Design and Synthesis of 2,6-Disubstituted-4′-Selenoadenosine-5′-N,N-Dimethyluronamide Derivatives as Human A3 Adenosine Receptor Antagonists

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Abstract: A new series of 4′-selenoadenosine-5′-N,N-dimethyluronamide derivatives as highly potent and selective human A3 adenosine receptor (hA3AR) antagonists, is described. The highly selective A2AR agonists, 4′-selenoadenosine-5′-N-methyluronamides were successfully converted into selective antagonists by adding a second N-methyl group to the 5′-uronamide position. All the synthesized compounds showed medium to high binding affinity at the hA3AR. Among the synthesized compounds, 2-H-N6-3-iodobenzylationamine derivative 9f exhibited the highest binding affinity at hA3AR. (Ki = 22.7 nM). The 2-H analogues generally showed better binding affinity than the 2-Cl analogues. The CAMP functional assay with 2-Cl-N6-3-iodobenzylationamine derivative 9i demonstrated hA3AR antagonist activity. A molecular modelling study suggests an important role of the hydrogen of 5′-uronamide as an essential hydrogen bonding donor for hA3AR activation.

Keywords: A3 adenosine receptor; structure-activity relationship; 4′-Selenonucleosides; antagonist.

1. Introduction

Adenosine, which is the endogenous ligand of the adenosine receptors (ARs), is an important neuromodulator and mediates through activation of its four receptors, consisting of A1, A2A, A2B, and A3 subtypes. These receptors are widely distributed in tissues and involved in various physiological activities [1]. Each subtype couples to a preferred type of G protein; A1 and A3ARs primarily couple to the Gi/o proteins, and A2A and A2BARs couple to Gi proteins. A2B and A3ARs are also known to be coupled to Gq proteins. AR signaling and their physiological roles have been extensively studied [2,3]. Among them, the A3AR is an important receptor to regulate cardioprotection in cardiac ischemia [4], degranulation of neutrophils [5], and cell proliferation [6]. These results led to the development of A3AR agonists as anticancer agents [7]. Selective A3AR antagonists are also promising ligands to modulate inflammation [8] and cerebroprotection [9,10]. Some studies showed that A3AR antagonists could enhance cancer treatment via the inhibition of HIF-1α and VEGF protein accumulation in hypoxia and in tumors [11] and are potential anti-glaucoma therapeutics as they reduce intraocular pressure in mouse and monkey [12].

In the past decades, a variety of approaches have been followed to discover novel drug candidates targeting A3AR. 2-Chloro-N6-(3-iodobenzyl)adenosine-5′-N-methyluronamide (Cl-IB-MECA, 1) and its 4′-thio analogue 2 were discovered as potent and selective A3AR agonists from the extensive structure-activity relationships based on the structure of adenosine [13] (Figure 1). The 5′-uronamide hydrogen of 1, required for full agonism, forms a putative hydrogen bond with T94 (3.36) at hA3AR as modeled, suggesting that this interaction is essential for receptor activation by adenosine agonists [14]. Consistent with these
findings, the 4′-truncated analogues, such as 3 and 4, lacking this hydrogen bond donor were discovered to act as A3AR antagonists or low-efficacy agonists, demonstrating that a hydrogen bond donating ability of the 5′-uronamide promotes A3AR activation [15,16]. The removal of the hydrogen bond donor ability by appending another methyl group to the 5′-uronamide, e.g., 5′-N,N-dimethyluronamidine derivatives 5 and 6, similarly reduced A3AR efficacy. These compounds were characterized as potent and selective A3AR antagonists [17,18].

On the basis of a bioisosteric rationale, we recently reported that 4′-seleno analogues of 1 and 2, i.e., 7 and 8, were discovered as potent and selective hA3AR agonists [19] (Figure 2). They exhibited comparable A3AR binding affinity as the corresponding 4′-oxo- and 5′-thio nucleosides 1 and 2. However, X-ray analysis indicated that in the pure crystalline state they preferred a syn nucleobase orientation and a South sugar conformation, unlike 1 and 2. As mentioned above, removal of the amide hydrogen of the 5′-uronamide of 1 and 2 by N-methylation, resulting in 5 and 6 successfully converted A3AR agonists into A3AR antagonists. Based on these findings, we hypothesized that the 4′-seleno analogue of 5 or 6, bearing a 5′-N,N-dimethyluronamidine moiety might be an A3AR antagonist (Figure 2). Thus, we analyzed the structure-activity relationship as A3AR ligands of this series by modifying N6- and C2 positions, by synthesizing novel 4′-selenonucleosides 9a-I. Herein, we report the synthesis and biological evaluation of 2,6-disubstituted-4′-selenoadenosine-5′-N,N-dimethyluronamidine derivatives 9a-I as potent and selective A3AR antagonists.
The rationale for the design of the target nucleosides 9a–l.

2. Results

2.1. Chemistry

For the synthesis of final compounds 9a–l, key intermediates, 4′-seleno purine nucleosides 15a–b were synthesized from D-ribose following the previously reported procedures [18,19] (Scheme 1). Briefly, D-ribose was converted to L-lyxono-lactone derivative 10 in three steps (oxidation to lactone, conversion of D-ribo configuration to L-lyxo configuration, and tert-butyldiphenylsilyl (TBDPS) protection). Reduction of 10 with NaBH₄, ring cyclization of resulting diol with selenide ion, and a Pummerer rearrangement of 4-seleno sugar afforded the glycosyl donor 11. A Vorbrüggen condensation of 11 with 6-chloropurine and 2,6-dichloropurine produced the N⁷-β-anomers 12a and 13a with concomitant formation of their corresponding N⁷-β-anomers 12b and 13b, respectively. Conversion of N⁷ isomers 12b and 13b to their corresponding N⁷ isomers 12a and 13a was achieved by using TMSOTf at high temperature. Removal of the TBDPS group of 12a and 13a yielded the 5′-CH₂OH derivatives 14a and 14b, respectively. Conversion of the 5′-hydroxymethyl group of 14a and 14b into a 5′-N,N-dimethyluronamide was successfully achieved, but the final deprotection of the acetonide group under strongly acidic conditions resulted in decomposition, instead of giving the desired final products. Thus, we exchanged the acetonide protecting group of 14a and 14b with a TBS group in four steps, giving 15a and 15b, respectively. Firstly, a PNB protecting group was attached to the 5′-position of 14a and 14b, followed by acetonide group deprotection with 50% aqueous TFA to give diols. The diols were then protected with a TBS group using TBSOTf followed by deprotection of the PNB group with sodium hydroxide in 1,4-dioxane to give 15a and 15b, respectively. The final deprotection with sodium hydroxide required mild reaction conditions (room temperature, overnight) because of the possible hydrolytic conversion of 6-chloropurine to hypoxanthine.
Scheme 1. Synthesis of intermediates 15a and 15b from D-ribose (a) i. Br₂, H₂O, K₂CO₃; ii. KOH, H₂O, rt, 15 h; (c) TBDPSCI, Et₃N, DMAP, CH₂Cl₂, rt, 4 h; (d) NaBH₄, MeOH, rt, 1 h; (e) i. MsCl, Et₃N, DMAP, CH₂Cl₂, rt, 1 h; ii. NaOH, 1,4-dioxane, rt, 15 h.

Synthesis of the final nucleosides, 9a–l from the key intermediates, 15a and 15b is shown in Scheme 2. The direct oxidation of the alcohols of 15a and 15b to the carboxylic acids have been tried with many oxidizing reagents, but none of them could give the desired acid. Thus, we employed a sequential oxidation method via aldehyde instead of direct oxidation to the carboxylic acid. Albright–Goldman oxidations of 15a and 15b, using DMSO as an oxidizing agent under mild condition afforded the aldehydes 16a and 16b, respectively. Tollens’ oxidation converted the aldehydes 16a and 16b to the corresponding carboxylic acids smoothly, which without purification underwent the amide coupling reaction with dimethylamine in the presence of DIPEA, and HATU to yield the 5′-N,N-dimethyluronamides 17a and 17b, respectively. The TBS deprotection of 17a and 17b with TBAF and acetic acid gave the diols 18a and 18b, respectively. The key intermediates...
18a and 18b were treated with various amines such as ammonia, alkylamines and 3-halobenzylamines to yield 2-H derivatives 9a–f and 2-Cl derivatives 9g–l, respectively.

![Scheme 2](image)

**Scheme 2.** Synthesis of 5′-N,N-dimethyluronamide 4′-selenonucleoside analogues 9a–l. (a) DMSO, Ac₂O, 100 °C, 1 h; (b) AgNO₃, NaOH, NH₄OH, THF, 0 °C, 30 min; (c) (CH₃)₂NH, AgNO₃, NaOH, NH₄OH, THF, 0 °C, 30 min; (d) TBAF, AcOH, THF, rt, 15 h; (e) RNH₂, Et₃N, EtOH, reflux, 15–30 h.

### 2.2. Biology

#### 2.2.1. Binding Affinity

The binding affinities of all the final compounds 9a–l were evaluated, using radioligand binding assays at four human AR subtypes (Table 1), by reported methods [20]. All of the final compounds 9a–l exhibited medium to high binding affinity at the hA₃AR, while no binding affinity at other subtypes such as hA₁, hA₂A, and hA₂B ARs was observed. Among the tested compounds, compound 9f exhibited the highest affinity (Kᵢ = 22.7 nM) at hA₃AR, which is comparable to the corresponding 4′-oxo- and 4′-thio analogues 5 (Kᵢ = 29.0 nM) and 6 (Kᵢ = 15.5 nM). The introduction of a 3-halobenzyl group at the N₆ position increased the hA₃AR binding affinity when compared to the N₆-unsubstituted adenine compounds 9a or 9g, indicating that a favorable hydrophobic interaction exits at the hA₃AR binding site. In the 2-H series, the binding affinity of 3-halobenzyl derivatives 9c–f was decreased in the following order: 3-Br-benzyl 9f > 3-Cl-benzyl 9d > 3-F-benzyl 9c. The halogen size correlated with hA₃AR binding affinity, whereas in the 2-Cl series, the binding affinity of 3-halobenzyl derivatives 9i–l was almost same within the range of 180–250 nM. In general, the 4′-seleno-5′-N,N-dimethyluronamide derivatives 9a–l exhibited
lower binding affinity than the 4′-seleno-5′-N-methyluronamide derivatives 7 and 8. It is interesting to note that 2-Cl-N6-3-iodobenzyl analogue 9i exhibited much weaker binding affinity than the corresponding 2-H analogue 9f. This tendency was also found in the 5′-N-methyluronamide 4′-seleno derivatives 7 and 8.

Table 1. Binding affinities of known A3AR ligands and 5′-N,N-dimethyluronamide-4′-selenonucleoside derivatives (9a-l) at human A1, A2A, A2B, and A3ARs.

| Compound | Affinity, K_i, nM ± SEM a,b (or % Inhibition at 10 uM) |
|----------|-----------------------------------------------------|
|          | X Y R^1 R^2 hA1AR hA2AAR hA2BAR hA3AR               |
| 5        | O Cl 3-I-Bn CH3 5870 ± 930 >10,000 >10,000 29.0 ± 4.9 |
| 6        | S Cl 3-I-Bn CH3 6220 ± 640 >10,000 >10,000 15.5 ± 3.1 |
| 7        | Se H 3-I-Bn H 480 ± 94 1080 ± 140 ND 0.57 ± 0.10  |
| 8        | Se Cl 3-I-Bn H 311 ± 47 1200 ± 70 ND 4.20 ± 0.73   |
| 9a       | Se H H CH3 16% ± 4 22% ± 3 17% ± 1 3710 ± 600     |
| 9b       | Se H CH3 CH3 9% ± 2 3% ± 2 9% ± 4 609 ± 47         |
| 9c       | Se H 3-F-Bn CH3 13% ± 2 12% ± 1 19% ± 5 2020 ± 170 |
| 9d       | Se H 3-Cl-Bn CH3 23% ± 5 4% ± 3 15% ± 1 1190 ± 160 |
| 9e       | Se H 3-Br-Bn CH3 43% ± 7 37% ± 8 ND 36.3 ± 12.7    |
| 9f       | Se H 3-I-Bn CH3 36% ± 6 32% ± 1 ND 22.7 ± 8.9      |
| 9g       | Se Cl H CH3 9% ± 1 3% ± 3 25% ± 5 3250 ± 370       |
| 9h       | Se Cl CH3 CH3 58% ± 7 5% ± 2 19% ± 5 1060 ± 140    |
| 9i       | Se Cl 3-F-Bn CH3 34% ± 2 9% ± 4 14% ± 2 238 ± 37   |
| 9j       | Se Cl 3-Cl-Bn CH3 63% ± 5 13% ± 1 27% ± 4 195 ± 40  |
| 9k       | Se Cl 3-Br-Bn CH3 58% ± 2 10% ± 4 27% ± 6 180 ± 12  |
| 9l       | Se Cl 3-I-Bn CH3 68% ± 2 10% ± 1 23% ± 3 253 ± 29   |

a All binding experiments were performed using adherent mammalian cells stably transfected with cDNA encoding the appropriate hAR (A1AR and A3AR in CHO cells, A2AAR in Hela cells and A2BAR in HEK-293 cells). Binding was carried out using 2 nM [3H]DPCPX, 3 nM [3H]ZM241385, 25 nM [3H]DPCPX or 0.5 nM [3H]NECA as radioligands for A1, A2A, A2B, and A3ARs, respectively. Values are expressed as mean ± SEM (n = 2). b When a 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 NECA. c Ref [16]. d Ref [19].

2.2.2. CAMP Functional Assay

In a cAMP functional assay at hA3AR expressed in CHO cells, compound 9l behaved as an antagonist, like compounds 5 and 6, with K_B value of 114.5 nM (Figure 3). Like 5 and 6, an additional methyl group on the 5′-N-methyluronamide converted an agonist into an antagonist, indicating that amide hydrogen is essential for receptor activation in this series, as well. However, the fact that the 5′-N,N-dimethyluronamide derivatives exhibited weaker binding affinity than the corresponding 5′-N-methyluronamide derivatives demonstrates that steric effects induced by 5′-N,N-dimethyluronamide reduce the binding affinity at the A3AR.
Figure 3. Concentration–response curve of 9l in a functional assay at human A3AR measuring inhibition of 10 μM NECA-induced cAMP accumulation. Points represent mean ± SD (vertical bars) of duplicate experiments.

2.3. Molecular Modelling Studies

To investigate how 5′-N,N-dimethyluronamide 4′-selenonucleoside derivatives bind at hA3AR, we docked our compounds into the reported homology model of hA3AR [21] using Autodock Vina [22]. The most potent compound 9f bound well at the orthosteric binding site with a South ring conformation (2′-endo/3′-exo), displaying H-bonds with Ser271 and His272 (Figure 4). Compared to the 5′-N-methyluronamide derivative 5, the adenine ring still maintained π–π interaction with Phe168 and the iodobenzene ring had interactions with Val169, Ile253 and Leu264 [19]. The glycosidic bond was in an anti conformation. However, either H-bonding of 5′-N-uronamide with Thr94 or the adenine with Asn250 was not observed (marked as a red circle in Figure 4), suggesting that this H-bonding plays a key role in discriminating an agonist from an antagonist.

Figure 4. Predicted binding mode of compound 9f in the homology model of hA3AR. Hydrogen bonds are depicted as green dashed line. Hydrophobic interactions are marked with a purple dashed line and π–π interactions are marked with a pink dashed line.
3. Materials and Methods

3.1. Chemical Synthesis

Proton (\(^{1}\)H) and carbon (\(^{13}\)C) NMR spectra were obtained on a Jeol JNM-ECA 300 (JEOL Ltd. Tokyo, Japan; 300/75 MHz), Bruker AV 400 (Bruker, Billerica, MA, USA; 400/100 MHz), and AMX 500 (Bruker, Billerica, MA, USA; 500/125 MHz) spectrometer. The \(^1\)H NMR data were reported as peak multiplicities: \(s\) for singlet; \(d\) for doublet; \(dd\) for doublet of doublets; \(t\) for triplet; \(td\) for triplet of doublet; \(q\) for quartet; \(qu\) for quintet; \(bs\) for broad singlet and \(m\) for multiplet. Coupling constants were reported in hertz. The chemical shifts were reported as ppm (\(\delta\)) relative to the solvent peak. All reactions were routinely carried out under an inert atmosphere of dry nitrogen. IKA RCT basic type heating mantle was used to provide a constant heat source. Microwave-assisted reactions were carried out in sealed vessels using a Biotage Initiator + US/JPN (Biotage, Uppsala, Sweden; part no. 356007) microwave reactor, and the reaction temperatures were monitored by an external surface IR sensor. High-resolution mass spectra were measured with electrospray-ionization quadrupole time-of-flight (ESI-Q-TOF) techniques. Melting points were recorded on a Barnstead electrothermal 9100 instrument and are uncorrected. Reactions were checked by thin layer chromatography (Kieselgel 60 F254, Merck, Kenilworth, NJ, US). Spots were detected by viewing under a UV light, and by colorizing with charring after dipping in a p-anisaldehyde solution. The crude compounds were purified by column chromatography on a silica gel (Kieselgel 60, 70–230 mesh, Merck). All the anhydrous solvents were redistilled over CaH\(_2\), or P\(_2\)O\(_5\), or sodium/benzophenone prior to the reaction.

\((2S,3S,4R,5R)-3,4-Bis(\text{tert-butyldimethylsilyl})oxy)-5-(6-chloro-9H-purin-9-yl)tetrahydrodelenophene-2-carbaldehyde (16a).\) To a solution of 15a \[19\] (348 mg, 0.60 mmol) in dimethyl sulfoxide (5 mL) was added acetic anhydride (0.11 mL, 1.2 mmol), and the reaction mixture was stirred at 0 \(\degree\) C 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 (MgSO\(_4\)), filtered, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (hexane–ethyl acetate = 4:1) to give 16a (288 mg, 83%) as a white foam: \(^1\)H NMR (400 MHz, CDCl\(_3\)) \(\delta\) 9.76 (d, \(J = 2.4\) Hz), 8.78 (s, 1 H), 8.32 (s, 1 H), 6.24 (d, \(J = 7.2\) 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); \(^{13}\)C NMR (100 MHz, CDCl\(_3\)) \(\delta\) 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, 0.21, –4.2, –4.45, –4.55, –5.3.

\((2S,3S,4R,5R)-3,4-Bis(\text{tert-butyldimethylsilyl})oxy)-5-(2,6-dichloro-9H-purin-9-yl)tetrahydrodelenophene-2-carbaldehyde (16b).\) Compound 15b \[19\] (1.49 g, 2.43 mmol) was converted to 16b (1.22 g, 82%) as a yellow foam, using a procedure similar to that used in the preparation of 16a: \(^1\)H NMR (500 MHz, CDCl\(_3\)) \(\delta\) 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); \(^{13}\)C NMR (75 MHz, CDCl\(_3\)) \(\delta\) 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.

\((2S,3S,4R,5R)-3,4-Bis(\text{tert-butyldimethylsilyl})oxy)-5-(6-chloro-9H-purin-9-yl)-N-methyltetrahydrodelenophene-2-carboxamide (17a).\) To a solution of AgNO\(_3\) (170 mg, 1.00 mmol) was added 1 N NaOH (1.0 mL, 1.00 mmol) at 0 \(\degree\) C. After Ag\(_2\)O was precipitated, 24% ammonia–water (0.3 mL) was added to the reaction mixture until the mixture was clear. The solution of 16a (288 mg, 0.50 mmol) in THF (8 mL) was added to the above Tollens’ reagent at 0 \(\degree\) C, and the reaction mixture was stirred at the same temperature for 30 min and diluted with water (10 mL). The aqueous layer was extracted with ethyl acetate (10 mL × 2), and the aqueous layer was acidified with 1 N HCl solution (3 mL) and extracted with ethyl acetate (10 mL × 2). The combined organic layers were washed with brine, dried (MgSO\(_4\)), filtered, and evaporated under reduced pressure to give the crude acid. To a
solution of the crude acid in THF (5 mL) were added 1-[bis(dimethylamino)methylene]-1H-1,2,3-triazolo[4,5-b]pyridinium 3-oxide hexafluorophosphate (HATU) (190 mg, 0.50 mmol), dimethylamino hydrochloride (82 mg, 1.00 mmol), and diisopropylamine (0.19 mL, 1.10 mmol) at room temperature. The reaction mixture was stirred at same temperature for 1 h and diluted with ethyl acetate (10 mL). The organic layer was washed with water (5 mL x 2), and the aqeous layer was further extracted with ethyl acetate (10 mL x 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 = 3:1) to give 17a (120 mg, 39%) as a white foam; ¹H NMR (400 MHz, CDCl₃) δ 8.76 (s, 1 H), 8.67 (bs, 1 H), 6.62 (d, J = 6.8 Hz, 1 H), 4.93 (bs, 1 H), 4.63 (m, 1 H), 4.21 (m, 1 H), 3.04 (s, 3 H), 2.99 (s, 3 H), 0.92 (s, 9 H), 0.71 (s, 9 H), 0.08 (d, J = 3.6 Hz, 6 H), 0.01 (s, 3 H).

(2S,3S,4R,5R)-3,4-Bis((tert-butylidimethylsilyl)oxy)-5-(2,6-dichloro-9H-purin-9-yl)-N-methyltetrahydroselenophene-2-carboxamide (17b). Compound 16b (623 mg, 1.02 mmol) was converted to 17b (260 mg, 58%) as a yellow foam, using a procedure similar to that used in the preparation of 17a: ¹H NMR (400 MHz, MeOD) δ 8.93 (s, 1 H), 8.73 (s, 1 H), 6.37 (d, J = 6.4 Hz, 1 H), 4.95 (dd, J = 6 Hz, 3.2 Hz, 1 H), 4.67 (t, J = 3.8 Hz, 1 H), 4.50 (d, J = 4 Hz, 1 H), 3.03 (s, 3 H), 2.99 (s, 3 H).

General Procedure for the Synthesis of 9a–l

To a stirred solution of 18a (80 mg, 0.13 mmol) in THF (3 mL) was added 1 M tetra-n-butylammonium fluoride in THF solution (0.13 mL, 0.13 mmol) and acetic acid (7 µL, 0.13 mmol), and the reaction mixture was stirred at room temperature for 15 h. The solvent was evaporated, and the resulting residue was purified by silica gel column chromatography (dichloromethane–methanol = 100:1–10:1) to give 18a (45 mg, 85%) as a white solid; ¹H NMR (400 MHz, CDCl₃) δ 8.93 (s, 1 H), 8.73 (s, 1 H), 6.37 (d, J = 6.4 Hz, 1 H), 4.95 (dd, J = 6 Hz, 3.2 Hz, 1 H), 4.67 (t, J = 3.8 Hz, 1 H), 4.50 (d, J = 4 Hz, 1 H), 3.03 (s, 3 H), 2.99 (s, 3 H).

To a stirred solution of 18a − 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 were evaporated, and the residue was purified by silica gel column chromatography (dichloromethane–methanol = 100:1–10:1) to give 9a–l as a white solid.

(2S,3S,4R,5R)-5-(6-Chloro-9H-purin-9-yl)-3,4-dihydroxy-N-methyltetrahydro selenophene-2-carboxamide (18b). Compound 17b (83 mg, 0.127 mmol) was converted to 18b (41 mg, 75%) as a white solid, using a procedure similar to that used in the preparation of 18a: ¹H NMR (400 MHz, MeOD) δ 8.93 (s, 1 H), 6.27 (d, J = 6 Hz, 1 H), 4.88 (m, 1 H), 4.65 (t, J = 4 Hz, 1 H), 4.50 (d, J = 4.4 Hz, 1 H), 3.04 (s, 3 H), 2.99 (s, 3 H).
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1 H) 4.48 (d, J = 4.8 Hz, 1 H) 3.02 (s, 3 H), 2.97 (s, 3 H); 13C NMR (100 MHz, MeOD) δ 173.9, 165.8, 163.3, 156.2, 154.2, 150.7, 143.5, 141.6, 131.4, 124.3, 120.8, 115.1, 82.1, 77.6, 56.8, 42.4, 38.3, 36.4; HRMS (FAB) found 481.0894 (calculated for C19H22F6N3O2Se (M + H)^+ 481.0903).

(2S,3S,4R,5R)-5-(6-((3-chlorobenzyl)amino)-9H-purin-9-yl)-3,4-dihydroxy-N,N-dimethyltetrahydrodroselenophene-2-carboxamide (9d). White solid; yield: 68%; 1H NMR (400 MHz, MeOD) δ 8.46 (s, 1 H), 8.24 (s, 1 H), 7.36 (s, 1 H), 7.18–7.28 (m, 4 H), 6.24 (d, J = 5.2 Hz, 1 H), 4.68 (dd, J = 4.4 Hz, 4 Hz, 1 H), 4.48 (d, J = 4.8 Hz, 1 H), 3.02 (s, 3 H), 2.97 (s, 3 H); 13C NMR (100 MHz, MeOD) δ 173.8, 156.1, 154.2, 143.1, 141.7, 135.5, 131.2, 128.6, 128.3, 128.3, 127.0, 120.8, 82.0, 77.6, 56.9, 42.4, 38.2, 36.4; HRMS (FAB) found 497.0599 (calculated for C19H22ClN2O2Se (M + H)^+ 497.0607).

(2S,3S,4R,5R)-5-(6-((3-bromobenzyl)amino)-9H-purin-9-yl)-3,4-dihydroxy-N,N-dimethyltetrahydrodroselenophene-2-carboxamide (9e). White solid; yield: 75%; 1H NMR (400 MHz, MeOD + CDCl3 = 1:3) δ 8.42 (s, 1 H), 8.29 (s, 1 H), 7.50 (s, 1 H), 7.36 (d, J = 7.6 Hz, 1 H), 7.28 (d, J = 7.6 Hz, 1 H), 7.17 (t, J = 8 Hz, 1 H), 6.17 (d, J = 4.8 Hz, 1 H), 4.76 (bs, 2 H), 4.73–4.69 (m, 1 H), 4.44 (d, J = 4.8 Hz, 1 H), 3.02 (s, 3 H), 2.98 (s, 3 H); 13C NMR (100 MHz, MeOD + CDCl3 = 1:3) δ 172.1, 155.0, 153.3, 141.2, 140.6, 130.8, 130.7, 130.5, 126.5, 122.9, 119.8, 81.3, 77.8, 76.6, 55.9, 41.5, 38.0, 36.3; HRMS (FAB) found 541.0106 (calculated for C19H19BrN2O2Se (M + H)^+ 541.0102).

(2S,3S,4R,5R)-5-(6-((3-iodobenzyl)amino)-9H-purin-9-yl)-N,N-dimethyltetrahydrodroselenophene-2-carboxamide (9f). White solid; yield: 73%; 1H NMR (400 MHz, MeOD) δ 8.45 (s, 1 H), 8.27 (s, 1 H), 7.71 (s, 1 H), 7.56 (d, J = 7.4 Hz, 1 H), 7.33 (d, J = 7.7 Hz, 1 H), 7.04 (t, J = 7.8 Hz, 1 H), 6.21 (d, J = 5.4 Hz, 1 H), 4.78–4.76 (m, 1 H), 4.74 (bs, 2 H), 4.71–4.69 (m, 1 H), 4.46 (d, J = 4.9 Hz, 1 H), 3.03 (s, 3 H), 2.99 (s, 3 H); 13C NMR (100 MHz, DMSO-d6) δ 173.5, 156.1, 154.3, 142.1, 137.8, 137.7, 131.6, 128.1, 120.9, 95.6, 82.3, 79.2, 78.8, 77.7, 57.0, 42.5, 38.9, 37.2; HRMS (FAB) found 588.9971 (calculated for C19H19I2N2O2Se (M + H)^+ 588.9963).
(2S,3S,4R,5R)-5-(6-((3-bromobenzyl)amino)-2-chloro-9H-purin-9-yl)-3,4-dihydroxy-N,N-dimethyltetrahydroselenophene-2-carboxamide (9k). White solid; yield: 94%; $^1$H NMR (400 MHz, MeOD) $\delta$ 8.43 (s, 1 H), 7.54 (s, 1 H), 7.36 (m, 2 H), 7.33–7.39 (t, $J$ = 7.6 Hz, 1 H), 6.15 (d, $J$ = 5.6 Hz, 1 H), 4.76 (dd, $J$ = 6.4 Hz, 3.2 Hz, 1 H), 4.71 (bs, 1 H), 4.65 (dd, $J$ = 3.2 Hz, 1 H), 4.48 (d, $J$ = 4.8 Hz, 1 H), 3.03 (s, 3 H), 2.98 (s, 3 H); $^{13}$C NMR (100 MHz, MeOD) $\delta$ 174.5, 157.3, 156.5, 152.7, 143.6, 142.7, 132.6, 132.1, 128.4, 124.2, 82.8, 78.3, 57.6, 45.3, 43.0, 38.9, 37.1; HRMS (FAB) found 574.9708 (calculated for C$_{19}$H$_{21}$BrClN$_5$O$_3$Se (M + H)$^+$ 574.9712).

(2S,3S,4R,5R)-5-(2-chloro-6-((3-iodobenzyl)amino)-9H-purin-9-yl)-3,4-dihydroxy-N,N-dimethyltetrahydroselenophene-2-carboxamide (9l). White solid; yield: 78%; $^1$H NMR (400 MHz, MeOD) $\delta$ 8.43 (s, 1 H), 7.75 (s, 1 H), 7.57 (d, $J$ = 4 Hz, 1 H), 7.36 (d, $J$ = 3.6 Hz, 1 H), 7.07 (t, $J$ = 8 Hz, 1 H), 6.15 (d, $J$ = 5.6 Hz, 1 H), 4.76 (dd, $J$ = 5.2 Hz, 3.2 Hz, 1 H), 4.64–4.68 (m, 2 H), 4.48 (d, $J$ = 4.8 Hz, 1 H), 3.03 (s, 3 H), 2.98 (s, 3 H); $^{13}$C NMR (100 MHz, MeOD) $\delta$ 173.8, 156.8, 155.9, 152.0, 142.8, 142.0, 138.0, 137.6, 131.5, 128.3, 95.1, 82.1, 77.6, 57.0, 44.5, 42.4, 38.3, 36.5.; HRMS (FAB) found 622.9584 (calculated for C$_{19}$H$_{21}$ClIN$_6$O$_3$Se (M + H)$^+$ 622.9574).

3.2. Biological Evaluation

3.2.1. Binding Assay at hA$_1$AR

Adenosine A$_1$ receptor competition binding experiments were carried out in membranes from CHO-A$_1$ cells (Euroscreen, Gosselies, Belgium). On the day of assay, membranes were defrosted and re-suspended in incubation buffer 20 mM Hepes, 100 mM NaCl, 10 mM MgCl$_2$, 2 UI/mL adenosine deaminase (pH = 7.4). Each reaction well of a GF/C multiscreen plate (Millipore, Madrid, Spain), prepared in duplicate, contained 15 $\mu$g of protein, 2 nM [³H]DPCPX and test compound. Nonspecific binding was determined in the presence of 10 $\mu$M (R)-PIA. The reaction mixture was incubated at 25 °C for 60 min, after which samples were filtered and measured in a microplate beta scintillation counter (Microbeta Trilux, Perkin Elmer, Madrid, Spain).

3.2.2. Binding Assay at hA$_2$AAR

Adenosine A$_2$A receptor competition binding experiments were carried out in membranes from HeLa-A$_2$A cells. On the day of assay, membranes were defrosted and re-suspended in incubation buffer 50 mM Tris-HCl, 1 mM EDTA, 10 mM MgCl$_2$ and 2 UI/mL adenosine deaminase (pH = 7.4). Each reaction well of a GF/C multiscreen plate (Millipore, Madrid, Spain), prepared in duplicate, contained 10 $\mu$g of protein, 2 nM [³H]DPCPX and test compound. Nonspecific binding was determined in the presence of 50 $\mu$M NECA. The reaction mixture was incubated at 25 °C for 30 min, after which samples were filtered and measured in a microplate beta scintillation counter (Microbeta Trilux, Perkin Elmer, Madrid, Spain).

3.2.3. Binding Assay at hA$_2$BAR

Adenosine A$_2$B receptor competition binding experiments were carried out in membranes from HEK-293-A$_2$B cells (Euroscreen, Gosselies, Belgium) prepared following the provider’s protocol. On the day of assay, membranes were defrosted and re-suspended in incubation buffer 50 mM Tris-HCl, 1 mM EDTA, 10 mM MgCl$_2$ and 2 UI/mL adenosine deaminase (pH = 7.4). Each reaction well of a GF/C multiscreen plate (Millipore, Madrid, Spain), prepared in duplicate, contained 10 $\mu$g of protein, 3 nM [³H]ZM241385 and test compound. Nonspecific binding was determined in the presence of 50 $\mu$M NECA. The reaction mixture was incubated at 25 °C for 30 min, after which samples were filtered and measured in a microplate beta scintillation counter (Microbeta Trilux, Perkin Elmer, Madrid, Spain).

3.2.4. Binding Assay at hA$_3$AR

Adenosine A$_3$ receptor competition binding experiments were carried out in a multiscreen GF/B 96-well plate (Millipore, Madrid, Spain) pretreated with binding buffer (Tris-HCl 50 mM, EDTA 1 mM, MgCl$_2$ 5 mM, 2 U/mL adenosine deaminase, pH = 7.4).
In each well was incubated 30 µg of membranes from Hela-A₃ cell line and prepared in laboratory (Lot: A005/05-07-2019, protein concentration = 3925 µg/mL), 10 nM [³H]-NECA (26.3 Ci/mmol, 1 mCi/mL, Perkin Elmer NET811250UC) and compounds studied in standard methods. Nonspecific binding was determined in the presence of R-PIA 100µM (Sigma P4532, Sigma-Aldrich, St. Louis, MO, USA). The reaction mixture (Vt: 200 µL/well) was incubated at 25 °C for 180 min, after filtered and washed six times with 250 µL wash buffer (Tris-HCl 50mM pH = 7.4), before measuring in a microplate beta scintillation counter (Microbeta Trilux, PerkinElmer, Madrid, Spain).

3.2.5. Cyclic AMP Accumulation Assay

Human adenosine A₃ receptor functional experiments were carried out in CHO-A₃#18 cell line. The day before the assay, the cells were seeded on the 96-well culture plate (Falcon 353072, Corning, Glendale, AZ, USA). The cells are washed with wash buffer (Dulbecco’s modified eagle’s medium nutrient mixture F-12 ham (Sigma D8062), 25 mM Hepes; pH = 7.4). Wash buffer is replaced by incubation buffer (Dulbecco’s modified eagle’s medium nutrient mixture F-12 ham (Sigma D8062), 25 mM Hepes, 30 µM Rolipram (Sigma R6520); pH = 7.4). Test compounds and MRS1220 as reference compound (Sigma M228) are added and the cells incubated at 37 °C for 15 min. After, 0.1 µM of 5′-N-ethylcarboxamido-adenosine (NECA) (Sigma E2387) is added and the cells incubated at 37 °C for 10 min. Forskolin (Sigma F3917, Sigma-Aldrich, St. Louis, MO, USA) is added and incubated at 37 °C for 5 min. After incubation, the amount of cAMP is determined using a cAMP Biotrak Enzymeimmunoassay (EIA) System Kit (GE Healthcare RPN225, GE Healthcare, Chicago, IL, USA).

3.3. Molecular Modelling

The hA₃AR homology model was obtained from reference 20. Autodock Vina 4 (The Scripps Research Institute, La Jolla, CA, USA) was used as the docking tool to generate ligand-protein complex using the following settings: center_x = −7.288, center_y = −8.071, center_z = 51.576, size_x = 40, size_y = 40, size_z = 40, energy_range = 4, exhaustiveness = 8. The ligand–protein complex with the best IFD score were selected and analyzed. The molecular graphic figures were generated by Biovia Discovery Studio Visualizer software (https://3dsbiovia.com/) (accessed on 13 April 2021.).

4. Conclusions

On the basis of potent and selective antagonist 5 and 6 at the human A₃AR, N⁶-, substituted-5′-N,N-dimethylcarbamoyl-4′-selenonucleoside derivatives (9a–l) were synthesized from D-ribose and evaluated for their binding affinity toward hARs. All final compounds exhibited medium to high binding affinity toward A₃AR with high selectivity compared to other subtypes. Among these derivatives, compound 9f was found to show the highest binding affinity (Kᵢ = 22.7 nM) at hA₃AR, comparable to the corresponding 4′-oxo- and 4′-thio analogues 5 (Kᵢ = 29.0 nM) and 6 (Kᵢ = 15.5 nM). As in the case of 4′-oxo- and 4′-thio analogues 5 and 6, addition of another methyl group to the 5′-N-methyluronamide converted an A₃AR agonist into an A₃AR antagonist, demonstrating the importance of amide hydrogen for receptor activation, which was supported by the molecular modelling study.

We believe that this study helps to define the pharmacophore needed for receptor activation or inactivation and will aid in the design of selective A₃AR ligands by medicinal chemists.

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