Research paper

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

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ABSTRACT

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 ($IC_{50} = 0.36$ μM). Among the compounds tested, 6′-β-fluoro-homoaristeromycin **3a** showed potent antiviral activity ($EC_{50} = 0.12$ 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 IC_{50} 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

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responses. 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 several RNA viruses, that result from the inhibition of the 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 inhibitory 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′-hydroxyl 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 nucleoside **3** was 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 S_N2 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 D-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-alcohol **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-diazoniabicyclo[2.2.2]
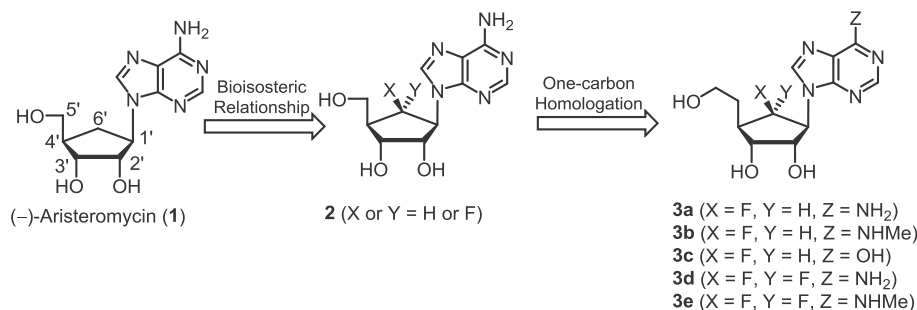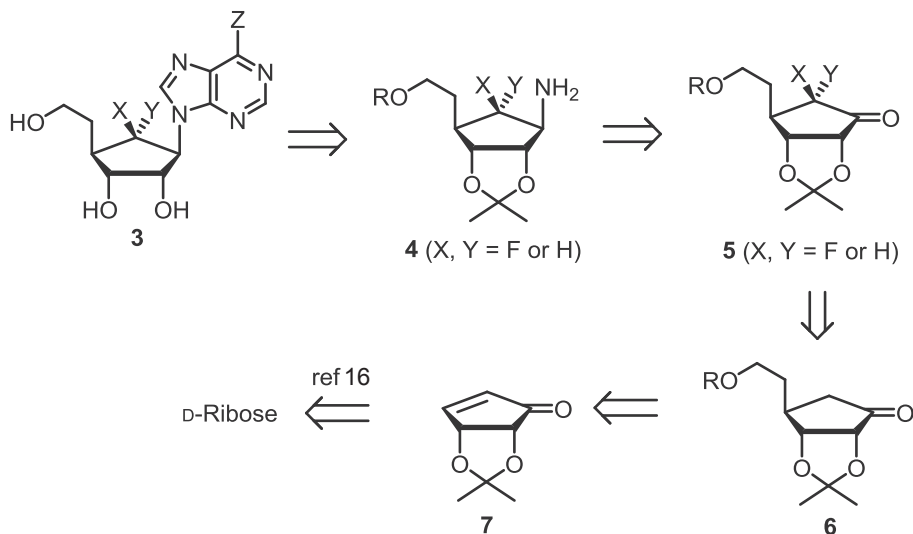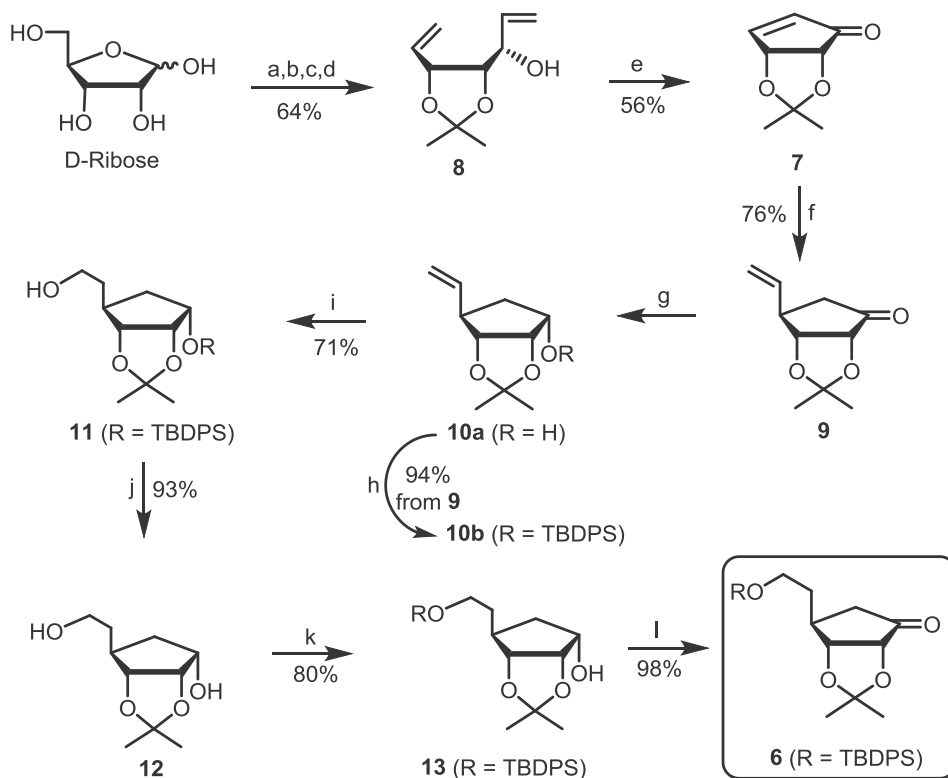

Fig. 1. Rationale for the design of 6′-fluorinated-5′-homoaristeromycin analogues **3a**−**e**.

Scheme 1. Retrosynthetic analysis of target nucleoside **3**.

Reagents and conditions: (a) acetone, c-H$_2$SO$_4$, rt, 3 h; (b) vinylMgBr, THF, -78 $^{\circ}$C to 0 $^{\circ}$C, 3 h; (c) NaIO$_4$, H$_2$O, 0 $^{\circ}$C to rt, 40 min; d) NaH, DMSO, CH$_3$PPh$_3$Br, THF, 0 $^{\circ}$C to reflux, 15 h; (e) (i) Neolyst M2, CH$_2$Cl$_2$, rt, 2 d; (ii) PDC, CH$_2$Cl$_2$, rt, 6 h; (f) vinylMgBr, CuBr-CH$_3$SCH$_3$, TMSCl, HMPA, THF, -78 $^{\circ}$C, 2 h; (g) NaBH$_4$, MeOH, 0 $^{\circ}$C, 1 h; (h) TBDPSCl, imidazole, DMF, 0 $^{\circ}$C to rt, 3 h; (i) (i) BH$_3$-CH$_3$SCH$_3$, THF, 0 $^{\circ}$C to rt, 1 h; (ii) NaBO$_3$-H$_2$O, H$_2$O, 0 $^{\circ}$C to rt, 16 h; (j) n-Bu$_4$NF, THF, 0 $^{\circ}$C to rt, 16 h; (k) Et$_3$N, DMAP, TBDPSCl, CH$_2$Cl$_2$, 0 $^{\circ}$C to rt, 2 h; (l) NMO, 4A MS, TPAP, CH$_2$Cl$_2$, rt, 1 h.

Scheme 2. Synthesis of key intermediate **6** [17].
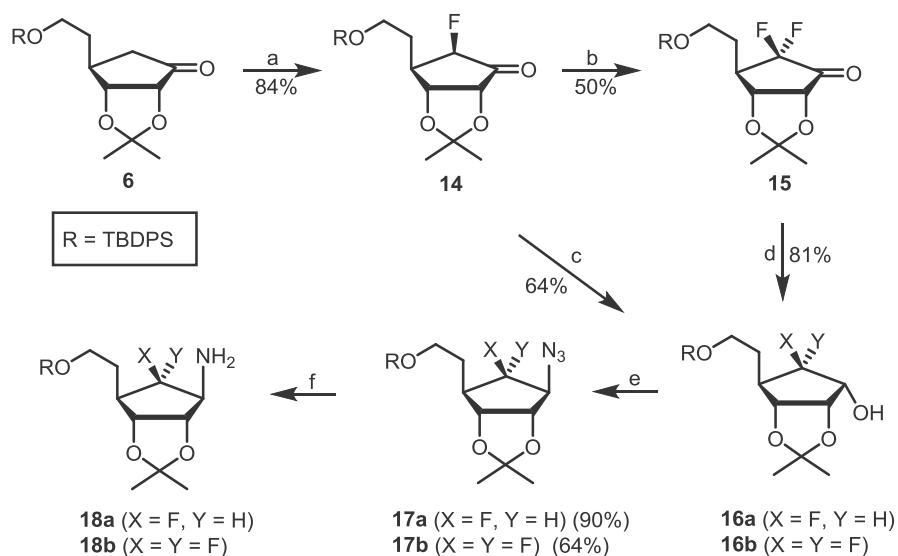
Reagent and Conditions: (a) (i) TESCl, LiHMDS, THF, -78 °C, 1 h; (ii) Selectfluor, CH₃CN, 0 °C, 16 h; (b) (i) TESCl, LiHMDS, THF, -78 °C, 1 h; (ii) Selectfluor, CH₃CN, 0 °C, 16 h; (c) NaBH₄, THF, -40 °C to 0 °C, 3 h; (d) NaBH₄, THF, 0 °C, 3 h; (e) (i) Tf₂O, pyridine, 0 °C, 1 h; (ii) NaN₃, DMF, 100 °C, 12 h for **16a**; 60 °C, 12 h for **16b**, 12 h; (f) H₂, Pd/C, MeOH, rt, 3 h for **17a**; 12 h for **17b**.

Scheme 3. Synthesis of precursors **18a** and **18b** for purine base build-up.

octane ditetrafluoroborate) 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 stereochemistry 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 of **16a** and **16b** with a linear, less bulky and more powerful nucleophile, NaN₃ 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-dichloropyrimidine and triethylamine in *n*-butanol under microwave irradiation to yield **19a** (Scheme 4) [14,16]. However, weaker nucleophile, 6,6-difluoroamine **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-dichloropyrimidine, 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 **3e**, 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 **3b** 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′-β-fluoroadenosine analogue **3a** was 5.6 times more potent than 6,6′-difluoroadenosine analogue **3d**. A similar trend was observed with 6′-fluorinated-adenosine analogues (n = 1), **2a** and **2b**. 6′-β-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 (EC₅₀ = 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
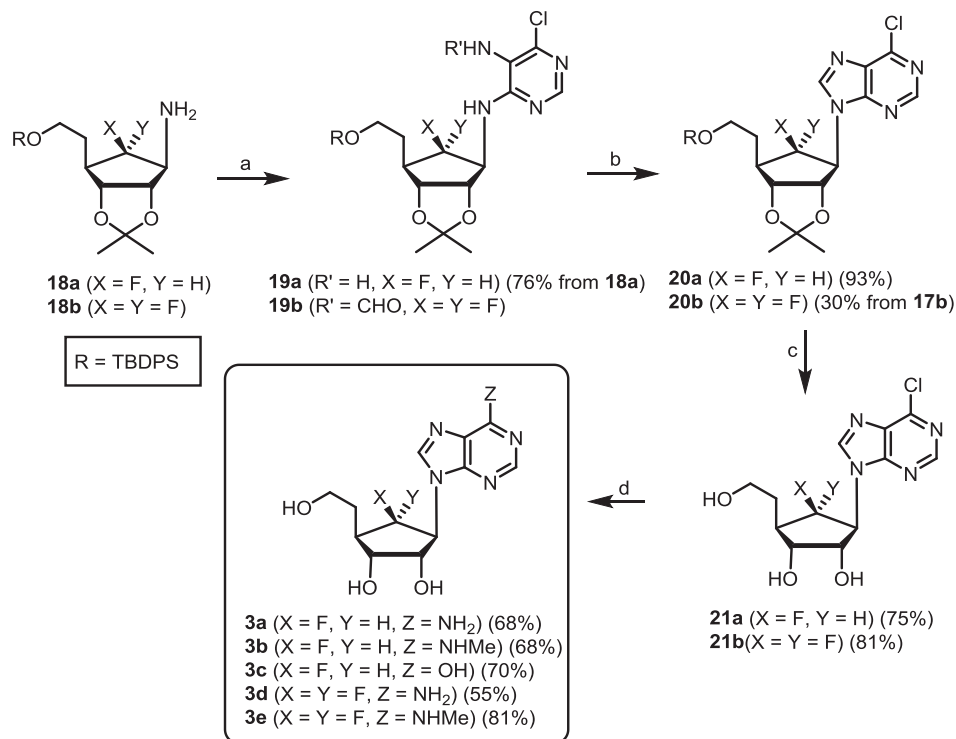
Reagent and Conditions: (a) 5-amino-4,6-dichloropyrimidine for **19a** or 4,6-dichloro-5-foramidopyrimidine for **19b**, *N,N*-diisopropylethylamine or triethylamine, *n*-BuOH, MW, 170 °C, 10 h; (b) CH$_3$C(O)OCH(OEt)$_2$, 120 °C, 14 h; (c) 50% aq. TFA for **21a**, 70% aq. TFA for **21b**, MeOH, 0 °C to rt, 2 h;(d) NH$_3$/*t*-BuOH, 90 °C, 3 h for **3a** or NH$_3$/MeOH, 70 °C, overnight, for **3d**, or 40% aq. MeNH$_2$, 80 °C, 5 h for **3b** and **3e**, or 1 *N* HCl, 1,4-dioxane, reflux, overnight for **3c**.

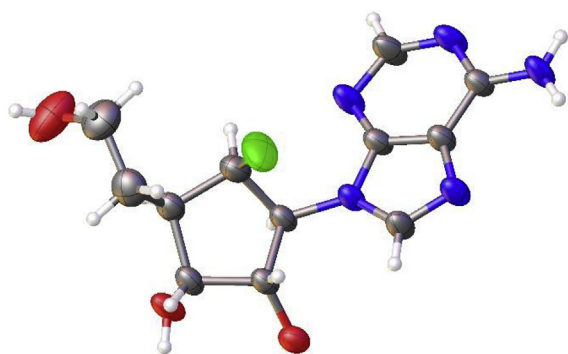**Scheme 4.** Synthesis of 6′-fluorinated homoaristeromycin analogues **3a**–**3e**.



Fig. 2. The X-ray crystal structure of **3a**.

similar IC$_{50}$ values (0.36 and 0.37 μM, respectively), although only **3a** displayed anti-CHIKV activity. Regular 6′-β-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 homoaristeromycin 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′-β-fluoro-homoaristeromycin (**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′-β-fluoro-homoaristeromycin (**3a**) is a promising new template for the development of anti-CHIKV agents.

Table 1
Inhibitory activity towards SAH hydrolase and the anti-CHIKV activity in Vero E6 cells of the nucleosides **3a**-**3e** and **2a**-**2b**.
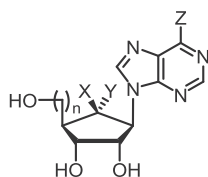
Compound No	SAH hydrolase IC$_{50}$ (μM)	CHIKV	
		EC$_{50}$ (μM)	CC$_{50}$ (μM)
3a (n = 2, X = F, Y = H, Z = NH$_2$)	0.36	0.12	>250
3b (n = 2, X = F, Y = H, Z = NHMe)	0.37	>100	>100
3c (n = 2, X = F, Y = H, Z = OH)	>100	>100	>100
3d (n = 2, X = F, Y = F, Z = NH$_2$)	2.03	>100	>25
3e (n = 2, X = F, Y = F, Z = NHMe)	3.05	>100	>100
2a (n = 1, X = F, Y = H, Z = NH$_2$)	0.37	>100	>100
2b (n = 1, X = F, Y = F, Z = NH$_2$)	1.06	0.13	>1.25

4. Experimental section

4.1. General methods

Proton (^{1}H) and carbon ^{13}C{^{1}H} 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 ^{1}H 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; quin for quintet; bs for 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+ 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 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 CaH$_2$, or P$_2$O$_5$, 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 chlorotrietylsilane (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 NH$_4$Cl (30 mL). The solution was extracted with ethyl acetate (2 × 100 mL). The organic layer was dried using anhydrous MgSO$_4$ 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 × 100). The organic layer was dried over anhydrous MgSO$_4$, filtered and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (hexanes:ethyl acetate = 5.6:1) to give **14** [17] (3.7 g, 84%) as colorless oil, which was equilibrated to the 6-fluorogeminal diol.

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 × 100 mL). The organic layer was dried over anhydrous MgSO$_4$, filtered and concentrated *in vacuo*. The residue was purified by silica gel chromatography (hexanes:ethyl acetate = 11.5:1) to give **16a** as colorless oil (2.62 g, 64%); [α]$_D^{25}$ − 43.5 (*c* 0.14, CH$_3$OH): ^{1}H NMR (400 MHz, CDCl$_3$) δ 7.68 (m, 4H), 7.40 (m, 6H), 4.9 (ddd, *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); ^{13}C NMR (100 MHz, CDCl$_3$) δ 135.8, 133.9, 129.8, 113.8, 101.6, 99.8, 84.0, 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; ^{19}F NMR (376 MHz, CDCl$_3$) δ −204.7; HRMS (FAB) found 459.2366 [calcd for C$_{26}$H$_{36}$FO$_4$Si$^+$ (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 N$_2$ 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 × 100 mL). The organic layer was dried over anhydrous MgSO$_4$ 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 × 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 × 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%): $[\alpha]_D^{25}$ +18.8 (*c* 0.340, CHCl$_3$); ^{1}H NMR (500 MHz, CDCl$_3$) δ 7.69−7.66 (m, 4H), 7.44−7.36 (m, 6H), 4.54 (m, 1H), 4.36−4.34 (m, 1H), 3.97 (ddd, *J* = 6.4, 12.5 Hz, 1H), 3.79−3.74 (m, 2H), 2.87 (d, *J* = 5.6 Hz, 1H), 2.65−2.60 (m, 1H), 1.97−1.91 (m, 1H), 1.58−1.52 (m, 4H), 1.32 (s, 3H), 1.05 (s, 9H); ^{13}C NMR (125 MHz, CDCl$_3$) δ 135.6, 133.5, 129.6, 128.0 (*J* = 248.8, 263.5 Hz), 127.6, 112.9, 81.1, 75.2, 71.0 (*J* = 12.4, 19.8 Hz), 61.5, 44.9 (*J* = 20.5 Hz), 28.8, 26.8, 26.0, 24.4, 19.1; ^{19}F NMR (CDCl$_3$, 376 MHz) δ −112.7 (dd, *J* = 16.1, 241.9 Hz), −115.8 (dd, *J* = 24.8, 242.7 Hz); HRMS (FAB) found 494.2540 [calcd for C$_{26}$H$_{38}$F$_2$NO$_4$Si$^+$ (M + NH$_4$)$^+$ 494.2533].

4.1.3. (2-((3aR,4R,5R,6S,6aS)-6-Azido-5-fluoro-2,2-dimethyltetrahydro-4H-cyclopenta[d][1,3]dioxol-4-yl)ethoxy)(tert-butyl)diphenylsilane (17a)

To a stirred solution of **16a** (2.35 g, 5.12 mmol) in anhydrous pyridine (50 mL) was added triflic anhydride (1.72 mL, 10.24 mmol) at 0 °C and the reaction mixture was stirred at 0 °C for 1 h, quenched with water (3 mL) and concentrated *in vacuo*. The residue was diluted with ethyl acetate (100 mL) and washed with 15% aqueous CuSO$_4$ solution (3 × 30 mL). The organic layer was dried over anhydrous MgSO$_4$, filtered and concentrated *in vacuo* to get the crude triflate, which was used immediately for the next step without further purification.

To a stirred solution of the crude triflate in anhydrous DMF (50 mL) was added sodium azide (3.08 g, 51.2 mmol) at room temperature and the reaction mixture was stirred at 100 °C for 12 h, cooled to room temperature and diluted with water (10 mL). The solution was extracted with diethyl ether (100 mL). The organic layer was washed with water (5 × 50 mL), dried anhydrous MgSO$_4$, filtered and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (hexanes:ethyl acetate = 32.3:1) to give **17a** as colorless oil (0.36 g, 90%): $[\alpha]_D^{25}$ − 98.7 (*c* 0.155, CH$_3$OH); ^{1}H NMR (400 MHz, CDCl$_3$) δ 7.69 (m, 4H), 7.41 (m, 6H), 4.92 (dt, *J* = 3.2, 52.8 Hz, 1 H), 4.68 (m, 1H), 4.45 (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 (100 MHz, CDCl$_3$) δ 135.8, 133.8, 129.9, 127.8, 114.2, 99.4, 97.6, 83.6, 82.3, 68.3 (*J* = 15.7 Hz), 61.9, 45.5 (*J* = 17.7 Hz), 30.3 (*J* = 4.6 Hz), 27.4, 24.9, 19.3; ^{19}F NMR (376 MHz, CDCl$_3$) δ −205.5; HRMS (FAB) found 506.2254 [calcd for C$_{26}$H$_{34}$FN$_3$NaO$_3$Si$^+$ (M + Na)$^+$ 506.2246].

4.1.4. (2-((3aR,4R,6S,6aS)-6-azido-5,5-difluoro-2,2-dimethyltetrahydro-4H-cyclopenta[d][1,3]dioxol-4-yl)ethoxy)(tert-butyl)diphenylsilane (17b)

To a stirred solution of **16b** (1.65 g, 3.46 mmol) in anhydrous pyridine (30 mL) was added triflic anhydride (1.16 mL 6.92 mmol) at 0 °C. The reaction mixture was stirred at 0 °C for 1 h, quenched with saturated aqueous sodium bicarbonate solution (5 mL), concentrated *in vacuo* to get the crude residue, which was diluted with ethyl acetate (40 mL), washed with 15% aqueous CuSO$_4$ solution and extracted with ethyl acetate (2 × 30 mL). The organic

layer was washed with water, dried over MgSO$_4$ and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (hexanes:ethyl acetate = 2.5:1) to give the triflate, which was used immediately for the next step.

To a stirred solution of the triflate in anhydrous DMF (30 mL) was added sodium azide (0.62 g, 10.38 mmol) at room temperature and the reaction mixture was stirred at 60 °C for 12 h, cooled to room temperature and diluted with water (10 mL). The mixture was extracted with diethyl ether (2 × 40 mL) and the organic layer was washed with water (5 × 30 mL), dried over anhydrous MgSO$_4$ and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (hexanes:ethyl acetate = 19:1) to give **17b** as colorless oil (1.12 g, 64%): $[\alpha]_D^{25}$ +14.6 (*c* 0.335, CHCl$_3$); ^{1}H NMR (500 MHz, CDCl$_3$) δ 7.69−7.67 (m, 4H), 7.44−7.36 (m, 6H), 4.40−4.38 (m, 1H), 4.30−4.27 (m, 1H), 3.94 (dt, *J* = 5.3, 17.6 Hz, 1H), 3.77 (t, *J* = 6.2 Hz, 2H), 2.66−2.59 (m, 1H), 2.02−1.96 (m, 1H), 1.75−1.68 (m, 1H), 1.52 (s, 3H), 1.28 (s, 3H), 1.04 (s, 9H); ^{13}C NMR (125 MHz, CDCl$_3$) δ 135.6, 133.5, 129.6, 127.6, 127.3(*J* = 270.0, 275.0 Hz), 113.5, 80.1 (*J* = 8.6 Hz), 79.5 (*J* = 7.0 Hz), 68.6 (*J* = 18.9, 22.8 Hz), 60.9, 46.5 (*J* = 20.1 Hz), 28.7 (*J* = 4.3 Hz), 29.0, 26.8, 24.7, 19.2; ^{19}F NMR (376 MHz, CDCl$_3$) δ −104.5, −120.2; HRMS (FAB) found 524.2161 [calcd for C$_{26}$H$_{33}$F$_2$N$_3$NaO$_3$Si$^+$ (M + Na)$^+$ 524.2151].

4.1.5. N [4]-((3aS,4S,5R,6R,6aR)-6-(2-((tert-Butyldiphenylsilyl)oxy)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. 55% 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 *N,N*-diisopropylethylamine at room temperature and the reaction mixture was subjected to microwave irradiation 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$_3$, dried over anhydrous MgSO$_4$, filtered, and concentrated *in vacuo*. The residue was purified by silica gel chromatography (hexanes:ethyl acetate = 4.6:1) to give **19a** as colorless oil (0.91 g, 76%): $[\alpha]_D^{25}$ − 19.7 (*c* 0.167, CHCl$_3$); ^{1}H NMR (500 MHz, CDCl$_3$) δ 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, 1H), 4.75−4.64 (m, 1H), 4.61 (merged dd, *J*$_1$ = *J*$_2$ = 6.7 Hz, 1H), 4.44 (merged dd, *J*$_1$ = *J*$_2$ = 6.7 Hz, 1H), 3.82−3.71 (m, 2H), 3.60−3.45 (brs, 2 H), 2.51−2.37 (m, 1H), 1.94−1.80 (m, 2H), 1.54 (s, 3H), 1.30 (s, 3H), 1.03 (s, 9H); ^{13}C NMR (150 MHz, CDCl$_3$) δ 154.3, 149.3, 143.2, 135.5, 133.6, 129.6, 127.6, 122.3, 114.1, 98.3 (*J* = 211.8 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, 19.1 ^{19}F NMR (376 MHz, CDCl$_3$) δ −208.0; HRMS (FAB) found 585.2468 [calcd for C$_{30}$H$_{39}$ClFN$_4$O$_3$Si$^+$ (M + H)$^+$ 585.2458].

4.1.6. 9-((3aS,4S,5R,6R,6aR)-6-(2-((tert-Butyldiphenylsilyl)oxy)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 chromatography (hexanes:ethyl acetate = 9:1) to give **20a** as a white foam (0.87 g, 93%): $[\alpha]_D^{25}$ − 12.3 (*c* 0.162, CH$_3$OH); UV (CH$_3$OH) λ_{max} 263 nm; ^{1}H NMR (400 MHz, CDCl$_3$) δ 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),

2.64 (m, 1H), 1.95 (m, 2H), 1.59 (s, 3H), 1.34 (s, 3H), 1.06 (s, 9H); ^{13}C NMR (100 MHz, CDCl$_3$) δ 152.3, 144.2, 135.7, 133.7, 129.9, 127.9, 115.3, 99.2, 97.4, 83.4, 83.0, 63.3, 61.7, 46.0, 30.5, 27.6, 27.0, 25.1, 19.3; ^{19}F NMR (376 MHz, CDCl$_3$) δ −200.72; HRMS (FAB) found 595.2290 [calcd for C$_{31}$H$_{37}$ClFN$_4$O$_3$Si$^+$ (M + H)$^+$ 595.2302].

4.1.7. 9-((3aS,4S,6R,6aR)-6-(2-((tert-Butyldiphenylsilyl)oxy)ethyl)-5,5-difluoro-2,2-dimethyltetrahydro-4H-cyclopenta[d][1,3]dioxol-4-yl)-6-chloro-9H-purine (20b)

To solution of **17b** (0.93 g, 1.85 mmol) in methanol (18 mL) was added 10% palladium on carbon (wet with ca. 55% water) (200 mg) at room temperature and the reaction mixture was stirred under H$_2$ 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 **19b** was used for the next step without further purification. To the crude **19b** was added diethoxymethyl acetate (15 mL) and the reaction mixture was stirred at 120 °C overnight. 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: [α]$_D^{25}$ − 10.1 (c 0.250, CHCl$_3$); ^{1}H NMR (600 MHz, CDCl$_3$) δ 8.78 (s, 1H), 8.24 (d, J = 1.9 Hz, 1H), 7.70−7.67 (m, 4H), 7.43−7.36 (m, 6H), 5.38−5.32 (m, 1H), 5.14 (t, J = 6.9 Hz, 1H), 4.52 (t, J = 6.4 Hz, 1H), 3.85−3.80 (m, 2H), 2.98−2.90 (m, 1H), 2.09−2.03 (m, 1H), 1.86−1.81 (m, 1H), 1.59 (s, 3H), 1.31 (s, 3H), 1.05 (s, 9H); ^{13}C NMR (150 MHz, CDCl$_3$) δ 152.5, 152.4, 151.6, 143.7 (J = 4.3 Hz), 135.6, 133.4, 131.4, 129.7, 127.7, 126.1(J = 247.5, 262.5 Hz), 114.5, 79.9 (J = 10.1 Hz), 78.8 (J = 7.2 Hz), 63.8 (J = 22.3 Hz), 60.6, 46.6 (J = 20.1 Hz), 28.6 (J = 4.3 Hz), 27.2, 26.8, 24.9, 19.2; ^{19}F NMR (376 MHz, CDCl$_3$) δ −107.7, −117.1; HRMS (FAB) found 613.2213 [calcd for C$_{31}$H$_{36}$ClF$_2$N$_4$O$_3$Si$^+$ (M + H)$^+$ 613.2208].

4.1.8. (1R,2S,3S,4R,5R)-3-(6-Chloro-9H-purin-9-yl)-4-fluoro-5-(2-hydroxyethyl)cyclopentane-1,2-diol (21a)

To a stirred solution of **20a** (0.57 g, 0.966 mmol) in methanol (3 mL) was added 50% aqueous trifluoroacetic acid (15 mL) at 0 °C. The reaction mixture was stirred at room temperature for 2 h. The solvent was removed under reduced pressure and the residue was purified by column chromatography (methylene chloride:methanol = 9:1) to give **21a** (0.23 g, 75%), which was crystallized from diethyl ether/methanol as a white solid: mp 147−149 °C; [α]$_D^{25}$ − 32.7 (c 0.107, CH$_3$OH); UV (CH$_3$OH) λ_{max} 263.5 nm; ^{1}H NMR (400 MHz, CD$_3$OD) δ 8.75 (s, 1H), 8.71 (d, J = 2.4 Hz, 1H), 5.18 (dt, J = 4.0, 51.6 Hz, 1H), 5.15, (m, 1H), 4.80 (m, 1H), 4.06 (t, 5.4 Hz, 1H), 3.72 (m, 2H), 2.41 (m, 1H), 1.9 (m, 2H); ^{13}C NMR (100 MHz, CD$_3$OD) δ 154.0, 153.2, 151.4, 147.4 (J = 3.62 Hz), 94.3, 92.5, 74.5, 73.7, 64.3 (J = 17.3 Hz), 31.8 (J = 7.1 Hz); ^{19}F NMR (376 MHz, CD$_3$OD) δ −204.34; HRMS (FAB) found 317.0810 [calcd for C$_{12}$H$_{12}$ClFN$_4$O$_3^+$ (M + H)$^+$ 317.0811].

4.1.9. 2-((3aR,4R,6S,6aS)-6-(6-Chloro-9H-purin-9-yl)-5,5-difluoro-2,2-dimethyltetrahydro-4H-cyclopenta[d][1,3]dioxol-4-yl)ethan-1-ol (21b)

To a stirred solution of **20b** (0.28 g, 0.45 mmol) in 1,4-dioxane (4 mL) was added 70% aqueous trifluoroacetic acid solution (12 mL) at 0 °C and the reaction mixture was stirred at room temperature for 2 h. The mixture was concentrated in vacuo and the residue was purified by silica gel column chromatography (methylene chloride:methanol = 9:1) to give **21b** (0.12 g, 81%), which was crystallized from diethyl ether/methanol as a white solid: mp 80−82 °C [α]$_D^{25}$ +53.2 (c 0.130, CH$_3$OH); UV (CH$_3$OH) λ_{max} 264 nm; ^{1}H NMR (500 MHz, CD$_3$OD) δ 8.77 (s, 1H), 8.76 (d, J = 1.9 Hz, 1H), 5.45 (ddd, J$_1$ = J$_2$ = 8.7 Hz, J$_3$ = 17.9 Hz, 1H), 4.91−4.88 (m, 1H), 4.10−4.09 (m, 1H), 3.74−3.69 (m, 2H), 2.70−2.67 (m, 1H), 2.01−1.95 (m, 1H), 1.83, 1.78 (m, 1H); ^{13}C NMR (125 MHz, CDCl$_3$) δ 155.0, 154.13, 152.42, 148.46, 133.03, 126.16 (J = 251.0, 259.1 Hz), 74.3 (J = 7.1 Hz), 72.8 (J = 6.6 Hz), 65.9 (J = 23.0 Hz), 61.2, 31.3 (J = 6.8 Hz), 61.2, 31.3 (J = 6.8 Hz) (1 carbon hidden by methanol peak); ^{19}F NMR (376 MHz, CD$_3$OD) δ −103.3, −115.9; HRMS (FAB) found 335.0713 [calcd for C$_{12}$H$_{14}$ClF$_2$N$_4$O$_3^+$ (M + H)$^+$ 335.0717].

4.1.10. (1R,2S,3S,4R,5R)-3-(6-Amino-9H-purin-9-yl)-4-fluoro-5-(2-hydroxyethyl)cyclopentane-1,2-diol (3a)

A solution of **21a** (70 mg, 0.22 mmol) in saturated tert-butanolic ammonia 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** (44 mg, 68%), which was crystallized from diethyl ether/methanol as a white solid: mp 168−170 °C; [α]$_D^{25}$ − 49.3 (c 0.146, CH$_3$OH); UV (CH$_3$OH) λ_{max} 259 nm, ^{1}H NMR (400 MHz, CD$_3$OD) δ 8.26 (d, J = 2.0 Hz, 1H), 8.20 (s, 1H), 5.11 (dt, J = 3.6, 55.2 Hz, 1H), 4.99 (ddd, J = 3.2, 9.6, 26.4 Hz, 1H), 4.75 (m, 1H), 4.04 (m, 1H), 3.71 (m, 2H), 2.38 (m, 1H), 1.89 (m, 2H); ^{13}C NMR (100 MHz, CD$_3$OD) δ 157.4, 153.9, 151.5, 141.7 (J = 4.4 Hz), 94.2, 92.6, 74.5, 73.7, 63.7 (J = 17.1 Hz), 61.1, 47.8, (J = 17.9 Hz), 31.8 (J = 6.6 Hz); ^{19}F NMR (376 MHz, CD$_3$OD) δ −204.9; HRMS (FAB) found 298.1310 [calcd for C$_{12}$H$_{17}$FN$_5$O$_3^+$ (M + H)$^+$ 298.1310].

4.1.11. (1S,2R,3R,4R,5S)-4-fluoro-3-(2-hydroxyethyl)-5-(6-(methylamino)-9H-purin-9-yl)cyclopentane-1,2-diol (3b)

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; [α]$_D^{25}$ − 1.06 (c 0.160, CH$_3$OH); UV (CH$_3$OH) λ_{max} 265 nm; ^{1}H NMR (400 MHz, CD$_3$OD) δ 8.25 (s, 1H), 8.2 (d, J = 1.4 Hz, 1H), 5.09 (dt, J = 3.6, 54.8 Hz, 1H), 4.96 (ddd, J = 3.0, 9.4, 26.7 Hz, 1H), 4.74 (t, J = 7.4 Hz, 1H), 4.02 (t, J = 6.0 Hz, 1H), 3.74−3.67 (m, 2H), 3.20−3.00 (brs, 3H), 2.42−2.31 (m, 1H), 1.94−1.84 (m, 2H); ^{13}C NMR (125 MHz, CD$_3$OD) δ 157.5, 154.6, 151.1, 141.8 (J = 3.6 Hz), 121.1, 94.2 (J = 180.5 Hz), 75.3, 74.4, 64.4 (J = 17.0 Hz), 61.8, 48.5 (J = 18.5 Hz), 32.6 (J = 6.6 Hz), 28.6; ^{19}F NMR (376 MHz, CD$_3$OD) δ −206.4; HRMS (FAB) found 312.1460 [calcd for C$_{13}$H$_{19}$FN$_5$O$_3^+$ (M + H)$^+$ 312.1466].

4.1.12. 9-((1S,2R,3R,4R,5S)-2-fluoro-4,5-dihydroxy-3-(2-hydroxyethyl)cyclopentyl)-1,9-dihydro-6H-purin-6-one (3c)

To a stirred 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 C-18 reverse-phase silica gel column chromatography (H$_2$O) to give **3c** (33 mg, 70%), which was crystallized from diethyl ether/methanol as a white solid: mp 184−186 °C; [α]$_D^{25}$ − 33.25 (c 0.160, H$_2$O); UV (H$_2$O) λ_{max} 250 nm; ^{1}H NMR (800 MHz, D$_2$O) δ 8.20 (s, 1H), 8.08 (s, 1H), 5.07 (dt, J = 3.7, 54.2 Hz, 1H), 4.91 (ddd, J = 3.2, 9.8, 30.2 Hz, 1H), 4.74−4.72 (m, 1H), 4.05 (t, J = 5.9 Hz, 1H), 3.65 (t, J = 6.6 Hz, 2H), 2.33−2.25 (m, 1H), 1.87−1.77 (m, 2H); ^{13}C NMR (150 MHz, D$_2$O)

δ 158.4, 149.4, 145.7, 141.0, 123.0, 91.9 ($J = 179.9$ Hz), 72.5, 72.1, 61.8 ($J = 16.9$ Hz), 59.6, 45.5 ($J = 18.5$ Hz), 29.4 ($J = 6.9$ Hz)); ^{19}F NMR (376 MHz, CD$_3$OD) δ -204.9; HRMS (FAB) found 299.1150 [calcd for C$_{12}$H$_{16}$FN$_4$O$_5^+$ (M + H)$^+$ 299.1150].

4.1.13. 2-((3aR,4R,6S,6aS)-6-(6-Amino-9H-purin-9-yl)-5,5-difluoro-2,2-dimethyltetrahydro-4H-cyclopenta[d][1,3]dioxol-4-yl) ethan-1-ol (3d)

A solution of **21b** (33 mg g, 0.099 mmol) in saturated methanolic 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 as a white solid: mp 109−111 °C; $[\alpha]_D^{25}$ +131.8 (c 0.040, CH$_3$OH); UV (CH$_3$OH) λ_{max} 261 nm; ^{1}H NMR (500 MHz, CD$_3$OD) δ 8.27 (d, $J = 1.8$ Hz, 1H), 8.20 (s, 1H), 5.29 (ddd, $J_1 = J_2 = 8.4$ Hz, $J_3 = 17.8$ Hz, 1H), 4.77 (dd, $J = 6.6$, 8.8 Hz, 1H), 4.07 (t, $J = 4.8$ Hz, 1H), 3.74−3.69 (m, 2H), 2.69−2.62 (m, 1H), 2.00−1.94 (m, 1H), 1.83−1.78 (m, 1H); ^{13}C NMR (125 MHz, CDCl$_3$) δ 158.2, 154.8, 152.6, 142.7, 126.1 ($J = 251.3$, 258.5 Hz), 120.6, 74.0 ($J = 7.9$ Hz), 73.0 ($J = 6.5$ Hz), 65.2 ($J = 21.6$ Hz), 61.3, 50.7, 31.3 ($J = 6.5$ Hz); ^{19}F NMR (376 MHz, CD$_3$OD) δ -103.3, -115.9; HRMS (FAB) found 316.1220 [calcd for C$_{12}$H$_{16}$F$_2$N$_5$O$_3^+$ (M + H)$^+$ 316.1216].

4.1.14. 2-((3aR,4R,6S,6aS)-5,5-difluoro-2,2-dimethyl-6-(6-(methylamino)-9H-purin-9-yl)tetrahydro-4H-cyclopenta[d][1,3] dioxol-4-yl)ethan-1-ol (3e)

A solution of **21b** (10 mg, 0.03 mmol) in methanol (2 mL) was added 40% methylamine aqueous solution (0.25 mL, 3.22 mmol). The reaction mixture was heated at 50 °C for 5 h 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 give **3e** (0.008 g, 81%), which was crystallized from diethyl ether/methanol as a white solid: mp 89−91 °C; $[\alpha]_D^{25}$ +2.0 (c 0.160, CH$_3$OH); UV (CH$_3$OH) λ_{max} 265 nm; ^{1}H NMR (500 MHz, CD$_3$OD) δ 8.25 (s, 1H), 8.21 (s, 1H), 5.27 (ddd, $J_1 = J_2 = 8.4$, 17.8 Hz, 1H), 4.76 (dd, $J = 6.6$, 8.7 Hz, 1H), 4.06 (t, $J = 4.7$ Hz, 1H), 3.74−3.68 (m, 2H), 3.11 (brs, 3H), 2.66−2.61 (m, 1H), 2.00−1.94 (m, 1H), 1.83−1.77 (m, 1H); ^{13}C NMR (125 MHz, CDCl$_3$) δ 157.6, 154.8, 151.5, 142.0, 126.1 ($J = 250.1$, 258.38 Hz), 121.2, 74.2 ($J = 7.5$ Hz), 73.0 ($J = 7.0$ Hz), 65.1 ($J = 21.4$ Hz), 61.3, 50.7, 31.3 ($J = 6.5$ Hz), 28.6; ^{19}F NMR (376 MHz, CD$_3$OD) δ -103.3, -115.9; HRMS (FAB) found 330.1386 [calcd for C$_{13}$H$_{18}$F$_2$N$_5$O$_3^+$ (M +H)$^+$ 330.1372].

4.2. Antiviral activity and inhibition of purified SAH hydrolase

The determination of the antiviral activity of the synthesized compounds and the calculation of the EC$_{50}$ and CC$_{50}$ was done as described before [14].

The inhibition of purified SAH hydrolase by the different compounds and determination of IC$_{50}$ values was done as previously described [14].

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

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, ρcalc = 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 maxmin-1 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.kr) using no. CCDC 1963491.