Synthesis and Bioactivity of Novel Trisubstituted Triazole Nucleosides

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ABSTRACT
A series of novel trisubstituted 1,2,3-triazole purine nucleosides were efficiently synthesized via Huisgen 1,3-dipolar cycloaddition in good yields. Bioactivity against cytomegalovirus (CMV) and varicella-zoster virus (VZV) in human embryonic lung cell cultures was evaluated and all compounds show low antiviral activity.

Introduction
More than half of the clinic drugs for treating viral disease are nucleoside analogues.[1] Certain acyclic nucleosides, in which the ribose sugar is replaced with a chain structure, have outstanding biological activity,[2] an example is acyclovir, the first acyclic nucleoside drug approved for treating HSV-1, HSV-2 and VZV.[1,3] Other acyclic nucleoside drugs include ganciclovir-treating cytomegalovirus (CMV),[4] penciclovir-treating varicella-zoster virus (VZV),[4] adefovir-treating herpesviruses, retroviruses and HBV,[5] tenofovir used as an anti-HIV and anti-HBV agent,[6] cidofovir-treating herpesviruses (CMV), and adenoviruses (Figure 1).[7]

Ribavirin, the first example of a triazole-containing nucleoside drug, presents a broad spectrum of antiviral activity against many DNA and RNA viruses.[8] Among various structural modifications of nucleosides, 1,2,3-triazole-nucleoside analogues have been extensively studied and exhibit broad bioactivity,[9] such as antibacterial[10], antifungal[10b,10c,11], anticancer[12], and antiviral[13]. These compounds include the replacement of sugar[9b,14] or nucleobases[15] with a 1,2,3-triazole moiety as well as an additional linker of 1,2,3-triazoles between a phosphonoalkyl unit and a nucleobase.[16]
In view of the fact that moderate activity is shown by compounds PTriaT, PTriaU, and PtoriaA\textsuperscript{[16h]} against HCV and by compound PTriaP towards HSV-1 and HSV-2 (Figure 2),\textsuperscript{[16j]} we assumed that 4,5-double substituted 1,2,3-triazole-nucleoside analogues might be biologically active.

### Results and Discussion

#### Chemistry

Azidation of ethyl bromoacetate 1 with NaN\textsubscript{3} gave ethyl 2-azidoacetate 2, which was directly used to be cyclized with 1,4-butynediol at 110\textdegree C without any catalysts and the corresponding 4,5-bis(hydroxymethyl)-1,2,3-triazole 3 was formed in 51% yields.\textsuperscript{[17]} Then selectively protecting one hydroxyl group in 3 was successfully performed in 73% yield via treatment with triphenylmethyl chloride. 4 was mesylated with methanesulfonyl chloride and then treated with nucleobases in DMF at room temperature to give the expected product 5 in good yields.\textsuperscript{[18]} Removal of the trityl group was smoothly carried out in the presence of trifluoroacetic acid (TFA) to give the product 6a and 6b (Scheme 1).

The structures of target compounds 6a and 6b were proven on the basis of \textsuperscript{1}H NMR and \textsuperscript{13}C NMR spectroscopic analysis. The \textsuperscript{1}H NMR spectrum of 6a showed singlets at $\delta$ 8.80 and 8.67 ppm for CH of the 6-chloropurine group, with signals...
at $\delta$ 3.79 and 1.03 ppm as quartet and triplet for CH$_2$ and CH$_3$ of the ethoxycarbonyl group, in addition to singlets at $\delta$ 5.78, 5.57 and 4.74 ppm for three other CH$_2$, respectively. Its $^{13}$C NMR spectra showed the characteristic signals at $\delta$ 166.82, 61.56 and 13.63 ppm corresponding to C=O, CH$_2$CH$_3$ and CH$_2$CH$_3$ of the ethoxycarbonyl group, signals at $\delta$ 151.72, 151.67, 149.12, 147.13, 146.87, 130.58, and 130.01 ppm for the 6-chloropurine group and triazole ring, in addition with signals
at $\delta$ 54.63, 49.20 and 34.54 ppm for three methylene groups, respectively. The spectroscopic analysis for $6b$ was similar with that of $6a$ and the data was in agreement with the structure.

After treatment of $6a$ or $6b$ with aqueous ammonia, the ester group was converted into the corresponding amides and the 6-chloropurine group was transformed into adenine, e.g. $6a$ converted to $7d$. The 6-chloro atom in $6b$, however, is intact under this condition. In aqueous TFA solution ($V(\text{TFA})/V(\text{H}_2\text{O}) = 3:1$), compounds $6b$ and $7b$ were converted to the corresponding guanine nucleosides $6c$ and $7c$, respectively. $^{[19]}$ The $^1\text{H}$ NMR spectrum of $6c$ showed the characteristic signal at $\delta$ 10.67 ppm as a singlet, which could prove this conversion.

Treatment of ester $4$ with methylamine at room temperature formed the amidic compound $8$, which converted to nucleoside $9$ in a good yield under similar condition for compound $5$. The $^1\text{H}$ NMR spectrum of $8$ showed the characteristic signals at $\delta$ 8.28 and 2.66 ppm corresponding to the presence of the CH$_3$NH group. After deprotecting the trityl group of $9a$ and $9b$ in TFA-CH$_2$Cl$_2$ solution, $10a$ and $10b$ were obtained, respectively. The conversion of 6-chloroguanine $10b$ to guanine $10c$ was smoothly performed in TFA aqueous solution at room temperature.

Selective substitution of the chlorine atom in $5b$ by amine was efficiently conducted via treatment with cyclopropylamine at $0^\circ\text{C}$ to form $11$ (Scheme 2). At $60^\circ\text{C}$, however, both the chlorine atom and the ester group were transformed to give $12$. The $^1\text{H}$ NMR spectrum of $11$ showed signals at $\delta$ 3.19–3.17, 0.68–0.62, and 0.61–0.54 ppm as multiplets for the CH and CH$_2$ of the cyclopropyl group, with signals at $\delta$ 3.01 ppm as singlet for the NH of cyclopropylamino group, respectively. Its $^{13}\text{C}$
NMR spectrum showed the characteristic signals at $\delta$ 24.17 and 6.80 ppm corresponding to CH and CH$_2$ of the cyclopropyl group, respectively. $^1$H and $^{13}$C NMR spectra of 12 showed the characteristic signals corresponding to the presence of two cyclopropylamino groups.

The final product 14 was obtained after treating 12 with TFA to remove the trityl group. Under similar deprotecting conditions, 13 was formed. Treatment of 13 with aqueous ammonia gave the target compound 15.

**Antiviral activity evaluation**

All the synthesized compounds 6a–c, 7b–d, 10a–c, and 13–15 were evaluated for antiviral activities against herpes viruses, using human embryonic lung (HEL) cell-based assays, cytomegalovirus (AD-169 strain and Davis strain), varicella-zoster virus (TK$^+$ VZV strain and TK$^-$ VZV strain). Ganciclovir, cidofovir, acyclovir, and brivudin were used as the reference compounds. The antiviral activity was expressed as the EC$_{50}$: the compound concentration required to reduce virus plaque formation by 50%. Unfortunately, none of these compounds demonstrated activity against these tested viruses as shown by the EC$_{50}$ values (>20 $\mu$M, Table 1). The cytotoxicity of the tested compounds toward the uninfected host cells was defined as the minimum cytotoxic concentration (MCC) that causes a microscopically detectable alteration of normal cell morphology.

**Table 1.** Anti-CMV and anti-VZV activities of the synthesized compounds.

<table>
<thead>
<tr>
<th>Compound</th>
<th>CMV</th>
<th>VZV</th>
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<tbody>
<tr>
<td></td>
<td>EC$_{50}$ ($\mu$M)$^a$</td>
<td>Cytotoxicity ($\mu$M)</td>
</tr>
<tr>
<td>6a</td>
<td>&gt;20</td>
<td>&gt;100</td>
</tr>
<tr>
<td>6b</td>
<td>&gt;20</td>
<td>100</td>
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<tr>
<td>6c</td>
<td>&gt;20</td>
<td>&gt;100</td>
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<tr>
<td>7b</td>
<td>&gt;20</td>
<td>&gt;100</td>
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<tr>
<td>7c</td>
<td>&gt;20</td>
<td>&gt;100</td>
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<tr>
<td>7d</td>
<td>&gt;20</td>
<td>&gt;100</td>
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<tr>
<td>10a</td>
<td>ND</td>
<td>ND</td>
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<tr>
<td>10b</td>
<td>&gt;20</td>
<td>&gt;100</td>
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<td>&gt;20</td>
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</tr>
<tr>
<td>15</td>
<td>&gt;100</td>
<td>&gt;100</td>
</tr>
<tr>
<td>Ganciclovir</td>
<td>7.0</td>
<td>7.0</td>
</tr>
<tr>
<td>Cidofovir</td>
<td>0.70</td>
<td>1.02</td>
</tr>
<tr>
<td>Acyclovir</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td>Brivudin</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

$^a$Effective concentration required to reduce virus plaque formation by 50%. Virus input was 100 plaque forming units (PFU) for HCMV. Virus input was 20 plaque forming units (PFU).

$^b$Minimum cytotoxic concentration that causes a microscopically detectable alteration of cell morphology.

$^c$Not determined.
Conclusion

In conclusion, a number of 1,4,5-trisubstituted 1H-1,2,3-triazole purines were effectively synthesized. Antiviral activity against CMV and VZV in human embryonic lung (HEL) cell cultures was evaluated and none show significant activity towards these selected virus.

Experimental

$^1$H NMR, $^{13}$C NMR spectra were recorded on a Bruker AM-400 nuclear magnetic resonance spectrometer. Chemical shifts (δ) are reported in ppm relative to the TMS peak. Melting points are uncorrected and were determined on a XT4A type melting point apparatus. High-resolution mass spectra were acquired with an Agilent Accurate-Mass-Q-TOF MS 6520 system equipped with an electrospray ionization (ESI) source. Thin-layer chromatography (TLC) was carried out on silica gel precoated plates purchased from Yantai Institute of Chemical Industry. Column chromatography separation was carried out on silica gel (40~50 μm, Yantai Institute of Chemical Industry). All the chemical reagents were analytical or chemical pure reagents.

**Ethyl 2-(4,5-bis(hydroxymethyl)-1H-1,2,3-triazol-1-yl)acetate (3)**

A solution of ethyl 2-bromoacetate 1 (11.40 mL, 100 mmol) and NaN$_3$ (8.60 g, 100 mmol) in acetone / H$_2$O (50 mL, V/V = 3:2) was heated at 60°C overnight. The residue was dissolved in EtOAc and washed with brine, the organic layer was dried over MgSO$_4$, filtered, and evaporated under reduced pressure to afford ethyl 2-azidoacetate 2 (9.67 g, 75%) as an colorless oil, which was used in the next step without being further purified.

A solution of 2 (9.67 g, 75 mmol) and 1,4-butynediol (5.86 g, 68 mmol) was stirred at 110°C overnight. The reaction was monitored by TLC (stained with iodine). When the reaction was completed, the mixture was cooled to room temperature and diluted in EtOAc. The organic layer was evaporated under reduced pressure. The residue was purified by silica gel column chromatography using petroleum ether—ethyl acetate (3:7, v/v) as an eluent to afford compound 3 (7.20 g, 51%) as a white solid, m.p.105°C ~ 107°C.$^1$H NMR (400 MHz, DMSO-d$_6$) δ 5.38 (t, $J = 5.5$ Hz, 1 H), 5.12 (t, $J = 5.5$ Hz, 1 H), 4.58 (d, $J = 5.3$ Hz, 2 H), 4.51 (d, $J = 5.4$ Hz, 2 H), 4.17 (q, $J = 7.0$ Hz, 2 H), 1.21 (t, $J = 7.2$ Hz, 3 H)$^{13}$C NMR (400 MHz, DMSO-d$_6$) δ 167.16, 144.36, 134.71, 61.38, 54.12, 51.19, 49.24, 13.96. HRMS calculated for [C$_8$H$_{14}$N$_3$O$_4$+H]$^+$: 216.0984, Found: 216.0987.

**Ethyl 2-(5-(hydroxymethyl)-4-((trityloxy)methyl)-1H-1,2,3-triazol-1-yl)acetate (4)**

To a solution of 3 (4.30 g, 20 mmol) in CH$_2$Cl$_2$ (25 mL) was added TEA (2.90 mL, 22 mmol) and triphenylmethyl chloride (6.05 g, 22 mmol) at 0°C. The mixture was
stirred at 0°C for 5 h and subsequently quenched with saturated aqueous NaHCO₃. The mixture was extracted with CH₂Cl₂ (50 mL). The organic layer was dried over MgSO₄, filtered, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography using the mixture petroleum ether—ethyl acetate (1:1, v/v) as an eluent to afford compound 4 (6.67 g, 73%) as a white solid, m.p.107°C ~ 109°C.¹H NMR (400 MHz, DMSO-d₆) δ 7.44 – 7.26 (m, 15H), 5.42 (t, J = 5.4 Hz, 1H), 5.38 (s, 2H), 4.47 (d, J = 5.3 Hz, 2H), 4.05 (s, 2H), 1.21 (t, J = 7.1 Hz, 3H).

¹³C NMR (100 MHz, DMSO-d₆) δ 167.05, 143.47, 140.76, 135.55, 128.27, 127.99, 127.15, 86.55, 61.41, 56.71, 51.17, 49.38, 13.92. HRMS calculated for [C₂₇H₂₈N₃O₄]+: 458.2080, Found: 458.2078.

**Ethyl 2-((5-((6-chloro-9H-purin-9-yl)methyl)-4-((trityloxy)methyl)-1H-1,2,3-triazol-1-yl)acetate (5a)**

To a solution of 4 (4.57 g, 10 mmol) in CH₂Cl₂ (25 mL) was added TEA (1.46 mL, 11 mmol) and methanesulfonyl chloride (850 µL, 11 mmol) at 0°C. The mixture was stirred at 0°C for 10 min and subsequently quenched with saturated aqueous NaHCO₃. The mixture was extracted with CH₂Cl₂ (50 mL). The organic layer was dried over MgSO₄, filtered, and concentrated under reduced pressure afforded crude product 4. A mixture of crude methane sulfate 4 (4.16 g, 8.0 mmol), K₂CO₃ (1.32 g, 9.60 mmol) and 6-chloropurine (1.47 g, 9.6 mmol) in DMF (20 mL) was stirred at room temperature for 5 h. The reaction mixture was quenched with distilled water. The residue was dissolved in CH₂Cl₂ (100 mL) and washed with H₂O (80 mL). The organic layer was dried over MgSO₄, filtered, and concentrated in vacuo. The crude residue was purified on a silica gel column using petroleum ether – EtOAc (1:1, v/v) as eluent, giving pure compound 5a (3.32 g, 70%) as a white solid, m.p.110°C ~ 111°C.¹H NMR (400 MHz, DMSO-d₆) δ 8.66 (s, 1H), 8.46 (s, 1H), 7.35 – 7.25 (m, 15 H), 5.64 (s, 4 H), 4.23 (s, 2 H), 3.97 – 3.90 (m, 2 H), 1.09 (t, J = 7.1 Hz, 3 H).¹³C NMR (100 MHz, DMSO-d₆) δ 166.82, 151.63, 151.58, 149.18, 146.60, 143.29, 130.69, 130.60, 128.18, 127.93, 127.12, 86.77, 61.69, 56.96, 49.34, 34.95, 13.66. HRMS calculated for [C₃₂H₂₉ClN₇O₃]+: 594.2020, Found: 594.2025.

**Ethyl 2-((5-((2-amino-6-chloro-9H-purin-9-yl)methyl)-4-((trityloxy)methyl)-1H-1,2,3-triazol-1-yl)acetate (5b)**

Compound 5b was synthesized from 4 by a similar procedure to that described for 5a as a white solid (3.6 g, 69%), m. p. 115°C ~ 117°C.¹H NMR (400 MHz, DMSO-d₆) δ 7.76 (s, 1H), 7.34 – 6.90 (m, 15 H), 5.64 (s, 4 H), 4.23 (s, 2 H), 3.97 – 3.90 (m, 2 H), 1.09 (t, J = 7.1 Hz, 3 H).¹³C NMR (100 MHz, DMSO-d₆) δ 166.87, 159.67, 153.69, 149.52, 143.30, 142.89, 141.92, 131.13, 128.20, 127.89, 127.08, 122.99, 86.72, 61.65, 56.73, 49.46, 34.23, 13.66. HRMS calculated for [C₃₂H₃₀ClN₈O₃]+: 609.2129, Found: 609.2130.
Ethyl 2-(5-((6-chloro-9H-purin-9-yl)methyl)-4-(hydroxymethyl)-1H-1,2,3-triazol-1-yl)acetate (6a)

To a solution of 5a (3.00 g, 5.06 mmol) in CH₂Cl₂ (15 mL) at 0°C (ice bath) was added TFA (2.28 mL, 25.30 mmol). After stirred 7 h at 0°C, the mixture was neutralized to pH 7 with saturated aqueous NaHCO₃. The residue was concentrated in vacuo and purified on a silica gel column using petroleum ether – EtOAc (2:3, v/v) as eluent to afford compound 6a (0.78 g, 74%) as a white solid, m.p. 172°C ∼ 173°C. 

$^1$H NMR (400 MHz, DMSO-d₆) δ 8.80 (s, 1 H), 8.67 (s, 1 H), 5.78 (s, 2 H), 5.57 (s, 2 H), 4.74 (s, 2 H), 3.79 (q, J = 7.2 Hz, 2 H), 1.03 (t, J = 7.1 Hz, 3 H).

$^{13}$C NMR (400 MHz, DMSO-d₆) δ 166.82, 151.72, 151.67, 149.12, 147.13, 146.87, 130.58, 130.01, 61.56, 54.63, 49.20, 34.54, 13.63. HRMS calculated for [C₁₃H₁₄ClN₇O₃]+: 352.0918, Found: .352.0919.

Ethyl 2-(5-((2-amino-6-chloro-9H-purin-9-yl)methyl)-4-(hydroxymethyl)-1H-1,2,3-triazol-1-yl)acetate (6b)

Compound 6b was synthesized from 5b by a similar procedure to that described for 6a as a white solid (0.94 g, 81%), m.p. 185°C ∼ 187°C. 

$^1$H NMR (400 MHz, DMSO-d₆) δ 7.64 (s, 1 H), 6.52 (s, 2 H), 5.08 (d, J = 15.4 Hz, 2 H), 5.01 (s, 2 H), 4.23 (s, 2 H), 3.39 (q, J = 7.1 Hz, 2 H), 0.62 (t, J = 7.1 Hz, 3 H).

$^{13}$C NMR (100 MHz, DMSO-d₆) δ 166.80, 159.79, 153.79, 149.47, 146.69, 142.69, 130.42, 122.95, 61.65, 54.62, 49.31, 33.90, 13.67. HRMS calculated for [C₁₃H₁₅ClN₈O₃]+: 367.1028, Found: .367.1028.

Ethyl 2-(5-((2-amino-6-oxo-9H-purin-9-yl)methyl)-4-(hydroxymethyl)-1H-1,2,3-triazol-1-yl)acetate (6c)

To a solution of 6b (183 mg, 0.50 mmol) in CH₂Cl₂ at room temperature was drop-wise added TFA/H₂O = 3:1 (4 mL, v/v) and stirred overnight. The residue was concentrated in vacuo and purified on a silica gel column using CH₃OH – CH₂Cl₂ (1:9, v/v) as eluent to afford compound 6c (78 mg, 45%) as a white solid, m. p. 175°C ∼ 177°C. 

$^1$H NMR (400 MHz, DMSO-d₆) δ 10.67 (s, 1 H), 7.68 (s, 1 H), 6.48 (s, 2 H), 5.53 (s, 2 H), 5.40 (d, J = 5.5 Hz, 1 H), 5.34 (s, 2 H), 4.66 (d, J = 5.5 Hz, 2 H), 3.93 (q, J = 7.1 Hz, 2 H), 1.11 (t, J = 7.1 Hz, 3 H).

$^{13}$C NMR (100 MHz, DMSO-d₆) δ 166.81, 156.74, 153.63, 150.84, 146.29, 137.08, 130.82, 116.11, 61.62, 54.54, 49.22, 33.56, 13.73. HRMS calculated for [C₁₃H₁₆N₈O₄]+: 349.1367, Found: 349.1369.

2-(5-((6-Chloro-9H-purin-9-yl)methyl)-4-(hydroxymethyl)-1H-1,2,3-triazol-1-yl)acetamide (7d)

A solution of 6a (121 mg, 0.35 mmol) and NH₃·H₂O (10.00 mL) was stirred at room temperature overnight. The residue was concentrated in vacuo and purified with
CH$_3$OH to give pure 7d (73 mg, 65%) as a white solid, m.p. 189°C ∼ 191°C. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 8.15 (s, 2 H), 7.97 (s, 1 H), 7.27 (s, 2 H), 5.58 (s, 2 H), 5.44 (m, 1 H), 5.29 (s, 2 H), 4.62 (s, 2 H). $^{13}$C NMR (100 MHz, DMSO-$d_6$) $\delta$ 167.62, 156.44, 152.95, 149.62, 146.50, 141.08, 131.36, 118.84, 54.98, 50.62, 34.61. HRMS calculated for [C$_{11}$H$_{13}$N$_9$O$_2$]$:^+$: 304.1270, Found: 304.1272.

**2-(5-(2-Amino-6-chloro-9H-purin-9-yl)methyl)-4-(hydroxymethyl)-1H-1,2,3-triazol-1-yl)acetamide (7b)**

Compound 7b was synthesized from 6b by a similar procedure to that described for 7d as a white solid (75 mg, 67%), m.p. 215°C ∼ 217°C. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 8.11 (s, 1 H), 7.78 (s, 1 H), 7.45 (s, 1 H), 6.98 (s, 2 H), 5.37 (s, 2 H), 5.29 (s, 2 H), 4.60 (s, 2 H). $^{13}$C NMR (100 MHz, DMSO-$d_6$) $\delta$ 167.34, 159.69, 153.81, 149.41, 146.19, 142.85, 130.44, 123.06, 54.61, 50.38, 34.36. HRMS calculated for [C$_{11}$H$_{13}$N$_9$O$_3$]$:^+$: 338.08752, Found: 338.08765.

**2-(5-(2-Amino-6-oxo-9H-purin-9-yl)methyl)-4-(hydroxymethyl)-1H-1,2,3-triazol-1-yl)acetamide (7c)**

A solution of 7b (188 mg, 0.56 mmol) and TFA/H$_2$O = 3:1 (4 mL, v/v) was heated to 60°C and stirred overnight. The residue was concentrated in vacuo and purified with CH$_3$OH to give pure 7c (72 mg, 41%) as a white solid, m.p. 237°C ∼ 239°C. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 10.72 (s, 1 H), 7.77 (s, 1 H), 6.48 (s, 2 H), 5.44 (s, 2 H), 5.40–5.31 (m, 3 H), 4.62 (s, 2 H). $^{13}$C NMR (100 MHz, DMSO-$d_6$) $\delta$ 168.32, 156.57, 153.68, 150.81, 146.16, 137.08, 130.49, 115.72, 54.61, 50.38, 34.36. HRMS calculated for [C$_{11}$H$_{13}$N$_9$O$_3$]$:^+$: 320.1214, Found: 320.1216.

**2-(5-(Hydroxymethyl)-4-((trityloxy)methyl)-1H-1,2,3-triazol-1-yl)-N-methylacetamide (8)**

To a solution of 4 (914 mg, 2.0 mmol) in CH$_2$Cl$_2$ at 0°C (ice bath) was added methylamine (2 mL) and stirred for 10 min. The residue was concentrated in vacuo and purified on a silica gel column using petroleum ether – EtOAc (3:7, v/v) as eluent to afford compound 8 (831 mg, 94%) as a white solid, m.p. 109°C ∼ 110°C. $^1$H NMR (400 MHz, DMSO-$d_6$) $\delta$ 8.28 (d, $J$ = 4.5 Hz, 1 H), 7.52–7.26 (m, 15 H), 5.34 (t, $J$ = 5.4 Hz, 1 H), 5.13 (s, 2 H), 4.48 (d, $J$ = 5.4 Hz, 2 H), 4.07 (s, 2H), 2.66 (d, $J$ = 4.6 Hz, 3 H). $^{13}$C NMR (101 MHz, DMSO-$d_6$) $\delta$ 166.43, 144.02, 141.43, 136.22, 128.79, 128.46, 127.62, 87.04, 57.29, 51.57, 50.80, 26.13. HRMS calculated for [C$_{26}$H$_{27}$N$_4$O$_3$]$:^+$: 443.2083, Found: 443.2084.

**2-(5-((6-Chloro-9H-purin-9-yl)methyl)-4-((trityloxy)methyl)-1H-1,2,3-triazol-1-yl)-N-methylacetamide (9a)**

Compound 9a was synthesized from 8 by a similar procedure to that described for 5a as a white solid (464 mg, 68%), m.p. 105°C ∼ 106°C. $^1$H NMR (400 MHz,
DMSO-\textit{d}6 δ 8.65 (s, 1 H), 8.47 (s, 1 H), 8.28 (d, \textit{J} = 4.6 Hz, 1 H), 7.31 – 7.22 (m, 15 H), 5.59 (s, 2 H), 5.31 (s, 2 H), 4.08 (s, 2 H), 2.54 (d, \textit{J} = 4.5 Hz, 3 H). $^{13}$C NMR (101 MHz, DMSO-\textit{d}6) δ 166.05, 152.04, 151.96, 149.52, 147.16, 143.72, 143.29, 131.44, 131.09, 128.59, 128.33, 127.54, 87.14, 57.41, 50.90, 35.75, 26.02. HRMS calculated for [C$_{31}$H$_{28}$ClN$_8$O$_2$+H]$^+$: 579.2024, Found: 579.2023.

2-(5-((2-Amino-6-chloro-9H-purin-9-yl)methyl)-4-((trityloxy)methyl)-1H-1,2,3-triazol-1-yl)-N-methylacetamide (9b)

Compound 9b was synthesized from 8 by a similar procedure to that described for 5a as a white solid (456 mg, 65%), m.p. 112 °C ∼ 114 °C. $^1$H NMR (400 MHz, DMSO-\textit{d}6) δ 8.29 (d, \textit{J} = 4.6 Hz, 1 H), 7.79 (s, 1 H), 7.33 – 7.24 (m, 15 H), 6.86 (s, 2 H), 5.34 (s, 2 H), 5.31 (s, 2 H), 4.01 (s, 2 H), 2.62 (d, \textit{J} = 4.5 Hz, 3 H). $^{13}$CNMR (101 MHz, DMSO-\textit{d}6) δ 166.21, 160.16, 154.22, 149.94, 143.76, 142.99, 142.52, 131.75, 128.63, 128.33, 127.51, 123.54, 87.14, 57.27, 51.03, 35.19, 26.11. HRMS calculated for [C$_{31}$H$_{29}$ClN$_9$O$_2$+H]$^+$: 594.2133, Found: 594.2136.

2-(5-((6-Chloro-9H-purin-9-yl)methyl)-4-(hydroxymethyl)-1H-1,2,3-triazol-1-yl)-N-methylacetamide (10a)

Compound 10a was synthesized from 9a by a similar procedure to that described for 6a as a white solid (129 mg, 70%), m.p. 168 °C ∼ 169 °C. $^1$H NMR (400 MHz, DMSO-\textit{d}6) δ 8.76 (s, 1 H), δ 8.63 (s, 1 H), 8.14 (d, \textit{J} = 4.4 Hz, 1 H), 5.72 (s, 2 H), 5.30 (t, \textit{J} = 5.2 Hz, 1 H), 5.24 (s, 2 H), 4.64 (d, \textit{J} = 5.2 Hz, 2 H), 2.42 (d, \textit{J} = 4.4 Hz, 3 H). $^{13}$C NMR (400 MHz, DMSO-\textit{d}6) δ 165.92, 152.26, 151.96, 149.35, 147.63, 146.93, 131.07, 130.60, 55.04, 50.77, 35.33, 25.90. HRMS calculated for [C$_{12}$H$_{13}$ClN$_8$O$_2$+H]$^+$: 337.0923, Found: 337.0922.

2-(5-((2-Amino-6-chloro-9H-purin-9-yl)methyl)-4-(hydroxymethyl)-1H-1,2,3-triazol-1-yl)-N-methylacetamide (10b)

Compound 10b was synthesized from 9b by a similar procedure to that described for 6a as a white solid (148 mg, 65%), m.p. 175°C ~ 177°C. $^1$H NMR (400 MHz, DMSO-\textit{d}6) δ 8.12 (d, \textit{J} = 4.3 Hz, 1 H), 8.02 (s, 1 H), 6.87 (s, 2 H), 5.35 (s, 2 H), 5.25 (s, 2 H), 5.21 (t, \textit{J} = 5.4 Hz, 1 H), 4.54 (d, \textit{J} = 5.2 Hz, 2 H), 2.49 (d, \textit{J} = 4.4 Hz, 2 H). $^{13}$C NMR (100 MHz, DMSO-\textit{d}6) δ 165.71, 159.67, 153.78, 149.36, 146.24, 142.79, 130.42, 123.03, 54.61, 50.49, 34.27, 25.62. HRMS calculated for [C$_{12}$H$_{14}$ClN$_9$O$_2$+H]$^+$: 352.1037, Found: 352.1033.

2-(5-((2-Amino-6-oxo-9H-purin-9(6H)-yl)methyl)-4-(hydroxymethyl)-1H-1,2,3-triazol-1-yl)-N-methylacetamide (10c)

Compound 10c was synthesized from 10b by a similar procedure to that described for 6c as a white solid (68 mg, 42%), m.p. 159°C ~ 161°C. $^1$H NMR (400 MHz,
DMSO-d6) δ 10.78 (s, 1 H), 8.24 (s, 1 H), 7.84 (s, 1 H), 6.56 (s, 2 H), 5.64 – 4.96 (m, 5 H), 4.59 (s, 2 H), 2.61 (s, 3 H). 13C-NMR (100 MHz, DMSO-d6) δ 165.78, 156.42, 153.78, 146.05, 130.67, 115.17, 54.55, 50.46, 34.14, 25.68. HRMS calculated for [C12H15N9O3+H]+: 334.1371, Found: 334.1373.

Ethyl 2-((2-amino-6-(cyclopropylamino)-9H-purin-9-yl)methyl)-4-((trityloxy)methyl)-1H-1,2,3-triazol-1-yl)acetate (11)

To a solution of 5b (1.15 g, 1.89 mmol) in DMF (5 mL) was added cyclopropylamine (654 µL) and K2CO3 (312 mg, 2.23 mmol) at 0 °C. The mixture was stirred at 0 °C for 7 h and subsequently quenched with saturated aqueous NaHCO3. The mixture was extracted with CH2Cl2 (50 mL). The organic layer was dried over MgSO4, filtered, and concentrated under reduced pressure. The residue was purified by silica gel column chromatography using the CH3OH – CH2Cl2 (5:95, v/v) as eluent to afford compound 11 (816 mg, yield 69%) as a white solid, m.p. 123 °C ∼ 125 °C. 1H NMR (400 MHz, DMSO-d6) δ 7.44 – 7.22 (m, 16 H), 5.78 (s, 2 H), 5.62 (s, 2 H), 5.19 (s, 2 H), 4.16 (s, 2 H), 3.99 (q, J = 7.1 Hz, 2 H), 3.19 – 3.17 (m, 1 H), 3.01 (s, 1 H), 1.13 (t, J = 7.1 Hz, 3 H), 0.68 – 0.62 (m, 2 H), 0.61 – 0.54 (m, 2 H). 13C NMR (101 MHz, DMSO-d6) δ 167.29, 160.61, 156.33, 143.86, 143.08, 136.34, 132.35, 128.76, 128.44, 127.60, 113.43, 87.23, 62.07, 57.20, 49.94, 33.87, 24.17, 14.15, 6.80. HRMS calculated for [C35H36N9O3+H]+: 630.2941, Found: 630.2944.

2-((2-Amino-6-(cyclopropylamino)-9H-purin-9-yl)methyl)-N-cyclopropylacetamide (12)

A solution of 5b (383 mg, 0.63 mmol) and cyclopropylamine (3 mL) was heated at 60°C overnight in a seal tube. The residue was concentrated under reduced pressure and purified by silica gel column chromatography using the CH3OH – CH2Cl2 (5:95, v/v) as eluent to afford compound 12 (290 mg, 72%) as a white solid, m.p.167°C ∼ 169°C. 1H NMR (400 MHz, DMSO-d6) δ 8.49 (d, J = 3.9 Hz, 1 H), 7.42 – 7.21 (m, 16 H), 5.84 (s, 2 H), 5.42 (s, 2 H), 5.18 (s, 2 H), 4.06 (s, 2 H), 3.19 – 3.17 (m, 1 H), 3.01 (s, 1 H), 2.68 – 2.61 (m, 1 H), 0.68 – 0.62 (m, 4 H), 0.60 – 0.54 (m, 2 H), 0.49 – 0.44 (m, 2 H). 13C NMR (100 MHz, DMSO-d6) δ 166.96, 160.61, 156.33, 143.86, 143.08, 136.43, 132.35, 128.76, 128.44, 127.60, 113.43, 87.23, 62.07, 57.20, 49.94, 33.87, 24.17, 14.15, 6.80; HRMS calculated for [C36H37N10O2+H]+: 641.3101, Found: 641.3108.

Ethyl 2-((2-amino-6-(cyclopropylamino)-9H-purin-9-yl)methyl)-4-(hydroxy methyl)-1H-1,2,3-triazol-1-yl)acetate (13)

Compound 13 was synthesized from 11 by a similar procedure to that described for 6a as a white solid (67 mg, 35%), m.p.165°C ∼ 167°C. 1H NMR (400 MHz, DMSO-d6) δ 7.98 (s, 1 H), 7.39 (s, 2 H), 5.55 (s, 2 H), 5.44 (s, 2 H), 4.67 (s, 2 H), 3.95 (q, J = 7.1 Hz, 2 H), 2.88 (m, 1 H), 1.12 (t, J = 7.1 Hz, 3 H), 0.91 – 0.80 (m, 2 H), 0.77 – 0.67 (m, 2 H). 13C NMR (100 MHz, DMSO-d6) δ 166.82, 158.10, 157.79, 146.26,
130.98, 118.74, 115.76, 112.78, 61.55, 54.54, 49.25, 33.26, 21.13, 13.69, 6.48. HRMS calculated for [C_{16}H_{21}N_{9}O_{3}+H]^+: 388.1846, Found: 337.1848.

2-(5-{(2-Amino-6-(cyclopropylamino)-9H-purin-9-yl)methyl}-4-(hydroxymethyl)-1H-1,2,3-triazol-1-yl)-N-cyclopropylacetamide (14)

Compound 14 was synthesized from 12 by a similar procedure to that described for 6a as a white solid (105 mg, 65%), m.p. 178°C ∼ 180°C. $^1$H NMR (400 MHz, DMSO-d$_6$) δ 8.43 (d, J = 4.0 Hz, 1 H), 7.73 (s, 1 H), 7.37 (s, 2 H), 5.40 (s, 2 H), 5.31 (s, 2 H), 4.61 (d, J = 5.3 Hz, 2 H), 3.01 (s, 1 H), 2.60–2.66 (m, 1 H), 0.66 – 0.63 (m, 4 H), 0.58 – 0.57 (m, 2 H), 0.48 – 0.44 (m, 2 H). $^{13}$C NMR (100 MHz, DMSO-d$_6$) δ 166.50, 160.09, 155.94, 145.88, 136.81, 131.15, 112.94, 54.60, 50.43, 33.58, 22.34, 6.38, 5.62. HRMS calculated for [C$_{17}$H$_{22}$N$_{10}$O$_2$+H]^+: 399.2000, Found: 399.2000.

2-(5-{(2-Amino-6-(cyclopropylamino)-9H-purin-9-yl)methyl}-4-(hydroxymethyl)-1H-1,2,3-triazol-1-yl)acetamide (15)

Compound 15 was synthesized from 13 by a similar procedure to that described for 7d as a white solid (66 mg, 60%), m.p. 202°C ∼ 204°C. $^1$H NMR (400 MHz, DMSO-d$_6$) δ 8.46 (s, 1 H), 7.74 (s, 1 H), 7.40 (s, 1 H), 5.95 (s, 2 H), 5.48 (s, 2 H), 5.31 (s, 2 H), 4.62 (s, 2 H), 2.99 (s, 1 H), 2.63 (m, 1 H), 0.65 – 0.61 (m, 2 H), 0.57 – 0.46 (m, 2 H). $^{13}$C NMR (100 MHz, DMSO-d$_6$) δ 166.56, 160.13, 155.96, 145.91, 136.82, 131.21, 112.95, 54.62, 50.46, 33.59, 22.38, 6.41. HRMS calculated for [C$_{14}$H$_{18}$N$_{10}$O$_2$+H]^+: 359.1690, Found: 359.1687.

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References


