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Synthesis and anti-HIV activity of C4-modified pyrimidine nucleosides

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Abstract

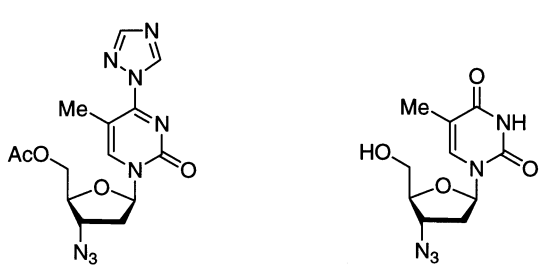
One-pot syntheses provided a series of triazole- and pentafluorophenyloxy-substituted pyrimidine nucleosides. Most of the compounds in the series displayed anti-HIV activities but none as potent as AZT **2**. 1-(β-D-*Erythro*-pentofuranosyl)-4-pentafluorophenyloxy-2(1*H*)-pyrimidinone **14** was the most potent and the most selective compound in the series with $EC_{50} = 1.6$ μM.

Keywords: Anti-HIV; Reverse transcriptase; Nucleoside; Pyrimidine; ATAZT; Triazole

1. Introduction

In this communication we report on the synthesis and biological evaluation of a series of C4-substituted pyrimidine nucleosides. Pyrimidine and purine nucleosides still comprise a significant proportion of marketed drugs against the human immunodeficiency virus (HIV). Emergence of drug-resistant virus [1] presents a serious drawback to the sustained clinical use of nucleoside [2] and non-nucleoside [3] inhibitors of the viral reverse transcriptase (RT) [4]. Inhibition of the viral protease (PR) [5] is achievable using peptidic [6] and cyclic non-peptide compounds [7] with every expectation that recent successes will be augmented by discoveries of new PR inhibitors. Clinical trials seek to demonstrate the value of combining nucleosides and PR inhibitors to maintain reduction of viral load and increase in CD4+ lymphocytes in HIV-infected patients [8]. Thus, there remains an important role for pyrimidine nucleosides and their analogues in HIV chemotherapy encouraging searches for potential, new antiviral nucleosides.

We previously reported on the structure of the pyrimidine nucleoside ATAZT [9] **1** (Fig. 1). This compound **1** represents an acylated, C4-triazole derivative of the anti-HIV drug zidovudine (AZT) **2**. Although the original synthesis of ATAZT **1** had been reported in the literature [10], the compound remained uncharacterised both structurally and biologically. Our preliminary evaluation of ATAZT **1** revealed promising anti-HIV activity, providing a lead compound for development through the synthesis of analogues. In attempts to improve antiviral activity, a series of derivatives **9–18** were prepared (Scheme 1) by varying the substituents at positions C4 and C5 (conventional numbering [11]) of the pyrimidine ring, and at positions O2′, O3′ and O5′ of the sugar ring (Table 1).



1 **2**

Fig. 1. Structures of ATAZT **1** and AZT **2**.

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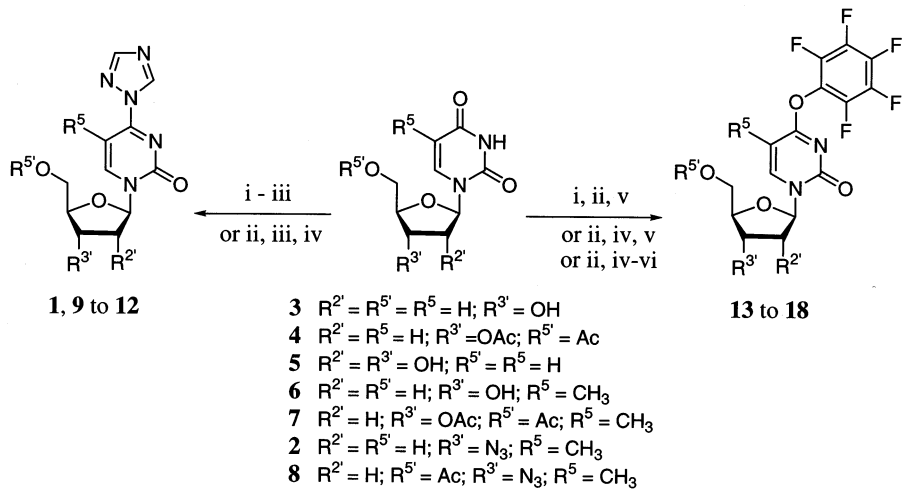


Scheme 1. Reagents and conditions: i, $(CF_3CO)_2O$; ii, pyridine; iii, 1,2,4-1*H*-triazole; iv, $ClC_6H_4P(O)Cl_2$; v, PfpOH; vi, $(CH_3)_3SiCl$.

2. Chemistry

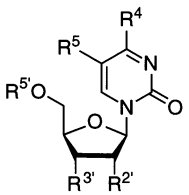
One-pot syntheses provided a series of C4-modified nucleosides from 2′-deoxyuridine **3**, uridine **5**, thymidine **6**, AZT **2** and *O*-acetates **4**, **7** and **8** (Scheme 1). Treatment of 2′-deoxyuridine **3** with trifluoroacetic anhydride [12–14] in pyridine facilitated transient protection at O5′ and O3′. Subsequent addition of 1,2,4-1*H*-triazole to the same reaction pot attached triazole to **3**, via a transient C4-pyridinium intermediate [12]. The C4-triazolyl nucleoside **9** was isolated in 79% yield after de-acylation of the sugar hydroxyl groups on flash chromatographic purification of the reaction product. Under the same reaction conditions but with pentafluorophenol (PfpOH) instead of triazole, **3** gave PfpO-substituted nucleoside **13** in comparable yield (78%). Although product yields for the ribose analogues were lower than those for the corresponding 2′-deoxyribose analogues, the presence of the additional hydroxyl group at the C2′ position did not seriously impede the progress of reactions. Thus, uridine **5** gave C4-substituted ribose nucleosides **10** (56%) and **14** (59%). X-ray crystal structure analysis of **13** and **14** provided the first example of isomorphous ribose and 2′-deoxyribose nucleosides with the same pyrimidine base [15].

Acetylated derivatives **11**, **12**, **15** and **16** were prepared in adequate yields from protected nucleosides **4** and **7**, using 4-chlorophenyl phosphodichloridate [16] and triazole or PfpOH in pyridine. Reaction of 5′-*O*-acetyl-AZT [10] **8** gave ATAZT **1** (88% yield). Similarly, reaction of **8** with PfpOH in place of triazole gave **17** (62% yield). Attempted reaction of AZT **2** with trifluoroacetic anhydride in the presence of PfpOH and pyridine failed to yield **18**. However, transient protection of **2** as its 5′-*O*-trimethylsilyl ether, and activation at C4 using 4-chlorophenyl phosphodichloridate in the presence of pyridine and PfpOH, was successful. Compound **18** was isolated in 62% yield although attempts to prepare its C4-triazolyl analogue failed using either the transient trifluroacetate or trimethylsilyl protection methods. Successful preparation of C5-methylated analogues of **9** and **13** from **6** was not achievable using the methods detailed here. The lability of the triazole and PfpO substitutents in compounds **12** and **16** precluded selective de-acylation, frustrating attempts to prepare C5-methylated analogues of **9** and **13** from these protected precursors.

3. Biological results and discussion

With the exception of triazole derivative **9** all of the compounds evaluated displayed some activity against HIV-1 (Table 1). None of the compounds was as potent as AZT **2** against HIV-1 in C8166 cells [17] and none without cytotoxicity. Replacement of the triazole ring of **9** by PfpO imparts weak antiviral activity to **13**. However, compound **10**, the ribose analogue of **9**, did display moderate antiviral activity and replacement of its triazole group by PfpO in **14** provided the most selective compound of the series ($EC_{50} = 1.6$ μM), equipotent with ATAZT **1**. Replacement of the triazole ring in ATAZT **1** by PfpO resulted in a marginal loss of antiviral activity with **17** having $EC_{50} = 2.0$ μM but with no improvement in cytotoxicity. Removal of the acetyl group from **17** resulted in a substantial loss of antiviral potency. Although their selectivity was still poor, protected triazole- and PfpO-substituted nucleosides **11** and **15** did show improved antiviral activities compared to their unprotected analogues **9** and **13**, as did derivatives **12** and **16** compared to their demethylated analogues.

Table 1
Antiviral activity of C4-modified pyrimidine nucleosides against HIV-1 in C1866 cells



	R^4	R^5	$R^{5'}$	$R^{3'}$	$R^{2'}$	EC_{50} (μM)[a]	TC_{50} (μM)[b]
9	1,2,4-Triazole	H	H	OH	H	>1000	>1000
10	1,2,4-Triazole	H	H	OH	OH	100	400
11	1,2,4-Triazole	H	Ac	OAc	H	400	>1000
12	1,2,4-Triazole	Me	Ac	OAc	H	40	80
1	1,2,4-Triazole	Me	Ac	N_3	H	1.6	80
13	C_6F_5O	H	H	OH	H	400	>1000
14	C_6F_5O	H	H	OH	OH	1.6	200
15	C_6F_5O	H	Ac	OAc	H	100	100
16	C_6F_5O	Me	Ac	OAc	H	>80	80
17	C_6F_5O	Me	Ac	N_3	H	2	80
18	C_6F_5O	Me	H	N_3	H	400	400
AZT						0.016	>1000

[a] EC_{50} represents the concentration of compound which reduced HIV-1 gp120 formation by 50% in infected cell cultures.
[b] TC_{50} represents the concentration of compound which reduced cell growth by 50%.

4. Conclusions

The series of C4-modified pyrimidine nucleosides described here possessed only moderate antiviral activity compared to AZT **2** with no substantial improvement in potency compared with the lead compound ATAZT **1**. Replacement of triazole at C4 by PfpO neither compromised nor provided substantial improvements in antiviral potency, although acylation at C5′ provided a marginal improvement in antiviral activity. The cytotoxocity and the lack of pronounced antiviral potency in the compounds evaluated did not warrant detailed investigations of their precise biological modes of action at this stage.

5. Experimental

5.1. Chemistry

NMR spectra were recorded on a Bruker AC-250 spectrometer at ^1H (250.1 MHz), ^{13}C (62.9 MHz) and ^{19}F (235.3 MHz) with positive chemical shifts downfield of TMS (^1H and ^{13}C) and negative chemical shifts upfield of CF_3CCl_3 (^{19}F). Proton and carbon assignments are reported using conventional numbering [11]. The ^{19}F NMR spectra of compounds **13**–**18** were nearly identical, therefore only data for a representative compound (**13**) are given. Mass spectra were recorded using VG Quatro II or AutoSpec instruments. IR spectra were recorded on a Mattson Galaxy 2020 FT-IR instrument and UV spectra were recorded on Varian-Cary 1E UV–Vis or Unicam PU8730 spectrophotometers. The UV spectra of compounds **1**, and **9**–**12** were nearly identical as were the UV spectra for compounds **13**–**18**, therefore only data for two representative compounds (**9** and **13**) are given. Melting points were determined with a Gallenkamp electrothermal digital apparatus and are uncorrected. Flash column chromatography [18] was performed using Sorbsil C60 silica gel and TLC was performed using plastic-backed Kieselgel 60 silica gel plates containing a fluorescent indicator and visualised under UV (254 nm) and with acidic anisaldehyde solution. Elemental analyses were performed by Butterworth Laboratories (Middlesex) and the results from C, H and N analyses were within ±0.4% of calculated values.

5.1.1. 1-(2-Deoxy-β-D-erythro-pentofuranosyl)-4-(1,2,4-triazol-1-yl)-2(1H)-pyrimidinone (9)

To 2′-deoxyuridine **3** (1.00 g, 4.40 mmol) in dry pyridine (40 ml) under Ar at 0°C, trifluoroacetic anhydride (1.86 ml, 13.16 mmol) was added and the solution stirred at room temperature (r.t.) for 24 h after which time 1,2,4-1*H*-triazole (3.03 g, 43.86 mmol) in pyridine (5 ml) was added and the mixture stirred at r.t. for a further 72 h. The product solution was then concentrated under vacuum. Flash column chromatography,

eluting with CH_2Cl_2–CH_3OH 4:1, gave **9** (904 mg, 79%) as a colourless solid; m.p. > 300°C; TLC (CH_2Cl_2–CH_3OH 4:1): R_f 0.76.

UV (95% EtOH): λ_{max} 249 nm (8200), 315 nm (4900).

IR (KBr disc): ν_{max} 3303, 3145, 3106, 2951, 2900, 1660, 1550, 1515, 1425, 1282, 1184 cm^{-1}.

^1H NMR [$(CD_3)_2SO$]: δ 2.10 (m, 1H, 2′-CH), 2.38 (m, 1H, 2′-CH), 3.62 (m, 2H, 5′-CH_2), 3.91 (m, 1H, 4′-CH), 4.23 (m, 1H, 3′-CH), 5.13 (t, 1H, *J* 5.1 Hz, 5′-OH), 5.31 (d, 1H, *J* 4.3 Hz, 3′-OH), 6.10 (t, 1H, *J* 6.0 Hz, 1′-CH), 6.96 (d, 1H, *J* 7.2 Hz, 5-CH), 8.38 (s, 1H, 3-CH), 8.69 (d, 1H, *J* 7.2 Hz, 6-CH), 9.41 ppm (s, 1H, 5-CH (triazole)).

^{13}C NMR [$(CD_3)_2SO$]: δ 41.2 (2′-CH_2), 60.9 (5′-CH_2), 69.8 (3′-CH), 87.4 (1′-CH), 88.5 (4′-CH), 93.8 (5-CH), 143.8 (5-CH (triazole)), 148.3 (6-CH), 153.8 (4-C), 154.3 (3-CH), 158.7 ppm (2-CO).

MS (FAB^+): *m*/*z* (rel. int.) 302 (*M* + Na, 28%), 280 (*M* + H, 66%), 260 (24%), 164 (67%), 150 (32%), 121 (100%), 108 (79%), 105 (65%).

HRMS Calc. for $C_{11}H_{14}N_5O_4$ (*M* + H): 280.105. Found: 280.105.

The following compounds were prepared in a similar manner:

5.1.2. 1-(2-Deoxy-β-D-erythro-pentofuranosyl)-4-pentafluorophenyloxy-2(1H)-pyrimidinone ***(13)***

From 2′-deoxyuridine **3** (1.00 g, 4.40 mmol), anhydrous pyridine (40 ml), trifluoroacetic anhydride (2.5 ml, 17.5 mmol) and PfpOH (8.10 g, 43.90 mmol) in pyridine (10 ml). Flash column chromatography, eluting with EtOAc–CH_3OH 9:1, gave **13** (1.35 g, 78%) as a colourless solid; m.p. 176°C; TLC (EtOAc–CH_3OH 9:1): R_f 0.50.

UV (95% EtOH): λ_{max} 286 nm (6730).

IR (KBr) ν_{max} 3479, 3403, 3083, 2940, 1654, 1556, 1450, 1295, 1114 cm^{-1}.

^1H NMR [$(CD_3)_2SO$]: δ 2.07 (m, 1H, 2′-CH), 2.29 (m, 1H, 2′-CH), 3.62 (s, 2H, 5′-CH_2), 3.88 (d, 1H, *J* 3.4 Hz, 4′-CH) 4.23 (s, 1H, 3′-CH), 5.14 (s, 1H, 5′-OH), 5.28 (s, 1H, 3′-OH), 6.06 (t, 1H, *J* 6.1 Hz, 1′-CH), 6.59 (d, 1H, *J* 7.3 Hz, 5-CH), 8.59 ppm (d, 1H, *J* 7.3 Hz, 6-CH).

^{13}C NMR [$(CD_3)_2SO$]: δ 1.2 (2′-CH_2), 61.0 (5′-CH_2), 70.0 (3′-CH), 87.2 (1′-CH), 88.5 (4′-CH), 93.0 (5-CH), 148.0 (6-CH), 154.0 (4-C), 169.3 ppm (2-CO).

^{19}F NMR [$(CD_3)_2SO$]: δ −14.7 (t, 2F, *J* 20.2 Hz, *meta* CF), −20.0 (t, 1F, *J* 21.2 Hz, *para* CF), −24.1 ppm (d, 2F, *J* 24.2 Hz, *ortho* CF).

MS (FAB^+): *m*/*z* (rel. int.) 417 (*M* + Na, 15%), 395 (*M* + H, 22%), 305 (14%), 279 (100%).

HRMS Calc. for $C_{15}H_{12}F_5N_2O_5$ (*M* + H): 395.068. Found: 395.067.

5.1.3. 1-(β-D-Erythro-pentofuranosyl-4-(1,2,4-triazol-1-yl)-2(1H)-pyrimidinone ***(10)***

From uridine **5** (1.01 g, 4.45 mmol), anhydrous pyridine (50 ml), trifluoroacetic anhydride (3.1 ml, 22.3 mmol) and 1,2,4-1*H*-triazole (3.10 g, 43.90 mmol) in pyridine (10 ml). Flash column chromatography, eluting with CH_2Cl_2–CH_3OH 4:1 followed by recrystallization from DMF, gave **10** (684 mg, 56%) as colourless crystals; m.p. 216°C; TLC (CH_2Cl_2–CH_3OH 4:1): R_f 0.45.

IR (KBr disc): ν_{max} 3357, 3300, 3118, 3095, 2933, 1697, 1630, 1562, 1523, 1417, 1285, 1107, 1068 cm^{-1}.

^1H NMR [$(CD_3)_2SO$]: δ 4.32 (m, 1H, 3′-CH), 4.34 (m, 1H, 4′-CH), 4.53 (m, 3H, 2′-CH, 5′-CH_2), 5.41 (d, 1H, *J* 6.0 Hz, 2′-OH), 5.72 (t, 1H, *J* 5.0 Hz, 5′-OH), 6.10 (d, 1H, *J* 4.5 Hz, 3′-OH), 6.31 (d, 1H, *J* 1.4 Hz, 1′-CH), 7.43 (d, 1H, *J* 7.3 Hz, 5-CH), 8.81 (s, 1H, 3-CH), 9.42 (d, 1H, *J* 7.3 Hz, 6-CH), 9.85 ppm (s, 1H, 5-CH (triazole)).

^{13}C NMR [$(CD_3)_2SO$]: δ 58.6 (5′-CH_2), 74.0 (2′-CH), 70.0 (3′-CH), 87.2 (1′-CH), 88.5 (4′-CH), 93.9 (5-CH), 142.9 (5-CH (triazole)), 147.5 (6-CH), 153.1 (4-C), 153.3 (3-CH), 157.8 ppm (2-CO).

MS (FAB^+): *m*/*z* (rel. int.) 318 (*M* + Na, 22%), 296 (*M* + H, 37%), 242 (20%), 165 (86%), 150 (49%), 115 (81%), 105 (100%).

HRMS Calc. for $C_{11}H_{14}N_5O_5$ (*M* + H): 296.099. Found: 296.097.

5.1.4. 1-(β-D-Erythro-pentofuranosyl)-4-pentafluorophenyloxy-2(1H)-pyrimidinone ***(14)***

From uridine **5** (2.0 g, 8.2 mmol), dry pyridine (30 ml), trifluoroacetic anhydride (5.8 ml, 41.0 mmol) and PfpOH (8.1 g, 43.9 mmol) in pyridine (20 ml). Flash column chromatography, eluting with EtOAc–CH_3OH (4:1), followed by recrystallization from CH_3OH gave **14** (1.98 g, 59%) as a colourless solid; m.p. 238°C; TLC (EtOAc–CH_3OH 4:1): R_f 0.53.

IR (KBr disc): ν_{max} 3465, 3413, 3123, 2987, 2932, 1745, 1677, 1661, 1627, 1550, 1515, 1291, 1230, 1199, 1179 cm^{-1}.

^1H NMR [$(CD_3)_2SO$]: δ 3.68 (m, 2H, 5′-CH_2), 3.96 (m, 3H, 2′-CH, 3′-CH, 4′-CH), 5.18 (d, 1H, *J* 6.0 Hz, 2′-OH), 5.28 (t, 1H, *J* 5.0 Hz, 5′-OH), 5.57 (d, 1H, *J* 4.5 Hz, 3′-OH), 5.72 (d, 1H, *J* 1.6 Hz, 1′-CH), 6.60 (d, 1H, *J* 7.4 Hz, 5-CH), 8.70 ppm (d, 1H, *J* 7.4 Hz, 6-CH).

^{13}C NMR [$(CD_3)_2SO$]: δ 59.6 (5′-CH_2), 74.5 (2′-CH), 68.4 (3′-CH), 84.3 (1′-CH), 90.6 (4′-CH), 92.7 (5-CH), 147.9 (6-CH), 153.7 (4-C), 168.5 ppm (2-CO).

MS (FAB^+): *m*/*z* (rel. int.) 411 (*M* + H, 70%), 308 (17%), 297 (100%), 156 (15%), 121 (19%), 108 (10%).

HRMS Calc. for $C_{15}H_{12}F_5N_2O_6$ (*M* + H): 411.062. Found: 411.063.

5.1.5. 1-(3,5-Di-O-acetyl-2-deoxy-β-D-erythro-pentofuranosyl)-4-(1,2,4-triazol-1-yl)-2(1H)-pyrimidinone ***(11)*** *[11]*

To a solution of 3′,5′-di-*O*-acetyl-2′-deoxyuridine **4** (3.08 g, 9.9 mmol) in anhydrous pyridine (100 ml) under Ar at 0°C, 4-chlorophenyl phosphodichloridate (3.20 ml, 19.7 mmol) was added with stirring. After 5

min 1,2,4-1*H*-triazole (2.70 g, 39.4 mmol) was added and the mixture allowed to warm to r.t. After a further 72 h the product solution was concentrated under vacuum and the residue dissolved in CH_2Cl_2 (600 ml), extracted with water (3 × 300 ml), dried ($MgSO_4$) and the solvent evaporated. Flash column chromatography eluting with EtOAc, followed by recrystallization from EtOAc, gave **11** (1.89 g, 53%) as colourless crystals; m.p. 151°C; TLC (EtOAc): R_f 0.17.

IR (KBr disc): ν_{max} 3121, 1751, 1675, 1670, 1552, 1515, 1239, 1059 cm^{-1}.

^1H NMR [$(CD_3)_2SO$]: δ 2.02 (s, 3H, CH_3), 2.07 (s, 3H, CH_3), 2.41 (m, 1H, 2′-CH), 2.61 (m, 1H, 2′-CH), 4.27 (d, 2H, *J* 4.4 Hz, 5′-CH_2), 4.38 (m, 1H, 4′-CH), 5.21 (m, 1H, 3′-CH), 6.14 (t, 1H, *J* 6.7 Hz, 1′-CH), 7.04 (d, 1H, *J* 7.3 Hz, 5-CH), 8.41 (s, 1H, 3-CH), 8.45 (d, 1H, *J* 7.3 Hz, 6-CH), 9.44 ppm (s, 1H, 5-CH (triazole)).

^{13}C NMR [$(CD_3)_2SO$]: δ 22.1 (CH_3), 22.3 (CH_3), 38.9 (2′-CH_2), 65.1 (5′-CH_2), 75.6 (3′-CH), 84.0 (1′-CH), 89.2 (4′-CH), 95.6 (5-CH), 145.2 (5-CH (triazole)), 149.5 (6-CH), 155.0 (4-C), 155.6 (3-CH), 160.3 (2-CO), 171.5 (CO), 171.6 ppm (CO).

MS (FAB^+): *m*/*z* (rel. int.) 386 (*M* + Na, 10%), 364 (*M* + H, 63%), 201 (32%), 164 (100%), 81 (96%), 69 (90%).

The following compounds were prepared in a similar manner:

5.1.6. 1-(3,5-Di-O-acetyl-2-deoxy-β-D-erythro-pentofuranosyl)-4-pentafluorophenyloxy-2(1H)-pyrimidinone ***(15)***

From 2′-deoxy-3′,5′-di-*O*-acetyl-2′-deoxyuridine **4** (1.00 g, 3.18 mmol), anhydrous pyridine (40 ml), 4-chlorophenyl phosphodichloridate (0.99 ml, 6.37 mmol) and PfpOH (2.34 g, 12.73 mmol) in pyridine (10 ml). Flash column chromatography, eluting with EtOAc–hexane 4:1, gave **15** (0.93 g, 61%) as a colourless semi-solid; TLC (EtOAc–hexane, 4:1): R_f 0.51.

IR (KBr disc): ν_{max} 3124, 2987, 2933, 1745, 1661, 1550, 1452, 1297, 1199, 1112, 1072 cm^{-1}.

^1H NMR [$(CD_3)_2SO$]: δ 2.06 (s, 6H, CH_3), 2.41 (m, 2H, 2′-CH_2), 4.25 (m, 2H, 5′-CH_2), 4.31 (m, 1H, 4′-CH), 5.17 (m, 1H, 3′-CH), 6.07 (t, 1H, *J* 6.5 Hz, 1′-CH), 6.62 (d, 1H, *J* 7.3 Hz, 5-CH), 8.32 ppm (d, 1H, *J* 7.3 Hz, 6-CH).

^{13}C NMR [$(CD_3)_2SO$]: δ 20.6 (CH_3), 20.8 (CH_3), 37.3 (2′-CH_2), 63.6 (5′-CH_2), 74.1 (3′-CH), 82.4 (1′-CH), 87.4 (4′-CH), 93.2 (5-CH), 147.7 (6-CH), 153.5 (4-C), 168.7 (2-CO) 170.1 (CO), 170.2 ppm (CO).

MS (FAB^+): *m*/*z* (rel. int.) 501 (*M* + Na, 66%), 479 (*M* + H, 54%), 279 (100%), 201 (75%).

HRMS Calc. for $C_{19}H_{16}F_5N_2O_7$ (*M* + H): 479.088. Found: 479.088.

5.1.7. 1-(3,5-Di-O-acetyl-2-deoxy-β-D-erythro-pentofuranosyl)-5-methyl-4-(1,2,4-triazol-1-yl)-2(1H)-pyrimidinone ***(12)***

From 3′,5′-di-*O*-acetylthymidine **7** (1.00 g, 3.09 mmol), anhydrous pyridine (50 ml), 4-chlorophenyl phosphodichloridate (1.00 ml, 6.17 mmol), and 1,2,4-1*H*-triazole (0.85 g, 12.30 mmol). Flash column chromatography, eluting with EtOAc, gave **12** (550 mg, 49%) as a colourless semi-solid; TLC (EtOAc): R_f 0.36.

IR (KBr disc): ν_{max} 3465, 3104, 2933, 2902, 1745, 1679, 1525, 1238, 1068 cm^{-1}.

^1H NMR [$(CD_3)_2SO$]: δ 2.03 (s, 3H, CH_3), 2.08 (s, 3H, CH_3), 2.32 (s, 3H, 5-CH_3), 2.49 (m, 2H, 2′-CH_2), 4.35 (m, 3H, 4′-CH, 5′-CH_2), 5.22 (m, 1H, 3′-CH), 6.15 (t, 1H, *J* 6.5 Hz, 1′-CH), 8.25 (s, 1H, 6-CH), 8.39 (s, 1H, 3-CH), 9.33 ppm (s, 1H, 5-CH).

^{13}C NMR [$(CD_3)_2SO$]: δ 16.5 (5-CH_3), 21.0 (CH_3), 21.2 (CH_3), 37.8 (2′-CH_2), 63.9 (5′-CH_2), 74.4 (3′-CH), 82.5 (1′-CH), 87.8 (4′-CH), 105.3 (5-C), 145.8 (5-CH), 148.0 (6-CH), 153.4 (4-C), 153.9 (3-CH), 158.5 (2-CO), 170.5 (CO), 170.6 ppm (CO).

MS (FAB^+): *m*/*z* (rel. int.) 400 (*M* + Na, 48%), 378 (*M* + H, 89%), 201 (27%), 178 (100%).

HRMS Calc. for $C_{16}H_{20}N_5O_6$ (*M* + H): 378.141; Found: 378.142.

5.1.8. 1-(3,5-Di-O-acetyl-2-deoxy-β-D-erythro-pentofuranosyl)-5-methyl-4-pentafluorophenyloxy-2(1H)-pyrimidinone ***(16)***

From 3′,5′-di-*O*-acetylthymidine **7** (1.00 g, 3.09 mmol), anhydrous pyridine (40 ml), 4-chlorophenyl phosphodichloridate (1.00 ml, 6.17 mmol) and PfpOH (2.27 g, 12.30 mmol) in pyridine (10 ml). Flash column chromatography, eluting with EtOAc–hexane 1:1, gave **16** (0.99 g, 67%) as a semi-solid; TLC (EtOAc–hexane 1:1): R_f 0.55.

IR (KBr disc): ν_{max} 3123, 2987, 2932, 1739, 1666, 1550, 1515, 1230, 1223, 1029 cm^{-1}.

^1H NMR ($CDCl_3$): δ 2.16 (s, 6H, CH_3), 2.20 (s, 3H, 5-CH_3), 2.75 (dd, 1H, *J* 5.6, 2.2 Hz, 2′-CH), 2.81 (dd, 1H, *J* 6.6, 2.2 Hz, 2′-CH), 4.36 (m, 1H, 4′-CH), 4.41 (m, 2H, 5′-CH_2), 5.22 (m, 1H, 3′-CH), 6.25 (t, 1H, *J* 5.6 Hz, 1′-CH) 7.86 ppm (s, 1H, 6-CH).

^{13}C NMR [$(CD_3)_2SO$]: δ 11.8 (5-CH_3), 20.9 (CH_3), 21.3 (CH_3), 37.5 (2′-CH_2), 63.9 (5′-CH_2), 74.4 (3′-CH), 82.6 (1′-CH), 87.2 (4′-CH), 102.3 (5-C), 145.2 (6-CH), 153.7 (4-C), 168.0 (2-CO), 170.4 (CO), 170.6 ppm (CO).

MS (FAB^+): *m*/*z* (rel. int.) 515 (*M* + Na, 50%), 493 (*M* + H, 37%), 293 (100%), 201 (62%).

HRMS Calc. for $C_{20}H_{18}F_5N_2O_7$ (*M* + H): 493.103. Found: 493.105.

*5.1.9. 1-(5-O-Acetyl-3-azido-2,3-dideoxy-β-D-erythro-pentofuranosyl)-5-methyl-4-(1,2,4-triazol-1-yl)-2 (1H)-pyrimidinone (**1**)*

From 5′-*O*-acetyl-AZT [10] **8** (3.08 g, 9.90 mmol), dry pyridine (50 ml), 4-chlorophenyl phosphodichloridate (1.85 ml, 11.34 mmol) and 1,2,4-1*H*-triazole (2.70 g, 39.40 mmol). Flash column chromatography, eluting with EtOAc, followed by recrystallization from EtOAc, gave **1** (1.79 g, 88%) as colourless crystals; m.p. 137°C; TLC (EtOAc): R_f 0.24.

IR (KBr disc): ν_{max} 3126, 2970, 2112, 1730, 1662, 1625, 1525, 1425, 1237, 977 cm^{-1}.

^1H NMR [$(CD_3)_2SO$]: δ 2.09 (s, 3H, CH_3), 2.35 (s, 3H, 5-CH_3), 2.59 (m, 2H, 2′-CH_2), 4.19 (m, 1H, 3-CH), 4.39 (m, 3H, 4′-CH, 5′-CH_2), 6.12 (t, 1H, *J* 5.6 Hz, 1′-CH), 8.23 (s, 1H, 6-CH), 8.40 (d, 1H, 3-CH), 9.35 ppm (s, 1H, 5-CH).

^{13}C NMR [$(CD_3)_2SO$]: δ 17.5 (CH_3), 21.0 (5-CH_3), 35.6 (2′-CH_2), 60.0 (3′-CH), 63.5 (5′-CH_2), 82.4 (1′-CH), 87.3 (4′-CH), 105.1 (5-C), 145.7 (5-CH), 148.0 (6-CH), 153.5 (4-C), 153.9 (3-CH), 158.4 (2-CO), 170.6 ppm (CO).

MS (FAB^+): *m*/*z* (rel. int.) 383 ($M+Na$, 1%), 361 ($M+H$, 37%), 178 (100%), 135 (5%).

HRMS Calc. for $C_{14}H_{17}N_8O_4$ ($M+H$): 361.137. Found: 361.138.

*5.1.10. 1-(5-O-Acetyl-3-azido-2,3-dideoxy-β-D-erythro-pentofuranosyl)-5-methyl-4-pentafluorophenyloxy-2(1H)-pyrimidinone (**17**)*

From 5′-*O*-acetyl-AZT [10] **8** (1.00 g, 3.24 mmol) dry pyridine (30 ml), 4-chlorophenyl phosphodichloridate (1.05 ml, 6.47 mmol) and PfpOH (2.38 g, 12.9 mmol) in pyridine (10 ml). Flash column chromatography, eluting with EtOAc–hexane 1:1, gave **17** as a colourless semi-solid (0.95 g, 62%); TLC (EtOAc–hexane 1:1): R_f 0.51.

IR (KBr disc): ν_{max} 3095, 2940, 2100, 1680, 1550, 1515, 1398, 1330, 1232, 1111, 992, 727 cm^{-1}.

^1H NMR ($CDCl_3$): δ 2.17 (s, 3H, CH_3), 2.25 (s, 3H, 5-CH_3), 2.40 (m, 1H, 2′-CH), 2.73 (m, 1H, 2′-CH), 4.16 (m, 2H, 5-CH_2), 4.42 (m, 2H, 3′-CH, 4′-CH), 6.09 (dd, 1H, *J* 4.8, 1.7 Hz, 1′-CH), 7.86 ppm (s, 1H, 6-CH).

^{13}C NMR [$(CD_3)_2SO$]: δ 11.9 (5-CH_3), 21.0 (CH_3), 37.3 (2′-CH_2), 50.0 (3′-CH), 63.4 (5′-CH2), 82.1 (1′-CH), 86.7 (4′-CH), 102.1 (5-C), 145.4 (6-CH), 153.7 (4-C), 167.9 (2-CO), 170.5 ppm (CO).

MS (FAB^+): *m*/*z* (rel. int.) 498 ($M+Na$, 56%), 293 (100%), 184 (31%).

HRMS Calc. for $C_{18}H_{15}F_5N_5O_5$ ($M+H$): 476.099. Found: 476.100.

*5.1.11. 1-(3-Azido-2,3-dideoxy-β-D-erythro-pentofuranosyl)-5-methyl-4-pentafluorophenyloxy-2(1H)-pyrimidinone (**18**)*

To AZT **2** (500 mg, 1.87 mmol) dissolved in pyridine (10 ml), trimethylsilyl chloride (0.48 ml, 3.74 mmol) was added and the mixture stirred for 30 min before cooling to 0°C. 4-Chlorophenyl phosphodichloridate (0.76 ml, 4.68 mmol) was then added and after stirring for 15 min, PfpOH (1.73 g, 9.36 mmol) in pyridine (10 ml) was added and the mixture left to stir at r.t. After 24 h, water (50 ml) was added and the mixture stirred for 1 h at r.t. before concentration under vacuum. Flash column chromatography, eluting with EtOAc–hexane 7:1, followed by recrystallization from EtOAc–CH_3OH, gave **18** (502 mg, 62%) as colourless crystals; m.p. 181°C; TLC (EtOAc–hexane 7:1): R_f 0.61.

IR (KBr disc): ν_{max} 3449, 2931, 2120, 1664, 1521, 1411, 1330, 1112, 988, 778 cm^{-1}.

^1H NMR [$(CD_3)_2SO$]: δ 2.13 (s, 3H, 5-CH_3), 2.42 (t, 2H, *J* 6.4 Hz, 2′-CH_2), 3.70 (m, 2H, 5′-CH_2), 3.91 (m, 1H, 4′-CH), 4.40 (q, 1H, *J* 6.7 Hz, 3′-CH), 5.40 (s, 1H, 5′-OH), 6.61 (t, 1H, *J* 4.3 Hz, 1′-CH), 8.42 ppm (s, 1H, 6-CH).

^{13}C NMR [$(CD_3)_2SO$]: δ 12.3 (5-CH_3), 38.8 (2′-CH_2), 59.2 (3′-CH), 60.4 (5′-CH_2), 85.3 (1′-CH), 86.4 (4′-CH), 101.6 (5-C), 143.3 (6-CH), 153.7 (4-C), 167.9 ppm (2-CO).

MS (FAB^+): *m*/*z* (rel. int.) 434 ($M+H$, 27%), 319 (10%), 293 (100%), 165 (42%), 128 (37%), 115 (34%), 105 (58%).

HRMS Calc. for $C_{16}H_{13}F_5N_5O_4$ ($M+H$): 434.081. Found: 434.083.

5.2. Antiviral and cytotoxicity assays

The antiviral activities and cytotoxicities of all of the compounds tested were determined using C8166 cells infected with HIV-1IIIB as described previously [17]. The inhibitory activity against HIV was determined by examining syncytia by XTT-Formazan assay for cell viability [19] and by measuring antigen gp120 as reported previously [20]. The EC_{50} value is the concentration of compound which reduces the production of viral antigen by 50% and the TC_{50} value is the concentration of compound which reduces the viability of uninfected cells by 50%. Values are the mean of two different experiments performed in triplicate (Table 1).

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