40 (s, 1 H), 7.88 (t, *J* = 1.4 Hz, 1 H), 7.57 (d, *J* = 7.6 Hz, 1 H), 7.49 (d, 1 H, *J* = 8.0 Hz), 7.07 (t, 1 H, *J* = 7.6 Hz), 6.34 (d, *J* = 9.2 Hz, 1 H), 5.02 (d, 1 H, *J* = 14.8 Hz), 4.97 (dd, 1 H, *J* = 3.6, 9.6 Hz), 4.69 (d, 1 H, *J* = 15.2 Hz), 4.34 (dd, *J* = 1.2, 3.6 Hz, 1 H), 4.05 (d, *J* = 1.2 Hz, 1 H), 2.70 (s, 3 H); ¹³C NMR (100 MHz, CD₃OD) δ 174.6, 163.1, 155.5, 152.3, 149.2, 143.1, 138.3, 137.2, 131.2, 128.5, 106.2, 94.8,

82.7, 76.3, 60.7, 47.8, 45.3, 26.8; HRMS (ESI) found 608.9404 [calcd for $C_{18}H_{18}ClIN_6O_3Se$ (M+H)⁺ 608.9417]; Anal. ($C_{18}H_{18}ClIN_6O_3Se$) C, H, N.

9-[6-(*tert*-Butyl-diphenyl-silanyloxymethyl)-2,2-dimethyl-tetrahydro-1,3-dioxo-5-selena-pentalen-4-yl]-6-chloro-9*H*-purine (6a) and its *N*⁷-isomer 6b—A suspension of 6-chloropurine (0.89 g, 5.76 mmol) and BSA (1.95 g, 2.35 mL, 9.60 mmol) in dry toluene (100 mL) was stirred at 95 °C for 1 h under nitrogen. To this clear solution were added a solution of **5** (2.56 g, 4.80 mmol) in dry toluene (50 mL) and TMSOTf (0.54 g, 0.45 mL, 2.40 mmol) at 95 °C, and the reaction mixture was stirred at same temperature for 15 h, cooled to room temperature, diluted with saturated aqueous NaHCO₃ solution (10 mL), and stirred for 15 min. The organic layer was separated, and the aqueous layer was extracted with ethyl acetate (70 mL × 2). The combined organic layers were washed with brine, dried (MgSO₄), filtered, and concentrated under reduced pressure. The crude residue was purified by silica gel column chromatography (hexane : ethyl acetate = 4:1) to give *N*⁹-isomer **6a** (1.47 g, 49%) as a pale yellow foam and *N*⁷-isomer **6b** (0.44 g, 15%) as a pale yellow foam.

Compound 6a—[α]_D²⁰ -6.78 (*c* 5.4, CH₂Cl₂); UV (CH₂Cl₂) λ_{max} 265.0 nm; ¹H NMR (400 MHz, CDCl₃) δ 8.65 (s, 1H), 8.31 (s, 1H), 7.66-7.61 (m, 4H), 7.43-7.32 (m, 6H), 6.27 (d, 1H, *J* = 3.2 Hz), 5.04 (dd, 1H, *J* = 3.2, 5.6 Hz), 4.97 (dd, 1H, *J* = 2.8, 5.6 Hz), 4.13 (td, 1H, *J* = 2.9, 7.3 Hz), 4.10-3.96 (m, 1H), 1.61 (s, 3H), 1.30 (s, 3H), 1.06 (s, 9H); ¹³C NMR (100 MHz, CDCl₃) δ 152.2, 151.6, 151.4, 144.7, 135.75, 135.71, 133.0, 132.9, 132.6, 130.17, 130.15, 128.00, 127.98, 112.4, 90.4, 85.7, 65.0, 58.2, 51.6, 27.8, 27.0, 25.3, 19.4; Anal. (C₂₉H₃₃ClN₄O₃SeSi) C, H, N.

Compound 6b—[α]_D²⁰ 0.69 (*c* 5.5, CH₂Cl₂); UV (CH₂Cl₂) λ_{max} 253.5 nm; ¹H NMR (400 MHz, CDCl₃) δ 8.89 (s, 1H), 8.65 (s, 1H), 7.68-7.65 (m, 4H), 7.45-7.37 (m, 6H), 6.74 (d, 1H, *J* = 3.6 Hz), 4.85-4.78 (m, 2H), 4.10 (td, 1H, *J* = 3.1, 6.8 Hz), 4.00-3.90 (m, 2H), 1.60 (s, 3H), 1.29 (s, 3H), 1.09 (s, 9H); ¹³C NMR (100 MHz, CDCl₃) δ 162.5, 152.9, 148.3, 143.3, 135.81, 135.77, 132.9, 132.8, 130.4, 130.3, 128.2, 122.6, 112.8, 91.5, 85.3, 66.0, 59.4, 51.2, 27.8, 27.1, 25.3, 19.5; Anal. (C₂₉H₃₃ClN₄O₃SeSi) C, H, N.

9-[6-(*tert*-Butyl-diphenyl-silanyloxymethyl)-2,2-dimethyl-tetrahydro-1,3-dioxo-5-selena-pentalen-4-yl]-2,6-dichloro-9*H*-purine (7a) and its *N*⁷-isomer 7b—A suspension of 2,6-dichloropurine (87 mg, 0.46 mmol) and BSA (0.19 mL, 0.76 mmol) in dry acetonitrile (10 mL) was stirred at 60 °C for 1 h under nitrogen. To this clear solution were added a solution of **5** (205 mg, 0.38 mmol) in dry acetonitrile (5 mL) and TMSOTf (34 μL, 0.19 mmol) at 60 °C, and the reaction mixture was stirred at same temperature for 15 h, cooled to room temperature, diluted with saturated aqueous NaHCO₃ solution (3 mL), and stirred for 15 min. The organic layer was separated, and the aqueous layer was extracted with ethyl acetate (7 mL × 2). The combined organic layers were washed with brine, dried (MgSO₄), filtered, and concentrated under reduced pressure. The crude residue was purified by silica gel column chromatography (hexane : ethyl acetate = 4:1) to give **6b** (50 mg, 20%) as a pale yellow foam and *N*⁷-isomer **7b** (75 mg, 29%) as a pale-yellow foam.

Compound 7a— $[\alpha]_D^{20}$ 13.55 (*c* 8.6, CH₂Cl₂); UV (CH₂Cl₂) $\lambda_{\max}$ 273.0 nm; ¹H NMR (500 MHz, CDCl₃) δ 8.22 (s, 1 H), 7.65–7.61 (m, 4 H), 7.42–7.31 (m, 6 H), 6.24 (d, *J* = 3.7 Hz, 1 H), 5.01–5.00 (m, 1 H), 4.94–4.92 (m, 1 H), 4.13–4.09 (m, 2 H), 3.97 (dd, *J* = 10.5, 12.8 Hz, 1 H), 1.59 (s, 3 H), 1.28 (s, 3 H), 1.05 (s, 9 H); ¹³C NMR (75 MHz, CDCl₃) δ 153.0, 152.3, 152.0, 144.9, 135.5, 135.5, 132.8, 132.6, 131.3, 129.9, 127.8, 127.7, 112.4, 90.1, 85.3, 77.2, 65.7, 57.1, 51.5, 27.7, 26.8, 25.2, 19.2; Anal. (C₂₉H₃₂Cl₂N₄O₃SeSi) C, H, N.

Compound 7b— $[\alpha]_D^{20}$ -15.94; (*c* 0.32, CH₂Cl₂); UV (CH₂Cl₂) $\lambda_{\max}$ 258.0 nm; ¹H NMR (400 MHz, CDCl₃) δ 8.66 (s, 1 H), 7.64–7.67 (m, 4 H), 7.37–7.48 (m, 6 H), 6.64 (d, 1 H, *J* = 3.6 Hz), 4.81–4.83 (dd, *J* = 3.2, 5.6 Hz, 1 H), 4.73–4.75 (dd, *J* = 3.6, 5.6 Hz, 1 H), 4.08–4.15 (m, 1 H), 3.89–3.98 (m, 2 H), 1.59 (s, 3 H), 1.28 (s, 3 H), 1.09 (s, 9 H); ¹³C NMR (100 MHz, CDCl₃) δ 164.1, 153.7, 149.8, 144.1, 135.79, 135.75, 132.8, 132.7, 130.4, 130.3, 128.2, 128.0, 121.8, 112.8, 91.5, 85.3, 65.9, 59.8, 51.4, 27.7, 27.1, 25.2, 19.5; Anal. (C₂₉H₃₂Cl₂N₄O₃SeSi) C, H, N.

Conversion of 6b to 6a—A solution of **6b** (3.93 g, 6.26 mmol) in toluene (50 mL) was added TMSOTf (0.71 g, 0.59 mL, 3.15 mmol) at 95 °C, and the reaction mixture was stirred at the same temperature for 30 min, cooled to room temperature, and diluted with saturated aqueous solution of NaHCO₃ (10 mL). The aqueous layer was extracted with ethylene acetate (10 mL × 2) and the organic layer was dried (MgSO₄), filtered and evaporated under reduced pressure. The crude residue was purified on flash silica gel column chromatography (hexane : ethyl acetate = 4 : 1) to give **6a** (1.95 g, 50%) as a pale yellow foam.

Conversion of 7b to 7a—A solution of **7b** (56 mg, 0.085 mmol) in toluene (5 mL) was added TMSOTf (8 μ L, 0.042 mmol) at 70 °C, and the reaction mixture was stirred at the same temperature for 30 min, cooled to room temperature, and diluted with saturated aqueous solution of NaHCO₃ (5 mL). The aqueous layer was extracted with ethylene acetate (10 mL × 2) and the organic layer was dried (MgSO₄), filtered and evaporated under reduced pressure. The crude residue was purified on flash silica gel column chromatography (hexane : ethyl acetate = 4 : 1) to give **7a** (26 mg, 46%) as a pale yellow foam.

((3a*S*,4*R*,6*R*,6a*R*)-6-(6-Chloro-9*H*-purin-9-yl)-2,2-dimethyltetrahydroselenopheno[3,4-*d*][1,3]dioxol-4-yl)methanol (8a)—To a stirred solution of **7a** (745 mg, 1.19 mmol) in THF (5 mL) was added HF·Et₃N (0.29 mL, 1.78 mmol) and the reaction mixture stirred at room temperature for 15 h and evaporated under reduced pressure. The residue was purified by silica gel column chromatography (hexane : ethyl acetate = 1 : 1) to give **8a** (372 mg, 80%) as a yellow form: $[\alpha]_D^{20}$ -43.57 (*c* 5.10, CH₂Cl₂); UV (CH₂Cl₂) $\lambda_{\max}$ 265.5 nm; ¹H NMR (400 MHz, CDCl₃) δ 9.10 (s, 1 H), 8.76 (s, 1 H), 6.35 (d, 1 H, *J* = 2.8 Hz), 5.27 (bs, 1 H), 5.12 (dd, 1 H, *J* = 2.8, 4.8 Hz), 5.03 (dd, 1 H, *J* = 1.2, 5.2 Hz), 4.25 (dd, 1 H, *J* = 3.4, 11 Hz), 4.18 (bs, 1 H), 4.07 (dd, 1 H, *J* = 2.6, 11 Hz), 1.62 (s, 3 H), 1.31 (s, 3 H); ¹³C NMR (100 MHz, CDCl₃) δ 152.1, 151.3, 151.0, 146.5, 131.9, 111.6, 92.9, 89.0, 65.3, 61.8, 51.8, 27.9, 25.2; Anal. (C₁₃H₁₅ClN₄O₃Se) C, H, N.

((3a*S*,4*R*,6*R*,6a*R*)-6-(2,6-dichloro-9*H*-purin-9-yl)-2,2-dimethyltetrahydroselenopheno[3,4-*d*][1,3]dioxol-4-yl)methanol (8b)—

Compound **7b** (7.28 g, 10.99 mmol) was converted to **8b** (3.77 g, 81%) using similar procedure used in the preparation of **8a**: mp 181–186 °C; $[\alpha]_D^{20}$ -53.7 (*c* 1.8, CH₂Cl₂+CH₃OH); UV (CH₂Cl₂) $\lambda_{\max}$ 274.0 nm; ¹H NMR (500 MHz, CDCl₃) δ 8.79 (s, 1 H), 6.28 (d, *J* = 3.2 Hz, 1 H), 5.15 (t, *J* = 8.1 Hz, 1 H), 5.03 (d, *J* = 4.9 Hz, 1 H), 4.22–4.19 (m, 2 H), 4.13–4.12 (m, 1 H), 4.07–4.04 (m, 1 H), 1.63 (s, 3 H), 1.33 (s, 3 H); ¹³C NMR (75 MHz, CDCl₃) δ 152.9, 152.2, 151.8, 146.3, 131.1, 111.7, 92.0, 88.3, 77.2, 65.2, 61.2, 51.2, 27.9, 25.1; Anal. (C₁₃H₁₄Cl₂N₄O₃Se) C, H, N.

((3a*S*,4*R*,6*R*,6a*R*)-6-(6-Chloro-9*H*-purin-9-yl)-2,2-dimethyltetrahydroselenopheno[3,4-*d*][1,3]dioxol-4-yl)methyl 4-nitrobenzoate (9a)—

To a stirred solution of **8a** (372 mg, 0.96 mmol) in dry dichloromethane (5 mL) were added *p*-nitrobenzoyl chloride (266 mg, 1.43 mmol) and triethylamine (0.27 mL, 1.91 mmol) at room temperature, and the reaction mixture was stirred at room temperature for 1 h and diluted with water (5 mL × 2). The aqueous layer was extracted with ethyl acetate (10 mL × 2), and the combined organic layers were washed with brine (5 mL), dried (MgSO₄), filtered, and evaporated under reduced pressure. The residue was purified by silica gel column chromatography (hexane : ethyl acetate = 3 : 1) to give **9a** (500 mg, 97%) as a yellow form: $[\alpha]_D^{20}$ -160.3 (*c* 2.10, CH₂Cl₂+CH₃OH); UV (CH₂Cl₂) $\lambda_{\max}$ 262.0 nm; ¹H NMR (400 MHz, CDCl₃) δ 8.80 (s, 1 H), 8.29 (s, 1 H), 8.28 (d, *J* = 8.5 Hz, 2 H), 8.17 (d, *J* = 9.0 Hz, 2 H), 6.35 (d, *J* = 2.7 Hz, 1 H), 5.41 (dd, *J* = 2.8, 5.5 Hz, 1 H), 5.22 (d, *J* = 3.5, 5.5 Hz, 1 H), 5.00 (dd, *J* = 8.1, 11.5 Hz, 1 H), 4.70 (dd, *J* = 7.0, 11.5 Hz, 1 H), 4.29 (td, *J* = 1.7, 7.5 Hz, 1 H), 1.63 (s, 3 H), 1.34 (s, 3 H); ¹³C NMR (75 MHz, CDCl₃) δ 167.3, 151.8, 151.4, 150.8, 150.5, 144.6, 134.6, 132.3, 130.8, 123.6, 123.5, 112.5, 90.2, 86.8, 66.7, 58.8, 47.9, 29.5, 27.5, 25.0; Anal. (C₂₀H₁₈ClN₅O₆Se) C, H, N.

((3a*S*,4*R*,6*R*,6a*R*)-6-(2,6-Dichloro-9*H*-purin-9-yl)-2,2-dimethyltetrahydroselenopheno[3,4-*d*][1,3]dioxol-4-yl)methyl 4-nitrobenzoate (9b)—

Compound **8b** (1.50 g, 3.54 mmol) was converted to **9b** (1.98 g, 98%) as a white solid, using similar procedure used in the preparation of **9a**: mp 177–182 °C; $[\alpha]_D^{20}$ -3.86 (*c* 12.6, CH₂Cl₂); UV (CH₂Cl₂) $\lambda_{\max}$ 268.0 nm; ¹H NMR (500 MHz, CDCl₃) δ 8.26 (d, *J* = 8.6 Hz, 2 H), 8.24 (s, 1H), 8.18 (d, *J* = 8.5 Hz, 2 H), 6.31 (d, *J* = 8.5 Hz, 1 H), 5.33–5.31 (m, 1 H), 5.27–5.25 (m, 1 H), 4.98 (dd, *J* = 8.3, 11.4 Hz, 1 H), 4.74 (dd, *J* = 7.2, 11.4 Hz 1 H), 1.60 (s, 3 H), 1.33 (s, 3 H); ¹³C NMR (75 MHz, CDCl₃) δ 164.1, 152.9, 152.4, 152.0, 150.6, 144.9, 134.7, 131.6, 130.8, 123.6, 112.8, 90.3, 87.0, 66.7, 58.3, 48.2, 27.7, 25.2; Anal. (C₂₀H₁₇Cl₂N₅O₆Se) C, H, N.

((2*R*,3*S*,4*R*,5*R*)-5-(6-Chloro-9*H*-purin-9-yl)-3,4-

dihydroxytetrahydroselenophen-2-yl)methyl 4-nitrobenzoate (10a)—A solution of **9a** (2.15 g, 4.35 mmol) in aqueous trifluoroacetic acid (60 mL) and THF (10 mL) was stirred at room temperature for 2 h and evaporated under reduced pressure. The residue was dissolved in ethyl acetate (50 mL) and the organic layer was washed with water (30 mL × 2). The aqueous layer was again extracted with ethyl acetate (30 mL × 3). The combined organic layers were washed with brine (5 mL), dried (MgSO₄), filtered and evaporated under

reduced pressure. The residue was purified by silica gel column chromatography (hexane : ethyl acetate = 1 : 1 to 1 : 5) to give **10a** (1.50 g, 76%) as a white solid: $[\alpha]_D^{20}$ -108.9 (*c* 2.53, CH₂Cl₂+CH₃OH); UV (CH₂Cl₂) $\lambda_{\max}$ 262.0 nm; ¹H NMR (500 MHz, CD₃OD) δ 8.83 (s, 1 H), 8.71 (s, 1 H), 8.30 (d, *J* = 8.8 Hz, 2 H), 8.21 (d, *J* = 8.7 Hz, 2 H), 6.31 (d, *J* = 6.9 Hz, 1 H), 5.05 (dd, *J* = 3.2, 6.7 Hz, 1 H), 4.99 (d, *J* = 8.2, 11.5 Hz, 1 H), 4.73 (dd, *J* = 6.7, 11.5 Hz, 1 H), 4.56 (t, *J* = 3.3 Hz, 1 H), 4.00-3.97 (m, 1 H); ¹³C NMR (75 MHz, CD₃OD) δ 166.4, 154.0, 153.6, 152.8, 152.1, 148.7, 137.2, 133.7, 132.6, 125.5, 80.5, 77.2, 68.8, 57.0, 46.3; Anal. (C₁₇H₁₄ClN₅O₆Se) C, H, N.

((2R,3S,4R,5R)-5-(2,6-Dichloro-9H-purin-9-yl)-3,4-dihydroxytetrahydroselenophen-2-yl)methyl 4-nitrobenzoate (10b)—Compound **9b** (1.98 g, 3.45 mmol) was converted to **10b** (1.60 g, 87%) as a yellow foam, using similar procedure used in the preparation of **10a**: $[\alpha]_D^{20}$ -11.59 (*c* 12.7, CH₂Cl₂+CH₃OH); UV (CH₂Cl₂) $\lambda_{\max}$ 267.0 nm; ¹H NMR (500 MHz, CD₃OD+CDCl₃) δ 8.78 (s, 1 H), 8.26 (d, *J* = 8.7 Hz, 2 H), 8.17 (d, *J* = 8.7 Hz, 2 H), 6.23 (d, *J* = 6.6 Hz, 1 H), 4.98-4.93 (m, 2 H), 4.76-4.71 (m, 1 H), 4.54-4.53 (t, *J* = 3.5 Hz, 1 H), 4.01-3.98 (m, 1 H), 3.63 (d, *J* = 6.2 Hz, 1 H), 3.08 (d, *J* = 4.8 Hz, 1 H); ¹³C NMR (75 MHz, CD₃OD +CDCl₃) δ 166.1, 154.9, 154.2, 152.7, 152.4, 149.0, 136.7, 132.8, 132.4, 125.2, 80.5, 79.8, 77.0, 68.7, 56.9, 46.2; Anal. (C₁₇H₁₃Cl₂N₅O₆Se) C, H, N.

((2R,3S,4R,5R)-3,4-Bis((tert-butyldimethylsilyl)oxy)-5-(6-chloro-9H-purin-9-yl)tetrahydroselenophen-2-yl)methyl 4-nitrobenzoate (11a)—To a stirred solution of **10a** (1.07 g, 2.36 mmol) in dry dichloromethane (30 mL) were added TMSOTf (1.87 g, 1.62 mL, 7.07 mmol), triethylamine (1.43 g, 1.97 mL, 14.15 mmol) and 4-dimethylaminopyridine (29 mg, 0.24 mmol), the reaction mixture was stirred at room temperature for 1 h and diluted with NaHCO₃ (60 mL). The aqueous layer was extracted with dichloromethane (30 mL × 2). The organic layer was washed with brine (5 mL), dried (MgSO₄), filtered and evaporated under reduced pressure. The residue was purified by silica gel column chromatography (hexane : ethyl acetate = 9 : 1) to give **11a** (1.54 g, 96%) as a white foam: $[\alpha]_D^{20}$ -85.6 (*c* 4.00, CH₂Cl₂); UV (CH₂Cl₂) $\lambda_{\max}$ 263.0 nm; ¹H NMR (400 MHz, CDCl₃) δ 8.77 (s, 1 H), 8.34 (s, 1 H), 8.31 (d, *J* = 8.8 Hz, 1 H), 8.20 (d, *J* = 8.8 Hz, 2 H), 6.08 (d, *J* = 6.3 Hz, 1 H), 5.09 (dd, *J* = 8.3, 11.7 Hz, 1 H), 4.99 (broad s, 1 H), 4.66 (dd, *J* = 6.5, 11.7 Hz, 1 H), 4.33 (t, *J* = 3.2 Hz, 1 H), 3.88 (broad s, 1 H), 0.91 (s, 9 H), 0.73 (s, 9 H), 0.066 (s, 3 H), 0.042 (s, 3 H), -0.077 (s, 3 H), -0.34 (s, 3 H); ¹³C NMR (75 MHz, CDCl₃) δ 164.3, 151.8, 151.6, 151.5, 150.8, 145.5, 134.7, 132.6, 130.7, 123.8, 78.8, 75.9, 66.5, 55.5, 44.7, 25.7, 25.5, 18.0, 17.7, -4.4, -4.7, -4.8, -5.3; Anal. (C₂₉H₄₂ClN₅O₆SeSi₂) C, H, N.

((2R,3S,4R,5R)-3,4-Bis((tert-butyldimethylsilyl)oxy)-5-(2,6-dichloro-9H-purin-9-yl)tetrahydroselenophen-2-yl)methyl 4-nitrobenzoate (11b)—Compound **10b** (1.47 g, 2.76 mmol) was converted to **11b** (1.76 g, 84%) as a yellow foam, using similar procedure used in the preparation of **11a**: $[\alpha]_D^{20}$ -8.07 (*c* 7.4, CH₂Cl₂); UV (CH₂Cl₂) $\lambda_{\max}$ 266.0 nm; ¹H NMR (500 MHz, CDCl₃) δ 8.35 (s, 1 H), 8.27 (d, *J* = 8.8 Hz, 2 H), 8.18 (d, *J* = 8.9 Hz, 2 H), 5.98 (s, 1 H), 5.06 (dd, *J* = 8.2, 11.7 Hz, 1 H), 4.88 (broad s, 1 H), 4.62 (dd, *J* = 6.5, 11.5 Hz, 1 H), 4.30-4.29 (m, 1 H), 3.88 (broad s, 1 H), 0.87 (s, 9 H), 0.73 (s, 9 H), 0.015 (s, 3 H), -0.018 (s, 3 H), -0.061 (s, 3 H), -0.30 (s, 3 H); ¹³C NMR (75 MHz, CDCl₃) δ 64.1,

152.8, 152.7, 152.1, 150.7, 146.1, 134.6, 131.6, 130.6, 123.7, 78.9, 75.9, 66.2, 55.5, 44.8, 25.6, 25.5, 17.9, 17.7, -4.42, -4.72, -4.95, -5.34; Anal. (C₂₉H₄₁Cl₂N₅O₆SeSi₂) C, H, N.

((2R,3S,4R,5R)-3,4-Bis((*tert*-butyldimethylsilyl)oxy)-5-(6-chloro-9H-purin-9-yl)tetra hydroselenophen-2-yl)methanol (12a)—To a stirred solution of **11a** (400 mg, 0.55 mmol) in dioxane (10 mL) was added 1N NaOH solution in water (1 mL) at room temperature, and the mixture was stirred at same temperature for 3 h. The reaction mixture was neutralized with glacial acetic acid and diluted with saturated NaHCO₃ solution (5 mL × 2). The aqueous layer was extracted with ethyl acetate (10 mL × 2), and the organic layer was washed with brine, dried (MgSO₄), filtered and evaporated under reduced pressure. The residue was purified by silica gel column chromatography (hexane : ethyl acetate = 5 : 1) to give **12a** (277 mg, 87%) as a white foam: [α]_D²⁰ -25.52 (c 4.62, CH₂Cl₂); UV (CH₂Cl₂) λ_{max} 265.0 nm; ¹H NMR (400 MHz, CDCl₃) δ 8.79 (s, 1 H), 8.27 (s, 1 H), 5.97 (d, 1 H, *J* = 7.2 Hz), 5.18 (bs, 1 H), 4.29 (t, 1 H, *J* = 2.8 Hz), 4.06-3.95 (m, 2 H), 3.67 (d, 1 H, *J* = 0.8 Hz), 0.95 (s, 9 H), 0.73 (s, 9 H), 0.12 (s, 3 H), 0.1 (s, 3 H), -0.13 (s, 3 H), -0.61 (d, 3 H, *J* = 1.2 Hz); ¹³C NMR (100 MHz, CDCl₃) δ 152.1, 151.5, 151.4, 146.3, 133.4, 78.9, 78.6, 64.2, 57.5, 51.9, 26.0, 25.94, 25.89, 25.85, 25.82, 18.2, 17.9, -4.19, -4.51, -5.53; Anal. (C₂₂H₃₉ClN₄O₃SeSi₂) C, H, N.

((2R,3S,4R,5R)-3,4-Bis((*tert*-butyldimethylsilyl)oxy)-5-(2,6-dichloro-9H-purin-9-yl)tetra hydroselenophen-2-yl)methanol (12b)—Compound **11b** (1.62 g, 2.13 mmol) was converted to **12b** (1.07 g, 83%) as a yellow foam, using similar procedure used in the preparation of **12a**: [α]_D²⁰ -9.11 (c 15.8, CH₂Cl₂); UV (CH₂Cl₂) λ_{max} 276.0 nm; ¹H NMR (400 MHz, CDCl₃) δ 8.33 (s, 1 H), 5.89 (d, *J* = 6.9 Hz, 1 H), 5.00 (broad s, 1 H), 4.25 (t, *J* = 2.9 Hz, 1 H), 4.02-3.94 (m, 2 H), 3.66 (s, 1 H), 0.90 (s, 9 H), 0.72 (s, 9 H), 0.080 (s, 3 H), 0.046 (s, 3 H), -0.11 (s, 3 H), -0.50 (s, 3 H); ¹³C NMR (75 MHz, CDCl₃) δ 152.5, 152.2, 146.7, 131.9, 79.7, 77.8, 77.2, 63.5, 56.9, 51.3, 25.7, 25.6, 18.0, 17.7, -4.40, -4.68, -4.78, -5.58; Anal. (C₂₂H₃₈Cl₂N₄O₃SeSi₂) C, H, N.

(2S,3S,4R,5R)-3,4-Bis((*tert*-butyldimethylsilyl)oxy)-5-(6-chloro-9H-purin-9-yl)tetra hydroselenophene-2-carbaldehyde (13a)—To a solution of **12a** (558 mg, 0.97 mmol) in dimethyl sulfoxide (5 mL) was added acetic anhydride (0.18 mL, 1.93 mmol) and the reaction mixture was stirred at 100 ° for 1 h, cooled to room temperature, and diluted with dichloromethane (20 mL). The mixture was washed with water (10 mL × 2), and the aqueous layer was further extracted with dichloromethane (20 mL × 2). The combined organic layers were washed with brine (5 mL), dried (MgSO₄), filtered, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (hexane : ethyl acetate = 4 : 1) to give **13a** (483 mg, 87%) as a white foam: [α]_D²⁰ -74.88 (c 1.23, CH₂Cl₂); UV (CH₂Cl₂) λ_{max} 265.0 nm; ¹H NMR (400 MHz, CDCl₃) δ 9.76 (d, 1 H, *J* = 2.4 Hz), 8.78 (s, 1 H), 8.32 (s, 1 H), 6.24 (d, 1 H, *J* = 7.2 Hz), 4.86 (dd, 1 H, *J* = 2.6, 7.0 Hz), 4.68 (t, 1 H, *J* = 3.0 Hz), 4.12 (dd, 1 H, *J* = 2.4, 2.8 Hz), 0.95 (s, 9 H), 0.71 (s, 9 H), 0.12 (s, 6 H), -0.05 (s, 3 H), -0.4 (s, 3 H); ¹³C NMR (100 MHz, CDCl₃) δ 194.6, 152.3, 152.1, 151.7, 145.3, 132.5, 81.0, 77.4, 74.6, 56.9, 56.8, 53.0, 25.9, 25.7, 18.3, 17.9, 0.21, -4.2, -4.45, -4.55, -5.3; Anal. (C₂₂H₃₇ClN₄O₃SeSi₂) C, H, N.

(2S,3S,4R,5R)-3,4-Bis((*tert*-butyldimethylsilyl)oxy)-5-(2,6-dichloro-9H-purin-9-yl)tetra hydroselenophene-2-carbaldehyde (13b)—Compound **12b** (3.99 g, 5.50 mmol) was converted to **13b** (2.98 g, 75%) as a yellow foam, using similar procedure used in the preparation of **13a**: $[\alpha]_D^{20}$ -33.2 (*c* 10.8, CH₂Cl₂); UV (CH₂Cl₂) $\lambda_{\max}$ 276.0 nm; ¹H NMR (500 MHz, CDCl₃) δ 9.73 (d, *J* = 2.1 Hz, 1 H), 8.40 (s, 1 H), 6.13 (d, *J* = 6.8 Hz, 1 H), 4.74-4.73 (m, 1 H), 4.63 (t, *J* = 2.9 Hz, 1 H), 4.11 (s, 1 H), 0.91 (s, 9 H), 0.71 (s, 9 H), 0.090 (s, 6 H), -0.043 (s, 3 H), -0.38 (s, 3 H); ¹³C NMR (75 MHz, CDCl₃) δ 194.3, 153.2, 153.0, 152.2, 145.7, 131.3, 81.0, 74.4, 56.7, 52.9, 25.69, 25.66, 25.6, 18.0, 17.6, -4.42, -4.70, -4.81, -4.86, -5.55; Anal. (C₂₂H₃₆Cl₂N₄O₃SeSi₂) C, H, N.

(2S,3S,4R,5R)-3,4-Bis((*tert*-butyldimethylsilyl)oxy)-5-(6-chloro-9H-purin-9-yl)-N-methyltetrahydroselenophene-2-carboxamide (14a)—To a solution of AgNO₃ (51 mg, 0.30 mmol) was added 1 N NaOH (0.30 mL, 0.30 mmol) at 0 °C. After Ag₂O was precipitated, 24% ammonia water (0.09 mL) was added to reaction mixture until the mixture was clear. The solution of **13a** (87 mg, 0.15 mmol) in THF (2 mL) was added to above Tollens' reagent at 0 °C, and the reaction mixture was stirred at same temperature for 30 min and diluted with water (6 mL). The aqueous layer was extracted with ethyl acetate (10 mL $\times$ 2), and the aqueous layer was acidified with 1 N HCl solution (3 mL) and extracted with ethyl acetate (10 mL $\times$ 2). The combined organic layers were washed with brine, dried (MgSO₄), filtered and evaporated under reduced pressure to give the crude acid. To this dried the residue were added a solution of crude acid in THF (5 mL), 1-[bis(dimethylamino)methylene]-1*H*-1,2,3-triazolo[4,5-*b*]pyridinium 3-oxid hexafluoro phosphate (HATU) (50 mg, 0.13 mmol), methylamine hydrochloride (20 mg, 0.30 mmol), and diisopropylamine (0.06 mL, 0.34 mmol) at room temperature. The reaction mixture was stirred at same temperature for 1 h, and diluted with ethyl acetate (5 mL). The organic layer was washed with water (3 mL $\times$ 2) and the aqueous layer was further extracted with ethyl acetate (10 mL $\times$ 2). The combined organic layers were washed with brine (2 mL), dried (MgSO₄), filtered and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (hexane : ethyl acetate = 3 : 1) to give **14a** (50 mg, 54%) as a white foam: $[\alpha]_D^{20}$ -36.04 (*c* 1.92, CH₂Cl₂); UV (CH₂Cl₂) $\lambda_{\max}$ 265.5 nm; ¹H NMR (400 MHz, CDCl₃) δ 8.77 (s, 1 H), 8.40 (bs, 1 H), 6.06 (d, 1 H, *J* = 7.6 Hz), 4.1 (bs, 1 H), 4.55 (t, 1 H, *J* = 2.4 Hz), 3.96 (d, 1 H, *J* = 2.8 Hz), 2.98 (d, 3 H, *J* = 4.8 Hz), 0.94 (s, 9 H), 0.7 (s, 9 H), 0.16 (s, 3 H), -0.17 (s, 3 H), -0.53 (s, 3 H); ¹³C NMR (100 MHz, CDCl₃) δ 170.2, 152.1, 151.5, 146.6, 133.2, 79.5, 78.3, 56.6, 49.9, 27.2, 25.9, 25.7, 18.2, 17.9, -4.3, -4.6, -5.4; Anal. (C₂₃H₄₀ClN₅O₃SeSi₂) C, H, N.

(2S,3S,4R,5R)-3,4-Bis((*tert*-butyldimethylsilyl)oxy)-5-(2,6-dichloro-9H-purin-9-yl)-N-methyltetrahydroselenophene-2-carboxamide (14b)—Compound **13b** (86 mg, 0.14 mmol) was converted to **14b** (56 mg, 62%) as a yellow foam, using similar procedure used in the preparation of **14a**: ¹H NMR (400 MHz, CDCl₃) δ 8.35 (s, 1 H), 5.97 (d, *J* = 7.6 Hz, 1 H), 4.83 (broad s, 1 H), 4.52 (s, 1 H), 3.95 (d, *J* = 2.7 Hz, 1 H), 2.99 (d, *J* = 4.7 Hz, 3 H), 0.91 (s, 9 H), 0.69 (s, 9 H), 0.15 (s, 3 H), 0.096 (s, 3 H), -0.16 (s, 3 H), -0.50 (s, 3 H); Anal. (C₂₃H₃₉Cl₂N₅O₃SeSi₂) C, H, N.

(2*S*,3*S*,4*R*,5*R*)-5-(6-Chloro-9*H*-purin-9-yl)-3,4-dihydroxy-*N*-methyltetrahydroseleno phene-2-carboxamide (15a)—To a stirred solution of **14a** (78 mg, 0.13 mmol) in THF (3 mL) was added tetra-*n*-butylammonium fluoride hydrate (34 mg, 0.13 mmol) and the reaction mixture was stirred at room temperature for 1 h. The solvent was evaporated and the resulting residue was purified by silica gel column chromatography (dichloromethane : methanol = 100 : 1 - 10 : 1) to give **15a** (34 mg, 70%) as a white solid: mp 212 °C (decomposed); $[\alpha]_D^{20}$ -19.05 (*c* 0.20, CH₂Cl₂); UV (CH₂Cl₂) $\lambda_{\max}$ 265.0 nm; ¹H NMR (400 MHz, CD₃OD) δ 9.03 (s, 1 H), 8.77 (s, 1 H), 6.35 (d, 1 H, *J* = 6.4 Hz), 4.95 (dd, 1 H, *J* = 3.6, 6.0 Hz), 4.60 (t, 1 H, *J* = 3.6 Hz), 4.12 (d, 1 H, *J* = 4.4 Hz), 2.81 (s, 3 H); ¹³C NMR (100 MHz, CD₃OD) δ 173.4, 152.8, 152.5, 151.4, 147.4, 132.5, 81.5, 78.6, 77.2, 56.5, 47.1, 26.9; Anal. (C₁₁H₁₂ClN₅O₃Se) C, H, N.

(2*S*,3*S*,4*R*,5*R*)-5-(2,6-Dichloro-9*H*-purin-9-yl)-3,4-dihydroxy-*N*-methyltetrahydroseleno phene-2-carboxamide (15b)—Compound **14b** (290 mg, 0.450 mmol) was converted to **15b** (155 mg, 83%) as a white solid, using similar procedure used in the preparation of **15a**: mp 141-144 °C; $[\alpha]_D^{20}$ -7.58 (*c* 2.3, CH₃OH); UV (CH₂Cl₂) $\lambda_{\max}$ 276.0 nm; ¹H NMR (500 MHz, CD₃OD) δ 9.03 (s, 1 H), 6.23 (d, *J* = 5.9 Hz, 1 H), 4.88 (dd, *J* = 3.2, 5.9 Hz, 1 H), 4.58 (t, *J* = 3.7 Hz, 1 H), 4.11 (d, *J* = 4.4 Hz, 1 H), 2.81 (s, 3 H); ¹³C NMR (75 MHz, CD₃OD+CDCl₃) δ 173.9, 154.7, 154.1, 148.7, 132.4, 82.1, 77.8, 57.3, 48.0, 41.1, 27.8; Anal. (C₁₁H₁₁Cl₂N₅O₃Se) C, H, N.

7-((3*aR*,4*R*,6*R*,6*aS*)-6-(((*tert*-Butyldiphenylsilyl)oxy)methyl)-2,2-dimethyltetrahydro selenopheno[3,4-*d*][1,3]dioxol-4-yl)-*N*-(3-iodobenzyl)-7*H*-purin-6-amine (16a)—A solution of **7a** (1.49 g, 2.39 mmol), 3-iodobenzylamine hydrochloride (840 mg, 3.10 mmol), and triethylamine (1.05 mL, 7.16 mmol) in ethanol (50 mL) was stirred at room temperature for 15 h. The reaction mixture was evaporated under reduced pressure, and the resulting residue was purified by silica gel chromatography (hexane : ethyl acetate = 3 : 1) to give **16a** (1.70 g, 87%) as a white foam: $[\alpha]_D^{20}$ 8.00 (*c* 0.20, CH₂Cl₂); UV (CH₂Cl₂) $\lambda_{\max}$ 280.5 nm; ¹H NMR (400 MHz, CDCl₃) δ 8.57 (s, 1 H), 8.37 (s, 1 H), 7.73 (t, *J* = 3.2 Hz, 1 H), 7.64-7.61 (m, 1 H), 7.57-7.52 (m, 4 H), 7.37-7.21 (m, 7 H), 7.06 (t, *J* = 7.8 Hz, 1 H), 6.60 (t, *J* = 10.0 Hz, 1 H), 5.93 (d, *J* = 3.2 Hz, 1 H), 4.79 (m, 2 H), 4.64 (dd, *J* = 5.2, 14.4 Hz, 1 H), 4.20 (dd, *J* = 3.2, 5.6 Hz, 1 H), 4.02-3.93 (m, 2 H), 3.76 (dd, *J* = 8.0, 10.0 Hz, 1 H), 1.31 (s, 3 H), 1.13 (s, 3 H), 1.05 (s, 9 H); ¹³C NMR (100 MHz, CDCl₃) δ 161.5, 153.6, 150.5, 144.4, 141.0, 137.5, 136.9, 135.71, 135.66, 132.7, 132.6, 130.6, 130.3, 130.2, 128.11, 128.08, 128.0, 113.2, 112.3, 94.7, 91.3, 86.1, 65.9, 63.0, 51.4, 44.8, 27.1, 24.9, 19.4; Anal. (C₃₆H₄₀IN₅O₃SeSi) C, H, N.

7-((3*aR*,4*R*,6*R*,6*aS*)-6-(((*tert*-Butyldiphenylsilyl)oxy)methyl)-2,2-dimethyltetrahydro selenopheno[3,4-*d*][1,3]dioxol-4-yl)-2-chloro-*N*-(3-iodobenzyl)-7*H*-purin-6-amine (16b)—Compound **7b** (1.80 g, 2.72 mmol) was converted to **16b** (1.39 g, 60%) as a white foam, using similar procedure used in the preparation of **16a**: $[\alpha]_D^{20}$ 12.17 (*c* 0.23, CH₂Cl₂); UV (CH₂Cl₂) $\lambda_{\max}$ 285.5 nm; ¹H NMR (400 MHz, CDCl₃) δ 8.35 (s, 1 H), 7.74 (t, *J* = 1.6 Hz, 1 H), 7.66-7.64 (m, 1 H), 7.57-7.51 (m, 4 H), 7.40-7.21 (m, 7 H), 7.09 (t, *J* = 7.6 Hz, 1 H), 6.79 (t, *J* = 5.0 Hz, 1 H), 5.84 (d, *J* = 2.8 Hz, 1 H), 4.81-4.73 (m, 2 H), 4.59 (dd, *J* = 5.2, 14.4 Hz, 1 H), 4.13-4.01 (m, 1 H),

4.01-3.94 (m, 2 H), 3.74-3.69 (m, 1 H), 1.29 (s, 3 H), 1.12 (s, 3 H), 1.04 (s, 9 H); ^{13}C NMR (100 MHz, CDCl_3) δ 163.1, 154.8, 151.0, 145.2, 140.3, 137.1, 135.74, 135.68, 132.7, 132.6, 130.7, 130.2, 128.4, 128.2, 128.1, 113.2, 111.1, 94.8, 91.3, 86.4, 65.9, 63.6, 51.4, 45.2, 27.1, 27.0, 24.9, 19.4; Anal. ($\text{C}_{36}\text{H}_{39}\text{ClIN}_5\text{O}_3\text{SeSi}$) C, H, N.

((3a*R*,4*R*,6*R*,6a*S*)-6-(6-((3-iodobenzyl)amino)-7*H*-purin-7-yl)-2,2-dimethyltetrahydro selenopheno[3,4-*d*][1,3]dioxol-4-yl)methanol (17a)—To a solution of **16a** (1.71 g, 2.06 mmol) in THF (20 mL) was added tetra-*n*-butylammonium fluoride (0.9 mL, 3.10 mmol) and the reaction mixture stirred at room temperature for 1 h. The solvent was evaporated and the residue was purified by silica gel chromatography (dichloromethane : methanol = 20 : 1) to give **17a** (1.11 g, 90%) as a white solid: $[\alpha]_{\text{D}}^{20}$ 73.66 (*c* 0.30, CH_2Cl_2); UV (CH_2Cl_2) λ_{max} 279.5 nm; ^1H NMR (400 MHz, CDCl_3) δ 9.04 (s, 1 H), 8.47 (s, 1 H), 7.76 (t, *J* = 1.6 Hz, 1 H), 7.66-7.63 (m, 1 H), 7.37 (d, *J* = 7.6 Hz, 1 H), 7.09 (t, *J* = 7.8 Hz, 1 H), 6.82 (t, *J* = 5.0 Hz, 1 H), 6.07 (d, *J* = 2.4 Hz, 1 H), 5.05 (d, *J* = 5.2 Hz, 1 H), 4.95 (d, *J* = 5.2 Hz, 1 H), 4.23-4.09 (m, 3 H), 1.28 (s, 3 H), 1.22 (s, 3 H); ^{13}C NMR (100 MHz, CDCl_3) δ 160.3, 153.4, 150.7, 146.1, 140.8, 137.7, 136.9, 130.6, 128.1, 111.9, 94.7, 93.6, 90.1, 66.4, 64.5, 52.8, 45.1, 27.2, 25.1; Anal. ($\text{C}_{20}\text{H}_{22}\text{IN}_5\text{O}_3\text{Se}$) C, H, N.

((3a*R*,4*R*,6*R*,6a*S*)-6-(2-Chloro-6-((3-iodobenzyl)amino)-7*H*-purin-7-yl)-2,2-dimethyltetrahydro selenopheno[3,4-*d*][1,3]dioxol-4-yl)methanol (17b)—Compound **16b** (1.39 g, 1.62 mmol) was converted to **17b** (0.86 g, 86%) as a white solid, using similar procedure used in the preparation of **17a**: $[\alpha]_{\text{D}}^{20}$ 83.33 (*c* 0.10, CH_2Cl_2); UV (CH_2Cl_2) λ_{max} 283.5 nm; ^1H NMR (400 MHz, CDCl_3) δ 8.93 (s, 1 H), 7.76 (t, 1 H, *J* = 1.2 Hz), 7.68-7.65 (m, 1 H), 7.40-7.38 (m, 1 H), 7.11 (t, 1 H, *J* = 15.6 Hz), 6.98 (t, 1 H, *J* = 5.2 Hz), 5.97 (d, 1 H, *J* = 2 Hz), 5.06 (d, 1 H, *J* = 4.8 Hz), 4.98 (dd, 1 H, *J* = 2.0, 4.8 Hz), 4.68-4.67 (m, 2 H), 4.18 (dd, *J* = 3.2, 10.8 Hz, 1 H), 4.12 (t, *J* = 3.0 Hz, 1 H), 4.04 (dd, *J* = 3.4, 10.6 Hz, 1 H), 1.26 (s, 3 H), 1.23 (s, 3 H); ^{13}C NMR (100 MHz, CDCl_3) δ 161.8, 154.5, 151.1, 146.9, 140.1, 137.1, 130.7, 128.5, 112.1, 94.7, 93.5, 66.8, 64.6, 53.0, 45.5, 27.1, 25.1; Anal. ($\text{C}_{20}\text{H}_{21}\text{ClIN}_5\text{O}_3\text{Se}$) C, H, N.

((3a*R*,4*R*,6*R*,6a*S*)-6-(6-((3-iodobenzyl)amino)-7*H*-purin-7-yl)-2,2-dimethyltetrahydro selenopheno[3,4-*d*][1,3]dioxol-4-yl)methyl benzoate (18a)—To a solution of **17a** (1.10 g, 1.88 mmol) in pyridine (20 mL) was added benzoyl chloride (0.26 mL, 2.25 mmol) and the reaction mixture stirred at room temperature for 3 h. The reaction mixture was evaporated under reduced pressure, and the resulting residue was purified by silica gel chromatography (hexane : ethyl acetate = 3 : 1) to give **18a** (1.20 g, 96%) as a white foam: $[\alpha]_{\text{D}}^{20}$ 76.57 (*c* 0.10, CH_2Cl_2); UV (CH_2Cl_2) λ_{max} 275.0 nm; ^1H NMR (400 MHz, CDCl_3) δ 8.54 (s, 1 H), 8.45 (s, 1 H), 7.87 (d, *J* = 7.52 Hz, 2 H), 7.74 (s, 1 H), 7.62 (d, *J* = 7.9 Hz, 1 H), 7.54 (t, *J* = 7.4 Hz, 1 H), 7.39-7.35 (m, 3 H), 7.08 (t, *J* = 7.7 Hz, 1 H), 6.66 (s, 1 H), 6.13 (d, *J* = 3.2 Hz, 1 H), 5.02-5.00 (m, 1 H), 4.94-4.91 (m, 1H), 4.70 (d, *J* = 4.96 Hz, 2 H), 4.57 (d, *J* = 6.4 Hz, 2 H), 4.24-4.20 (m, 1 H), 1.33 (s, 3 H), 1.20 (s, 3 H); ^{13}C NMR (75 MHz, CDCl_3) δ 165.9, 161.3, 153.5, 150.4, 144.4, 140.6, 137.3, 136.8, 133.7, 130.5, 129.6, 128.9, 128.6, 127.8, 113.8, 112.0, 94.6, 91.5, 86.4, 77.2, 65.6, 62.7, 47.3, 44.9, 27.0, 24.9; Anal. ($\text{C}_{20}\text{H}_{22}\text{IN}_5\text{O}_3\text{Se}$) C, H, N.

((3*aR*,4*R*,6*R*,6*aS*)-6-(2-Chloro-6-((3-iodobenzyl)amino)-7*H*-purin-7-yl)-2,2-dimethyl tetrahydroselenopheno[3,4-*d*][1,3]dioxol-4-yl)methyl benzoate (18b)

—Compound **17b** (0.86 g, 1.39 mmol) was converted to **18b** (0.91 g, 99 %) as a white foam, using similar procedure used in the preparation of **18a**: $[\alpha]_D^{20}$ 96.59 (*c* 0.10, CH₂Cl₂); UV (CH₂Cl₂) $\lambda_{\max}$ 283.5 nm; ¹H NMR (400 MHz, CDCl₃) δ 8.56 (s, 1 H), 7.86 (d, *J* = 7.4 Hz, 2 H), 7.77 (s, 1 H), 7.67 (d, *J* = 8.0 Hz, 1 H), 7.56 (t, *J* = 8.0 Hz, 1 H), 7.38 (dd, *J* = 8.8, 20.0 Hz, 3 H), 7.12 (t, 1 H, *J* = 8.0 Hz), 6.93 (t, *J* = 4.4 Hz, 1 H), 6.08 (s, 1 H), 5.09-5.01 (m, 2 H), 4.69 (d, *J* = 4.8 Hz, 2 H), 4.67-4.58 (m, 2 H), 4.23-4.27 (m, 1 H), 1.32 (s, 3 H), 1.22 (s, 3 H); ¹³C NMR (100 MHz, CDCl₃) δ 166.2, 162.2, 155.0, 151.1, 145.3, 137.9, 137.3, 133.9, 130.8, 129.8, 129.1, 128.5, 114.0, 111.0, 94.8, 91.9, 87.3, 66.0, 64.0, 48.0, 45.6, 27.1, 25.1; Anal. (C₂₀H₂₁ClIN₅O₃Se) C, H, N.

((2*R*,3*S*,4*R*,5*R*)-3,4-Dihydroxy-5-(6-((3-iodobenzyl)amino)-7*H*-purin-7-yl)tetrahydro selenophen-2-yl)methyl benzoate (19a)

—A solution of **18a** (1.25 g, 1.81 mmol) in 88 % formic acid (10 mL) was stirred at room temperature for 12 h and the reaction mixture was evaporated. The residue was purified by silica gel chromatography (dichloromethane : methanol = 20 : 1) to give **19a** (860 mg, 73%) as a white foam: $[\alpha]_D^{20}$ 29.60 (*c* 0.10, CH₃OH); UV (CH₃OH) $\lambda_{\max}$ 274.5 nm; ¹H NMR (400 MHz, CDCl₃) δ 8.22 (s, 1 H), 8.02 (s, 1 H), 7.99 (d, *J* = 2.8 Hz, 2 H), 7.78 (s, 1 H), 7.58-7.52 (m, 2 H), 7.44-7.35 (m, 3 H), 7.02 (t, *J* = 7.6 Hz, 1 H), 6.24 (d, *J* = 8.4 Hz, 1 H), 5.04 (d, *J* = 7.2 Hz, 1 H), 4.71-4.59 (m, 5 H), 4.01 (t, *J* = 8.0 Hz, 1 H); ¹³C NMR (100 MHz, CDCl₃) δ 166.2, 158.1, 152.2, 151.1, 145.5, 140.8, 137.0, 136.8, 133.7, 130.6, 129.9, 129.5, 128.7, 127.3, 112.3, 94.8, 80.8, 75.4, 66.0, 59.1, 44.6; Anal. (C₂₄H₂₂IN₅O₄Se) C, H, N.

((2*R*,3*S*,4*R*,5*R*)-5-(2-Chloro-6-((3-iodobenzyl)amino)-7*H*-purin-7-yl)-3,4-dihydroxytetra hydroselenophen-2-yl)methyl benzoate (19b)

—Compound **18b** (0.91 g, 1.25 mmol) was converted to **19b** (0.77 mg, 89%) as a white foam, using similar procedure used in the preparation of **19a**: $[\alpha]_D^{20}$ 20.53 (*c* 0.20, CH₃OH); UV (CH₃OH) $\lambda_{\max}$ 280.0 nm; ¹H NMR (400 MHz, CD₃OD) δ 8.68 (s, 1 H), 8.04-8.02 (m, 2 H), 7.84 (t, *J* = 1.6 Hz, 1 H), 7.65-7.61 (m, 1 H), 7.59-7.56 (m, 1 H), 7.51-7.48 (m, 2 H), 7.45-7.43 (m, 1 H), 7.07 (t, *J* = 7.6 Hz, 1 H), 6.32 (d, *J* = 8.0 Hz, 1 H), 4.80-4.70 (m, 3 H), 4.61-4.56 (m, 2 H), 4.44-4.30 (m, 1 H), 3.96-3.92 (m, 1 H); ¹³C NMR (100 MHz, CDCl₃) δ 166.1, 160.2, 153.8, 151.4, 140.1, 137.4, 136.9, 133.7, 130.6, 130.0, 129.4, 128.7, 127.8, 110.8, 94.8, 80.6, 75.2, 65.8, 59.5, 45.2; Anal. (C₂₄H₂₁ClIN₅O₄Se) C, H, N.

((2*R*,3*S*,4*R*,5*R*)-3,4-Bis((*tert*-butyldimethylsilyl)oxy)-5-(6-((3-iodobenzyl)amino)-7*H*-purin-7-yl)tetrahydroselenophen-2-yl)methyl benzoate (20a)

—To a solution of **19a** (860 mg, 1.32 mmol) in dichloromethane (30 mL) were added TMSOTf (1.5 mL, 6.61 mmol) and 2,6-lutidine (1.5 mL, 13.2 mmol) at 0 °C, and the reaction mixture was stirred at room temperature for 15 h. The reaction mixture was diluted with water (20 mL) and extracted with dichloromethane (20 mL $\times$ 2). The organic layer was washed with brine, dried (MgSO₄), filtered, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (hexane : ethyl acetate = 3 : 1) to give **20a** (1.14 g, 98%) as a white foam: $[\alpha]_D^{20}$ 2.67 (*c* 0.10, CH₂Cl₂); UV (CH₂Cl₂) $\lambda_{\max}$ 280.0 nm; ¹H NMR (400 MHz, CDCl₃) δ 8.60 (s, 1 H), 8.02 (d, *J* = 7.2 Hz, 2 H), 7.77 (s, 1

H), 7.64-7.60 (m, 1 H), 7.56 (d, $J = 7.6$ Hz, 1 H), 7.50-7.46 (m, 2 H), 7.39 (d, $J = 7.6$ Hz, 1 H), 7.03 (t, $J = 7.8$ Hz, 1 H), 6.20 (broad s, 1 H), 4.92 (broad s, 2 H), 4.62-4.53 (m, 2 H), 4.42 (dd, $J = 2.6, 9.4$ Hz, 1 H), 4.31 (broad s, 1 H), 3.73-3.71 (m, 1 H); ^{13}C NMR (100 MHz, CDCl_3) δ 166.0, 153.5, 151.0, 141.2, 137.4, 136.8, 133.9, 130.6, 129.8, 129.3, 128.9, 127.7, 94.8, 44.6, 25.8, 125.7, 18.1, 14.4, -4.3, -4.5, -4.8, -5.7; Anal. ($\text{C}_{36}\text{H}_{50}\text{IN}_5\text{O}_4\text{SeSi}_2$) C, H, N.

((2R,3S,4R,5R)-3,4-Bis((*tert*-butyldimethylsilyl)oxy)-5-(2-chloro-6-((3-iodobenzyl) amino)-7H-purin-7-yl)tetrahydroselenophen-2-yl)methyl benzoate (20b)—Compound **19b** (0.77 g, 1.12 mmol) was converted **20b** (0.94 g, 92 %) as a white foam, using similar procedure used in the preparation of **20a**: $[\alpha]_D^{20}$ 44.44 (c 0.10, CHCl_3); UV (CH_2Cl_2) λ_{max} 282.0 nm; ^1H NMR (400 MHz, CDCl_3) δ 8.52 (broad s, 1 H), 8.02 (d, $J = 7.8$ Hz, 1 H), 7.78 (s, 1 H), 7.65-7.61 (m, 1 H), 7.58 (d, $J = 8.0$ Hz, 1 H), 7.50-7.47 (m, 2 H), 7.41 (d, $J = 8.0$ Hz, 1 H), 7.05 (t, 1 H, $J = 4.0$ Hz), 6.13 (broad s, 1 H), 4.88 (broad s, 1 H), 4.58 (broad s, 2 H), 4.36 (dd, $J = 2.8, 6$ Hz, 1 H), 4.21 (broad s, 1 H), 3.96 (s, 1 H) 3.70 (s, 1 H), 0.89 (s, 9 H), 0.65 (s, 9 H), 0.05 (s, 3 H), 0.02 (s, 3 H), -0.16 (s, 3 H), -0.46 (broad s, 3 H); ^{13}C NMR (100 MHz, CDCl_3) δ 166.0, 154.7, 154.7, 151.6, 140.4, 137.8, 137.1, 133.9, 130.7, 129.9, 129.2, 128.9, 128.2, 112.2, 96.3, 94.8, 80.6, 74.6, 64.7, 58.3, 45.0, 25.9, 25.85, 25.77, 18.1, 0.2, -4.3, -4.5, -4.8, -5.6; Anal. ($\text{C}_{36}\text{H}_{49}\text{ClIN}_5\text{O}_4\text{SeSi}_2$) C, H, N.

((2R,3S,4R,5R)-3,4-Bis((*tert*-butyldimethylsilyl)oxy)-5-(6-((3-iodobenzyl)amino)-7H-purin-7-yl)tetrahydroselenophen-2-yl)methanol (21a)—To a solution of **20a** (1.14 g, 1.30 mmol) in methanol (25 mL) was added sodium methoxide (105 mg, 1.95 mmol) and the mixture was stirred at room temperature for 1 h. The reaction mixture was evaporated under reduced pressure and the residue was purified by silica gel column chromatography (hexane : ethyl acetate = 1 : 1) to give **21a** (1.00 g, 99%) as a white foam: $[\alpha]_D^{20}$ 26.00 (c 0.10, CH_2Cl_2); UV (CH_2Cl_2) λ_{max} 278.5 nm; ^1H NMR (400 MHz, CDCl_3) δ 8.48 (s, 1 H), 7.77 (s, 1 H), 7.55 (d, $J = 8.0$ Hz, 1 H), 7.39 (d, $J = 8.4$ Hz, 1 H), 7.02 (t, $J = 7.6$ Hz, 1 H), 6.08 (broad s, 1 H), 4.87 (broad s, 2 H), 4.61 (broad s, 1 H), 4.30 (broad s, 1 H), 4.01 (dd, $J = 4.2, 11.4$ Hz, 1 H), 3.77 (broad s, 1 H), 3.62 (t, $J = 4.4$ Hz, 1 H), 0.94 (s, 9 H), 0.64 (s, 9 H), 0.15 (s, 3 H), 0.12 (s, 3 H), -0.20 (s, 3 H), -0.62 (s, 3 H); ^{13}C NMR (75 MHz, CDCl_3) δ 152.6, 151.0, 141.8, 137.1, 136.5, 130.4, 127.5, 94.6, 50.5, 44.6, 26.0, 25.9, 18.2, -4.17, -4.21, -4.50, -5.67; Anal. ($\text{C}_{29}\text{H}_{46}\text{IN}_5\text{O}_3\text{SeSi}_2$) C, H, N.

((2R,3S,4R,5R)-3,4-Bis((*tert*-butyldimethylsilyl)oxy)-5-(2-chloro-6-((3-iodobenzyl) amino)-7H-purin-7-yl)tetrahydroselenophen-2-yl)methanol (21b)—Compound **20b** (0.86 g, 0.94 mmol) was converted to **21b** (0.65 mg, 90%) as a white foam, using similar procedure used in the preparation of **21a**: $[\alpha]_D^{20}$ 8.42 (c 0.10, CDCl_3); UV (CH_2Cl_2) λ_{max} 281.5 nm; ^1H NMR (400 MHz, CDCl_3) δ 8.49 (broad s, 1 H), 7.78 (s, 1 H), 7.74 (broad s, 1 H), 7.53 (d, $J = 8.0$ Hz, 1 H), 7.41 (d, $J = 8.0$ Hz, 1 H), 7.00 (t, $J = 8.0$ Hz, 1 H), 6.00 (broad s, 1 H), 4.89 (broad s, 1 H), 4.75 (broad s, 1 H), 4.64 (broad s, 1 H), 4.29 (broad s, 1 H), 4.14 (d, $J = 8.8$ Hz, 1 H), 3.79 (broad s, 1 H), 3.64-3.63 (m, 1 H), 0.95 (s, 9 H), 0.64 (s, 9 H), 0.15 (s, 3 H), 0.12 (s, 3 H), -0.20 (s, 3 H), -0.63 (broad s, 3 H); ^{13}C NMR (100 MHz, CDCl_3) δ 154.6, 151.3, 147.7, 141.5, 137.5, 136.4, 130.3, 127.9, 94.5, 81.3,

77.1, 63.0, 59.5, 50.6, 44.7, 26.0, 25.9, 18.2, 17.9, -4.2, -4.2, -4.5, -5.7; Anal. (C₂₉H₄₅ClIN₅O₃SeSi₂) C, H, N.

(2S,3S,4R,5R)-3,4-Bis((*tert*-butyldimethylsilyl)oxy)-5-(6-((3-iodobenzyl)amino)-7H-purin-7-yl)-N-methyltetrahydroselenophene-2-carboxamide (22a)—To a solution of **21a** (997 mg, 1.28 mmol) in dry DMF (10 mL) was added PDC (4.7 g, 12.8 mmol) and the reaction mixture was stirred at room temperature for 12 h and diluted with water (100 mL), and stirred for 1 h until obtaining the precipitate. The mixture was filtered, washed with water, and dissolved in dichloromethane. The aqueous layer was extracted with dichloromethane. The combining organic layers were washed with brine, dried, filtered, and concentrated under reduced pressure to give the crude carboxylic acid, which was used for the next reaction without further purification. To a stirred solution of crude acid (900 mg, 1.14 mmol) in dry dichloromethane (50 mL) were added 1-hydroxybenzotriazole hydrate (HOBt) (309 mg, 2.28 mmol), *N*-(3-dimethylaminopropyl) *N*'-ethylcarbodiimide (EDC) hydrochloride (438 mg, 2.28 mmol), methylamine hydrochloride (156 mg, 2.28 mmol), *N,N*-diisopropylethylamine (0.6 mL, 3.42 mmol) and the reaction mixture was stirred at room temperature for 15 h. The mixture was diluted with ethyl acetate and the organic layer was washed with water, dried, filtered, and evaporated. The residue was purified by silica gel chromatography (hexane : ethyl acetate = 3 : 1) to give **22a** (480 mg, 37%) as a white foam: $[\alpha]_D^{20}$ 55.87 (*c* 0.20, CH₂Cl₂); UV (CH₂Cl₂) $\lambda_{\max}$ 277.5 nm; ¹H NMR (400 MHz, CDCl₃) δ 8.51 (s, 1 H), 7.84 (s, 2 H), 7.67 (broad s, 1 H), 7.53 (d, *J* = 8.4 Hz, 1 H), 7.45 (d, *J* = 7.6 Hz, 1 H), 7.00 (t, *J* = 7.8 Hz, 1 H), 6.40 (d, *J* = 3.6 Hz, 1 H), 6.10 (s, 1 H), 5.19 (s, 2 H), 4.75 (s, 1 H), 4.30 (s, 1 H), 3.80 (d, *J* = 0.8 Hz, 1 H), 2.74 (s, 3 H), 0.95 (s, 9 H), 0.67 (s, 9 H), 0.15 (s, 6 H), -0.13 (s, 3 H), -0.58 (s, 3 H); ¹³C NMR (100 MHz, CDCl₃) δ 174.0, 172.0, 160.8, 153.2, 150.5, 146.8, 142.5, 136.7, 135.8, 134.8, 130.0, 129.6, 127.7, 127.2, 109.5, 94.3, 81.5, 76.1, 75.7, 75.6, 72.0, 70.2, 67.4, 63.9, 61.0, 59.7, 46.8, 44.1, 29.7, 26.8, 26.7, 26.6, 25.7, 25.7, 25.3, 24.8, 18.0, 17.7, -0.0, -4.4, -4.6, -4.7, -6.0; Anal. (C₃₀H₄₇IN₆O₃SeSi₂) C, H, N.

(2S,3S,4R,5R)-3,4-Bis((*tert*-butyldimethylsilyl)oxy)-5-(2-chloro-6-((3-iodobenzyl)amino)-7H-purin-7-yl)-N-methyltetrahydroselenophene-2-carboxamide (22b)—Compound **21b** (0.69 g, 0.741 mmol) was converted to **22b** (254 mg, 41%) as a white foam, using similar procedure used in the preparation of **21a**: $[\alpha]_D^{20}$ -7.33 (*c* 0.10, CDCl₃); UV (CH₂Cl₂) $\lambda_{\max}$ 282.5 nm; ¹H NMR (400 MHz, CDCl₃) δ 8.06 (broad s, 1 H), 7.90 (broad s, 1 H), 7.79 (broad s, 1 H), 7.32-7.54 (m, 2 H), 7.10 (broad s, 1 H), 7.01 (t, *J* = 8.0 Hz, 1 H), 6.00 (d, *J* = 8.0 Hz, 1 H), 5.15 (d, *J* = 9.2 Hz, 1 H), 5.07-5.05 (m, 1 H), 4.72 (d, *J* = 12.0 Hz, 1 H), 4.27 (s, 1 H), 3.94 (s, 1 H), 2.78 (d, *J* = 3.6 Hz, 3 H), 0.91 (s, 9 H), 0.67 (s, 9 H), 0.14 (s, 3 H), 0.12 (s, 3 H), -0.13 (s, 3 H)-0.60 (s, 3 H); ¹³C NMR (100 MHz, CDCl₃) δ 172.5, 162.8, 154.7, 151.1, 147.6, 141.8, 137.7, 136.1, 130.2, 128.1, 110.9, 94.3, 81.5, 76.0, 59.7, 47.7, 44.8, 26.9, 25.92, 25.88, 18.2, 17.9, -4.3, -4.4, -5.8; Anal. (C₃₀H₄₆ClIN₆O₃SeSi₂) C, H, N.

Biological assays

Cell culture and membrane preparation: CHO or HEK293 cells expressing the recombinant hA₁, hA_{2A} or A₃AR were cultured in Dulbecco's modified Eagle's 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. Cells were harvested after reaching confluency. After homogenization and suspension, cells were centrifuged at 1000 g for 10 min, and the pellet was discarded. The suspension was then recentrifuged at 20,000 g for 60 min at 4 °C. The resultant pellets were resuspended in buffer containing 3 Units/mL adenosine deaminase (from bovine spleen, Worthington Biochemical Corp., Lakewood, NJ) and incubated at 37 °C for 30 min. The aliquots of membrane preparation were stored at -80 °C until the binding experiments. The protein concentration was measured using the Bradford assay.³¹

Binding Assays at the hA₁ and hA_{2A}ARs: For binding to the hA₁AR, 50 µL of increasing concentrations of a test ligand and 50 µL of [³H]R-PIA (1 nM, PerkinElmer, Boston, MA) were incubated with membrane preparations (40 µg/tube) at 25 °C for 60 min in 50 mM Tris·HCl buffer (pH 7.4; MgCl₂, 10 mM) in a total assay volume of 200 µL. Nonspecific binding was determined using 10 µM of **23**. For hA_{2A}AR binding, membrane preparations (20 µg/tube) were incubated at 25 °C for 60 min with a final concentration of 10 nM [³H]2-[p-(2-carboxyethyl)phenyl-ethylamino-5'-N-ethylcarboxamidoadenosine (American Radiolabeled Chemicals, Inc., St. Louis, MO) in a mixture containing 50 µL of increasing concentrations of a test ligand and 200 µL of 50 mM Tris·HCl, pH 7.4, containing 10 mM MgCl₂. Compound **23** (10 µM) was used to define nonspecific binding. The reaction was terminated by filtration with GF/B filters. Filters were placed in scintillation vials containing 5 mL of Hydrofluor scintillation buffer and counted using a PerkinElmer Tricarb 2810TR Liquid Scintillation Analyzer.

Binding Assay at the hA₃AR: Each tube in the binding assay contained 100 µL membrane suspension (20 µg protein), 50 µL [¹²⁵I]N⁶-(4-amino-3-iodobenzyl)adenosine-5'-N-methyluronamide (0.2 nM, PerkinElmer, Boston, MA), and 50 µL of increasing concentrations of the test ligands in TrisHCl buffer (50 mM, pH 8.0) containing 10 mM MgCl₂, 1 mM EDTA. Nonspecific binding was determined using 10 µM of **23** in the buffer. The mixtures were incubated at 25 °C for 60 min. Binding reaction was terminated by filtration through Whatman GF/B filters under reduced pressure using a MT-24 cell harvester (Brandell, Gaithersburgh, MD, USA). Filters were washed three times with 9 mL ice-cold buffer. Filters were counted using a PerkinElmer Cobra II γ-counter.

Cyclic AMP accumulation assay: Cells were first treated with assay buffer containing rolipram (10 µM) and adenosine deaminase (3 units/mL) for 30 min. Agonists and/or test compounds were then added for another 10 min followed by the addition of forskolin (10 µM) and incubate for another 15 min. The reaction was terminated upon removal of the supernatant, and addition of 100 µL of Tween-20 (0.03%). Intramolecular cAMP levels were measured with an ALPHAScreen cAMP assay kit as instructed by manual from manufacturer.

Statistical 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.³² Data were expressed as mean $\pm$ standard error of the mean.

Molecular Modeling

Homology Modeling: To predict the 3D structure of hA₃AR, homology modeling study was performed. The primary sequence of hA₃AR (accession: P0DMS8) was used as the query sequence and the X-ray crystal structure of the agonist-bound hA_{2A}AR (PDB ID: 3QAK) was utilized as the template for homology modeling. The homology model was built and validated by SWISS-MODEL server.³³

Protein Preparation: The hA₃AR model went through using the Protein Preparation Wizard in Maestro v10.2 (Schrodinger, LLC, NY, USA). During the preparation process, the bond orders were assigned, hydrogen atoms were added, and the appropriate protonation states of the residues were generated at pH 7.4. All the hydrogen atoms were energy minimized with the optimized potential for liquid simulation (OPLS) 2005 force field until the average root mean square deviation for hydrogen atoms reached 0.30 Å.

Induced-fit docking: The X-ray crystal structure of **3p** was utilized and the charges were calculated with Merck molecular force field (MMFF94) in SYBYL-X 2.0 (Tripos Int., St. Louis, MO, USA). Then, it was docked into the homology model of hA₃AR using Induced Fit Docking (IFD) module in Maestro v10.2 (Schrodinger, LLC, NY, USA) with the following steps: (i) The grid for the binding site was defined as a 26 Å³ box from the centroid of selected residues (Thr94, Phe168, Asn250, Ser271, His272).³⁴ (ii) For the initial docking stage, Glide SP (standard precision) docking was performed retaining a maximum number of 20 poses per ligand. (iii) Residues within 5 Å from the ligand were allowed to move during the Prime refinement stage and further energy minimization was applied to optimize the side chain orientations. (iv) Then, re-docking of the ligands using Glide XP (extra precision) was performed within 30 kcal/mol of the best structure. The protein-ligand complexes with the best IFD score were selected and analyzed. All the molecular graphic figures were generated by PyMOL software (<http://www.pymol.org>). All the computational studies were undertaken on an Intel Xeon 16-Core 3.10 GHz workstation with Linux CentOS release 5.9.

Chemotaxis assay

The chemotaxis assay was performed as described in previous publication.¹⁰ Microphotographs of migrating microglial cells in the live cell chamber (Live Cell Instrument, Seoul, Korea) were monitored with inverted confocal microscopy system (Leica DMIRE2, TLC_SP2) and differential interference contrast transmission image programs (Leica, Ver. 2.5). Fifteen min after **1b** or **3d** was applied by micromanipulator (Narishige, Japan), the MCP-1 gradient (100 ng/mL; BD Pharmingen, San Jose, CA) was generated using a Harvard apparatus microinjection device (0.1 µL/min; PHD 2000 rogrammable). MRS1523 (1 µmol/L) or SCH58261 (100 mol/L) was applied to the cells for 20 min before treatment with MCP-1. For quantitative analysis, MCP-1 was laced in the bottom chamber of a chemotaxis chamber Neuro Probe, Cabin John, MD) and microglia (5×10^4 cells/mL in serum-free MEM) in the upper chamber were allowed to migrate to the bottom part for 2

hours, through membrane pore (8 mm² filter area; Neuro Probe). Migrated cells on the bottom-side filter were stained for nuclei with Harris's hematoxylin and then counted. The movement or morphological change of microglia was recorded for 20 min starting from the application of MCP-1.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

SAR	structure-activity relationship
AR	adenosine receptor

cAMP	cyclic adenosine-5'-monophosphate
IP₃	inositol triphosphate
DAG	diacylglycerol
Cl-IB-MECA	2-chloro- <i>N</i> ⁶ -(3-iodobenzyl)-5'- <i>N</i> -methylcarbamoyladenine
thio-Cl-IB-MECA	2-chloro- <i>N</i> ⁶ -(3-iodobenzyl)-5'- <i>N</i> -methylcarbamoyl-4'-thioadenine
<i>m</i>CPBA	<i>meta</i> -chloroperbenzoic acid
TMSOTf	trimethylsilyl trifluoromethanesulfonate
TBSOTf	<i>t</i> -butyldimethylsilyltrifluoromethanesulfonate
TBS	<i>t</i> -butyldimethylsilyl
TBDPS	<i>t</i> -butyldiphenylsilyl
NOE	nuclear Overhauser effect
PNB	<i>p</i> -nitrobenzoyl
TFA	trifluoroacetic acid
PDC	pyridinium dichromate
HOBt	1-hydroxybenzotriazole
EDC	1-ethyl-3-(3-dimethylaminopropyl)carbodiimide
TBAF	tetra- <i>n</i> -butylammonium fluoride
CHO	Chinese hamster ovary
HEK	human embryonic kidney
I-AB-MECA	<i>N</i> ⁶ -(3-iodo-4-aminobenzyl)-5'- <i>N</i> -methylcarboxamidoadenosine
<i>R</i>-PIA	(-)- <i>N</i> ⁶ -2-phenylisopropyladenosine
CGS21680	2-[<i>p</i> -(2-carboxyethyl)phenylethylamino]-5'- <i>N</i> -ethylcarboxamidoadenosine NECA, 5'- <i>N</i> -ethylcarboxamidoadenosine
MCP-1	monocyte chemotactic protein 1
BSA	<i>N</i> , <i>O</i> -bis(trimethylsilyl)acetamide
HATU	1-[bis(dimethylamino)methylene]-1 <i>H</i> -1,2,3-triazolo[4,5- <i>b</i>]pyridinium 3-oxid hexafluoro phosphate

DMEM	Dulbecco's modified Eagle's medium
OPLS	optimized potentials for liquid simulations
MMFF	Merck molecular force field
IFD	Induced Fit Docking

Figure 1.
Rational design of next generation 4'-selenonucleoside derivatives **3** based on a bioisosteric rationale.

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Figure 3. Inhibitory effect of compound **3d** on forskolin-induced stimulation of cAMP production at the hA₃AR expressed in CHO cells, compared to **23** as reference full agonist (= 100%). A representative curve from 3 determinations is shown.

Figure 4.

Binding mode of **3p** in hA₃AR and its comparison with the X-ray crystal structure. (A) Binding interactions of **3p** at the agonist binding site of hA₃AR. Compound **3p** is displayed as ball-and-stick with carbon atoms in red-pink. The interacting residues are depicted as capped sticks with carbon atoms in white. Hydrogen bonds are depicted as black dashed lines. (B) 2D interaction diagram of **3p** in hA₃AR. Hydrogen bonds are shown as red- and green-dotted arrows with their directionality. Hydrophobic interactions are marked in light brown. The π - π interactions are marked as purple discs and arrows. (C) The X-ray crystal structure of **3p**. It is displayed as capped sticks with carbon and hydrogen atoms in pale pink and cyan, respectively. A hydrogen bond is marked as a black dashed line. (D) Superimposition of X-ray crystal structure of **3p** with the docked conformation.

Figure 5.

A) Inhibition of MCP-1 induced microglial chemotaxis (N = 4) by **2b** and **3d**. B) PrestoBlue cell viability test (N = 4) after treatment of pure microglia with **2b** and **3d** for 24 h.

Scheme 1. Reagents and conditions

a) *m*CPBA, CH₂Cl₂, -78 °C, 45 min; b) Ac₂O, 100 °C, 15 h; c) 6-chloropurine, BSA, TMSOTf, toluene, 95 °C, 15 h or 2,6-dichloropurine, BSA, TMSOTf, CH₃CN, 60 °C, 2 h; d) TMSOTf, toluene, 95 ° for **6a** (50%) or 70 ° for **7a** (46%).

Scheme 2. Reagents and conditions

a) HF·Et₃N, THF, rt, 15 h; b) PNBCl, Et₃N, CH₂Cl₂, rt, 1 h; c) 50% aqueous TFA, THF, rt, 2 h; d) TBSOTf, Et₃N, DMAP, CH₂Cl₂, rt, 1 h; e) 1 *N*NaOH, 1,4-dioxane, rt, 15 h.

Scheme 3. Reagents and conditions

a) DMSO, Ac_2O , 100 °C, 1 h; b) $AgNO_3$, NaOH, NH_4OH , THF, 0 °C, 30 min; c)
 $CH_3NH_2 \cdot HCl$, DIPEA, HATU, THF, rt, 1 h; d) TBAF or $HF \cdot Et_3N$, THF, rt, 1 h or 15 h; e)
 R_1NH_2 , Et_3N , EtOH, reflux, 15-30 h.

Scheme 4. Reagents and conditions

a) 3-I-BnNH₂·HCl, Et₃N, EtOH, rt, 15 h; b) TBAF, THF, rt, 1 h; c) BzCl, pyridine, rt, 3 h; d) 88% HCOOH, rt, 12 h; e) TBSOTf, CH₂Cl₂ 0 ° to rt, 15 h; f) NaOMe, MeOH, rt, 1 h; g) i) PDC, DMF, rt, 12 h; ii) CH₃NH₂·HCl, HOBT, DIPEA, EDCCl, rt, 15 h; h) TBAF, THF, rt, 1 h.

Table 1

Binding Affinities of Known A₃AR Agonists (**1** and **2**) and N⁶-Substituted -4'-Selenoadenosine Derivatives **3a-u** at Three Subtypes of the hARs

Compound	affinity, <i>K_i</i> , nM ± SEM (or % inhibition) ^{a,b}			A ₃ Activation %, 28 = 100%
	hA ₁	hA _{2A}	hA ₃	
1a (X = O, R ¹ = 3-I-Bn, R ² = H) ^c	1620 ± 760	2910 ± 580	1.8 ± 0.7	100
1b (X = O, R ¹ = 3-I-Bn, R ² = Cl) ^c	1240 ± 320	5370 ± 2470	1.4 ± 0.2	99 ± 6
2a (X = S, R ¹ = 3-I-Bn, R ² = H) ^c	20.2 ± 2.9	475 ± 144	0.25 ± 0.06	100
2b (X = S, R ¹ = 3-I-Bn, R ² = Cl) ^d	193 ± 46	223 ± 36	0.38 ± 0.07	114 ± 9
3a (X = Se, R ¹ = 3-F-Bn, R ² = H)	3310 ± 520	1350 ± 210	2.30 ± 0.61	74 ± 8
3b (X = Se, R ¹ = 3-Cl-Bn, R ² = H)	570 ± 160	555 ± 108	3.45 ± 2.46	80 ± 7
3c (X = Se, R ¹ = 3-Br-Bn, R ² = H)	220 ± 39	560 ± 72	0.90 ± 0.33	91 ± 5
3d (X = Se, R ¹ = 3-I-Bn, R ² = H)	480 ± 94	1080 ± 140	0.57 ± 0.10	91 ± 16
3e (X = Se, R ¹ = 2-OMe-Bn, R ² = H)	1880 ± 410	1260 ± 270	3.75 ± 0.71	97 ± 12
3f (X = Se, R ¹ = 2-Cl-Bn, R ² = H)	1090 ± 190	1160 ± 340	4.41 ± 0.82	85 ± 13
3g (X = Se, R ¹ = 1-naphthyl, R ² = H)	440 ± 75	195 ± 31	1.76 ± 0.40	109 ± 9
3h (X = se, R ¹ = <i>trans</i> -2-phenylcyclopropyl, R ² = H)	420 ± 66	2480 ± 1040	3.93 ± 0.22	89 ± 9
3i (X = Se, R ¹ = cyclopropyl, R ² = H)	83.5 ± 16.6	54% ± 4	2.49 ± 0.49	92 ± 17
3j (X = Se, R ¹ = cyclobutyl, R ² = H)	37.5 ± 5.0	1080 ± 190	1.39 ± 0.01	82 ± 2
3k (X = Se, R ¹ = cyclopentyl, R ² = H)	25.2 ± 6.7	1090 ± 100	7.07 ± 2.06	77 ± 12
3l (X = Se, R ¹ = methyl, R ² = H)	2060 ± 510	18% ± 12	1.49 ± 0.54	108 ± 1
3m (X = Se, R ¹ = 3-F-Bn, R ² = Cl)	37.3% ± 4.1	42% ± 9	3.35 ± 0.66	106 ± 2
3n (X = Se, R ¹ = 3-Cl-Bn, R ² = Cl)	232 ± 47	238 ± 333	1.96 ± 0.17	99.7 ± 6.3
3o (X = Se, R ¹ = 3-Br-Bn, R ² = Cl)	156 ± 32	1230 ± 256	1.99 ± 0.33	106 ± 12
3p (X = Se, R ¹ = 3-I-Bn, R ² = Cl)	311 ± 47	1200 ± 70	4.20 ± 0.73	94.1 ± 3.4
3q (X = Se, R ¹ = cyclopropyl, R ² = Cl)	186 ± 57	30.3% ± 7.6	1.90 ± 0.14	83.6 ± 3.5
3r (X = Se, R ¹ = cyclobutyl, R ² = Cl)	61.7 ± 23.2	899 ± 142	1.00 ± 0.15	86.7 ± 6.2
3s (X = Se, R ¹ = cyclopentyl, R ² = Cl)	59.4 ± 21.0	1120 ± 153	3.56 ± 0.19	92.5 ± 4.6
3t (X = Se, R ¹ = 3-I-Bn, R ² = H)	26% ± 7	13% ± 7	4180 ± 1560	0.2 ± 2.5
3u (X = Se, R ¹ = 3-I-Bn, R ² = Cl)	7% ± 6	23% ± 8	7360 ± 1330	14 ± 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 human embryonic kidney (HEK-293) cells). Binding was carried out using 1 nM [³H]R-N⁶.

(phenylisopropyl)adenosine 10 nM [^3H]2-[p-(2-carboxyethyl)phenylethylamino]-5'-N-ethylcarboxamidoadenosine, or 0.5 nM [^{125}I]N⁶-(4-amino-3-iodobenzyl)adenosine-5'-N-methyluronamide as radioligands for A₁, A_{2A}, and A₃ARs, respectively. Values are expressed as mean $\pm$ sem, $n \pm 3$ -4 (outliers eliminated), and are normalized against a nonspecific binder, **23** (10 μM).

^b When value is expressed as a percentage, it refers to the percent inhibition of a specific radioligand binding at 10 μM , with nonspecific binding defined using 10 μM **23**.

^c Ref 27;

^d Ref 28.