

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

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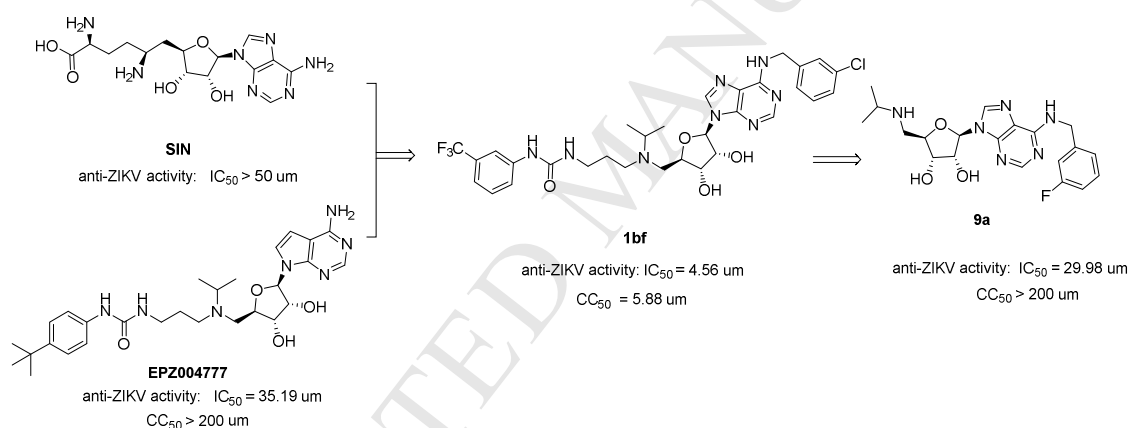
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KEYWORDS: anti-Zika virus, Sinefungin, EPZ004777, structure-activity relationships, methyltransferases



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).

This is the author's manuscript of the article published in final edited form as:

Tao, Z., Cao, R., Yan, Y., Huang, G., Lv, K., Li, W., ... & Yang, J. (2018). Design, synthesis and in vitro anti-Zika virus evaluation of novel Sinefungin derivatives. *European journal of medicinal chemistry*, 157, 994-1004. <https://doi.org/10.1016/j.ejmech.2018.08.057>

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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 ($IC_{50} = 4.56-20.16 \mu M$) than EPZ004777 ($IC_{50} = 35.19 \mu M$). Surprisingly, SIN was founded to be not as active ($IC_{50} > 50 \mu M$) as we and other research groups predicted. Interestingly, the intermediates **9a-b** and **11b** display potent anti-ZIKV potency ($IC_{50} = 6.33-29.98 \mu M$), and compound **9a** also exhibits acceptable cytotoxicity ($CC_{50} > 200 \mu M$), 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 Microesia 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]

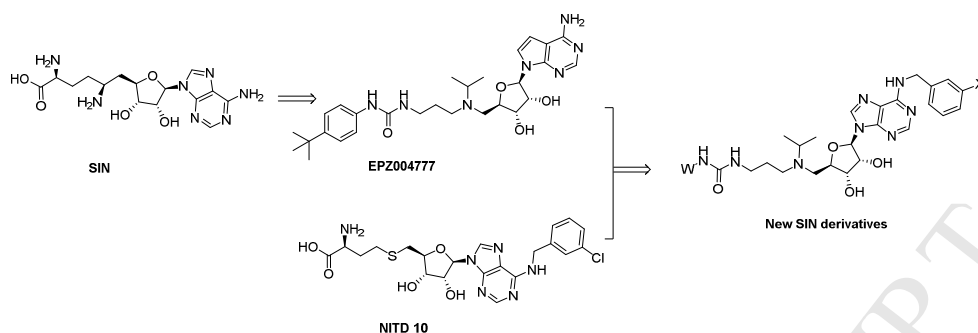
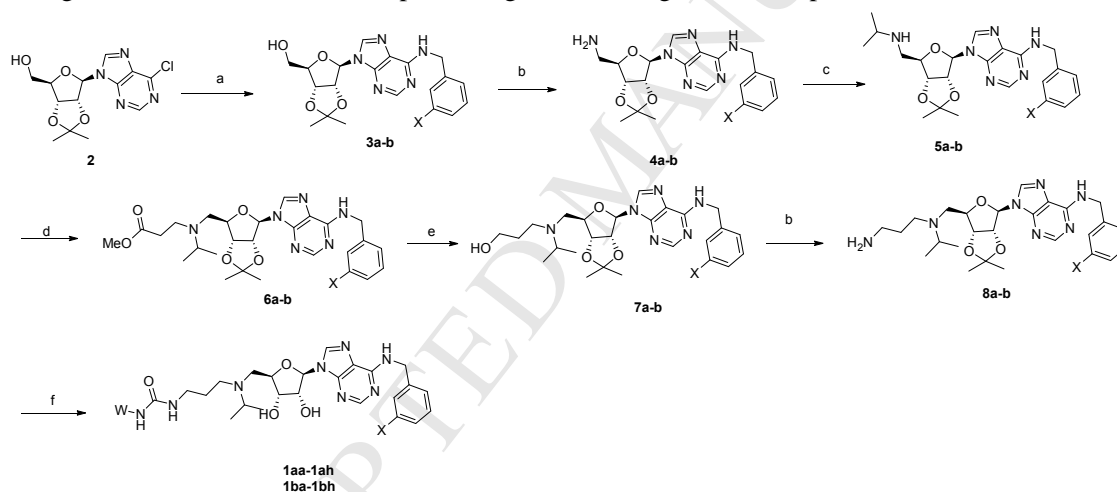


Figure 1. Design of new SIN derivatives

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 griseoleus* 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.



Reagents and conditions: a) amines, Et₃N, EtOH, reflux; b) i: DIAD, phthalimide, Ph₃P, THF, rt; ii: 85% NH₂NH₂, H₂O, EtOH, reflux; c) NaCNBH₃, acetone, AcOH, MeOH, rt; d) Methyl acrylate, MeOH, microwave, 80 °C; e) LiAlH₄, THF, 0 °C; f) i: isocyanate, DCM; ii: TFA, DCM.

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.

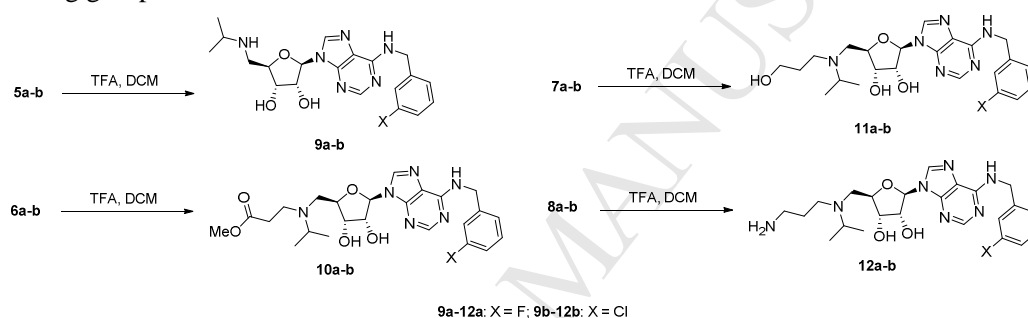
Recently, the crystal structural of ZIKV Mtase with SIN was resolved by Kamil Hercik et al. Based on the structural data, they suggested modification of the adenine moiety of SIN to increase selectivity and binding affinity of ZIKV Mtase. [14] Additionally, researchers from Novartis Institute for Tropical 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 (K_i = 0.002-0.24 μM) and WNV Mtase (K_i = 0.044-5.68 μM) without suppressing host MTases (K_i > 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 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 *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.

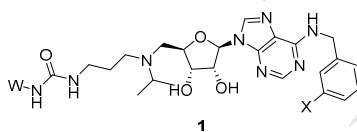
Surprisingly, SIN (IC₅₀ > 50 μM) is not as active against the ZIKV as we predicted, whereas EPZ004777 displays considerable anti-ZIKV activity (IC₅₀ = 35.19 μM). Among the target compounds, **1ad-af**, **1ba-bb**, and **1bf-bh** exhibit potent activity (IC₅₀ < 30 μM); **1aa-ac**, **1ag-ah**, and **1bc** 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 fluorine 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-*t*-Bu-phenyl, 4-CF₃-phenyl and 3-CF₃-phenyl as the aryl W group show excellent anti-ZIKV potency (IC₅₀ = 6.46-6.99 μM). In contrast, introduction of other aryl W substituents leads to a complete loss of activity (**1aa-ac** and **1ag-ah**, IC₅₀ > 50 μM). When the aryl X substituent is chlorine, apart from a few exceptions (**1bc-be**), all of these compounds display potent activity (IC₅₀ < 30 μM), and the contribution of aryl W group to the activity is in this order: 3-CF₃-phenyl > 4-Me-phenyl > cyclohexyl > 4-F-phenyl > *t*-Bu > 4-methoxyphenyl.

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 (IC₅₀ > 50 μM) but **9a**, **9b** and **11b** (IC₅₀ = 6.33-29.98 μM) fortunately display potent anti-ZIKV activity. Among them, compound **9b** (IC₅₀ = 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



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

ND, not detected; NA, not available.

2.3. Cytotoxicity

The SIN derivatives and additional intermediates that displayed considerable anti-ZIKV activity (IC₅₀ < 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 (CC₅₀ = 5.88-58.49 μM) exhibit higher cytotoxicity than EPZ004777 (CC₅₀ > 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 (CC₅₀ = 5.88 μM), the least **1bg** also exhibits the lowest cytotoxicity (CC₅₀ = 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 (CC₅₀ > 200 μM) and potent anti-ZIKV activity (IC₅₀ = 29.98 μM), suggesting compound **9a** might be a potent

candidate for further development. Compounds **9b** and **11b** with higher cytotoxicity ($CC_{50} = 29.39-70.61 \mu\text{M}$) 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 ($IC_{50} = 35.19 \mu\text{M}$). 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 ($IC_{50} = 4.56-20.16 \mu\text{M}$) than EPZ004777, but their CC_{50} values are lower than EPZ004777 ($CC_{50} > 200 \mu\text{M}$). We also observed that intermediates **9a-b** and **11b** display potent anti-ZIKV activity ($IC_{50} = 6.33-29.98 \mu\text{M}$). 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 ($CC_{50} > 200 \mu\text{M}$), 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**

Compds.	IC_{50} (μM)	CC_{50} (μM)	SI
9a	29.98 ± 6.93	>200	>6.67
9b	6.33 ± 1.93	29.39 ± 8.55	4.64
10a	>50	ND	NA
10b	>50	ND	NA
11a	>50	ND	NA
11b	27.47 ± 4.32	70.61 ± 0.05	2.57
12a	>50	ND	NA
12b	>50	ND	NA
SIN	>50	ND	NA
EPZ004777	35.19	>200	>5.68

ND, not detected; NA, not available.

4. Experimental protocols

4.1. Chemistry

^1H NMR spectra were determined on a Varian Mercury-400 or Bruker 500 M spectrometer in MeOD or CDCl_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_3N (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 : $\text{NH}_3\text{H}_2\text{O} = 200 : 10 : 0.1$) to yield compound **3a** (3.13 g, 82%) as a colorless oil; $[\alpha]_D^{20} = -167.77$ (c 0.78, MeOH); ^1H NMR (500 MHz, CDCl_3) δ 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 = 9 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_2), 4.58 (s, 1H, tetrahydrofuro-H), 4.00 (d, J = 12.6 Hz, 1H, $\text{CH}_2\text{OH-CH}$), 3.84 (d, J = 12.6 Hz, 1H, $\text{CH}_2\text{OH-CH}$), 1.68 (s, 3H, CH_3), 1.42 (s, 3H, CH_3); ^{13}C NMR (400 MHz, CDCl_3) δ

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 (CH₂-C), 43.8 (benzyl-CH₂-C), 27.6 (CH₃), 25.2 (CH₃); 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, $[\alpha]_D^{20} = -59.03$ (c 0.93, MeOH); ¹H NMR (500 MHz, CDCl₃) 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-CH₂), 4.58 (s, 1H), 4.00 (d, $J = 12.6$ Hz, 1H, CH₂OH-CH), 3.84 (d, $J = 12.6$ Hz, 1H, CH₂OH-CH), 1.68 (s, 3H, CH₃), 1.42 (s, 3H, CH₃); 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 Ph₃P (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 : NH₃H₂O = 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 : NH₃H₂O = 100 : 10 : 0.2) to yield compound **4a** (680 mg, 68%) as a colorless oil, ¹H NMR (500 MHz, CDCl₃) 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-CH₂), 4.36 (brs, 1H, tetrahydrofuro-H), 3.16-3.10 (m, 2H, CH₂), 1.64 (s, 3H, CH₃), 1.40 (s, 3H, CH₃); ¹³C NMR (400 MHz, CDCl₃) 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), 123.1 (d, $J = 2.7$ Hz, Ar-C), 120.5 (purin-C), 114.7 (acetamide-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 (CH₂-C), 41.8 (benzyl-CH₂-C), 27.2 (CH₃-C), 25.3 (CH₃-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, $[\alpha]_D^{20} = -23.43$ (c 1.05, MeOH); ¹H NMR (500 MHz, CDCl₃) 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-CH₂), 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 : NH₃H₂O = 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, 1H, CH₃), 1.08 (d, J = 6.2 Hz, 3H, isopropyl-CH₃), 1.05 (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 (acetone-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 (acetone-CH₃-C), 25.4 (acetone-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-((3-fluorobenzyl)amino)-9H-purin-9-yl)-2,2-dimethyltetrahydrofuro[3,4-d][1,3]dioxol-4-yl)methyl(isopropyl)amino)propanoate **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, acetone-CH₃), 1.43 (s, 3H, acetone-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-CH₂), 27.1 (acetone-CH₃-C), 25.4 (acetone-CH₃-C), 18.8 (isopropyl-CH₃-C), 16.7 (isopropyl-CH₃-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 LiAlH₄ (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 H₂O (20 μL), NaOH solution (20 μL, 15% solution), and H₂O (60 μL) successively. The mixture was stirred for 15 minutes. To the mixture was added anhydrous MgSO₄, 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, ¹H NMR (500 MHz, CDCl₃) 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-CH₂), 3.79-3.73 (m, 3H, CH₂ and CH), 3.10-2.78 (m, 4H, CH₂CH₂), 1.82-1.78 (m, 2H, CH₂), 1.66 (s, 3H, acetone-CH₃), 1.43 (s, 3H, acetone-CH₃), 1.12 (s, 3H, isopropyl-CH₃), 0.87 (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), 140.1 (purin-C), 130.1 (d, J = 8.2 Hz, Ar-C), 123.0 (d, J = 2.7 Hz, Ar-C), 120.5 (purin-C), 114.9 (acetone-C), 114.5 (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 (CH₂OH-C), 51.4 (isopropyl-CH-C), 49.3 (CH₂-C), 43.8 (benzyl-CH₂-C), 27.1 (acetone-CH₃-C), 25.4 (acetone-CH₃-C), 17.6 (isopropyl-CH₃-C), 15.8 (isopropyl-CH₃-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); $^1\text{H NMR}$ (500 MHz, CDCl_3) δ 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_2 -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): $m/z = 530$ $[\text{M} + \text{H}]^+$.

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_2O (0.5 mL) at 0 °C. The mixture was stirred overnight and concentrated. The residue was purified by preparing TLC (DCM : MeOH : $\text{NH}_3\text{H}_2\text{O} = 70 : 10 : 1$) 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); $^1\text{H NMR}$ (500 MHz, MeOD) δ 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), 4.37-4.35 (m, 1H, tetrahydrofuran-H), 4.25-4.22 (m, 1H, tetrahydrofuran-H), 3.28-3.21 (m, 3H, CH_2 and isopropyl-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); $^{13}\text{C NMR}$ (400 MHz, MeOD) δ 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), 37.7 (CH_2 -C), 27.3 (CH_2 -C), 16.5 (isopropyl- CH_3), 16.0 (isopropyl- CH_3); LRMS (ESI): $m/z = 633$ $[\text{M} + \text{Na}]^+$; HRMS-ESI (m/z): Calcd. For $\text{C}_{30}\text{H}_{37}\text{F}_2\text{N}_8\text{O}_4$ ($\text{M} + \text{H}$) $^+$: 611.2900; Found: 611.2897.

4.2.13.2.

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-(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); $^1\text{H NMR}$ (500 MHz, MeOD) δ 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), 3.26-3.20 (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); $^{13}\text{C NMR}$ (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-CH₃-C), 15.7 (isopropyl-CH₃-C); LRMS (ESI): $m/z = 607$ [M + H]⁺; HRMS-ESI (m/z): Calcd. For C₃₁H₄₀N₈O₄F (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; $[\alpha]_{\text{D}}^{20} = 14.34$ (c 0.45, MeOH); ¹H NMR (500 MHz, CDCl₃) δ 8.30 (s, 1H, purin-H), 7.92 (s, 1H, purin-H), 7.66 (s, 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, CH₂ 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-CH₃-C), 16.2 (isopropyl-CH₃-C); LRMS (ESI): $m/z = 645$ [M + Na]⁺; HRMS-ESI (m/z): Calcd. For C₃₁H₄₀N₈O₅F (M+H)⁺: 623.3100; Found: 623.3078.

4.2.13.4.

1-(4-(tert-butyl)phenyl)-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 **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; $[\alpha]_{\text{D}}^{20} = 10.12$ (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),

82.3 (tetrahydrofuran-C), 74.2 (tetrahydrofuran-C), 72.6 (tetrahydrofuran-C), 52.0 (CH₂-C), 50.7 (CH₂-C), 48.5 (isopropyl-CH-C), 43.8 (benzyl-CH₂-C), 38.2 (CH₂-C), 34.2 (*t*-Bu-C), 31.3 (*t*-Bu-CH₃-C), 26.8 (CH₂-C), 18.2 (isopropyl-CH₃-C), 16.1 (isopropyl-CH₃-C); LRMS (ESI): *m/z* = 671 [M + Na]⁺; HRMS-ESI (*m/z*): Calcd. For C₃₄H₄₆N₈O₄F (M+H)⁺: 649.3621; Found: 649.3611.

4.2.13.5. 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-(trifluoromethyl)phenyl)urea **1ae**

According to the general procedure, employing **8a** and 1-isocyanato-4-(trifluoromethyl)benzene afforded compound **1ae** as a solid, 85% yield, HPLC purity: 95.8%, method B; mp: 119-120°C; [α]_D²⁰ = 10.73 (c 1.38, MeOH); ¹H NMR (500 MHz, CDCl₃) δ 8.29 (s, 1H, purin-H), 8.19 (brs, 1H, NH), 7.92 (s, 1H, purin-H), 7.45 (d, *J* = 8.1 Hz, 2H, CF₃-phenyl-H), 7.40 (d, *J* = 8.1 Hz, 2H, CF₃-phenyl-H), 7.29-7.24 (m, 1H, F-phenyl-H), 7.14 (d, *J* = 7.5 Hz, 1H, F-phenyl-H), 7.09 (d, *J* = 9.5 Hz, 1H, F-phenyl-H), 6.96 (t, *J* = 7.5 Hz, 1H, F-phenyl-H), 6.69 (brs, 2H, NH and NH), 5.97 (d, *J* = 3.3 Hz, 1H, tetrahydrofuran-H), 4.83 (brs, 2H, benzyl-CH₂), 4.64 (brs, 1H, tetrahydrofuran-H), 4.49 (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), 2.97-2.83 (m, 2H, CH₂), 2.67 (brs, 2H, CH₂), 1.71-1.68 (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), 156.1 (urea-C), 154.6 (purin-C), 152.9 (purin-C), 148.2 (F-phenyl-C), 142.6 (CF₃-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 Hz, CF₃-C), 123.5 (q, *J* = 32.7 Hz, CF₃-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 (CH₂-C), 51.3 (CH₂-C), 43.8 (benzyl-CH₂-C), 38.4 (CH₂-C), 26.3 (CH₂-C), 18.1 (isopropyl-CH₃-C), 16.0 (isopropyl-CH₃-C); LRMS (ESI): *m/z* = 661 [M + H]⁺; HRMS-ESI (*m/z*): Calcd. For C₃₁H₃₇N₈O₄F₄ (M+H)⁺: 661.2868; Found: 661.2866.

4.2.13.6. 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-(3-(trifluoromethyl)phenyl)urea **1af**

According to the general procedure, employing **8a** and 1-isocyanato-3-(trifluoromethyl)benzene afforded compound **1af** as an oil, 68% yield, HPLC purity: 98.1%, method A; [α]_D²⁰ = 9.54 (c 0.71, MeOH); ¹H NMR (500 MHz, CDCl₃) δ 8.29 (s, 1H, purin-H), 7.96 (s, 1H, NH), 7.78 (s, 1H, purin-H), 7.51 (d, *J* = 7.8 Hz, 1H, CF₃-phenyl-H), 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, NH), 6.50 (brs, 1H, NH), 5.99 (d, *J* = 3.3 Hz, 1H, tetrahydrofuran-H), 4.83 (brs, 2H, benzyl-CH₂), 4.65 (brs, 2H, tetrahydrofuran-H), 4.51 (brs, 1H, tetrahydrofuran-H), 3.32-2.87 (m, 7H, 3 × CH₂ and isopropyl-CH), 1.87 (brs, 2H, CH₂), 1.19 (d, *J* = 5.6 Hz, 3H, isopropyl-CH₃), 1.07 (d, *J* = 5.6 Hz, 3H, isopropyl-CH₃); ¹³C NMR (400 MHz, CDCl₃) δ 162.1 (d, *J* = 244.5 Hz, F-phenyl-C), 156.1 (urea-C), 154.6 (purin-C), 152.9 (purin-C), 141.0 (purin-C), 140.0 (CF₃-phenyl-C), 139.2 (purin-C), 131.0 (q, *J* = 32.5 Hz, CF₃-phenyl-C), 130.2 (d, *J* = 8.4 Hz, F-phenyl-C), 129.2 (Ar-C), 123.2 (q, *J* = 272.1 Hz, CF₃-C), 123.1 (Ar-C), 121.6 (Ar-C), 118.6 (Ar-C), 115.0 (Ar-C), 114.5 (d, *J* = 21.7 Hz, F-phenyl-C), 114.3 (d, *J* = 21.5 Hz, F-phenyl-C), 90.2 (tetrahydrofuran-C), 82.3 (tetrahydrofuran-C), 74.1 (tetrahydrofuran-C), 72.5 (tetrahydrofuran-C), 52.0 (CH₂-C), 48.9 (CH₂-C), 43.2 (benzyl-CH₂-C), 37.6 (CH₂-C), 26.0 (CH₂-C), 17.7 (isopropyl-CH₃-C), 15.9 (isopropyl-CH₃-C); LRMS (ESI): *m/z* = 661 [M + Na]⁺; HRMS-ESI (*m/z*): Calcd. For C₃₁H₃₇N₈O₄F₄ (M+H)⁺: 661.2868; Found: 661.2867.

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; $[\alpha]_D^{20} = 1.43$ (c 0.77, MeOH); $^1\text{H NMR}$ (500 MHz, CDCl_3) 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- CH_2), 4.74 (brs, 1H, tetrahydrofuran-H), 4.60 (brs, 1H, tetrahydrofuran-H), 4.35-4.32 (m, 1H, tetrahydrofuran-H), 3.27 (brs, 2H, CH_2), 3.14-3.10 (m, 1H, isopropyl-CH), 3.04-2.86 (m, 2H, CH_2), 2.72 (brs, 2H, CH_2), 1.75-1.72 (m, 2H, CH_2), 1.32 (s, 9H, *t*-Bu), 1.16 (d, J = 5.6 Hz, 3H, isopropyl- CH_3), 1.03 (d, J = 5.6 Hz, 3H, isopropyl- CH_3); $^{13}\text{C NMR}$ (400 MHz, CDCl_3) δ 162.1 (d, J = 244.5 Hz, F-phenyl-C), 158.8 (urea-C), 154.6 (purin-C), 152.9 (purin-C), 148.6 (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 (CH_2 -C), 50.3 (*t*-Bu-C), 48.6 (CH_2 -C), 43.8 (benzyl- CH_2 -C), 38.6 (CH_2 -C), 29.7 (*t*-Bu- CH_3 -C), 26.7 (CH_2 -C), 18.6 (isopropyl- CH_3 -C), 15.8 (isopropyl- CH_3 -C); LRMS (ESI): $m/z = 573$ [$\text{M} + \text{H}$] $^+$; HRMS-ESI (m/z): Calcd. For $\text{C}_{28}\text{H}_{42}\text{N}_8\text{O}_4\text{F}$ ($\text{M} + \text{H}$) $^+$: 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-methoxybenzene afforded compound **1ah** as an oil, 76% yield, HPLC purity: 96.8%, method B; $[\alpha]_D^{20} = 5.05$ (c 0.69, MeOH); $^1\text{H NMR}$ (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- CH_2), 4.79 (brs, 1H, tetrahydrofuran-H), 4.37 (brs, 1H, tetrahydrofuran-H), 4.23 (brs, 2H, tetrahydrofuran-H), 0.98 (brs, 1H, isopropyl-CH), 3.46-3.43 (m, 1H, cyclohexyl-H), 3.24-2.72 (m, 7H, cyclohexyl-CH-H and 3 \times CH_2), 1.85-1.61 (m, 8H, CH_2 and cyclohexyl-6H), 1.23-1.10 (m, 10H, 2 \times CH_3 and cyclohexyl-4H); $^{13}\text{C NMR}$ (400 MHz, MeOD) δ 162.1 (d, J = 244.5 Hz, Ar-H), 159.1 (urea-C), 154.6 (purin-C), 152.5 (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-C), 82.7 (tetrahydrofuran-C), 73.1 (tetrahydrofuran-C), 72.0 (tetrahydrofuran-C), 52.0 (CH_2 -C), 48.5 (CH_2 -C), 43.2 (benzyl- CH_2 -C), 37.5 (CH_2 -C), 33.2 (cyclohexyl- CH_2 -C), 26.6 (CH_2 -C), 25.4 (cyclohexyl- CH_2 -C), 25.2 (cyclohexyl- CH_2 -C), 16.7 (isopropyl- CH_3 -C), 15.8 (isopropyl- CH_3 -C); LRMS (ESI): $m/z = 599$ [$\text{M} + \text{Na}$] $^+$; HRMS-ESI (m/z): Calcd. For $\text{C}_{30}\text{H}_{44}\text{N}_8\text{O}_4\text{F}_4$ ($\text{M} + \text{H}$) $^+$: 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-methoxybenzene afforded compound **1ba** as a solid, 75% yield, HPLC purity: 99.6%, method A; mp: 189-191°C; $[\alpha]_D^{20} = -25.34$ (c 0.23, DMSO); $^1\text{H NMR}$ (500 MHz, DMSO- d_6) 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

Hz, 1H, tetrahydrofuran-H), 5.18 (d, J = 5.8 Hz, 1H, tetrahydrofuran-H), 4.76-4.70 (m, 3H, tetrahydrofuran-H and benzyl-CH₂), 4.15 (brs, 1H, CH₂-1H), 3.93 (brs, 1H, CH₂-1H), 3.08-3.06 (m, 2H, CH₂), 2.98-2.92 (m, 1H, isopropyl-CH), 2.86-2.78 (m, 1H, NH), 2.46-2.40 (m, 2H, CH₂), 1.54-1.50 (m, 2H, CH₂), 0.97 (d, J = 6.3 Hz, 3H, isopropyl-CH₃), 0.88 (d, J = 6.3 Hz, 3H, isopropyl-CH₃); ¹³C NMR (400 MHz, DMSO-*d*₆) δ 157.2 (d, J = 237.5 Hz, F-phenyl-C), 155.7 (urea-C), 154.7 (purin-C), 152.9 (purin-C), 148.3 (Cl-phenyl-C), 143.2 (purin-C), 140.6 (purin-C), 137.4 (F-phenyl-C), 133.3 (Ar-C), 130.5 (Ar-C), 127.3 (Ar-C), 127.0 (Ar-C), 126.2 (Ar-C), 119.6 (d, J = 7.4 Hz, F-phenyl-C), 115.6 (Ar-C), 115.3 (Ar-C), 87.8 (tetrahydrofuran-C), 84.3 (tetrahydrofuran-C), 72.8 (tetrahydrofuran-C), 72.1 (tetrahydrofuran-C), 52.6 (CH₂-C), 50.7 (CH₂-C), 48.1 (isopropyl-CH-C), 42.8 (benzyl-CH₂-C), 37.8 (CH₂-C), 29.4 (CH₂-C), 19.4 (isopropyl-CH₃-C), 17.1 (isopropyl-CH₃-C); LRMS (ESI): m/z = 627 [M + H]⁺; HRMS-ESI (m/z): Calcd. For C₃₀H₃₇N₈O₄FCl (M+H)⁺: 627.2605; Found: 627.2622.

4.2.13.10.

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-(p-tolyl)urea **1bb**

According to the general procedure, employing **8b** and 1-isocyanato-4-methylbenzene afforded compound **1bb** as a solid, 65% yield, HPLC purity: 92.0%, method A; mp: 180-181°C; [α]_D²⁰ = 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), 51.9 (CH₂-C), 48.6 (CH₂-C), 42.9 (benzyl-C), 26.6 (CH₂-C), 22.2 (isopropyl-CH₃-C), 19.3 (isopropyl-CH₃-C); LRMS (ESI): m/z = 623 [M + H]⁺; HRMS-ESI (m/z): Calcd. For C₃₁H₄₀N₈O₄Cl (M+H)⁺: 623.2856; Found: 623.2858.

4.2.13.11. 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-methoxyphenyl)urea **1bc**

According to the general procedure, employing **8b** and 1-isocyanato-4-methoxybenzene afforded compound **1bc** as a solid, 77% yield, HPLC purity: 93.6%, method A; mp: 170-171°C; [α]_D²⁰ = 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, MeO-phenyl-H), 6.84 (d, J = 8.8 Hz, 2H, MeO-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) 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-CH₃-C), 22.3 (isopropyl-CH₃-C); LRMS (ESI): m/z

= 639 [M + H]⁺; HRMS-ESI (m/z): Calcd. For C₃₁H₄₀N₈O₅Cl (M+H)⁺: 639.2805; Found: 639.2812.

4.2.13.12.

1-(4-(tert-butyl)phenyl)-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 **1bd**

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]_D²⁰ = 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), 4.8 (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), 3.29-3.20 (m, 2H, CH₂), 3.09 (brs, 1H, isopropyl-CH), 2.96 (d, J = 12.9 Hz, 1H, CH₂-1H), 2.79 (brs, 1H, CH₂-1H), 2.62 (brs, 2H, CH₂), 1.70-1.68 (m, 2H, CH₂), 1.07 (d, J = 5.7 Hz, 3H, isopropyl-CH₃), 1.02 (d, 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), 133.9 (Cl-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-CH₃-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 C₃₄H₄₆N₈O₄Cl (M+H)⁺: 665.3325; Found: 665.3339.

4.2.13.13. 1-(3-((((2R,3S,4R,5R)-5-(6-((3-chlorobenzyl)amino)-9H-purin-9-yl)-3,4-dihydroxy tetrahydrofuran-2-yl)methyl)(isopropyl)amino)propyl)-3-(4-(trifluoromethyl)phenyl)urea **1be**

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]_D²⁰ = 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), 3.30-3.20 (m, 2H, CH₂), 3.09 (brs, 1H, isopropyl-CH), 2.96 (d, J = 12.9 Hz, 1H, CH₂-1H), 2.79 (brs, 1H, CH₂-1H), 2.62 (brs, 2H, CH₂), 1.70-1.68 (m, 2H, CH₂), 1.08 (d, J = 5.7 Hz, 3H, isopropyl-CH₃), 1.04 (d, J = 5.7 Hz, 3H, isopropyl-CH₃); ¹³C NMR (400 MHz, MeOD) 156.1 (urea-C), 154.5 (purin-C), 152.4 (purin-C), 148.5 (Cl-phenyl-C), 143.3 (purin-C), 141.4 (purin-C), 139.5 (CF₃-phenyl-C), 133.9 (Cl-phenyl-C), 129.5 (Cl-phenyl-C), 126.9 (Cl-phenyl-C), 126.7 (Cl-phenyl-C), 125.4 (q, J = 3.8 Hz, CF₃-phenyl-C), 125.3 (CF₃-phenyl-C), 124.5 (q, J = 273 Hz, CF₃-C), 12.9 (q, J = 32.3 Hz, CF₃-phenyl-C), 119.6 (purin-C), 117.5 (CF₃-phenyl-C), 89.0 (tetrahydrofuran-C), 83.1 (tetrahydrofuran-C), 73.2 (tetrahydrofuran-C), 72.1 (tetrahydrofuran-C), 52.2 (CH₂-C), 50.6 (CH₂-C), 42.9 (benzyl-CH₂-C), 38.0 (CH₂-C), 27.5 (CH₂-C), 16.8 (isopropyl-CH₃-C), 16.2 (isopropyl-CH₃-C); LRMS (ESI): m/z = 699 [M + Na]⁺; HRMS-ESI (m/z): Calcd. For C₃₁H₃₇N₈O₄F₃Cl (M+H)⁺: 677.2573; Found: 677.2585.

4.2.13.14. 1-(3-((((2R,3S,4R,5R)-5-(6-((3-chlorobenzyl)amino)-9H-purin-9-yl)-3,4-dihydroxy tetrahydrofuran-2-yl)methyl)(isopropyl)amino)propyl)-3-(3-(trifluoromethyl)phenyl)urea **1bf**

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]_D²⁰ = 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, CF₃-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 C₃₁H₃₇N₈O₄F₃Cl (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-dihydroxy tetrahydrofuran-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-CH₃-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 C₂₈H₄₂N₈O₄Cl (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 (CH₂-C), 49.8 (CH₂-C), 43.0 (benzyl-CH₂-C), 37.5 (CH₂-C), 33.2 (cyclohexyl-CH₂), 25.2 (cyclohexyl-CH₂), 24.6 (cyclohexyl-CH₂), 16.7 (isopropyl-CH₃-C), 15.9 (isopropyl-CH₃-C); LRMS (ESI): m/z = 615 [M + H]⁺; HRMS-ESI (m/z): Calcd. For C₃₀H₄₄N₈O₄Cl (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 H₂O (0.5 mL) at 0 °C. The mixture was stirred overnight and concentrated. The residue was purified by preparing TLC (DCM : MeOH : NH₃ H₂O = 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]_D²⁰ = -2.55 (c 0.67, 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), 4.85-4.83 (m, 2H, benzyl-CH₂), 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, CH₂), 1.20-1.18 (m, 6H, isopropyl-CH₃); ¹³C 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 (CH₂-C), 48.0 (isopropyl-CH-C), 19.9 (isopropyl-CH₃-C), 19.8 (isopropyl-CH₃-C); LRMS (ESI): m/z = 439 [M + Na]⁺; HRMS-ESI (m/z): Calcd. For C₂₀H₂₆N₆O₃F (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]_D²⁰ = -4.13 (c 0.99, 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), 4.35 (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, CH₂ and isopropyl-CH), 1.19-1.17 (m, 6H, isopropyl-CH₃); ¹³C NMR (500 MHz, MeOD) δ 154.6 (purin-C), 152.5 (purin-C), 141.5 (purin-C), 140.4 (purin-C), 133.9 (Ar-C), 129.6 (Ar-C), 129.4 (Ar-C), 126.9 (Ar-C), 126.7 (Ar-C), 125.4 (Ar-C), 120.0 (purin-C), 89.7 (tetrahydrofuran-C), 82.6 (tetrahydrofuran-C), 73.1 (tetrahydrofuran-C), 72.0 (tetrahydrofuran-C), 49.2 (CH₂-C), 48.2 (isopropyl-CH-C), 20.1 (isopropyl-CH₃-C); LRMS (ESI): m/z = 433 [M + H]⁺; HRMS-ESI (m/z): Calcd. For C₂₀H₂₆N₆O₃Cl (M+H)⁺: 433.1749; Found: 433.1760.

4.2.14.3.

methyl

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

According to the general procedure, employing **6a** afforded compound **10a** as a foam solid, 93% yield, [α]_D²⁰ = -7.56 (c 1.73, 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), 4.85-4.83 (m, 2H,

benzyl-CH₂), 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, CH₃O), 3.02-2.92 (m, 2H, isopropyl-CH and CH₂-1H), 2.84-2.75 (m, 3H, CH₂ and CH₂-1H), 2.48 (t, J = 7.0 Hz, 2H, CH₂), 1.06 (d, J = 6.0 Hz, 3H, isopropyl-CH₃), 1.01 (d, J = 6.0 Hz, 3H, isopropyl-CH₃); ¹³C NMR (500 MHz, MeOD) δ 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 (CH₂-C), 50.6 (CH₂-C), 49.1 (isopropyl-CH-C), 33.4 (isopropyl-CH₃-C), 17.6 (isopropyl-CH₃-C), 16.3; LRMS (ESI): m/z = 525 [M + Na]⁺; HRMS-ESI (m/z): Calcd. For C₂₄H₃₂N₆O₅F (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, [α]_D²⁰ = -2.65 (c 0.57, MeOH); ¹H NMR (500 MHz, MeOD) δ 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-CH₂), 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, CH₃O), 3.02-2.94 (m, 2H, isopropyl-CH and CH₂-1H), 2.85-2.82 (m, 3H, CH₂ and CH₂-1H), 2.50-2.47 (t, J = 7.0 Hz, 2H, CH₂), 1.06 (d, J = 6.6 Hz, 3H, isopropyl-CH₃), 1.01 (d, J = 6.6 Hz, 3H, isopropyl-CH₃); ¹³C NMR (500 MHz, MeOD) δ 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 (CH₂-C), 50.6 (CH₂-C), 46.1 (isopropyl-CH-C), 33.3 (CH₂-C), 17.5 (isopropyl-CH₃-C), 16.3 (isopropyl-CH₃-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; [α]_D²⁰ = 0.45 (c 0.67, MeOH); ¹H NMR (500 MHz, MeOD) δ 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-CH₂), 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, CH₂), 3.17-3.13 (m, 1H, isopropyl-CH), 2.99-2.96 (m, 1H, CH₂-1H), 2.89-2.85 (m, 1H, CH₂-1H), 2.73 (brs, 2H, CH₂) 1.72 (t, J = 6.8 Hz, 2H, CH₂), 1.10 (d, J = 6.5 Hz, 3H, isopropyl-CH₃), 1.04 (d, J = 6.5 Hz, 3H, isopropyl-CH₃); ¹³C NMR (500 MHz, MeOD) δ 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 (CH₂-C), 52.2 (CH₂-C), 50.9 (CH₂-C), 48.9 (isopropyl-CH-C), 29.5 (CH₂-C), 16.8 (isopropyl-CH₃-C), 16.1 (isopropyl-CH₃-C); LRMS (ESI): m/z = 497 [M + Na]⁺; HRMS-ESI (m/z): Calcd. For C₂₃H₃₂N₆O₄F (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; $[\alpha]_{\text{D}}^{20} = -0.43$ (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, 1 H, 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), 1.00 (d, $J = 6.5$ Hz, 3H, isopropyl-CH₃); ^{13}C 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 C₂₃H₃₂N₆O₄Cl (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, $[\alpha]_{\text{D}}^{20} = 10.53$ (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); ^{13}C 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 C₂₃H₃₃N₇O₃F (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, $[\alpha]_{\text{D}}^{20} = 8.74$ (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); ^{13}C 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 C₂₃H₃₃N₇O₃Cl (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 × 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.

Funding Sources

This work was financially supported by NSFC (81502923, 81773631, 81302702), National S&T Major Special Project on Major New Drug Innovations (2018ZX09711001-007-002).

Acknowledgement

Thanks to Dr. Luke A. Kassekert (University of Minnesota) for help in the preparation of this manuscript.

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.

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Highlights:

1. SIN ($IC_{50} > 50 \mu\text{m}$) proved to be inactive against ZIKV.
2. **1ad-af**, **1ba-bb**, and **1bf-bh** displays potent anti-ZIKV activity ($IC_{50}=4.56-20.16\mu\text{M}$).
3. **9a** exhibits good activity ($IC_{50}=29.98\mu\text{M}$) and acceptable cytotoxicity ($CC_{50}>200 \mu\text{M}$).