Synthesis and Binding Affinity of Homologated Adenosine Analogues as A3 Adenosine Receptor Ligands

Hyuk Woo Lee,† Won Jun Choi,‡ Kenneth A. Jacobson,§ and Lak Shin Jeong†,*

†Department of Bioinspired Science and Laboratory of Medicinal Chemistry, College of Pharmacy, Ewha Womans University, Seoul 120-750, Korea. *E-mail: lakjeong@ewha.ac.kr
‡College of Pharmacy, Dongguk University, Kyungki-do 410-774, Korea
§Molecular Recognition Section, Laboratory of Bioorganic Chemistry, National Institute of Diabetic, Digestive Disease and Kidney Disease, National Institutes of Health, Bethesda MD 20892-0810, USA

Received March 10, 2011, Accepted March 25, 2011

Homologated analogues 3a and 3b of potent and selective A3 adenosine receptor ligands, IB-MECA and dimethyl-IB-MECA were synthesized from commercially available 1-O-acetyl-2,3,5-tri-O-benzoyl-β-D-ribofuranose (4) via Co2(CO)8-catalyzed siloxymethylation as a key step. Unfortunately, homologated analogues 3a and 3b did not show significant binding affinities at three subtypes of adenosine receptors, indicating that free rotation, resulting from homologation, induced unfavorable interactions in the binding site of the receptor maybe due to the presence of many conformations.

Key Words : Co2(CO)8-catalyzed siloxymethylation, A3 adenosine receptor, Homologation

Introduction

Adenosine receptors (ARs) consisting of four subtypes, A1, A2A, A2B, and A3 play an important roles in regulating many physiological functions through binding with an endogenous adenosine.1 Thus, A3AR has been promising targets for the developments of new therapeutic agents against cancer, ischemia, inflammation, asthma, and glaucoma related to the signal transduction of cell.2

On the basis of the structure of adenosine, a number of adenosine analogues have been synthesized as AR ligands.2 Among these, IB-MECA (1, N6-(3-iodobenzyl)-5'-N-methyl-carboxamidoadenosine)3 has been known as one of the representative A3AR agonists. This compound showed high binding affinity (Ki = 1.0 nM) at the human A3AR with high selectivity to other subtypes. Compound 1 exhibited potent anticancer activity by inhibiting Wnt signaling pathway.4

Molecular modeling study indicates that NH of the 5'-uronamide served as a key hydrogen bonding donor in the binding site of A3AR, which was essential for the conformational change of the binding site required for receptor activation.5 Thus, the addition of methyl group on the 5'-uronamide of A3AR agonist 1, resulting in the formation of 2 (Dimethyl-Cl-IB-MECA) converted A3AR agonist 1 into potent and selective A3AR antagonist (Ki = 15.5 nM) because of the removal of a hydrogen bonding donor essential for the receptor activation.6

Introduction of the single bond between the purine base and the sugar makes the molecule adopt many conformations by free rotation, which can give a good chance to induce maximum favorable interactions in the binding site of the receptor.7 Thus, on the basis of potent binding affinity of compounds 1 and 2 at the A3AR, we designed and synthesized the homologated analogues 3a and 3b of compounds 1 and 2, using Co2(CO)8-catalyzed siloxymethylation8 as a key step. Herein, we report the synthesis of the homologated adenosine analogues 3a and 3b and their binding affinity at the A3AR.

Results and Discussion

Our synthetic strategy was to synthesize the homologated glycosyl donor and then to condense with 6-chloropurine. The homologated glycosyl donor 6 was synthesized from
commercially available 1-O-acetyl-2,3,5-tri-O-benzoyl-β-D-ribofuranose (4), as shown in Scheme 1.

1-O-Acetyl-2,3,5-tri-O-benzoyl-β-D-ribofuranose (4) was treated with carbon monoxide and a hydrosilane (HSiEt₂Me) in the presence of catalytic amounts of Co₂(CO)₈ to give the siloxymethylated compound 5. Treatment of 5 with tetra-n-butylammonium fluoride (TBAF) afforded glycosyl donor 6 which is ready for the Mitsunobu condensation. Condensation of 6 with 6-chloropurine under the Mitsunobu conditions produced 6-chloropurine derivative 7. Treatment of 7 with 3-iodobenzyl amine afforded the N₆-(3-iodobenzyl)amine derivative 8, which was treated with sodium methoxide to give triol 9. Compound 9 was treated with 2,2-dimethoxypropane under acidic conditions to give acetonide 10.

Scheme 2 illustrates the conversion of 4'-hydroxymethyl moiety into amide. Treatment of 10 with PDC in DMF afforded acid 11, which was coupled with methylamine and dimethylamine in the presence of HOBt and EDC to give the methylamido derivative 12 and dimethylamido derivative 13, respectively. Removal of the isopropylidene moiety in 12 and 13 under acidic conditions produced the final nucleo-

---

**Scheme 1**

**Scheme 2**
Table 1. Binding affinities of homologated adenosine derivatives 3a and 3b at three subtypes of ARs

<table>
<thead>
<tr>
<th>Compounds</th>
<th>$K_i$ (hA1AR)</th>
<th>$K_i$ (hA2AAR)</th>
<th>$K_i$ (hA3AR)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>nM or</td>
<td>nM or</td>
<td>nM or</td>
</tr>
<tr>
<td></td>
<td>% displ.</td>
<td>% displ.</td>
<td>% displ.</td>
</tr>
<tr>
<td>at 10 µM</td>
<td>at 10 µM</td>
<td>at 10 µM</td>
<td>at 10 µM</td>
</tr>
<tr>
<td>3a (R1 = H, R2 = Me)</td>
<td>0.3 ± 0.3%</td>
<td>14.0 ± 5.6%</td>
<td>8.7 ± 6.0%</td>
</tr>
<tr>
<td>3b (R1 = R2 = Me)</td>
<td>3.3 ± 3.3%</td>
<td>13.0 ± 8.2%</td>
<td>8.9 ± 1.8%</td>
</tr>
<tr>
<td>IB-MECA (1)</td>
<td>1620 ± 760</td>
<td>2910 ± 580</td>
<td>1.8 ± 0.7</td>
</tr>
<tr>
<td>Dimethyl-IB-MECA (2)</td>
<td>5870 ± 930</td>
<td>&gt; 10,000</td>
<td>29.0 ± 4.9</td>
</tr>
</tbody>
</table>

*All AR experiments were performed using adherent CHO cells stably transfected with cDNA encoding the appropriate human ARs (A1 AR and A3 AR in CHO cells and A2A AR in HEK-293 cells), using 1 nM [3H]CCPA (2-chloro-N6-cyclopentyladenosine) for A1 AR, 10 nM [3H]CGS 21680 (2-[p-(2-carboxyethyl)phenylethylamino]-5'-O-ethylcarboxamido-adenosine) for A2A AR, or 0.5 nM [125I]I-AB-MECA [N8-(4-amino-3-iodobenzyl)-5'-O-methylcarboxamidoadenosine] for A3 AR as radioligands, respectively. Values from the present study are expressed as mean ± s.e.m., n = 3-5.*

Sides 3a and 3b, respectively.

Radioligand binding assay was performed using adherent mammalian CHO (Chinese hamster ovary) cells stably transfected with cDNA encoding the appropriate human ARs (A1 AR and A3 AR) in CHO cells and A2A AR in HEK-293 cells, using 1 nM [3H]CCPA (2-chloro-N6-cyclopentyladenosine) for A1 AR, 10 nM [3H]CGS 21680 (2-[p-(2-carboxyethyl)phenylethylamino]-5'-O-ethylcarboxamido-adenosine) for A2A AR, or 0.5 nM [125I]I-AB-MECA [N8-(4-amino-3-iodobenzyl)-5'-O-methylcarboxamidoadenosine] for A3 AR as radioligands, respectively. Values are expressed as mean ± sem, n = 3-4 (outliers eliminated), and normalized against a non-specific binder, 5'-O-ethylcarboxamidoadenosine (NECA, 10 µM). Percentage value indicates the percent inhibition at a fixed concentration of 10 µM.

As shown in Table 1, homologated compounds 3a and 3b exhibited very low binding affinities at all three subtypes of human ARs, when compared with those of IB-MECA (1) and dimethyl-IB-MECA (2), which showed potent and selective binding affinity at the human A2A AR. This result indicates that the homologation disrupted favorable binding interactions of the compound at the ARs despite free rotation. Loss of binding affinity may be attributed to many conformations caused by free rotation, some of which induced unfavorable interactions at the AR binding sites.

In summary, we have accomplished the synthesis of homologated adenosine analogues 3a and 3b of potent A1 A2A AR ligands, IB-MECA and dimethyl-IB-MECA. Homologation was achieved using Co2(CO)8-catalyzed siloxymethylation. Despite their poor binding affinities at the AR, the result obtained from this study may be utilized for the identification of the binding mode of ARs.

**Experimental Section**

1H NMR spectra (CDCl3, CD3OD, or DMSO-d6) were recorded on Varian Unity Inova 400 MHz. Chemical shifts were reported in ppm units with TMS as the internal standard. 13C NMR spectra (CDCl3, CD3OD, or DMSO-d6) were recorded on Varian Unity Inova 100 MHz. Optical rotations were determined on Jasco in methanol or DMSO. UV spectra were recorded on U-3000 made by Hitachi in methanol or DMSO. Elemental analyses were measured on EA1110. The crude products were purified using a silica gel 60 (230-400 mesh, Merck). Reagents were purchased from Sigma Aldrich Company. All the anhydrous solvents were distilled over CaH2 or P2O5 or Na/benzophenone prior to the reaction.

1-Diethylmethylsilyloxymethyl-2,3,5-trienzeyl-β-d-ribofuranose (5). To the 250 mL of round bottomed flask flushed with CO (1 atm from a stock balloon), Co2(CO)8 (752.3 mg, 1.98 mmol) and HSIEt2Me (17.2 mL, 118.8 mmol) were added at room temperature. After the reaction mixture was stirred for 10 min, a solution of 4 (20.0 g, 39.6 mmol) in anhydrous CH2Cl2 (80 mL) was added, and the reaction mixture was stirred at 30 °C for 15 h under CO (1 atm). After the removal of the solvent, the residue was purified by silica gel column chromatography to give 5 (13.2 g, 58%) as a colorless syrup: [α]D25 +166.9° (c 8.30, MeOH); HR-MS (ESI): m/z calcd for C27H36O3Si [M+H]+: 577.2258; Found: 577.2230; 1H NMR (CDCl3) δ 0.10 (s, 3H), 0.62 (m, 4H), 0.96 (m, 6H), 3.88 (d, 2H, J = 3.6 Hz), 4.39 (q, 1H, J = 3.6 Hz), 4.56 (dd, 1H, J = 5.6, 11.4 Hz), 4.62 (m, 1H), 4.67 (dd, 1H, J = 3.6, 9.2 Hz), 5.69 (m, 2H), 7.31-8.08 (m, 15H); 13C NMR (CDCl3) δ =-48.9, 62.3, 6.87, 62.93, 64.91, 73.14, 73.32, 79.02, 83.63, 128.50, 128.56, 129.36, 129.62, 129.84, 129.86, 132.91, 133.22, 133.43, 133.46, 165.49, 165.69, 166.41; Anal. Calcd for C27H36O3Si: C, 66.4; H, 6.29. Found: C, 66.71; H, 6.31.

1-Hydroxymethyl-2,3,5-trienzeyl-β-d-ribofuranose (6). To a solution of 5 (12.0 g, 20.8 mmol) in anhydrous THF (100 mL) was added TBFA (25.0 mL, 25.0 mmol, 1.0 M in THF) at 0 °C, and the reaction mixture was stirred at room temperature for 1 h. The reaction mixture was evaporated, and the residue was purified by silica gel column chromatography to give 6 (8.35 g, 84%) as a colorless syrup: [α]D25 +70.92° (c 6.50, MeOH); HR-MS (ESI): m/z calcd for C27H35O3 [M+H]+: 477.1549; Found: 477.1521; 1H NMR (CDCl3) δ = 2.48 (br s, 1H), 3.82 (m, 1H), 3.94 (m, 1H), 4.38 (m, 1H), 4.59-4.72 (m, 3H), 5.69 (m, 2H), 7.36-7.96 (m, 15H); 13C NMR (CDCl3) δ = 61.93, 64.33, 72.18, 73.04, 80.18, 82.73, 128.60, 128.69, 129.28, 129.34, 129.74, 129.80, 129.88, 129.89, 129.92, 133.33, 133.46, 133.62, 165.61, 165.79, 166.67; Anal. Calcd for C27H35O3: C, 68.06; H, 5.08. Found: C, 68.11; H, 5.06.

6-Chloro-(2,3,5-trienzeyl-β-d-ribofuranosyl-9-methyl-yl)purine (7). To a solution of 6-chloropurine (1.77 g, 11.4 mmol) and Ph3P (4.13 g, 15.74 mmol) in anhydrous THF (50 mL) was added DIAD (3.10 mL, 15.74 mmol) at 0 °C under N2, and the reaction mixture was stirred for 30 min. To this mixture was added a solution of 3 (5.0 g, 10.5 mmol) in anhydrous THF (20 mL), and the reaction mixture was stirred at room temperature for 12 h. The reaction mixture...
was evaporated, and the residue was purified by silica gel column chromatography to give 7 (4.39 g, 68%) as a white foam: UV (MeOH) \( \lambda_{\text{max}} \) 265.0 nm (pH 7); [\( \alpha \)]\(_D^{25} \) = -35.2° (c 14.0, MeOH); HR-MS (ESI): \( m/z \) calc for \( \text{C}_8\text{H}_8\text{N}_3\text{O}_4 \) [M+H]: 498.0638; Found: 498.0615; \( ^1\)H NMR (CDCl\(_3\)) \( \delta \) 4.47 (dd, 1H, J = 4.0, 12.0 Hz), 4.55-4.76 (m, 5H), 5.34 (dd, 1H, J = 6.0, 7.6 Hz), 5.60 (dd, 1H, J = 3.2, 5.8 Hz), 7.36-7.91 (m, 15H), 8.22 (s, 1H), 8.61 (s, 1H); \( ^{13}\)C NMR (CDCl\(_3\)) \( \delta \) 45.02, 64.05, 72.33, 72.84, 78.68, 81.45, 128.73, 128.85, 129.07, 129.26, 129.74, 129.93, 131.30, 133.72, 133.86, 133.91, 146.33, 151.26, 152.20, 152.25, 165.53, 165.58, 166.17; Anal. Calcd for \( \text{C}_8\text{H}_8\text{N}_3\text{O}_4 \): C, 62.70; H, 4.11; N, 9.14. Found: C, 62.83; H, 4.16; N, 9.18.

**N\(^3\)-(3-Iodobenzylamino)-(2,3,5-tribenzoyl-\( \beta \)-d-ribufuranosyl-9-ymethyl)adenine (8).** To a solution of 6-chloropurine derivative 7 (4.1 g, 6.68 mmol) and 3-iodobenzylamine (1.34 mL, 10.03 mmol) in EtOH (50 mL) was added Et\( _3 \)N (2.79 mL, 20.04 mmol), and the reaction mixture was stirred overnight at room temperature. After evaporating the solvent, the residue was purified by silica gel column chromatography to give 8 (4.45 g, 82%) as a white foam: UV (MeOH) \( \lambda_{\text{max}} \) 268.5 nm (pH 7); [\( \alpha \)]\(_D^{25} \) = -60.46° (c 6.50, MeOH); HR-MS (ESI): \( m/z \) calc for \( \text{C}_{23}\text{H}_{22}\text{N}_5\text{O}_5 \) [M+H]: 538.0951; Found: 538.0923; \( ^1\)H NMR (CDCl\(_3\)) \( \delta \) 1.36 (s, 3H), 1.52 (s, 3H), 3.62 (dd, 1H, J = 2.0, 12.8 Hz), 3.71 (dd, 1H, J = 2.4, 12.8 Hz), 4.15 (m, 1H), 4.19 (dd, 1H, J = 3.2, 14.0 Hz), 4.52 (m, 1H), 4.60 (dd, 1H, J = 2.4, 6.2 Hz), 4.82 (brs, 2H), 4.92 (dd, 1H, J = 2.8, 6.4 Hz), 4.92 (dd, 1H, J = 2.8, 6.4 Hz), 6.07 (brs, 1H), 6.32 (brs, 1H), 7.05 (t, 1H, J = 8.0 Hz), 7.34 (d, 1H, J = 7.6 Hz), 7.60 (d, 1H, J = 7.6 Hz), 7.72 (s, 1H), 7.76 (s, 1H), 8.36 (s, 1H); \( ^{13}\)C NMR (CDCl\(_3\)) \( \delta \) 25.58, 27.30, 44.01, 46.67, 62.33, 82.83, 83.56, 85.11, 88.12, 94.79, 113.66, 127.10, 130.61, 136.78, 138.62, 139.11, 141.03, 153.07, 155.02; Anal. Calcd for \( \text{C}_{23}\text{H}_{22}\text{N}_5\text{O}_5 \): C, 49.64; H, 4.50; N, 13.03. Found: C, 49.68; H, 4.52; N, 13.06.

**N\(^3\)-(3-Iodobenzylamino)-(2',3',5'-O-isopropylidene-5'-N-methylcarboxamido-\( \beta \)-d-ribufuranosyl-9-ymethyl)adenine (12).** To a solution of 10 (2.0 g, 3.72 mmol) in anhydrous DMF (30 mL) was added pyridinium dichromate (14.0 g, 57.22 mmol), and the reaction mixture was stirred at room temperature for 20 h. The reaction mixture was poured into water (200 mL) and stirred at room temperature for 1 h. The precipitate was filtered, and the filter cake was washed with water (50 mL) and dried under high vacuum to give brownish solid 11 (1.70 g, 83%), which was used in the next step without further purification.

To a solution of 11 (1.0 g, 1.81 mmol), EDC (551 mg, 2.72 mmol), HO\( _3 \)B (367 mg, 2.72 mmol), and methylamine-HCl (183.7 mg, 2.72 mmol) in \( \text{CHCl}_3 \) (50 mL) was added DIPEA (0.946 mL, 5.43 mmol), and the mixture was stirred at room temperature for 12 h. The reaction mixture was evaporated, and the residue was purified by a silica gel column chromatography (hexane/EtOAc = 2:1-1:1) to give 12 (641.6 mg, 64%) as a white foam: UV (MeOH) \( \lambda_{\text{max}} \) 265.5 nm (pH 7); [\( \alpha \)]\(_D^{25} \) = -49.88° (c 8.20, MeOH); HR-MS (ESI): \( m/z \) calc for \( \text{C}_{78}\text{H}_{62}\text{N}_4\text{O}_{16} \) [M+H]: 1356.1060; Found: 1356.1034; \( ^1\)H NMR (CDCl\(_3\)) \( \delta \) 1.32 (s, 3H), 1.52 (s, 3H), 2.84 (d, 3H, J = 4.8 Hz), 4.28-4.36 (m, 3H), 4.47 (m, 1H), 4.52 (dd, 1H, J = 2.4, 4.8 Hz), 4.80 (brs, 2H), 4.94 (dd, 1H, J = 2.4, 6.2 Hz), 6.85 (brs, 1H), 7.00 (t, 1H, J = 7.6 Hz), 7.30 (d, 1H, J = 8.0 Hz), 7.55 (d, 1H, J = 8.0 Hz), 7.68 (brs, 2H), 8.36 (brs, 2H); \( ^{13}\)C NMR (CDCl\(_3\)) \( \delta \) 25.46, 25.92, 27.44, 43.74, 46.73, 81.27, 84.18, 84.50, 86.10, 94.69, 114.75, 119.79, 126.94, 130.47, 136.60, 140.11, 141.17, 149.86, 153.24, 154.99, 170.20; Anal. Calcd for \( \text{C}_{78}\text{H}_{62}\text{N}_4\text{O}_{16} \): C, 49.42; H, 4.46; N, 14.89. Found: C, 49.88; H, 4.50; N, 14.92.

**N\(^3\)-(3-Iodobenzylamino)-(2',3',5'-O-isopropylidene-5'-N-dimethylcarboxamido-\( \beta \)-d-ribufuranosyl-9-ymethyl)adenine (13).** Compound 13 (756.9 mg, 72%) was synthesized from 11 (1.0 g, 1.81 mmol) using dimethylamine-HCl (221.7 mg,
2.72 mmol) according to the procedure used in the preparation of compound 12.

UV (MeOH) \( \lambda_{\text{max}} \) 267.0 nm (pH 7); \( [\alpha]_D^{25} = -44.67^\circ \) (c 10.7, MeOH); HR-MS (ESI): \( m/z \) calead for \( C_{136}H_{153}NO_{12} [M+H]^+ \): 579.1217; Found: 579.1191; \( ^1H \) NMR (CDCl\( _3 \)) \( \delta \) 1.32 (s, 3H), 1.48 (s, 3H), 2.97 (s, 3H), 3.04 (s, 3H), 4.32 (dd, 1H, \( J = 9.2, 14.2 \) Hz), 4.40 (dd, 1H, \( J = 4.0, 14.0 \) Hz), 4.56 (m, 1H), 4.69 (dd, 1H, \( J = 2.4, 6.2 \) Hz), 4.80 (brs, 2H), 4.83 (d, 2H, \( J = 2.0 \) Hz), 6.59 (t, 1H, \( J = 6.0 \) Hz), 7.00 (t, 1H, \( J = 8.0 \) ), 7.30 (d, 1H, \( J = 7.2 \) Hz), 7.54 (d, 1H, \( J = 8.4 \) Hz), 7.68 (s, 1H), 7.86 (s, 1H), 8.36 (s, 1H); \( ^{13}C \) NMR (CDCl\( _3 \)) \( \delta \) 25.25, 27.07, 35.95, 37.27, 43.95, 45.72, 82.63, 83.32, 83.71, 84.71, 96.68, 113.89, 119.58, 126.96, 130.44, 134.52, 136.62, 141.42, 149.56, 153.17, 154.67, 162.47, 169.85; Anal. Calead for \( C_{136}H_{153}NO_{12} \): C, 83.71; H, 8.21; N, 15.05.

**Acknowledgments.** This work was supported by RP-Grant (2010-2011) of Ewha Womans University.

**References**