

# Structure–Activity Relationships of Truncated C2- or C8-Substituted Adenosine Derivatives as Dual Acting A<sub>2A</sub> and A<sub>3</sub> Adenosine Receptor Ligands

Xiyan Hou,<sup>†</sup> Mahesh S. Majik,<sup>†</sup> Kyunglim Kim,<sup>†</sup> Yuna Pyee,<sup>‡</sup> Yoonji Lee,<sup>†</sup> Varughese Alexander,<sup>†</sup> Hwa-Jin Chung,<sup>‡</sup> Hyuk Woo Lee,<sup>†</sup> Girish Chandra,<sup>†</sup> Jin Hee Lee,<sup>†</sup> Seul-gi Park,<sup>†</sup> Won Jun Choi,<sup>†,§</sup> Hea Ok Kim,<sup>†</sup> Khai Phan,<sup>||</sup> Zhan-Guo Gao,<sup>||</sup> Kenneth A. Jacobson,<sup>||</sup> Sun Choi,<sup>†</sup> Sang Kook Lee,<sup>‡</sup> and Lak Shin Jeong<sup>\*,†</sup>

<sup>†</sup>Laboratory of Medicinal Chemistry, College of Pharmacy, and Department of Bioinspired Science, Ewha Womans University, Seoul 120-750, Korea

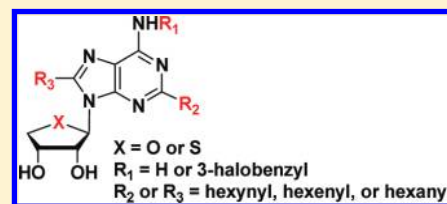
<sup>‡</sup>College of Pharmacy, Seoul National University, Seoul 151-742, Korea

<sup>§</sup>College of Pharmacy, Dongguk University, Kyungki-do 410-774, Korea

<sup>||</sup>Molecular Recognition Section, Laboratory of Bioorganic Chemistry, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, Maryland 20892, United States

## S Supporting Information

**ABSTRACT:** Truncated N<sup>6</sup>-substituted-4'-oxo- and 4'-thioadenosine derivatives with C2 or C8 substitution were studied as dual acting A<sub>2A</sub> and A<sub>3</sub> adenosine receptor (AR) ligands. The lithiation-mediated stannyl transfer and palladium-catalyzed cross-coupling reactions were utilized for functionalization of the C2 position of 6-chloropurine nucleosides. An unsubstituted 6-amino group and a hydrophobic C2 substituent were required for high affinity at the hA<sub>2A</sub>AR, but hydrophobic C8 substitution abolished binding at the hA<sub>2A</sub>AR. However, most of synthesized compounds displayed medium to high binding affinity at the hA<sub>3</sub>AR, regardless of C2 or C8 substitution, and low efficacy in a functional cAMP assay. Several compounds tended to be full hA<sub>2A</sub>AR agonists. C2 substitution probed geometrically through hA<sub>2A</sub>AR docking was important for binding in order of hexynyl > hexenyl > hexanyl. Compound **4g** was the most potent ligand acting dually as hA<sub>2A</sub>AR agonist and hA<sub>3</sub>AR antagonist, which might be useful for treatment of asthma or other inflammatory diseases.



## INTRODUCTION

The structure of endogenous adenosine, which binds to four subtypes (A<sub>1</sub>, A<sub>2A</sub>, A<sub>2B</sub>, and A<sub>3</sub>) of adenosine receptors (ARs)<sup>1,2</sup> to induce physiological effects, has been extensively modified, particularly on N<sup>6</sup> and/or 4'-hydroxymethyl positions for the development of potent and selective AR ligands.<sup>3,4</sup> Among these, 2-chloro-N<sup>6</sup>-(3-iodobenzyl)-5'-N-methylcarbamoyl-adenosine (Cl-IB-MECA, **1a**, X = O, R = 3-iodobenzyl; K<sub>i</sub> = 1.0 nM for hA<sub>3</sub>AR) is a versatile A<sub>3</sub>AR agonist, which is in clinical trial as an anticancer agent (Figure 1).<sup>5</sup> 2-Chloro-N<sup>6</sup>-(3-iodobenzyl)-5'-N-methylcarbamoyl-4'-thioadenosine (thio-Cl-IB-MECA, **1b**, X = S, R = 3-iodobenzyl; K<sub>i</sub> = 0.38 nM for hA<sub>3</sub>AR), which has a bioisosteric relationship to **1a**, was also found to be a potent and selective A<sub>3</sub>AR agonist.<sup>6</sup> Compounds **1a** and **1b** showed antiproliferative effects in various cancer cell lines, resulting from down-regulation of the Wnt signaling pathway and other mechanisms.<sup>7</sup>

A molecular modeling study<sup>8</sup> indicated that the NH of the 5'-uronamide of **1** serves as a hydrogen bonding donor in the A<sub>3</sub>AR binding site and is associated with the induced fit for the receptor activation. On this basis, we designed and synthesized truncated adenosine derivatives **2**,<sup>9</sup> removing the 5'-uronamide

of **1**, to bind potently but with altered ability to induce the conformational change essential for receptor activation. As expected, members of the series of compound **2** proved to be potent and selective A<sub>3</sub>AR antagonists.<sup>9</sup> These A<sub>3</sub>AR antagonists **2** also showed species-independent binding affinity, making them suitable for efficacy evaluation for drug development in small animal models.<sup>9</sup> Compound **2** (X = S, R = 3-iodobenzyl) exhibited a potent antiglaucoma effect in vivo.<sup>10</sup>

Recently, truncated C2- or C8-substituted 4'-thioadenosine derivatives **3** containing an appended hydrophobic hexenyl or hexynyl chain at the C2 or C8 position of **2** were designed and synthesized.<sup>11</sup> Among these, a C2-hexynyl derivative **3** (R = hexynyl) was discovered as a new template (K<sub>i</sub> = 7.19 ± 0.6 nM) for the development of potent A<sub>2A</sub>AR agonists<sup>12</sup> while maintaining antagonism at the A<sub>3</sub>AR (K<sub>i</sub> = 11.8 ± 1.3 nM).<sup>11</sup> However, C8-substituted derivatives displayed greatly reduced binding affinity at the A<sub>2A</sub>AR while maintaining high affinity at the A<sub>3</sub>AR, indicating that the binding pocket for C8 substituents in the A<sub>2A</sub>AR was relatively small when compared with that for C2 substituents,

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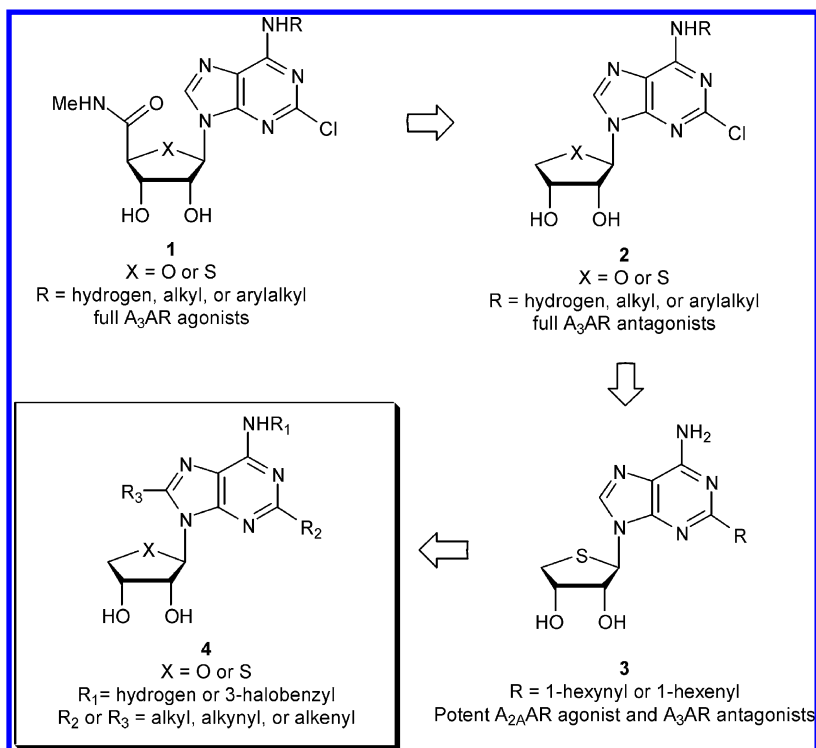


Figure 1. Design of the target nucleosides acting dually at the A<sub>2A</sub> and A<sub>3</sub>ARs.

which could accommodate steric bulk in both A<sub>2A</sub> and A<sub>3</sub>ARs.<sup>11</sup> This dual activity at both of these subtypes might be beneficial for developing therapeutic drugs against diseases such as asthma and inflammation.

Thus, it was of great interest to extend the structure–activity relationship study of the truncated 4'-thio series 3 to the 4'-oxo series 4 with various substituents at C2 or C8 positions. Truncated 4'-oxoadenosine derivatives containing C2-H or C2-Cl were previously found to interact potently and selectively with the A<sub>3</sub>AR.<sup>9c,d</sup> It was also interesting to determine the effects of N<sup>6</sup> substituents on binding affinity to the A<sub>2A</sub>AR because 3-halobenzyl substituents at the N<sup>6</sup> position generally increased the binding affinity and selectivity at the A<sub>3</sub>AR.<sup>3,4</sup> Herein, we report a full account of truncated C2-, C8-, or N<sup>6</sup>-modified adenosine derivatives acting dually at the A<sub>2A</sub> and A<sub>3</sub>ARs.

## RESULTS AND DISCUSSION

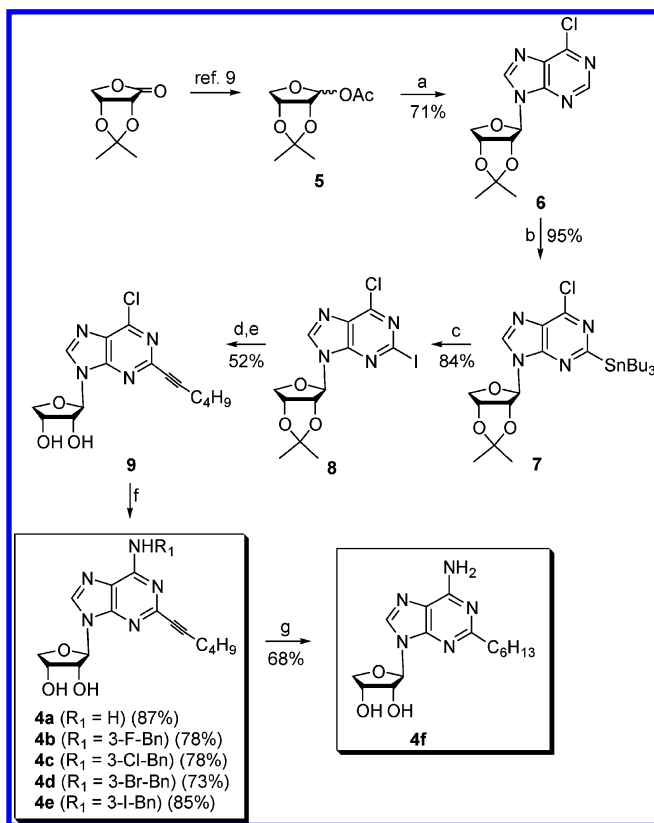
**Chemistry.** First, 2-hexynyl-N<sup>6</sup>-substituted-adenosine derivatives 4a–f were synthesized from glycosyl donor 5,<sup>9</sup> which was easily synthesized from commercially available 2,3-O-isopropylidene-D-erythronic  $\gamma$ -lactone in two steps, as shown in Scheme 1.

Condensation of glycosyl donor 5 with silylated 6-chloropurine in the presence of trimethylsilyl trifluoromethanesulfonate (TMSOTf) as a Lewis acid yielded the  $\beta$ -anomer 6<sup>9c</sup> (71%) as a single diastereomer. The anomeric configuration in 6 was easily confirmed by a <sup>1</sup>H NMR nuclear Overhauser effect (NOE) experiment between H-8 and 3'-H. For the functionalization at the C2 position of 6-chloropurine in 6, lithiation-mediated stannyl transfer of 6-chloropurine nucleosides reported by Tanaka and co-workers<sup>13a</sup> was utilized. Treatment of 6 with a freshly prepared lithium 2,2,6,6-tetramethylpiperidide (LiTMP) initially formed the C8-lithiated species because the C8-hydrogen is more acidic than the C2-hydrogen. Reaction of the C8-lithiated species with

*n*-tributyltin chloride produced C2-stannyl derivative 7 as a sole regioisomer, which resulted from an anionic transfer of the stannyl group from C8 to the C2 position (see Scheme 1S in the Supporting Information for a reaction mechanism).<sup>13a</sup> Treatment of 7 with iodine gave 2-iodo-6-chloropurine derivative 8.<sup>13</sup> Sonogashira<sup>14</sup> coupling of 8 with 1-hexyne in the presence of tetrakis(triphenylphosphine)palladium and cesium carbonate yielded C2-hexynyl derivative, which was hydrolyzed with 1 N HCl to give the 2-hexynyl-6-chloro derivative 9. Compound 9 was converted into 2-hexynyladenosine derivative 4a and 2-hexynyl-N<sup>6</sup>-substituted-adenosine derivatives 4b–e by treatment with ammonia and 3-halobenzyl amines, respectively. 2-Hexynyladenosine derivative 4a was subjected to hydrogenation with 10% Pd on carbon to give saturated analogue 4f.

The corresponding 4'-thioadenosine derivatives 4g–k were synthesized from glycosyl donor 10, which was easily synthesized from D-mannose according to our previously published procedure,<sup>9</sup> as illustrated in Scheme 2.

The lithiation-mediated stannyl transfer<sup>13</sup> of 6-chloropurine nucleosides used in Scheme 1 was first tried for the synthesis of 4'-thioadenosine derivatives 4g–k but resulted in poor formation of 2-iodo-6-chloropurine derivative 11 (5%), due to the presence of acidic 1' and 4' protons (conventional nucleoside numbering) in the thiophene ring. Thus, alternative method of directly condensing glycosyl donor 10 with freshly prepared 2-iodo-6-chloropurine<sup>13b</sup> was attempted, as shown in Scheme 2. Condensation of glycosyl donor 10 with silylated 2-iodo-6-chloropurine in the presence of TMSOTf afforded the  $\beta$ -anomer 11 (60%) along with a trace amount of its  $\alpha$ -anomer. The anomeric configuration was also confirmed by a <sup>1</sup>H NMR NOE experiment between H-8 and 3'-H. Sonogashira<sup>14</sup> coupling reaction of 11 with 1-hexyne in the presence of tetrakis(triphenylphosphine)palladium {(Ph<sub>3</sub>P)<sub>4</sub>Pd} and cesium carbonate yielded the C2-hexynyl derivative 12.

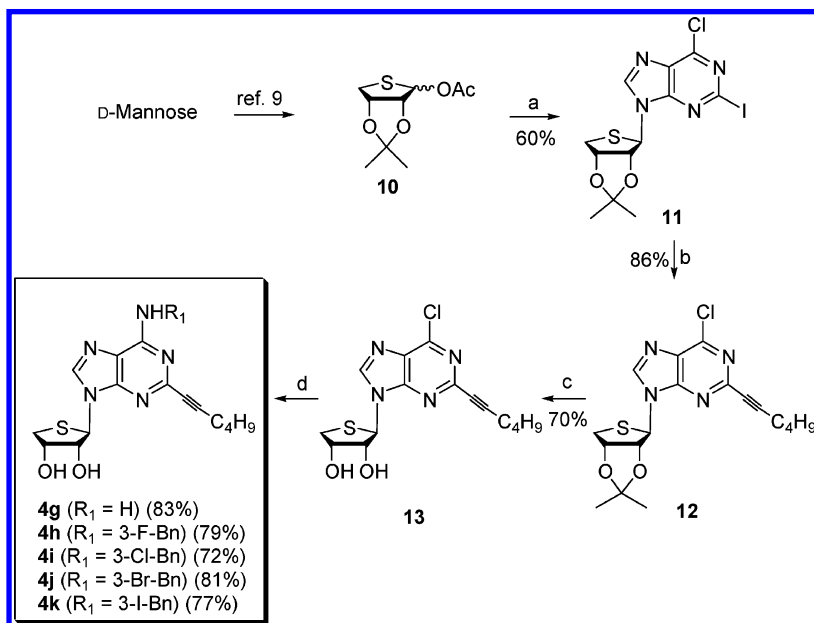
Scheme 1<sup>a</sup>

<sup>a</sup>Reagents and conditions: (a) silylated 6-chloropurine, TMSOTf, DCE, room temp to 80 °C, 5 h; (b) LiTMP, Bu<sub>3</sub>SnCl, HF, hexane, -78 °C, 1 h; (c) I<sub>2</sub>, THF, room temp, 24 h; (d) 1-hexyne, Cs<sub>2</sub>CO<sub>3</sub>, (Ph<sub>3</sub>P)<sub>4</sub>Pd, CuI, DMF, room temp, 3 h; (e) 1 N HCl, THF, room temp, 15 h; (f) NH<sub>3</sub>/*t*-BuOH, 100 °C, 8 h or R<sub>1</sub>NH<sub>2</sub>, Et<sub>3</sub>N, EtOH, room temp, 24–48 h; (g) Pd/C, H<sub>2</sub>, MeOH.

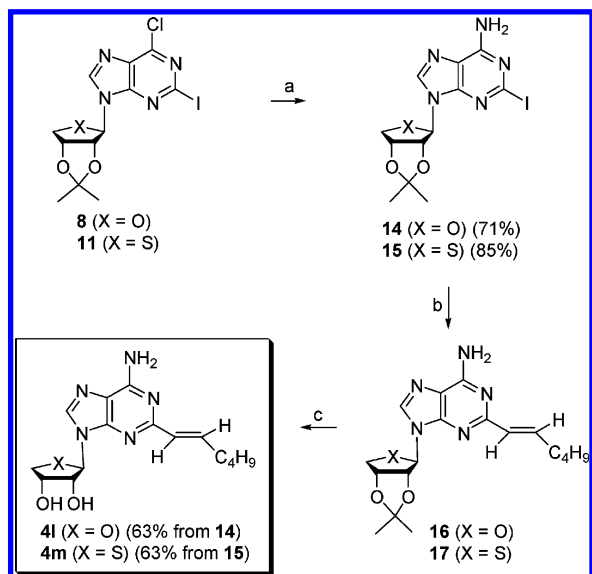
Treatment of **12** with 1 N HCl yielded the 2-hexynyl-6-chloro derivative **13**, which was treated with ammonia and 3-halobenzyl amines to afford 2-hexynyl-4'-thioadenosine derivative **4g**<sup>11</sup> and 2-hexynyl-*N*<sup>6</sup>-substituted-4'-thioadenosine derivatives **4h–k**, respectively.

After the successful introduction of the hexynyl group at the C2 position, we decided to extend the synthesis to other hydrophobic C2-hexenyl and C2-hexynyl derivatives. Synthesis of 2-hexenyl derivatives was accomplished using a Suzuki<sup>15</sup> coupling reaction as a key step (Scheme 3). Treatment of 2-iodo-6-chloro derivatives **8** and **11** with methanolic ammonia gave 2-iodo-6-amino derivatives **14** and **15**, respectively. Suzuki coupling of C2-iodo derivatives **14** and **15** with (*E*)-1-catecholboranylhexene,<sup>16</sup> prepared by treating with 1-hexyne and catecholborane in the presence of (Ph<sub>3</sub>P)<sub>4</sub>Pd, produced 2-hexenyl derivatives **16** and **17**, respectively. Treatment of **16** and **17** with 1 N HCl gave 2-hexenyl-adenosine derivative **4l** and 2-hexenyl-4'-thioadenosine derivative **4m**,<sup>11</sup> respectively.

By use of a synthetic strategy similar to Scheme 1, C8-substituted adenosine derivatives **4n–p** were synthesized from glycosyl donors **5** and **10** (Scheme 4). Condensation of **5**<sup>9</sup> with silylated 6-chloropurine under Lewis acid conditions followed by treatment with methanolic ammonia afforded adenine derivative **18**. Treatment of **18** with bromine and sodium acetate in methanol yielded 8-bromo derivative **19**. However, in the case of 4'-thioadenosine derivatives, the same method was tried but resulted in the extensive decomposition of starting material. Thus, direct condensation of **10** with 8-bromo-adenine<sup>17</sup> under Lewis acid conditions provided 8-bromo derivative **20** but only in 20% yield. Sonogashira coupling of **19** and **20** with 1-hexyne using bis(triphenylphosphine) palladium dichloride {(Ph<sub>3</sub>P)<sub>4</sub>PdCl<sub>2</sub>} gave C8-hexynyl derivatives **21** and **22**, respectively. Treatment of **21** and **22** with 1 N HCl yielded the final 8-hexynyl derivatives **4n** and **4o**,<sup>11</sup>

Scheme 2<sup>a</sup>

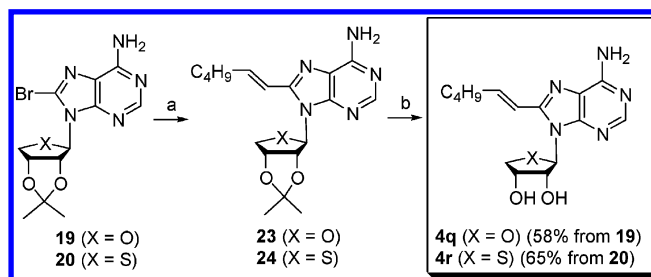
<sup>a</sup>Reagents and conditions: (a) 2-iodo-6-chloropurine, BSA, TMSOTf, CH<sub>3</sub>CN, room temp to 80 °C, 3 h; (b) 1-hexyne, Cs<sub>2</sub>CO<sub>3</sub>, (Ph<sub>3</sub>P)<sub>4</sub>Pd, CuI, DMF, room temp, 3 h; (c) 1 N HCl, THF, room temp, 20 h; (d) NH<sub>3</sub> in *t*-BuOH, 100 °C, 8 h or R<sub>1</sub>NH<sub>2</sub>, Et<sub>3</sub>N, EtOH, room temp, 48 h.

Scheme 3<sup>a</sup>

<sup>a</sup>Reagents and conditions: (a)  $\text{NH}_3/\text{MeOH}$ , 80 °C, 2 h; (b) (*E*)-1-catecholboranylhexene,  $(\text{Ph}_3\text{P})_4\text{Pd}$ ,  $\text{Na}_2\text{CO}_3$ , DMF,  $\text{H}_2\text{O}$ , 90 °C, 15 h; (c) 1 N HCl, THF, room temp, 15 h.

respectively. Compound **4n** was converted to *n*-hexenyl derivative **4p** using catalytic hydrogenation.

For the synthesis of 8-hexenyl derivatives **4q** and **4r**, 8-bromo derivatives **19** and **20** were coupled with (*E*)-1-catecholboranylhexene<sup>16</sup> under Suzuki conditions<sup>15</sup> to give C2-hexenyl derivatives **23** and **24**, respectively (Scheme 5). Removal of the isopropylidene group in **23** and **24** under acidic conditions

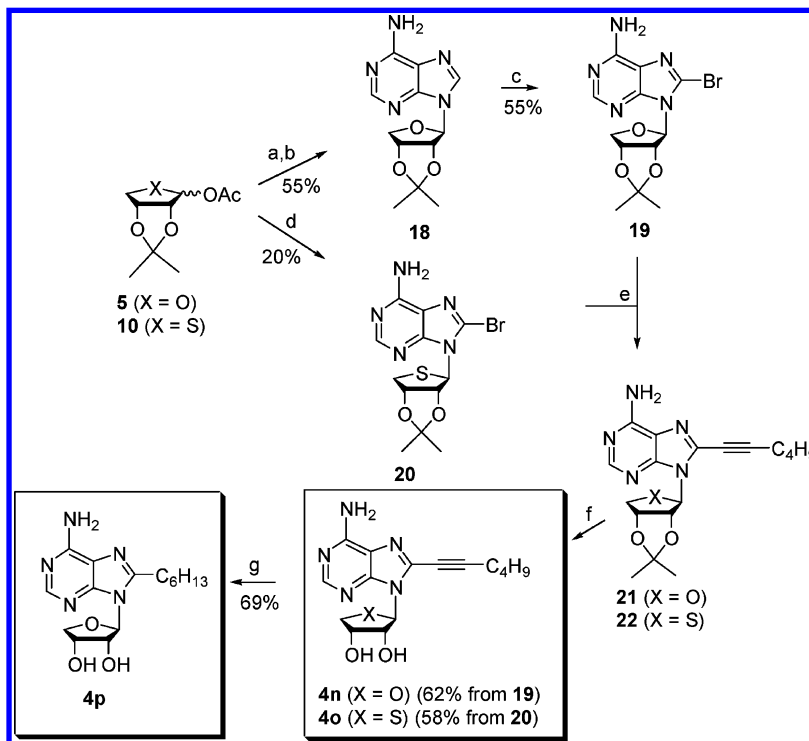
Scheme 5<sup>a</sup>

<sup>a</sup>Reagents and conditions: (a) (*E*)-1-catecholboranylhexene,  $(\text{Ph}_3\text{P})_4\text{Pd}$ ,  $\text{Na}_2\text{CO}_3$ , DMF,  $\text{H}_2\text{O}$ , 90 °C, 15 h; (b) 1 N HCl, THF, room temp, 15 h.

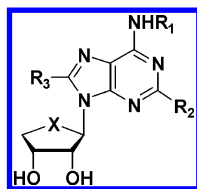
afforded the 8-hexenyladenosine derivative **4q** and the 8-hexenyl-4'-thioadenosine derivative **4r**.<sup>11</sup>

**Biological Evaluation.** Binding assays were carried out using standard radioligands and membrane preparations from Chinese hamster ovary (CHO) cells stably expressing the human (h)  $\text{A}_1$  or  $\text{A}_3\text{AR}$  or human embryonic kidney (HEK)-293 cells expressing the  $\text{hA}_{2\text{A}}\text{AR}$ .<sup>18</sup> Nonspecific binding was defined using 10  $\mu\text{M}$  of 5'-*N*-ethylcarboxamidoadenosine (**25**, NECA).

As shown in Table 1, 4'-thio derivatives generally exhibited higher binding affinities to  $\text{hA}_{2\text{A}}$  and  $\text{hA}_3\text{AR}$ s than the corresponding 4'-oxo derivatives. In case of *N*<sup>6</sup>-amino derivatives, most of the C2-substituted analogues ( $\text{R}_2$  = hexynyl and hexenyl) showed moderate to high binding affinities at the  $\text{hA}_{2\text{A}}$  and  $\text{hA}_3\text{AR}$ s, while most of C8-substitutions ( $\text{R}_3$  = hexynyl, hexenyl, and hexanyl) reduced binding affinity at the  $\text{hA}_{2\text{A}}\text{AR}$  but maintained high affinity at the  $\text{hA}_3\text{AR}$ .

Scheme 4<sup>a</sup>

<sup>a</sup>Reagents and conditions: (a) silylated 6-chloropurine, TMSOTf, DCE, room temp to 80 °C, 5 h; (b)  $\text{NH}_3$ , MeOH, 80 °C, 2 h; (c)  $\text{Br}_2$ , 1 N NaOAc, MeOH, room temp, 40 min; (d) silylated 8-bromo-adenine, TMSOTf, DCE, room temp to 90 °C, 2 h; (e) 1-hexyne, CuI, TEA, DMF,  $(\text{PPh}_3)_2\text{PdCl}_2$ , room temp, 3 h; (f) 1 N HCl, THF, room temp, 15 h; (g)  $\text{H}_2$ , Pd/C, MeOH, 15 h.

**Table 1.** Binding Affinities of Known A<sub>3</sub>AR Antagonist 2 and Truncated C2- and C8-Substituted Derivatives 4a–r at Three Subtypes of hARs and A<sub>3</sub>AR-Mediated Inhibition of cAMP Production

compd (R <sub>1</sub> , R <sub>2</sub> , R <sub>3</sub> )	affinity (K <sub>i</sub> ± SEM (nM) or % inhibition) <sup>a</sup>			relative efficacy (% inhibition ± SEM of cAMP) <sup>c</sup> hA <sub>3</sub>
	hA <sub>1</sub>	hA <sub>2A</sub>	hA <sub>3</sub>	
2 (X = S, R <sub>1</sub> = 3-I-Bn, R <sub>2</sub> = Cl, R <sub>3</sub> = H) <sup>b</sup>	2490 ± 940	341 ± 75	4.16 ± 0.50	4.2 ± 2.3
4a (X = O, R <sub>1</sub> = H, R <sub>2</sub> = 2-hexynyl, R <sub>3</sub> = H)	740 ± 430	63.2 ± 15	138 ± 44	17.4 ± 2.4
4b (X = O, R <sub>1</sub> = 3-F-Bn, R <sub>2</sub> = 2-hexynyl, R <sub>3</sub> = H)	9 ± 1%	25 ± 3%	570 ± 130	29.5 ± 5.5
4c (X = O, R <sub>1</sub> = 3-Cl-Bn, R <sub>2</sub> = 2-hexynyl, R <sub>3</sub> = H)	9 ± 5%	42 ± 13%	150 ± 140	39.7 ± 3.8
4d (X = O, R <sub>1</sub> = 3-Br-Bn, R <sub>2</sub> = 2-hexynyl, R <sub>3</sub> = H)	12 ± 4%	27 ± 4%	67 ± 18	37.2 ± 2.9
4e (X = O, R <sub>1</sub> = 3-I-Bn, R <sub>2</sub> = 2-hexynyl, R <sub>3</sub> = H)	18 ± 2%	32 ± 9%	220 ± 50	31.5 ± 2.8
4f (X = O, R <sub>1</sub> = H, R <sub>2</sub> = 2-hexenyl, R <sub>3</sub> = H)	10 ± 5%	2160 ± 270	39 ± 5%	22.3 ± 4.5
4g (X = S, R <sub>1</sub> = H, R <sub>2</sub> = 2-hexynyl, R <sub>3</sub> = H) <sup>d</sup>	39 ± 10%	7.19 ± 0.6	11.8 ± 1.3	2.8 ± 1.6
4h (X = S, R <sub>1</sub> = 3-F-Bn, R <sub>2</sub> = 2-hexynyl, R <sub>3</sub> = H)	24 ± 5%	46 ± 5%	150 ± 80	47.6 ± 4.9
4i (X = S, R <sub>1</sub> = 3-Cl-Bn, R <sub>2</sub> = 2-hexynyl, R <sub>3</sub> = H)	25 ± 2%	3730 ± 200	24.0 ± 6.0	47.2 ± 3.4
4j (X = S, R <sub>1</sub> = 3-Br-Bn, R <sub>2</sub> = 2-hexynyl, R <sub>3</sub> = H)	12 ± 1%	3910 ± 970	24.0 ± 5.0	25.2 ± 3.0
4k (X = S, R <sub>1</sub> = 3-I-Bn, R <sub>2</sub> = 2-hexynyl, R <sub>3</sub> = H)	15 ± 3%	4890 ± 840	39 ± 5	40.0 ± 5.6
4l (X = O, R <sub>1</sub> = H, R <sub>2</sub> = 2-hexenyl, R <sub>3</sub> = H)	31.9 ± 1.2%	178 ± 26	218 ± 79	42.6 ± 3.1
4m (X = S, R <sub>1</sub> = H, R <sub>2</sub> = 2-hexenyl, R <sub>3</sub> = H) <sup>d</sup>	16.2 ± 8.4%	72.0 ± 19.1	13.2 ± 0.8	10.7 ± 4.1
4n (X = O, R <sub>1</sub> = H, R <sub>2</sub> = H, R <sub>3</sub> = 2-hexynyl)	290 ± 70	27.2 ± 2.9%	31.7 ± 7.4	24.5 ± 2.9
4o (X = S, R <sub>1</sub> = H, R <sub>2</sub> = H, R <sub>3</sub> = 2-hexynyl) <sup>d</sup>	49.3 ± 4.9%	46.5 ± 4.3%	20.0 ± 4.0	1.7 ± 3.9
4p (X = O, R <sub>1</sub> = H, R <sub>2</sub> = H, R <sub>3</sub> = 2-hexenyl)	18.1 ± 5.0%	5.8 ± 4.6%	24.7 ± 2.1%	12.9 ± 2.2
4q (X = O, R <sub>1</sub> = H, R <sub>2</sub> = H, R <sub>3</sub> = 2-hexenyl)	500 ± 140	27.3 ± 6.3%	94.2 ± 30.0	19.5 ± 2.4
4r (X = S, R <sub>1</sub> = H, R <sub>2</sub> = H, R <sub>3</sub> = 2-hexenyl) <sup>d</sup>	3.7 ± 2.9%	22.8 ± 6.4%	259 ± 10	6.1 ± 1.7

<sup>a</sup>All binding experiments were performed using adherent mammalian cells stably transfected with cDNA encoding the appropriate hAR (A<sub>1</sub>AR and A<sub>3</sub>AR in CHO cells and A<sub>2A</sub>AR in HEK-293 cells). Binding was carried out using 1 nM [<sup>3</sup>H]R-(−)-N<sup>6</sup>-2-phenylisopropyladenosine (R-PIA), [<sup>3</sup>H]2-[p-(2-carboxyethyl)phenylethylamino]-5'-N-ethylcarboxamidoadenosine (26, CGS21680, 10 nM), or 0.5 nM [<sup>125</sup>I]N<sup>6</sup>-(3-iodo-4-aminobenzyl)-5'-N-methylcarboxamidoadenosine (27, I-AB-MECA) as radioligands for A<sub>1</sub>, A<sub>2A</sub>, and A<sub>3</sub>ARs, respectively. Values are expressed as mean ± SEM, *n* = 3–4 (outliers eliminated) and normalized against a nonspecific binder 25 (10 μM). Values expressed as a percentage in italics refer to percent inhibition of specific radioligand binding at 10 μM, with nonspecific binding defined using 10 μM 25. <sup>b</sup>Reference 9. <sup>c</sup>Maximal efficacy (at 10 μM) in an A<sub>3</sub>AR functional assay, determined by inhibition of forskolin-stimulated cAMP production in AR-transfected CHO cells, expressed as percent inhibition (mean ± standard error, *n* = 3–5) in comparison to effect (100%) of full agonist 1a at 10 μM. <sup>d</sup>Binding data from ref 11.

These findings indicate that bulky hydrophobic pockets exist in the binding sites of A<sub>2A</sub>AR and A<sub>3</sub>AR, allowing the C2-substituent to form favorable hydrophobic interactions, but a bulky hydrophobic group at C8 position could be tolerated only at the binding site of the hA<sub>3</sub>AR but not hA<sub>2A</sub>AR. The ability to enhance affinity at the A<sub>2A</sub>AR in the truncated series by extending an unsaturated carbon chain at the C2 position implies a mode of receptor binding in common with the riboside series.<sup>19</sup> However, introduction of a hydrophobic 3-halobenzyl substituent on the N<sup>6</sup>-amino group of 4a and 4g, resulting in 4b–e and 4h–k, respectively dramatically decreased the binding affinity at the hA<sub>2A</sub>AR but maintained the binding affinity at the hA<sub>3</sub>AR. These results indicate that an unsubstituted primary amino group at the N<sup>6</sup> position is conducive to high binding affinity at the hA<sub>2A</sub>AR while a bulky hydrophobic substituent at the N<sup>6</sup> position is essential for high affinity at the hA<sub>3</sub>AR. By correlation of pharmacological behavior with the nucleoside substitution pattern, a hexynyl substituent generally produced higher binding affinity than the corresponding hexenyl substituent, and two hexenyl derivatives (4f and 4p) were greatly reduced in binding affinity at the three subtypes of hARs, indicating that the geometry of the substituent is important for the binding affinity. All compounds

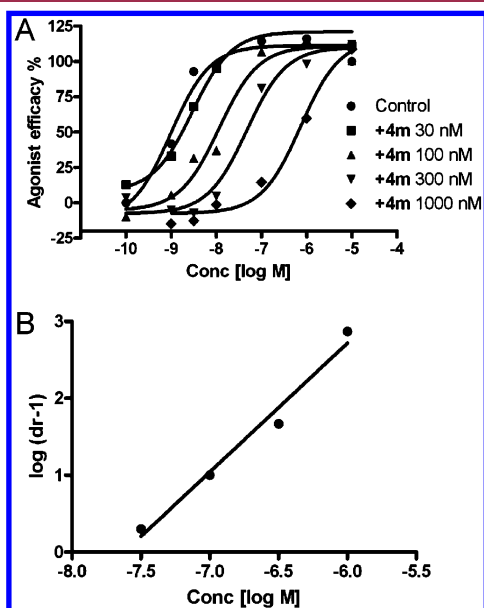
showed weak binding affinity at the hA<sub>1</sub>AR, although 4a, 4n, and 4q displayed measurable K<sub>i</sub> values less than 1 μM at this subtype. Thus, in some cases hydrophobic substitution at the C8 position permitted moderate binding affinity (K<sub>i</sub> < 500 nM) at the hA<sub>1</sub>AR. 4'-Oxo derivative 4d and 4'-thio derivatives 4i, 4j, 4k, and 4o displayed high selectivity for the hA<sub>3</sub>AR in comparison to hA<sub>1</sub>AR and hA<sub>2A</sub>AR, with K<sub>i</sub> values in the range of 20–67 nM. From the SAR study, compound 4g was discovered to be the most potent dually acting ligand, binding with high affinity at both the hA<sub>2A</sub>AR (K<sub>i</sub> = 7.19 ± 0.6 nM) and hA<sub>3</sub>AR (K<sub>i</sub> = 11.8 ± 1.3 nM) to the exclusion of the hA<sub>1</sub>AR.<sup>11</sup>

The functional screening protocol used at the hA<sub>3</sub>AR consisted of examining the effects of a single concentration of each compound (10 μM), which in each case except for 4f and 4p far exceeded the K<sub>i</sub> values in binding and therefore was interpreted as a roughly maximal effect under ligand saturating conditions. As shown in Table 1, most of the derivatives are low efficacy partial agonists at the hA<sub>3</sub>AR, with relative percent inhibition of cyclic adenosine 5'-monophosphate (cAMP) formation not exceeding 50%. The 4'-thio C2-substituted derivatives 4i, 4j, and 4k appear to be hA<sub>3</sub>AR-selective partial agonists. Some of the C2-substituted derivatives, 4c, 4h, 4i, 4k, and 4l, displayed between 40% and 50% maximal efficacy in



comparison to **1a**. This was in contrast to another C2 derivative **4g**, which did not have significant residual efficacy at the hA<sub>3</sub>AR. The C8-substituted derivatives **4n–r** did not exceed 25% of the maximal efficacy to **1a**. A C8-hexynyl derivative **4o** in the 4'-thio series, which was highly potent in hA<sub>3</sub>AR binding ( $K_i = 20$  nM) and selective in comparison to A<sub>1</sub>AR and A<sub>2A</sub>AR did not have significant residual efficacy at the hA<sub>3</sub>AR.

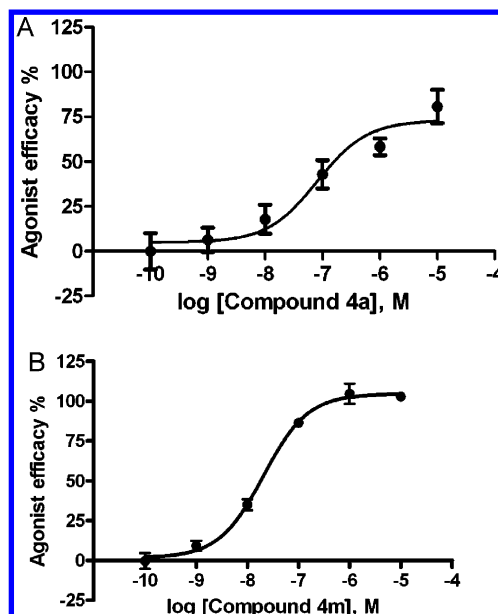
In a set of full concentration–response experiments, compound **4g** induced parallel right shifts of the concentration–response curve of a full agonist **1a** in the inhibition of cAMP production at the hA<sub>3</sub>AR expressed in CHO cells, indicating that it is as a full, competitive antagonist ( $K_B = 1.69$  nM) at the hA<sub>3</sub>AR,<sup>11</sup> as our previously reported truncated N<sup>6</sup>-substituted 4'-thioadenosine derivatives.<sup>9</sup> Similarly, by the same criteria compound **4m** was a competitive antagonist ( $K_B = 23.9$  nM) at the hA<sub>3</sub>AR (Figure 2).



**Figure 2.** Effects of compound **4m** on inhibition of cAMP production induced by full agonist **1a** at the hA<sub>3</sub>AR expressed in CHO cells (A). The data were transformed to a linear Schild plot (B) indicating competitive antagonism with a  $K_B$  of 23.9 nM.

However, in a cAMP functional assay at the hA<sub>2A</sub>AR expressed in CHO cells, several of these nucleoside agonists demonstrated a higher degree of relative efficacy than at the hA<sub>3</sub>AR. For example, the most potent compound **4g** behaved as a full agonist compared to the standard CGS21680 and displayed an  $EC_{50}$  of 12 nM,<sup>11</sup> and compound **4a** displayed a maximal stimulation of cAMP formation of  $89.9 \pm 3.8\%$ , relative to the full agonist NECA (=100%), with an  $EC_{50}$  of 80.3 nM (Figure 3A).

Compounds **4l** and **4m** were also agonists at the hA<sub>2A</sub>AR, with maximal percent activity at 10  $\mu$ M relative to NECA of  $83.0 \pm 5.2\%$  and  $108.8 \pm 5.1\%$ , respectively. In a full concentration–response curve (Figure 3B), compound **4m** displayed an  $EC_{50}$  value of  $20.6 \pm 1.58$  nM in stimulation of cAMP formation via the hA<sub>2A</sub>AR. As reported by us previously, compound **4g** was a potent full agonist in stimulation of cAMP formation via the hA<sub>2A</sub>AR with an  $EC_{50}$  of 12 nM.<sup>11</sup> The findings that truncated nucleosides **4g** and **4m** serve as dual ligands acting at the hA<sub>2A</sub> and hA<sub>3</sub>ARs are similar to the pharmacological profile of a more heavily 2,5'-substituted



**Figure 3.** Effects of compounds **4a** (A) and **4m** (B) in stimulation of cAMP production at the hA<sub>2A</sub>AR expressed in CHO cells, compared to NECA as reference full agonist (=100%).

adenosine derivative, which was also a hA<sub>2A</sub>AR agonist and hA<sub>3</sub>AR antagonist.<sup>20</sup>

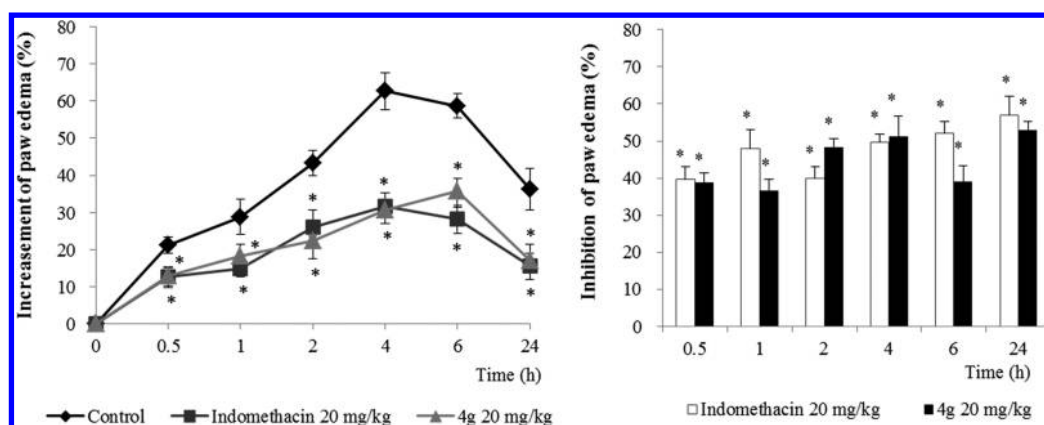
We then examined the effects of several compounds in stimulation of cAMP production at the hA<sub>2B</sub>AR expressed in CHO cells, compared to NECA as reference full agonist (=100%). The most potent compound **4g** was a weak partial agonist in cyclic AMP accumulation ( $EC_{50} \approx 10$   $\mu$ M).<sup>11</sup> As indicated by the percent stimulation at 10  $\mu$ M, none of other potent truncated derivatives (4'-oxo **4a**  $1.9 \pm 4.0\%$ , **4l**  $1.8 \pm 4.9\%$ ; 4'-thio **4m**  $3.6 \pm 2.9\%$ , **4o**  $7.0 \pm 4.4\%$ ) displayed significant activity at this AR subtype.

Before examination of the pharmacological effect of the most potent compound **4g**, the partition coefficient (log  $P$ ) was first calculated using Tripos SYBYL X-1.2 for the druggability of **4g**. The log  $P$  of **4g** was 0.51, which is sufficient to penetrate cell membranes, suggesting the possibility of bioavailability by oral as well as other administration routes.

Acute inflammation is a short-term process that is characterized by the typical signs of inflammation, such as swelling, pain, and loss of function due to the infiltration of the tissues by plasma and leukocytes. Among them, edema is one of the fundamental actions of acute inflammation and it is an essential parameter to be considered when evaluating compounds with a potential anti-inflammatory activity.<sup>21</sup> Therefore, we tested the anti-inflammatory activity of **4g** on carrageenan-induced paw edema model in rats.<sup>22</sup> Paw edema was induced by the injection of carrageenan (0.1 mL in 1% solution), and the volume of paw edema was monitored for 24 h. The paw edema was increased and reached its maximum at 4 h after treatment of carrageenan.

As shown in Figure 4, the treatment of **4g** significantly ( $P < 0.01$ ) reduced the paw edema formation. The inhibition rate at 4 h was 51.1% with the treatment of **4g** (20 mg/kg). In the same experimental condition, indomethacin (20 mg/kg) was shown as 49.7% inhibition at 4 h.

The potency of the anti-inflammatory effect of **4g** was similar to that of indomethacin. In this study, the formation of edema reached a maximum at 4 h after carrageenan injection and the



**Figure 4.** Inhibitory effect of **4g** on the carrageenan-induced paw edema. Paw edema was induced as described in the Experimental Section. The paw volume was measured before (0 h) and at intervals of 0.5, 1, 2, 4, 6, and 24 h after carrageenan injection using a plethysmometer. Data represent the mean  $\pm$  SD ( $n = 6$ ). \* indicates statistically significant differences from the control group ( $P < 0.01$ ).

administration of **4g** inhibited the paw edema induced by carrageenan (Figure 4). These findings demonstrated that **4g** has a potent in vivo anti-inflammatory activity in an acute inflammation model system.

**Molecular Docking Study.** The truncated C2-substituted derivative **4g** showed excellent binding affinity at the  $hA_{2A}$ AR with a  $K_i$  of 7.19 nM. Substituting the  $N^6$  position with a chlorobenzyl group, however, dramatically decreased the  $hA_{2A}$ AR binding affinity of **4i** by 520-fold in comparison to **4g**. Our flexible docking study showed that **4g** binds readily into the antagonist-bound X-ray crystal structure of human  $A_{2A}$ AR (PDB code 3EML)<sup>23</sup> (Figure 5A).<sup>11</sup> Its adenine and sugar moieties make tight interactions with the binding site residues via the  $\pi$ - $\pi$  stacking with Phe168 and H-bonding with Glu169, Asn253, and Ser277. The C2-hexynyl group also contributes to the ligand binding through favorable hydrophobic interactions. In contrast, the  $N^6$ -chlorobenzyl derivative **4i** appeared to have various binding modes and did not maintain some key interactions for binding (Figure 5B). It seems that **4i** would lose important interactions at the binding site in order to accommodate both the bulky  $N^6$ -chlorobenzyl and C2-hexynyl groups. This result could explain why appending a halobenzyl group at the  $N^6$  position decreased the  $A_{2A}$ AR binding affinity.

The binding modes of a series of the C2-substituted and  $N^6$ -unsubstituted derivatives (i.e., **4a**, **4f**, **4g**, **4l**, and **4m**) were also compared. As shown in Figure 6A, the adenine and sugar moieties in this series of truncated nucleosides maintained almost exactly the same binding interactions. This docking study demonstrated that the different binding modes of the C2-substituents directly influenced their activities and a good correlation ( $r^2 = 0.86$ ) was shown between the binding affinities and docking scores (Figure 6B). The activity of the compounds decreased as the flexibility of their C2-substituents increased. Containing the most flexible hexanyl group at the C2 position, **4f** showed a relatively low binding affinity and docking score. Taken altogether, the appropriate size of the functional group at the  $N^6$  position and rigidity of the C2-substituents appear to contribute to the ligand binding.

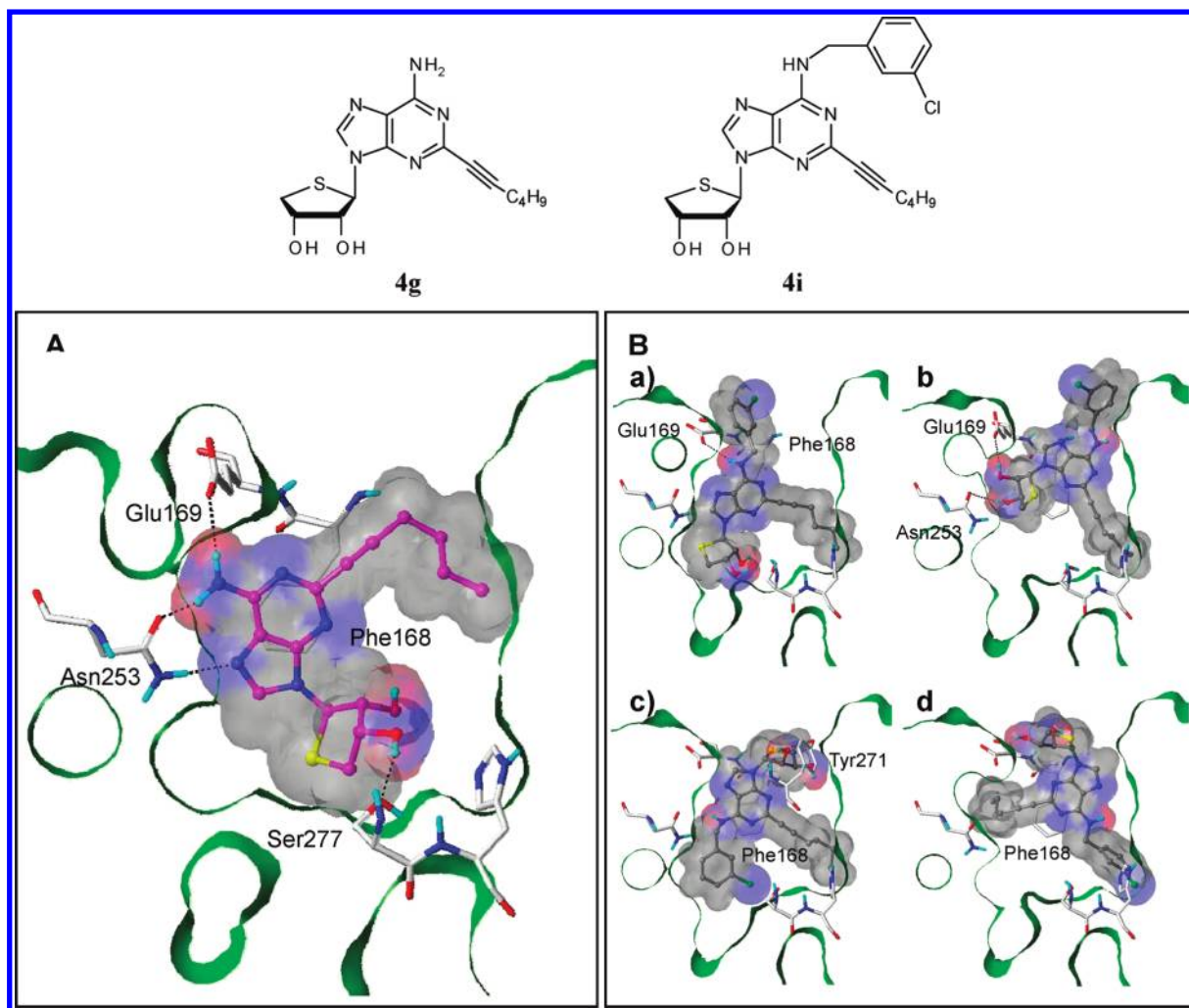
## CONCLUSIONS

In summary, the series of truncated  $N^6$ -substituted 4'-oxo- and 4'-thioadenosine derivatives, **4a-r** with substitution at C2 or C8 position, were synthesized in order to examine the

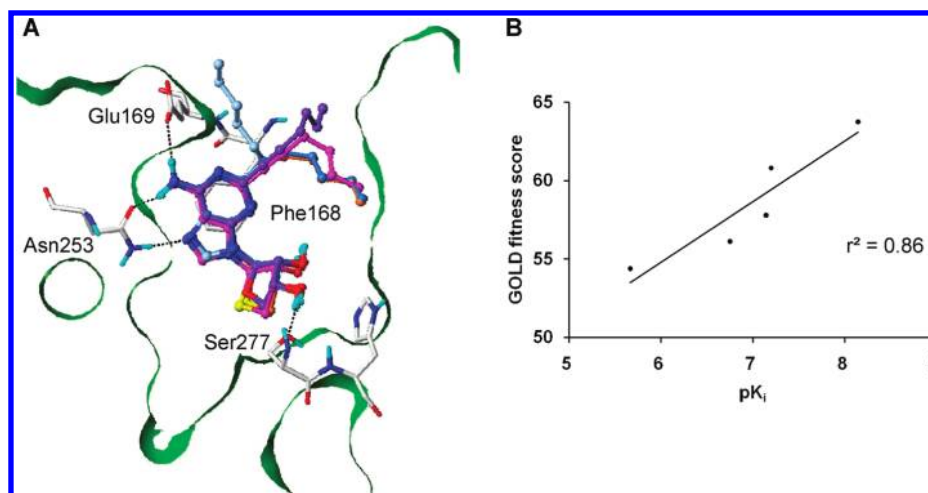
structure-activity relationships of this class of nucleosides as dual acting  $A_{2A}$  and  $A_3$ AR ligands. The corresponding 4'-thio- and 4'-oxonucleosides were prepared starting from D-mannose and D-erythronic  $\gamma$ -lactone, respectively. The highlight of our synthetic endeavor is the functionalization at the C2 position of 6-chloropurine nucleosides using lithiation-mediated stannyl transfer and palladium-catalyzed cross-coupling reactions. From the study, it was revealed that an unsubstituted amino group at the  $N^6$  position and a hydrophobic substituent at the C2 position were required for high binding affinity at the  $hA_{2A}$ AR, but hydrophobic substitution at the C8 position abolished binding affinity at the  $hA_{2A}$ AR. However, most of the synthesized compounds displayed medium to high binding affinity at the  $hA_3$ AR, regardless of the C2- or C8-substitution. Additionally, it was also found that the geometry of the C2-substituent is crucial for the binding affinity in order of hexynyl > hexenyl > hexanyl. From this study, compounds **4g** and **4m** were discovered as a preferred ligand to act dually at the  $hA_{2A}$ AR and  $hA_3$ AR with high affinity. This mixed activity as  $A_{2A}$ AR agonist/ $A_3$ AR antagonist might be advantageous for antiasthmatic activity. Moreover, these findings may facilitate the discrimination of the modes of binding of nucleoside derivatives at all subtypes of ARs.

## EXPERIMENTAL SECTION

**General Methods.**  $^1\text{H}$  NMR spectra ( $\text{CDCl}_3$ ,  $\text{CD}_3\text{OD}$  or  $\text{DMSO}-d_6$ ) were recorded on Varian Unity Inova 400 MHz instrument. The  $^1\text{H}$  NMR data are reported as peak multiplicities: s for singlet, d for doublet, dd for doublet of doublets, t for triplet, q for quartet, br s for broad singlet, and m for multiplet. Coupling constants are reported in hertz.  $^{13}\text{C}$  NMR spectra ( $\text{CDCl}_3$ ,  $\text{CD}_3\text{OD}$ , or  $\text{DMSO}-d_6$ ) were recorded on Varian Unity Inova 100 MHz instrument.  $^{19}\text{F}$  NMR spectra ( $\text{CDCl}_3$ ,  $\text{CD}_3\text{OD}$ ) were recorded on Varian Unity Inova 376 MHz instrument. The chemical shifts were reported as parts per million ( $\delta$ ) relative to the solvent peak. 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 measured on B-540 made by Buchi. Elemental analyses (C, H, and N) were used to determine the purity of all synthesized compounds, and the results were within  $\pm 0.4\%$  of the calculated values, confirming  $\geq 95\%$  purity. Reactions were checked with TLC (Merck precoated 60F<sub>254</sub> plates). Spots were detected by viewing under a UV light, colorizing with charring after dipping in anisaldehyde solution with acetic acid, sulfuric acid, and methanol. Column chromatography was performed on silica gel 60



**Figure 5.** Predicted binding modes of the C2-substituted derivative **4g** and its  $N^6$ -chlorobenzyl derivative **4i** in an  $A_{2A}AR$  crystal structure.<sup>23</sup> (A) Compound **4g** binds very well to the receptor, interacting with important residues at the binding site. (B) Compound **4i** shows various binding modes and does not maintain the key interactions. Compounds **4g** and **4i** are depicted in ball-and-stick with carbon atoms in magenta and gray, respectively. The key residues are displayed as capped-stick with carbon atoms in white, and the hydrogen bonds are marked in black dashed lines. The van der Waals surfaces of the ligands are colored by hydrogen bonding capability (red, H-bond donating region; blue, H-bond accepting region). The Fast Connolly surface of the protein is Z-clipped, and the nonpolar hydrogens are undisplayed for clarity.



**Figure 6.** Predicted binding modes of the five C2-substituted and  $N^6$ -unsubstituted derivatives in an  $hA_{2A}AR$  crystal structure<sup>23</sup> and the correlation between their experimental binding affinities and docking scores. (A) The adenine and sugar moieties of the molecules showed almost exactly the same binding modes maintaining the key interactions. Compounds **4a**, **4f**, **4g**, **4l**, and **4m** are depicted as ball-and-stick with carbon atoms in purple, light-blue, magenta, skyblue, and orange, respectively. (B) The scatter plot of the pK<sub>i</sub> values and GOLD fitness scores for the five compounds showed a good correlation with  $r^2$  of 0.86.



(230–400 mesh, Merck). Reagents were purchased from Aldrich Chemical Co. Solvents were obtained from local suppliers. All the anhydrous solvents were distilled over  $\text{CaH}_2$ ,  $\text{P}_2\text{O}_5$ , or sodium/benzophenone prior to the reaction.

**Synthesis.** (–)-6-Chloro-9-((3a*S*,6*R*,6a*S*)-tetrahydro-2,2-dimethylfuro[3,4-*d*][1,3]dioxol-6-yl)-9*H*-purine (**6**). 6-Chloropurine (0.618 g, 4.00 mmol), ammonium sulfate (0.079 g, 0.60 mmol), and hexamethyldisilazane (HMDS) (15 mL) were refluxed for 15 h under dry and inert conditions. The volatile was evaporated under high vacuum, and the resulting solid was dissolved in 1,2-dichloroethane (10 mL) and cooled at 0 °C. To this solution, a solution of **5**<sup>9</sup> (0.404 g, 2.00 mmol) in 1,2-dichloroethane (10 mL) and TMSOTf (0.72 mL, 4.00 mmol) were added dropwise, and the reaction mixture was stirred at 0 °C for 30 min at room temperature for 1 h and finally heated at 80 °C for 5 h. The mixture was cooled and diluted with  $\text{CH}_2\text{Cl}_2$ . The organic layer was washed with saturated  $\text{NaHCO}_3$  solution, dried over anhydrous  $\text{MgSO}_4$ , and filtered. The solvent was evaporated under reduced pressure. The crude syrup was subjected to flash silica gel column chromatography (hexane/EtOAc = 2:1) to give **6** (0.420 g, 71%) as a white solid: mp 118–119 °C; UV (MeOH)  $\lambda_{\text{max}}$  = 264 nm;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  8.73 (s, 1 H), 8.18 (s, 1 H), 6.10 (s, 1 H), 5.49 (d, 1 H,  $J$  = 6.0 Hz), 5.27–5.29 (m, 1 H), 4.25–4.31 (m, 2 H), 1.58 (s, 3 H), 1.41 (s, 3 H);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ )  $\delta$  152.3, 151.8, 151.4, 145.1, 132.5, 113.7, 92.2, 84.7, 81.5, 76.3, 26.6, 25.0;  $[\alpha]_D^{25}$  –50.69 (c 0.80, MeOH); (ESI+) ( $M + H^+$ )  $m/z$  297.0752. Anal. Calcd for  $\text{C}_{12}\text{H}_{13}\text{ClN}_4\text{O}_3$ : C, 48.58; H, 4.42; N, 18.88. Found: C, 48.59; H, 4.12; N, 18.08.

(–)-2-(Tributylstannyl)-6-chloro-9-((3a*S*,6*R*,6a*S*)-tetrahydro-2,2-dimethylfuro[3,4-*d*][1,3]dioxol-6-yl)-9*H*-purine (**7**). To a stirred solution of 2,2,6,6-tetramethylpiperidine (TMP, 10.7 mL, 63.53 mmol) in dry hexane (15 mL) and dry THF (30 mL) was added *n*-butyllithium (41.6 mL, 1.5 M solution in hexanes, 66.7 mmol) dropwise at –78 °C over 30 min, and the mixture was stirred at the same temperature for 1 h. To this mixture, a solution of **6** (3.77 g, 12.7 mmol) in dry THF (30 mL) was added dropwise, and the mixture was stirred at –78 °C for 30 min. *n*-Tributyltin chloride (17.23 mL, 63.53 mmol) was added dropwise to the dark reaction mixture, and the mixture was stirred at the same temperature for 1 h. The resulting dark solution was quenched by dropwise addition of a saturated aqueous  $\text{NH}_4\text{Cl}$  solution (50 mL). After the mixture was stirred at room temperature for 15 h, the mixture was diluted with  $\text{CH}_2\text{Cl}_2$  (100 mL). The organic layer was washed with saturated  $\text{NaHCO}_3$  solution, dried over anhydrous  $\text{MgSO}_4$ , and filtered. The solvent was evaporated under reduced pressure. The crude syrup was subjected to flash silica gel column chromatography (hexane/EtOAc = 5:1) to give **7** (7.10 g, 95%) as a colorless syrup: UV (MeOH)  $\lambda_{\text{max}}$  = 269 nm;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  8.06 (s, 1 H), 6.06 (s, 1 H), 5.58 (d, 1 H,  $J$  = 6.0 Hz), 5.30–5.32 (m, 1 H), 4.24–4.30 (m, 2 H), 1.55–1.66 (m, 9 H), 1.41 (s, 3 H), 1.27–1.38 (m, 7 H), 1.19–1.23 (m, 5 H), 0.86–0.92 (m, 9 H);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ )  $\delta$  182.4, 150.6, 150.1, 144.1, 131.1, 113.5, 91.9, 84.6, 81.9, 76.3, 29.3, 29.2, 29.1, 27.8, 27.5, 27.2, 26.6, 24.9, 13.9, 12.7, 12.6, 10.9, 9.3, 9.2;  $[\alpha]_D^{25}$  –8.18 (c 0.33, MeOH); (ESI+) ( $M + H^+$ )  $m/z$  587.1804. Anal. Calcd for  $\text{C}_{24}\text{H}_{39}\text{ClN}_4\text{O}_3\text{Sn}$ : C, 49.21; H, 6.71; N, 9.56. Found: C, 49.03; H, 6.66; N, 9.16.

(–)-6-Chloro-9-((3a*S*,6*R*,6a*S*)-tetrahydro-2,2-dimethylfuro[3,4-*d*][1,3]dioxol-6-yl)-2-iodo-9*H*-purine (**8**). To a stirred solution of **7** (7.10 g, 12.1 mmol) in dry THF (100 mL) was added iodine (4.60 g, 18.2 mmol), and the reaction mixture was stirred for 24 h under a nitrogen atmosphere. The mixture was diluted with saturated sodium metabisulfite, stirred for 1 h, and then extracted with  $\text{CH}_2\text{Cl}_2$  (3 × 30 mL). The organic layer was dried over anhydrous  $\text{MgSO}_4$  and filtered. The solvent was evaporated under reduced pressure. The crude syrup was subjected to flash silica gel column chromatography (hexane/EtOAc = 3:1) to give **8** (4.30 g, 84%) as a white foam: UV ( $\text{CH}_2\text{Cl}_2$ )  $\lambda_{\text{max}}$  = 282.5 nm;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  8.07 (s, 1 H), 6.05 (s, 1 H), 5.40 (d, 1 H,  $J$  = 6.0 Hz), 5.25–5.28 (m, 1 H), 4.27–4.28 (m, 2 H), 1.57 (s, 3 H), 1.42 (s, 3 H);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ )  $\delta$  152.1, 151.3, 145.1, 132.4, 116.8, 113.8, 92.1, 84.7, 81.5, 76.8, 26.6, 25.1;  $[\alpha]_D^{25}$  –21.88 (c 0.16, MeOH); (ESI+) ( $M + H^+$ )  $m/z$

422.9715. Anal. Calcd for  $\text{C}_{12}\text{H}_{12}\text{ClIN}_4\text{O}_3$ : C, 34.10; H, 2.86; N, 13.26. Found: C, 33.98; H, 2.77; N, 13.00.

(–)-(2*R*,3*S*,4*S*)-2-(6-Chloro-2-(hex-1-ynyl)-9*H*-purin-9-yl)-tetrahydrofuran-3,4-diol (**9**). To a solution of **8** (1.00 g, 2.37 mmol) in anhydrous DMF (20 mL) were added tetrakis-(triphenylphosphine)palladium (0.68 g, 0.59 mmol), copper iodide (0.054 g, 0.28 mmol), cesium carbonate (0.77 g, 2.37 mmol), and 1-hexyne (0.3 mL, 2.61 mmol) at room temperature, and the reaction mixture was stirred at room temperature for 3 h. The reaction mixture was evaporated, and the crude product was used in the next step without further purification. To a stirred ice-cooled solution of above crude product in THF (5 mL) was added 1 N HCl (5 mL), and the mixture was stirred at room temperature for 15 h, neutralized with 1 N NaOH solution, and then evaporated under reduced pressure. The mixture was subjected to flash silica gel column chromatography ( $\text{CH}_2\text{Cl}_2/\text{MeOH}$  = 30:1) to give **9** (0.42 g, 52%) as a white solid: mp 135–137 °C; UV (MeOH)  $\lambda_{\text{max}}$  = 281.0 nm;  $^1\text{H}$  NMR ( $\text{CD}_3\text{OD}$ )  $\delta$  8.70 (s, 1 H), 6.05 (d, 1 H,  $J$  = 6.4 Hz), 4.90–4.92 (m, 1 H), 4.55 (dd, 1 H,  $J$  = 4.0, 9.6 Hz), 4.43–4.45 (m, 1 H), 4.02 (dd, 1 H,  $J$  = 1.6, 9.6 Hz), 2.50 (t, 2 H,  $J$  = 7.2 Hz), 1.61–1.69 (m, 2 H), 1.50–1.58 (m, 2 H), 0.98 (t, 3 H,  $J$  = 7.2 Hz);  $^{13}\text{C}$  NMR ( $\text{CD}_3\text{OD}$ )  $\delta$  153.1, 151.1, 147.9, 147.2, 132.1, 91.5, 91.1, 80.5, 76.6, 75.7, 72.3, 31.4, 23.1, 19.5, 13.9;  $[\alpha]_D^{25}$  –65.83 (c 0.12, MeOH); (ESI+) ( $M + H^+$ )  $m/z$  337.1062. Anal. Calcd for  $\text{C}_{15}\text{H}_{17}\text{ClIN}_4\text{O}_3$ : C, 53.50; H, 5.09; N, 16.64. Found: C, 53.56; H, 5.01; N, 16.34.

(–)-6-Chloro-9-((3a*S*,4*R*,6a*R*)-2,2-dimethyltetrahydrothieno[3,4-*d*][1,3]dioxol-4-yl)-2-iodo-9*H*-purine (**11**). To a stirred solution of 2-iodo-6-chloropurine (1.90 g, 6.88 mmol) in dry  $\text{CH}_3\text{CN}$  (30 mL) was added *N*,*O*-bis(trimethylsilyl)acetamide (BSA, 2.24 mL, 9.16 mmol) under dry and inert conditions, and the reaction mixture was stirred at 40 °C for 45 min to obtain a clear solution. To this solution, a solution of **10**<sup>9</sup> (1.00 g, 4.58 mmol) in dry  $\text{CH}_3\text{CN}$  (5 mL) was added dropwise, followed by the addition of TMSOTf (0.91 mL) at room temperature, and the mixture was heated to 80 °C for 3 h. The mixture was cooled to room temperature, quenched with saturated  $\text{NaHCO}_3$  solution (40 mL), and diluted with EtOAc (50 mL), and then organic layers were separated. The aqueous layer was again extracted with EtOAc (3 × 30 mL). The combined organic layers were dried over anhydrous  $\text{MgSO}_4$  and filtered. The solvent was evaporated under reduced pressure. The residue was subjected to flash silica gel column chromatography (hexane/EtOAc = 3:1) to give compound **11** as a white foam (1.20 g, 60%): UV ( $\text{CH}_2\text{Cl}_2$ )  $\lambda_{\text{max}}$  = 282.0 nm;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  8.06 (s, 1 H), 5.84 (s, 1 H), 5.33 (t, 1 H,  $J$  = 4.8 Hz), 5.21 (d, 1 H,  $J$  = 5.6 Hz), 3.80 (dd, 1 H,  $J$  = 4.4, 12.8 Hz), 3.25 (d, 1 H,  $J$  = 12.8 Hz), 1.59 (s, 3 H), 1.37 (s, 3 H);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ )  $\delta$  151.9, 151.2, 144.4, 132.7, 116.7, 112.1, 90.0, 84.8, 70.7, 41.5, 26.6, 24.8;  $[\alpha]_D^{25}$  –66.33 (c 0.10,  $\text{CH}_2\text{Cl}_2$ ); (ESI+) ( $M + H^+$ )  $m/z$  438.9486. Anal. Calcd for  $\text{C}_{12}\text{H}_{12}\text{ClIN}_4\text{O}_3\text{S}$ : C, 32.86; H, 2.76; N, 12.77; S, 7.31. Found: C, 32.89; H, 2.89; N, 12.47; S, 7.01.

(–)-6-Chloro-9-((3a*S*,4*R*,6a*R*)-2,2-dimethyltetrahydrothieno[3,4-*d*][1,3]dioxol-4-yl)-2-(hex-1-ynyl)-9*H*-purine (**12**). Compound **11** (0.60 g, 1.37 mmol) was converted to **12** (0.46 g, 86%) as a thick syrupy liquid, using the same Sonogashira conditions used in the preparation of **9**: UV (MeOH)  $\lambda_{\text{max}}$  = 285.0 nm;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  8.16 (s, 1 H), 5.90 (s, 1 H), 5.32 (t, 1 H,  $J$  = 4.8 Hz), 5.21 (d, 1 H,  $J$  = 5.2 Hz), 3.79 (dd, 1 H,  $J$  = 4.4, 12.8 Hz), 3.23 (d, 1 H,  $J$  = 12.8 Hz), 2.48 (t, 2 H,  $J$  = 7.2 Hz), 1.61–1.70 (m, 2 H), 1.58 (s, 3 H), 1.47–1.54 (m, 2 H), 1.35 (s, 3 H), 0.95 (t, 3 H,  $J$  = 7.2 Hz);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ )  $\delta$  151.3, 151.2, 146.3, 144.7, 130.8, 112.1, 91.3, 89.9, 843.7, 79.7, 70.2, 41.2, 30.3, 26.6, 24.8, 22.3, 19.3, 13.8;  $[\alpha]_D^{25}$  –57.35 (c 0.469, MeOH); (ESI+) ( $M + H^+$ )  $m/z$  393.1145. Anal. Calcd for  $\text{C}_{18}\text{H}_{21}\text{ClIN}_4\text{O}_3\text{S}$ : C, 55.02; H, 5.39; N, 14.26; S, 8.16. Found: C, 55.41; H, 5.21; N, 14.47; S, 8.11.

(–)-(2*R*,3*S*,4*R*)-2-(6-Chloro-2-(hex-1-ynyl)-9*H*-purin-9-yl)-tetrahydrothiophene-3,4-diol (**13**). Compound **12** (0.92 g, 2.34 mmol) was converted to **13** (0.58 g, 70%) as a white solid, using the same hydrolysis conditions used in the preparation of **9**: mp 76–78 °C; UV (MeOH)  $\lambda_{\text{max}}$  = 284.5 nm;  $^1\text{H}$  NMR ( $\text{CD}_3\text{OD}$ )  $\delta$  8.87 (s, 1 H), 6.11 (d, 1 H,  $J$  = 6.4 Hz), 4.69 (dd, 1 H,  $J$  = 3.2, 6.4 Hz), 4.48 (dd, 1 H,  $J$  = 3.6, 7.6 Hz), 3.56 (dd, 1 H,  $J$  = 4.4, 10.8 Hz), 2.97 (dd, 1 H,

$J = 3.6, 10.8 \text{ Hz}$ ), 2.51 (t, 2 H,  $J = 6.8 \text{ Hz}$ ), 1.61–1.67 (m, 2 H), 1.50–1.56 (m, 2 H), 0.98 (t, 3 H,  $J = 7.2 \text{ Hz}$ );  $^{13}\text{C}$  NMR ( $\text{CD}_3\text{OD}$ )  $\delta$  153.6, 151.0, 148.0, 147.1, 131.9, 91.5, 80.9, 80.5, 74.4, 64.6, 35.5, 31.4, 23.1, 19.5, 14.0;  $[\alpha]^{25}_{\text{D}} -32.49$  (c 0.634, MeOH); (ESI+) ( $\text{M} + \text{H}^+$ )  $m/z$  353.0835. Anal. Calcd for  $\text{C}_{15}\text{H}_{17}\text{ClN}_4\text{O}_2\text{S}$ : C, 51.06; H, 4.86; N, 15.88; S, 9.09. Found: C, 51.09; H, 4.89; N, 15.47; S, 9.21.

(–)-9-((3aS,6R,6aS)-Tetrahydro-2,2-dimethylfuro[3,4-*d*][1,3]-dioxol-6-yl)-2-iodo-9H-purin-6-amine (14). A solution of 8 (0.303 g, 0.72 mmol) in methanolic ammonia (5 mL) was stirred for 2 h at 80 °C. The reaction mixture was evaporated and the crude residue was subjected to flash silica gel column chromatography (hexane/EtOAc = 1:1) to give 14 (0.205 g, 71%) as a colorless syrup: UV ( $\text{CH}_2\text{Cl}_2$ )  $\lambda_{\text{max}} = 262.0 \text{ nm}$ ;  $^1\text{H}$  NMR ( $\text{DMSO}-d_6$ )  $\delta$  8.16 (s, 1 H), 7.74 (br s, 2 H,  $\text{D}_2\text{O}$  exchangeable), 6.11 (s, 1 H), 5.27 (d, 1 H,  $J = 5.6 \text{ Hz}$ ), 5.13–5.15 (m, 1 H), 4.06–4.12 (m, 2 H), 1.47 (s, 3 H), 1.33 (s, 3 H);  $^{13}\text{C}$  NMR ( $\text{DMSO}-d_6$ )  $\delta$  155.9, 149.3, 139.8, 120.9, 118.9, 112.0, 89.2, 84.0, 80.7, 74.5, 26.2, 24.6;  $[\alpha]^{25}_{\text{D}} -13.25$  (c 0.08,  $\text{CH}_2\text{Cl}_2$ ); (ESI+) ( $\text{M} + \text{H}^+$ )  $m/z$  404.0225. Anal. Calcd for  $\text{C}_{12}\text{H}_{14}\text{IN}_5\text{O}_3$ : C, 35.75; H, 3.50; N, 17.37. Found: C, 35.89; H, 3.89; N, 17.47.

(–)-9-((3aS,4R,6aR)-Tetrahydro-2,2-dimethylthieno[3,4-*d*]-[1,3]dioxol-4-yl)-2-iodo-9H-purin-6-amine (15). Compound 11 (0.535 g, 1.22 mmol) was converted to 15 (0.439 g, 85%) as a colorless syrup, according to the same procedure used in the preparation of 14: UV ( $\text{CH}_2\text{Cl}_2$ )  $\lambda_{\text{max}} = 267.0 \text{ nm}$ ;  $^1\text{H}$  NMR ( $\text{CD}_3\text{OD}$ )  $\delta$  8.20 (s, 1 H), 5.97 (s, 1 H), 5.30 (pseudo t, 1 H,  $J = 5.2 \text{ Hz}$ ), 5.23 (d, 1 H,  $J = 5.6 \text{ Hz}$ ), 3.80 (dd, 1 H,  $J = 4.4, 12.8 \text{ Hz}$ ), 3.14 (d, 1 H,  $J = 12.8 \text{ Hz}$ ), 1.54 (s, 3 H), 1.35 (s, 3 H);  $^{13}\text{C}$  NMR ( $\text{CD}_3\text{OD}$ )  $\delta$  156.1, 150.4, 141.9, 120.0, 118.0, 112.7, 91.2, 86.4, 71.3, 41.6, 26.8, 24.9;  $[\alpha]^{25}_{\text{D}} -61.90$  (c 0.08,  $\text{CH}_2\text{Cl}_2$ ); (ESI+) ( $\text{M} + \text{H}^+$ )  $m/z$  419.998. Anal. Calcd for  $\text{C}_{12}\text{H}_{14}\text{IN}_5\text{O}_2\text{S}$ : C, 34.38; H, 3.37; N, 16.70; O, 7.63; S, 7.65. Found: C, 34.11; H, 3.22; N, 16.40; S, 7.45.

(–)-9-((3aS,6R,6aS)-Tetrahydro-2,2-dimethylfuro[3,4-*d*][1,3]-dioxol-6-yl)-9H-purin-6-amine (18). A solution of 6 (0.37 g, 1.25 mmol) in methanolic ammonia (5 mL) was heated for 2 h at 80 °C. The reaction mixture was evaporated and the crude residue was subjected to flash silica gel column chromatography (hexane/EtOAc = 1:1) to give 18 (0.27 g, 78%) as a white foam: UV ( $\text{CH}_2\text{Cl}_2$ )  $\lambda_{\text{max}} = 255 \text{ nm}$ ;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  8.32 (s, 1 H), 7.89 (s, 1 H), 6.19 (brs, 2 H), 6.03 (s, 1 H), 5.48 (d, 1 H,  $J = 6.0 \text{ Hz}$ ), 5.27 (dd, 1 H,  $J = 3.2, 5.6 \text{ Hz}$ ), 4.24–4.31 (m, 2 H), 1.58 (s, 3 H), 1.41 (s, 3 H);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ )  $\delta$  154.7, 151.3, 149.5, 141.1, 120.3, 113.5, 91.9, 84.8, 81.7, 76.1, 26.6, 25.1;  $[\alpha]^{25}_{\text{D}} -35.48$  (c 0.09,  $\text{CH}_2\text{Cl}_2$ ); (ESI+) ( $\text{M} + \text{H}^+$ )  $m/z$  278.1257. Anal. Calcd for  $\text{C}_{12}\text{H}_{13}\text{N}_5\text{O}_3$ : C, 51.98; H, 5.45; N, 25.26. Found: C, 51.98; H, 5.87; N, 25.47.

(–)-8-Bromo-9-((3aS,6R,6aS)-tetrahydro-2,2-dimethylfuro[3,4-*d*][1,3]dioxol-6-yl)-9H-purin-6-amine (19). To a solution of 18 (89 mg, 0.32 mmol) in MeOH (10 mL) and 1 N sodium acetate (1.7 mL) was added bromine (0.033 mL, 0.64 mmol), and the mixture was stirred at room temperature for 40 min. The mixture was diluted with saturated sodium metabisulfite solution and stirred until the red color disappeared. The volatile was evaporated, and the resulting aqueous layer was extracted with EtOAc (3 × 30 mL). The combined organic layers were washed with water, dried over anhydrous  $\text{MgSO}_4$ , and filtered. The solvent was evaporated to give a pale yellowish residue. The residue was purified by flash silica gel column chromatography (hexane/EtOAc = 2:1) to give 19 (63 mg, 55%) as a white foam: UV ( $\text{CH}_2\text{Cl}_2$ )  $\lambda_{\text{max}} = 262 \text{ nm}$ ;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  8.26 (s, 1 H), 6.20 (brs, 2 H), 6.16 (s, 1 H), 5.61 (d, 1 H,  $J = 5.6 \text{ Hz}$ ), 5.36–5.38 (m, 1 H), 4.20–4.21 (m, 2 H), 1.59 (s, 3 H), 1.42 (s, 3 H);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ )  $\delta$  153.8, 151.9, 150.9, 128.8, 120.0, 113.2, 92.4, 84.3, 82.2, 76.5, 26.6, 24.9;  $[\alpha]^{25}_{\text{D}} -28.08$  (c 0.26,  $\text{CH}_2\text{Cl}_2$ ); (ESI+) ( $\text{M} + \text{H}^+$ )  $m/z$  356.0365. Anal. Calcd for  $\text{C}_{12}\text{H}_{14}\text{BrN}_5\text{O}_3$ : C, 40.47; H, 3.96; N, 19.66. Found: C, 40.12; H, 3.88; N, 19.48.

(–)-8-Bromo-9-((3aR,6R,6aS)-tetrahydro-2,2-dimethylthieno[3,4-*d*][1,3]dioxol-6-yl)-9H-purin-2-amine (20). 8-Bromoadenine (0.40 g, 1.84 mmol) and 10 (0.20 g, 0.92 mmol) were condensed to give 20 (69 mg, 20%) as colorless syrup, according to similar procedure used in the preparation of 6: UV (MeOH)  $\lambda_{\text{max}} = 263.5 \text{ nm}$ ;  $^1\text{H}$  NMR ( $\text{CDCl}_3$ )  $\delta$  8.25 (s, 1 H), 5.91 (s, 1 H), 5.81 (brs, 2 H,  $\text{NH}_2$ ), 5.54 (d, 1 H,  $J = 5.2 \text{ Hz}$ ), 5.50 (pseudo t, 1 H,  $J = 5.2 \text{ Hz}$ ),

3.87 (dd, 1 H,  $J = 4.0, 12.4 \text{ Hz}$ ), 3.14 (d, 1 H,  $J = 12.4 \text{ Hz}$ ), 1.60 (s, 3 H), 1.38 (s, 3 H);  $^{13}\text{C}$  NMR ( $\text{CDCl}_3$ )  $\delta$  154.3, 153.0, 150.7, 127.8, 120.4, 111.4, 89.0, 86.0, 71.9, 41.4, 26.6, 24.7;  $[\alpha]^{25}_{\text{D}} -77.07$  (c 0.16,  $\text{CH}_2\text{Cl}_2$ ); (ESI+) ( $\text{M} + \text{H}^+$ )  $m/z$  373.0151. Anal. Calcd for  $\text{C}_{12}\text{H}_{14}\text{BrN}_5\text{O}_2\text{S}$ : C, 38.72; H, 3.79; N, 18.81; S, 8.61. Found: C, 38.88; H, 3.88; N, 18.48; S, 8.41.

(–)-(2R,3S,4S)-2-(6-Amino-2-(hex-1-ynyl)-9H-purine-9-yl)-tetrahydrofuran-3,4-diol (4a). A solution of 9 (0.125 g, 0.42 mmol) in  $\text{NH}_3/\text{BuOH}$  (5 mL) was stirred at 100 °C for 8 h. The reaction mixture was evaporated and the residue was subjected to flash silica gel column chromatography ( $\text{CH}_2\text{Cl}_2/\text{MeOH} = 20:1$ ) to give 4a (0.103 g, 87%) as a white solid: mp 164–166 °C; UV (MeOH)  $\lambda_{\text{max}} = 269.5 \text{ nm}$ ;  $^1\text{H}$  NMR ( $\text{CD}_3\text{OD}$ )  $\delta$  8.26 (s, 1 H), 5.94 (d, 1 H,  $J = 6.4 \text{ Hz}$ ), 4.86–4.87 (m, 1H), 4.51 (dd, 1 H,  $J = 4.0, 9.6 \text{ Hz}$ ), 4.40–4.42 (m, 1 H), 3.98 (dd, 1 H,  $J = 2.0, 9.6 \text{ Hz}$ ), 2.45 (t, 2 H,  $J = 7.2 \text{ Hz}$ ), 1.61–1.65 (m, 2 H), 1.49–1.55 (m, 2 H), 0.98 (t, 3 H,  $J = 7.6 \text{ Hz}$ );  $^{13}\text{C}$  NMR ( $\text{CD}_3\text{OD}$ )  $\delta$  157.2, 151.1, 148.1, 142.3, 120.1, 99.4, 88.4, 81.4, 76.6, 75.4, 72.3, 31.7, 23.2, 19.6, 14.1;  $[\alpha]^{25}_{\text{D}} -39.50$  (c 0.16, MeOH); (ESI+) ( $\text{M} + \text{H}^+$ )  $m/z$  318.1557. Anal. Calcd for  $\text{C}_{15}\text{H}_{19}\text{N}_5\text{O}_3$ : C, 56.77; H, 6.03; N, 22.07. Found: C, 56.98; H, 5.88; N, 22.01.

**General Procedure for the Synthesis of 4b–e.** To a solution of diol 9 in EtOH (5 mL) were added  $\text{Et}_3\text{N}$  (3 equiv) and 3-halobenzylamine (1.5 equiv) at room temperature, and the mixture was stirred at room temperature for 24–48 h. The solvent was evaporated and the residue was purified by a flash silica gel column chromatography ( $\text{CH}_2\text{Cl}_2/\text{MeOH} = 20:1$ ) to give 4b–e as white solids.

(–)-(2R,3S,4S)-2-(6-(3-Fluorobenzylamino)-2-(hex-1-ynyl)-9H-purin-9-yl)tetrahydrofuran-3,4-diol (4b). Yield, 78%; mp 206–207 °C; UV (MeOH)  $\lambda_{\text{max}} = 272.0 \text{ nm}$ ;  $^1\text{H}$  NMR ( $\text{DMSO}-d_6$ )  $\delta$  8.47 (brs, 1 H,  $\text{D}_2\text{O}$  exchangeable), 8.44 (s, 1 H), 7.32–7.37 (m, 1 H), 7.12–7.17 (m, 2 H), 7.02–7.07 (m, 1 H), 5.85 (d, 1 H,  $J = 6.8 \text{ Hz}$ ), 5.45 (d, 1 H,  $J = 6.4 \text{ Hz}$ ,  $\text{D}_2\text{O}$  exchangeable), 5.20 (d, 1 H,  $J = 4.0 \text{ Hz}$ ,  $\text{D}_2\text{O}$  exchangeable), 4.69–4.75 (m, 3 H), 4.33 (dd, 1 H,  $J = 3.6, 9.2 \text{ Hz}$ ), 4.24–4.25 (m, 1 H), 3.79 (dd, 1 H,  $J = 1.6, 9.2 \text{ Hz}$ ), 2.41 (t, 2 H,  $J = 7.2 \text{ Hz}$ ), 1.49–1.56 (m, 2 H), 1.38–1.47 (m, 2 H), 0.91 (t, 3 H,  $J = 7.2 \text{ Hz}$ );  $^{13}\text{C}$  NMR ( $\text{DMSO}-d_6$ )  $\delta$  163.4, 161.0, 154.0, 149.1, 145.8, 142.9, 140.6, 130.2 (d), 123.1, 119.0, 113.6 (q), 87.1, 85.6, 81.6, 74.4, 73.5, 70.2, 42.4, 29.8, 21.5, 17.9, 13.4;  $[\alpha]^{25}_{\text{D}} -58.87$  (c 0.23, MeOH); (ESI+) ( $\text{M} + \text{H}^+$ )  $m/z$  426.1948. Anal. Calcd for  $\text{C}_{22}\text{H}_{24}\text{FN}_5\text{O}_3$ : C, 62.11; H, 5.69; N, 16.46. Found: C, 61.98; H, 5.89; N, 16.23.

(–)-(2R,3S,4S)-2-(6-(3-Chlorobenzylamino)-2-(hex-1-ynyl)-9H-purin-9-yl)tetrahydrofuran-3,4-diol (4c). Yield, 78%; mp 210–212 °C; UV (MeOH)  $\lambda_{\text{max}} = 271.5 \text{ nm}$ ;  $^1\text{H}$  NMR ( $\text{DMSO}-d_6$ )  $\delta$  8.47 (brs, 1 H,  $\text{D}_2\text{O}$  exchangeable), 8.44 (s, 1 H), 7.27–7.39 (m, 4 H), 5.84 (d, 1 H,  $J = 6.8 \text{ Hz}$ ), 5.45 (d, 1 H,  $J = 6.4 \text{ Hz}$ ,  $\text{D}_2\text{O}$  exchangeable), 5.20 (d, 1 H,  $J = 4.0 \text{ Hz}$ ,  $\text{D}_2\text{O}$  exchangeable), 4.67–4.75 (m, 3 H), 4.33 (dd, 1 H,  $J = 3.6, 9.2 \text{ Hz}$ ), 4.24–4.25 (m, 1 H), 3.79 (dd, 1 H,  $J = 1.6, 9.2 \text{ Hz}$ ), 2.41 (t, 2 H,  $J = 6.8 \text{ Hz}$ ), 1.49–1.57 (m, 2 H), 1.38–1.47 (m, 2 H), 0.91 (t, 3 H,  $J = 7.2 \text{ Hz}$ );  $^{13}\text{C}$  NMR ( $\text{DMSO}-d_6$ )  $\delta$  154.0, 149.2, 145.8, 142.5, 140.6, 132.9, 130.1, 128.3, 127.0, 126.6, 125.8, 87.1, 85.7, 81.6, 74.4, 73.5, 70.2, 42.3, 29.8, 21.5, 17.9, 13.4;  $[\alpha]^{25}_{\text{D}} -57.46$  (c 0.13, MeOH); (ESI+) ( $\text{M} + \text{H}^+$ )  $m/z$  442.1647. Anal. Calcd for  $\text{C}_{22}\text{H}_{24}\text{ClN}_5\text{O}_3$ : C, 59.79; H, 5.47; N, 15.85. Found: C, 59.99; H, 5.55; N, 15.90.

(–)-(2R,3S,4S)-2-(6-(3-Bromobenzylamino)-2-(hex-1-ynyl)-9H-purin-9-yl)-tetrahydrofuran-3,4-diol (4d). Yield, 73%; mp 217–218 °C; UV (MeOH)  $\lambda_{\text{max}} = 273.0 \text{ nm}$ ;  $^1\text{H}$  NMR ( $\text{DMSO}-d_6$ )  $\delta$  8.47 (brs, 1 H,  $\text{D}_2\text{O}$  exchangeable), 8.44 (s, 1 H), 7.54 (s, 1 H), 7.42 (d, 1 H,  $J = 7.6 \text{ Hz}$ ), 7.25–7.34 (m, 2 H), 5.84 (d, 1 H,  $J = 6.4 \text{ Hz}$ ), 5.45 (d, 1 H,  $J = 6.4 \text{ Hz}$ ,  $\text{D}_2\text{O}$  exchangeable), 5.20 (d, 1 H,  $J = 4.0 \text{ Hz}$ ,  $\text{D}_2\text{O}$  exchangeable), 4.67–4.74 (m, 3 H), 4.32 (dd, 1 H,  $J = 3.6, 9.2 \text{ Hz}$ ), 4.24–4.25 (m, 1 H), 3.79 (dd, 1 H,  $J = 1.6, 9.2 \text{ Hz}$ ), 2.41 (t, 2 H,  $J = 7.2 \text{ Hz}$ ), 1.49–1.56 (m, 2 H), 1.38–1.47 (m, 2 H), 0.91 (t, 3 H,  $J = 7.2 \text{ Hz}$ );  $^{13}\text{C}$  NMR ( $\text{DMSO}-d_6$ )  $\delta$  154.0, 145.8, 142.8, 140.6, 130.5, 130.0, 129.6, 126.3, 121.5, 87.1, 85.7, 81.6, 78.8; 74.4, 73.5, 70.2, 42.3, 29.9, 21.5, 17.9, 13.4;  $[\alpha]^{25}_{\text{D}} -49.63$  (c 0.14, MeOH); (ESI+) ( $\text{M} + \text{H}^+$ )  $m/z$  486.1136. Anal. Calcd for  $\text{C}_{22}\text{H}_{24}\text{BrN}_5\text{O}_3$ : C, 54.33; H, 4.97; N, 14.40. Found: C, 54.23; H, 5.01; N, 14.21.



(-)-(2*R*,3*S*,4*S*)-2-(6-(3-Iodobenzylamino)-2-(hex-1-ynyl)-9*H*-purin-9-yl)tetrahydrofuran-3,4-diol (**4e**). Yield, 85%; mp 224–226 °C; UV (MeOH)  $\lambda_{\text{max}}$  = 273.0 nm;  $^1\text{H}$  NMR (DMSO- $d_6$ )  $\delta$  8.47 (brs, 1 H, D<sub>2</sub>O exchangeable), 8.43 (s, 1 H), 7.73 (pseudo t, 1 H,  $J$  = 1.6 Hz), 7.59 (d, 1 H,  $J$  = 7.6 Hz), 7.34 (d, 1 H,  $J$  = 8.0 Hz), 7.11 (pseudo t, 1 H,  $J$  = 8.0 Hz), 5.84 (d, 1 H,  $J$  = 6.4 Hz), 5.44 (brs, 1 H, D<sub>2</sub>O exchangeable), 5.19 (d, 1 H,  $J$  = 2.8 Hz, D<sub>2</sub>O exchangeable), 4.63–4.72 (m, 3 H), 4.33 (dd, 1 H,  $J$  = 3.6, 9.2 Hz), 4.25 (brs, 1 H), 3.79 (dd, 1 H,  $J$  = 1.6, 9.2 Hz), 2.41 (t, 2 H,  $J$  = 7.2 Hz), 1.50–1.57 (m, 2 H), 1.38–1.47 (m, 2 H), 0.91 (t, 3 H,  $J$  = 7.2 Hz);  $^{13}\text{C}$  NMR (DMSO- $d_6$ )  $\delta$  153.9, 149.1, 145.8, 142.6, 140.6, 135.9, 135.4, 130.5, 126.6, 118.9, 94.7, 87.1, 85.7, 81.6, 74.4, 73.5, 70.2, 42.2, 29.8, 21.5, 17.9, 13.4;  $[\alpha]_{\text{D}}^{25}$  –58.09 (c 0.14, MeOH); (ESI+) ( $\text{M} + \text{H}^+$ )  $m/z$  534.0998. Anal. Calcd for C<sub>22</sub>H<sub>24</sub>IN<sub>5</sub>O<sub>3</sub>: C, 49.54; H, 4.54; N, 13.13. Found: C, 49.14; H, 4.13; N, 13.01.

(-)-(2*R*,3*S*,4*S*)-2-(6-Amino-2-hexyl-9*H*-purine-9-yl)-tetrahydrofuran-3,4-diol (**4f**). A mixture of **4a** (22 mg, 0.069 mmol), absolute ethanol (5 mL), and 10% palladium on carbon (5 mg) was hydrogenated on a Parr apparatus at 40 psi for 15 h. The mixture was filtered and the solvent was evaporated to give a residue. The residue was subjected to flash silica gel column chromatography (CH<sub>2</sub>Cl<sub>2</sub>/MeOH = 20:1) to give **4f** (15 mg, 68%) as a white solid: mp 105–107 °C; UV (MeOH)  $\lambda_{\text{max}}$  = 261.5 nm;  $^1\text{H}$  NMR (CD<sub>3</sub>OD)  $\delta$  8.19 (s, 1 H), 5.95 (d, 1 H,  $J$  = 6.4 Hz), 4.93–4.96 (m, 1 H), 4.52 (dd, 1 H,  $J$  = 4.0, 9.6 Hz), 4.42–4.44 (m, 1 H), 3.99 (dd, 1 H,  $J$  = 1.6, 9.6 Hz), 2.74 (t, 2 H,  $J$  = 7.6 Hz), 1.74–1.80 (m, 2 H), 1.30–1.39 (m, 6 H), 0.88–0.92 (m, 3 H);  $^{13}\text{C}$  NMR (CD<sub>3</sub>OD)  $\delta$  167.2, 157.1, 151.8, 141.5, 119.0, 90.5, 76.6, 75.5, 72.5, 40.0, 33.0, 30.3, 30.0, 23.8, 14.6;  $[\alpha]_{\text{D}}^{25}$  –54.05 (c 0.11, MeOH); (ESI+) ( $\text{M} + \text{H}^+$ )  $m/z$  322.1883. Anal. Calcd for C<sub>15</sub>H<sub>23</sub>N<sub>5</sub>O<sub>3</sub>: C, 56.06; H, 7.21; N, 21.79. Found: C, 56.44; H, 7.02; N, 21.49.

(-)-(2*R*,3*S*,4*R*)-2-(6-Amino-2-(hex-1-ynyl)-9*H*-purin-9-yl)-tetrahydrothiophene-3,4-diol (**4g**). Compound **13** (0.065 g, 0.18 mmol) was converted to **4g** (0.051 g, 83%) as a white solid, using the same procedure in the preparation of **4a**: mp 234–235; UV (MeOH)  $\lambda_{\text{max}}$  = 271.5 nm;  $^1\text{H}$  NMR (DMSO- $d_6$ )  $\delta$  8.47 (s, 1 H), 7.35 (br s, 2 H, D<sub>2</sub>O exchangeable), 5.85 (d, 1 H,  $J$  = 7.2 Hz), 5.53 (d, 1 H,  $J$  = 6.4 Hz, D<sub>2</sub>O exchangeable), 5.34 (d, 1 H,  $J$  = 4.0 Hz, D<sub>2</sub>O exchangeable), 4.57–4.61 (m, 1 H), 4.33–4.35 (m, 1 H), 3.40 (dd, 1 H,  $J$  = 4.4, 10.8 Hz), 2.80 (dd, 1 H,  $J$  = 2.8, 10.8 Hz), 2.41 (t, 2 H,  $J$  = 6.8 Hz), 1.49–1.55 (m, 2 H), 1.40–1.46 (m, 2 H), 0.91 (t, 3 H,  $J$  = 7.2 Hz);  $^{13}\text{C}$  NMR (DMSO- $d_6$ )  $\delta$  155.7, 149.9, 145.7, 140.4, 118.3, 85.4, 81.3, 78.5, 72.2, 61.1, 34.3, 29.9, 21.5, 17.9, 13.4;  $[\alpha]_{\text{D}}^{25}$  –28.36 (c 0.20, MeOH); (ESI+) ( $\text{M} + \text{H}^+$ )  $m/z$  334.1336. Anal. Calcd for C<sub>15</sub>H<sub>19</sub>N<sub>5</sub>O<sub>2</sub>S: C, 54.04; H, 5.74; N, 21.01; S, 9.62. Found: C, 54.44; H, 5.89; N, 21.21; S, 9.41.

**General Procedure for the Synthesis of 4h–k.** To a solution of diol **13** in EtOH (20 mL) were added Et<sub>3</sub>N (3 equiv) and 3-halobenzylamine (1.5 equiv) at room temperature, and the mixture was stirred at room temperature for 48 h. The solvent was evaporated and the residue was purified by a flash silica gel column chromatography (CH<sub>2</sub>Cl<sub>2</sub>/MeOH = 20:1) to give **4h–k** as white solids.

(-)-(2*R*,3*S*,4*R*)-2-(6-(3-Fluorobenzylamino)-2-(hex-1-ynyl)-9*H*-purin-9-yl)tetrahydrothiophene-3,4-diol (**4h**). Yield, 79%; mp 200–202 °C; UV (MeOH)  $\lambda_{\text{max}}$  = 274.0 nm;  $^1\text{H}$  NMR (DMSO- $d_6$ )  $\delta$  8.52 (s, 1 H), 8.45 (br s, 1 H, D<sub>2</sub>O exchangeable), 7.32–7.37 (m, 1 H), 7.12–7.17 (m, 2 H), 7.02–7.07 (m, 1 H), 5.87 (d, 1 H,  $J$  = 7.2 Hz), 5.54 (d, 1 H,  $J$  = 6.0 Hz, D<sub>2</sub>O exchangeable), 5.36 (d, 1 H,  $J$  = 4.4 Hz, D<sub>2</sub>O exchangeable), 4.68 (br s, 2 H), 4.58–4.62 (m, 1 H), 4.34–4.35 (m, 1 H), 3.41 (dd, 1 H,  $J$  = 4.4, 10.8 Hz), 2.80 (dd, 1 H,  $J$  = 2.8, 10.8 Hz), 2.40 (t, 2 H,  $J$  = 7.2 Hz), 1.49–1.55 (m, 2 H), 1.39–1.47 (m, 2 H), 0.91 (t, 3 H,  $J$  = 7.2 Hz);  $^{13}\text{C}$  NMR (DMSO- $d_6$ )  $\delta$  163.4, 160.9, 154.0, 149.4, 145.6, 143.0, 140.5, 130.2 (d), 123.1, 118.6, 113.6 (q), 85.7, 81.5, 78.6, 72.2, 61.1, 42.4, 34.4, 29.8, 21.5, 17.9, 13.4;  $[\alpha]_{\text{D}}^{25}$  –48.91 (c 0.184, MeOH); (ESI+) ( $\text{M} + \text{H}^+$ )  $m/z$  442.1708. Anal. Calcd for C<sub>22</sub>H<sub>24</sub>FN<sub>5</sub>O<sub>2</sub>S: C, 59.85; H, 5.48; N, 15.86; S, 7.26. Found: C, 59.84; H, 5.88; N, 15.47; S, 7.21.

(-)-(2*R*,3*S*,4*R*)-2-(6-(3-Chlorobenzylamino)-2-(hex-1-ynyl)-9*H*-purin-9-yl)tetrahydrothiophene-3,4-diol (**4i**). Yield, 72%; mp

199–201 °C; UV (MeOH)  $\lambda_{\text{max}}$  = 275.0 nm;  $^1\text{H}$  NMR (DMSO- $d_6$ )  $\delta$  8.52 (s, 1 H), 8.46 (br s, 1 H, D<sub>2</sub>O exchangeable), 7.27–7.39 (m, 4 H), 5.87 (d, 1 H,  $J$  = 7.6 Hz), 5.54 (d, 1 H,  $J$  = 6.4 Hz, D<sub>2</sub>O exchangeable), 5.35 (d, 1 H,  $J$  = 4.4 Hz, D<sub>2</sub>O exchangeable), 4.58–4.67 (m, 3 H), 4.33–4.35 (m, 1 H), 3.41 (dd, 1 H,  $J$  = 4.0, 10.8 Hz), 2.80 (dd, 1 H,  $J$  = 3.2, 10.8 Hz), 2.41 (t, 2 H,  $J$  = 7.2 Hz), 1.50–1.57 (m, 2 H), 1.38–1.47 (m, 2 H), 0.91 (t, 3 H,  $J$  = 7.2 Hz);  $^{13}\text{C}$  NMR (DMSO- $d_6$ )  $\delta$  153.9, 149.4, 145.7, 142.5, 140.5, 132.9, 130.1, 127.0, 126.6, 125.8, 118.7, 85.7, 81.6, 78.6, 72.2, 61.1, 42.3, 34.4, 29.8, 21.5, 17.9, 13.4;  $[\alpha]_{\text{D}}^{25}$  –46.67 (c 0.30, MeOH); (ESI+) ( $\text{M} + \text{H}^+$ )  $m/z$  458.1415. Anal. Calcd for C<sub>22</sub>H<sub>24</sub>ClN<sub>5</sub>O<sub>2</sub>S: C, 57.70; H, 5.28; N, 15.29; S, 7.00. Found: C, 57.41; H, 5.68; N, 15.47; S, 7.00.

(-)-(2*R*,3*S*,4*R*)-2-(6-(3-Bromobenzylamino)-2-(hex-1-ynyl)-9*H*-purin-9-yl)tetrahydrothiophene-3,4-diol (**4j**). Yield, 81%; mp 206–208 °C; UV (MeOH)  $\lambda_{\text{max}}$  = 275.5 nm;  $^1\text{H}$  NMR (DMSO- $d_6$ )  $\delta$  8.52 (s, 1 H), 8.46 (br s, 1 H, D<sub>2</sub>O exchangeable), 7.54 (s, 1 H), 7.42 (d, 1 H,  $J$  = 8.0 Hz), 7.33 (d, 1 H,  $J$  = 7.6 Hz), 7.27 (t, 1 H,  $J$  = 7.6 Hz), 5.87 (d, 1 H,  $J$  = 7.2 Hz), 5.84 (d, 1 H,  $J$  = 6.4 Hz, D<sub>2</sub>O exchangeable), 5.36 (d, 1 H,  $J$  = 4.0 Hz, D<sub>2</sub>O exchangeable), 4.60–4.66 (m, 3 H), 4.34–4.35 (m, 1 H), 3.40 (dd, 1 H,  $J$  = 4.0, 10.8 Hz), 2.80 (dd, 1 H,  $J$  = 3.2, 10.8 Hz), 2.41 (t, 2 H,  $J$  = 7.2 Hz), 1.51–1.55 (m, 2 H), 1.39–1.45 (m, 2 H), 0.91 (t, 3 H,  $J$  = 7.2 Hz);  $^{13}\text{C}$  NMR (DMSO- $d_6$ )  $\delta$  153.9, 149.4, 145.6, 142.7, 140.5, 130.4, 129.9, 129.5, 126.2, 121.5, 118.6, 85.7, 81.5, 78.6, 72.2, 61.1, 42.3, 34.4, 29.8, 21.5, 17.9, 13.4;  $[\alpha]_{\text{D}}^{25}$  –52.17 (c 0.207, MeOH); (ESI+) ( $\text{M} + \text{H}^+$ )  $m/z$  504.0890. Anal. Calcd for C<sub>22</sub>H<sub>24</sub>BrN<sub>5</sub>O<sub>2</sub>S: C, 52.59; H, 4.81; N, 13.94; S, 6.38. Found: C, 52.22; H, 4.89; N, 13.57; S, 5.99.

(-)-(2*R*,3*S*,4*R*)-2-(6-(3-Iodobenzylamino)-2-(hex-1-ynyl)-9*H*-purin-9-yl)tetrahydrothiophene-3,4-diol (**4k**). Yield, 77%; mp 225–227 °C; UV (MeOH)  $\lambda_{\text{max}}$  = 274.5 nm;  $^1\text{H}$  NMR (DMSO- $d_6$ )  $\delta$  8.52 (s, 1 H), 8.45 (br s, 1 H, D<sub>2</sub>O exchangeable), 7.73 (s, 1 H), 7.58 (d, 1 H,  $J$  = 8.0 Hz), 7.34 (d, 1 H,  $J$  = 7.6 Hz), 7.11 (t, 1 H,  $J$  = 7.6 Hz), 5.87 (d, 1 H,  $J$  = 7.2 Hz), 5.54 (d, 1 H,  $J$  = 6.0 Hz, D<sub>2</sub>O exchangeable), 5.36 (d, 1 H,  $J$  = 4.0 Hz, D<sub>2</sub>O exchangeable), 4.57–4.62 (m, 3 H), 4.32–4.36 (m, 1 H), 3.40 (dd, 1 H,  $J$  = 4.0, 10.8 Hz), 2.80 (dd, 1 H,  $J$  = 3.2, 10.8 Hz), 2.42 (t, 2 H,  $J$  = 7.2 Hz), 1.50–1.57 (m, 2 H), 1.38–1.47 (m, 2 H), 0.91 (t, 3 H,  $J$  = 7.2 Hz);  $^{13}\text{C}$  NMR (DMSO- $d_6$ )  $\delta$  153.9, 149.4, 145.6, 142.6, 140.5, 135.9, 135.4, 130.5, 126.6, 118.6, 94.7, 85.7, 81.5, 78.6, 72.1, 61.1, 42.2, 34.3, 29.8, 21.5, 17.9, 13.4;  $[\alpha]_{\text{D}}^{25}$  –43.33 (c 0.18, MeOH); (ESI+) ( $\text{M} + \text{H}^+$ )  $m/z$  550.0764. Anal. Calcd for C<sub>22</sub>H<sub>24</sub>IN<sub>5</sub>O<sub>2</sub>S: C, 48.09; H, 4.40; N, 12.75; S, 5.84. Found: C, 48.09; H, 4.21; N, 12.47; S, 6.01.

(-)-(2*R*,3*S*,4*S*)-2-(6-Amino-2-(*E*-hex-1-enyl)-9*H*-purin-9-yl)-tetrahydrofuran-3,4-diol (**4l**). A mixture of **14** (0.096 g, 0.24 mmol), tetrakis(triphenylphosphine)palladium (28 mg, 0.024 mmol), sodium carbonate (76 mg, 0.72 mmol), and (*E*)-1-catecholboranylhexene (67 mg, 0.33 mmol) in DMF and H<sub>2</sub>O (8:1, 5 mL) was stirred at 90 °C for 15 h. The reaction mixture was filtered over a Celite bed, and the residue was evaporated to give the crude compound **16**. To a solution of **16** in THF (5 mL) was added 1 N HCl (5 mL), and the mixture was stirred at room temperature for 15 h. The mixture was neutralized with 1 N NaOH and then carefully evaporated under reduced pressure. The crude residue was subjected to flash silica gel column chromatography (CH<sub>2</sub>Cl<sub>2</sub>/MeOH = 20:1) to give **4l** (48 mg, 63%) as a white solid: mp 165–167 °C; UV (MeOH)  $\lambda_{\text{max}}$  = 293.0 nm;  $^1\text{H}$  NMR (CD<sub>3</sub>OD)  $\delta$  8.20 (s, 1 H), 6.99–7.06 (m, 1 H), 6.36 (tt, 1 H,  $J$  = 1.6, 15.6 Hz), 5.97 (d, 1 H,  $J$  = 6.4 Hz), 4.94 (dd, 1 H,  $J$  = 4.8, 6.0 Hz), 4.53 (dd, 1 H,  $J$  = 4.0, 9.6 Hz), 4.43–4.46 (m, 1 H), 3.99 (dd, 1 H,  $J$  = 2.0, 9.6 Hz), 2.25–2.31 (m, 2 H), 1.47–1.54 (m, 2 H), 1.36–1.45 (m, 2 H), 0.95 (t, 3 H,  $J$  = 7.2 Hz);  $^{13}\text{C}$  NMR (CD<sub>3</sub>OD)  $\delta$  160.9, 156.9, 151.7, 141.6, 141.3, 130.7, 90.3, 76.4, 75.4, 72.4, 57.9, 33.3, 32.3, 23.4, 14.3;  $[\alpha]_{\text{D}}^{25}$  –47.05 (c 0.12, MeOH); (ESI+) ( $\text{M} + \text{H}^+$ )  $m/z$  320.1747;  $[\alpha]_{\text{D}}^{25}$  –44.80 (c 0.12, MeOH). Anal. Calcd for C<sub>15</sub>H<sub>21</sub>N<sub>5</sub>O<sub>3</sub>: C, 56.41; H, 6.63; N, 21.90. Found: C, 56.78; H, 6.89; N, 21.77.

(-)-(2*R*,3*S*,4*R*)-2-(6-Amino-2-(*E*-hex-1-enyl)-9*H*-purine-9-yl)-tetrahydrothiophene-3,4-diol (**4m**). Compound **15** (46 mg, 0.11 mmol) was converted to **4m** (23 mg, 63%) as a white solid, according to the same procedure used in the preparation of **4l**: mp 209–210 °C; UV (MeOH)  $\lambda_{\text{max}}$  = 274.5 nm;  $^1\text{H}$  NMR (DMSO- $d_6$ )  $\delta$  8.37 (s, 1 H),

7.11 (brs, 2 H, NH<sub>2</sub>), 6.87–6.94 (m, 1 H), 6.29 (d, 1 H, *J* = 15.2 Hz), 5.89 (d, 1 H, *J* = 7.2 Hz), 5.52 (d, 1 H, OH, *J* = 6.0 Hz), 5.36 (d, 1 H, OH, *J* = 4.0 Hz), 4.63–4.67 (m, 1 H), 4.37 (pseudo t, 1 H, *J* = 3.2 Hz), 3.42 (dd, 1 H, *J* = 4.4, 10.8 Hz), 2.80 (dd, 1 H, *J* = 2.8, 10.8 Hz), 2.20–2.26 (m, 2 H), 1.41–1.48 (m, 2 H), 1.29–1.38 (m, 2 H), 0.90 (t, 3 H, *J* = 7.2 Hz); <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>) δ 158.2, 155.5, 150.6, 139.6, 138.2, 130.4, 117.7, 78.5, 72.3, 61.0, 34.4, 31.4, 30.5, 21.7, 13.8; [α]<sup>25</sup><sub>D</sub> –44.80 (c 0.12, MeOH); (ESI+) (M + H<sup>+</sup>) *m/z* 336.1499. Anal. Calcd for C<sub>15</sub>H<sub>21</sub>N<sub>5</sub>O<sub>3</sub>S: C, 53.71; H, 6.31; N, 20.88; S, 9.56. Found: C, 53.89; H, 6.71; N, 20.49; S, 9.32.

**(–)-(2R,3S,4S)-2-(6-Amino-8-(hex-1-ynyl)-9H-purine-9-yl)-tetrahydrofuran-3,4-diol (4n).** Compound **19** (0.605 g, 0.19 mmol) was dissolved in Et<sub>3</sub>N (10 mL) and DMF (10 mL). After the solution was purged with N<sub>2</sub>, (Ph<sub>3</sub>P)<sub>2</sub>PdCl<sub>2</sub> (0.238 g, 0.34 mmol), CuI (0.065 g, 0.34 mmol), and 1-hexyne (0.49 mL, 4.25 mmol) were subsequently added dropwise. The mixture was stirred at room temperature for 3 h. The volatiles were evaporated to give the crude compound **21**. To a solution of **21** in THF (10 mL) was added 1 N HCl (10 mL), and the mixture was stirred at room temperature for 15 h. The mixture was neutralized with 1 N NaOH and then evaporated under reduced pressure. The crude residue was subjected to flash silica gel column chromatography (CH<sub>2</sub>Cl<sub>2</sub>/MeOH = 10:1) to give **4n** (0.334 g, 62%) as a white solid: mp 228–232 °C; UV (MeOH) λ<sub>max</sub> = 291.5 nm; <sup>1</sup>H NMR (CD<sub>3</sub>OD) δ 8.19 (s, 1 H), 6.14 (d, 1 H, *J* = 7.2 Hz), 5.32 (dd, 1 H, *J* = 4.8, 6.8 Hz), 4.57 (dd, 1 H, *J* = 3.2, 9.6 Hz), 4.43–4.45 (m, 1 H), 3.99 (dd, 1 H, *J* = 1.2, 9.6 Hz), 2.59 (t, 2 H, *J* = 6.8 Hz), 1.64–1.70 (m, 2 H), 1.52–1.58 (m, 2 H), 1.00 (t, 3 H, *J* = 7.2 Hz); <sup>13</sup>C NMR (CD<sub>3</sub>OD) δ 156.9, 154.4, 150.6, 136.7, 120.3, 99.9, 91.1, 76.1, 75.1, 72.9, 70.8, 31.2, 23.1, 19.7, 13.9; [α]<sup>25</sup><sub>D</sub> –42.75 (c 0.14, MeOH); (ESI+) (M + H<sup>+</sup>) *m/z* 318.1568. Anal. Calcd for C<sub>15</sub>H<sub>19</sub>N<sub>5</sub>O<sub>3</sub>S: C, 56.77; H, 6.03; N, 22.07. Found: C, 56.89; H, 6.23; N, 22.47.

**(–)-(2R,3S,4R)-2-(6-Amino-8-(hex-1-ynyl)-9H-purine-9-yl)-tetrahydrothiophene-3,4-diol (4o).** Compound **20** (69 mg, 0.19 mmol) was converted to **4o** (36 mg, 58%) as a white solid, according to the same procedure used in the preparation of **4n**: mp 234–235 °C; UV (MeOH) λ<sub>max</sub> = 294.0 nm; <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>) δ 8.16 (s, 1 H), 7.40 (brs, 2 H, NH<sub>2</sub>), 6.03 (d, 1 H, *J* = 7.6 Hz), 5.44 (d, 1 H, OH, *J* = 6.4 Hz), 5.33 (d, 1 H, OH, *J* = 4.0 Hz), 5.24–5.29 (m, 1 H), 4.40 (dd, 1 H, *J* = 1.6, 3.2 Hz), 3.42 (dd, 1 H, *J* = 3.6, 11.2 Hz), 2.80 (dd, 1 H, *J* = 2.0, 11.2 Hz), 2.59 (t, 2 H, *J* = 6.8 Hz), 1.55–1.62 (m, 2 H), 1.44–1.51 (m, 2 H), 0.93 (t, 3 H, *J* = 7.2 Hz); <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>) δ 155.7, 153.1, 149.1, 133.7, 118.9, 98.0, 76.5, 72.3, 70.7, 63.2, 35.5, 29.6, 21.4, 18.3, 13.4; [α]<sup>25</sup><sub>D</sub> –112.2 (c 0.14, MeOH); (ESI+) (M + H<sup>+</sup>) *m/z* 334.1339. Anal. Calcd for C<sub>15</sub>H<sub>19</sub>N<sub>5</sub>O<sub>2</sub>S: C, 54.04; H, 5.74; N, 21.01; S, 9.62. Found: C, 54.43; H, 5.88; N, 19.94; S, 9.31.

**(–)-(2R,3S,4S)-2-(6-Amino-8-hexyl-9H-purine-9-yl)-tetrahydrofuran-3,4-diol (4p).** A mixture of **4n** (0.116 g, 0.36 mmol), absolute ethanol (20 mL), and 10% palladium on carbon (20 mg) was hydrogenated on a Parr apparatus at 40 psi for 15 h. The mixture was filtered, and the solvent was evaporated. The mixture was subjected to flash silica gel column chromatography (CH<sub>2</sub>Cl<sub>2</sub>/MeOH = 20:1) to give **4p** (81 mg, 69%) as a white solid: mp 228–229 °C; UV (MeOH) λ<sub>max</sub> = 259.5 nm; <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>) δ 8.14 (s, 1 H), 7.41 (brs, 2 H, D<sub>2</sub>O exchangeable), 5.76 (d, 1 H, *J* = 6.8 Hz), 5.37 (brs, 1 H, D<sub>2</sub>O exchangeable), 5.25 (brs, 1 H, D<sub>2</sub>O exchangeable), 5.13–5.15 (m, 1 H), 4.40 (dd, 1 H, *J* = 3.2, 9.2 Hz), 4.28 (pseudo t, 1 H, *J* = 3.2 Hz), 3.83 (d, 1 H, *J* = 9.2 Hz), 2.82–2.87 (m, 2 H), 1.69–1.77 (m, 2 H), 1.32–1.39 (m, 2 H), 1.26–1.31 (m, 4 H), 0.85–0.88 (m, 3 H); <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>) δ 154.3, 152.9, 150.5, 150.1, 118.1, 88.3, 74.3, 73.2, 70.7, 30.9, 28.4, 27.6, 27.4, 22.0, 13.9; [α]<sup>25</sup><sub>D</sub> –36.50 (c 0.13, MeOH); (ESI+) (M + H<sup>+</sup>) *m/z* 322.1883. Anal. Calcd for C<sub>15</sub>H<sub>23</sub>N<sub>5</sub>O<sub>3</sub>S: C, 56.06; H, 7.21; N, 21.79. Found: C, 56.45; H, 7.22; N, 21.77.

**(–)-(2R,3S,4S)-2-(6-Amino-8-(hex-1-enyl)-9H-purine-9-yl)-tetrahydrofuran-3,4-diol (4q).** A mixture of **19** (78.8 mg, 0.22 mmol), tetrakis(triphenylphosphine)palladium(0) (26 mg, 0.022 mmol), sodium carbonate (70 mg, 0.66 mmol), and (*E*)-1-catecholboranylhexene (134 mg, 0.66 mmol) in DMF and H<sub>2</sub>O (8:1, 5 mL) was stirred for 15 h at 90 °C. The reaction mixture was filtered by a bed of

Celite, and the volatiles were evaporated to give the crude compound **23**. To a solution of **23** in THF (3 mL) was added 1 N HCl (3 mL), and the mixture was stirred at room temperature for 15 h. The mixture was neutralized with 1 N NaOH solution and then carefully evaporated under reduced pressure. The mixture was subjected to flash silica gel column chromatography (CH<sub>2</sub>Cl<sub>2</sub>/MeOH = 20:1) to give **4q** (41 mg, 58%) as a white solid: mp 204–206 °C; UV (MeOH) λ<sub>max</sub> = 296.5 nm; <sup>1</sup>H NMR (DMSO-*d*<sub>6</sub>) δ 8.08 (s, 1 H), 7.18 (brs, 2 H, D<sub>2</sub>O exchangeable), 6.84–6.92 (m, 1 H), 6.58 (tt, 1 H, *J* = 1.6, 15.6 Hz), 5.91 (d, 1 H, *J* = 6.8 Hz), 5.36 (d, 1 H, *J* = 6.8 Hz, D<sub>2</sub>O exchangeable), 5.21 (d, 1 H, *J* = 4.0 Hz, D<sub>2</sub>O exchangeable), 5.05–5.06 (m, 1 H), 4.37 (dd, 1 H, *J* = 3.6, 9.6 Hz), 4.28 (d, 1 H, *J* = 3.6 Hz), 3.82 (dd, 1 H, *J* = 1.2, 9.6 Hz); 2.28–2.34 (m, 2 H), 1.43–1.50 (m, 2 H), 1.31–1.40 (m, 2 H), 0.91 (t, 3 H, *J* = 7.6 Hz); <sup>13</sup>C NMR (DMSO-*d*<sub>6</sub>) δ 155.4, 151.9, 150.2, 148.0, 140.8, 118.8, 116.6, 87.6, 74.1, 73.3, 70.5, 32.0, 30.3, 21.7, 13.7; [α]<sup>25</sup><sub>D</sub> –24.76 (c 0.11, MeOH); (ESI+) (M + H<sup>+</sup>) *m/z* 320.1717. Anal. Calcd for C<sub>15</sub>H<sub>21</sub>N<sub>5</sub>O<sub>3</sub>S: C, 56.41; H, 6.63; N, 21.93. Found: C, 56.32; H, 6.85; N, 21.89.

**(–)-(2R,3S,4R)-2-(6-Amino-8-(hex-1-enyl)-9H-purine-9-yl)-tetrahydrothiophene-3,4-diol (4r).** Compound **20** (42 mg, 0.12 mmol) was converted to **4r** (25 mg, 65%) as a white solid, according to the same procedure used in the preparation of **4q**: mp 248–249 °C; UV (MeOH) λ<sub>max</sub> = 297.5 nm; <sup>1</sup>H NMR (CD<sub>3</sub>OD) δ 8.15 (s, 1 H), 6.94–7.01 (m, 1 H), 6.60 (d, 1 H, *J* = 15.2 Hz), 6.15 (d, 1 H, *J* = 8.0 Hz), 5.27 (dd, 1 H, *J* = 3.6, 8.0 Hz), 4.48–4.50 (m, 1 H), 3.65 (dd, 1 H, *J* = 3.6, 11.6 Hz), 2.93 (dd, 1 H, *J* = 1.6, 11.6 Hz), 2.36–2.42 (m, 2 H), 1.53–1.58 (m, 2 H), 1.42–1.50 (m, 2 H), 0.98 (t, 3 H, *J* = 7.2 Hz); <sup>13</sup>C NMR (CD<sub>3</sub>OD) δ 153.5, 152.9, 151.8, 151.2, 143.9, 120.1, 117.4, 78.8, 74.4, 63.9, 36.3, 32.0, 32.1, 23.4, 14.3; [α]<sup>25</sup><sub>D</sub> –71.56 (c 0.10, MeOH); (ESI+) (M + H<sup>+</sup>) *m/z* 336.1494. Anal. Calcd for C<sub>15</sub>H<sub>21</sub>N<sub>5</sub>O<sub>2</sub>S: C, 53.71; H, 6.31; N, 20.88; S, 9.56. Found: C, 53.56; H, 6.33; N, 20.67; S, 9.45.

**Pharmacology. In Vitro Assays. Cell Culture and Membrane Preparation.** CHO cells stably expressing either the recombinant hA<sub>1</sub> or hA<sub>2A</sub>AR and HEK-293 cells stably expressing the human A<sub>2A</sub>AR 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. In addition, we added 800 μg/mL Geneticin to the hA<sub>2A</sub>AR medium and 500 μg/mL hygromycin B to the hA<sub>1</sub>AR, hA<sub>2B</sub>AR, and hA<sub>3</sub>AR media. After harvesting the cells, we centrifuged them at 250g for 5 min at 4 °C. The pellet was resuspended in 50 mM Tris-HCl buffer (pH 7.5), containing 10 mM MgCl<sub>2</sub>. The suspension was homogenized with an electric homogenizer for 10 s and was then recentrifuged at 20000g for 30 min at 4 °C. The resultant pellet was homogenized again, resuspended in the buffer mentioned above in the presence of 3 U/mL adenosine deaminase, finally pipetted into 1 mL vials, and stored at –80 °C until the binding experiments were conducted. The concentration of protein was determined using a BCA protein assay kit from Pierce Biotechnology (Rockford, IL).<sup>24</sup>

**Radioligand Binding Assay.** Radioligand binding assays with A<sub>1</sub>, A<sub>2A</sub>, and A<sub>3</sub>ARs were performed according to the procedures described previously.<sup>25–27</sup> Briefly, for binding to human A<sub>1</sub> receptors, [<sup>3</sup>H]PIA (1 nM) was incubated with membranes (40 μg/tube) from CHO cells stably expressing human A<sub>1</sub> receptors at 25 °C for 60 min in 50 mM Tris-HCl buffer (pH 7.4; MgCl<sub>2</sub>, 10 mM) in a total assay volume of 200 μL. Nonspecific binding was determined using 10 μM 25. For human A<sub>2A</sub> receptor binding, membranes (20 μg/tube) from HEK-293 cells stably expressing human A<sub>2A</sub>ARs were incubated with 15 nM [<sup>3</sup>H]26 at 25 °C for 60 min in 200 μL of 50 mM Tris-HCl, pH 7.4, containing 10 mM MgCl<sub>2</sub>. Reaction was terminated by filtration with GF/B filters. For competitive binding assay to human A<sub>3</sub>ARs, each tube contained 100 μL suspension of membranes (20 μg of protein) from CHO cells stably expressing the human A<sub>3</sub>AR, 50 μL of [<sup>125</sup>I]27 (0.5 nM), and 50 μL of increasing concentrations of the nucleoside derivative in Tris-HCl buffer (50 mM, pH 7.4) containing 10 mM MgCl<sub>2</sub> and 1 mM EDTA. Nonspecific binding was determined using 10 μM 25 in the buffer. The mixtures were incubated at 25 °C for 60 min. Binding reactions were terminated by filtration through Whatman



GF/B filters under reduced pressure using a MT-24 cell harvester (Brandell, Gaithersburg, MD, U.S.). Filters were washed three times with 9 mL of ice-cold buffer. Radioactivity was determined in a Beckman 5500B  $\gamma$ -counter.

For binding at all three subtypes,  $K_i$  values are expressed as the mean  $\pm$  SEM,  $n = 3-5$  (outliers eliminated), and normalized against a nonspecific binder, 10  $\mu$ M 25. Alternatively, for weak binding a percent inhibition of specific radioligand binding at 10  $\mu$ M, relative to inhibition by 10  $\mu$ M 25 assigned as 100%, is given.

**cAMP Accumulation Assay.** Intracellular cAMP levels were measured with a competitive protein binding method.<sup>28</sup> CHO cells that expressed the recombinant hA<sub>2A</sub>AR, hA<sub>2B</sub>AR, or hA<sub>3</sub>AR were harvested by trypsinization. After centrifugation and resuspension in medium, cells were planted in 24-well plates in 1.0 mL of medium. After 24 h, the medium was removed and cells were washed three times with 1 mL of DMEM containing 50 mM HEPES, pH 7.4. Cells were then treated with the test agonist in the presence of rolipram (10  $\mu$ M) and adenosine deaminase (3 units/mL). For the hA<sub>2A</sub>AR and the hA<sub>2B</sub>AR, incubation with agonist was performed for 30 min. For the hA<sub>3</sub>AR, after 30 min forskolin (10  $\mu$ M) was added to the medium, and incubation was continued for an additional 15 min. The reaction was terminated by removing the supernatant, and cells were lysed upon the addition of 200  $\mu$ L of 0.1 M ice-cold HCl. The cell lysate was resuspended and stored at  $-20$  °C. For determination of cAMP production, 100  $\mu$ L of the HCl solution was used in the Sigma Direct cAMP enzyme immunoassay following the instructions provided with the kit. The results were interpreted using a Bio-Tek Instruments ELx808 Ultra microplate reader at 405 nm.

**Data Analysis.** Binding and functional parameters were calculated using the Prism 5.0 software (GraphPAD, San Diego, CA, U.S.). IC<sub>50</sub> values obtained from competition curves were converted to  $K_i$  values using the Cheng–Prusoff equation.<sup>29</sup> Data were expressed as the mean  $\pm$  standard error.

**In Vivo Assay of Anti-Inflammatory Effect.** *Animals.* Male Sprague–Dawley (SD) rats (140–160 g, 5 weeks old) were purchased from Central Laboratory Animal Inc. (Seoul, Korea). Animals were housed under standard laboratory conditions with free access to food and water. The temperature was thermostatically regulated to  $22 \pm 2$  °C, and a 12 h light/dark schedule was maintained. Prior to their use, they were allowed 1 week for acclimatization within the work area environment. All animal experiments were carried out in accordance with Institutional Animal Care and Use Committee Guidelines of Seoul National University (SNU-201110-4).

**Carrageenan-Induced Paw Edema.** Carrageenan-induced hind paw edema model in rats was used for the assessment of anti-inflammatory activity.<sup>30</sup> Test compounds **4g** (20 mg/kg) and indomethacin (20 mg/kg) were administered by an intraperitoneal injection dissolved in 5% Cremophor and 5% ethanol in PBS, and solvent alone was served as a vehicle control. Thirty minutes after the administration of **4g**, vehicle, or indomethacin, paw edema was induced by subplantar injection of 0.1 mL of 1% freshly prepared carrageenan suspension in normal saline into the right hind paw of each rat. The left hind paw was injected with 0.1 mL of normal saline. The paw volume was measured before (0 h) and at intervals of 0.5, 1, 2, 4, 6, and 24 h after carrageenan injection using a plethysmometer (Ugo Basile, Comerio, Italy).

**Statistics.** All experiments were repeated at least three times. Data were presented as the mean  $\pm$  SD for the indicated number of independently performed experiments. The statistical significances within a parameter were evaluated by one-way and multiple analysis of variation (ANOVA).

**Molecular Modeling.** The 3D structures of the molecules were generated with Concord and energy minimized using MMFF94s force field and MMFF94 charge until the rms of Powell gradient was 0.05 kcal mol<sup>-1</sup> Å<sup>-1</sup> in SYBYL-X 1.2 (Tripos International, St. Louis, MO, U.S.). The X-ray crystal structure of the A<sub>2A</sub>AR (PDB code 3EML) was prepared by Biopolymer Structure Preparation Tool in SYBYL. The docking study was performed using GOLD, version 5.0.1 (Cambridge Crystallographic Data Centre, Cambridge, U.K.), which employs a genetic algorithm (GA) and allows for full ligand flexibility

and partial protein flexibility. The region of 9 Å around the cocrystallized ligand was defined as the binding site. The side chains of the eight residues (i.e., Thr88, Phe168, Glu169, Trp246, Leu249, Asn253, Ser277, and His278), which are important for ligand binding, were set to be flexible with “crystal mode”. GoldScore scoring function was used, and other parameters were set as default except the number of GA runs, set as 30. The Fast Connolly surface of the receptor and the van der Waals surface of each ligand were generated by MOLCAD in SYBYL. All computational calculations were undertaken on Intel Xeon Quad-core 2.5 GHz workstation with Linux Cent OS, release 5.5.

## ■ ASSOCIATED CONTENT

### Supporting Information

Scheme 1S for the mechanism of lithiation mediated stannyl transfer for the formation of **7**. This material is available free of charge via the Internet at <http://pubs.acs.org>.

## ■ AUTHOR INFORMATION

### Corresponding Author

\*Phone: 82-2-3277-3466. Fax: 82-2-3277-2851. E-mail: [lakjeong@ewha.ac.kr](mailto:lakjeong@ewha.ac.kr).

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## ■ ABBREVIATIONS USED

AR, adenosine receptor; Cl-IB-MECA, 2-chloro-N<sup>6</sup>-(3-iodobenzyl)-5'-N-methylcarbamoyl-adenosine; thio-Cl-IB-MECA, 2-chloro-N<sup>6</sup>-(3-iodobenzyl)-5'-N-methylcarbamoyl-4'-thioadenosine; TMSOTf, trimethylsilyl trifluoromethanesulfonate; NOE, nuclear Overhauser effect; R-PIA, (–)-N<sup>6</sup>-2-phenylisopropyladenosine; I-AB-MECA, N<sup>6</sup>-(3-iodo-4-aminobenzyl)-5'-N-methylcarboxamidoadenosine; NECA, 5'-N-ethylcarboxamidoadenosine; HMDS, hexamethyldisilazane; BSA, N,O-bis(trimethylsilyl)acetamide; DMEM, Dulbecco's modified Eagle's medium; CHO, Chinese hamster ovary; HEK, human embryonic kidney; cAMP, cyclic adenosine 5'-monophosphate

## ■ REFERENCES

- (1) Olah, M. E.; Stiles, G. L. The role of receptor structure in determining adenosine receptor activity. *Pharmacol. Ther.* **2000**, *85*, 55–75.
- (2) Fredholm, B. B.; Cunha, R. A.; Svenningsson, P. Pharmacology of adenosine receptors and therapeutic applications. *Curr. Top. Med. Chem.* **2002**, *3*, 413–426.
- (3) Baraldi, P. G.; Cacciari, B.; Romagnoli, R.; Merighi, S.; Varani, K.; Borea, P. A.; Spalluto, G. A<sub>3</sub> adenosine receptor ligands: history and perspectives. *Med. Res. Rev.* **2000**, *20*, 103–128.
- (4) Jacobson, K. A.; Gao, Z.-G. Adenosine receptors as therapeutic targets. *Nat. Rev. Drug Discovery* **2006**, *5*, 247–264.
- (5) Kim, H. O.; Ji, X.-d.; Siddiqi, S. M.; Olah, M. E.; Stiles, G. L.; Jacobson, K. A. 2-Substitution of N<sup>6</sup>-benzyladenosine-5'-uronamides enhances selectivity for A<sub>3</sub> adenosine receptors. *J. Med. Chem.* **1994**, *37*, 3614–3621.
- (6) (a) Jeong, L. S.; Jin, D. Z.; Kim, H. O.; Shin, D. H.; Moon, H. R.; Gunaga, P.; Chun, M. W.; Kim, Y.-C.; Melman, N.; Gao, Z.-G.;

- Jacobson, K. A.  $N^6$ -Substituted D-4'-thioadenosine-5'-methyluronamides: potent and selective agonists at the human  $A_3$  adenosine receptor. *J. Med. Chem.* **2003**, *46*, 3775–3777. (b) Jeong, L. S.; Lee, H. W.; Jacobson, K. A.; Kim, H. O.; Shin, D. H.; Lee, J. A.; Gao, Z.-G.; Lu, C.; Duong, H. T.; Gunaga, P.; Lee, S. K.; Jin, D. Z.; Chun, M. W.; Moon, H. R. Structure–activity relationships of 2-chloro- $N^6$ -substituted-4'-thioadenosine-5'-uronamides as highly potent and selective agonists at the human  $A_3$  adenosine receptor. *J. Med. Chem.* **2006**, *49*, 273–281.
- (7) (a) Lee, E. J.; Min, H. Y.; Chung, H. J.; Park, E. J.; Shin, D. H.; Jeong, L. S.; Lee, S. K. A novel adenosine analog, thio-Cl-IB-MECA, induces  $G_0/G_1$  cell cycle arrest and apoptosis in human promyelocytic leukemia HL-60 cells. *Biochem. Pharmacol.* **2005**, *70*, 918–924. (b) Bar-Yehuda, S.; Stemmer, S. M.; Madi, L.; Castel, D.; Ochaion, A.; Cohen, S.; Barer, F.; Zabutti, A.; Perez-Liz, G.; Del Valle, L.; Fishman, P. The  $A_3$  adenosine receptor agonist CF102 induces apoptosis of hepatocellular carcinoma via de-regulation of the Wnt and NF- $\kappa$ B signal transduction pathways. *Int. J. Oncol.* **2008**, *33*, 287–295. (c) Kohno, Y.; Sei, Y.; Koshiba, M.; Kim, H. O.; Jacobson, K. A. Induction of apoptosis in HL-60 human promyelocytic leukemia cells by selective adenosine  $A_3$  receptor agonists. *Biochem. Biophys. Res. Commun.* **1996**, *219*, 904–910.
- (8) Kim, S. K.; Jacobson, K. A. Three-dimensional quantitative structure–activity relationship of nucleosides acting at the  $A_3$  adenosine receptor: analysis of binding and relative efficacy. *J. Chem. Inf. Model.* **2007**, *47*, 1225–1231.
- (9) (a) Jeong, L. S.; Choe, S. A.; Gunaga, P.; Kim, H. O.; Lee, H. W.; Lee, S. K.; Tosh, D. K.; Patel, A.; Palaniappan, K. K.; Gao, Z.-G.; Jacobson, K. A.; Moon, H. R. Discovery of a new nucleoside template for human  $A_3$  adenosine receptor ligands: D-4'-thioadenosine derivatives without 4'-hydroxymethyl group as highly potent and selective antagonists. *J. Med. Chem.* **2007**, *50*, 3159–3162. (b) Jeong, L. S.; Pal, S.; Choe, S. A.; Choi, W. J.; Jacobson, K. A.; Gao, Z.-G.; Klutz, A. M.; Hou, X.; Kim, H. O.; Lee, H. W.; Tosh, D. K.; Moon, H. R. Structure–activity relationships of truncated D- and L-4'-thioadenosine derivatives as species-independent  $A_3$  adenosine receptor antagonists. *J. Med. Chem.* **2008**, *51*, 6609–6613. (c) Pal, S.; Choi, W. J.; Choe, S. A.; Heller, C. L.; Gao, Z.-G.; Chinn, M.; Jacobson, K. A.; Hou, X.; Lee, S. K.; Kim, H. O.; Jeong, L. S. Structure–activity relationships of truncated adenosine derivatives as highly potent and selective human  $A_3$  adenosine receptor antagonists. *Bioorg. Med. Chem.* **2009**, *17*, 3733–3738. (d) Jacobson, K. A.; Siddiqi, S. M.; Olah, M. E.; Ji, X. d.; Melman, N.; Bellamkonda, K.; Meshulam, Y.; Stiles, G. L.; Kim, H. O. Structure–activity relationships of 9-alkyladenine and ribose-modified adenosine derivatives at rat  $A_3$  adenosine receptors. *J. Med. Chem.* **1995**, *38*, 1720–1735.
- (10) Wang, Z.; Do, C. W.; Avila, M. Y.; Peterson-Yantorno, K.; Stone, R. A.; Gao, Z.-G.; Joshi, B.; Besada, P.; Jeong, L. S.; Jacobson, K. A.; Civan, M. M. Nucleoside-derived antagonists to  $A_3$  adenosine receptors lower mouse intraocular pressure and act across species. *Exp. Eye Res.* **2010**, *90*, 146–154.
- (11) Hou, X.; Kim, H. O.; Alexander, V.; Kim, K.; Choi, S.; Park, S.; Lee, J. H.; Yoo, L. S.; Gao, Z.; Jacobson, K. A.; Jeong, L. S. Discovery of a new human  $A_{2A}$  adenosine receptor agonist, truncated 2-hexynyl-4'-thioadenosine. *ACS Med. Chem. Lett.* **2010**, *1*, 516–520.
- (12) (a) Sitkovsky, M. V.; Lukashev, D.; Apasov, S.; Kojima, H.; Koshiba, M.; Cladwell, C.; Ohta, A.; Thiel, M. Physiological control of immune response and inflammatory tissue damage by hypoxia-inducible factors and adenosine  $A_{2A}$  receptors. *Annu. Rev. Immunol.* **2004**, *22*, 657–682. (b) Lappas, C. M.; Sullivan, G. W.; Linden, J. Adenosine  $A_{2A}$  agonists in development for the treatment of inflammation. *Expert Opin. Invest. Drugs* **2005**, *14*, 797–806.
- (13) (a) Kato, K.; Hayakawa, H.; Tanaka, H.; Kumamoto, H.; Shindoh, S.; Shuto, S.; Miyasaka, T. A new entry to 2-substituted purine nucleosides based on lithiation-mediated stannyl transfer of 6-chloropurine nucleosides. *J. Org. Chem.* **1997**, *62*, 6833–6841. (b) Brun, V.; Legraverend, M.; Grierson, D. S. Cyclin-dependent kinase (CDK) inhibitors: development of a general strategy for the construction of 2,6,9-trisubstituted purine libraries. Part 1. *Tetrahedron Lett.* **2001**, *42*, 8161–8164. (c) Taddei, D.; Kilian, P.; Slawin, A. M. Z.; Woollins, D. Synthesis and full characterization of 6-chloro-2-iodopurine, a template for the functionalisation of purines. *Org. Biomol. Chem.* **2004**, *2*, 665–670.
- (14) Chinchilla, R.; Nájera, C. The Sonogashira reaction: a booming methodology in synthetic organic chemistry. *Chem. Rev.* **2007**, *107*, 874–922.
- (15) Suzuki, A. Carbon–carbon bonding made easy. *Chem. Commun.* **2005**, *38*, 4759–4763.
- (16) Miyaura, N.; Suzuki, A. Palladium-Catalyzed Reaction of 1-Alkenylboronates with Vinylic Halides: (1Z,3E)-1-Phenyl-1,3-octadiene. In *Organic Syntheses*; Wiley & Sons: New York, 1993; Collect. Vol. 8, pp 532–534.
- (17) Laxer, A.; Major, D. T.; Gottlieb, H. E.; Fischer, B. ( $^{15}\text{N}_5$ )-Labeled adenine derivatives: synthesis and studies of tautomerism by  $^{15}\text{N}$  NMR spectroscopy and theoretical calculations. *J. Org. Chem.* **2001**, *66*, 5463–5481.
- (18) (a) Perreira, M.; Jiang, J. K.; Klutz, A. M.; Gao, Z. G.; Shainberg, A.; Lu, C.; Thomas, C. J.; Jacobson, K. A. Reversine and its 2-substituted adenine derivatives as potent and selective  $A_3$  adenosine receptor antagonists. *J. Med. Chem.* **2005**, *48*, 4910–4918. (b) Jarvis, M. F.; Schutz, R.; Hutchison, A. J.; Do, E.; Sills, M. A.; Williams, M. [ $^3\text{H}$ ]CGS 21680 a selective  $A_2$  adenosine receptor agonist directly labels  $A_2$  receptors in rat brain. *J. Pharmacol. Exp. Ther.* **1989**, *251*, 888–893. (c) Olah, M. E.; Gallo-Rodriguez, C.; Jacobson, K. A.; Stiles, G. L.  $^{125}\text{I}$ -4-Aminobenzyl-5'-N-methylcarboxamidoadenosine a high affinity radioligand for the rat  $A_3$  adenosine receptor. *Mol. Pharmacol.* **1994**, *45*, 978–982. (d) Nordstedt, C.; Fredholm, B. B. A modification of a protein-binding method for rapid quantification of cAMP in cell-culture supernatants and body fluid. *Anal. Biochem.* **1990**, *189*, 231–234.
- (19) (a) Matsuda, A.; Shinozaki, M.; Yamaguchi, T.; Homma, H.; Nomoto, R.; Miyasaka, T.; Watanabe, Y.; Abiru, T. 2-Alkynyladenosines: a novel class of selective adenosine  $A_2$  receptor agonists with potent antihypertensive effects. *J. Med. Chem.* **1992**, *35*, 241–252. (b) Cristalli, G.; Volpini, R.; Vittori, S.; Camaioni, E.; Monopoli, A.; Conti, A.; Dionisotti, S.; Zocchi, C.; Ongini, E. 2-Alkynyl derivatives of adenosine-5'-N-ethyluronamide (NECA): selective  $A_2$  adenosine receptor agonists with potent inhibitory activity on platelet aggregation. *J. Med. Chem.* **1994**, *37*, 1720–1726. (c) Lambertucci, C.; Costanzi, S.; Vittori, S.; Volpini, R.; Cristalli, G. Synthesis and adenosine receptor affinity and potency of 8-alkynyl derivatives of adenosine. *Nucleosides, Nucleotides Nucleic Acids* **2001**, *20*, 1153–1157.
- (20) Bevan, N.; Butchers, P. R.; Cousins, R.; Coates, J.; Edgar, E. V.; Morrison, V.; Sheehan, M. J.; Reeves, J.; Wilson, D. J. Pharmacological characterisation and inhibitory effects of (2R,3R,4S,5R)-2-(6-amino-2-[[[(1S)-2-hydroxy-1-(phenylmethyl)ethyl]amino]-9H-purin-9-yl]-5-(2-ethyl-2H-tetrazol-5-yl)tetrahydro-3,4-furandiol, a novel ligand that demonstrates both adenosine  $A_{2A}$  receptor agonist and adenosine  $A_3$  receptor antagonist activity. *Eur. J. Pharmacol.* **2007**, *564*, 219–225.
- (21) Morris, C. J. Casrageenan-induced pawedema in the rat and mouse. *Methods Mol. Biol.* **2003**, *225*, 115–121.
- (22) Otterness, I. G.; Moore, P. F. Carrageenan foot edema test. *Methods Enzymol.* **1988**, *162*, 320–327.
- (23) Jaakola, V. P.; Griffith, M. T.; Hanson, M. A.; Cherezov, V.; Chien, E. Y. T.; Lane, J. R.; Ijzerman, A. P.; Stevens, R. C. The 2.6 angstrom crystal structure of a human  $A_{2A}$  adenosine receptor bound to an antagonist. *Science* **2008**, *322*, 1211–1217.
- (24) Bradford, M. M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein–dye binding. *Anal. Biochem.* **1976**, *72*, 248–254.
- (25) Kecskés, A.; Tosh, D. K.; Wei, Q.; Gao, Z. G.; Jacobson, K. A. GPCR ligand dendrimer (GLiDe) conjugates: adenosine receptor interactions of a series of multivalent xanthine antagonists. *Bioconjugate Chem.* **2011**, *22*, 1115–1127.
- (26) Jarvis, M. F.; Schutz, R.; Hutchison, A. J.; Do, E.; Sills, M. A.; Williams, M. J. [ $^3\text{H}$ ]CGS 21680, a selective  $A_2$  adenosine receptor agonist directly labels  $A_2$  receptors in rat brain. *Pharmacol. Exp. Ther.* **1989**, *251*, 888–893.

(27) Olah, M. E.; Gallo-Rodriguez, C.; Jacobson, K. A.; Stiles, G. L.  $^{125}\text{I}$ -4-Aminobenzyl-5'-N-methylcarboxamidoadenosine, a high affinity radioligand for the rat  $A_3$  adenosine receptor. *Mol. Pharmacol.* **1994**, *45*, 978–982.

(28) (a) Nordstedt, C.; Fredholm, B. B. A modification of a protein-binding method for rapid quantification of cAMP in cell-culture supernatants and body fluid. *Anal. Biochem.* **1990**, *189*, 231–234.

(b) Post, S. R.; Ostrom, R. S.; Insel, P. A. Biochemical methods for detection and measurement of cyclic AMP and adenylyl cyclase activity. *Methods Mol. Biol.* **2000**, *126*, 363–374.

(29) Cheng, Y. C.; Prusoff, H. R. Relationship between the inhibition constant ( $K_i$ ) and the concentration of inhibitor which causes 50 per cent inhibition ( $I_{50}$ ) of an enzymatic reaction. *Biochem. Pharmacol.* **1973**, *22*, 3099–3108.

(30) Yeşilada, E.; Küpeli, E. *Berberis crataegina* DC. root exhibits potent anti-inflammatory, analgesic and febrifuge effects in mice and rats. *J. Ethnopharmacol.* **2002**, *79*, 237–248.