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Identification of 6′-β-fluoro-homoaristeromycin as a potent inhibitor of chikungunya virus replication

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We have reported on aristeromycin (1) and 6′-fluorinated-aristeromycin analogues (2), which are active against RNA viruses such as Middle East respiratory syndrome coronavirus (MERS-CoV), severe acute respiratory syndrome coronavirus (SARS-CoV), Zika virus (ZIKV), and Chikungunya virus (CHIKV). However, these exhibit substantial cytotoxicity. As this cytotoxicity may be attributed to 5′-phosphorylation, we designed and synthesized one-carbon homologated 6′-fluorinated-aristeromycin analogues. This modification prevents 5′-phosphorylation by cellular kinases, whereas the inhibitory activity towards S-adenosyl-L-homocysteine (SAH) hydrolase will be retained. The enantiomerically pure 6′-fluorinated-5′-homoaristeromycin analogues 3a-e were synthesized via the electrophilic fluorination of the silyl enol ether with Selectfluor, using a base-build up approach as the key steps. All synthesized compounds exhibited potent inhibitory activity towards SAH hydrolase, among which 6′-β-fluoroadenosine analogue 3a was the most potent (IC50 = 0.36 μM). Among the compounds tested, 6′-β-fluoro-homoaristeromycin 3a showed potent antiviral activity (EC50 = 0.12 μM) against the CHIKV, without noticeable cytotoxicity up to 250 μM. Only 3a displayed anti-CHIKV activity, whereas both 3a and 3b inhibited SAH hydrolase with similar IC50 values (0.36 and 0.37 μM, respectively), which suggested that 3a’s antiviral activity did not merely depend on the inhibition of SAH hydrolase. This is further supported by the fact that the antiviral effect was specific for CHIKV and some other alphaviruses and none of the homologated analogues inhibited other RNA viruses, such as SARS-CoV, MERS-CoV, and ZIKV. The potent inhibition and high selectivity index make 6′-β-fluoro-homoaristeromycin (3a) a promising new template for the development of antivirals against CHIKV, a serious re-emerging pathogen that has infected millions of people over the past 15 years.

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1. Introduction

RNA viruses have RNA as their genetic material and many important human pathogens have single-stranded RNA (ssRNA) in their genome [1]. The Baltimore classification distinguishes three groups of RNA viruses based on the nature of their genome and mode of replication: double-stranded RNA viruses, negative-sense ssRNA viruses, and positive-sense ssRNA (+RNA) viruses [1,2]. The +RNA viruses form the largest group and contain many important human pathogens, such as dengue virus, hepatitis C virus, severe acute respiratory syndrome (SARS) coronavirus [3], middle east respiratory syndrome (MERS) coronavirus [4], Zika virus (ZIKV) [5], and chikungunya virus (CHIKV) [6]. Antiviral therapy is still lacking for most of these viruses that have a serious impact on human health. Thus, it is desirable to develop new antiviral agents for the treatment of viral diseases caused by RNA viruses.

The genome of a +RNA virus functions as messenger RNA (mRNA) and can be directly translated by host ribosomes into viral (poly)proteins that ultimately form the replication complexes that drive the replication of viral RNA. The 5′-end of the genomes of most +RNA viruses contain a cap structure that is important for translation, RNA stability and evasion of host innate immune
Capping of viral RNA is usually carried out by one or multiple viral proteins that use S-adenosylmethionine (SAM) as the methyl donor.

Cellular S-adenosylhomocysteine (SAH) hydrolase is a pivotal enzyme in the regeneration cycle of SAM, which serves as a methyl donor in cellular methylation reactions and is required for viral mRNA capping [7]. Inhibition of SAH hydrolase leads to the accumulation of SAH, which in turn inhibits SAM-dependent methylation reactions, including those required for the maturation of viral RNA [7]. Thus, SAH hydrolase is a promising target for the development of broad-spectrum antiviral agents [8].

A variety of carbocyclic adenosine analogues with antiviral activity, including aristeromycin are assumed to exert their antiviral action via the inhibition of SAH hydrolase [9]. A close correlation has been detected between the antiviral effects of various carbocyclic and acyclic adenosine analogues and their inhibitory effects on SAH hydrolase in biochemical assays with purified protein [9]. These compounds were observed to be weak inhibitors of flavivirus replication in plaque reduction assays [10], whereas they exhibited potent antiviral activity against the replication of ss(+)RNA viruses such as Ebola [11]. Among these, aristeromycin (1) (Fig. 1) is the representative carbocyclic nucleoside, which was first synthesized in racemic form in 1966 and shortly thereafter, isolated from the fermentation broth of Streptomyces citricolor [12]. It displays potent antiviral activity against SAH hydrolase [8]. Although it is a potent SAH hydrolase inhibitor, its therapeutic utility is limited, because of its significant toxicity [13]. This cytotoxicity may be caused by 5'-phosphorylation of the compound and its inhibition of cellular RNA polymerases and/or incorporation into cellular RNA [13].

Based on the potent antiviral activity of 1, we recently reported the synthesis of enantiomerically pure 6'-fluorinated-aristeromycin analogues and their potent anti-RNA virus activities [14]. Among these, 6,6'-difluoro-aristeromycin 2 exhibits potent antiviral activities against MERS-CoV, SARS-CoV, ZIKV, and CHIKV [14]. However, compound 2 still exhibited substantial cytotoxicity, although it was less cytotoxic than 1. This cytotoxicity may be attributed to 5'-phosphorylation, similar to what is presumed for 1. Therefore, it is desirable to design aristeromycin analogues that retain their inhibitor activity towards SAH hydrolase without being 5'-phosphorylated by cellular kinases. Therefore, our strategy was to design 5'-homologated analogues that are expected to displace the phosphate-susceptible 5'-hydroxy from the phosphate-transfer zone in the kinases. The feasibility of this strategy is supported by the fact that (−)-homoaristeromycin is inactive against HSV-1 and HSV-2, possibly, due to its failure to be phosphorylated [15]. Thus, we designed and synthesized 6'-fluorinated-5'-homoaristeromycin analogues 3a−e, which combined the one-carbon homologation at the 5'-position with the bioisosteric displacement of the hydrogen with the fluorine at the 6'-position. It was expected that compounds 3a−e would retain the inhibitory activity against SAH hydrolase, whereas the lack of 5'-phosphorylation would lead to lower cytotoxicity.

Here, we report the synthesis of enantiomerically pure 6'-fluorinated-5'-homoaristeromycin analogues 3a−e via the electrophilic fluorination [14,16,17] of the silyl enol ether with Selectfluor as the key step and assessment of their antiviral activity.

2. Results and discussion

To synthesize the final nucleosides 3a−e, we designed the retrosynthetic analysis, as illustrated in Scheme 1. Final nucleosides 3 were derived from amino intermediate 4 by the linear purine base build-up approach. Amino derivative 4 was easily derived from ketone 5 via the stereoselective reduction of the ketone and S2 displacement with azide as key steps. 6-Fluoroketone 5 was synthesized using stereoselective electrophilic fluorination of silyl enol ether, which was obtained from key intermediate 6. Ketone 6 was derived from α-cyclopentenone 7, using a Michael reaction as a key step. Intermediate 7 was efficiently derived from D-ribose according to our previously published procedure with a minor modification [16,17].

Key intermediate 6 was synthesized according to our previously published procedure, as shown in Scheme 2 [17]. Briefly, D-ribose was converted to diene 8 in four steps without purification in a 64% yield. The ring-closing metathesis (RCM) reaction of 6 with Neolyst M2 instead of Grubbs' catalyst, followed by pyridinium dichromate (PDC) oxidation afforded the cyclopentenone 7 in a 56% yield. This modified procedure created desired intermediate 7 in six steps with a 36% overall yield [16].

The Michael addition of 7 with vinylmagnesium bromide in the presence of copper(I) bromide afforded vinyl ketone 9 [18] in a 76% yield. Reduction of 9 with sodium borohydride yielded α-alkanol 10a as the single stereoisomer, which was protected with a tert-butyldiphenylsilyl (TBDPS) group to yield 10b. The hydroboration-oxidation of 10b with a borane-dimethyl sulphide complex afforded primary alcohol 11. The removal of the TBDPS group of 11 with tetra-n-butylammonium fluoride (TBAF) yielded diol 12, in which the primary hydroxyl group was selectively protected with a TBDPS group to afford 13. Oxidation of the remaining secondary alcohol in 13 with tetra-n-propylammonium perruthenate (TPAP) and N-methylmorpholine N-oxide (NMO) afforded ketone 6 [17], which served as the key starting material for the electrophilic fluorination.

Ketone 6 was converted to the amines 18a and 18b, which were the key precursors for the synthesis of the final nucleosides, as shown in Scheme 3. Ketone 6 was treated with lithium bis(trimethylsilyl)amide (LiHMDS), followed by trapping the resulting enolate with chlorotriethylsilane (TESCl) to produce silyl enol ether. The silyl enol ether was treated with electrophilic fluorine. Selectfluor (1-chloromethyl-4-fluoro-1,4-diazabicyclo[2.2.2]
Scheme 1. Retrosynthetic analysis of target nucleoside 3.

Scheme 2. Synthesis of key intermediate 6 [17].

Reagents and conditions: (a) acetone, c-H₂SO₄, rt, 3 h; (b) vinyLMgBr, THF, -78 °C to 0 °C, 3 h; (c) NaO₄, H₂O, 0 °C to rt, 40 min; (d) NaH, DMSO, CH₃PPh₃Br, THF, 0 °C to reflux, 15 h; (e) (i) Neolyist M2, CH₂Cl₂, rt, 2 d; (ii) PDC, CH₂Cl₂, rt, 6 h; (f) vinyLMgBr, CuBr-CH₂SCH₃, TMSCl, HMPA, THF, -78 °C, 2 h; (g) NaBH₄, MeOH, 0 °C, 1 h; (h) TBDPSi, imidazole, DMF, 0 °C to rt, 3 h; (i) (i) BH₃·SMe₂, THF, 0 °C to rt, 1 h; (ii) NaBO₂·H₂O, H₂O, 0 °C to rt, 16 h; (j) n-Bu₄NF, THF, 0 °C to rt, 16 h; (k) Et₃N, DMAP, TBDPSi, CH₂Cl₂, 0 °C to rt, 2 h; (l) NMO, 4 Å MS, TPAP, CH₂Cl₂, rt, 1 h.
octane difluoroborate) to produce desired 6-β-fluoro-cyclopentanone 14 as the single stereoisomer, which was equilibrated to geminal diol because of the electronegative fluorine atom [14,16,17]. The β stereochirality of fluorine was obtained because of the steric hindrance by the 2,3-isopropylidene group, which was confirmed by long-range coupling between H-8 and 6'-β-F and X-ray crystallography after conversion to adenosine derivative 3a. 6,6-Difluoro ketone 15 was synthesized from 14 in a 50% yield using the same procedure. Fluoro ketones 14 and 15 were reduced with sodium borohydride to produce alcohols 16a and 16b, respectively. First, we attempted a direct condensation reaction with 6-chloropurine under the Mitsunobu conditions to obtain the desired nucleoside, but it failed to yield the desired condensed product. Direct S_N2 reactions of triflates synthesized from 16a and 16b with a 6-chloropurine anion were also unsuccessful. Thus, we decided to utilize the linear purine base build-up approach [14]. The S_N2 displacement of triflates 16a and 16b with a linear, less bulky and more powerful nucleophile, NaH, proceeded smoothly to yield azido derivatives 17a and 17b, respectively. Catalytic reduction of 17a and 17b with 10% Pd/C yielded amines 18a and 18b, respectively.

6-Fluoroamine 18a was treated with 5-amino-4,6-dichloropurine and triethylamine in n-butanol under microwave irradiation to yield 19a (Scheme 4) [14,16]. However, weaker nucleophile, 6,6-difluorooamine 18b did not give desired product 19b under the same microwave conditions. Thus, we used a stronger electrophile, 4,6-dichloro-5-formamidopyrimidine rather than 5-amino-4,6-dichloropurine, which afforded the desired product 19b in a good yield. The cyclisation of 19a and 19b with diethoxymethyl acetate yielded 6-chloropurine nucleosides 20a and 20b, respectively. The H-8 proton in the 1H NMR spectrum of 20a was split as a doublet with a small coupling constant (J = 2.4 Hz), which resulted from the long-range coupling between H-8 and 6'-β-F, which confirmed the desired stereochemistry of 6'-F, as well as 6-chloropurine.

Treatment of 20a and 20b with 50% aqueous trifluoroacetic acid yielded 6-chloropurine nucleosides 21a and 21b, respectively. For the synthesis of adenosine derivatives, 21a was treated with saturated tert-butanolic ammonia to yield 3a, whereas 21b was converted to 3d using saturated methanolic ammonia solution in a steel bomb. Treatment of 21a and 21b with 40% aqueous methylamine afforded N-[6]-methyladenosine nucleosides 3b and 3c, respectively. Hydrolysis of 21a with 1 N HCl in 1,4-dioxane yielded inosine derivative 3c. The structure of 3a was confirmed by single-crystal X-ray crystallography [19] (Fig. 2).

2.1. Biological activity

The synthesized nucleosides 3a-3e were assayed for their inhibitory activity against SAH hydrolase as well as their antiviral activity against the +RNA viruses, MERS-CoV, SARS-CoV, ZIKV, and CHIKV using cytopathic effect (CPE) reduction assays (Table 1) [14]. The 6'-fluorinated adenosine analogues (n = 2) 3a and 13b exhibited potent inhibitory activity against SAH hydrolase. 6,6'-Difluoroadenosine analogues 3d and 3e also inhibited SAH hydrolase, but to a lesser extent. For example, 6'-fluoroadenosine analogue 3a was 5.6 times more potent than 6,6'-difluoroadenosine analogue 3d. A similar trend was observed with 6'-fluoroadenosine analogues (n = 1), 2a and 2b. 6'-Fluoro-N-[6]-methyladenosine 3b showed similar inhibitory activity to that of 6'-β-fluoroadenosine 3a. A similar trend was observed with 6,6'-difluoro-adenosine 3d and 6,6'-difluoro-N-[6]-methyladenosine 3e. However, the inosine analogue 3c did not inhibit SAH hydrolase, as no inhibitory activity was observed at the highest dose tested (100 μM).

Among the compounds tested, 6'-β-fluoro-homoaristeromycin 3a showed potent antiviral activity (EC_{50} = 0.12 μM) against CHIKV without noticeable cytotoxicity up to 250 μM (highest dose tested). This antiviral activity did not merely seem to depend on the inhibition of SAH hydrolase, as 3a and 3b inhibited the enzyme with...
similar IC$_{50}$ values (0.36 and 0.37 µM, respectively), although only 3a displayed anti-CHIKV activity. Regular 6'-fluoro-aristeromycin 2a was inactive against CHIKV. In addition, although 6,6'-difluoro-aristeromycin 2b displayed broad-spectrum antiviral activity against several RNA viruses, including SARS-CoV, MERS-CoV, CHIKV, and ZIKV, the corresponding homologated analogue 3d did not exhibit antiviral activity, despite its potent inhibitory activity towards SAH hydrolase. These results demonstrate that other factors besides the inhibitory activity towards SAH hydrolase may be involved in the antiviral activity of this series. More detailed studies on the antiviral activity of 3a and its mode of action will be published elsewhere (Kovacikova et al., submitted).

3. Conclusion

To reduce the cytotoxicity of 6'-fluorinated aristeromycin analogues 2a and 2b, which is likely due to their 5'-phosphorylation by cellular kinases, we designed and synthesized 6'-fluorinated homoaaristeromycin analogues 3a–3e. These nucleosides were synthesized from D-ribose, using the Michael reaction, stereoselective electrophilic fluorination, and linear purine base build-up as key steps. Among the compounds tested, 6'-fluoro-homoaaristeromycin (3a) showed potent anti-CHIKV activity (EC$_{50}$ = 0.12 µM) without noticeable cytotoxicity at concentrations up to 250 µM, which resulted in a high selectivity index (SI) of more than 2083. This indicates that one carbon homologation may prevent 5'-phosphorylation, which results in low cytotoxicity. This homologation had no effect on the inhibitory activity towards SAH hydrolase, as compounds 3a and 2a inhibited this enzyme with similar IC$_{50}$ values (0.36 and 0.37 µM). The antiviral activity of 3a was specific for CHIKV and to a lesser extent some other alphaviruses, whereas other viruses have been shown to be sensitive to the SAH hydrolase inhibitors 2a and 2b. This suggests that the potent anti-CHIKV activity of 3a is based on more than merely the inhibition of SAH hydrolase. This study shows that 6'-fluoro-homoaaristeromycin (3a) is a promising new template for the development of anti-CHIKV agents.

![Diagram](image)

Fig. 2. The X-ray crystal structure of 3a.
4. Experimental section

4.1. General methods

Proton (1H) and carbon 13C[1H] NMR spectra were obtained on a Bruker AV 400 (400/100 MHz). AMX 500 (500/125 MHz), Jeol JNM-ECA 600 (600/150 MHz) or Bruker AVANCE III 800 (800/200 MHz) spectrometer. The 1H NMR data were reported as peak multiplicities: s for singlet; d for doublet; dd for doublet of doublets; t for triplet; td for triplet of doublet; q for quartet; qn for quintet; bs or broad singlet and m for multiplet. Coupling constants were reported in Hertz. The chemical shifts were reported as ppm (δ) relative to the solvent peak. All reactions were routinely carried out under an inert atmosphere of dry nitrogen. IKA RCT basic type heating mantle was used to provide a constant heat source. Microwave-assisted reactions were carried out in sealed vessels using a Biotage Initiator. Microwave reactions were checked by thin layer chromatography (Kieselgel 60 FF230 mesh, Merck). All the crude compounds were purified by column chromatography (hexanes:ethyl acetate 5:1) to give colorless oil. Micro- wave-assisted reactions were carried out in sealed vessels using a Biotage Initiator- US/JPN (part no. 356007) microwave reactor and the reaction temperatures were monitored by an external surface IR sensor. High resolution mass spectra were measured with electrospray-ionization quadrupole time-of-flight (ESI-Q-TOF) techniques. Melting points were recorded on a Barnstead electrothermal 9100 instrument and are uncorrected. Reactions were checked by thin layer chromatography (Kieselgel 60 F254, Merck). Spots were detected by viewing under a UV light, and by colorizing with charring after dipping in a p-anisaldehyde solution. The crude compounds were purified by column chromatography on a silica gel (Kieselgel 60, 70–230 mesh, Merck). All the anhydrous solvents were redistilled over CaH2, P2O5, or sodium/benzophenone prior to the reaction.

4.1.1. 3aS,4R,5R,6R,6aR)-6-(2-((tert-Butyldiphenylsilyl)oxy)ethyl)-5-fluoro-2,2-dimethyltetrahydro-4H-cyclopenta[d][1,3]dioxol-4-ol (16a)

To a stirred solution of 6 [17] (6.4 g, 14.5 mmol) in anhydrous tetrahydrofuran (105 mL) at –78 °C, under nitrogen atmosphere were added chlorotriethylsilane (9.73 mL, 58.0 mmol) and lithium bis (trimethylsilyl)amide (1.0 M solution in tetrahydrofuran, 29.0 mL, 29.0 mmol) and the reaction mixture was stirred at –78 °C for 1 h, warmed to 0 °C and quenched using saturated aqueous NH4Cl (30 mL). The solution was extracted with ethyl acetate (2 x 100 mL). The organic layer was dried using anhydrous MgSO4 and concentrated in vacuo to give the crude silyl enol ether, which was used immediately for the next step without further purification.

To a stirred solution of the crude silyl enol ether in anhydrous acetonitrile (120 mL) was added Selectfluor® (7.7 g, 21.8 mmol) at 0 °C and the reaction mixture was stirred for 16 h at 0 °C, diluted with brine (50 mL) and extracted with ethyl acetate (2 x 100). The organic layer was dried over anhydrous MgSO4, filtered and concentrated in vacuo. The residue was purified by silica gel column chromatography (hexanes:ethyl acetate = 5:1) to give 14 [17] (3.7 g, 84%) as colorless oil, which was equilibrated to the 6-fluorogeminal diol.

| Compound No | SAH hydrolase EC50 (μM) | CHIKV EC50 (μM) | CC50 (μM) |
|-------------|------------------------|----------------|-----------|
| 3a (n = 2, X = F, Y = H, Z = NH3) | 0.36 | 0.12 | >250 |
| 3b (n = 2, X = F, Y = H, Z = NHMe) | 0.37 | >100 | >100 |
| 3c (n = 2, X = F, Y = H, Z = OH) | >100 | >100 | >100 |
| 3d (n = 2, X = F, Y = F, Z = NHMe) | 3.05 | >100 | >100 |
| 3e (n = 2, X = F, Y = F, Z = NH2) | 0.37 | >100 | >100 |
| 2a (n = 1, X = F, Y = H, Z = NH2) | 1.06 | 0.13 | 1.25 |
| 2b (n = 1, X = F, Y = F, Z = NH2) | 1.00 | 0.13 | 1.25 |

To a stirred solution of 14 [4.06 g, 8.89 mmol] in tetrahydrofuran (120 mL) was slowly added sodium borohydride (0.5 mg, 13.34 mmol) at −40 °C and the reaction mixture was stirred at −40 °C for 1 h and then at 0 °C for 3 h. The mixture was diluted with water (50 mL) and extracted with ethyl acetate (2 x 100 mL). The organic layer was dried over anhydrous MgSO4, filtered and concentrated in vacuo. The residue was purified by silica gel chromatography (hexanes:ethyl acetate = 11:1.5) to give 16a as colorless oil (2.62 g, 64%); [α]20D = 43.5 (c 0.14, CH3OH); 1H NMR (400 MHz, CDCl3) δ 7.68 (m, 4H), 7.40 (m, 6H), 4.59 (dd, J = 3.6, 4.4, 50.8 Hz, 1H), 4.64 (m, 1H), 4.54 (m, 1H), 4.08 (m, 1H), 3.75 (m, 1H), 2.73 (dd, J = 2.8, 4.4 Hz, 1H), 2.48 (m, 1H), 1.89 (m, 1H), 1.66 (m, 1H), 1.51 (s, 3H), 1.34 (s, 3H), 1.05 (s, 9H); 13C NMR (100 MHz, CDCl3) δ 135.8, 133.9, 129.8, 113.8, 101.6, 99.8, 84.0, 80.4, 78.2, 72.3 (J = 27.5 Hz), 62.5, 43.5 (J = 72.0 Hz), 29.6 (J = 5.9 Hz), 26.5, 24.7, 19.3; 19F NMR (376 MHz, CDCl3) δ −204.7; HRMS (FAB) found 459.2366 [calcd for C26H36FO4Si (M + H)+ 459.2361].

4.1.2. (3aS,4R,6R,6aR)-6-(2-((tert-Butyldiphenylsilyl)oxy)ethyl)-5,5-difluoro-2,2-dimethyltetrahydro-4H-cyclopenta[d][1,3]dioxol-4-ol (16b)

To a stirred solution of 14 [17] (5.7 g, 10.03 mmol) in anhydrous tetrahydrofuran (100 mL) at 0 °C, under N2 atmosphere were added triethylamine (7.0 mL, 50.15 mmol), chlorotriethylsilane (8.4 mL, 50.15 mmol) and lithium bis trimethylsilyl amide (1.0 M solution in tetrahydrofuran, 25.1 mL, 25.1 mmol) and the reaction mixture was stirred at room temperature for 1 h, warmed to 0 °C and quenched using saturated aqueous ammonium chloride solution. The solution was extracted with ethyl acetate (2 x 100 mL). The organic layer was dried over anhydrous MgSO4 and concentrated in vacuo to give the crude silyl enol ether, which was used immediately for the next step without further purification.

To a stirred solution of the crude silyl enol ether in anhydrous acetonitrile (100 mL) was added a solution of Selectfluor® (7.11 g, 20.06 mmol) in acetonitrile (85 mL) at 0 °C. The reaction mixture
was stirred overnight at room temperature, concentrated in vacuo and diluted with ethyl acetate (2 x 100 mL). The organic layer was dried over anhydrous MgSO\(_4\) and concentrated in vacuo. The residue was purified by silica gel column chromatography (hexanes:ethyl acetate = 2:3:1) to give 15 (2.37 g, 50%) as the 6,6-difluorogeminal diol.

To a stirred solution of 15 (2.19 g, 4.61 mmol) in tetrahydrofuran (45 mL) was added sodium borohydride (0.21 g, 5.53 mmol) at 0 °C over a period of 15 min. The reaction mixture was stirred at 0 °C for 3 h, neutralized with glacial acetic acid, diluted with water (30 mL), and extracted with ethyl acetate (2 x 40 mL). The organic layer was washed with brine (30 mL), dried over anhydrous MgSO\(_4\) and concentrated in vacuo. The residue was purified by silica gel column chromatography (hexanes:ethyl acetate = 9:1) to give 16b as colorless oil (1.79 g, 81%); \([\delta]_{D}^{1} = +18.8 \) (c 0.340, CHC\(_3\)); \( ^{1}H\) NMR (300 MHz, CDCl\(_3\)) \( \delta 7.69-7.66 (m, 4H), 4.40-4.38 (m, 1H), 4.30-4.27 (m, 1H), 3.94 (dt, \( J = 5.3, 17.6 \) Hz), 3.77 (t, \( J = 6.2 \) Hz), 2.66-2.59 (m, 1H), 2.02-1.96 (m, 1H), 1.75-1.68 (m, 1.5), 1.52 (s, 3H), 1.28 (s, 3H), 1.04 (s, 9H); \( ^{13}C\) NMR (75 MHz, CDCl\(_3\)) \( \delta 181.1, 175.2, 71.0, 135.8, 133.8, 129.6, 127.6, 127.3 \) (M, 770, 275.0 Hz), 113.5, 80.1 (J = 8.6, 77.5 Hz, 79.5 Hz, 68.9 (J = 18.9 Hz), 60.9, 46.5 (J = 20.1 Hz), 28.7 (J = 43.8 Hz), 29.0, 26.8, 24.7, 19.2; \( ^{19}F\) NMR (376 MHz, CDCl\(_3\)) \( \delta -104.5, -120.2 \) (M, 524.2161 [calcld for C\(_{26}H\_33F\_2N\_3NaO\_3Si\_2 (M + Na)^+ 524.2151].

4.1.5. \( \text{N}\)[(3aR,4R,5R,6aR)-5-azido-5-fluoro-2,2-dimethyltetrahydro-4H-cyclopenta[d][1,3][dioxol-4-yl]ethoxy]ethyl-5-fluoro-2,2-dimethyltetrahydro-4H-cyclopenta[d][1,3]-dioxol-4-yl]-6-chloropyrimidine-4,5-diamine (19a)

To a stirred solution of 17a (0.991 g, 2.04 mmol) in methanol (50 mL) was added 10% palladium on carbon (wet with ca. 5% water) (250 mg) at room temperature and the reaction mixture was stirred under H\(_2\) for 3 h. After completion of reaction (TLC), the suspension was filtered through a pad of Celite and concentrated to give the amine 18a as colorless syrup, which was used for the next step without further purification.

To a solution of the crude 18a (0.933 g, 2.04 mmol) in n-butanol (20 mL) were added 5-amino-4,6-dichloropyrimidine and NN-diisopropylethylamine at room temperature and the reaction mixture was subjected to microwave irrigation at 170 °C for 10 h. After cooled to room temperature, the reaction mixture was concentrated in vacuo, diluted with ethyl acetate (30 mL), washed with saturated aqueous NaHCO\(_3\) and filtered through a pad of Celite and concentrated to give the residue. The residue was purified by silica gel column chromatography (hexanes:ethyl acetate = 2:1:9) to give 19a as colorless oil (0.91 g, 76%); \([\delta]_{D}^{1} = -18.7 \) (c 0.167, CHC\(_3\)); \( ^{1}H\) NMR (500 MHz, CDCl\(_3\)) \( \delta 8.07 \) (s, 1H), 7.60-7.64 (m, 4H), 7.44-7.34 (m, 6H), 5.33 (d, \( J = 7.9 \) Hz), 5.05 (dt, \( J = 3.0, 53.3 \) Hz, 3.79 (m, 1H), 3.79 (m, 2H), 3.67 (m, 1H), 2.34 (m, 1H), 1.87 (m, 1H), 1.52 (s, 3H), 1.32 (s, 3H), 1.07 (s, 9H); \( ^{13}C\) NMR (125 MHz, CDCl\(_3\)) \( \delta 153.8, 135.8, 129.9, 127.8, 114.2, 99.4, 97.6, 83.6, 82.3, 68.8 (J = 15.7 Hz), 61.9, 45.5 (J = 17.7 Hz), 30.3 (J = 4.8 Hz), 27.4, 24.9, 19.3; \( ^{19}F\) NMR (376 MHz, CDCl\(_3\)) \( \delta -205.5 \) (M, 506.2254 [calcld for C\(_{26}H\_33F\_2N\_3NaO\_3Si\_2 (M + Na)^+ 506.2246].

4.1.6. 9-[(3aS,4R,5R,6aS)-6-azido-5,5-difluoro-2,2-dimethyltetrahydro-4H-cyclopenta[d][1,3][dioxol-4-yl]ethoxy]ethyl]-5-fluoro-2,2-dimethyltetrahydro-4H-cyclopenta[d][1,3]-dioxol-4-yl]-6-chloro-9H-purine (20a)

A stirred mixture of 19a (0.91 g, 1.56 mmol) and diethoxymethyl acetate (15 mL) was heated at 120 °C for 14 h and cooled to room temperature. The solvent was removed under reduced pressure and the residue was purified by silica gel column chromatography (hexanes:ethyl acetate = 9:1) to give 20a as a white foam (0.87 g, 93%); \([\delta]_{D}^{1} = -12.3 \) (c 0.162, CH\(_3\)OH); UV (CH\(_3\)OH) \( \lambda_{max} \) 263 nm; \( ^{1}H\) NMR (400 MHz, CDCl\(_3\)) \( \delta 8.78 \) (s, 1H), 8.32 (d, \( J = 2.4 \) Hz, 1H), 7.68 (m, 4H), 7.4 (m, 6H), 5.18 (m, 1H), 5.08 (m, 2H), 4.62 (m, 1H), 3.81 (m, 2H), 3.71 (m, 2H), 3.60 (m, 1H), 3.52 (t, \( J = 11.3 \) Hz, 2H), 3.45 (brs, 2H), 2.51-2.37 (m, 1H), 1.94--1.80 (m, 2H), 1.54 (s, 3H), 1.30 (s, 3H), 0.93 (s, 9H); \( ^{13}C\) NMR (150 MHz, CDCl\(_3\)) \( \delta 154.3, 149.3, 143.2, 135.3, 133.6, 129.6, 127.6, 127.3, 114.1, 98.3 (J = 211.9 Hz), 84.1, 83.4, 61.8, 60.3 (J = 19.4 Hz), 45.6 (J = 21.8 Hz), 30.4 (J = 5.6), 27.3, 26.8, 24.9, 19.2; \( ^{19}F\) NMR (376 MHz, CDCl\(_3\)) \( \delta -208.0 \) (M, 585.2468 [calcld for C\(_{30}H\_39F\_2N\_3O\_4Si\_2 (M + H)^+ 585.2458].

To solution of 17b (0.93 g, 1.85 mmol) in methanol (18 mL) was added 10% palladium on carbon (with cat. 5% water) (200 mg) at room temperature and the reaction mixture was stirred under H2 atmosphere at room temperature overnight. The suspension was filtered through a Celite and concentrated to give the amine 18b as colorless syrup which was used for the next step without further purification.

To a stirred solution of the crude 18b in 1,4-dioxane (15 mL) were added 4,6-dichloro-5-formamidopyrimidine (0.71 g, 3.70 mmol) and triethylamine (1.9 mL, 13.60 mmol) and the reaction mixture was heated at reflux for 2 d and cooled to room temperature. The solvent was removed under reduced pressure and the residue was purified by silica gel column chromatography (hexane:ethyl acetate = 5:1) to give 20b (0.35 g, 30%) as a white foam: \[ \text{[M} + \text{H]}^+ \text{ HRMS (FAB) found 298.1310 [calcd for C}_{12}\text{H}_{17}\text{FN}_5\text{O}_3 \]

To a stirred solution of the crude 20b in methanol (18 mL) was added 10% palladium on carbon (with cat. 5% water) (200 mg) at room temperature and the reaction mixture was stirred under H2 atmosphere at room temperature overnight. The suspension was filtered through a Celite and concentrated to give the amine 21b as colorless syrup which was used for the next step without further purification.

To a stirred solution of the crude 21b in 1,4-dioxane (15 mL) were added 4,6-dichloro-5-formamidopyrimidine (0.71 g, 3.70 mmol) and triethylamine (1.9 mL, 13.60 mmol) and the reaction mixture was heated at reflux for 2 d and cooled to room temperature. The solvent was removed under reduced pressure and the residue was purified by silica gel column chromatography (hexane:ethyl acetate = 5:1) to give 22b (0.35 g, 30%) as a white foam: \[ \text{[M} + \text{H]}^+ \text{ HRMS (FAB) found 298.1310 [calcd for C}_{12}\text{H}_{17}\text{FN}_5\text{O}_3 \]

A solution of 21a (70 mg, 0.22 mmol) in saturated tert-butanol in steel bomb was heated at 90 °C for 3 h. After cooling to room temperature, the solvent was removed under reduced pressure and the residue was purified by silica gel chromatography (methylene chloride:methanol = 5:1) to give 3a (34 mg, 68%), which was crystallized from diethyl ether/methanol as a white solid: mp 147–149 °C; [\text{M} + \text{H}]^+ \text{ HRMS (FAB) found 613.2222 [calcd for C}_{25}\text{H}_{31}\text{ClF}_{2}\text{N}_4\text{O}_3 \]

A solution of 21a (55 mg, 0.17 mmol) in methanol was added 40% methylamine aqueous solution and the reaction mixture was heated at 80 °C for 5 h in steel bomb. After cooling to room temperature, the solvent was removed under reduced pressure and the residue was purified by silica gel chromatography (methylene chloride:methanol = 5:1) to give 3b (37 mg, 68%), which was crystallized from diethyl ether/methanol as a white solid: mp 171–173 °C; [\text{M} + \text{H}]^+ \text{ HRMS (FAB) found 613.2222 [calcd for C}_{25}\text{H}_{31}\text{ClF}_{2}\text{N}_4\text{O}_3 \]

A solution of 21a (50 mg, 0.185 mmol) in 1,4-dioxane (3 mL) was added 1 N HCl (3 mL) at room temperature and the reaction mixture was heated at reflux overnight, cooled to room temperature and concentrated in vacuo. The residue was purified by C18 reverse-phase silica gel column chromatography (H2O) to give 3c (3 mg, 70%), which was crystallized from diethyl ether/methanol as a white solid: mp 184–186 °C; [\text{M} + \text{H}]^+ \text{ HRMS (FAB) found 613.2222 [calcd for C}_{25}\text{H}_{31}\text{ClF}_{2}\text{N}_4\text{O}_3 \]

A solution of 21a (50 mg, 0.158 mmol) in 1,4-dioxane (3 mL) was added 1 N HCl (3 mL) at room temperature and the reaction mixture was heated at reflux overnight, cooled to room temperature and concentrated in vacuo. The residue was purified by C18 reverse-phase silica gel column chromatography (H2O) to give 3c (3 mg, 70%), which was crystallized from diethyl ether/methanol as a white solid: mp 184–186 °C; [\text{M} + \text{H}]^+ \text{ HRMS (FAB) found 613.2222 [calcd for C}_{25}\text{H}_{31}\text{ClF}_{2}\text{N}_4\text{O}_3 \]

A solution of 21a (70 mg, 0.22 mmol) in saturated tert-butanol in steel bomb was heated at 90 °C for 3 h. After cooling to room temperature, the solvent was removed under reduced pressure and the residue was purified by silica gel chromatography (methylene chloride:methanol = 5:1) to give 3a (34 mg, 68%), which was crystallized from diethyl ether/methanol as a white solid: mp 147–149 °C; [\text{M} + \text{H}]^+ \text{ HRMS (FAB) found 613.2222 [calcd for C}_{25}\text{H}_{31}\text{ClF}_{2}\text{N}_4\text{O}_3 \]

A solution of 21a (55 mg, 0.17 mmol) in methanol was added 40% methylamine aqueous solution and the reaction mixture was heated at 80 °C for 5 h in steel bomb. After cooling to room temperature, the solvent was removed under reduced pressure and the residue was purified by silica gel chromatography (methylene chloride:methanol = 5:1) to give 3b (37 mg, 68%), which was crystallized from diethyl ether/methanol as a white solid: mp 171–173 °C; [\text{M} + \text{H}]^+ \text{ HRMS (FAB) found 613.2222 [calcd for C}_{25}\text{H}_{31}\text{ClF}_{2}\text{N}_4\text{O}_3 \]

A solution of 21a (50 mg, 0.185 mmol) in 1,4-dioxane (3 mL) was added 1 N HCl (3 mL) at room temperature and the reaction mixture was heated at reflux overnight, cooled to room temperature and concentrated in vacuo. The residue was purified by C18 reverse-phase silica gel column chromatography (H2O) to give 3c (3 mg, 70%), which was crystallized from diethyl ether/methanol as a white solid: mp 184–186 °C; [\text{M} + \text{H}]^+ \text{ HRMS (FAB) found 613.2222 [calcd for C}_{25}\text{H}_{31}\text{ClF}_{2}\text{N}_4\text{O}_3 \]
A solution of 21b (33 mg, 0.099 mmol) in saturated methanol ammonia (20 mL) was heated at 70 °C overnight in steel bomb. After cooled to room temperature, the solvent was removed under reduced pressure and the residue was purified by silica gel column chromatography (methylene chloride: methanol = 9:1) to obtain 3d (0.012 g, 55% based on recovered starting material), which was crystallized from diethyl ether/methanol (4:1, v/v). This work was supported by a grant from the Mid-career Research program (NRF-2016R1A2B3010164) of National Research Foundation (NRF), Korea.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ejmech.2019.111956.

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[19] Crystal structure data for C12H16FN5O3 (3a) are as follows: Mr = 297.30, T = 296.5(2) K, orthorhombic, space group P212121, a = 10.34289(14) Å, b = 11.70521(15) Å, c = 15.2643(3) Å, α = 90°, β = 90°, γ = 90°, V = 1847.98(5) Å³, Z = 4, pcac = 1.069 g/cm³, μ = 0.728 mm⁻¹, F(000) = 624.0, crystal size 0.146 × 0.086 × 0.013 mm³, radiation CuKα (λ = 1.54184). Of 22855 reflections collected in the 2θ range from 9.522 to 153.208° using an ω scan on a SuperNova, Dual, Cu at zero, AtlasS2 diffractometer, 3856 were unique reflections (Rint = 0.0310, Rsigma = 0.0180). Using Olex2, the structure was solved with the ShelXT structure solution program using Direct Methods and refined with the ShelXL refinement package using Least Squares minimization. Final R indexes [all data] R1 = 0.0367, wR2 = 0.1054, GOF = 1.057, and max/min residual electron density 0.23/-0.18 eÅ⁻³. Flack parameter = 0.12(7). Further details of the crystal structure investigation(s) may be obtained from the Cambridge Crystallographic Data Centre (CCDC, 12 Union Road, Cambridge, CB2 1EZ, UK); Tel: (+44)1223-336-408, Fax: (+44)1223-336033, e-mail: deposit@ccdc.cam.ac.uk) using no. CCDC 1963491.