Chikungunya virus nonstructural protein 1 as an antiviral target

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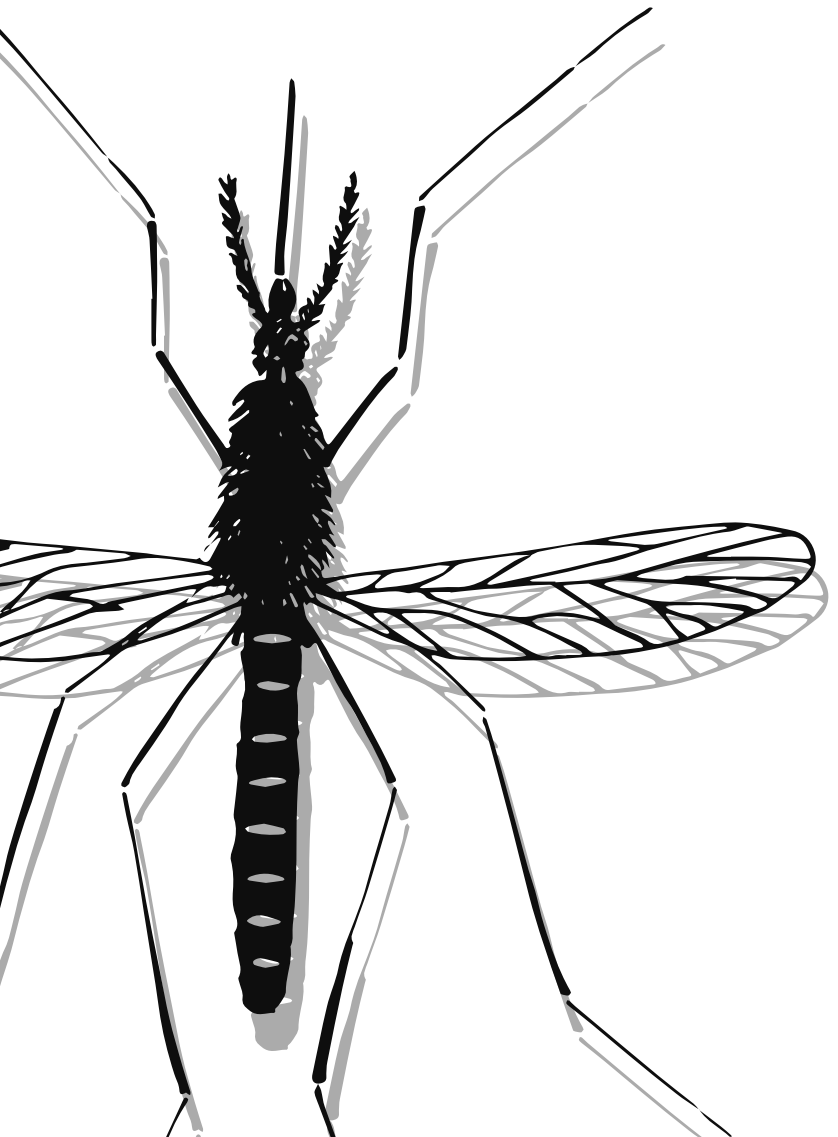

CHAPTER 3

Identification of 6'-fluorinated-aristeromycin and 6'-fluorinated-homoaristeromycin analogues as Chikungunya virus inhibitors

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Abstract

6'-fluorinated-aristeromycin analogues were designed as dual-target antiviral compounds aimed at inhibiting both Chikungunya virus (CHIKV) RNA-dependent RNA polymerase and the host cell S-adenosyl-L-homocysteine (SAH) hydrolase. Our results demonstrated that the introduction of a fluorine at the 6'-position of aristeromycin to yield 6'-fluorinated-aristeromycin analogues enhanced the inhibition of SAH hydrolase as well as the antiviral activity of these compounds. Aristeromycin and the *N6*-mehyltaristeromycin analogues **2a-e** showed potent inhibition of SAH hydrolase, while only the aristeromycin derivatives **2b-c** exhibited antiviral activity against CHIKV. 6',6'-Difluoro-aristeromycin **(2c)** showed the strongest anti-CHIKV effect among the 6'-fluorinated-aristeromycin analogues, with more than 2 $\log_{10}$ reduction of the infectious progeny titer. Due to their substantial cytotoxicity, likely attributable to 5'-phosphorylation, one-carbon homologated 6'-fluorinated-aristeromycin analogues were designed. This approach was intended to prevent 5'-phosphorylation by cellular kinases while retaining the inhibitory activity against SAH hydrolase. Four out of five synthesized 6'-fluorinated-homoaristeromycin analogues **4a-e** exhibited potent inhibitory activity towards SAH hydrolase, among which 6'-β-fluoro-homoaristeromycin **(4a)** was the most potent (IC_{50} = 0.36 μM). 6'-β-Fluoro-homoaristeromycin **(4a)** also potently inhibited CHIKV replication (EC_{50} = 0.12 μM) in cytopathic effect (CPE)-reduction assays without inducing noticeable cytotoxicity. Remarkably, only **4a** displayed anti-CHIKV activity, whereas both **4a** and **4b** inhibited SAH hydrolase with similar IC_{50} values (0.36 μM and 0.37 μM, respectively), suggesting that **4a**'s antiviral activity did not merely depend on the inhibition of SAH hydrolase. This was supported by the fact that the antiviral effect was specific for CHIKV and the related alphavirus Semliki Forest virus, while none of the homologated analogues inhibited other plus-stranded RNA viruses, such as Severe acute respiratory syndrome coronavirus, Middle East respiratory syndrome coronavirus or Zika virus. The chemical synthesis of these compounds has been published elsewhere.

Introduction

Chikungunya virus (CHIKV) is an alphavirus belonging to the *Togaviridae* family that is transmitted by mosquitoes and causes a painful arthritis that can persist for months to years (1-3). CHIKV belongs to the + sense single stranded (ss) RNA virus group (Baltimore class IV) (4, 5), which means that their genomic RNA has the same polarity as mRNA and can be directly translated by host ribosomes in the infected cell. The CHIKV genome is translated into a polyprotein that is subsequently cleaved into individual nonstructural proteins 1-4 (nsPs) by a protease domain in nsP2. The nsPs 1-4 harbour a variety of enzymatic activities that are required for the replication of the viral RNA and invariably include an RNA-dependent RNA polymerase (RdRp) encoded by CHIKV nsP4 (6), an enzyme which is not present in uninfected cells. The alphavirus nsP4 transcribes the genomic RNA into a complementary- strand RNA that subsequently serves as the template for the synthesis of new + strand genomic and subgenomic RNA (7).

Many + strand RNA viruses, including CHIKV, encode methyltransferases (MTases) that are required for methylation of viral RNA to produce a cap structure at the 5' terminus. Because this capping process is crucial for the stability and translation of the viral RNA and evasion of the host innate immune response, viral MTases are considered promising targets for the development of antiviral therapy (8-10). Inhibition of MTases, such as the CHIKV MTase encoded by nsP1, can also be achieved indirectly, by the inhibition of S-adenosyl-L-homocysteine (SAH) hydrolase (11, 12). Under normal physiological conditions, this cellular enzyme catalyses the conversion of SAH into adenosine and L-homocysteine. Inhibition of SAH hydrolase leads to the accumulation of SAH in cells, which in turn inhibits S-adenosyl-L-methionine (SAM)-dependent transmethylase reactions by feedback inhibition (13, 14). The CHIKV nsP1-catalysed methylation of viral RNA is dependent on SAM. More specifically, CHIKV nsP1 uses SAM as a methyl donor to transfer a methyl group onto an acceptor molecule of guanosine-5'-triphosphate (GTP) to produce methylated GTP (m^7GTP). In the alphavirus RNA capping process, the GTP molecule is methylated before it is transferred onto the 5' end of viral RNA, which differs from the mechanism used by the host cell (8).

Compounds targeting cellular proteins might exhibit a broader spectrum of antiviral activity and less likelihood of developing drug resistance, although they are more likely to be toxic to the host cell. Thus, the approach of targeting cellular proteins such as SAH hydrolase can be considered as a promising strategy for the development

of broad-spectrum antiviral agents (13, 14). A number of compounds have been reported to act as SAH hydrolase inhibitors. Type I inhibitors act through inactivation of the NAD^+ cofactor of the enzyme, and their inhibitory effect on its catalytic activity can be reversed by the addition of an excess of NAD^+. Type II inhibitors are irreversible inhibitors of the SAH hydrolase that form covalent bonds with amino acid residues in the active site of the enzyme, a process which cannot be reversed by the addition of NAD^+ or adenosine, or by dialysis (13).

Because both CHIKV nsP1 and nsP4 are critical for virus replication, broad-spectrum nucleoside analogue inhibitors that could directly target nsP4 activity and/or indirectly inhibit the methylation of viral RNA through their effect on the host SAH hydrolase were designed (15). Modified nucleosides are usually taken up by cells via nucleoside transporters and can be successively converted into mono-, di-, and triphosphates by cellular kinases. Then, these modified nucleoside triphosphates (NTPs) can compete with natural NTPs during RNA synthesis or can be incorporated into the nascent viral RNA, leading to chain termination or detrimental mutations (16).

(-)-Aristeromycin **(1)** is a naturally occurring carbocyclic nucleoside that was originally identified as a metabolite of *Streptomyces citricolor* in 1967 (17). It is a type I SAH hydrolase inhibitor and exhibits potent antiviral activity against many viruses (13). However, it could not be further advanced into clinical development because of its cytotoxicity, which is likely linked to its conversion into a triphosphate form (18, 19). Therefore, **1** was used as a prototype for the design and synthesis of 6'-fluorinated-aristeromycin analogues as dual-target compounds (15). 6',6'-difluoro-aristeromycin, designated as compound **2c**, exhibited potent antiviral activities against CHIKV (EC_{50} 0.13 μM, CC_{50} >1.25 μM) (Table 1) and also against other emerging + strand RNA viruses such as Middle East Respiratory Syndrome coronavirus (MERS-CoV), Severe acute respiratory syndrome coronavirus (SARS-CoV) or Zika virus (ZIKV) (15). However, because compound **2c** still exhibited substantial cytotoxicity, presumably due to its 5'-phosphorylation, 6'-fluorinated-homoaristeromycin analogues **4a-e** were synthesized (20). These compounds were expected to retain their inhibitory activity towards SAH hydrolase without being 5'-phosphorylated by cellular kinases. The prevention of 5'-phosphorylation would be achieved by a one-carbon homologation step at the 5' position that is expected to displace the susceptible 5'-hydroxyl from the phosphate-transfer zone in the kinases (Fig. 1) (20). The chemical synthesis of both 6'-fluorinated-aristeromycin and 6'-fluorinated-homoaristeromycin analogues is described in (15) and (20). It is based on stereoselective electrophilic fluorination of silyl enol ether with Selectfluor as the fluorine source.

Lastly, to test the hypothesis whether 6'-fluorinated-aristeromycin analogues act as dual-target compounds and inhibit CHIKV RdRp, a phosphoramidate prodrug **3** of nucleoside **2**, was synthesized using the McGuigan ProTides approach (21-28). This method is designed to overcome the limitations associated with the parental nucleosides such as inefficient conversion to their corresponding 5'-monophosphate form and poor cellular uptake. In this chapter, the ability of all nucleosides to inhibit recombinant human SAH hydrolase is reported. Furthermore, the antiviral effect of 6'-fluorinated-aristeromycin and 6'-fluorinated-homoaristeromycin analogues on CHIKV is reported from cytophathic effect (CPE)-based assays and viral load reduction assays. These data were essential for providing more insight into the structure-activity relationships (SAR) of these CHIKV inhibitors.

Materials and Methods

SAH hydrolase assay

SAH hydrolase assays were performed as described previously in (29-32). The gene encoding human placental SAH hydrolase was cloned into expression plasmid pPROKcd20. Recombinant SAH hydrolase proteins was produced in *E. coli* JM109 in 50 mM Tris-HCl (pH 7.5) containing 2 mM ethylenediaminetetraacetic acid and was purified by DEAE-cellulose column (2.8 cm x 6 cm), ammonium sulphate fractionation and precipitation (35 – 60%), Sephacryl S-300HR (1.0 cm x 105 cm), and DEAE cellulose (2.8 cm x 24 cm). The protein purity was confirmed by 10 % sodium dodecyl sulphate polyacrylamide gel electrophoresis and Coomassie staining. The protein concentration was determined by using the Bradford method using a standard curve with different concentrations of bovine serum albumin. Enzyme activity was determined in reaction mixtures (250 µl) that contained 50 mM sodium phosphate (pH 8.0), 2 µM SAH hydrolase (0.5 µM tetrameric form), and varying concentrations of compounds. The reaction mixtures were pre-incubated with the compound for 10 min at 37 °C, after which the reaction was initiated by adding 100 µM SAH. The reaction was allowed to proceed for 20 min, followed by the addition of 5,5'-dithiobis-2-nitrobenzoate (DNTB) to a final concentration of 200 µM. The absorbance of the reaction product 5-thio-2-nitrobenzoic acid (TNB) was measured at 412 nm using a spectrophotometer (Varian, Cary 100). The molar extinction coefficient for TNB (ε_{412} = 13 700 M^{-1} cm^{-1}) was used in calculations to quantify TNB formation.

Cells, viruses and compounds

VeroE6 cells were maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 8% foetal calf serum (FCS), 2 mM L-glutamine, 100 IU/ml of penicillin and 100 µg/ml of streptomycin, and were grown at 37 °C in a humidified incubator with 5% CO_2. Infections were performed in Eagle's minimal essential medium (EMEM) with 25 mM HEPES supplemented with 2% FCS, L-glutamine and antibiotics. Infectious clone-derived CHIKV (CHIKV-LS3) was generated as described by Scholte at al. (33). The compounds were dissolved in dimethylsulfoxide (DMSO) to obtain 20 mM stock solutions. All work with infectious CHIKV was performed inside biosafety cabinets in the BSL-3 facilities of the Leiden University Medical Center.

Antiviral CPE-reduction assay

VeroE6 cells were seeded at a density of 5000 cells/well in a total volume of 100 µl/well in 96-well plates. The following day, compound dilutions with concentrations of 150 µM, 50 µM, 16.7 µM and 5.6 µM were prepared in the infection medium by 3-fold serial dilution of the 150 µM solution. After replacing the culture medium with the respective dilutions of the compound, the cells were infected with CHIKV at a MOI of 0.005. Viability assays were conducted in parallel. Each compound was tested at each concentration in quadruplicate (4 biological replicates per concentration). The CellTiter 96 AQueous One Solution Cell Proliferation Assay was conducted at 96 hours post-infection (hpi) for CHIKV by adding 20 µl/well of the MTS/PMS reagent (Promega). The assay was stopped after 2 h by fixing the cells with 37% formaldehyde. The absorbance was measured at 495 nm in a Berthold Mithras LB 940 plate reader, and the values were expressed relative to uninfected (infection) or untreated (viability) controls. The results represent the average of quadruplicate samples expressed as the mean ± SD. Compounds that were found to be protective were further evaluated in CPE reduction assays by testing 8 different concentrations to determine the EC_{50} as previously described (33). The cytotoxicity (CC_{50}) of the compounds was determined in parallel, and all experiments were performed in quadruplicate. GraphPad Prism v8.0.1 was used for EC_{50} and CC_{50} determination by nonlinear regression.

Viral load reduction assay

VeroE6 cells were seeded at a density of 7.5 x 10^4 cells/well in 0.5 ml DMEM/8% FCS in 24-well cell culture plates and allowed to adhere overnight. The next day, compound

dilutions (0 – 1.5 μM) were prepared in EMEM/2% FCS to which CHIKV was added at a MOI of 0.1 to yield inoculum for infecting the cells. Cells were incubated at 37 °C with 250 μl/well of the inoculum for 1 h. After the infection, the cells were washed twice with 1 ml/well of warm phosphate-buffered saline (PBS) and 0.5 ml/well of fresh EMEM/2% FCS with different concentration of compound **2c** (0 – 1.5 μM) was added. The cells were incubated for 30 h at 37 °C, after which supernatants were harvested and stored at-80 °C to determine the infectious virus titer by plaque assay. Viability assays were conducted in parallel as described in the previous paragraph. Plaque assays with CHIKV were performed on VeroE6 cells as described previously (33). Compound **2c** was tested at each concentration in duplicate in two independent experiments (n = 4). GraphPad Prism v8.0.1 was used for statistical analysis with a one-way ANOVA multiple comparison test.

Results and Discussion

Inhibition of SAH hydrolase

The synthesized 6'-fluorinated-aristeromycin and 6'-fluorinated-homoaristeromycin analogues were assayed for their inhibitory activity against SAH hydrolase using enzymatic assays with recombinant SAH hydrolase (Table 1 and 2). All adenosine derivatives **2a-e** potently inhibited SAH hydrolase, but none of the pyrimidine analogues **2f-j** showed any inhibitory activity at concentrations up to 100 μM. In addition, none of the prodrugs **3a-c** exhibited inhibitory activity at concentrations up to 100 μM (Table 1). This result was expected because adenosine, and not the prodrug, is the substrate for SAH hydrolase. The 6'-fluorinated-homoaristeromycin analogues **4a** and **4b** also exhibited potent inhibitory activity against SAH hydrolase while 6,6'-difluoro-homoaristeromycin analogues **4d** and **4e** inhibited the SAH hydrolase to a lesser extent (Table 2). Among the aristeromycin analogues, 6'-β-fluoroaristeromycin **(2a)** was the most potent inhibitor (IC_{50} = 0.37 μM), which was 3.6-fold more potent than the control **1** (IC_{50} = 1.32 μM) (Table 1). However, 6'-α-fluoroaristeromycin (**2b**, IC_{50} = 9.70 μM) was 26-fold less potent than the corresponding 6'-β-fluoro analogue **2a** and 7.4-fold less active than the 6'-unsubstituted compound **1**. This indicates that the stereochemistry at the 6'-position is important for inhibitory activity. Interestingly, the introduction of two fluorines at the 6'-position resulting in 6',6'-difluoro-aristeromycin (**2c**, IC_{50} = 1.06 μM), yielded an analogue that was slightly more potent than the

control **1**. The inhibitory activity of the 6'-fluoroaristeromycin series can be ranked in the following order: 6'-β-F > 6',6'-F, F > 6' H > 6'-α-F. The introduction of a methyl group at the *N6*-amino group of **2a**, resulting in 6'-β-fluoro-*N6*-methyl-aristeromycin **2d**, decreased the inhibitory activity (IC_{50} = 4.39 μM) 11.9-fold, while the addition of a methyl group to the *N6*-amino group of **2c**, resulting in 6',6'-difluoro- *N6*-methyl-aristeromycin **2e**, increased the inhibitory activity (IC_{50} = 0.76 μM) 1.7-fold.

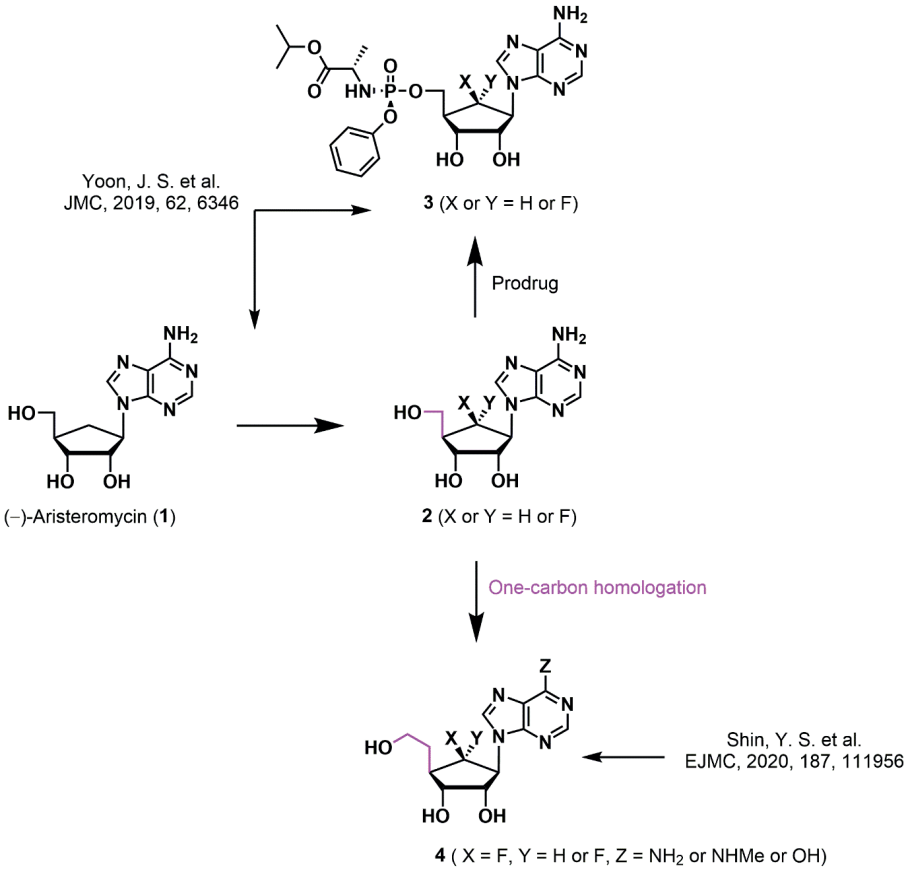


Figure 1. Rationale for the design of the target nucleosides **2**, **3** and **4.** The numbering of compounds **4** corresponds to **3** in (20). Adapted from (15) and (20).

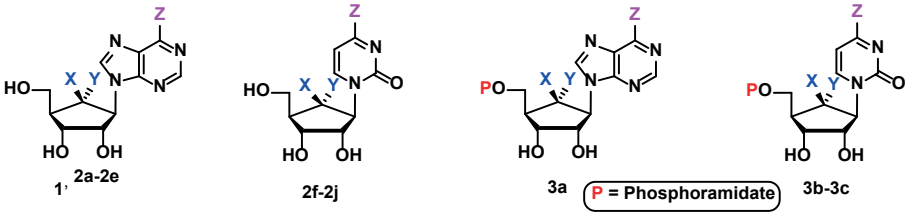
These results demonstrate that the *N6*-methyladenine and adenine moieties do not lead to a decrease in inhibitory activity. The inhibitory activity for 6'-β-fluoro-homoaristeromycin **(4a)** and 6'-β-fluoro-*N6*-methyl-homoaristeromycin **(4b)** were comparable to that of **2a** (IC_{50}s of 0.36 μM and 0.37 μM, respectively). Nevertheless, the SAH hydrolase inhibition by 6,6'-difluoro-homoaristeromycin **(4d)**

and 6,6'-difluoro- *N6*-methyl-homoaristeromycin **(4e)** was 5.6-fold and 8.5-fold lower, respectively, compared to the most potent compounds. The inosine analogue **4c** did not inhibit SAH hydrolase, as no inhibitory activity was observed at the highest dose tested of 100 µM (Table 2).

Anti-CHIKV activity

The novel 6'-fluoro-aristeromycin analogues **2a-j** and **3a-c** as well as 6'-fluoro-homoaristeromycin analogues **4a-e** were screened for antiviral activity against CHIKV. The compounds were tested in CPE-reduction assays at 4 concentrations, that is, 150 µM, 50 µM, 16.7 µM and 5.6 µM by preparing 3-fold serial dilutions. Compounds that

Table 1. Inhibition of SAH hydrolase and CHIKV replication by aristeromycin analogues **2a-j** and **3a-c** [a, b, c]. Adapted from (15).



Compound no.	X	Y	Z	SAH hydrolase IC_{50} (µM)	CHIKV EC_{50} (µM)[a]	CHIKV CC_{50} (µM)[b]	CHIKV SI
1	H	H	NH_2	1.32	0.8	6.3	7.9
2a	F	H	NH_2	0.37	>100	>100	
2b	H	F	NH_2	9.70	0.53	1.32	2.49
2c	F	F	NH_2	1.06	0.13	>1.25	>9.6
2d	F	H	NHMe	4.39	>100	>100	
2e	F	F	NHMe	0.76	>100	>100	
2f	F	H	OH	>100	>100	>100	
2g	H	F	OH	>100	>100	>100	
2h	F	F	OH	>100	>100	>100	
2i	F	H	NH_2	>100	>100	>100	
2j	F	F	NH_2	>100	>100	>100	
3a	F	F	NH_2	>100	1.95	>12.5	>6.4
3b	F	H	OH	>100	>100	>100	
3c	F	F	OH	>100	>100	>100	

[a]EC_{50}: effective concentration to inhibit CHIKV replication in VeroE6 cells by 50%. [b]CC_{50}: cytotoxic concentration that reduces cell viability by 50%. EC_{50} >100 indicates that no antiviral activity was observed at the highest concentration tested because there was no protection. SI = CC_{50}/EC_{50}.

demonstrated antiviral activity in this primary screen were tested more extensively in dose-response experiments at 8 different concentrations to determine the half maximal effective concentration (EC_{50}). The half maximal cytotoxic concentration (CC_{50}) was determined in parallel in uninfected cell cultures (Table 1 and 2).

As shown in Table 1, aristeromycin derivatives **2b-c** demonstrated potent anti-CHIKV activity (EC_{50} = 0.13-0.53 μM), while aristeromycin derivative **2a**, other purine *N6*-methylaristeromycin derivatives **2d** and **2e** and pyrimidine derivatives **2f-j** did not show significant antiviral activities, not even at a dose of 100 μM. This result suggests that the antiviral activity might be due to an (indirect) effect on the CHIKV nsP1 through the inhibition of host SAH hydrolase. 6',6'-difluoro-aristeromycin **(2c)** was the most active aristeromycin analogue against CHIKV with an EC_{50} of 0.13 μM. 6'-α-fluoroaristeromycin **(2b)** also showed some inhibitory effects on CHIKV replication, but this was likely due to pleiotropic effects, as the selectivity index (SI) was <3. Among the phosphoramidate prodrugs **3a-c**, only the prodrug **3a** exhibited anti-CHIKV activity (Table 1). It may inhibit CHIKV nsP4 after conversion into the triphosphate form, although it remains to be determined in biochemical assays

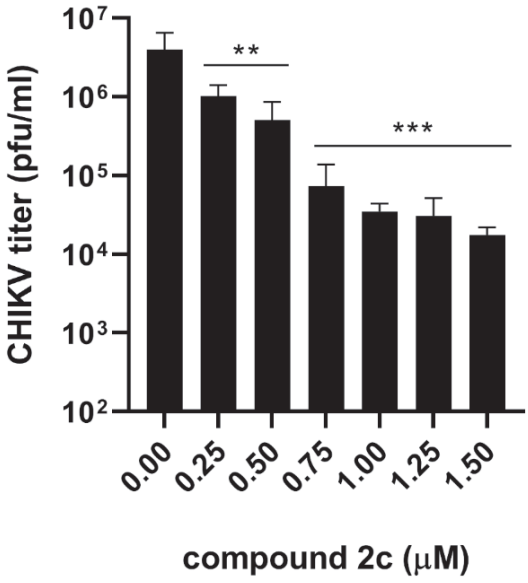
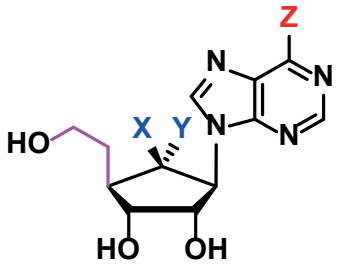


Figure 2. Effect of compound **2c** on the release of infectious progeny from CHIKV-infected VeroE6 cells. Cells were infected with CHIKV in medium with various concentrations of compound **2c** (x-axis). Infectious progeny titers were determined by plaque assay (n = 4). Significant differences are indicated by *: *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$; ****, $p < 0.0001$.

whether the triphosphate form affects the RdRp activity. Interestingly, the prodrug **3a** was less potent, but also much less cytotoxic than the parent compound **2c**, which is unusual as phosphoramidates are typically more potent than their parental drug (21-28). The phosphoramidate **3a** might be slowly hydrolysed to its 5'-monophosphate by metabolic enzymes, or to the parent drug **2c** by a phosphatase, which could inhibit SAH hydrolase, explaining the observed antiviral effect. A viral load reduction assay was performed with compound **2c** by infecting cells with CHIKV followed by treatment with different concentrations of **2c**. At 30 hpi, infectious progeny titers in the medium were determined by plaque assay. Treatment with concentrations higher than 1 μM of **2c** reduced infectious CHIKV titers by more than 2 $\log_{10}$ (Fig. 2).

Among the 6'-fluoro-homoaristeromycin analogues tested (Table 2), 6'-β-fluoro-homoaristeromycin **(4a)** showed potent anti-CHIKV activity (EC_{50} = 0.12 μM) without noticeable cytotoxicity up to 250 μM. This antiviral activity did not

Table 2. Inhibition of SAH hydrolase and CHIKV replication by 6'-fluorinated-5'-homoaristeromycin analogues **4a-e** [a, b, c]. The numbering of compounds **4a-e** corresponds to **3a-e** in (20). Adapted from (20).



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Compound no.	X	Y	Z	SAH hydrolase IC_{50} (μM)	CHIKV EC_{50} (μM)[a]	CHIKV CC_{50} (μM)[b]	CHIKV SI
4a	F	H	NH_2	0.36	0.12	>250	>1000
4b	F	H	NHMe	0.37	>100	>100	
4c	F	H	OH	>100	>100	>100	
4d	F	F	NH_2	2.03	ND[c]	>25	
4e	F	F	NHMe	3.05	>100	>100	

[a]EC_{50}: effective concentration to inhibit CHIKV replication in VeroE6 cells by 50%. [b]CC_{50}: cytotoxic concentration that reduces cell viability by 50%. [c]ND: not determined due to cytotoxicity of the compound. EC_{50} >100 indicates that no antiviral activity was observed at the highest concentration tested because there was no protection. SI = CC_{50}/EC_{50}.

merely seem to depend on the inhibition of SAH hydrolase, as **4a** and **4b** inhibited the enzyme with similar IC_{50} values (0.36 μM and 0.37 μM, respectively), although only **4a** displayed anti-CHIKV activity. In addition, although 6',6'-difluoro-aristeromycin **(2c)** displayed antiviral activity against CHIKV and several other + strand RNA viruses, including SARS-CoV, MERS-CoV, and ZIKV (15), the corresponding homologated analogue **4d** did not exhibit anti-CHIKV activity (Table 2). These results demonstrated that besides SAH hydrolase inhibition other effects may be involved in the antiviral activity of the homologated **4a-e** series.

Conclusions

6'-fluorinated-aristeromycin analogues **2a-j** were designed as dual-target antiviral compounds aimed at inhibiting both CHIKV nsP4 and the host SAH hydrolase. Phosphoramidate prodrugs **3a-c** were synthesized to determine whether these would inhibit CHIKV replication through an effect on CHIKV nsP4. The introduction of fluorine at the 6'-position was found to increase the inhibition of both SAH hydrolase activity and the replication of CHIKV and several other + strand RNA viruses (15). The 6'-fluorinated aristeromycin analogue **2c** inhibited SAH hydrolase activity and CHIKV replication more potently compared to the 6'-unsubstituted compound **1** (Table 1). Considering the results for all viruses tested, there was a correlation between the inhibition of SAH hydrolase and the antiviral activity of the compounds, suggesting that the latter was mainly due to the indirect inhibition of viral methylation reactions. The SAR studies and the lack of antiviral effects of several purine and pyrimidine analogues suggest that the antiviral effect of **1**, **2a** and **2c** is unlikely due to targeting of the viral RdRp (15). Compound **2c** appears to be interesting for further development and evaluation as a broad-spectrum antiviral agent, as it inhibited SARS-CoV, MERS-CoV and ZIKV in addition to CHIKV.

To reduce the cytotoxicity of 6'-fluorinated-aristeromycin analogues **2a** and **2c**, which is likely due to their 5'-phosphorylation by cellular kinases, 6'-fluorinated-homoaristeromycin analogues **4a-e** were designed. Among the compounds tested, 6'-β-fluoro-homoaristeromycin **(4a)** 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 (Table 2). This indicates that one-carbon homologation may prevent 5'-phosphorylation and consequently cytotoxicity. This homologation had no effect on the inhibitory activity towards SAH hydrolase, as compounds **2a**

and **4a** inhibited this enzyme with very similar IC_{50} values (0.37 μM and 0.36 μM, respectively) (Table 1 and 2). The antiviral activity of **4a** was specific for CHIKV and to a lesser extent for some other alphaviruses (34), whereas other viruses have been shown to be sensitive to the SAH hydrolase inhibitors **2a** and **2c** (15). As mentioned earlier, it suggests that the potent anti-CHIKV activity of **4a** is based on more than the mere inhibition of SAH hydrolase. A detailed molecular study of the antiviral activity of 6'-β-fluoro-homoaristeromycin **(4a)** is discussed in Chapter 4 of this thesis.

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