Design, Synthesis and in vitro Anti-Zika virus evaluation of novel Sinefungin derivatives

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KEYWORDS: anti-Zika virus, Sinefungin, EPZ004777, structure-activity relationships, methyltransferases

ABSTRACT: 1bf shows better activity (IC₅₀ = 4.56 µM) than EPZ004777 (IC₅₀ = 35.19 µM). Intermediate 9a displays good activity (IC₅₀ = 29.98 µM) and acceptable cytotoxicity (CC₅₀ > 200 µM).

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KEYWORDS: anti-Zika virus, Sinefungin, EPZ004777, structure-activity relationships, methyltransferases

ABSTRACT: We report herein the design and synthesis of a series of novel Sinefungin (SIN) derivatives, based on the structures of SIN and its analogue EPZ004777. Our results reveal that target compounds 1ad-af, 1ba-bb and 1bf-bh show better activity (IC$_{50}$ = 4.56-20.16 µM) than EPZ004777 (IC$_{50}$ = 35.19 µM). Surprisingly, SIN was founded to be not as active (IC$_{50}$ > 50 µM) as we and other research groups predicted. Interestingly, the intermediates 9a-b and 11b display potent anti-ZIKV potency (IC$_{50}$ = 6.33-29.98 µM), and compound 9a also exhibits acceptable cytotoxicity (CC$_{50}$ > 200 µM), suggesting their promising potential to be leads for further development.

1. Introduction

Zika virus (ZIKV) was first identified in 1947 in the Zika forest of Uganda, where it was isolated from the blood of sentinel rhesus macaques. [1] It belongs to the flavivirus genus of the Flaviviridae family, is related to yellow fever virus (YFV), dengue virus (DENV) and west nile virus (WNV). In the decades following its discovery, ZIKV posed little concern to the general public as it remained relatively dormant. The first outbreak occurred on the island of Yap in the federated states of Micronesia in April 2007. [2-3] Subsequently, ZIKV strains have become more prevalent, leading to an increase in global epidemics. More than 60 countries and territories had reported ZIKV infection since the first epidemic. [4] Importantly, the ZIKV can lead to the rare birth defect microcephaly and other neurological disorders in infants and adults. [5] It was reported that over 3500 babies were born with microcephaly between Oct. 2015 and Jan. 2016 in Brazil. The world health organization (WHO) declared in November 2016 that the ZIKV is a highly significant and a long-term problem. [6]
infection. Sinefungin (SIN, Figure 1) was isolated from the fermentation broth of *Streptomyces griseoletus* NRRL 3739 by Eli Lilly as a potential antifungal antibiotic. [7-8] It is structurally similar to S-Adenosyl methionine (SAM) and acts as a competitive inhibitor of numerous Methyltransferases (Mtases). It was reported that SIN exhibited antitumor, [9] antiviral [10-11] and antiparasitic activity [12]. Recently, SIN was demonstrated to show considerable anti-flavivirus activity (WNV, DENV-2 and YFV). Since ZIKV belongs to the flavivirus family, we agreed with others [13-15] in speculating that SIN might also be a potential ZIKV inhibitor.

**Figure 1.** Design of new SIN derivatives

To date, there are no clinically approved vaccines or antiviral drugs for the treatment of ZIKV infection. Sinefungin (SIN, Figure 1) was isolated from the fermentation broth of *Streptomyces griseoletus* NRRL 3739 by Eli Lilly as a potential antifungal antibiotic. [7-8] It is structurally similar to S-Adenosyl methionine (SAM) and acts as a competitive inhibitor of numerous Methyltransferases (Mtases). It was reported that SIN exhibited antitumor, [9] antiviral [10-11] and antiparasitic activity [12]. Recently, SIN was demonstrated to show considerable anti-flavivirus activity (WNV, DENV-2 and YFV). Since ZIKV belongs to the flavivirus family, we agreed with others [13-15] in speculating that SIN might also be a potential ZIKV inhibitor.

**Scheme 1.** Synthesis of the target compounds

SIN is water soluble, and displays a lower log P value of -3.01 (calculated by chemdraw 16.0) which limits its membrane permeability. [13, 16] EPZ004777, a SIN structural analogue containing a lipophilicity side chain, was developed as a potent histone methyltransferase inhibitor. [17] We hypothesized that EPZ004777 could address the issue of SIN membrane permeability while retaining the anti-ZIKV activity.

Recently, the crystal structural of ZIKV Mtase with SIN was resolved by Kamil Hercik et al. Based on the structural data, they suggested modification of the adenine moiety of SIN to increase selectivity and binding affinity of ZIKV Mtase. [14] Additionally, researchers from Novartis Institute for Tropical Disease (NITD) discovered NITD 10, a S-adenosyl-l-homocysteine (SAH) analogue with 3-chloro-benzyl at the N-6 position of adenine. It selectively inhibited DENV-3 Mtase (Ki = 0.002-0.24 μM) and WNV Mtase (Ki = 0.044-5.68 μM) without suppressing host MTases (Ki > 50 μM). [18] Thus, we sought to design, synthesis
and evaluate a new series of SIN derivatives containing 3-fluoro- or chloro-benzyl moieties at N-6 position of adenine and a lipophilicity side chain (aryl W) while integrating the structure features of \textit{NITD 10} and EPZ004777.

2. Results and discussion

2.1. Chemistry

The synthesis of new SIN derivatives 1 is shown in scheme 1. Coupling of the chloropurine 2 with substituted benzyl amines in EtOH at reflux resulted in adenines 3a-b. The primary alcohol of 3a-b was converted to the corresponding amines 4a-b via standard Mitsunobu displacement with phthalimide followed by hydrazinolysis in 75-77% yields over two steps. Reductive amination of 4a-b with acetone in the presence of NaCNBH₃ and acetic acid provided isopropyl amines 5a-b in good yields. Michael addition of compounds 5a-b with methyl acrylate in MeOH at reflux over 24 hours furnished 6a-b in a low yield, but under microwave radiation at 90 °C over 3 hours gave 6a-b in 76-80% yields. Reduction of methyl esters 6a-b with LiAlH₄ in THF afforded alcohols 7a-b, which upon Mitsunobu reaction and hydrazinolysis generated amines 8a-b. Treatment of 8a-b with isocyanate in DCM followed by removal of the acetonide protecting group with TFA furnished the SIN derivatives 1ab-af, and 1ba-bf.

Scheme 2. Synthesis of compounds 9-12

To achieve structurally diverse targets, the acetonide protecting group of the intermediates 5-8 was also removed by TFA in DCM, as outlined in scheme 2. The resulting compounds 9-12 were also tested for their anti-ZIKV activity.

2.2. Anti-ZIKV activity

All new SIN derivatives 1aa-ah, 1ba-bh, and deprotected intermediates 9-12 were evaluated for their \textit{in vitro} anti-ZIKV activity in an infection-based cell culture model that utilizes a ZIKV strain SMGC and BHK cell line by Cell Titer-Glo Luminescent Cell Viability Assay (Promega). The ZIKV was obtained from the Institute of Microbiology, Chinese Academy of Sciences, Beijing, China. The IC₅₀ values of the SIN derivatives 1aa-ah, 1ba-bh and intermediates 9-12 along with SIN and EPZ004777 for comparison were summarized in μM in Tables 1 and 2, respectively.

Surprisingly, SIN (IC₅₀ > 50 μM) is not as active against the ZIKV as we predicted, whereas EPZ004777 displays considerable anti-ZIKV activity (IC₅₀ = 35.19 μM). Among the target compounds, 1ad-af, 1ba-bb, and 1bf-bh exhibit potent activity (IC₅₀ < 30 μM); 1aa-ac, 1ag-ah, and 1bc are not active against ZIKV; 1bd-be are failure to acquire anti-ZIKV activity in this model, possibly due to their high toxicity (CC₅₀ = 6.35-6.73 μM) in BHK cell line. In particular, compounds 1ad-af, 1bb, and 1bf (IC₅₀ = 4.56-6.99 μM) were found to be 5.0-7.7-fold more potent than the reference EPZ004777.

Generally, compounds with chlorine as the aryl X substituent display higher activity than the corresponding fluorne analogues (1aa-ab vs 1ba-bb; 1af-ah vs 1bf-bh). Notably, this SAR result is consistent with an earlier report on the DENV-related SAR observations by NITD, [18] suggesting that the Mtase structures of ZIKV and DENV might be similar. When the aryl X
substituent is fluorine, compounds 1ad-af with 4-\text{Bu}-phenyl, 4-\text{CF}_3\text{-phenyl} and 3-\text{CF}_3\text{-phenyl} as the aryl \text{W} group show excellent anti-ZIKV potency (IC$_{50}$ = 6.46-6.99 µM). In contrast, introduction of other aryl \text{W} substituents leads to a compete loss of activity (1aa-ac and 1ag-ah, IC$_{50}$ > 50 µM). When the aryl X substituent is chloride, apart from a few exceptions (1bc-be), all of these compounds display potent activity (IC$_{50}$ < 30 µM), and the contribution of aryl \text{W} group to the activity is in this order: 3-\text{CF}_3\text{-phenyl} > 4-\text{Me-phenyl} > cyclohexyl > 4-\text{F-phenyl} > 4-\text{Bu-phenyl}.

Subsequently, intermediates 9-12 were tested for their anti-ZIKV activity (Table 2). Most of the intermediates 10a-b, 11a and 12a-b are not active (IC$_{50}$ > 50 µM) but 9a, 9b and 11b (IC$_{50}$ = 6.33-29.98 µM) fortunately display potent anti-ZIKV activity. Among them, compound 9b (IC$_{50}$ = 6.33 µM) demonstrated to be more potent than its fluorine analogue 10a. Interestingly, this SAR is similar as above in the SIN derivatives.

**Table 1. Structures and anti-ZIKV activity of the target compounds**

<table>
<thead>
<tr>
<th>Compds.</th>
<th>X</th>
<th>W</th>
<th>IC$_{50}$ (µM)</th>
<th>CC$_{50}$ (µM)</th>
<th>SI</th>
</tr>
</thead>
<tbody>
<tr>
<td>1aa</td>
<td>F</td>
<td>4-F-phenyl</td>
<td>&gt;50</td>
<td>ND</td>
<td>NA</td>
</tr>
<tr>
<td>1ab</td>
<td>F</td>
<td>4-Me-phenyl</td>
<td>&gt;50</td>
<td>ND</td>
<td>NA</td>
</tr>
<tr>
<td>1ac</td>
<td>F</td>
<td>4-MeO-phenyl</td>
<td>&gt;50</td>
<td>ND</td>
<td>NA</td>
</tr>
<tr>
<td>1ad</td>
<td>F</td>
<td>4-\text{t-Bu-phenyl}</td>
<td>6.65 ± 0.07</td>
<td>18.71±0.76</td>
<td>2.81</td>
</tr>
<tr>
<td>1ae</td>
<td>F</td>
<td>4-\text{CF}_3\text{-phenyl}</td>
<td>6.46±0.22</td>
<td>20.27±0.93</td>
<td>3.14</td>
</tr>
<tr>
<td>1af</td>
<td>F</td>
<td>3-\text{CF}_3\text{-phenyl}</td>
<td>6.99±0.39</td>
<td>12.88±0.28</td>
<td>1.84</td>
</tr>
<tr>
<td>1ag</td>
<td>F</td>
<td>\text{t-Bu}</td>
<td>&gt;50</td>
<td>ND</td>
<td>NA</td>
</tr>
<tr>
<td>1ah</td>
<td>F</td>
<td>Cyclohexyl</td>
<td>&gt;50</td>
<td>ND</td>
<td>NA</td>
</tr>
<tr>
<td>1ba</td>
<td>Cl</td>
<td>4-F-phenyl</td>
<td>15.29 ± 8.37</td>
<td>20.01±7.83</td>
<td>1.31</td>
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<tr>
<td>1bb</td>
<td>Cl</td>
<td>4-Me-phenyl</td>
<td>5.80 ± 1.42</td>
<td>19.11±0.76</td>
<td>3.29</td>
</tr>
<tr>
<td>1bc</td>
<td>Cl</td>
<td>4-MeO-phenyl</td>
<td>&gt;50</td>
<td>ND</td>
<td>NA</td>
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<td>1bd</td>
<td>Cl</td>
<td>4-\text{t-Bu-phenyl}</td>
<td>NA</td>
<td>6.73±0.02</td>
<td>NA</td>
</tr>
<tr>
<td>1be</td>
<td>Cl</td>
<td>4-\text{CF}_3\text{-phenyl}</td>
<td>NA</td>
<td>6.35±1.13</td>
<td>NA</td>
</tr>
<tr>
<td>1bf</td>
<td>Cl</td>
<td>3-\text{CF}_3\text{-phenyl}</td>
<td>4.56±3.84</td>
<td>5.88±1.62</td>
<td>1.29</td>
</tr>
<tr>
<td>1bg</td>
<td>Cl</td>
<td>\text{t-Bu}</td>
<td>20.61±0.38</td>
<td>58.49±1.16</td>
<td>2.84</td>
</tr>
<tr>
<td>1bh</td>
<td>Cl</td>
<td>Cyclohexyl</td>
<td>12.24±4.02</td>
<td>57.57±1.42</td>
<td>4.70</td>
</tr>
<tr>
<td>SIN</td>
<td></td>
<td></td>
<td>&gt;50</td>
<td>ND</td>
<td>NA</td>
</tr>
<tr>
<td>EPZ004777</td>
<td></td>
<td></td>
<td>35.19±7.02</td>
<td>&gt;200</td>
<td>&gt;5.68</td>
</tr>
</tbody>
</table>

ND, not detected; NA, not available.

### 2.3. Cytotoxicity

The SIN derivatives and additional intermediates that displayed considerable anti-ZIKV activity (IC$_{50}$ < 50 µM) were further evaluated for cytotoxicity test against BHK cell line by Cell Titer-Glo Luminescent Cell Viability Assay (Promega). Unfortunately, all the tested SIN derivatives (CC$_{50}$ = 5.88-58.49 µM) exhibit higher cytotoxicity than EPZ004777 (CC$_{50}$ > 200 µM), and their selective indexes (SI = 1.29-4.70) are lower than EPZ004777 (SI > 5.68). The best compound 1bf displays the highest cytotoxicity (CC$_{50}$ = 5.88 µM), the least 1bg also exhibits the lowest cytotoxicity (CC$_{50}$ = 58.49 µM). Considering both of the activity and cytotoxicity, compound 1bh which holds the highest selective index (SI = 4.70) among these analogues could be selected as a lead compound for further modification.

To our delight, we observed that compound 9a shows acceptable cytotoxicity (CC$_{50}$ > 200 µM) and potent anti-ZIKV activity (IC$_{50}$ = 29.98 µM), suggesting compound 9a might be a potent
candidate for further development. Compounds 9b and 11b with higher cytotoxicity (CC\textsubscript{50} = 29.39-70.61 µM) deserve further modification in the future.

3. Conclusion

In summary, the natural product SIN was selected as our lead compound but proved to be inactive in this study, whereas its structural analogue EPZ004777 displays considerable anti-ZIKV activity (IC\textsubscript{50} = 35.19 µM). A series of structural unique SIN derivatives with a 3-fluoro or chloro-benzyl group and a lipophilicity side chain were designed, synthesized and evaluated for anti-ZIKV activity. SIN derivatives 1ad-af, 1ba-bb, and 1bf-bh show more potent activity (IC\textsubscript{50} = 4.56-20.16 µM) than EPZ004777, but their CC\textsubscript{50} values are lower than EPZ004777 (CC\textsubscript{50} > 200 µM). We also observed that intermediates 9a-b and 11b display potent anti-ZIKV activity (IC\textsubscript{50} = 6.33-29.98 µM). These intermediates, possessing a simplified structure, could be selected as new lead compounds for further studies. In addition, we found that compound 9a displays acceptable cytotoxicity (CC\textsubscript{50} > 200 µM), suggesting its promising potential as a candidate for further development. Studies to determine the in vivo efficacy of 9a are currently underway.

Table 2. Anti-ZIKV activity and cytotoxicity of compounds 9-12

<table>
<thead>
<tr>
<th>Compds.</th>
</tr>
</thead>
<tbody>
<tr>
<td>IC\textsubscript{50} (µM)</td>
</tr>
<tr>
<td>9a</td>
</tr>
<tr>
<td>9b</td>
</tr>
<tr>
<td>10a</td>
</tr>
<tr>
<td>10b</td>
</tr>
<tr>
<td>11a</td>
</tr>
<tr>
<td>11b</td>
</tr>
<tr>
<td>12a</td>
</tr>
<tr>
<td>12b</td>
</tr>
<tr>
<td>SIN</td>
</tr>
<tr>
<td>EPZ004777</td>
</tr>
</tbody>
</table>

ND, not detected; NA, not available.

4. Experimental protocols

4.1. Chemistry

\(^1\text{H}\) NMR spectra were determined on a Varian Mercury-400 or Bruker 500 M spectrometer in MeOD or CDCl\textsubscript{3} using tetramethylsilane as an internal standard. Electrospray ionization (ESI) mass spectra was obtained on an Agilent 1260-6420 Mass spectrum instruments. The reagents were all of analytical grade or chemically pure. TLC was performed on silica gel plates (Merck, ART5554 60F254). All the anhydrous solvents were purchased from J&K Scientific.

4.2. Synthesis

4.2.1. ((3aR,4R,6R,6aR)-6-(6-((3-fluorobenzyl)amino)-9H-purin-9-yl)-2,2-dimethyl tetrahydrofuro[3,4-d][1,3]dioxol-4-yl)methanol 3a

To a stirred solution of compound 2 (3 g, 9.2 mmol) in EtOH (50 mL) was added Et\textsubscript{3}N (2.6 mL, 18.4 mmol) and 3-fluorobenzylamine (2.1 mL, 18.4 mmol) at room temperature. The mixture was stirred for 5 hours at 40 °C and concentrated. The residue was purified by column chromatography over silica gel (DCM : MeOH : NH\textsubscript{3}H\textsubscript{2}O = 200 : 10 : 0.1) to yield compound 3a (3.13 g, 82%) as a colorless oil; [\(\alpha\)]\textsubscript{D}\textsuperscript{20} = -167.77 (c 0.78, MeOH); \(^1\text{H}\) NMR (500 MHz, CDCl\textsubscript{3}) \(\delta\) 8.39 (s, 1H, purin-H), 7.79 (s, 1H, purin-H), 7.35-7.30 (m, 1H, Ar-H), 7.17 (d, J = 7.5 Hz, 1H, Ar-H), 7.11 (d, J = 8 Hz, 1H, Ar-H), 6.59 (brs, 1H, NH), 5.89 (s, 1H, tetrahydrofuro-H), 5.24 (t, J = 5.1 Hz, 1H, tetrahydrofuro-H), 5.14 (d, J = 5.5 Hz, 1H, tetrahydrofuro-H), 4.89 (brs, 2H, benzyl-CH\textsubscript{2}), 4.58 (s, 1H, tetrahydrofuro-H), 4.00 (d, J = 12.6 Hz, 1H, CH\textsubscript{2}OH-CH), 3.84 (d, J = 12.6 Hz, 1H, CH\textsubscript{2}OH-CH), 1.68 (s, 3H, CH\textsubscript{3}), 1.36 (s, 3H, CH\textsubscript{3}); \(^1\text{C}\) NMR (400 MHz, CDCl\textsubscript{3}) \(\delta\)
162.5 (d, J = 242.6 Hz, Ar-C), 154.8 (purin-C), 152.8 (purin-C), 147.5 (purin-C), 140.7 (purin-C), 139.8 (Ar-C), 130.2 (d, J = 8.1 Hz, Ar-C), 123.12 (d, J = 2.5 Hz, Ar-H), 121.2 (purin-C), 114.5 (d, J = 21.8 Hz, Ar-C), 114.0 (Ar-C), 94.4 (tetrahydrofuro-C), 86.1 (tetrahydrofuro-C), 83.1 (tetrahydrofuro-C), 81.7 (tetrahydrofuro-C), 63.4 (CH$_2$-CH$_3$), 43.8 (benzyl-CH$_2$-C), 27.6 (CH$_3$), 25.2 (CH$_3$); LRMS (ESI): m/z = 438 [M + Na]$^+$. 

4.2.2. ((3aR,4R,6R,6aR)-6-(6-((3-chlorobenzyl)amino)-9H-purin-9-yl)-2,2-dimethyl tetrahydrofuro[3,4-d][1,3]dioxol-4-yl)methanol 3b

Following above synthetic procedure of compound 3a, replacing 3-fluorobenzylamine with 3-chlorobenzylamine afforded compound 3b (85%) as a colorless oil, $[\alpha]_{20}^D = -59.03$ (c 0.93, MeOH); $^1$H NMR (500 MHz, CDCl$_3$) δ 8.40 (s, 1H, purin-H), 7.79 (s, 1H, purin-H), 7.39 (s, 1H, Ar-H), 7.30-7.28 (m, 3H, Ar-H), 6.59 (brs, 1H, NH), 5.89 (s, 1H, tetrahydrofuro-H), 5.24 (t, J = 5.1 Hz, 1H, tetrahydrofuro-H), 5.14 (d, J = 5.5 Hz, 1H, tetrahydrofuro-H), 4.89 (brs, 2H, benzyl-CH$_2$), 4.58 (s, 1H), 4.00 (d, J = 12.6 Hz, 1H, CH$_2$OH-CH), 3.84 (d, J = 12.6 Hz, 1H, CH$_2$OH-CH), 1.68 (s, 3H, CH$_3$), 1.42 (s, 3H, CH$_3$); LRMS (ESI): m/z = 454 [M + Na]$^+$. 

4.2.3. 9-((3aR,4R,6R,6aR)-6-(aminomethyl)-2,2-dimethyltetrahydrofuro[3,4-d][1,3]dioxol-4-yl)-N-(3-fluorobenzyl)-9H-purin-6-amine 4a

To a stirred solution of compound 3a (1 g, 2.4 mmol) in THF (20 mL) was added Ph$_3$P (1.26 g, 4.8 mmol), DIAD (0.94 mL, 4.8 mmol) and phthalimide (0.53 g, 3.6 mmol) at room temperature. The mixture was stirred for 3 hours and concentrated. The residue was purified by column chromatography over silica gel (DCM : MeOH : NH$_3$H$_2$O = 200 : 10 : 0.1) to yield a brown oil, which was used directly for the next step.

To a stirred solution of above oil in EtOH (50 mL) was added hydrazine hydrate (1 mL) at room temperature. The mixture was refluxed for 2 hours and filtered. The filtrate was concentrated. The residue was purified by column chromatography over silica gel (DCM : MeOH : NH$_3$H$_2$O = 100 : 10 : 0.2) to yield compound 4a (680 mg, 68%) as a colorless oil, $^1$H NMR (500 MHz, CDCl$_3$) δ 8.44 (s, 1H, purin-H), 7.88 (s, 1H, purin-H), 7.35-7.30 (m, 1H, Ar-H), 7.19 (d, J = 7.5 Hz, 1H, Ar-H), 7.12 (d, J = 8.2 Hz, 1H, Ar-H), 7.0 (t, J = 5.1 Hz, 1H, Ar-H), 6.34 (brs, 1H, NH), 6.04 (d, J = 2.6 Hz, 1H, tetrahydrofuro-H), 5.48-5.46 (m, 1H, tetrahydrofuro-H), 5.16-5.14 (m, 1H, tetrahydrofuro-H), 4.91 (brs, 2H, benzyl-CH$_2$), 4.36 (brs, 1H, tetrahydrofuro-H), 3.16-3.10 (m, 2H, CH$_2$), 1.64 (s, 3H, CH$_3$), 1.40 (s, 3H, CH$_3$); $^{13}$C NMR (400 MHz, CDCl$_3$) δ 162.5 (d, J = 242.5 Hz, Ar-C), 154.7 (purin-C), 153.2 (purin-C), 141.1 (d, J = 7.1 Hz, Ar-C), 140.0 (purin-C), 139.41 (purin-C), 130.1 (d, J = 8.2 Hz, Ar-C), 123.1 (d, J = 2.7 Hz, Ar-C), 120.5 (purin-C), 114.7 (acetónide-C), 114.5 (d, J = 21.6 Hz, Ar-C), 114.4 (d, J = 21.5 Hz, Ar-C), 91.2 (tetrahydrofuro-C), 86.1 (tetrahydrofuro-C), 83.5 (tetrahydrofuro-C), 81.6 (tetrahydrofuro-C), 43.3 (CH$_2$-C), 41.8 (benzyl-CH$_2$-C), 27.2 (CH$_2$-C), 26.5 (CH$_3$-C); LRMS (ESI): m/z = 415 [M + H]$^+$. 

4.2.4. 9-((3aR,4R,6R,6aR)-6-(aminomethyl)-2,2-dimethyltetrahydrofuro[3,4-d][1,3]dioxol-4-yl)-N-(3-chlorobenzyl)-9H-purin-6-amine 4b

Following above synthetic procedure of compound 4a, replacing 3a with 3b afforded compound 4b (75%) as a colorless oil, $[\alpha]_{20}^D = -23.43$ (c 1.05, MeOH); $^1$H NMR (500 MHz, CDCl$_3$) δ 8.44 (s, 1H, purin-H), 7.88 (s, 1H, purin-H), 7.40 (s, 1H, Ar-H), 7.35-7.30 (m, 3H, Ar-H), 6.34 (brs, 1H, NH), 6.06 (d, J = 2.6 Hz, 1H, tetrahydrofuro-H), 5.48-5.46 (m, 1H, tetrahydrofuro-H), 5.16-5.14 (m, 1H, tetrahydrofuro-H), 4.91 (brs, 2H, benzyl-CH$_2$), 4.30 (brs, ...
1H, tetrahydrofuro-H), 3.10-2.99 (m, 2H, CH₂), 1.64 (s, 3H, CH₃), 1.43 (s, 3H, CH₃); LRMS (ESI): m/z = 431 [M + H⁺].

4.2.5. N-(3-fluorobenzyl)-9-((3aR,4R,6R,6aR)-6-((isopropylamino)methyl)-2,2-dimethyl tetrahydrofuro[3,4-d][1,3]dioxol-4-yl)-9H-purin-6-amine 5a

To a stirred solution of compound 4a (1 g, 2.4 mmol) in MeOH (30 mL) was added acetone (3 mL) and NaCNBH₃ (0.6 g, 9.6 mmol) at room temperature. The mixture was stirred for 15 minutes, adjusted to pH 7 by acetic acid. The mixture was stirred 4 hours at the same temperature, quenched by 1M NaOH solution (10 mL) at 0°C, diluted by H₂O (50 mL), and extracted by DCM (30 mL × 3). The organic layer was washed by brine, dried over anhydrous MgSO₄, filtered and concentrated. The residue was purified by column chromatography over silica gel (DCM : MeOH : NH₃·H₂O = 100 : 10 : 1) to yield compound 5a (850 mg, 77%) as an oil, ¹H NMR (500 MHz, CDCl₃) δ 8.43 (s, 1H, purin-H), 7.89 (s, 1H, purin-H), 7.35-7.30 (m, 1H, Ar-H), 7.18 (d, J = 7.5 Hz, 1H, Ar-H), 7.12 (d, J = 9.5 Hz, Ar-H), 7.01 (t, J = 7.0 Hz, 1H, Ar-H), 6.48 (brs, 1H, NH), 6.05 (s, 1H, tetrahydrofuro-H), 5.51 (brs, 1H, tetrahydrofuro-H), 5.10-5.08 (m, 1H, tetrahydrofuro-H), 4.90 (brs, 2H, benzyl-CH₂), 4.40 (brs, 1H, tetrahydrofuro-H), 2.98-2.90 (m, 2H, CH₂), 2.80-2.78 (m, 1H, isopropyl-CH), 1.65 (s, 3H, CH₃), 1.43 (s, 3H, CH₃), 1.08 (d, J = 6.2 Hz, 3H, isopropyl-CH₃); ¹³C NMR (400 MHz, CDCl₃) δ 162.5 (d, J = 242.5 Hz, Ar-C), 154.7 (purin-C), 153.2 (purin-C), 148.6 (purin-C), 141.1 (d, J = 6.8 Hz, Ar-C), 139.4 (purin-C), 130.1 (d, J = 8.2 Hz, Ar-C), 123.0 (d, J = 2.7 Hz, Ar-C), 120.5 (purin-C), 114.6 (acetonide-C), 114.5 (d, J = 21.6 Hz, Ar-C), 114.4 (d, J = 21.5 Hz, Ar-C), 90.9 (tetrahydrofuro-C), 85.7 (tetrahydrofuro-C), 83.5 (tetrahydrofuro-C), 82.2 (tetrahydrofuro-C), 48.9 (isopropyl-CH-C), 48.8 (CH₂-C), 43.9 (benzyl-C), 27.3 (acetonide-CH₃-C), 25.4 (acetonide-CH₃-C), 22.7 (isopropyl-CH₃-C), 22.6 (isopropyl-CH₃-C); LRMS (ESI): m/z = 457 [M + H⁺].

4.2.6. N-(3-chlorobenzyl)-9-((3aR,4R,6R,6aR)-6-((isopropylamino)methyl)-2,2-dimethyl tetrahydrofuro[3,4-d][1,3]dioxol-4-yl)-9H-purin-6-amine 5b

Following above synthetic procedure of compound 5a, replacing 4a with 4b afforded compound 5b (75%) as an oil, LRMS (ESI): m/z = 473 [M + H⁺].

4.2.7. methyl 3-(((3aR,4R,6R,6aR)-6-(6-((3-fluorobenzyl)amino)-9H-purin-9-yl)-2,2-dimethyltetrahydrofuro[3,4-d][1,3]dioxol-4-yl)methyl)(isopropyl)amino)propionate 6a

A solution of 5a (1 g, 2.2 mmol) and methyl acrylate (0.4 mL) in MeOH (10 mL) was sealed in a microwave tube, heated to 90°C under microwave radiation for 3 hours. The mixture was concentrated and purified by column chromatography over silica gel (DCM : MeOH = 40 : 1) to yield compound 6a (0.9 g, 76%) as an oil, ¹H NMR (500 MHz, CDCl₃) δ 8.43 (s, 1H, purin-H), 7.90 (s, 1H, purin-H), 7.35-7.30 (m, 1H, Ar-H), 7.18 (d, J = 7.5 Hz, 1H, Ar-H), 7.12 (d, J = 9.5 Hz, Ar-H), 7.01 (t, J = 7.0 Hz, 1H, Ar-H), 6.32 (brs, 1H, NH), 6.07 (s, 1H, tetrahydrofuro-H), 5.57 (brs, 1H, tetrahydrofuro-H), 5.08 (brs, 1H, tetrahydrofuro-H), 4.90 (brs, 2H, benzyl-CH₂), 4.34 (brs, 1H, tetrahydrofuro-H), 3.69 (s, 3H, MeO-CH₃), 2.94-2.48 (m, 7H, CH₂CH₂, CH₂, and isopropyl-CH), 1.65 (s, 3H, acetonide-CH₃), 1.43 (s, 3H, acetonide-CH₃), 1.05 (s, 3H, isopropyl-CH₃), 0.92 (s, 3H, isopropyl-CH₃); ¹³C NMR (400 MHz, CDCl₃) δ 162.5 (d, J = 242.5 Hz, Ar-C), 154.7 (purin-C), 153.2 (purin-C), 148.6 (purin-C), 141.1 (d, J = 6.8 Hz, Ar-C), 139.8 (purin-C), 130.1 (d, J = 8.2 Hz, Ar-C), 123.0 (d, J = 2.7 Hz, Ar-C), 120.5 (purin-C), 114.5 (d, J = 21.6 Hz, Ar-C), 114.4 (d, J = 21.5 Hz, Ar-C), 91.2 (tetrahydrofuro-C), 83.6 (tetrahydrofuro-C), 83.2 (tetrahydrofuro-C), 77.2 (tetrahydrofuro-C), 51.9 (MeO-C), 51.7 (CH₂-C), 46.3 (CH₂-C),
43.8 (benzyl-CH$_2$), 27.1 (acetonide-CH$_3$-C), 18.8 (isopropyl-CH$_3$-C), 16.7 (isopropyl-CH$_3$-C); LRMS (ESI): m/z = 543 [M + H]$^+$. 

4.2.8. methyl 3-(((3aR,4R,6R,6aR)-6-(6-((3-fluorobenzyl)amino)-9H-purin-9-yl)-2,2-dimethyltetrahydrofuro[3,4-d][1,3]dioxol-4-yl)methyl)(isopropyl)amino)propanoate 6b
Following above synthetic procedure of compound 6a, replacing 5a with 5b afforded compound 6b (80%) as an oil, LRMS (ESI): m/z = 559 [M + H]$^+$. 

4.2.9. 3-(((3aR,4R,6R,6aR)-6-(6-((3-fluorobenzyl)amino)-9H-purin-9-yl)-2,2-dimethyl tetrahydrofuro[3,4-d][1,3]dioxol-4-yl)methyl)(isopropyl)amino)propan-1-ol 7a
To a stirred solution of 6a (900 mg, 1.66 mmol) in anhydrous THF (10 mL) was added LiAlH$_4$ (3.3 mL, 1 N solution in THF) at 0°C. The mixture was stirred for 1 hour at 0°C, diluted by ether (20 mL), and slowly quenched by H$_2$O (20 µL), NaOH solution (20 µL, 15% solution), and H$_2$O (60 µL) successively. The mixture was stirred for 15 minutes. To the mixture was added anhydrous MgSO$_4$, filtered and concentrated. The residue was purified by column chromatography over silica gel (DCM : MeOH = 40 : 1) to yield compound 7a (680 mg, 80%) as an oil, $^1$H NMR (500 MHz, CDCl$_3$) δ 8.43 (s, 1H, purin-H), 7.92 (s, 1H, purin-H), 7.35 - 7.30 (m, 1H, Ar-H), 7.18 (d, J = 7.5 Hz, 1H, Ar-H), 7.12 (d, J = 9.5 Hz, Ar-H), 7.01 (t, J = 7.0 Hz, 1H, Ar-H), 6.28 (brs, 1H, NH), 6.12 (s, 1H, tetrahydrofuro-H), 5.54 (brs, 1H, tetrahydrofuro-H), 5.11 (brs, 1H, tetrahydrofuro-H), 4.92 (brs, 2H, benzyl-CH$_2$), 3.79-3.73 (m, 3H, CH$_2$ and CH), 3.10-2.78 (m, 4H, CH$_2$CH$_2$), 1.82-1.78 (m, 2H, CH$_2$), 1.66 (s, 3H, acetonide-CH$_3$), 1.43 (s, 3H, acetonide-CH$_3$), 1.12 (s, 3H, isopropyl-CH$_3$), 0.87 (s, 3H, isopropyl-CH$_3$); $^{13}$C NMR (400 MHz, CDCl$_3$) δ 162.5 (d, J = 242.5 Hz, Ar-C), 154.7 (purin-C), 153.2 (purin-C), 148.6 (purin-C), 141.1 (d, J = 8.2 Hz, Ar-C), 140.1 (purin-C), 130.1 (d, J = 2.7 Hz, Ar-C), 120.5 (purin-C), 114.9 (acetonide-C), 114.9 (d, J = 21.6 Hz, Ar-C), 114.4 (d, J = 21.5 Hz, Ar-C), 90.7 (tetrahydrofuro-C), 83.8 (tetrahydrofuro-C), 83.4 (tetrahydrofuro-C), 77.2 (tetrahydrofuro-C), 65.6 (tetrahydrofuro-C), 62.7 (CH$_2$OH-C), 51.4 (isopropyl-CH$_3$), 49.3 (CH$_2$-C), 43.8 (benzyl-CH$_2$-C), 27.1 (acetonide-CH$_3$-C), 25.4 (acetonide-CH$_3$-C), 17.6 (isopropyl-CH$_3$-C), 15.8 (isopropyl-CH$_3$-C); LRMS (ESI): m/z = 515 [M + H]$^+$. 

4.2.10. 3-(((3aR,4R,6R,6aR)-6-(6-((3-chlorobenzyl)amino)-9H-purin-9-yl)-2,2-dimethyl tetrahydrofuro[3,4-d][1,3]dioxol-4-yl)methyl)(isopropyl)amino)propan-1-ol 7b
Following above synthetic procedure of compound 7a, replacing 6a with 6b afforded compound 7b (85%) as an oil, LRMS (ESI): m/z = 531 [M + H]$^+$. 

4.2.11. N1-(((3aR,4R,6R,6aR)-6-(6-((3-fluorobenzyl)amino)-9H-purin-9-yl)-2,2-dimethyltetrahydrofuro[3,4-d][1,3]dioxol-4-yl)methyl)-N1-isopropylpropane-1,3-diamine 8a
Following above synthetic procedure of compound 4a, replacing 3a with 7a afforded crude 8a (80% percent pure from LC-MS, yield 76%) which was used directly for the next step without further purification, LRMS (ESI): m/z = 514 [M + H]$^+$. 

4.2.12. N1-(((3aR,4R,6R,6aR)-6-(6-((3-fluorobenzyl)amino)-9H-purin-9-yl)-2,2-dimethyltetrahydrofuro[3,4-d][1,3]dioxol-4-yl)methyl)-N1-isopropylpropane-1,3-diamine 8b
Following above synthetic procedure of compound 4a, replacing 3a with 7b afforded 8b (yield 78%) as an oil which was used directly for the next step without further purification, $[\alpha]_D^{20} = -2.15$ (c 1.35, MeOH); $^1H$ NMR (500 MHz, CDCl$_3$) $\delta$ 8.41 (s, 1H, purin-H), 8.01 (s, 1H, purin-H), 7.37 (s, 1H, Ar-H), 7.30-7.28 (m, 3H, Ar-H), 6.66 (brs, 1H, NH), 6.12 (s, 1H, tetrahydrofuran-H), 5.59 (d, $J = 5.8$ Hz, 1H, tetrahydrofuran-H), 5.05-5.03 (m, 1H, tetrahydrofuran-H), 4.87 (brs, 2H, benzyl-CH$_2$), 4.32 (brs, 1H, tetrahydrofuran-H), 2.97-2.95 (m, 1H, isopropyl-CH), 2.81-2.79 (m, 2H, CH$_2$), 2.68-2.65 (m, 1H, CH$_3$-H), 2.53-2.50 (m, 1H, CH$_2$-H), 1.82-1.78 (m, 2H, CH$_2$), 1.59-1.54 (m, 2H, CH$_2$), 1.42 (s, 3H ), 0.98 (d, $J = 6.5$ Hz, 3H, CH$_3$), 0.78 (d, $J = 6.5$ Hz, 3H, CH$_3$); LRMS (ESI): $m/z = 530$ [M + H]*.

4.2.13. General procedure for the synthesis of SIN derivatives 1aa-ah, 1ba-bh.

To a stirred solution of 8a-b (0.15 mmol) in acetonitrile (5 mL) was added isocyanate (0.2 mmol) at room temperature. The mixture was stirred for 1 hour and concentrated. The residue was purified by column chromatography over silica gel (DCM : MeOH = 20 : 1) to yield an oil.

To a stirred solution of above solid in DCM (6 mL) was added TFA (1 mL) and H$_2$O (0.5 mL) at 0 °C. The mixture was stirred overnight and concentrated. The residue was purified by preparing TLC (DCM : MeOH : NH$_3$H$_2$O = 70 : 10 : 1) to yield SIN derivatives 1aa-ah, 1ba-bh.

4.2.13.1. 1-(3-(((2R,3S,4R,5R)-5-(6-((3-fluorobenzyl)amino)-9H-purin-9-yl)-3,4-dihydroxy tetrahydrofuran-2-yl)methyl)(isopropyl)amino)propyl)-3-(4-fluorophenyl)urea 1aa

According to the general procedure, employing 8a and 1-fluoro-4-isocyanatobenzene afforded compound 1aa as a solid, 80% yield, HPLC purity: 97.6%, method A; mp: 180-182 °C; $[\alpha]_D^{20} = 12.91$ (c 0.55, MeOH); $^1H$ NMR (500 MHz, MeOD) $\delta$ 8.28 (s, 1H, purin-H), 8.24 (s, 1H, purin-H), 7.35-7.29 (m, 3H, Ar-H), 7.22 (d, $J = 7.5$ Hz, 1H, Ar-H), 7.15 (d, $J = 9.8$ Hz, 1H, Ar-H), 7.01-6.95 (m, 3H, Ar-H), 6.02 (d, $J = 4.0$ Hz, 1H, tetrahydrofuran-H), 4.88 (brs, 2H, benzyl-CH$_2$), 4.77 (brs, 1H, tetrahydrofuran-H), 4.37-3.45 (m, 1H, tetrahydrofuran-H), 3.28-3.21 (m, 3H, CH$_2$ and isoproyly-CH), 3.08-2.76 (m, 4H, CH$_2$ and CH$_2$), 1.76-1.73 (m, 2H, CH$_2$), 1.15 (s, 3H, isopropyl-CH$_3$), 1.10 (s, 3H, isopropyl-CH$_3$); $^13$C NMR (400 MHz, MeOD) $\delta$ 162.1 (d, $J = 244.5$ Hz, F-phenyl-C), 158.6 (d, $J = 240.1$ Hz, F-phenyl-C), 156.9 (urea-C), 154.5 (purin-C), 152.4. (purin-C), 141.9 (purin-C), 139.7 (purin-C), 135.5 (3-F-phenyl-C), 129.7 (d, $J = 8.3$ Hz, Ar-C), 129.3 (Ar-C), 122.7 (d, $J = 2.8$ Hz, Ar-C), 120.6 (d, $J = 7.7$ Hz, Ar-C), 119.6 (purin-C), 114.7 (d, $J = 22.6$ Hz, 4-F-phenyl-C), 113.6 (d, $J = 20.6$ Hz, 3-F-phenyl-C), 113.3 (d, $J = 20.3$ Hz, 3-F-phenyl-C), 89.2 (tetrahydrofuran-C), 82.7 (tetrahydrofuran-C), 73.2 (tetrahydrofuran-C), 72.1 (tetrahydrofuran-C), 52.1 (CH$_2$-C), 43.0 (benzyl-CH$_2$-C), 37.8 (CH$_2$-C), 27.3 (CH$_2$-C), 16.5 (isopropyl-CH$_3$), 16.0 (isopropyl-CH$_3$); LRMS (ESI): $m/z = 633$ [M + Na]+; HRMS-ESI (m/z): Calcd. For C$_{30}$H$_{37}$F$_2$N$_4$O$_3$ (M+H)$^+$: 611.2900; Found: 611.2897.

4.2.13.2. 1-(3-(((2R,3S,4R,5R)-5-((3-fluorobenzyl)amino)-9H-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl)(isopropyl)amino)propyl)-3-(p-tolyl)urea 1ab

According to the general procedure, employing 8a and 1-isocyanato-4-methylbenzene afforded compound 1ab as a solid, 76% yield, HPLC purity: 98.9%, method A; mp: 190-192 °C; $[\alpha]_D^{20} = 17.36$ (c 0.67, MeOH); $^1H$ NMR (500 MHz, MeOD) $\delta$ 8.28 (s, 1H, purin-H), 8.20 (s, 1H, purin-H), 7.36-7.30 (m, 1H, F-phenyl-H), 7.22-7.18 (m, 3H, Ar-H), 7.13 (d, $J = 9.8$ Hz, 1H, F-phenyl-H), 7.04 (d, $J = 7.6$ Hz, 1H, tolyl-H), 6.96 (t, $J = 7.5$ Hz, 1H, F-phenyl-H), 6.02 (d, $J = 4.0$ Hz, 1H, tetrahydrofuran-H), 4.88 (brs, 2H, benzyl-CH$_2$), 4.77 (brs, 1H, tetrahydrofuran-H), 4.41-4.38 (m, 1H, tetrahydrofuran-H), 4.30-4.25 (m, 1H, tetrahydrofuran-H), 3.44-3.41 (m, 1H, isopropyl-CH$_3$), 3.26-3.20 (m, 4H, CH$_2$ and CH$_2$), 2.92-2.90 (m, 2H, CH$_2$), 2.72 (s, 3H, tolyl-CH$_3$), 1.82-1.78 (m, 2H, CH$_2$), 1.22 (s, 3H, isopropyl-CH$_3$), 1.17 (s, 3H, isopropyl-CH$_3$); $^13$C NMR (400
MHz, MeOD) δ 162.1 (d, J = 244.5 Hz, F-phenyl-C), 157.3 (urea-C), 154.6 (purin-C), 152.4 (purin-C), 141.9 (purin-C), 139.8 (purin-C), 136.5 (Me-phenyl-C), 131.7 (Me-phenyl-C), 129.8 (d, J = 8.3 Hz, F-phenyl-C), 128.7 (Me-phenyl-C), 122.7 (d, J = 2.8 Hz, F-phenyl-C), 119.8 (purin-C), 119.1 (Me-phenyl-C), 113.6 (d, J = 22.1 Hz, F-phenyl-C), 113.3 (d, J = 21.9 Hz, F-phenyl-C), 89.7 (tetrahydrofuran-C), 81.5 (tetrahydrofuran-C), 73.1 (tetrahydrofuran-C), 72.0 (tetrahydrofuran-C), 52.0 (CH₂-C), 48.3 (CH₂-C), 43.0 (benzyl-CH₂-C), 37.2 (isopropyl-CH-C), 26.8 (CH₂-C), 16.1 (isopropyl-CH₂-C), 15.7 (isopropyl-CH₂-C); LRMS (ESI): m/z = 607 [M + H]+; HRMS-ESI (m/z): Calcd. For C₃₁H₄₀N₄O₆F(M+H)+: 607.3151; Found: 607.3129.

4.2.13.3
1-((3-(((2R,3S,4R,5R)-5-(6-((3-fluorobenzyl)amino)-9H-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl(isopropyl)amino)propyl)-3-(4-methoxyphenyl)urea 1ac

According to the general procedure, employing 8a and 1-isocyanato-4-methoxybenzene afforded compound 1ac as a solid, 79% yield, HPLC purity: 98.4%, method A; mp: 143-144 °C; [α]²⁰_D = 14.34 (c 0.45, MeOH); ¹H NMR (500 MHz, CDCl₃) δ 8.30 (s, 1H, purin-H), 7.92 (s, 1H, purin-H), 7.66 (d, 1H, NH), 7.29-7.24 (m, 1H, Ar-H), 7.21 (d, J = 8.5 Hz, 2H, MeO-phenyl-H), 7.14 (d, J = 7.5 Hz, 1H, F-phenyl-H), 7.09 (d, J = 9.5 Hz, 1H, F-phenyl-H), 6.95 (t, J = 7.5 Hz, 1H, F-phenyl-H), 6.75 (d, J = 8.5 Hz, 2H, MeO-phenyl-H), 6.66 (brs, 1H, NH), 6.31 (brs, 1H, NH), 5.95 (d, J = 3.3 Hz, 1H, tetrahydrofuran-H), 4.84 (brs, 2H, benzyl-CH₂), 4.65 (brs, 1H, tetrahydrofuran-H), 4.51 (brs, 1H, tetrahydrofuran-H), 4.32 (brs, 1H, tetrahydrofuran-H), 3.72 (s, 3H, MeO-CH₃), 3.24-3.17 (m, 3H, CH₃ and isopropyl-CH), 2.97-2.83 (m, 2H, CH₂), 2.67 (brs, 2H, CH₂), 1.69-1.65 (m, 2H, CH₂), 1.07 (d, J = 5.6 Hz, 3H, isopropyl-CH₃), 0.98 (d, J = 5.6 Hz, 3H, isopropyl-CH₃); ¹³C NMR (400 MHz, CDCl₃) δ 162.1 (d, J = 244.5 Hz, F-phenyl-C), 157.4 (MeO-phenyl-C), 155.8 (urea-C), 154.6 (purin-C), 152.9 (purin-C), 141.2 (purin-C), 139.0 (purin-C), 131.8 (MeO-phenyl-C), 130.1 (d, J = 8.3 Hz, F-phenyl-C), 123.1 (Ar-C), 122.3 (Ar-C), 120.1 (purin-C), 114.4 (d, J = 22.3 Hz, F-phenyl-C), 114.3 (MeO-phenyl-C), 114.1 (d, J = 22.0 Hz, F-phenyl-C), 89.8 (tetrahydrofuran-C), 82.0 (tetrahydrofuran-C), 74.1 (tetrahydrofuran-C), 72.5 (tetrahydrofuran-C), 55.4 (MeO-CH₃), 51.9 (CH₂-C), 48.4 (CH₂-C), 43.8 (benzyl-CH₂-C), 38.1 (isopropyl-CH-C), 29.7 (CH₂-C), 26.8 (CH₂-C), 17.9 (isopropyl-CH₂-C), 16.2 (isopropyl-CH₂-C); LRMS (ESI); m/z = 645 [M + Na]+; HRMS-ESI (m/z): Calcd. For C₃₁H₄₀N₄O₆F(M+Na)+: 623.3100; Found: 623.3078.

4.2.13.4.
1-((4-((tert-butyl)phenyl)-3-(((2R,3S,4R,5R)-5-(6-((3-fluorobenzyl)amino)-9H-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl(isopropyl)amino)propyl)urea 1ad

According to the general procedure, employing 8a and 1-(tert-butyl)-4-isocyanatobenzene afforded compound 1ad as a solid, 72% yield, HPLC purity: 96.5%, method B; mp: 110-112 °C; [α]²⁰_D = 10.12 (c 0.57, MeOH); ¹H NMR (500 MHz, CDCl₃) δ 8.32 (s, 1H, purin-H), 7.94 (s, 1H, purin-H), 7.71 (s, 1H, NH), 7.29-7.24 (m, 5H, Ar-H), 7.14 (d, J = 7.5 Hz, 1H, F-phenyl-H), 7.09 (d, J = 9.5 Hz, 1H, F-phenyl-H), 6.96 (t, J = 7.5 Hz, 1H, F-phenyl-H), 6.66 (brs, 1H, NH), 6.36 (brs, 1H, NH), 5.95 (d, J = 3.3 Hz, 1H, tetrahydrofuran-H), 4.85 (brs, 2H, benzyl-CH₂), 4.66 (brs, 1H, tetrahydrofuran-H), 4.54 (brs, 1H, tetrahydrofuran-H), 4.35 (brs, 1H, tetrahydrofuran-H), 3.27 (brs, 2H, CH₂), 3.14-3.10 (m, 1H, isopropyl-CH), 2.97-2.83 (m, 2H, CH₂), 2.67 (brs, 2H, CH₂), 1.71-1.68 (m, 2H, CH₂), 1.27 (s, 9H, tBu), 1.07 (d, J = 5.6 Hz, 3H, isopropyl-CH₃), 0.98 (d, J = 5.6 Hz, 3H, isopropyl-CH₃); ¹³C NMR (400 MHz, CDCl₃) δ 162.1 (d, J = 244.5 Hz, F-phenyl-C), 156.9 (urea-C), 154.6 (purin-C), 152.9 (purin-C), 145.8 (t-Bu-phenyl-C), 141.9 (purin-C), 139.0 (purin-C), 136.3 (t-Bu-phenyl-C), 130.1 (d, J = 8.3 Hz, F-phenyl-C), 125.8 (t-Bu-phenyl-C), 123.1 (d, J = 2.8 Hz, F-phenyl-C), 120.1 (purin-C), 119.5 (t-Bu-phenyl-C), 114.5 (d, J = 21.6 Hz, F-phenyl-C), 114.2 (d, J = 20.8 Hz, F-phenyl-C), 89.8 (tetrahydrofuran-C),
\[ \delta \text{H NMR (500 MHz, CDCl}_3) \delta 8.29 (s, 1H, purin-H), 7.92 (s, 1H, NH), 7.29-7.24 (m, 1H, Ar-H), 7.17-7.14 (m, 2H, Ar-H), 7.09 (d, J = 7.5 Hz, 1H, F-phenyl-H), 7.09 (d, J = 9.5 Hz, 1H, F-phenyl-H), 6.96 (t, J = 7.5 Hz, 1H, purin-H), 6.69 (brs, 2H, NH and NH), 5.97 (d, J = 3.3 Hz, 1H, tetrahydrofuran-H), 4.83 (brs, 2H, benzyl-CH), 4.64 (brs, 1H, tetrahydrofuran-H), 4.49 (brs, 1H, tetrahydrofuran-H), 4.35-4.32 (m, 4H, t-But-C), 3.27 (brs, 2H, CH), 3.14-3.10 (m, 1H, isopropyl-CH), 2.97-2.83 (m, 2H, CH), 2.67 (brs, 2H, CH), 1.71-1.68 (m, 2H, CH), 1.07 (d, J = 5.6 Hz, 3H, isopropyl-CH), 0.98 (d, J = 5.6 Hz, 3H, isopropyl-CH); \]

According to the general procedure, employing 8a and 1-isocyanato-4-(trifluoromethyl)benzene afforded compound 1af as a solid, 85% yield, HPLC purity: 98.1%, method A; \([\alpha]^{20}_D = 9.54\) (c 0.71, MeOH); \(^1\)H NMR (500 MHz, CDCl\(_3\)) \(\delta 8.29\) (s, 1H, purin-H), 7.92 (s, 1H, NH), 7.29-7.24 (m, 1H, Ar-H), 7.17-7.14 (m, 2H, Ar-H), 7.09 (d, J = 9.5 Hz, 1H, Ar-H), 6.96 (t, J = 7.5 Hz, 1H, Ar-H), 6.75 (brs, 1H, CH), 6.50 (brs, 1H, NH), 5.99 (d, J = 3.3 Hz, 1H, tetrahydrofuran-H), 4.83 (brs, 2H, benzyl-CH\(_2\)), 4.65 (brs, 2H, tetrahydrofuran-H), 4.51 (brs, 1H, tetrahydrofuran-H), 3.32-2.87 (m, 7H, 3 × CH), 1.71-1.68 (m, 2H, CH), 1.07 (d, J = 5.6 Hz, 3H, isopropyl-CH), 1.07 (d, J = 5.6 Hz, 3H, isopropyl-CH); \(^1\)C NMR (400 MHz, CDCl\(_3\)) \(\delta 156.1\) (urea-C), 154.6 (purin-C), 152.9 (purin-C), 148.2 (CF\(_3\)-phenyl-C), 140.9 (d, J = 6.9 Hz, F-phenyl-C), 138.6 (Ar-C), 130.1 (d, J = 8.2 Hz, Ar-C), 126.0 (d, J = 3.5 Hz, Ar-C), 124.2 (q, J = 272.1 Hz, CF\(_3\)-phenyl-C), 123.5 (q, J = 32.7 Hz, CF\(_3\)-phenyl-C), 123.0 (d, J = 2.8 Hz, Ar-C), 120.04 (Ar-C), 114.5 (Ar-C), 114.2 (Ar-C), 89.8 (tetrahydrofuran-C), 82.3 (tetrahydrofuran-C), 74.3 (tetrahydrofuran-C), 72.8 (tetrahydrofuran-C), 52.0 (CH\(_2\)-C), 46.5 (brs, 2H, tetrahydrofuran-H), 4.51 (brs, 1H, tetrahydrofuran-H), 3.32-2.87 (m, 7H, 3 × CH), 1.71-1.68 (m, 2H, CH), 1.07 (d, J = 5.6 Hz, 3H, isopropyl-CH), 1.07 (d, J = 5.6 Hz, 3H, isopropyl-CH).
4.2.13.7.
1-(tert-butyl)-3-(3-((((2R,3S,4R,5R)-5-(6-((3-fluorobenzyl)amino)-9H-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl)(isopropyl)amino)propyl)urea 1ag

According to the general procedure, employing 8a and 2-isocyanato-2-methylpropane afforded compound 1ag as an oil, 73% yield, HPLC purity: 97.2%, method A; [α]20D = 1.43 (c 0.77, MeOH); 1H NMR (500 MHz, CDCl3) δ 8.37 (s, 1H, purin-H), 7.97 (s, 1H, purin-H), 7.29-7.24 (m, 1H, Ar-H), 7.14 (d, J = 7.5 Hz, 1H, Ar-H), 7.09 (d, J = 9.5 Hz, 1H, Ar-H), 6.96 (t, J = 7.5 Hz, 1H, Ar-H), 6.52 (brs, 1H, NH), 5.99 (d, J = 3.3 Hz, 1H, tetrahydrofuran-H), 4.89 (brs, 2H, benzyl-CH2), 4.74 (brs, 1H, tetrahydrofuran-H), 4.60 (brs, 1H, tetrahydrofuran-H), 4.35-4.32 (m, 1H, tetrahydrofuran-H), 3.27 (brs, 2H, CH2), 3.14-3.10 (m, 1H, isopropyl-CH), 3.04-2.86 (m, 2H, CH2), 2.72 (brs, 2H, CH2), 1.75-1.72 (m, 2H, CH2), 1.32 (s, 9H, t-Bu), 1.16 (d, J = 5.6 Hz, 3H, isopropyl-CH3), 1.03 (d, J = 5.6 Hz, 3H, isopropyl-CH3);

13C NMR (400 MHz, CDCl3) δ 162.1 (d, J = 244.5 Hz, F-phenyl-C), 158.8 (urea-C), 154.6 (purin-C), 152.9 (purin-C), 152.5 (purin-C), 141.2 (d, J = 6.9 Hz, F-phenyl-C), 139.2 (purin-C), 130.2 (d, J = 8.2 Hz, F-phenyl-C), 123.0 (d, J = 2.8 Hz, F-phenyl-C), 120.3 (purin-C), 114.5 (d, J = 21.7 Hz, F-phenyl-C), 114.3 (d, J = 21.8 Hz, F-phenyl-C), 90.0 (tetrahydrofuran-C), 82.6 (tetrahydrofuran-C), 74.0 (tetrahydrofuran-C), 72.3 (tetrahydrofuran-C), 51.8 (CH2), 50.3 (t-Bu), 48.6 (CH2), 43.8 (benzyl-CH2), 38.6 (CH2), 29.7 (t-Bu-CH3), 26.7 (CH2), 18.6 (isopropyl-CH2), 15.8 (isopropyl-CH3); LRMS (ESI): m/z = 573 [M + H]+; HRMS-ESI (m/z): Calcd. For C28H42N8O4F(M+H)+: 573.3308; Found: 573.3317.

4.2.13.8.
1-cyclohexyl-3-(3-((((2R,3S,4R,5R)-5-(6-((3-fluorobenzyl)amino)-9H-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl)(isopropyl)amino)propyl)urea 1ah

According to the general procedure, employing 8a and 1-isocyanato-4-methoxylbenzene afforded compound 1ah as an oil, 76% yield, HPLC purity: 96.8%, method B; [α]20D = 5.05 (c 0.69, MeOH);

1H NMR (500 MHz, MeOD) δ 8.31 (s, 1H, purin-H), 8.25 (s, 1H, purin-H), 7.40-7.34 (m, 1H, Ar-H), 7.20 (d, J = 7.5 Hz, 1H, Ar-H), 7.15 (d, J = 9.5 Hz, 1H, Ar-H), 7.00 (t, J = 7.5 Hz, 1H, Ar-H), 6.04 (d, J = 4.0 Hz, 1H, tetrahydrofuran-H), 4.88 (brs, 2H, benzyl-CH2), 4.79 (brs, 1H, tetrahydrofuran-H), 4.37 (brs, 1H, tetrahydrofuran-H), 4.23 (brs, 2H, tetrahydrofuran-H), 0.98 (brs, 1H, isopropyl-CH2), 3.46-3.43 (m, 1H, cyclohexyl-H), 3.24-2.72 (m, 7H, cyclohexyl-CH-H and 3 × CH2), 1.85-1.61 (m, 8H, cyclohexyl-CH2), 1.23-1.10 (m, 10H, 2 × CH3 and cyclohexyl-6H), 1.85-1.61 (m, 8H, CH2 and cyclohexyl-6H), 1.23-1.10 (m, 10H, 2 × CH3 and cyclohexyl-4H);

13C NMR (400 MHz, MeOD) δ 162.1 (d, J = 244.5 Hz, Ar-H), 159.1 (urea-C), 154.6 (purin-C), 152.9 (purin-C), 142.0 (Ar-C), 139.8 (purin-C), 129.7 (d, J = 8.2 Hz, Ar-C), 122.7 (d, J = 2.8 Hz, Ar-C), 119.7 (purin-C), 113.5 (d, J = 22.2 Hz, Ar-C), 113.3 (d, J = 22.4 Hz, Ar-C), 89.2 (tetrahydrofuran-H), 82.7 (tetrahydrofuran-H), 73.1 (tetrahydrofuran-H), 72.0 (tetrahydrofuran-H), 52.0 (CH2), 48.5 (CH2), 43.2 (benzyl-CH2), 37.3 (CH2), 33.2 (cyclohexyl-CH2-C), 26.6 (CH2-C), 25.4 (cyclohexyl-CH2-C), 25.2 (cyclohexyl-CH2-C), 16.7 (isopropyl-CH2-C), 15.8 (isopropyl-CH2-C); LRMS (ESI): m/z = 599 [M + Na]+; HRMS-ESI (m/z): Calcd. For C30H34N8O4F4(M+Na)+: 599.3464; Found: 599.3464.

4.2.13.9.
1-(3-((((2R,3S,4R,5R)-5-(6-((3-chlorobenzyl)amino)-9H-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl)(isopropyl)amino)propyl)-3-(4-fluorophenyl)urea 1ba

According to the general procedure, employing 8b and 1-isocyanato-4-methoxylbenzene afforded compound 1ba as a solid, 75% yield, HPLC purity: 99.6%, method A; mp: 189-191°C; [α]20D = -25.34 (c 0.23, DMSO); 1H NMR (500 MHz, DMSO-d6) δ 8.47 (brs, 1H, purin-H), 8.43 (s, 1H, NH), 8.22 (s, 1H, purin-H), 7.39-7.35 (m, 3H, Ar-H), 7.33-7.25 (m, 3H, Ar-H), 7.06-7.01 (m, 2H, Ar-H), 6.13 (brs, 1H, NH), 5.88 (d, J = 6.9 Hz, 1H, tetrahydrofuran-H), 5.44 (d, J = 7.4
According to the general procedure, employing 8b and 1-isocyanato-4-methoxybenzene afforded compound 1bb as a solid, 65% yield, HPLC purity: 92.0%, method A; mp: 180-181°C; [α]20D = 13.69 (c 0.45, MeOH); 1H NMR (500 MHz, MeOD) δ 8.30 (s, 1H, purin-H), 8.18 (s, 1H, purin-H), 7.41 (s, 1H, Ar-H), 7.33-7.28 (m, 3H, Ar-H), 7.20 (d, J = 8.8 Hz, 2H, Me-phenyl-H), 7.05 (d, J = 8.8 Hz, 2H, Me-phenyl-H), 6.01 (d, J = 4.0 Hz, 1H, tetrahydrofuran-H), 4.79 (brs, 2H, benzyl-CH2), 4.78-4.76 (m, 1H, tetrahydrofuran-H), 4.49 (brs, 1H, tetrahydrofuran-H), 4.40 (brs, 1H, tetrahydrofuran-H), 3.38-3.35 (m, 3H, CH2 and isopropyl-CH), 3.28-3.17 (m, 4H, 2 × CH2), 2.28 (s, 3H, tolyl-CH3), 1.92 (brs, 2H, CH2), 1.29 (s, 3H, isopropyl-CH3), 0.94 (s, 3H, isopropyl-CH3); 13C NMR (400 MHz, MeOD) δ 157.4 (urea-C), 152.5 (purin-C), 141.5 (Ar-C), 140.0 (purin-C), 136.4 (purin-C), 133.9 (Ar-C), 131.9 (Ar-C), 129.5 (Ar-C), 129.3 (Ar-C), 128.8 (Ar-C), 128.4 (Ar-C), 126.9 (Ar-C), 126.7 (Ar-C), 125.3 (Ar-C), 119.2 (Ar-C), 90.2 (tetrahydrofuran-C), 80.2 (tetrahydrofuran-C), 72.9 (tetrahydrofuran-C), 72.1 (tetrahydrofuran-C), 52.6 (CH2-C), 50.7 (CH2-C), 48.1 (isopropyl-CH-C), 37.8 (CH2-C), 29.4 (CH2-C), 19.4 (isopropyl-CH-C), 17.1 (isopropyl-CH2-C); LRMS (ESI): m/z = 627 [M + H]⁺; HRMS-ESI (m/z): Calcd. For C30H37N6O4FCl (M+H)⁺: 627.2605; Found: 627.2622.

4.2.13.10.

According to the general procedure, employing 8b and 1-isocyanato-4-methylbenzene afforded compound 1bc as a solid, 77% yield, HPLC purity: 93.6%, method A; mp: 170-171°C; [α]20D = 10.88 (c 0.38, MeOH); 1H NMR (500 MHz, MeOD) δ 8.29 (s, 1H, purin-H), 8.22 (s, 1H, purin-H), 7.41 (s, 1H, Ar-H), 7.33-7.28 (m, 3H, Ar-H), 7.20 (d, J = 8.8 Hz, 2H, Me-phenyl-H), 6.84 (d, J = 8.8 Hz, 2H, Me-phenyl-H), 6.02 (d, J = 4.0 Hz, 1H, tetrahydrofuran-H), 4.79 (brs, 2H, benzyl-CH2), 4.78-4.76 (m, 1H, tetrahydrofuran-H), 4.49 (brs, 1H, tetrahydrofuran-H), 4.34 (brs, 1H, tetrahydrofuran-H), 3.79 (s, 3H, MeO-CH3), 3.38-3.35 (m, 3H, CH2 and isopropyl-CH), 3.28-3.17 (m, 4H, 2 × CH2), 1.84 (brs, 2H, CH2), 1.29 (s, 3H, isopropyl-CH3), 0.94 (s, 3H, isopropyl-CH3); 13C NMR (400 MHz, MeOD) δ 157.7 (Meo-phenyl-C), 155.7 (urea-C), 152.5 (purin-C), 141.5 (purin-C), 139.9 (purin-C), 131.8 (Cl-phenyl-C), 129.5 (Cl-phenyl-C), 129.3 (purin-C), 126.9 (Meo-phenyl-C), 126.7 (Meo-phenyl-C), 125.3 (Cl-phenyl-C), 121.4 (Cl-phenyl-C), 113.6 (Meo-phenyl-C), 89.9 (tetrahydrofuran-C), 73.0 (tetrahydrofuran-C), 72.1 (tetrahydrofuran-C), 54.4 (CH2-C), 51.9 (CH2-C), 48.5 (isopropyl-CH-C), 42.9 (benzyl-CH2-C), 31.6 (CH2-C), 26.6 (CH2-C), 25.4 (isopropyl-CH2-C), 22.3 (isopropyl-CH2-C); LRMS (ESI): m/z
According to the general procedure, employing 8b and 1-isocyanato-4-methoxybenzene afforded compound 1bd as a solid, 73% yield, HPLC purity: 99.3%; method A; mp: 122-124°C; [α]D = 12.72 (c 0.78, MeOH); 1H NMR (500 MHz, MeOD) δ 8.27 (s, 1H, purin-H), 8.25 (s, 1H, purin-H), 7.41 (s, 1H, Cl-phenyl-H), 7.32-7.22 (m, 7H, Ar-H), 6.02 (d, J = 4.0 Hz, 1H, tetrahydrofuran-H), 4.88 (brs, 2H, benzyl-CH2), 4.87-4.76 (m, 1H, tetrahydrofuran-H), 4.22-4.19 (m, 1H, tetrahydrofuran-H), 3.97 (t, J = 5.7 Hz, 3H, isopropyl-CH3); 13C NMR (400 MHz, MeOD) 157.0 (urea-C), 152.4 (purin-C), 144.9 (Cl-phenyl-C), 141.4 (purin-C), 139.6 (purin-C), 136.6 (t-Bu-phenyl-C), 129.5 (Cl-phenyl-C), 126.9 (Cl-phenyl-C), 126.7 (Cl-phenyl-C), 125.3 (Cl-phenyl-C), 125.0 (t-Bu-phenyl-C), 118.8 (t-Bu-phenyl-C), 89.0 (tetrahydrofuran-C), 83.2 (tetrahydrofuran-C), 73.2 (tetrahydrofuran-C), 72.0 (tetrahydrofuran-C), 52.1 (CH2-C), 50.6 (CH2-C), 42.9 (benzyl-CH2-C), 39.0 (CH2-C), 30.4 (t-Bu-CH2-C), 27.8 (CH2-C), 17.0 (isopropyl-CH3-C), 16.2 (isopropyl-CH3-C); LRMS (ESI): m/z = 687 [M + Na]+; HRMS-ESI (m/z): Calcd. For C31H31N5O4Cl (M+H)+: 665.3325; Found: 665.3339.

According to the general procedure, employing 8b and 1-isocyanato-4-methoxybenzene afforded compound 1be as a solid, 80% yield, HPLC purity: 97.6%; method B; mp: 116-117°C; [α]D = 11.53 (c 0.76, MeOH); 1H NMR (500 MHz, MeOD) δ 8.25 (s, 2H, purin-H), 7.52-7.48 (m, 4H, CF3-phenyl-H), 7.41 (s, 1H, Cl-phenyl-H), 7.33-7.27 (m, 3H, Cl-phenyl-H), 6.02 (d, J = 4.0 Hz, 1H, tetrahydrofuran-H), 4.88 (brs, 2H, benzyl-CH2), 4.79-4.76 (m, 1H, tetrahydrofuran-H), 4.35-4.32 (m, 1H, tetrahydrofuran-H), 4.22-4.18 (m, 1H, tetrahydrofuran-H), 3.09 (t, J = 5.7 Hz, 3H, isopropyl-CH3); 13C NMR (400 MHz, MeOD) 156.1 (urea-C), 152.4 (purin-C), 144.9 (Cl-phenyl-C), 141.4 (purin-C), 139.6 (purin-C), 136.6 (t-Bu-phenyl-C), 129.5 (Cl-phenyl-C), 126.9 (Cl-phenyl-C), 126.7 (Cl-phenyl-C), 125.3 (Cl-phenyl-C), 125.0 (t-Bu-phenyl-C), 118.8 (t-Bu-phenyl-C), 89.0 (tetrahydrofuran-C), 83.2 (tetrahydrofuran-C), 73.2 (tetrahydrofuran-C), 72.0 (tetrahydrofuran-C), 52.1 (CH2-C), 50.6 (CH2-C), 42.9 (benzyl-CH2-C), 39.0 (CH2-C), 30.4 (t-Bu-CH2-C), 27.8 (CH2-C), 17.0 (isopropyl-CH3-C), 16.2 (isopropyl-CH3-C); LRMS (ESI): m/z = 699 [M + Na]+; HRMS-ESI (m/z): Calcd. For C32H35N5O5Cl (M+H)+: 677.2573; Found: 677.2585.

According to the general procedure, employing 8b and 1-isocyanato-4-methoxybenzene afforded compound 1bf as a foam solid, 74% yield, HPLC purity: 98.2%; method B; [α]D = 10.00 (c 0.53, MeOH); 1H NMR (500 MHz, MeOD) δ 8.27 (s, 1H, purin-H), 8.25 (s, 1H, purin-H), 7.48 (d, J = 2.9 Hz, 1H, Ar-H), 7.42 (s, 1H, Cl-phenyl-H), 7.38 (t, J = 7.8 Hz, 1H,
Ar-H), 7.33-7.30 (m, 2H, Ar-H), 7.28 (brs, 1H, Ar-H), 7.21 (d, J = 7.3 Hz, 1H, Ar-H), 6.02 (d, J = 4.0 Hz, 1H, tetrahydrofuran-H), 4.88 (brs, 2H, benzyl-CH₂), 4.79-4.76 (m, 1H, tetrahydrofuran-H), 4.35-4.32 (m, 1H, tetrahydrofuran-H), 4.22-4.18 (m, 1H, tetrahydrofuran-H), 3.30-3.20 (m, 2H, CH₂), 3.13 (brs, 1H, isopropyl-CH), 2.96 (d, J = 12.9 Hz, 1H, CH₂-1H), 2.84 (brs, 1H, CH₂-1H), 2.66 (brs, 2H, CH₂), 1.74-1.70 (m, 2H, CH₂), 1.10 (d, J = 5.7 Hz, 3H, isopropyl-CH₃), 1.05 (d, J = 5.7 Hz, 3H, isopropyl-CH₃); ¹³C NMR (400 MHz, MeOD) 156.3 (urea-C), 154.5 (purin-C), 152.4 (purin-C), 141.4 (purin-C), 140.5 (CF₃-phenyl-C), 139.6 (purin-C), 133.9 (Cl-phenyl-C), 130.5 (q, J = 31.8 Hz, CF₃-phenyl-C), 129.5 (Cl-phenyl-C), 128.9 (Cl-phenyl-C), 126.9 (Cl-phenyl-C), 126.7 (Cl-phenyl-C), 125.3 (CF₃-phenyl-C), 124.2 (q, J = 274.3 Hz, CF₂-C), 121.3 (CF₃-phenyl-C), 119.6 (purin-C), 117.3 (q, J = 3.5 Hz, CF₃-phenyl-C), 114.5 (q, J = 3.9 Hz, CF₃-phenyl-C), 89.0 (tetrahydrofuran-C), 83.0 (tetrahydrofuran-C), 73.2 (tetrahydrofuran-C), 72.1 (tetrahydrofuran-C), 52.2 (CH₂-C), 50.7 (CH₂-C), 42.9 (benzyl-CH₂-C), 37.9 (CH₂-C), 27.5 (CH₂-C), 16.8 (isopropyl-CH₃-C), 16.1 (isopropyl-CH₃-C); LRMS (ESI): m/z = 677 [M + H]⁺; HRMS-ESI (m/z): Calcd. For C₃₁H₅₁N₃O₇F₂Cl (M+H)⁺: 677.2573; Found: 677.2581.

4.2.13.15.
1-(tert-butyl)-3-(3-(((2R,3S,4R,5R)-5-(6-((3-chlorobenzyl)amino)-9H-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl)(isopropyl)amino)propyl)urea 1bg

According to the general procedure, employing 8b and 1-isocyanato-4-methoxybenzene afforded compound 1bg as a foam solid, 76% yield, HPLC purity: 94.7%, method A; [α]D²⁰ = 7.04 (c 0.54, MeOH); ¹H NMR (500 MHz, MeOD) δ 8.31 (s, 1H, purin-H), 8.28 (s, 1H, purin-H), 7.43 (s, 1H, Ar-H), 7.35-7.30 (m, 2H, Ar-H), 7.28-7.25 (m, 1H, Ar-H), 6.04 (d, J = 4.0 Hz, 1H, tetrahydrofuran-H), 4.88 (brs, 2H, benzyl-CH₂), 4.81-4.80 (m, 1H, tetrahydrofuran-H), 4.38-4.35 (m, 1H, tetrahydrofuran-H), 4.22-4.18 (m, 1H, tetrahydrofuran-H), 3.30-3.20 (m, 1H, isopropyl-CH), 3.13 (t, J = 6.1 Hz, 2H, CH₂), 3.10-2.98 (m, 2H, CH₂), 2.72 (brs, 2H, CH₂), 1.69 (brs, 2H, CH₂), 1.29 (s, 9H, t-Bu), 1.14 (d, J = 5.7 Hz, 3H, isopropyl-CH₃), 1.08 (d, J = 5.7 Hz, 3H, isopropyl-CH₃); ¹³C NMR (400 MHz, MeOD) 159.1 (urea-C), 154.5 (purin-C), 152.4 (purin-C), 141.5 (purin-C), 139.1 (purin-C), 133.9 (Ar-C), 129.5 (Ar-C), 127.0 (Ar-C), 126.7 (Ar-C), 125.3 (Ar-C), 119.7 (purin-C), 89.2 (tetrahydrofuran-C), 82.5 (tetrahydrofuran-C), 73.1 (tetrahydrofuran-C), 72.0 (tetrahydrofuran-C), 52.0 (CH₂-C), 49.3 (CH₂-C), 42.9 (benzyl-CH₂-C), 37.2 (CH₂-C), 28.3 (t-Bu-CH₂-C), 27.6(CH₂-C), 16.7 (isopropyl-CH₃-C), 15.8 (isopropyl-CH₃-C); LRMS (ESI): m/z = 589 [M + H]⁺; HRMS-ESI (m/z): Calcd. For C₃₂H₇₁N₃O₇F₂Cl (M+H)⁺: 589.3012; Found: 589.3039.

4.2.13.16.
1-(3-(((2R,3S,4R,5R)-5-(6-((3-chlorobenzyl)amino)-9H-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl)(isopropyl)amino)propyl)-3-cyclohexylurea 1bh

According to the general procedure, employing 8b and 1-isocyanato-4-methoxybenzene afforded compound 1bh as a solid, 68% yield, HPLC purity: 97.3%, method A; mp: 145-147°C; [α]D²⁰ = 4.71 (c 0.51, MeOH); ¹H NMR (500 MHz, MeOD) δ 8.31 (s, 1H, purin-H), 8.28 (s, 1H, purin-H), 7.43 (s, 1H, Ar-H), 7.35-7.30 (m, 2H, Ar-H), 7.28-7.25 (m, 1H, Ar-H), 6.04 (d, J = 4.0 Hz, 1H, tetrahydrofuran-H), 4.88 (brs, 2H, benzyl-CH₂), 4.81-4.80 (m, 1H, tetrahydrofuran-H), 4.38-4.35 (m, 1H, tetrahydrofuran-H), 4.22-4.18 (m, 1H, tetrahydrofuran-H), 3.46-3.42 (m, 1H, cyclohexyl-CH), 3.16-3.13 (m, 3H, CH₂ and isopropyl-CH), 3.10-2.98 (m, 2H, CH₂), 2.69 (brs, 1H, CH₂), 1.86 (brs, 2H, CH₂), 1.70-1.60 (m, 6H, 3 × cyclohexyl-CH₂), 1.21-1.07 (m, 10H, 2 × cyclohexyl-CH₂ and 2 × isopropyl-CH₃); ¹³C NMR (400 MHz, MeOD) 159.1 (urea-C), 154.5 (purin-C), 152.4 (purin-C), 148.6 (Ar-C), 141.5 (purin-C), 139.8 (purin-C), 133.9 (Ar-C), 129.5 (Ar-C), 126.9 (Ar-C), 126.7 (Ar-C), 125.3 (Ar-C), 119.7 (purin-C), 89.1 (tetrahydrofuran-C),
82.8 (tetrahydrofuran-C), 73.1 (tetrahydrofuran-C), 72.0 (tetrahydrofuran-C), 52.0 (CH$_2$-C), 49.8 (CH$_2$-C), 43.0 (benzyl-CH$_2$-C), 37.5 (CH$_2$-C), 33.2 (cyclohexyl-CH$_2$), 25.2 (cyclohexyl-CH$_2$), 24.6 (cyclohexyl-CH$_2$), 16.7 (isopropyl-CH$_3$-C), 15.9 (isopropyl-CH$_3$-C); LRMS (ESI): m/z = 615 [M + H]$^+$; HRMS-ESI (m/z): Calcd. For C$_{30}$H$_{44}$N$_8$O$_4$Cl (M+H)$^+$: 615.3169; Found: 615.3186.

4.2.14. General procedure for the preparation of compounds 9-12

To a stirred solution of compound 5-8 in DCM (6 mL) was added TFA (1 mL) and H$_2$O (0.5 mL) at 0 °C. The mixture was stirred overnight and concentrated. The residue was purified by preparing TLC (DCM : MeOH : NH$_3$H$_2$O = 70 : 10 : 1) to yield compound 9-12.

4.2.14.1. (2R,3R,4S,5R)-2-(6-((3-fluorobenzyl)amino)-9H-purin-9-yl)-5-((isopropylamino)methyl)tetrahydrofuran-3,4-diol 9a

According to the general procedure, employing 5a afforded compound 9a as a foam solid, 90% yield, HPLC purity: 97.8%, method C; [α]$^D_{20}$ = -2.55 (c 0.67, MeOH); $^1$H NMR (500 MHz, MeOD) δ 8.30 (s, 1H, purin-H), 8.26 (s, 1H, purin-H), 7.36-7.33 (m, 1H, Ar-H), 7.22 (d, J = 7.5 Hz, 1H, Ar-H), 7.15 (d, J = 9.8 Hz, 1H, Ar-H), 7.00-6.98 (m, 1H, Ar-H), 6.01 (d, J = 3.8 Hz, 1H, tetrahydrofuran-H), 4.85-4.83 (m, 2H, benzyl-CH$_2$), 4.37 (dd, J = 4.2, 4.7 Hz, 1 H, tetrahydrofuran-H), 4.28-4.26 (m, 1H, tetrahydrofuran-H), 3.24-3.20 (m, 1H, isopropyl-CH), 3.15-3.08 (m, 2H, CH$_2$), 1.20-1.18 (m, 6H, isopropyl-CH$_3$); $^{13}$C NMR (500 MHz, MeOD) δ 162.8 (d, J = 245 Hz, Ar-C), 154.7 (purin-C), 152.5 (purin-C), 142.0 (purin-C), 140.4 (purin-C), 129.8 (Ar-C), 128.7 (Ar-C), 122.7 (d, J = 3 Hz, Ar-C), 120.0 (purin-C), 113.6 (d, J = 21.6 Hz, Ar-C), 113.3 (d, J = 21.6 Hz, Ar-C), 89.7 (tetrahydrofuran-C), 82.4 (tetrahydrofuran-C), 73.1 (tetrahydrofuran-C), 72.0 (tetrahydrofuran-C), 49.3 (CH$_2$-C), 48.0 (isopropyl-CH-C), 20.1 (isopropyl-CH$_3$-C); LRMS (ESI): m/z = 439 [M + Na]$^+$; HRMS-ESI (m/z): Calcd. For C$_{20}$H$_{26}$N$_6$O$_3$F (M+H)$^+$: 417.2045; Found: 417.2062.

4.2.14.2. (2R,3R,4S,5R)-2-(6-((3-chlorobenzyl)amino)-9H-purin-9-yl)-5-((isopropylamino)methyl) tetrahydrofuran-3,4-diol 9b

According to the general procedure, employing 5b afforded compound 9b as an oil, 94% yield, HPLC purity: 97.9%, method C; [α]$^D_{20}$ = -4.13 (c 0.99, MeOH); $^1$H NMR (500 MHz, MeOD) δ 8.30 (s, 1H, purin-H), 8.26 (s, 1H, purin-H), 7.42 (s, 1H, Ar-H), 7.33-7.26 (m, 3H, Ar-H), 6.01 (d, J = 3.8 Hz, 1H, tetrahydrofuran-H), 4.85-4.83 (m, 2H, benzyl-CH$_2$), 4.35 (dd, J = 4.2, 4.7 Hz, 1 H, tetrahydrofuran-H), 4.18-4.16 (m, 1H, tetrahydrofuran-H), 3.20-3.04 (m, 3H, CH$_2$ and isopropyl-CH), 1.19-1.17 (m, 6H, isopropyl-CH$_3$); $^{13}$C NMR (500 MHz, MeOD) δ 154.6 (purin-C), 152.5 (purin-C), 142.0 (purin-C), 140.4 (purin-C), 129.8 (Ar-C), 128.7 (Ar-C), 122.7 (d, J = 3 Hz, Ar-C), 120.0 (purin-C), 113.6 (d, J = 21.6 Hz, Ar-C), 113.3 (d, J = 21.6 Hz, Ar-C), 89.7 (tetrahydrofuran-C), 82.4 (tetrahydrofuran-C), 73.1 (tetrahydrofuran-C), 49.3 (CH$_2$-C), 48.0 (isopropyl-CH-C), 19.9 (isopropyl-CH$_3$-C), 19.8 (isopropyl-CH$_3$-C); LRMS (ESI): m/z = 439 [M + Na]$^+$; HRMS-ESI (m/z): Calcd. For C$_{20}$H$_{26}$N$_6$O$_3$F (M+H)$^+$: 417.2045; Found: 417.2062.

4.2.14.3. methyl 3-((((2R,3S,4R,5R)-5-(6-((3-fluorobenzyl)amino)-9H-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)(isopropyl)amino)propanoate 10a

According to the general procedure, employing 6a afforded compound 10a as a foam solid, 93% yield, HPLC purity: 97.9%, method C; [α]$^D_{20}$ = -7.56 (c 1.73, MeOH); $^1$H NMR (500 MHz, MeOD) δ 8.30 (s, 1H, purin-H), 8.26 (s, 1H, purin-H), 7.36-7.33 (m, 1H, Ar-H), 7.22 (d, J = 7.5 Hz, 1H, Ar-H), 7.15 (d, J = 9.8 Hz, 1H, Ar-H), 7.00-6.98 (m, 1H, Ar-H), 6.01 (d, J = 3.8 Hz, 1H, tetrahydrofuran-H), 4.85-4.83 (m, 2H,
benzyl-CH$_2$), 4.35 (dd, $J = 4.2$, 4.7 Hz, 1 H, tetrahydrofuran-H), 4.13-4.10 (m, 1H, tetrahydrofuran-H), 3.63 (s, 3H, CH$_3$O), 3.02-2.92 (m, 2H, isopropyl-CH and CH$_2$-1H), 2.84-2.75 (m, 3H, CH$_3$ and CH$_2$-1H), 2.48 (t, $J = 7.0$ Hz, 2H, CH$_2$), 1.06 (d, $J = 6.0$ Hz, 3H, isopropyl-CH$_3$), 1.01 (d, $J = 6.0$ Hz, 3H, isopropyl-CH$_3$); $^{13}$C NMR (500 MHz, MeOD) $\delta$ 173.5 (ester-C), 162.5 (d, $J = 245.4$ Hz, Ar-C), 154.6 (purin-C), 152.5 (purin-C), 142.0 (purin-C), 139.9 (purin-C), 129.9 (Ar-C), 129.8 (Ar-C), 122.7 (d, $J = 3$ Hz, Ar-C), 119.6 (purin-C), 113.7 (d, $J = 21.6$ Hz, Ar-C), 113.3 (d, $J = 21.6$ Hz, Ar-C), 88.9 (tetrahydrofuran-C), 83.8 (tetrahydrofuran-C), 73.1 (tetrahydrofuran-C), 71.7 (tetrahydrofuran-C), 51.9 (MeO-C), 51.1 (CH$_2$-C), 50.6 (CH$_3$-C), 49.1 (isopropyl-CH-C), 33.4 (isopropyl-CH$_3$-C), 17.6 (isopropyl-CH$_3$-C), 16.3; LRMS (ESI): m/z = 525 [M + Na]$^+$; HRMS-ESI (m/z): Calcd. For C$_{29}$H$_{32}$N$_4$O$_5$F (M+H)$^+$: 503.2413; Found: 503.2429.

4.2.14.4. methyl 3-(((2R,3S,4R,5R)-5-((6-((3-chlorobenzyl)amino)-9H-purin-9-yl)-3,4-dihydroxytetrahydrofuran-2-yl)methyl)(isopropyl)amino)propanoate 10b

According to the general procedure, employing 6b afforded compound 10b as a foam solid, 85% yield, [$\alpha$]$^2_{D} = -2.65$ (c 0.57, MeOH); $^1$H NMR (500 MHz, MeOD) $\delta$ 8.30 (s, 2H, purin-C), 7.42 (s, 1H, Ar-H), 7.33-7.26 (m, 3H, Ar-H), 6.01 (d, $J = 3.8$ Hz, 1H, tetrahydrofuran-H), 4.85-4.83 (m, 2H, benzyl-CH$_2$), 4.35 (dd, $J = 4.2$, 4.7 Hz, 1 H, tetrahydrofuran-H), 4.13-4.10 (m, 1H, tetrahydrofuran-H), 3.63 (s, 3H, CH$_3$O), 3.02-2.94 (m, 2H, isopropyl-CH and CH$_2$-1H), 2.85-2.82 (m, 3H, CH$_3$ and CH$_2$-1H), 2.50-2.47 (t, $J = 7.0$ Hz, 2H, CH$_2$), 1.06 (d, $J = 6.6$ Hz, 3H, isopropyl-CH$_3$), 1.01 (d, $J = 6.6$ Hz, 3H, isopropyl-CH$_3$); $^{13}$C NMR (500 MHz, MeOD) $\delta$ 173.5 (ester-C), 154.6 (purin-C), 152.5 (purin-C), 141.5 (purin-C), 140.0 (purin-C), 133.9 (Ar-C), 129.6 (Ar-C), 129.4 (Ar-C), 127.0 (Ar-C), 126.7 (Ar-C), 125.4 (Ar-C), 119.8 (purin-C), 88.9 (tetrahydrofuran-C), 83.8 (tetrahydrofuran-C), 73.1 (tetrahydrofuran-C), 71.7 (tetrahydrofuran-C), 51.9 (MeO-C), 51.2 (CH$_3$-C), 50.6 (CH$_3$-C), 46.1 (isopropyl-CH$_3$-C), 33.3 (CH$_2$-C), 17.5 (isopropyl-CH$_3$-C), 16.3 (isopropyl-CH$_3$-C); LRMS (ESI): m/z = 519 [M + H]$^+$.

4.2.14.5. (2R,3R,4S,5R)-2-(6-((3-fluorobenzyl)amino)-9H-purin-9-yl)-5-(((3-hydroxypropyl)(isopropyl)amino)methyl)tetrahydrofuran-3,4-diol 11a

According to the general procedure, employing 7a afforded compound 11a as a white solid, 88% yield, HPLC purity: 99.5%, method A; mp: 152-154 °C; [$\alpha$]$^2_{D} = 0.45$ (c 0.67, MeOH); $^1$H NMR (500 MHz, MeOD) $\delta$ 8.30 (s, 2H, purin-H), 8.26 (s, 1H, purin-H), 7.36-7.33 (m, 1H, Ar-H), 7.22 (d, $J = 7.5$ Hz, 1H, Ar-H), 7.15 (d, $J = 9.8$ Hz, 1H, Ar-H), 7.00-6.98 (m, 1H, Ar-H), 6.01 (d, $J = 3.8$ Hz, 1H, tetrahydrofuran-H), 4.85-4.83 (m, 2H, benzyl-CH$_2$), 4.35 (dd, $J = 4.2$, 4.7 Hz, 1 H, tetrahydrofuran-H), 4.19-4.18 (m, 1H, tetrahydrofuran-H), 3.65 (t, $J = 5.5$ Hz, 2H, CH$_2$), 3.17-3.13 (m, 1H, isopropyl-CH), 2.99-2.96 (m, 1H, CH$_2$-1H), 2.89-2.85 (m, 1H, CH$_2$-1H), 2.73 (brs, 2H, CH$_2$) 1.72 (t, $J = 6.8$ Hz, 2H, CH$_2$), 1.10 (d, $J = 6.5$ Hz, 3H, isopropyl-CH$_3$), 1.04 (d, $J = 6.5$ Hz, 3H, isopropyl-CH$_3$); $^{13}$C NMR (500 MHz, MeOD) $\delta$ 162.5 (d, $J = 245$ Hz, Ar-C), 154.6 (purin-C), 152.5 (purin-C), 142.0 (purin-C), 139.9 (purin-C), 129.8 (Ar-C), 129.7 (Ar-C), 122.7 (Ar-C), 119.7 (purin-C), 113.6 (d, $J = 21.6$ Hz, Ar-C), 113.5 (d, $J = 21.6$ Hz, Ar-C), 89.1 (tetrahydrofuran-C), 83.0 (tetrahydrofuran-C), 73.2 (tetrahydrofuran-C), 72.1 (tetrahydrofuran-C), 60.8 (CH$_2$-C), 52.2(CH$_2$-C), 50.9 (CH$_3$-C), 48.9 (isopropyl-CH$_3$-C), 29.5 (CH$_2$-C), 16.8 (isopropyl-CH$_3$-C), 16.1 (isopropyl-CH$_3$-C); LRMS (ESI): m/z = 497 [M + Na]$^+$; HRMS-ESI (m/z): Calcd. For C$_{29}$H$_{32}$N$_4$O$_5$F (M+H)$^+$: 475.2464; Found: 475.2458.

4.2.14.6. (2R,3R,4S,5R)-2-(6-((3-chlorobenzyl)amino)-9H-purin-9-yl)-5-(((3-hydroxypropyl)(isopropyl)amino)methyl)tetrahydrofuran-3,4-diol 11b
According to the general procedure, employing 7b afforded compound 11b as a white solid, 89% yield, HPLC purity: 92.7%, method A; mp: 144-147°C; [α]20D =-0.43 (c 0.70, MeOH); 1H NMR (500 MHz, MeOD) δ 8.30 (s, 1H, purin-H), 8.26 (s, 1H, purin-H), 7.42 (s, 1H, Ar-H), 7.33-7.26 (m, 3H, Ar-H), 6.01 (d, J = 3.8 Hz, 1H, tetrahydrofuran-H), 4.85-4.83 (m, 2H, benzyl-CH2), 4.76 (brs, 1H, tetrahydrofuran-H), 4.30-4.28 (m, 1H, tetrahydrofuran-H), 4.18-4.16 (m, 1H, tetrahydrofuran-H), 3.62 (t, J = 6.8 Hz, 2H, CH2), 3.15-3.08 (m, 1H, isopropyl-CH), 2.94-2.92 (m, 1H, CH2-1H), 2.82-2.81 (m, 1H, CH2-1H), 2.68 (brs, 2H, CH2), 1.69 (t, J = 6.9 Hz, 2H, CH2), 1.06 (d, J = 6.5 Hz, 3H, isopropyl-CH3). 13C NMR (500 MHz, MeOD) δ 154.6 (purin-C), 152.5 (purin-C), 141.5 (purin-C), 139.9 (purin-C), 133.9 (Ar-C), 129.6 (Ar-C), 129.4 (Ar-C), 127.0 (Ar-C), 126.8 (Ar-C), 125.4 (Ar-C), 119.7 (purin-C), 89.1 (tetrahydrofuran-C), 83.2 (tetrahydrofuran-C), 73.2 (tetrahydrofuran-C), 72.1 (tetrahydrofuran-C), 60.9 (CH2-C), 52.1 (CH2-C), 50.7 (CH2-C), 48.2 (isopropyl-CH-C), 29.7 (CH2-C), 16.9 (isopropyl-CH3-C), 16.1 (isopropyl-CH3-C); LRMS (ESI): m/z = 513 [M + Na]+; HRMS-ESI (m/z): Calcd. For C23H19N1O3Cl (M+H)+: 491.2168; Found: 491.2170.

4.2.14.7.
(2R,3S,4R,5R)-2-(((3-aminopropyl)(isopropyl)amino)methyl)-5-6-((3-fluorobenzyl)amino)-9H-purin-9-yl)tetrahydrofuran-3,4-diol 12a

According to the general procedure, employing 8a afforded compound 12a as an oil, 79% yield, [α]20D = 10.53 (c 1.02, MeOH); 1H NMR (500 MHz, MeOD) δ 8.30 (s, 1H, purin-H), 8.26 (s, 1H, purin-H), 7.36-7.33 (m, 1H, Ar-H), 7.22 (d, J = 7.5 Hz, 1H, Ar-H), 7.15 (d, J = 9.8 Hz, 1H, Ar-H), 7.00-6.98 (m, 1H, Ar-H), 6.01 (d, J = 3.8 Hz, 1H, tetrahydrofuran-H), 4.82 (brs, 2H, benzyl-CH2), 4.81-4.79 (m, 1H, tetrahydrofuran-H), 4.40-4.38 (m, 1H, tetrahydrofuran-H), 4.32-4.30 (m, 1H, tetrahydrofuran-), 3.12-2.98 (m, 7H, 3 × CH2 and isopropyl-CH), 1.92-1.90 (brs, 2H, CH2), 1.19 (d, J = 5.3 Hz, 3H, isopropyl-CH3), 1.13 (d, J = 5.3 Hz, 3H, isopropyl-CH3); 13C NMR (500 MHz, MeOD) δ 162.5 (d, J = 245.1 Hz, Ar-C), 154.7 (purin-C), 152.6 (purin-C), 142.0 (purin-C), 139.8 (purin-C), 129.8 (d, J = 7.9 Hz, Ar-C), 129.4 (Ar-C), 122.8 (d, J = 3.2 Hz, Ar-C), 119.8 (purin-C), 113.6 (d, J = 21.8 Hz, Ar-C), 113.3 (d, J = 21.6 Hz, Ar-C), 89.5 (tetrahydrofuran-C), 81.4 (tetrahydrofuran-C), 73.1 (tetrahydrofuran-C), 72.1 (tetrahydrofuran-C), 52.1 (CH2-C), 48.8 (isopropyl-CH-C), 29.4 (CH2-C), 23.3 (CH2-C), 16.3 (isopropyl-CH3-C), 15.4 (isopropyl-CH3-C); LRMS (ESI); m/z = 496 [M + Na]+; HRMS-ESI (m/z): Calcd. For C23H19N1O3F (M+H)+: 474.2623; Found: 474.2625.

4.2.14.8.
(2R,3S,4R,5R)-2-(((3-aminopropyl)(isopropyl)amino)methyl)-5-6-((3-chlorobenzyl)amino)-9H-purin-9-yl)tetrahydrofuran-3,4-diol 12b

According to the general procedure, employing 8b afforded compound 12b as an oil, 85% yield, [α]20D = 8.74 (c 1.09, MeOH); 1H NMR (500 MHz, MeOD) δ 8.32 (s, 1H, purin-H), 8.27 (s, 1H, purin-H), 7.42 (s, 1H, Ar-H), 7.34-7.27 (m, 3H, Ar-H), 6.04 (d, J = 4.1 Hz, 1H, tetrahydrofuran-H), 4.85 (brs, 2H, benzyl-CH2), 4.81-4.80 (m, 1H, tetrahydrofuran-H), 4.34 (t, J = 5.3 Hz, 1H, tetrahydrofuran-H), 4.25-4.20 (m, 1H, tetrahydrofuran-H), 3.20-3.15 (m, 1H, isopropyl-CH), 3.07 (t, J = 6.0 Hz, 2H, CH2), 2.95-2.78 (m, 4H, 2 × CH2), 1.81-1.78 (m, 2H, CH2), 1.10 (d, J = 6.0 Hz, 3H, isopropyl-CH3), 1.04 (d, J = 6.0 Hz, 3H, isopropyl-CH3); 13C NMR (500 MHz, MeOD) δ 154.6 (purin-C), 152.5 (purin-C), 141.5 (purin-C), 139.9 (purin-C), 133.9 (Ar-C), 129.6 (Ar-C), 129.4 (Ar-C), 127.0 (Ar-C), 126.8 (Ar-C), 125.4 (Ar-C), 119.7 (purin-C), 89.1 (tetrahydrofuran-C), 82.3 (tetrahydrofuran-C), 73.2 (tetrahydrofuran-C), 72.1 (tetrahydrofuran-C), 52.0 (CH2-C), 49.1 (isopropyl-CH-C), 39.6 (CH2-C), 29.4 (CH2-C), 23.5 (CH2-C), 16.6 (isopropyl-CH3-C), 15.4 (isopropyl-CH3-C); LRMS (ESI): m/z = 512 [M + Na]+; HRMS-ESI (m/z): Calcd. For C23H19N1O3Cl (M+H)+: 490.2328; Found: 490.2327.
4.3. HPLC purity determination

All samples were performed on an Agilent 1260 HPLC-UV system, using Method A, B, or C as follows. Solvent A = Acetonitrile; solvent B = Tetramethylammonium hydroxide solution, solvent C = Methonal; column (Waters Xterra RP18, 4.6 mm x 250 mm, 5 µm), 40 °C; UV at 254 nm.

Preparation of solvent B: To a stirred solution tetramethylammonium hydroxide (4.53 g) in H₂O (1000 mL) was added triethylamine (0.1 mL), and then adjusted to pH 9.0 by phosphoric acid.

Method A: Solvent A : Solvent B = 50 : 50, flow: 0.7 mL/min, 16 min;
Method B: Solvent A : Solvent B = 50 : 50, flow: 1.5 mL/min, 16 min;
Method C: Solvent C : Solvent B = 60 : 40, flow:1.2 mL/min, 20 min.

4.4. Anti-ZIKV Assay

The reference drug Sinefungin and EPZ004777 were purchased from STEMCELL Technologies. The ZIKV was obtained from the Institute of Microbiology, Chinese Academy of Sciences, Beijing, China.

BHK cells were seeded into 96-well plate using DMEM supplemented with 10% FBS and penicillin and streptomycin (100 units/ml and 100 µg/ml, respectively) at a density of 5,000 cells per well. After 24 h incubation at 37 °C with 5% CO₂, original medium was changed into maintenance medium (DMEM supplemented with 2% FBS and P/S) in a volume of 100 µL. Zika virus stocks propagated in Vero cells were diluted to 100TCID₅₀ and added 50 µL to each virus control and administered well. The compounds that diluted from 50 µM by 3-fold dilution in maintenance medium were added to administered wells. The wells were supplemented with maintenance medium to 200 µL. The plates were incubated for 8 days until the CPE reached 100%. The cell viability was measured by Cell Titer-Glo® luminescent cell viability kit according to the manufacturer’s instructions. The IC₅₀ values were calculated by Origin 8.0.

4.5. Cytotoxicity determination

Various concentrations of compounds from 200 µM to 0.09 µM by 3-fold dilution were diluted in maintenance medium. BHK cells were seeded in 96-well plate at a density of 5,000 cells per well and allowed to recover for 24 h. Culture medium was replaced by maintenance medium containing the compound to be examined or drug-free. After 8 days’ exposure, cell viability was also determined with Cell Titer-Glo reagent. The CC₅₀ values were calculated by Origin 8.0.

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Notes

The authors declare no competing financial interest.
ABBREVIATIONS

ZIKV, Zika virus; YFV, yellow fever virus; DENV, dengue virus; WNV, west nile virus; WHO, world health organization; SIN, Sinefungin; SAM, S-Adenosyl methionine; Mtases, Methyltransferases; SAH, S-adenosyl-l-homocysteine; NITD, Novartis Institute for Tropical Disease.

REFERENCES


Highlights:

1. SIN (IC₅₀ > 50 µm) proved to be inactive against ZIKV.
2. 1ad-af, 1ba-bb, and 1bf-bh displays potent anti-ZIKV activity (IC₅₀=4.56-20.16µM).
3. 9a exhibits good activity (IC₅₀=29.98µM) and acceptable cytotoxicity (CC₅₀>200 µM).