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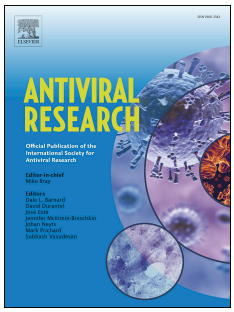

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Design, synthesis, and in vitro biological evaluation of novel 6-methyl-7-substituted-7-deaza purine nucleoside analogs as anti-influenza A agents

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Abstract

Among many subtypes of influenza A viruses, influenza A(H1N1) and A(H3N2) subtypes are currently circulating among humans (WHO report 2014-15). Therapeutically, the emergence of viral resistance to currently available drugs (adamantanes and neuraminidase inhibitors) has heightened alarms for developing novel drugs that could address diverse targets in the viral replication cycle in order to improve treatment outcomes. To this regard, the design and synthesis of nucleoside analog inhibitors as potential anti-influenza A agents is a very active field of research nowadays. In this study, we designed and synthesized a series of hitherto unknown 6-methyl-7-substituted-7-deaza purine nucleoside analogs, and evaluated for their biological activities against influenza A virus strains, H1N1 and H3N2. From the viral inhibition assay, we identified some effective compounds, among which, compounds **5x** (IC_{50} = 5.88 μM and 6.95 μM for H1N1 and H3N2, respectively) and **5z** (IC_{50} = 3.95 μM and 3.61 μM for H1N1 and H3N2, respectively) demonstrated potent anti-influenza A activity. On the basis of selectivity index, we conceive that compound **5x** may serve as a chemical probe of interest for further lead optimization studies with a general aim of developing novel and effective anti-influenza A virus agents.

Key words: H1N1, H3N2, Influenza A, Nucleoside analogs

1. Introduction

Influenza is one of the most burdensome and widespread human infectious diseases the impact of which is felt globally each year, affecting approximately 20% of world's population with an estimated annual epidemics of 3 to 5 million cases of severe illness, and about 250, 000 to 500, 000 deaths (WHO, 2014). Influenza A virus represents the most significant public health problem as estimated to have claimed 18,500 lives (laboratory-confirmed cases) in 2009 H1N1 global pandemic, and still consistently contributing to the death toll in pandemic countries across the globe (Dawood et al., 2012; WHO, 2014; WHO, 2015). According to World Health Organization (WHO) reports of 2014-15, H1N1 and H3N2 strains have spread quickly among humans.

Therapeutically, the two classes of anti-influenza drugs: M2 ion channel inhibitors, also called adamantanes (amantadine and rimantadine); and neuraminidase inhibitors (oseltamivir and zanamivir), remain the mainstay of combating influenza A spread and treating incident infections. But unfortunately, most of influenza A strains, including the currently circulating H1N1 and H3N2, have become resistant to adamantanes (Deyde et al., 2007; Sheu et al., 2011). Especially in recent years, the emergence of H1N1 resistance against oseltamivir and zanamivir has complicated the treatment outcomes (Correia et al., 2015; Han et al., 2012; Orozovic et al., 2014; Pizzorno et al., 2013). Nevertheless, the drug development pipeline to tackle this viral resistance remains woefully thin, including the recently developed pyrimido-pyrroloquinoxalinedione analogs targeting nucleoproteins (Lin et al., 2015), nucleoside analogs (Gao et al., 2015), daidzein analogs (Chung et al., 2015), and the hydrazide derivatives targeting non-structural protein 1 (NS1) (Barman et al., 2014). Clearly, there is a pressing need for developing novel chemical-based anti-influenza A drugs that could improve treatment outcomes. To this regard, the design and synthesis of nucleoside analog inhibitors as potential anti-influenza A agents is very active field of research (Al-Saad et al., 2014; Elayadi et al., 2014; Gao et al., 2015).

Recently, β-*D*-6-methyl-7-deaza-adenine ribonucleoside (compound A in Fig. 1) has been shown to possess good inhibitory activity against tuberculosis and dengue virus. However, this

compound causes significant cytotoxicity (Naus et al., 2014; Wu et al., 2010) and, thus, has limited clinical use as an antiviral agent. It is highly desirable to improve the toxicity profile of this compound. On the other hand, the introduction of a methyl group at the 2'-β-position on the ribose ring of some nucleoside analogs has been demonstrated to be an important sugar modification necessary to achieve excellent potency against various viruses without interfering with the function of host cell polymerases (Eldrup et al., 2004a; Eldrup et al., 2004b; Olsen et al., 2004). In addition, 2'-α-fluoro ribonucleosides have been shown to exhibit potent antiviral activity to suppress influenza A virus infections in Madin-Darby canine kidney (MDCK) cells (Tuttle et al., 1993). Collectively, the introduction of 2'-deoxy-2'-fluoro-2'-C-methyl ribofuranosyl moiety on the ribose ring may improve the antiviral activity and reduce toxicity of nucleoside analogs. One particular example is Sofosbuvir (Sovaldi®), shown in Fig. 1, which is a newly improved nucleoside inhibitor that possesses the same moiety in its structure, and has been found potent against hepatitis C virus. Based on these preliminary and intriguing findings from literature, we devised a rationale that combining the two moieties: 2'-deoxy-2'-fluoro-2'-C-methyl ribosyl sugars and 6-methyl-7-deazapurine bases, together in a single scaffold and then performing structure-activity relationship (SAR) studies may reveal a novel class of anti-influenza A virus nucleoside analogs that will be expected to possess less cytotoxicity. The biological evaluation of the designed compounds validated our rationale as we found a compound with more potent anti-influenza A activity and less cytotoxicity in comparison to the reference drug Ribavirin. Here, we report the synthesis and biological evaluation of a novel series of 6-methyl-7-substituted-7-deaza purine nucleoside analogs against influenza A virus with the reasonable SAR revealed.

2. Materials and methods

2.1. Experimental chemistry

Reagents were purchased from commercial suppliers and used without further purification unless otherwise noted. NMR spectra were recorded on Bruker Avance 400 MHz (^{1}H at 400MHz) and 500 MHz (^{1}H at 500 MHz, ^{13}C at 125 MHz) spectrometer. Chemical shifts values are given in ppm and referred as the internal standard to TMS (tetramethylsilane). The peak patterns are indicated as follows: s, singlet; d, doublet; t, triplet; q, quadruplet; m, multiplet and dd, doublet of doublets. The coupling constants (*J*) are reported in hertz (Hz). LC-MS data were recorded on

Agilent HPLC/MCD instrument. Silica gel column chromatography was performed over silica gel 200−300 mesh, and the eluent was a mixture of ethyl acetate and petroleum ether, or, a mixture of isopropanol and dichloromethane.

2.1.1. Inhibitor design and synthesis

The sugar and base moieties were synthesized by using the inexpensive and easily available material. Diisopropylidine derivative of D-mannitol was used as the starting material for the sugar moiety synthesis, which underwent symmetrical oxidative cleavage to afford the isopropylidine protected D-glyceraldehyde. Compound **1** was then synthesized by following the procedures described by Wang et al. (Wang et al., 2009). Detail procedures described by Kim et al. (Kim et al., 2012) were followed to obtain the base moiety, 4-Chloro-7*H*-pyrrolo[2,3-d]pyrimidine (13.2% overall yield) through a five steps process using ethyl cyanoacetate as the starting material. Coupling of the sugar moiety and base moiety gave the compound **3.** Various modifications were performed at 7-position to obtain the desired nucleoside analogs. Details regarding the syntheses and characterizations of the compounds can be found in the Supplementary data.

2.2. Biological experiments

2.2.1. Cells and virus

MDCK cells were cultured in Dulbecco's modified Eagle's medium (DMEM) (Gibco) supplemented with 10% fetal bovine serum, 100 U/ml penicillin and 100 μg/ml streptomycin at 37°C in 5% CO_2. The influenza A virus strains H1N1 (A/WSN/33) and H3N2 (A/Hong Kong/8/68) were propagated in the allantoic cavity of 9-day-old embryonated chicken eggs. The harvested viruses were then inoculated in MDCK cells for adaptation. The culture suspensions were collected and stored in multiple single-use aliquots at -80 °C for further use.

2.2.2. Cytotoxicity assay

MDCK cells were grown as monolayers in 96-well plates after seeding for 18–24 h. The medium was removed and rinsed twice with Hanks' solution and the compounds were serially diluted in 100 μl medium containing 0.3% BSA. After incubating for 72 h at 37 °C in a humidified 5%

CO_2 incubator, 50ul diluted CCK-8 reagent was added to each well and incubated for 2 h. The absorbance was measured at 450 nm using Envision Plate Reader (PerKin Elmer). The CC_{50} values were calculated by analyzing the data using Graph Pad Prism 5.

2.2.3. Viral inhibition assay

MDCK cells were grown to confluence in 96-well microtiter plates. The medium was removed, and fifty microliters, equal to approximately 0.01 MOI of H1N1 virus or H3N2 virus were added to the plates and incubated at 37 °C for 2 h. The cells were then covered with 50 μl of medium containing various amounts of Ribavirin or one of the synthesized compounds in the presence of 1 μg/ml TPCK trypsin and 0.3% BSA. After incubation in 5% CO_2 at 37 °C for 72 h, 50 μl of diluted CCK-8 reagent were added to each well, and the mixture was incubated for 2 h. The A450 was then measured using Envision Plate Reader (PerKin Elmer). Data were analyzed using Graph Pad Prism 5.

2.2.4. Plaque reduction assay

The plaque reduction assay was performed in 6 well tissue culture plates. The MDCK cells were seeded at 6×10^5 cells/well and incubated in 5% CO_2 at 37 °C for 18-24 h. The monolayer of MDCK cells was then infected with H1N1 or H3N2 virus (100 PFU/well) for 1h at 37 °C. The inoculums were removed, and the cells were washed twice with Hanks' solution. The cells were then overlaid with 1% agar MEM-containing Ribavirin or the synthesized compound **5x** in the presence of 1 μg/ml trypsin and 0.3% BSA. Three days after infection, the monolayers were fixed and stained with 0.1% crystal violet solution, and the plaques counted.

3. Results and discussion

3.1. Chemistry

The synthesis was focused to combine 2'-deoxy-2'-fluoro-2'-C-methyl ribosyl sugar and 6-methyl-7-deazapurine base to yield a novel nucleoside scaffold (**4**), and further evaluating the antiviral activities.

The novel scaffold (**4**) was synthesized by using a route described in **Scheme 1**. Initially, the coupling of 2'-C-methyl-2'-fluoro sugar with 6-chloro-7-deazapurine suffered from low yield

and poor anomeric stereoselectivity under Mitsunobu condition. Moreover, the desired product failed to be obtained via the Vorbruggen reaction. Therefore, we employed a previously reported method that was effective to couple 2'-C-methyl-2'-fluoro sugar part with purine bases through double stereoselective inversion of β-lactol (Reddy et al., 2011). Treatment of the known compound **1** with carbon tetra bromide and triphenylphosphine (CBr_4/PPh_3, Appel reaction) in anhydrous DCM gave the α-bromo ribofuranose **2** as a major product. The S_N2 coupling between **2** and 4-chloro-7H-pyrrolo [2, 3-d] pyrimidine with KO^{t}Bu was accomplished to give the key intermediate **3** in an acceptable yield (50%). The compound **4** was then obtained readily through a palladium-catalyzed reaction in 96% yield (**Scheme 1**).

Next, the halogenation of **4** with *N*-Iodosuccinimide (NIS), *N*-Bromosuccinimide (NBS) and *N*-Chlorosuccinimide (NCS) in DMF afforded the halogenated nucleoside intermediates **6a-c**, respectively, and then subsequent deprotection yielded **5a-d** (**Scheme 2**).

In the following steps, 7-iodo-7-deazapurine nucleoside (**5b**) served as a key intermediate for the synthesis of 7-substituted 7-deazapurine ribonucleosides via various palladium catalysts. Under the catalytic effect of $Pd(PPh_3)_4$, **5b** was first reacted with $Zn(CN)_2$ in dry DMF to obtain 7-cyano derivative **5e**, and then the subsequent oxidation reaction provided 7-formamido derivative **5f**. Next, using the same catalyst, the Stille reaction (Baillargeon and Stille, 1986) of **5b** with tributyl(vinyl)tin and 2-(tributylstannyl)thiophene, respectively, provided 7-ethyl and 7-thiophen-2'-yl substituted nucleosides **5h** and **5j** in acceptable yields. Further, the coupling of compound **5b** with corresponding substituted alkynyl reagents was performed by using $Pd(PPh_3)_4$ and CuI (Sonogashira reaction) (Sonogashira, 2002) at mild temperature to obtain the products **5g** and **5q** in 98% and 51% yields, respectively. Moreover, **5b** was also used as building block for the synthesis of the 7-(het)aryl 7-deazapurine nucleosides via the Suzuki cross-coupling reaction (Miyaura and Suzuki, 1995) with various heteroaryl boronic acids. The reactions were conducted in a water/acetonitrile 2:1 mixture with $Pd(OAc)_2$, PPh_3 and sodium carbonate to provide 7-heteroaryl derivatives **5k-p** in moderate yields. Finally, compound **5b** was converted to 7-carbomethoxyvinyl and 7-aromatic vinyl derivatives **5i** and **5r-z** (moderate to high yields) in the presence of $Pd(OAc)_2$ and PPh_3 by treatment with corresponding substituted vinyl reagents, called Heck reaction (**Scheme 3**).

3.2. Biological results

3.2.1. Evaluation of anti-influenza virus activity and cytotoxicity

All the synthesized nucleoside analogs were evaluated for their *in vitro* anti-influenza A activity against two viral strains, A/WSN/33 (H1N1) and A/Hong Kong/8/68 (H3N2), while using Ribavirin as a positive control. In addition, the cytotoxicity was also evaluated in parallel using MDCK cells. The considered measurements of the *in vitro* screening were IC_{50} (compound concentration that inhibits viral replication by 50%) and cytotoxic concentration CC_{50} (compound concentration that reduces cell viability by 50%). The selectivity index (SI = ratio of CC_{50} to IC_{50}) was also calculated where needed as an estimate of therapeutic window.

The antiviral activity data, as summarized in **Table 1**, revealed the significant resistance of both strains against most of the synthesized compounds (IC_{50} values > 100 μM) compared to the reference Ribavirin. However, among the twenty-six compounds (**Table 1**), ten compounds in case of H1N1, while eleven compounds in case of H3N2 showed certain level of antiviral activity with IC_{50} values < 100 μM. Especially, the compounds **5r** (IC_{50} = 7.11 μM and 10.53 μM for H1N1 and H3N2, respectively), **5x** (IC_{50} = 5.88 μM and 6.95 μM for H1N1 and H3N2, respectively) and **5z** (IC_{50} = 3.95 μM and 3.61 μM for H1N1 and H3N2, respectively) were more potent and active than Ribavirin (IC_{50} = 10.92 μM and 11.2 μM for H1N1 and H3N2, respectively). The inhibition data for these three compounds has been shown in Fig. 2.

As far as the cytotoxicity data is concerned, most of the novel nucleoside analogs with 2'-deoxy-2'-fluoro-2'-C-methyl ribosyl sugars demonstrated no significant inhibitory effect (CC_{50} > 100 μM) on the growth of MDCK cells, which indicates they possess low cytotoxic potential. Surprisingly, the cytotoxic evaluation of most active compounds revealed that **5r** and **5z** possess a certain level of cytotoxic potential having CC_{50} values 53.2 μM and 28.3 μM, respectively, in comparison to the compound **5x** and the reference Ribavirin (both having CC_{50} values > 100 μM). However, despite the lower CC_{50} values in comparison to the reference Ribavirin, the differential activity exhibited by these compounds in terms of SI values (SIs of **5r** = 7.48 and 5.05 for H1N1 and H3N2, respectively; SIs of **5z** = 7.16 and 7.83 for H1N1 and H3N2, respectively) is quite significant and remains an open question for further investigations. Interestingly, **5x** possesses SI values (> 17 in case of H1N1 and > 14 in case of H3N2) that are much better in comparison to **5r** and **5z**. The SI value demonstrates the differential

activity/selectivity of a compound towards its target. The greater the SI value of the compound is, the more selective it is. Usually, an SI value below 2 indicates general toxicity of a pure compound (Koch et al., 2005). Based on this assumption, the SI values of **5r**, **5x**, and **5z** exhibit a high degree of viral selectivity. Since **5x** has an IC_{50} very close to **5z** and SI values two times that of **5z**, we conceived **5x** has a higher potency than **5z** and thus selected it for further evaluation in plaque reduction assay.

Inspired by design strategy of target compounds and their anti-influenza inhibitory activity, we also performed anti-tuberculosis (anti-T.B) and anti-dengue virus assays. The results showed that all the compounds demonstrated IC_{50} values > 100 μM in anti-T.B assay. Whereas, some of the compounds demonstrated considerable IC_{50} values against dengue virus (detail will be reported in the future).

3.2.2. Structure-activity relationship (SAR) studies

A comparison of the effect of different C7-substituents on antiviral activity was made with Ribavirin. For the first SAR, to begin with, compounds **5j-r** bearing bulky substituents, including substituted and unsubstituted benzenes (**5k-n**), (hetero)aromatics (**5j**, **5o** and **5p**), styrene (**5r**) and phenylacetylene (**5q**), at the C7-position of the 7-deazapurine base demonstrated better inhibitory activities than the compounds with smaller substituents, such as halogen atoms (**5b-d)** and other electron-withdrawing groups (**5e-i**). The antiviral activity was further improved to be more potent when the phenyl group (**5k**, IC_{50} = 92.3 μM for H1N1) was replaced by substituted benzenes, such as 4-fluoro benzene (**5l**, IC_{50} = 72.2 μM and 65. 3 μM for H1N1 and H3N2, respectively), 4-trifluoromethyl benzene (**5m**, IC_{50} = 55.1 μM and 48.15 μM for H1N1 and H3N2, respectively) and 4-methoxyl benzene (**5n**, IC_{50} = 72.6 μM and 86 μM for H1N1 and H3N2, respectively). Whereas, the antiviral activity declined in the order of less to no when the phenyl group was changed to thienyl (**5j**, IC_{50} = 64.8 μM for H3N2), 4-pyridyl (**5o**, IC_{50} > 100 μM) and naphthyl (**5p**, IC_{50} > 100 μM) groups, respectively. It is noteworthy that the (E)-7-styryl derivative exhibited slightly stronger activity (**5r**, IC_{50} = 7.11 μM and 10.53 μM for H1N1 and H3N2, respectively) in comparison to Ribavirin, while 7-phenylethynyl analog (**5q**, IC_{50} = 50.6 μM and 47 μM for H1N1 and H3N2, respectively) demonstrated decreased activity.

From the first SAR, we conceived that heteroaryl groups could increase the anti-influenza activity, particularly, when the trans-vinyl was used as the linker at C7-position in the 7-deazapurine base. Therefore, in an effort to find a more active and potent compound, this series was further optimized by placing various substituents in the phenyl ring (**5s-u**) or replacing the phenyl group with heteroaryl groups (**5v-z**). Our results showed that introduction of electron-withdrawing groups such as fluoro and trifluoromethyl groups at the 4-position of phenyl ring could result in loss of antiviral activity of nucleoside analogs (**5s** and **5t**), while the addition of electron-donating groups, such as 4-methoxystyryl group (**5u**, IC_{50} = 28 μM and 26 μM for H1N1 and H3N2, respectively), could decrease the potency. Moreover, the (1, 1'-biphenyl)-4-ylvinyl nucleoside analog (**5v**) also exhibited loss of antiviral activity, most probably, because of the too bulkyl group that warrants further investigations.

Furthermore and interestingly, there was a distinct difference in the potency among nucleoside derivatives bearing heteroaryl substituents, even with the same hetero atom, at the diverse positions. For example, compound **5x** having the nitrogen atom of vinylpyridine ring at 2-position demonstrated improved antiviral activity (IC_{50} = 5.88 μM and 6.95 μM for H1N1 and H3N2, respectively), but the activity was completely lost when the nitrogen atom was at 4-position (**5w**). These results indicate that hetero atoms at the 2-position of vinylhetaryl-substituted series are required for antiviral activity, but their presence at 4-position may induce loss of activity. Nevertheless, compound **5y** containing 2-Vinylpyrazine demonstrated slightly potent activity (IC_{50} = 23.6 μM and 54.52 μM for H1N1 and H3N2, respectively), but not as potent as that of **5x**. Finally, encouraged by the improved activity and potency of heteroaryl substitution in case of compound **5x**, we decided to substitute it with 2-Vinylthiophene. As expected, (E)-2-(thiophen-2-yl)vinyl analog exhibited the most potent antiviral activity (**5z**, IC_{50} = 3.95 μM and 3.61 μM for H1N1 and H3N2, respectively) in comparison to **5r** and **5x**, and the reference Ribavirin. However, considering the cytotoxic potential (Table 1) and SI values, **5x** may be a promising lead in managing influenza A infections.

3.2.3. Plaque reduction assay

In order to validate the anti-influenza A activity, compound **5x** was further assessed in a plaque reduction assay using Ribavirin as reference. The images in Fig. 3 show that compound **5x** and Ribavirin inhibited plaque formation by influenza virus H1N1 and H3N2 in a concentration-

dependent manner, ranging from 1.1 μM to 30 μM. The plaque formation was effectively inhibited by **5x** at a concentration of 30 μM. Moreover, compared to our reference drug Ribavirin, compound **5x** showed better plaque suppressive effect, indicating that **5x** was an obviously potent inhibitor consistent with the viral inhibition results discussed in Table 1.

4. Conclusions

We have designed and synthesized a class of novel 6-methyl-7-substituted-7-deaza purine nucleoside analogs by using a stereoselective synthetic approach accompanied by some well-known cross-coupling reactions. We also successfully explored the SAR of synthesized compounds in relation to anti-influenza virus activity. From viral inhibition assay, we have identified compound **5x** as the most active inhibitor against wild-type influenza A strains, H1N1 (IC_{50} = 5.88 μM) and H3N2 (IC_{50} = 6.95 μM), which is more potent than Ribavirin. We conceive that compound **5x** may serve as a chemical probe of interest for further SAR and lead optimization studies with a general aim of developing novel and effective anti-influenza A virus agents.

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Conflict of interest

None

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Schemes

Scheme 1. Reagents and conditions: i) CBr_4, PPh_3, DCM, -20 to -15 °C; ii) 4-chloro-7H-pyrrolo [2, 3-d] pyrimidine, tBuOK, MeCN: tBuOH = 1.5: 1, r.t. to 50 °C; iii) $AlMe_3$, $Pd(PPh_3)_4$, THF, 80 °C.

Scheme 2. Reagents and conditions: i) for **6a**: NIS, DMF, 50°C; for **6b**: NBS, DMF, r.t.; for **6c**: NCS, DMF, 50°C; ii) NH_3/MeOH, r.t.

Scheme 3. Reagents and conditions: i) $Zn(CN)_2$, $Pd(PPh_3)_4$, DMF, 150 °C; ii） NH_4OH, H_2O_2, dioxane, 100 °C; iii) for **5h**: $nBu_3(CHCH_2)Sn$, $Pd(PPh_3)_4$, DMF, 100 °C; for **5j**: nBu_3(2-thienyl)Sn, $Pd(PPh_3)_4$, DMF, 100 °C; iv) for **5k-5p**: R_1-$B(OH)_2$, $Pd(OAc)_2$, PPh_3, Na_2CO_3, H_2O/MeCN, 100 °C; v) for **5g**: $Pd(PPh_3)_4$, CuI, TEA, triethylsilylacetelene, THF, 45 °C; and then K_2CO_3, MeOH, r.t; for **5q**: $Pd(PPh_3)_4$, CuI, TEA, arylacetelene, THF, 50 °C; vi) for **5r-5z**: vinyl compound, $Pd(OAc)_2$, PPh_3, TEA, DMF, 100 °C.

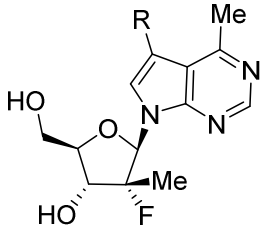
Figure Legends

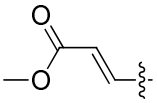
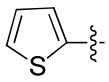
Figure 1. Structures with potent antiviral activity. The colored parts appear to be crucial for potent antiviral activity.

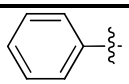
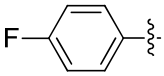
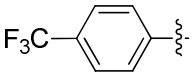
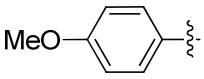
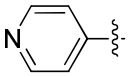
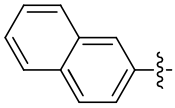
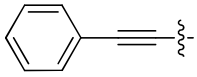
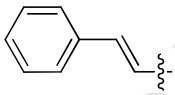
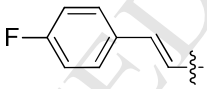
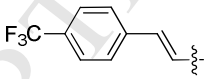
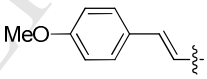
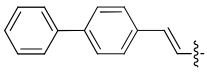
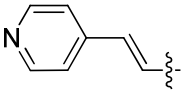
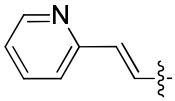
Figure 2. Dose-response curves for compounds **5r**, **5x** and **5z** against influenza A virus strains A/WSN/33 (H1N1) and A/Hong Kong/8/68 (H3N2).

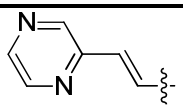
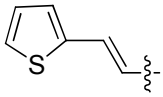
Figure 3. Reduction of plaque formation by A/WSN/33 (H1N1) and A/Hong Kong/8/68 (H3N2) virus in MDCK cells upon treatment with compound **5x** and Ribavirin. VC, virus control

Table 1. In vitro anti-influenza A virus activity of the test compounds in two strains, and the cytotoxic evaluation in MDCK cells



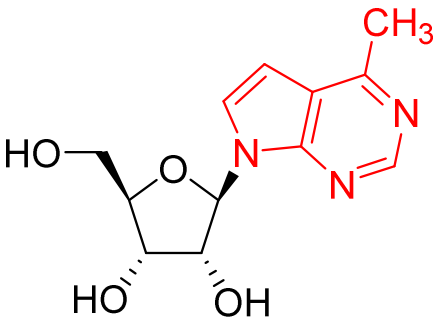
Entry	Compound	R	Antiviral activity IC_{50} (μM)[a]		Cytotoxicity CC_{50} (μM)[b]
			H1N1	H3N2	
1	**5a**	H	>100	>100	>100
2	**5b**	I	>100	>100	>100
3	**5c**	Br	>100	>100	>100
4	**5d**	Cl	>100	>100	>100
5	**5e**	CN	>100	>100	>100
6	**5f**	$CONH_2$	>100	>100	>100
7	**5g**		>100	>100	>100
8	**5h**		>100	52.6	>100
9	**5i**		>100	>100	>100
10	**5j**		>100	64.8	>100

11	**5k**		92.3	>100	>100
12	**5l**		72.2	65.3	>100
13	**5m**		55.1	48.15	>100
14	**5n**		72.6	86	>100
15	**5o**		>100	>100	>100
16	**5p**		>100	100	>100
17	**5q**		50.6	47	92.8
18	**5r**		7.11	10.53	53.2
19	**5s**		>100	>100	90.2
20	**5t**		>100	>100	28.8
21	**5u**		28	26	>100
22	**5v**		>100	>100	>100
23	**5w**		>100	>100	>100
24	**5x**		5.88	6.95	>100

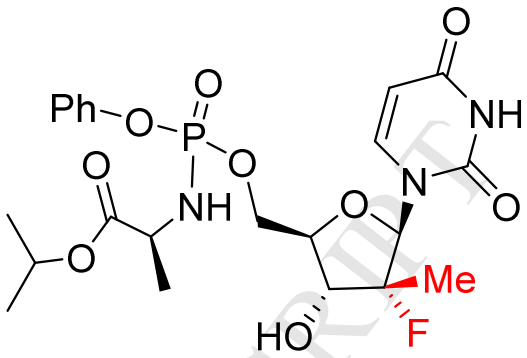
25	**5y**		23.6	54.52	>100
26	**5z**		3.95	3.61	28.3
27	**Ribavirin**	-	10.92	11.2	>100

[a] Concentration required to inhibit influenza A virus growth by 50%
[b] Cytotoxic concentration required to inhibit MDCK cells growth by 50%

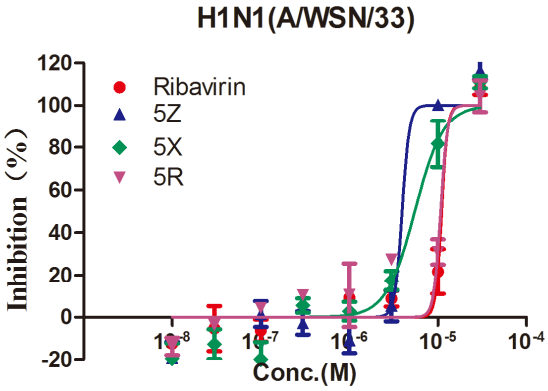
Figure 1

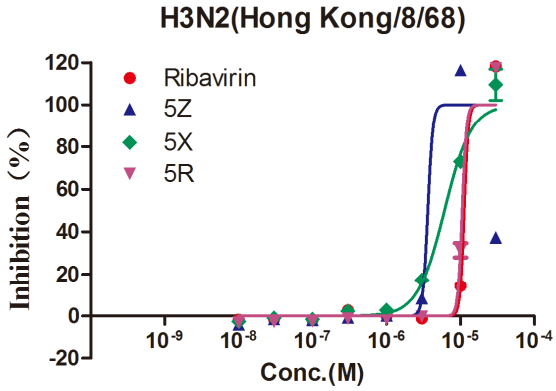


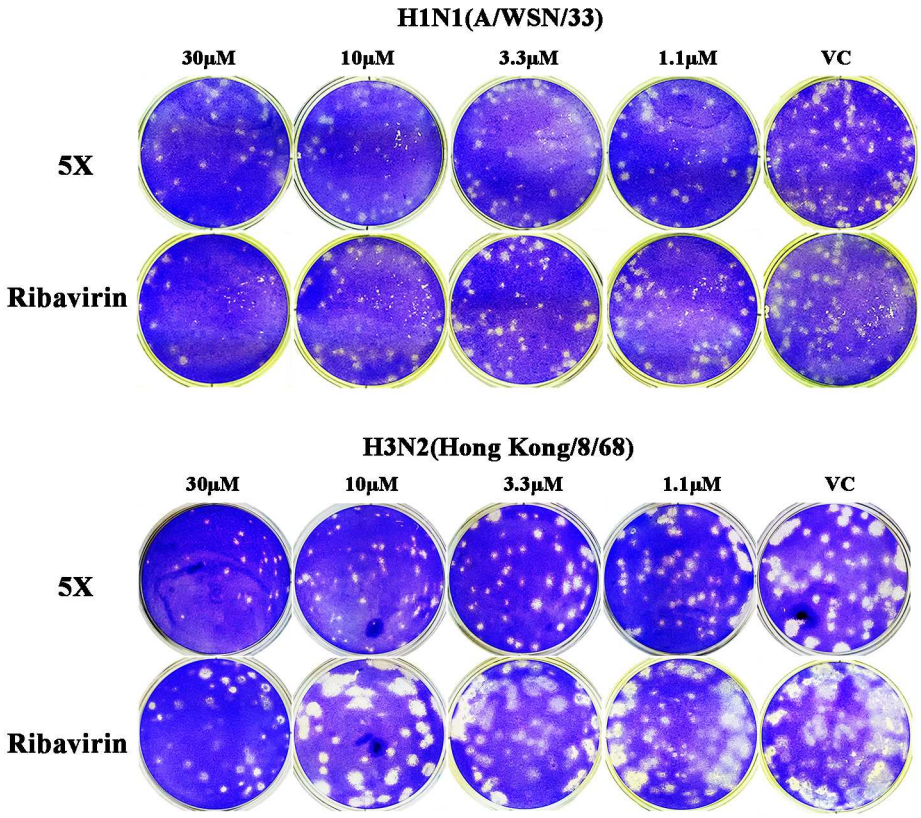
Compound A



Sofosbuvir







Highlights

- A novel nucleoside scaffold, compound **4,** was preparedwith high stereoselectivity
- 26 6-methyl 7-substituted-7-deaza purine nucleoside analogues were synthesized
- Anti-influenza A virus activity and cytotoxicity evaluation were conducted
- Three compounds **5r**, **5x** and **5z** showed more potent antiviral activity than Ribavirin
- **5x** may serve as a potential lead compound for anti-influenza A drug development