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Synthesis and anti-HIV-1 activity of 4-substituted-7-(2′-deoxy-2′-fluoro-4′-azido-β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine analogues

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ABSTRACT

Three novel 4-subsituted-7-(2′-deoxy-2′-fluoro-4′-azido-β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine analogues were designed, synthesized, and tested for their anti-HIV-1 activity. Initial biological studies indicated that among these pyrrolo[2,3-*d*]pyrimidine ribonucleoside analogues, 4-amino-7-(2′-deoxy-2′-fluoro-4′-azido-β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine **10** exhibited the most potent anti-HIV-1 activity (EC_{50} = 0.5±0.3 μM), while 4-hydroxy-7-(2′-deoxy-2′-fluoro-4′-azido-β-D-ribofuranosyl)pyrrolo[2,3-*d*] pyrimidine **9** and 4-amino-5-fluoro-7-(2′-deoxy-2′-fluoro-4′-azido-β-D-ribofuranosyl)pyrrolo[2,3-*d*] pyrimidine **11** showed moderate activity (EC_{50} = 13 ± 8 and 5.4 ± 0.3 μM, respectively). The cytotoxicity of these compounds has also been assessed. No significant cytotoxicities were found for any of these compounds with concentrations up to 25 μM.

Since 7-deazapurines are ideal shape mimics of the parent purines, the corresponding ribonucleosides can replace naturally occurring RNA-constituents as substrates or inhibitors. Thus, it can be expected that further modifications on the pyrrolo[2,3-*d*]pyrimidine moiety may generate new pharmacologically active compounds against human immunodeficiency virus.[1–3] On the other hand, several purine nucleosides containing the 2′-deoxy-2′-fluoro-β-D-arabinofuranosyl moiety have been synthesized as potential antiviral and anti-leukemic agents.[4–6] Specially, a 7-deazapurine analogue, 2,4-diamino-7-(2′-deoxy-2′-fluoro-β-D-arabinofuranosyl)pyrrolo[2,3-*d*] pyrimidine, has been reported to exhibit good in vitro anti-HBV activity.[7]

As a continuous research program of our laboratory searching for antiviral agents,[8] we now report the design and synthesis of novel 4-subsituted-7-(2′-deoxy-2′-fluoro-4′-azido-β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine analogues **9**, **10**, and **11** (Scheme 1), which can specifically inhibit HIV-1 replication.

As shown in Scheme 1, treatment of the known nucleoside analogue **1a**[9] with saturated methanolic ammonia gave **2a** in 88% isolated yield. Selective substitution of the primary hydroxy group with iodide,[10] followed by treatment with DBU furnished alkene **4a** in 37% yield over the two steps. Next, the key reaction, addition of iodine azide to the double bond of **4a** was attempted.[11] To our delight, when alkene **4a** was subjected to a premixed solution of iodine chloride and sodium azide in THF, the desired product **5a** was isolated in 89% yield. Subsequent protection of the 3′-hydroxy group and displacement of the iodide with *m*-chlorobenzoyloxy group provided **7a**. Treatment of **7a** with sodium methoxide resulted in the formation of **8** in 60% isolated yield, demethylation[1] of which then gave the 4-hydroxy pyrrolo[2,3-*d*]pyrimidine derivative **9**[12] in good yield. On the other hand, treatment of **7a** with saturated mathanolic ammonia at 70 °C would give 4-amino pyrrolo[2,3-*d*]pyrimidine derivative **10**[13] in moderate yield. Starting with **1b**[14] and following the same sequence as described for the synthesis of **10**, 4-amino-5-fluoropyrrolo[2,3-*d*]pyrimidine derivative **11**[15] could also be synthesized. It needed to be indicated that, during the deprotection of **1b**, apart from the desired product **2b**, substantial amount of the corresponding 4-methoxy derivative resulting from displacement of the chloride by methanol was also formed, which explained the relatively lower yield of the step compared to the synthesis of **2a**.

The anti-HIV-1 activity of compounds **9**, **10** and **11** were evaluated by MTT method[16] and the results were listed in Table 1. All three compounds tested could inhibit HIV-1 (wild type) replication. However, none was as effective as AZT. Among them, compound **10** exhibited the most potent anti-HIV-1 activity (EC_{50} = 0.5 ± 0.3 μM), while compounds **9** and **11** only showed moderate activity (EC_{50} = 13 ± 8 and 5.4 ± 0.3 μM, respectively). The cytotoxicity of these compounds had also been assessed. No

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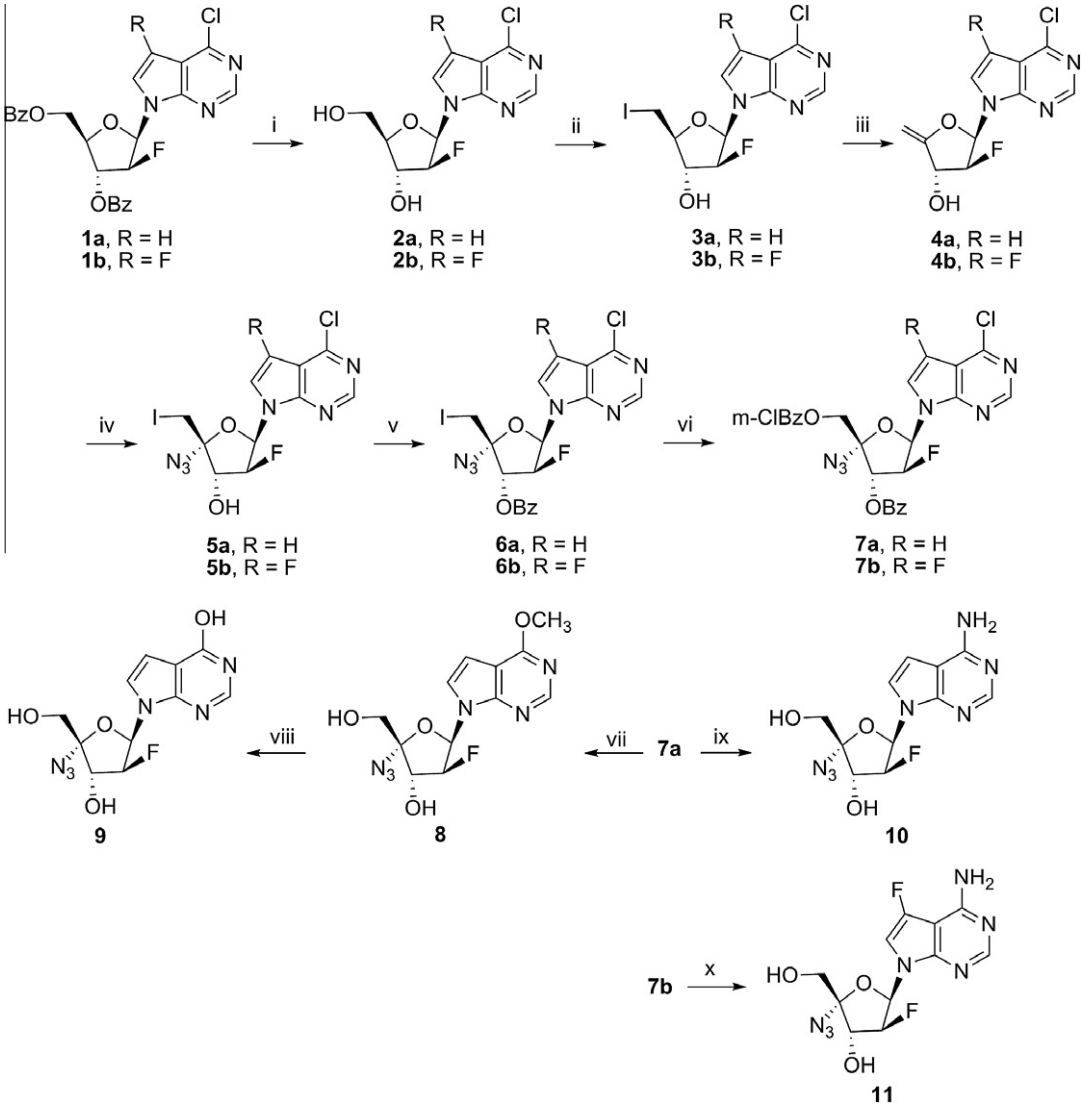

Scheme 1. Reagents and conditions: (i) $NH_3{\cdot}CH_3OH$, 0 °C to rt, 88% for **2a**, 46% for **2b**; (ii) I_2/PPh_3/imidazole, THF, rt, 59% for **3a**, 87% for **3b**; (iii) DBU, THF, 60 °C, 62% for **4a**, 45% for **4b**; (iv) ICl, NaN_3, THF, rt, 4 h, 89% for **5a**, 72% for **5b**; (v) BzCl, Et_3N, DMAP, CH_2Cl_2, 0 °C, 5 min, 83% for **6a**, 81% for **6b**; (vi) m-CPBA, m-CBA, K_2HPO_4, *n*-Bu_4NHSO_4, CH_2Cl_2/H_2O, rt, 36 h, 64% for **7a**, 13% for **7b**; (vii) CH_3ONa/CH_3OH, rt, 5 h, 60%; (viii) Me_3SiCl, NaI, CH_3CN, rt, 5 h, 60%; (ix) $NH_3{\cdot}CH_3OH$, 70 °C, 24 h, 33%; (x) $NH_3{\cdot}CH_3OH$, rt, 24 h, 40%.

Table 1
Anti-HIV-1 activity and cellular toxicity of compounds **9**, **10** and **11**.

Compounds	EC_{50}[a] (μM)	CC_{50}[b] (μM)
9	13 ± 8	⩾25
10	0.5 ± 0.3	⩾25
11	5.4 ± 0.3	⩾25
AZT	0.084 ± 0.0026	⩾25

[a] EC_{50} (μM) is the concentration that inhibits HIV-1 (wild type) by 50%.
[b] CC_{50} (μM) is the concentration of the compound at which 50% of the cells were destroyed.

significant cytotoxicities were found for any of these compounds with concentrations up to 25 μM.

In summary, three novel 4-subsituted-7-(2′-deoxy-2′-fluoro-4′-azido-β-D-ribofuranosyl)pyrrolo[2,3-*d*]pyrimidine analogues were designed, synthesized and their anti-HIV-1 activity evaluated in vitro. Initial biological studies indicated that compound **10** showed promising activity against HIV-1 replication. Further investigations of the biological activities of **10** are currently underway.

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12. ^{1}H NMR (300 MHz, CD_3OD) 7.95 (1H, s, H-2), 7.31 (1H, m, H-6), 6.82 (1H, dd, J = 9.6, 5.7 Hz, H-1′) 6.68 (1H, d, J = 3.6 Hz, H-5), 5.30 (1H, dt, J = 54, 5.7 Hz, H-2′), 4.74 (1H, dd, J = 20.7, 5.7 Hz, H-3′) and 3.89 (2H, s, H-5′); ^{13}C NMR (75 MHz, D_2O) 161.4, 149.9, 144.7, 124.3, 109.8, 103.9, 97.9 (J_{FC} = 8.9 Hz), 96.2 (J_{FC} = 193.9 Hz), 82.7 (J_{FC} = 17.2 Hz), 76.0 (J_{FC} = 23.7 Hz) and 63.7; m/z (ESI) 333 (M^++Na, 100%) and 311 (15) [found: M^++Na, 333.0738. $C_{11}H_{11}FN_6NaO_4$ requires 333.0724].
13. ^{1}H NMR (300 MHz, CD_3OD) 8.11 (1H, s, H-2), 7.31 (1H, m, H-6), 6.82 (1H, dd, J = 9.9, 5.7 Hz, H-1′), 6.64 (1H, d, J = 3.9 Hz, H-5), 5.30 (1H, dt, J = 54, 5.7 Hz, H-2′), 4.75 (1H, dd, J = 20.7, 5.7 Hz, H-3′) and 3.89 (2H, s, H-5′); ^{13}C NMR (75 MHz, CD_3OD) 159.1, 152.6, 151.4, 124.6, 104.5, 101.4, 97.8 (J_{FC} = 9.0 Hz), 96.3 (J_{FC} = 193.5 Hz), 82.5 (J_{FC} = 17.3 Hz), 76.1 (J_{FC} = 23.8 Hz) and 63.7; m/z (ESI) 310 (M^++H, 100%) [found: M^++H, 310.1084. $C_{11}H_{13}FN_7O_3$ requires 310.1064].
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15. ^{1}H NMR (300 MHz, CD_3OD) 8.11 (1H, s, H-2), 7.17 (1H, m, H-6), 6.86 (1H, m, H-1′), 5.27 (1H, dt, J = 54, 5.7 Hz, H-2′), 4.70 (1H, dd, J = 21.0, 5.7 Hz, H-3′) and 3.87 (2H, s, H-5′); ^{13}C NMR (75 MHz, CD_3OD) 157.6, 154.0, 147.7, 145.4 (J_{FC} = 244.8 Hz), 106.9 (J_{FC} = 28.0 Hz), 97.8 (J_{FC} = 9.2 Hz), 96.2 (J_{FC} = 193.6 Hz), 82.0 (J_{FC} = 17.3 Hz), 75.9 (J_{FC} = 23.7 Hz) and 63.6; m/z (ESI) 328 (M^++H, 100%) [found: M^++H, 328.0973. $C_{11}H_{12}F_2N_7O_3$ requires 328.0970].
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