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Novel nucleosides as potent influenza viral inhibitors

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ABSTRACT

Influenza virus infection constitutes a significant health problem in need of more effective therapies. We have recently identified ((2*R*,3*S*,4*R*,5*R*)-3-acetoxy-5-(4-benzamido-2-oxopyrimidin-1(2*H*)-yl)-4-fluoro-3,4-dimethyl-tetrahydrofuran-2-yl) methyl benzoate (**18c**) as a potent influenza virus inhibitor. We now here report the synthesis and evaluation of a series of C-3′ modified ribose nucleosides. These novel compounds were prepared, primarily by taking known ((2*R*,3*R*,4*R*)-3-benzoyloxy-4-fluoro-4-methyl-5-oxo-tetrahydrofuran-2-yl)methyl benzoate (**1**) and converting it in to C-3 keto sugar (**7**), reacting C-3 keto group with methyl magnesium bromide, followed by coupling these sugars with purine and pyrimidine bases. Anti influenza viral activity was determined by screening against both A and B viral strains.

1. Introduction

The outbreak of 2009 influenza A virus that is believed to have originated from Mexico is rapidly spreading across the globe. World wide more than 209 countries and overseas territories or communities have reported laboratory confirmed cases of pandemic influenza H1N1 2009, including at least 14711 deaths.[1] Thus impact of influenza infection is felt globally each year when the disease develops in approximately 20% of the world's population. Influenza A virus, in particular, represents a significant health risk to the public. This is due to both its ability to spread quickly within human populations and the high degree of mortality associated with infection.

Influenza viruses are enveloped RNA viruses that are classified into three types: A–C. Two major surface glycoproteins, hemaglutinin (HA) and neuraminidase (NA) are responsible for the antigenic properties of the viruses. For influenza A, there are at least 9 subtypes of NA and 16 subtypes of HA in contrast to only one subtype of influenza B, NA and HA. Influenza C contains both HA and a NA activity in a single surface glycoprotein and only one subtype is known. Influenza C is not considered a serious disease and most adults have protective antibodies immunity from influenza A and B, however, does not last long since virus is constantly undergoing variations.

Influenza A undergoes a progressive antigenic drift and in addition undergoes an antigenic shift in which a 'new' HA and sometimes 'NA' appears. Influenza B has not undergone antigenic shift, perhaps because it is not found in birds or animals. Since 1977, there have been three influenza A viruses circulating in humans. These are influenza A subtypes H3N2, H1N1 and H5N1. H5N1 avian influenza A viruses which have high human mortality rate,[2] since 1997 are prime candidates for the next pandemic influenza A virus.

In April 2009 a novel flu strain evolved initially, dubbed 'swine flu' and also known as influenza A/H1N1 emerged in Mexico, the United States, and several other nations. The world health organization (WHO) officially declared the outbreak to be a pandemic. The WHO's declaration of a pandemic level 6 was an indication of spread.

Only two classes of influenza virus antivirals are currently available, inhibitors of the viral M2 ion channel protein (Amantadine and Rimantadine) and inhibitors of the viral neuraminidase (Zanamivir and Oseltamivir) (see Fig. 1).

The emergence of influenza viruses resistant to the M2 inhibitors occurs at high frequency in treated patients and thus they are not recommended for a general and uncontrolled use.[3] Many of the human isolated of H5N1 viruses are already resistant to these inhibitors.[4] For addition, a recent study has shown influenza A virus is resistant to Oseltamivir occurred in 20% of the children treated with this drug.[5] In fact, H5N1 viruses that are partially resistant to Oseltamivir have recently been reported.[6] Similar information of influenza B virus is limited. Recently a study assessed the prevalence and transmissibility of influenza B viruses with reduced sensitivity to neuraminidase inhibitors.[6] The emergence of influenza virus resistant to existing classes of antiviral drugs highlights the need for additional antiviral drugs against influenza. Therefore, a need exists for novel antiviral agents that address one or more locations in the viral replication cycle.

Abbreviations: HA, hemaglutinin; NA, neuraminidase; WHO, world health organization; NIs, nucleoside inhibitors; MDCK cells, Madin-Darby canine kidney cells; AACF, Antimicrobial Acquisition and Coordinating facility.

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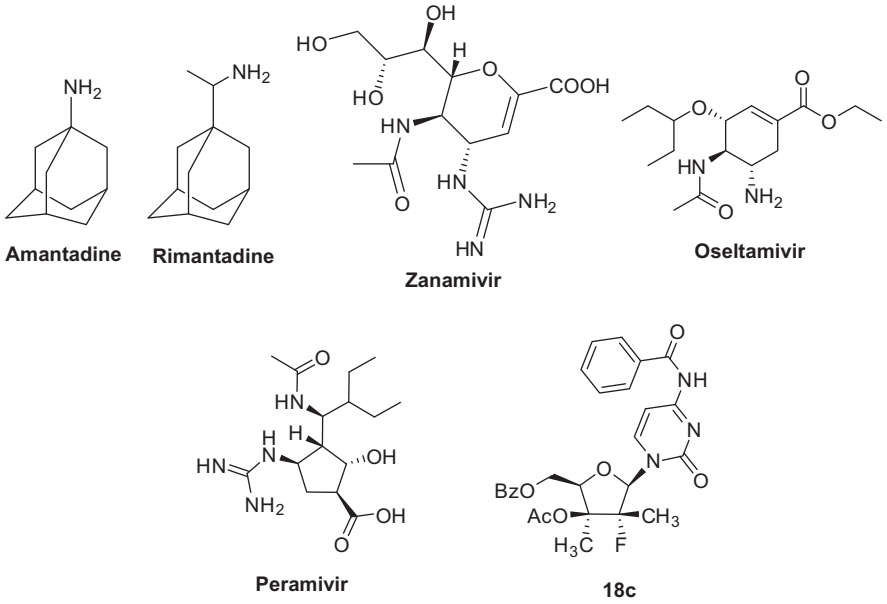


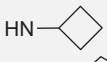
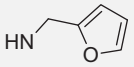
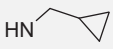
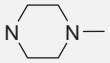
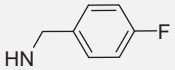
Figure 1. Structures of Amantadine, Rimantidine, Zanamivir, Oseltemivir, Peramivir and ((2*R*,3*S*,4*R*,5*R*)-3-acetoxy-5-(4-benzamido-2-oxopyrimidin-1(2*H*)-yl)-4-fluoro-3,4-dimethyltetrahydrofuran-2-yl)methyl benzoate (**18c**).

The design and synthesis of nucleoside analogues[7,8] as potential antiviral and anticancer agents is a very active field of research, within this area, systems of particular interest include 2′-deoxy nucleosides.[9] These nucleosides are compounds in which hydroxyl group at 2′-position has been replaced with different groups. In parallel fluorinated sugars have received a great interest for their biological medicinal applications[10] indeed the introduction of a C–F bond in a molecule is of great importance for its physicochemical properties. For example, fluorine substituted nucleoside, Gemcitabine is used in cancer and herpes therapies. Introduction of fluorine atom can improve the bioavailability of drug owing to increasing lipophilicity. Considering all these aspects and necessity to find novel nucleosides having antiviral activity, we decided to synthesize 3′ modified 2′-deoxy nucleoside analogues. Nucleoside inhibitors (NIs) described in literature, have involved some modifications at any of the 2′, 3′, 4′ or 5′-position, the present modification leading to di substitutions at each carbon of both 2′ and 3′ nucleosides are less prevalent. Most likely due to the added synthetic challenges associated with their synthesis.

2. Chemistry

Recently, there has been an intensive effort seeking influenza viral drugs with novel chemical structures,[11] or that hits new

Table 1
In vitro anti-influenza activity of **13**, **13a**–**i**, **22** and **24** against Flu A, Flu B and inhibitory activity of influenza virus infection of MDCK cell

	R	R_1	Flu A(H5N1) Vietnam/1203/2004H EC_{50} (μg/ml)	Flu B Florida/4/2006 EC_{50} (μg/ml)	MDCK IC_{50} (μg/ml)
13	H	Cl	7.1	3.7	9.2
13a	H	OH	33	65	>100
13b	H	OC_2H_5	48	>100	>100
13c	H		50	20	>100
13d	H		23	27	>100
13e	H		31	32	>100
13f	H		32	17	>100
13g	H		24	25	>100
13h	H		>100	42	>100
13i	H		32	30	36
22	H	Cl	>100	>100	>100
24	H	NH_2	>100	>100	>100

EC_{50}, virus inhibitory concentration, 50% endpoint; IC_{50}, cell inhibitory concentration 50% endpoint.

targets or work in new ways to inhibit influenza virus and to overcome resistance. As part of our effort to synthesize novel antivirals, we report here the synthesis and evaluation of nucleoside analogues against influenza virus. Our initial structure–activity relationship (SAR) was focused on the evaluation of modifications at C-3 position of sugar. A limited SAR was also performed on heterobases.

For the initial studies, a series of nucleosides modified at C-3′ position (Tables 1 and 2) were synthesized from novel sugar intermediates (**11**) and (**11A**). These intermediates were synthesized using a route described in Scheme 1.

The starting material **1** required for the synthesis of **11** and **11A** was prepared according to literature procedure.[12] Di benzoyl fluoro lactone **1** was reduced with lithium tri tertiary butoxy aluminum hydride ($Li(O^tBu)_3AlH$) in THF followed by methylation of anomeric hydroxyl group under boran trifluoride ethereate ($BF_3{\cdot}OEt_2$) conditions. Some quantity (20%) of mono debenzoylated product **4** was obtained under these conditions. In the next step **3** and **4** were together deprotected to afford compound **5**. C-5 hydroxy compound was then protected with TBDPS in subsequent step to furnish the compound **6**. As a next step to introduce methyl group at C-3 carbon, oxidation reaction was performed by oxidizing the hydroxyl group at this position to keto group with Dess–Martin periodinane, in quantitative yield. Addition of organometallics to 2-keto ribofuranose intermediates is known to proceed stereo selectively with addition of the alkyl groups to the β face, leading to the formation of the corresponding ribo derivatives. However, in our case the resultant 3-ketosugar **7** when treated with freshly prepared methyl magnesium bromide produced a mixture of α and β isomers **8** and **8A** in the ratio of 1.2:1.

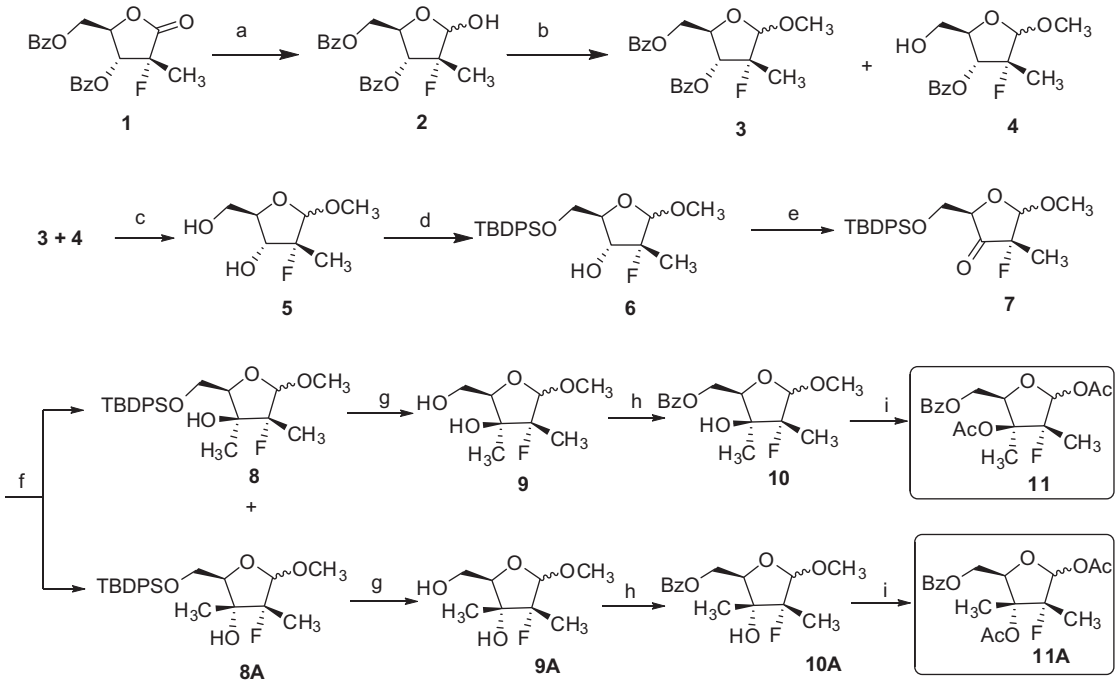
We studied the solvent effect in this reaction by taking THF instead of diethyl ether. Formation of C-3 β-hydroxy isomer, approximately four times more was observed in this solvent. The resulting tertiary alcohols were then converted **11** and **11A** by subsequent removal of silyl protecting group with Bu_4NF, followed by benzoylation gave compounds **10** and **10A**. Demethylation of anomeric methoxy group and acetylation of both the hydroxyls was achieved under $AC_2O/CH_3COOH/H_2SO_4$ conditions to give key intermediates **11** and **11A**.

The effect of modification of C-3 position of sugar on SAR was investigated by first coupling sugar with 6-chloropurine. Many glycosylation protocols for coupling are known in the literature. These include the silver salt, the chloromercury, the fusion, the sodium salt, the phase transfer, and the boron trifluoride etherate methods. We employed Vorbrüggen conditions (*N*,*O*-bis(trimethylsilyl)acetamide, ACN, $SnCl_4$ or ACN, DBU, TMSOTf) to yield a mixture of α/β diastereoisomers. Separation of individual isomers was accomplished by column chromatography. Coupling of sugar **11** with 6-chloropurine under the above conditions afforded nucleosides **12** with a configuration of α at anomeric carbon and **13** with β configuration. Similarly **11A** gave **21** and **22**. Under similar conditions 6-chloro-9*H*-purin-2-amine yielded only β-anomer **13j**, **22a** from **11** and **11A,** respectively. Nucleophilic displacement of chlorine atom of **12** and **13** with different amines gave compounds **12a–i** and **13a–i**.

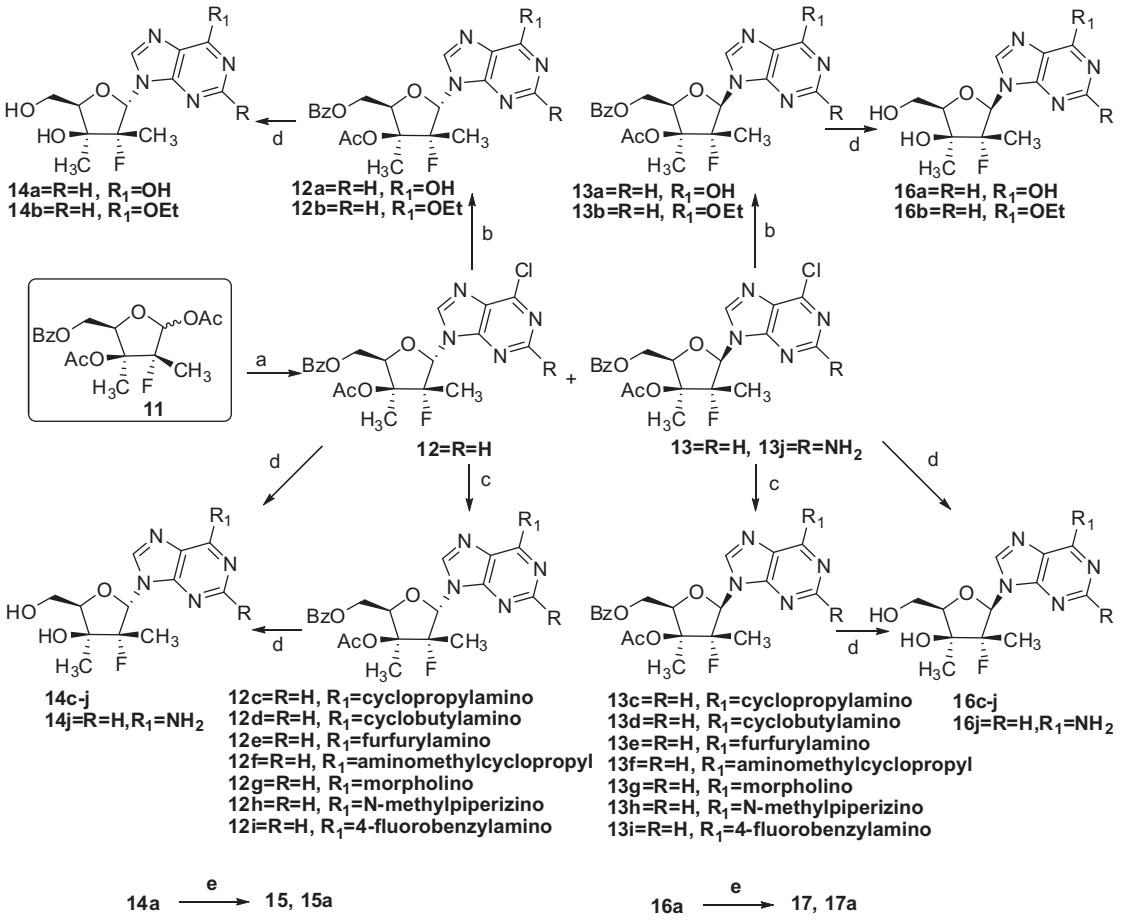
In an effort to further explore the structural effects on furanose we de protected **12**, **12a–i** and **13**, **13a–i** (Scheme 2) using methanolic ammonia at room temperature to give the desired β anomeric nucleoside compounds **16a–j** as well as α anomeric compounds **14a–j** with free hydroxyl groups at C-5′ and C-3′.

Table 2
In vitro anti-influenza activity of **18** and **18a–c**, against Flu A, Flu B and inhibitory activity of influenza virus infection of MDCK cells

	B	Flu A(H5N1) Vietnam/1203/ 2004H EC_{50} (μg/ml)	Flu B Florida/ 4/2006 EC_{50} (μg/ml)	MDCK IC_{50}, (μg/ml)
18	Uracil	25	34	>100
18a	Thymine	>100	>100	>100
18b	5-Fluoruracil	26	23	>100
18c	*N*-Benzoylcytosine	18	7.7	>100



Scheme 1. Synthesis of crucial sugar intermediates **11** and **11A**. Reagents and conditions: (a) lithium tri *tert*-butoxy aluminum hydride, THF; (b) $BF_3{\cdot}OEt_2$, MeOH; (c) NH_3/MeOH; (d) TBDPSCl, imidazole, dry DCM; (e) Dess–Martin periodinane, dry DCM; (f) MeMgI, dry diethyl ether; (g) dry THF, TBAF; (h) dry DCM, BzCl, DMAP, TEA; (i) H_2SO_4, acetic acid, acetic anhydride.



Scheme 2. Synthesis of nucleosides **12**, **12a**–**i**, **13j**, **13**, **13a**–**i**, **13j**, **14a**–**j**, **16a**–**j**, **15**, **15a**, **17** and **17a**. Reagents and conditions: (a) 6-chloropurine/6-chloro-9H-purin-2-amine, ACN, DBU, TMSOTf, 65 °C overnight/*N*,*O*-bis(trimethylsilyl)acetamide, ACN, $SnCl_4$, 70 °C, 2 h; (b) ethanol reflux, 16 h; (c) R_1NH/R_1NH_2, ethanol, reflux, 1 h; (d) NH_3/MeOH; overnight; (e) DCM, RCOCl, at 0 °C.

In order to evaluate the effect of the modification of heterobase, analogues **18**, **18a**–**c**, and **25** were prepared by coupling **11** and **11A** with commercially available pyrimidine bases. Coupling reaction was performed in the presence of *N*,*O*-bis(trimethylsilyl)acetamide, ACN, $SnCl_4$ by the methodology of Vorbrüggen. No α anomer was observed in these conditions. Removal of ester groups under methanolic ammonia conditions furnished compounds **19**, **19a**–**c** and **26**.

To study the effect of substitutions at the C5′–OH position on the above analogues, a series of ester prodrugs **15**–**15a**, **17**–**17a** and **20**–**20e** were prepared. The synthesis of these derivatives was accomplished by reacting C5′–OH with acid chlorides (see Scheme 3).

Configuration at newly created stereo center, that is, at C-3 carbon in **11** and **11A** was determined by observing cross peaks in NOESY between methyl at C-3 and hydrogen at C-4 in compound **11**. Similar observation was made between C-2-methyl and C-3-methyl for compound **11A**, inferring that methyl is α in compound **11** and β in **11A**. Likewise in nucleosides cross peak between a doublet at δ 1.9 (3′-CH_3) and multiplet at δ 4.45–4.52 (C4′-CH) in 2D NOESY confirmed that these protons are in proximity, indicating that they are in α plane. In the case of α-furanosides (**12**) stereochemistry at anomeric position was confirmed by observation of cross peak between C-2′-Me at δ 1.46 and anomeric proton doublet at δ 6.62. Such a cross peak was absent in β-furanosides **13**, **13j**, **18b** and **18c** (see Fig. 2).

The solid-state structure of representative compound in this series (**12**) (Fig. 3) was determined by single crystal X-ray crystallography, stereochemistry at different centers were found to be C(9)-R, C(10)-S, C(11)-R and C(12)-S.

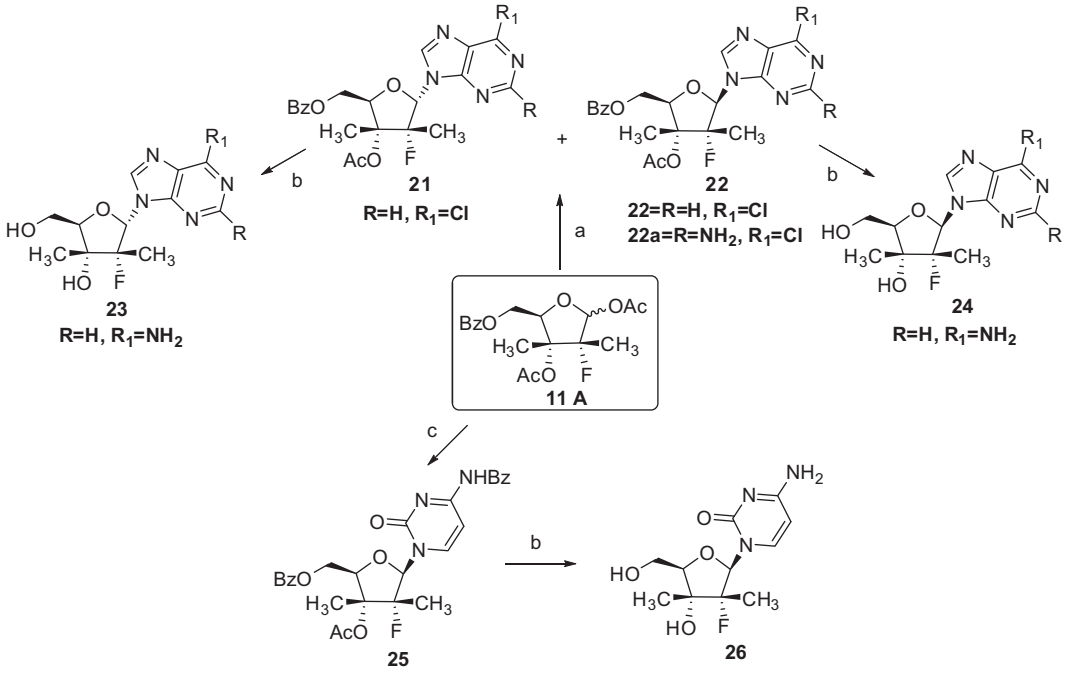
3. Results and Discussions

The modified C-3′-hydroxy nucleosides were evaluated for their antiviral effects on two flu viral strains, FluA (H5N1) Vietnam/1203/2004H and Flu B florida/4/2006 in MDCK cell line at the NIAID Antimicrobial Acquisition and Coordinating facility (AACF). In addition, cytotoxicity was evaluated in parallel in MDCK cell line. Selectivity index (SI) was determined by taking the ratio of cell inhibitory concentration, 50% endpoint (IC_{50}) to virus inhibitory concentration, 50% endpoint (EC_{50}): SI = (IC_{50}/EC_{50}).

3.1. Effect of purine/pyrimidine bases

As said earlier coupling of **11** and **11A** with different hetero bases yielded α nucleosides and their derivatives (**12**, **12a**–**i**, **14**, **14a**–**i**, **21** and **23**), as well as β nucleosides. However, all α nucleosides were found to be inactive even up to 100 μM concentration. Hence these compounds were omitted from the present discussion. For the initial SAR, to begin with, we tested the purine nucleosides obtained from **11** for their antiviral activity. Furanosides **13**, **13a**–**i** with β configuration at anomeric carbon were active with an IC_{50} values ranging from 7.1–50 μg/ml in flu-A and 3.7–65 μg/ml flu-B.

Many of the β nucleoside compounds (**13**, **13a**–**i**) were less toxic as shown (Table 1) with CC_{50} values of >100 μg/ml. Most active compound in this series is ((2*R*,3*S*,4*R*,5*R*)-3-acetoxy-5-(6-chloro-9H-purin-9-yl)-4-fluoro-3,4-dimethyltetrahydrofuran-2-yl)methyl benzoate **13**. However, we were not interested to evaluate this



Scheme 3. Synthesis of nucleosides **21**, **22**, **22a**, **23**, **24**, **25** and **26** from the sugar intermediate **11A**. Reagents and conditions: (a) 6-chloropurine/6-chloro-9H-purin-2-amine, ACN, DBU, TMSOTf, 65 °C overnight/*N*,*O*-bis(trimethylsilyl)acetamide, ACN, $SnCl_4$, 70 °C, 2 h; (b) NH_3/MeOH; room temperature, 16 h; (c) *N*-benzoylcytosine, *N*,*O*-bis(trimethylsilyl)acetamide, ACN, $SnCl_4$, 70 °C, 2 h.

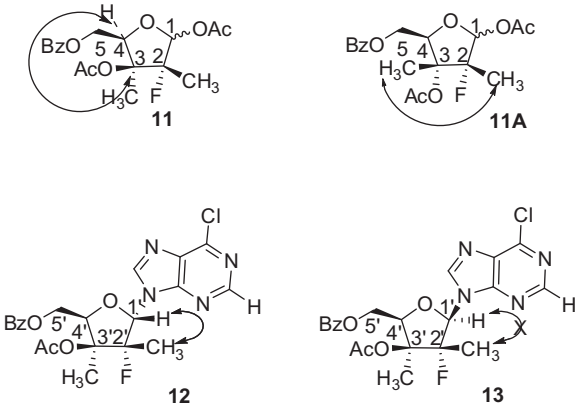


Figure 2. ^{1}H NMR NOE correlations of compound **11** and **11A**, **12**, and **13**.

compound further as it contained a reactive chloro group at the 6th position of purine nucleus. In an effort to find a compound with more activity, we screened the corresponding amino compounds by replacing chloro group with various amines. Cycloalkyl amino derivatives **13d**, **13f** heterocyclic alkyl derivative **13h** were slightly better active than their corresponding aromatic alkyl amine compounds **13e** and **13i**. NIs with guanine derivatives **13j** and **22a** were not active.

To evaluate the effect of other bases, we shifted our focus from purine to pyrimidine bases (Scheme 4; Table 3) uracil **18**, thymine **18a**, 5-fluorouracil **18b** and *N*-benzoylcytosine **18c**. The activity of these NIs were declined in the order of **18c**>**18b**>**18**>**18a**. The most active compound was *N*-benzoylcytosine analogue **18c**.

3.2. Effect of modification of the sugar

As we introduced a new stereo center at C-3′, two diastereomeric analogues were obtained. We separated both the isomers

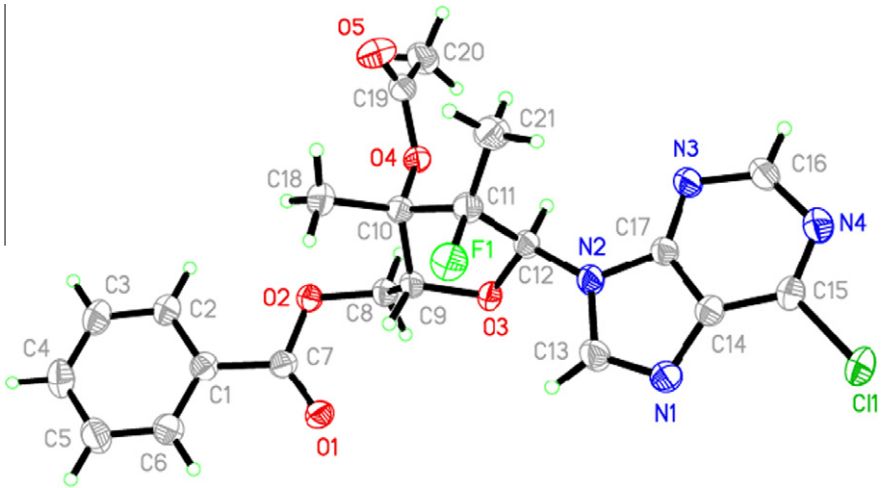
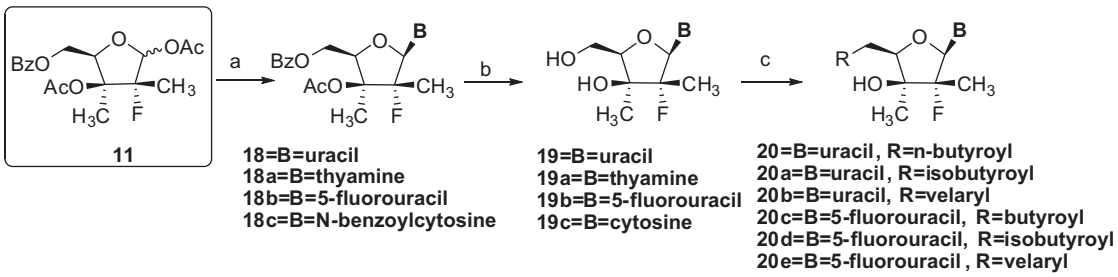
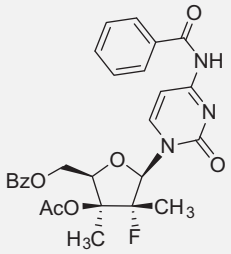
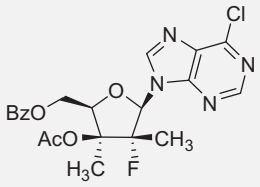


Figure 3. ORTEP drawing of (3-acetoxy-5-(6-chloro-9H-purin-9-yl)-4-fluoro-3,4-dimethyltetrahydrofuran-2-yl)methyl benzoate (**12**).



Scheme 4. Synthesis of compounds **18**, **18a–c**, **19**, **19a–c**, **20** and **20a–e**. Reagents and conditions: (a) pyrimidine base (uracil/thymine/6-fluorouracil/*N*-benzyoylcytosine), *N*,*O*-bis(trimethylsilyl)acetamide, ACN, $SnCl_4$, 70 °C, 2 h; (b) NH_3/MeOH; overnight; (c) acid chlorides DCM, at 0 °C.

Table 3
In vitro anti-influenza activity of **18c** and **13**, against Flu A, Flu B in various strains and inhibitory activity of influenza virus infection of MDCK cells

Compound	Assay	Virus	Cell line	EC_{50} (μg/ml)	IC_{50}	SI
 **18c**	Neutral red	FluA(H5N1) Vietnam/1203/2004H	MDCK	18	>100	>5.6
	Neutral red	FluB Florida/4/2006	MDCK	1.1	>100	>91
	Visual	FluB Florida/4/2006	MDCK	0.591	>100	>170
	Virus yield	FluB Florida/4/2006	MDCK	EC_{90}:1.9	>100	>53
	Visual-CONF	FluB Florida/4/2006	MDCK	0.59	>100	>170
 **13**	Neutral red	FluA(H5N1) Vietnam/1203/2004H	MDCK	1.76	19	11
	Virus yield	FluA(H5N1) Vietnam/1203/2004H	MDCK	EC_{90}:4.4	19	4.1
	Visual-CONF	FluA(H5N1) Vietnam/1203/2004H	MDCK	1.26	18	15
	Neutral red	FluB Florida/4/2006	MDCK	1.2	18	15
	Virus yield	FluB Florida/4/2006	MDCK	EC_{90}:6.3	18	2.9
	Visual-CONF	FluB Florida/4/2006	MDCK	1.2	18	15

Note: SI = $(IC_{50})/(EC_{50})$.

and tested them individually. Surprisingly all the isomers obtained from **11A**, irrespective of its configuration either at anomeric center or at C-3′ were not active, whereas compounds obtained from **11** are active.

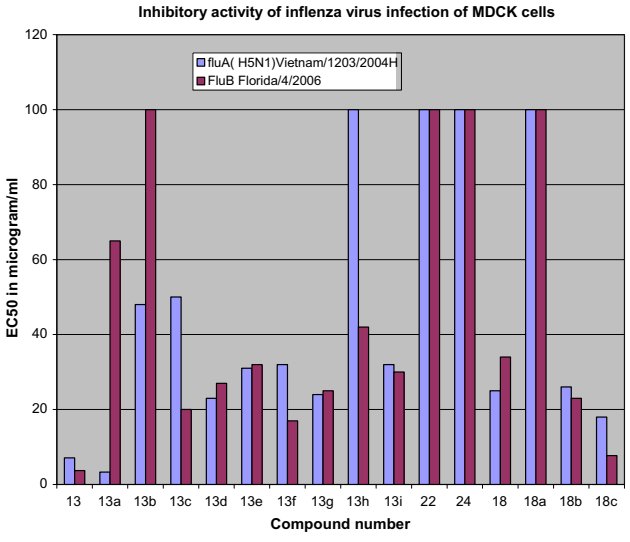
To probe the role of C-5′–OH and C-3′–OH, we prepared the deprotected analogues of **13**, **13a–i**, **18**, **18a**, **18b**, and **18c**. The corresponding nucleosides having sugar free hydroxyls of these compounds **16**, **16a–j** and **19**, **19a–c** (Schemes 2 and 4) failed to show activity.

To gain a better understanding of the reason for lacking of activity for these compounds, we synthesized and evaluated monoesters of C-5′–OH purine and pyrimidine NIs (**17**, **17a** and **20**, **20a–e**) (Schemes 2 and 4). Surprisingly all these compounds were found to be incapable of inhibiting virus (EC_{50} >100 μg/ml).

Based on these results we hypothesized that, since we used the cell based assay to screen the compounds, the ability of a compound to inhibit the influenza virus would be some extent dependent on the cellular uptake. That is why compounds with C-5′-benzoyl–C-2′-acetyl esters were active as they acted as prodrugs.

Thus the results suggest that *N*-benzoylcytosine analogue **18c** was the most active compound (Table 2). This activity was also confirmed in other standard assays like, increase in neutral red (NR) dye uptake, and decrease in virus yield assay (Table 3).

It is note worthy to mention that this novel analogue displayed significant activity with specificity towards Flu B strain.



4. Conclusion

In conclusion we demonstrated in the present study, the synthesis of new series of nucleoside analogues with significant bio-

logical activity and it is confidently expected that further SAR of compound **18c** will be found more potent and interesting compounds, having useful properties.

5. Experimental section

5.1. General experimental procedures

All reagents and solvents were used without further purification or drying. All reagents were purchased from Sigma–Aldrich, unless otherwise specified. Commercial grade anhydrous solvents were purchased from Rankem chemicals or Merck. All reactions were performed under nitrogen, unless otherwise specified. The NMR experiments were accomplished on a Bruker AVX300 (ultra shield™) at 300 K in $CDCl_3$ (calibrated to the residual nondeuterated solvent signals). MS analysis was performed on Shimadzu LCMS 2010, EV and Alliance Waters 2695 micromass QUATTRO MICRO API. Esquire 3000 plus ion-trap mass spectrometer equipped with electrospray ionization (ESI) in positive and negative mode: spray voltage, 3.5 kV; dissolvation gas 600 l/h. Dry gas, N2, 1.5 l/min; scanning range, *m*/*z* 50–1000. HRMS obtained on a QSTAR XL instrument. Column chromatography was performed using Merck silica gel 60, 40–63 μm and Pharmacia Sephadex LH-20, 20–100 μm. The obtained fractions from all chromatographic steps were analyzed by TLC (mobile phase: DCM/Methanol [9:1]; stationary phase: Merck silica gel 60 PF254, detected with staining reagents anisaldehyde at VIS, UV254, UV366). HPLC was performed on a Shimadzu 2010 auto sampler with a photodiode array detector (DAD). LC-parameters: stationary phase: Zorbax SB-C18, rapid resolution 3.5 μm (4.6–150 mm), Agilent, USA; temperature: 40 °C; mobile phase: water (A); methanol (B); flow rate 1.0 ml/min; UV detection wavelength: 205, 280, 360 nm; injection volume: 10 μl; gradient. A summary of the key crystallographic information for the compound **12**, intensity data were collected on a Bruker SMART CCD area detector diffractometer with graphite-monochromated radiation (λ = 0.71073 Å). Biological assays were performed at AACF as per their protocols. Visit: http://niaid-aacf.org/protocols/Respiratory.

5.1.1. ((2*R*,3*R*,4*R*)-3-Benzoyloxy-4-fluoro-5-hydroxy-4-methyltetrahydrofuran-2-yl)methyl benzoate (2)

To a stirred solution of compound *((2R,3R,4R)-3-benzoyloxy-4-fluoro-4-methyl-5-oxo-tetrahydrofuran-2-yl)methyl benzoate* (60 g, 161 mmol) in THF (300 ml) cooled to −10 °C, then Li(^{t}BuO)$_3$AlH (241.9 ml, 241 mmol) was added and stirred at 0 °C for 1 h. Completion of the reaction monitored by TLC, quenched with saturated ammonium chloride solution. THF was removed under reduced pressure and the aqueous layer was extracted with ethyl acetate. The combined organic extracts were washed with water, brine and dried over Na_2SO_4. Organic layer was removed under reduced pressure and the residue was purified by silica gel column chromatography using 16% ethyl acetate: hexane to give **2** (60 g, 99%) as a colorless liquid. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.11–8.00 (m, 4H), 7.61–7.37 (m, 6H), 5.65 (dd, 1H, *J* = 23.4 Hz, 7.8 Hz, 7.5 Hz), 5.34 (dd, 1H, *J* = 9.6 Hz, 2.7 Hz), 4.69–4.66 (m, 1H), 4.57–4.53 (m, 2H), 3.40 (br s, 1H, –OH), 1.60–1.56 (m, 3H, *J* = 22.5). MS (ESI) *m*/*z* 397 [M+Na]$^+$. IR cm^{-1}: 3439, 3070, 2940, 2868, 1733, 1729, 1453, 1274, 1115, 710.

5.1.2. ((2*R*,3*R*,4*R*)-3-Benzoyloxy-4-fluoro-5-methoxy-4-methyltetrahydrofuran-2-yl)methyl benzoate (3) and (2*R*,3*R*,4*R*)-4-fluoro-2-(hydroxymethyl)-5-methoxy-4-methyltetrahydrofuran-3-yl benzoate (4)

To a stirred solution of compound **2** (60 g, 160 mmol) in methanol (300 ml), $BF_3{\cdot}OEt_2$ (60.02 ml, 481 mmol) was added at room temperature then reaction mixture was heated to 80 °C for 5 h. After that reaction quenched with satd $NaHCO_3$ solution, methanol was removed under reduced pressure, extracted with ethyl acetate. The combined organic extracts were washed with water, brine and dried over Na_2SO_4. Organic layer was removed under reduced pressure and the residue was purified by silica gel column chromatography using 8% ethyl acetate: hexane as eluent furnished compound **3** (17 g), and 15% ethyl acetate eluded compound **4** (18 g) as a colorless liquid. The overall yield was 56%. *Compound* **3**: ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.01–7.93 (m, 4H), 7.56–7.48 (m, 6H), 5.53 (dd, 1H, *J* = 24.3 Hz, 7.80 Hz), 4.97 (d, 1H, *J* = 9.6 Hz), 4.58–4.53 (m, 2H), 4.41–4.38 (m, 1H), 3.30 (s, 3H), 1.44 (d, 3H, *J* = 22.8). MS (ESI) *m*/*z* 413 [M+Na]$^+$. IR cm^{-1}: 3439, 2961, 2939, 1728, 1602, 1452, 1270, 1117, 710. *Compound* **4**: ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.11–8.08 (m, 2H), 7.61–7.59 (m, 1H), 7.50–7.45 (m, 2H), 5.41 (dd, 1H, *J* = 23.7 Hz, 7.50 Hz), 4.85 (d, 1H, *J* = 10.5 Hz), 4.34–4.32 (m, 2H), 3.76–3.69 (m, 1H), 3.33 (s, 3H), 1.49 (d, 3H, *J* = 22.8). MS (ESI) *m*/*z*: 307 [M+NH$_4$]$^+$, 302 [M+H]$^+$.

5.1.3. (2*R*,3*R*,4*R*)-4-Fluoro-2-(hydroxymethyl)-5-methoxy-4-methyl-tetrahydrofuran-3-ol (5)

Compounds **3** and **4** (17 g, 43.8 mmol, 18 g, 63.3 mmol) in methanolic ammonia (25% w/w 500 ml) was stirred at rt for 36 h. Completion of the reaction monitored by TLC, methanol was removed under reduced pressure. Water added to the reaction mixture and extracted with ethyl acetate, the organic layer was dried over Na_2SO_4, concentrated under reduced pressure. Purified by silica gel column chromatography using 20% ethyl acetate in Hexane to give **5** (16 g, 99%) as an off-white solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ 4.79 (d, 1H, *J* = 10.8 Hz), 4.01–3.97 (m, 2H), 3.85 (d, 1H,), 3.71–3.66 (m, 1H), 3.44 (s, 3H), 2.05 (br s, 1H, –OH), 2.02 (br s, 1H, –OH), 1.47 (d, 3H, *J* = 23.4 Hz). MS (ESI) *m*/*z* 203 [M+Na]$^+$. IR cm^{-1}: 3437, 3372, 3294, 2924, 1459, 1102, 1049, 1006, 821, 731.

5.1.4. (2*R*,3*R*,4*R*)-2-((*tert*-Butyldiphenylsilyloxy)methyl)-4-fluoro-5-methoxy-4-methyl-tetrahydrofuran-3-ol (6)

To a magnetically stirred solution of **5** (16 g, 88.8 mmol) and imidazole (9.07 g, 133.2 mmol) in CH_2Cl_2 (200 ml) cooled to 0 °C, TBDPSCl (23.1 ml, 88.8 mmol) was added and stirred for 2 h at rt. Completion of the reaction monitored by TLC, water added to the reaction mixture and extracted with CH_2Cl_2. The combined organic extracts were washed with water, brine and dried over Na_2SO_4. Concentrated under reduced pressure, purification of the residue by silica gel column chromatography using 7% ethyl acetate: hexane as eluent to give **6** (16 g, 43%) as colorless liquid. ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.72–7.69 (m, 4H), 7.42–7.41 (m, 6H), 4.79 (d, 1H, *J* = 11.1.0 Hz), 3.97–3.87 (m, 4H), 3.35 (s, 3H), 1.86 (br s, 1H), 1.50 (d, 3H). MS (ESI) *m*/*z* 441 [M+Na]$^+$.

5.1.5. (2*R*,4*S*)-2-((*tert*-Butyldiphenylsilyloxy)methyl)-4-fluoro-5-methoxy-4-methyl-dihydrofuran-3(2*H*)-one (7)

A solution of compound **6** (16 g, 38.2 mmol) and Dess–Martin periodinane (24.3 g, 57.4 mmol) in CH_2Cl_2 (150 ml) stirred at 0 °C to room temperature under N_2 atmosphere. Completion of the reaction monitored by TLC and quenched by the saturated solution of $Na_2S_2O_3$ and $NaHCO_3$ in equal volumes (100 ml + 100 ml). The aqueous layers were extracted with CH_2Cl_2 and the combined organic extracts were washed with water, brine and dried over Na_2SO_4 and concentrated under reduced pressure to give **7** (24 g as a crude). ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.69–7.67 (m, 4H), 7.42–7.39 (m, 6H), 5.07 (d, 1H, *J* = 12.3 Hz), 4.38 (dd, 1H, *J* = 5.1 Hz, 3.3 Hz), 3.889–3.83 (m, 2H), 3.46 (s, 3H), 1.42 (d, 3H, *J* = 24 Hz), 1.03 (s, 9H). MS (ESI) *m*/*z* 439 [M+Na]$^+$. IR cm^{-1}: 2934, 2859, 1783, 1382, 1114, 998, 703.

5.1.6. (2*R*,3*S*,4*R*)-2-((*tert*-Butyldiphenylsilyloxy)methyl)-4-fluoro-5-methoxy-3,4-dimethyltetrahydrofuran-3-ol (8): and (2*R*,3*R*,4*R*)-2-((*tert*-butyldiphenylsilyloxy)methyl)-4-fluoro-5-methoxy-3,4-dimethyltetrahydrofuran-3-ol (8A)

To a solution of **7** (23 g, 55.2 mmol) in dry ether at 0 °C was added methyl magnesium bromide (60.8 ml, 121 mmol) drop wise for 30 min and stirred at rt for 4 h. Completion of the reaction monitored by TLC and quenched with saturated ammonium chloride solution. Aqueous layer was extracted with ethyl acetate and the combined organic layers were washed with water, brine and dried over Na_2SO_4. Organic layers were concentrated under reduced pressure and purified by silica gel column chromatography using 3% and 3.5% ethyl acetate in hexane gave **8** and **8A** (9.1 g and 1.5 g, overall yield 64.2%) as a colorless liquid. *Compound* **7**: ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.74–7.72 (m, 4H), 7.43–7.39 (m, 6H), 4.83 (d, 1H, J = 15.3 Hz), 4.08 (m, 1H), 3.84–3.80 (m, 2H), 3.39 (s, 3H), 1.34 (d, 3H, J = 24.6 Hz), 1.16 (s, 3H), 1.05 (s, 9H). MS (ESI) m/z 455 $[M+Na]^+$. *Compound* **8**: ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.74–7.72 (m, 4H), 7.42–7.39 (m, 6H), 4.81 (d, 1H, J = 11.4 Hz), 4.16–4.14 (m, 1H), 4.00–3.81 (m, 2H), 3.37 (s, 3H), 1.42 (d, 3H, J = 23.4 Hz), 1.27 (s, 3H), 1.06 (s, 9H). MS (ESI) m/z 455 $[M+Na]^+$.

5.1.7. (2*R*,3*S*,4*R*)-4-Fluoro-2-(hydroxymethyl)-5-methoxy-3,4-dimethyltetrahydrofuran-3-ol (9)

To a solution of **7** (6.3 g, 14.5 mmol) in THF (100 ml) at 0 °C was added 1 N TBAF (14.5 ml, 14.5 mmol) drop wise for 10 min and stirred at rt for 1.5 h. Completion of the reaction monitored by TLC and neutralized with satd $NaHCO_3$. Aqueous layer was extracted with ethyl acetate and the combined organic extracts were washed with water, brine and dried over Na_2SO_4 and concentrated under reduced pressure, purified by silica gel column chromatography using 50% ethyl acetate: hexane as eluent furnished **9** (2.8 g, 99%) as a colorless liquid. ^{1}H NMR (300 MHz, $CDCl_3$): δ 4.84 (d, 1H, J = 11.1 Hz), 4.11 (m, 1H), 3.88–3.87 (m, 2H), 3.46 (s, 3H), 3.35 (s, 1H), 2.05 (m, 1H), 1.40 (d, 3H, J = 23.4 Hz), 1.27 (d, 3H, J = 2.1 Hz). MS (ESI) m/z 217 $[M+Na]^+$.

5.1.8. ((2*R*,3*S*,4*R*)-4-Fluoro-3-hydroxy-5-methoxy-3,4-dimethyltetrahydrofuran-2-yl)methyl benzoate (10)

To a solution of **9** (2 g, 10.3 mmol) in dry DCM (40 ml) at 0 °C was added DMAP (2.5 g, 20.6 mmol), TEA (4.2 ml, 30.9 mmol), stirred for 15 min then drop wise addition of BzCl (5.9 ml, 51.5 mmol) for 10 min and stirred at rt for 18 h. Completion of the reaction monitored by TLC and quenched with water. Aqueous layer was extracted with ethyl acetate and the combined organic layers were washed with satd $NaHCO_3$ solution, brine and dried over Na_2SO_4. Concentrated under reduced pressure and purified by silica gel column chromatography using 3% ethyl acetate: hexane as eluent furnished compound **10** (2.1 g, 68.4%) as a colorless liquid. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.09–8.06 (m, 4H), 7.57–7.42 (m, 6H), 4.85 (d, 1H, J = 10.2 Hz), 4.60–4.59 (m, 2H), 4.50–4.44 (m, 1H), 3.46 (s, 3H), 1.46 (d, 3H, J = 23.4 Hz), 1.26 (s, 3H). MS (ESI) m/z: 425 $[M+Na]^+$. IR cm^{-1}: 3425, 2927, 1723, 1679, 1602, 1451, 1281, 1124, 710.

5.1.9. (3*R*,4*S*,5*R*)-5-(Benzoyloxymethyl)-3-fluoro-3,4-dimethyltetrahydrofuran-2,4-diyl diacetate (11)

To a solution of **10** (2.1 g, 7.04 mmol) in acetic acid (15 ml) at 0 °C was added H_2SO_4 (1.5 ml, 15.5 mmol) and acetic anhydride (2.79 ml, 29.5 mmol), and stirred the reaction at rt for 16 h. Completion of the reaction monitored by TLC, reaction mixture was slowly added to ice cold water, and extracted with ethyl acetate; organic layer was washed with satd $NaHCO_3$ and dried over Na_2SO_4 and concentrated under reduced pressure to yield **11** (1.9 g, 73.27%) as a colorless gummy mass. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.06–8.04 (m, 2H), 7.57–7.55 (m, 1H), 7.47–7.42 (m, 2H), 6.19 (d, 1H, J = 20.4 Hz), 4.73–4.67 (m, 1H), 4.67–4.44 (m, 2H), 2.06 (s, 3H), 1.80 (s, 3H), 1.51 (d, 3H, J = 22.8 Hz), 1.43 (s, 3H). MS (ESI) m/z: 391 $[M+Na]^+$.

5.1.10. (2*R*,3*R*,4*R*)-4-Fluoro-2-(hydroxymethyl)-5-methoxy-3,4-dimethyltetrahydrofuran-3-ol (9A)

To a solution of compound **8A** (1.5 g, 3.4 mmol) in THF (25 ml) at 0 °C was added 1 M TBAF (3.47 ml, 3.4 mmol) drop wise for 10 min and stirred at rt for 1.5 h. Completion of the reaction monitored by TLC and neutralized with satd $NaHCO_3$. Aqueous layer was extracted with ethyl acetate and the combined organic extracts were washed with water, brine and dried over Na_2SO_4 and concentrated under reduced pressure, purified by silica gel column chromatography using 50% ethyl acetate: hexane as eluent furnished compound **9A** (0.5 g, 75%) as a colorless liquid. ^{1}H NMR (300 MHz, $CDCl_3$): δ 4.84 (d, 1H, J = 15.3 Hz), 4.00–3.98 (m, 3H), 3.45 (s, 3H), 1.34 (d, 3H, J = 24.3 Hz), 1.17 (s, 3H). MS (ESI) m/z: 217 $[M+Na]^+$.

5.1.11. ((2*R*,3*R*,4*R*)-4-Fluoro-3-hydroxy-5-methoxy-3,4-dimethyltetrahydrofuran-2-yl)methyl benzoate (10A)

To a solution of compound **9A** (0.5 g, 2.5 mmol) in dry DCM (10 ml) at 0 °C was added DMAP (0.47 g, 3.8 mmol), TEA (1.42 ml, 10.3 mmol), stirred for 15 min then drop wise addition of BzCl (1.7 ml, 15.4 mmol) for 10 min and stirred at 45 °C for 48 h. Completion of the reaction monitored by TLC and quenched with water. Aqueous layer was extracted with ethyl acetate and the combined organic layers were washed with satd $NaHCO_3$ solution, brine and dried over Na_2SO_4. Concentrated under reduced pressure to yield compound **10A** (0.5 g, 65%). MS (ESI) m/z: 299 $[M+Na]^+$.

5.1.12. (3*R*,4*R*,5*R*)-5-(Benzoyloxymethyl)-3-fluoro-3,4-dimethyltetrahydrofuran-2,4-diyl diacetate (11A)

To a solution of compound **10A** (0.5 g, 1.67 mmol) in acetic acid (10 ml) at 0 °C was added H_2SO_4 (0.36 ml, 3.69 mmol) and acetic anhydride (0.66 ml, 7.04 mmol), then stirred the reaction at rt for 16 h. Completion of the reaction monitored by TLC, reaction mixture was slowly added to ice cold water, and extracted with ethyl acetate; organic layer was washed with satd $NaHCO_3$ and dried over Na_2SO_4 and concentrated under reduced pressure to yield compound **11A** (0.5 g, 81%) as a colorless liquid. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.08–8.04 (m, 2H), 7.56–7.41 (m, 3H), 6.17 (d, 1H, J = 12.6 Hz), 4.94 (dd, 1H, J = 11.4 Hz, 3 Hz), 4.66–4.65 (m, 1H), 4.55–4.49 (m, 1H), 2.09 (s, 3H), 2.06 (s, 3H), 1.58 (m, 6H). MS (ESI) m/z: 391 $[M+Na]^+$, 386 $[M+NH_4]^+$.

5.1.13. ((2*R*,3*S*,4*R*,5*S*)-3-Acetoxy-5-(6-chloro-9H-purin-9-yl)-4-fluoro-3,4-dimethyltetrahydrofuran-2-yl)methyl benzoate (12) and ((2*R*,3*S*,4*R*,5*R*)-3-acetoxy-5-(6-chloro-9H-purin-9-yl)-4-fluoro-3,4-dimethyltetrahydrofuran-2-yl)methyl benzoate (13)

To a stirred solution of 6-chloropurine (0.87 g, 5.7 mmol) in acetonitrile (20 ml) was added DBU (1.7 ml, 11.4 mmol) and TMSOTf (2.76 ml, 15.2 mmol) at 0 °C, stirred for 15 min then compound **11** (1.4 g, 3.8 mmol) dissolved in acetonitrile (20 ml) was added and reaction continued stirring at 65 °C for 8 h. Completion of reaction monitored by TLC and quenched with saturated $NaHCO_3$. Aqueous layer was extracted with ethyl acetate and the combined organic layers were washed with satd $NaHCO_3$ solution, brine and dried over Na_2SO_4. Concentrated under reduced pressure and purified by silica gel column chromatography using 20% (**12**) and 25% (**13**) ethyl acetate: hexane as eluent furnished compounds **12** (0.7 g) and **13** (0.3 g) as colorless solid, overall yield 57%. *Compound* **12**: ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.78 (s, 1H), 8.36 (d, 1H, J = 3.6 Hz), 8.08–8.04 (m, 2H), 7.60 (m, 1H), 7.49–7.44 (m, 2H), 6.63 (d, 1H, J = 22.5 Hz), 4.90–4.80 (m, 3H), 2.21 (s, 3H), 1.92 (s, 3H), 1.48 (d, 3H, J = 23.1 Hz). ^{13}C NMR: 13.72, 14.35, 29.58, 62.90,

84.39, 88.12, 88.32, 101.67, 121.86, 128.44 (2), 129.59 (3), 133.35, 141.64, 148.82, 152.55, 161.98, 166.07, 168.70. MS (ESI) *m*/*z*: 485 $[M+Na]^+$, 463 $[M+1]^+$. *Compound* **13**: ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.94 (s, 1H), 8.54 (d, 1H, *J* = 4.2 Hz), 8.06–8.03 (m, 2H), 7.59 (m, 1H), 7.49–7.43 (m, 2H), 6.97 (d, 1H, *J* = 21.3 Hz), 4.88–4.80 (m, 2H), 4.53–4.47 (m, 1H), 2.19 (s, 3H), 1.91 (s, 3H), 1.49 (d, 3H, *J* = 23.1 Hz), ^{13}C NMR: 13.61, 14.15, 29.54, 62.91, 84.42, 86.95, 88.21, 102.38, 128.37 (2), 129.35 (3), 131.24, 133.24, 144.41, 151.13, 151.71, 152.09, 166.04, 168.81. MS (ESI) *m*/*z*: 485 $[M+Na]^+$, 463 $[M+1]^+$.

5.1.14. ((2*R*,3*S*,4*R*,5*R*)-3-Acetoxy-4-fluoro-5-(6-hydroxy-9H-purin-9-yl)-3,4-dimethyltetrahydrofuran-2-yl)methyl benzoate (13a) and ((2*R*,3*S*,4*R*,5*R*)-3-acetoxy-5-(6-ethoxy-9H-purin-9-yl)-4-fluoro-3,4-dimethyltetrahydrofuran-2-yl)methyl benzoate (13b)

A stirred solution of **13** (1.0 g, 2.16 mmol) in ethanol (20 ml) was refluxed for 36 h. Completion of the reaction monitored by TLC, water added to the reaction mixture and the aqueous layer was extracted with ethyl acetate and the combined organic layers were washed with brine and dried over Na_2SO_4. Concentrated under reduced pressure and purified by silica gel column chromatography using 3% *methanol* in *dichloromethane* as eluent furnished compound **13b** (0.05 g) as a colorless solid and 5% methanol in dichloromethane as eluent to give compound **13a** (0.45 g). The overall yield was 52%. *Compound* **13a**: ^{1}H NMR (300 MHz, $CDCl_3$): δ 10.30 (bs, 1H), 8.22 (d, 1H, *J* = 3.9 Hz), 8.09–8.04 (m, 3H), 7.61–7.48 (m, 3H), 6.93 (d, 1H, *J* = 21.6 Hz), 4.84–4.77 (m, 2H), 4.49–4.47 (m, 1H), 2.17 (s, 3H), 1.89 (d, 3 h, *J* = 2.4 Hz), 1.46 (d, 3H, *J* = 23.1 Hz). MS (ESI) *m*/*z*: 445 $[M+1]^+$, 467 $[M+Na]^+$. *Compound* **13b**: ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.67 (s, 1H), 8.32 (d, 1H, *J* = 3.9 Hz), 8.07–8.03 (m, 2H), 7.60–7.59 (m, 1H), 7.48–7.43 (m, 2H), 6.71 (d, 1H, *J* = 21.9 Hz), 4.86–4.81 (m, 2H), 4.77–4.76 (m, 2H), 4.68–4.50 (m, 2H), 2.16 (s, 3H), 1.90 (d, 3H, *J* = 2.11 Hz), 1.50–1.25 (m, 6H). MS (ESI) *m*/*z*: 473 $[M+1]^+$, 495 $[M+Na]^+$.

5.1.15. ((2*R*,3*S*,4*R*,5*R*)-3-Acetoxy-5-(6-(cyclopropylamino)-9H-purin-9-yl)-4-fluoro-3,4-dimethyltetrahydrofuran-2-yl)methyl benzoate (13c)

To a stirred solution of compound **13** (1.0 g, 2.16 mmol) in ethanol (20 ml) was added compound *cyclopropylamine* (1.79 ml, 25.90 mmol) and refluxed for 30 min completion of the reaction monitored by TLC, water added to the reaction mixture and the aqueous layer was extracted with ethyl acetate and the combined organic layers were washed with brine and dried over Na_2SO_4. Concentrated under reduced pressure, purified by silica gel column chromatography using 40% ethyl acetate in hexane as eluent furnished compound **13c** (0.45 g, 44%) as a colorless solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.64 (s, 1H), 8.08–8.04 (m, 3H), 7.60–7.58 (m, 1H), 7.49–7.44 (m, 2H), 6.37 (s, 1H), 6.11 (d, 1H, *J* = 22.8 Hz), 4.89–4.84 (m, 1H), 4.75–4.71 (m, 1H), 4.56–4.49 (m, 1H), 2.92–2.88 (m, 1H), 2.19 (s, 3H), 1.88 (d, 3H, *J* = 2.1 Hz), 1.35 (d, 3H, *J* = 22.5 Hz), 0.88–0.86 (m, 2H), 0.53–0.46 (m, 2H). MS (ESI) *m*/*z*: 484 $[M+1]^+$.

5.2. Synthesis of compounds 13d–i will follow the above conditions

5.2.1. ((2*R*,3*S*,4*R*,5*R*)-3-Acetoxy-5-(6-(cyclobutylamino)-9H-purin-9-yl)-4-fluoro-3,4-dimethyltetrahydrofuran-2-yl)methyl benzoate (13d)

^{1}H NMR (300 MHz, $CDCl_3$): δ 8.52 (s, 1H), 8.08–8.05 (m, 3H), 7.71–7.60 (m, 1H), 7.48–7.43 (m, 2H), 6.29 (d, 1H, *J* = 7.5 Hz), 6.14 (d, 1H, *J* = 22.8 Hz), 4.94–4.82 (m, 1H), 4.68–4.65 (m, 1H), 4.59–4.55 (m, 1H), 4.23–4.20 (m, 1H), 2.36–1.36 (m, 15H). MS (ESI) *m*/*z*: 498 $[M+1]^+$, 520 $[M+Na]^+$.

5.2.2. ((2*R*,3*S*,4*R*,5*R*)-3-Acetoxy-4-fluoro-5-(6-(furan-2-ylmethylamino)-9H-purin-9-yl)-3,4-dimethyltetrahydrofuran-2-yl)methyl benzoate (13e)

^{1}H NMR (300 MHz, $CDCl_3$): δ 8.60 (s, 1H), 8.12–8.00 (m, 3H), 7.48–7.43 (m, 3H), 7.30 (d, 1H, *J* = 0.9 Hz), 6.46–6.43 (m, 1H), 6.27–6.21 (m, 2H), 6.11 (d, 1H, *J* = 22.5 Hz), 4.97–4.90 (dd, 1H, *J*1 = 15 Hz, *J*2 = 15 Hz), 4.76–4.71 (dd, 1H, *J*1 = 12 Hz, *J*2 = 12 Hz), 4.60–4.41 (m, 3H), 4.22–4.20 (m, 1H), 2.14 (s, 3H), 1.82 (d, 3H, *J* = 2.4 Hz), 1.34 (d, 3H, *J* = 22.2 Hz). MS (ESI) *m*/*z*: 546 $[M+Na]^+$.

5.2.3. ((2*R*,3*S*,4*R*,5*R*)-3-Acetoxy-5-(6-(cyclopropylmethylamino)-9H-purin-9-yl)-4-fluoro-3,4-dimethyltetrahydrofuran-2-yl)methyl benzoate (13f)

^{1}H NMR (300 MHz, $CDCl_3$): δ 8.52 (s, 1H), 8.06–8.01 (m, 3H), 7.60–7.57 (m, 1H), 7.49–7.44 (m, 2H), 6.20 (s, 1H), 6.15 (d, 1H, *J* = 22.8 Hz0, 4.90–4.86 (m, 2H), 4.59–4.53 (m, 1H), 3.40–3.34 (m, 2H), 2.19 (s, 3H), 1.91 (d, 3H, *J* = 2.4 Hz), 1.63 (s, 1H), 1.37 (d, 3H, *J* = 22.5 Hz), 0.89–0.88 (m, 1H), 0.49–0.44 (m, 2H), 0.25–0.23 (m, 2H). MS (ESI) *m*/*z*: 498 $[M+1]^+$, 499 $[M+Na]^+$.

5.2.4. (2*R*,3*S*,4*R*,5*R*)-3-Acetoxy-4-fluoro-3,4-dimethyl-5-(6-morpholino-9H-purin-9-yl)tetrahydrofuran-2-yl)methyl benzoate (13g)

^{1}H NMR (300 MHz, $CDCl_3$): δ 8.80 (s, 1H), 8.47 (d, 1H, *J* = 4.1 Hz), 8.06–8.03 (m, 2H), 7.59–7.56 (m, 1H), 7.48–7.43 (m, 2H), 6.58 (d, 1H, *J* = 23.4 Hz), 4.86–4.73 (m, 2H), 4.53–4.47 (m, 1H), 3.99–3.83 (m, 4H), 3.54–3.28 (m, 2H), 3.27–3.23 (m, 2H), 2.22 (s, 3H), 1.89 (d, 3H, *J* = 2.1 Hz), 1.26 (d, 3H, *J* = 22.8 Hz). MS (ESI) *m*/*z*: 536 $[M+Na]^+$, 514 $[M+1]^+$.

5.2.5. ((2*R*,3*S*,4*R*,5*R*)-3-Acetoxy-4-fluoro-3,4-dimethyl-5-(6-(4-methylpiperazin-1-yl)-9H-purin-9-yl)tetrahydrofuran-2-yl)methyl benzoate (13h)

^{1}H NMR (300 MHz, $CDCl_3$): δ 8.80 (s, 1H), 8.45 (d, 1H, *J* = 5.1 Hz), 8.07–8.04 (m, 2H), 7.59–7.57 (m, 1H), 7.49–7.44 (m, 2H), 6.54 (d, 1H, *J* = 23.1 Hz), 4.87–4.76 (m, 2H), 4.57–4.53 (m, 1H), 3.50–3.48 (m, 2H), 3.31 (s, 2H), 2.67–2.60 (m, 3H), 2.38 (s, 1H), 2.24 (s, 1H), 1.88 (d, 3H, *J* = 2.11 Hz), 1.23 (d, 3H, *J* = 22.8 Hz). MS (ESI) *m*/*z*: 527 $[M+1]^+$, 549 $[M+Na]^+$.

5.2.6. ((2*R*,3*S*,4*R*,5*R*)-3-Acetoxy-4-fluoro-5-(6-(4-fluorobenzylamino)-9H-purin-9-yl)-3,4-dimethyltetrahydrofuran-2-yl)methyl benzoate (13i)

^{1}H NMR (300 MHz, $CDCl_3$): δ 8.56 (s, 1H), 8.06 (s, 1H), 7.96–7.93 (m, 2H), 7.62–7.60 (m, 1H), 7.61–7.58 (m, 2H), 7.25–7.20 (m, 2H), 6.94–6.88 (m, 2H), 6.38 (bs, 1H), 6.10 (d, 1H, *J* = 22.8 Hz), 4.98–4.91 (m, 1H), 4.64–4.61 (m, 1H), 4.48–4.42 (m, 1H), 4.34–4.30 (m, 2H), 2.12 (s, 3H), 1.76 (s, 3H), 1.34 (d, 3H, *J* = 22.5 Hz). MS (ESI) *m*/*z*: 552 $[M+1]^+$.

5.2.7. ((2*R*,3*S*,4*R*,5*R*)-3-Acetoxy-5-(2-amino-6-chloro-9H-purin-9-yl)-4-fluoro-3,4-dimethyltetrahydrofuran-2-yl)methyl benzoate (13j)

To a stirred solution of 6-chloro-9H-purin-2-amine (0.18 g, 1.08 mmol) in acetonitrile (5 ml) was added *N*,*O*-bis(trimethylsilyl)acetamide (1.6 ml, 6.52 mmol) at rt and refluxed for 2 h. To this added compound **11** (0.2 g, 0.54 mmol) in acetonitrile (5 ml) drop wise at 0 °C, and added $SnCl_4$ (0.09 ml, 0.81 mmol) and refluxed for 15 h, Completion of the reaction monitored by TLC, neutralized the reaction mixture and with saturated NaHCO3 solution, aqueous layer was extracted with ethyl acetate and the combined organic layers were washed with brine and dried over Na_2SO_4. Concentrated under reduced pressure, purified by silica gel column chromatography using 5% methanol in dichloromethane as eluent to give compound **13j** (0.08 g, 30%) as a colorless solid. ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.29 (d, 1H, *J* = 4.2 Hz), 8.06–8.03 (m, 2H),

7.59–7.56 (m, 1H), 7.49–7.44 (m, 2H), 6.80 (d, 1H, *J* = 21.6 Hz), 5.09 (s, 2H), 4.83–4.78 (m, 2H), 4.51–4.44 (m, 1H), 2.17 (s, 3H), 1.89 (d, 3H, *J* = 2.4 Hz), 1.48 (d, 3H, *J* = 23.1 Hz). MS (ESI) *m*/*z*: 478 [M+1], 500 [M+Na].

5.2.8. ((2*R*,3*S*,4*R*,5*R*)-3-Acetoxy-5-(2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)-4-fluoro-3,4-dimethyltetrahydrofuran-2-yl)methyl benzoate (18)

To a stirred solution of uracil (0.30 g, 2.71 mmol) in acetonitrile (7 ml) was added *N*,*O*-bis(trimethylsilyl)acetamide (4 ml, 16.3 mmol) at rt and refluxed for 2 h. To this added compound **11** (0.5gm, 1.35 mmol) in acetonitrile (8 ml) drop wise at 0 °C, and added $SnCl_4$ (0.2 ml, 2.03 mmol) and refluxed for 15 h, Completion of the reaction monitored by TLC, neutralized the reaction mixture and with saturated NaHCO3 solution, aqueous layer was extracted with ethyl acetate and the combined organic layers were washed with brine and dried over Na_2SO_4. Concentrated under reduced pressure, purified by silica gel column chromatography using 35% ethyl acetate in hexane as eluent to give compound **18** (0.35 g, 61%) as a colorless solid. ^{1}H NMR: ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.67 (s, 1H), 8.06–8.03 (m, 2H), 7.59–7.33 (m, 4H), 6.39 (d, 1H, *J* = 22.8 Hz), 5.76, 5.55 (dd, 1H, *J* = 2.1 Hz, *J* = 2.4 Hz), 4.80–4.43 (m, 3H), 2.13 (s, 3H), 1.81 (d, 3H, *J* = 2.4 Hz), 1.49 (d, 3H, *J* = 23.4 Hz). ^{13}C NMR: 13.50, 14.61, 22.02, 63.08, 84.33, 87.18, 88.26, 102.21, 103.36, 128.48 (2), 129.50 (2), 129.67, 133.35, 140.94, 150.73, 163.01, 166.16, 168.98. MS (ESI) *m*/*z*: 443 $[M+Na]^+$. IR cm^{-1}: 3433, 2931, 2346, 1713, 1385, 1274, 952, 712.

5.3. Synthesis of compound 18a–c will follow the above conditions

5.3.1. ((2*R*,3*S*,4*R*,5*R*)-3-Acetoxy-4-fluoro-3,4-dimethyl-5-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)tetrahydrofuran-2-yl)methyl benzoate (18a)

^{1}H NMR (300 MHz, $CDCl_3$): δ 8.07 (s, 1H), 8.05–8.04 (m, 2H), 7.60–7.58 (m, 1H), 7.49–7.44 (m, 2H), 7.15–7.13 (m, 1H), 6.38 (d, 1H, *J* = 23.1 Hz), 4.74–4.65 (m, 2H), 4.49–4.41 (m, 1H), 2.12 (s, 3H), 1.94 (d, 3H, *J* = 0.9 Hz), 1.83 (d, 3H, *J* = 2.4 Hz), 1.48 (d, 3H, *J* = 23.4 Hz). ^{13}C NMR: 12.55, 13.60, 14.62, 22.05, 29.68, 63.04, 84.17, 86.97, 88.27, 110.50, 128.51 (2), 129.56 (2), 129.69, 133.37, 136.57, 150.72, 163.39, 166.20, 169.90. MS (ESI) *m*/*z*: 435 [M+1], 457 [M+Na]. IR cm^{-1}: 3432, 2926, 1753, 1692, 1602, 1547, 1384, 1276, 1115, 954, 833.

5.3.2. ((2*R*,3*S*,4*R*,5*R*)-3-Acetoxy-4-fluoro-5-(5-fluoro-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)-3,4-dimethyltetrahydrofuran-2-yl)methyl benzoate (18b)

^{1}H NMR: ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.56 (bs, 1H), 8.06–8.04 (m, 2H), 7.63–7.58 (m, 1H), 7.50–7.45 (m, 2H), 7.42–7.39 (dd, 1H, *J*1 = 6.3 Hz, *J*2 = 6.0 Hz), 6.39–6.32 (dd, 1H, *J*1 = 22.5 Hz, *J*2 = 22.5 Hz), 4.79–4.47 (m, 1H), 4.68–4.63 (m, 1H), 4.47–4.41 (m, 1H), 2.13 (s, 3H), 1.82 (d, 3H, *J* = 2.4 Hz), 1.50 (d, 3H, *J* = 23.4 Hz). ^{13}C NMR: 13.51, 14.56, 22.01, 63.01, 84.50, 87.43, 88.15, 125.14, 125.60 (2), 128.53 (2), 129.44, 129.69, 133.41, 138.61, 149.21, 156.48, 166.18, 168.95. MS (ESI) *m*/*z*: 461 [M+Na]. IR cm^{-1}: 3550, 3479, 3414, 3227, 2283, 1723, 1275, 1113, 780.

5.3.3. ((2*R*,3*S*,4*R*,5*R*)-3-Acetoxy-5-(4-benzamido-2-oxopyrimidin-1(2*H*)-yl)-4-fluoro-3,4-dimethyltetrahydrofuran-2-yl) methyl benzoate (18c)

^{1}H NMR (300 MHz, $CDCl_3$): δ 8.06 (d, 2H, *J* = 7.2 Hz), 7.90 (d, 2H, *J* = 3.78 Hz), 7.81–7.80 (m, 2H), 7.63–7.45 (m, 9H), 6.66 (d, 1H, *J* = 22.2 Hz), 4.81–4.49 (m, 3H), 2.31 (s, 3H), 2.12 (s, 3H), 1.83 (d, 3H, *J* = 2.4 Hz), 1.53 (d, 3H). ^{13}C NMR: 13.52, 14.88, 22.02, 63.12, 84.41, 88.54, 88.59, 96.58, 100.92, 103.44, 127.55 (2), 128.51 (2), 129.07 (3), 129.68 (2), 132.94 (2), 133.31, 145.94, 155.22, 162.48, 166.18, 169.08. MS (ESI) *m*/*z*: 524 $[M+1]^+$. IR cm^{-1}: 3409, 3070, 2924, 1744, 1718, 1665, 1617, 1485, 1393, 1234, 1061, 708.

5.4. Antiviral activity

5.4.1. Increase in neutral red (NR) dye uptake assay

Neutral red is added to the medium; cells not damaged by virus take up a greater amount of dye, which is read on a computerized micro plate auto reader. The method as described by McManus (*Appl. Environ. Microbiol.* **1976**, *31*, 35–38) is used. An EC_{50} is determined from this dye uptake.

5.4.2. Decrease in virus yield assay

Compounds considered active by NR dye uptake was re-tested using both CPE inhibition and, using the same plate, effect on reduction of virus yield by assaying frozen and thawed eluates from each cup for virus titer by serial dilution onto monolayers of susceptible cells. Development of CPE in these cells is the indication of presence of infectious virus. As in the initial tests, a known active drug is run in parallel as a positive control. The 90% effective concentration (EC_{90}), which is that test drug concentration that inhibits virus yield by 1 log10, is determined from these data.

5.5. Assay of cytotoxicity

5.5.1. Neutral red uptake

In the neutral red dye uptake phase of the antiviral test described above, the two toxicity control wells also receive neutral red and the degree of color intensity is determined spectrophotometrically. A neutral red IC_{50} (NR IC_{50}) is subsequently determined.

5.5.2. Visual observation

In the CPE inhibition tests, two wells of uninfected cells treated with each concentration of test compound will be run in parallel with the infected, treated wells. At the time CPE is determined microscopically, the toxicity control cells will also be examined microscopically for any changes in cell appearance compared to normal control cells run in the same plate. These changes may be enlargement, granularity, cells with ragged edges, a filmy appearance, rounding, detachment from the surface of the well, or other changes. A 50% cell inhibitory (cytotoxic) concentration (IC_{50}) is determined by regression analysis of these data.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2010.07.017. These data include MOL files and InChiKeys of the most important compounds described in this article.

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