Full Paper

Synthesis and Anti-HIV-1 Activity of New Fluoro-HEPT Analogues: An Investigation on Fluoro *versus* Hydroxy Substituents*

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Coupling of 6-benzyl-5-hydroxymethyluracil (**1**) with formaldehyde acetals followed by fluorination using (diethylamino)sulfur trifluoride (DAST) afforded 1-alkenyloxymethyl and 1-propargyloxymethyl 5-fluoromethyl-6-benzyluracils **3a–c**. 6-(3,5-Dimethylbenzyl)-5-ethyl-1-[(2-fluoroethoxy)methyl]pyrimidine-2,4(1*H*,3*H*)-dione (**6**) was synthesized by fluorination of the corresponding hydroxy derivative **5**. Sonogoshira reaction was performed on 6-(3,5-dimethylbenzyl)-5-ethyl-1-(4-iodobenzyl)uracil (**7**) with propargyl alcohol to afford **8** which was fluorinated to give the fluoro propargyl derivative **9**. Compound **7** was synthesized by N1-alkylation of the corresponding uracil. Significant activity was found against HIV-1 except for compounds with 5-hydroxymethyl and 5-fluoromethyl substituents.

Keywords: Anti-HIV-1 activity / DAST / MKC-442 analogues / Non-nucleoside reverse transcriptase inhibitors / Sonogashira cross-coupling

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Introduction

The enzyme HIV reverse transcriptase (RT) is responsible for the conversion of single stranded RNA viral genome into a double-stranded DNA copy. RT inhibitors are classified as nucleoside and non-nucleoside. Nucleoside reverse transcriptase inhibitors (NRTIs) were the first anti-HIV drugs discovered by Mitsuya *et al*. [1, 2]. They block HIV RT by chain termination. Non-nucleoside reverse transcriptase inhibitors (NNRTIs) represent another important group of HIV-1 RT inhibitors with proven efficacy in the clinic. Only compounds that bind to a specific pocket situated ~10 Å from the enzyme's polymerase active site are considered NNRTIs. 1-[(2-Hydroxyethoxy)methyl]-6-(phenylthio)thymine (HEPT) derivatives (Fig. 1) are the first non-nucleoside reverse transcriptase inhibitor analogs shown to have potent anti-HIV activity [3, 4]. Since the discovery of HEPT and MKC-442 (a HEPT analogue, Fig. 1) [5–7], different types of HEPT and MKC-442 analogues, have been synthesized in order to improve their activity against HIV-1 wild type and its resistant mutants [8–14]. In this paper, some fluoro HEPT analogues were synthesized by the reaction of DAST with the appropriate hydroxy compounds. All of the synthesized compounds were tested for their activity against HIV-1 wild type and mutant strains resistant against NNRTIs. We considered it interesting to compare equally bulky fluoro and hydroxy derivatives because of their different hydrophilic properties.

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Results and discussion

Chemistry

5-Hydroxmethyl-6-benzyluracil (**1**) [15] was silylated with *N*,*O*-bis(trimethylsilyl)acetamide (BSA) and treated with formaldehyde acetals [9] in dry acetonitrile in the presence of trimethylsilyl trifluoromethanesulfonate (TMS-triflate) as a

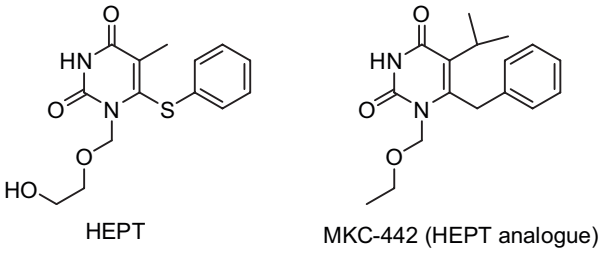
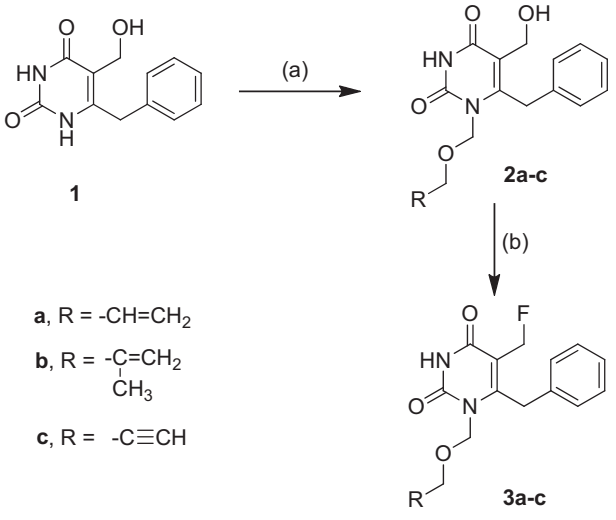


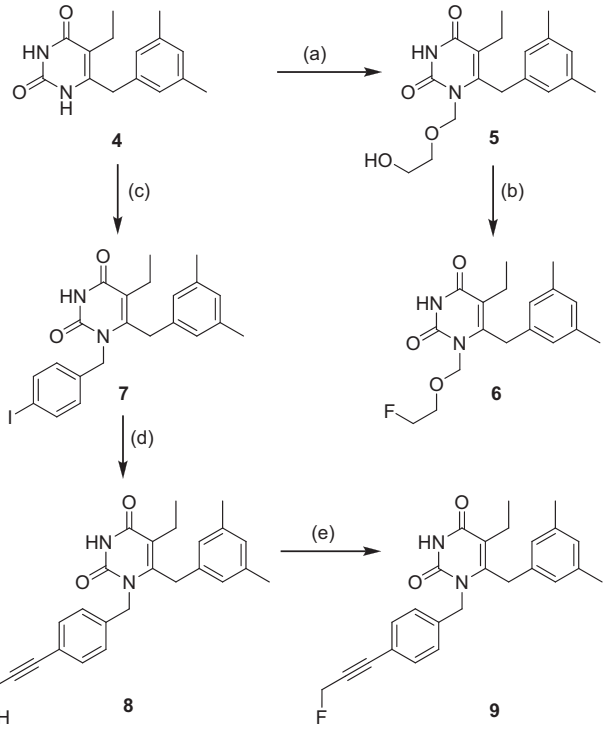
Figure 1. The structures of HEPT and MKC-442.

Lewis acid catalyst [16] at −50°C to afford 6-benzyl-5-hydroxymethyl-1-alkenyloxymethyl (**2a**,**b**) and propargyloxymethyluracil (**2c**). Fluorination of compounds **2a–c** was carried out with diethylaminosulfur trifluoride (DAST) in methylene chloride to furnish the fluoro derivatives **3a–c** (Scheme 1). ^{1}H-NMR of compounds **3a–c** showed the disappearance of the signal corresponding to the -OH group and the $-CH_2F$ appeared as doublets at 5.34 ppm with coupling constants of $J_{H,F} = 48.9$ Hz.

6-(3,5-Dimethylbenzyl)-5-ethyl-1-[(2-hydroxyethoxy)methyl]-pyrimidine-2,4(1*H*,3*H*)-dione (**5**) was prepared in 92% yield by silylation of 6-(3,5-dimethylbenzyl)-5-ethyluracil (**4**) using BSA followed by coupling with 1,3-dioxolane in the presence of TMS-triflate in dry acetonitrile. Compound **5** was fluorinated with DAST in dry methylene chloride to furnish 6-(3,5-dimethylbenzyl)-5-ethyl-1-[(2-fluoroethoxy)methyl]pyrimidine-2,4-(1*H*,3*H*)-dione (**6**) in 70% yield. The silylated compound of **4** was refluxed with 4-iodobenzyl bromide in acetonitrile for 90 h to give 6-(3,5-dimethylbenzyl)-5-ethyl-1-(4-iodobenzyl)-pyrimidine-2,4(1*H*,3*H*)-dione (**7**) in 45% yield. The coupling at N1 of uracil ring to afford compound **7** was confirmed by



Scheme 1. Reagents and conditions: (a) i) BSA, CH_3CN, ii) TMS-triflate, –50°C, $(RCH_2O)_2CH_2$; (b) DAST, 0°C, CH_2Cl_2.



Scheme 2. Reagents and conditions: (a) i) BSA, CH_3CN, ii) TMS-triflate, –50°C, 1,3-dioxane; (b) DAST, 0°C, CH_2Cl_2; (c) i) BSA, CH_3CN, RT, ii) *p*-iodobenzyl bromide, reflux; (d) propargyl alcohol, Et_3N, CuI, $PdCl_2(PPh_3)_2$; (e) DAST, 0°C, CH_2Cl_2.

nuclear Overhauser effects (NOE) which is based on through space transfer of spin polarization from one spin population to another *via* cross-relaxation in NMR. On irradiation of CH_2-C6 at 3.75 ppm, 3.43% NOE was detected with CH_2N at 4.85 ppm which also showed 2.57% NOE with CH_2-C6 by irradiation. Sonogashira cross-coupling [17–21] was applied on compound **7** and propargyl alcohol using copper(I) iodide and $PdCl_2(PPh_3)_3$ as catalysts to afford compound **8** in 73% yield. Fluorination of the hydroxyl compound **8** by DAST in dry methylene chloride furnishes the fluoromethyl derivative **9** in 75% yield (Scheme 2). According to ^{1}H-NMR the $-CH_2F$ appeared as a doublet at 5.18 ppm with a coupling constant of $J_{H,F} = 47.4$ Hz.

Antiviral activity

The HIV-1 strain HTLV-IIIB in MT-4 cells was used in our assay to investigate the anti-HIV-1 activity of MKC-442 analogues synthesized in the present study. The results are summarized in Table 1.

Neither HEPT analogues with 5-hydroxymethyl groups (**2a–c**) nor 5-fluoromethyl derivatives (**3a–c**) showed any significant activities against HIV-1. Compounds **5–9** showed higher

Table 1. Cytotoxicity and anti-HIV-1 activity of compounds **2a–c**, **3a-c**, **5–9**, and reference compounds MKC-442 and efavirenz (EFV).

Compound	CC_{50} (μM)[b]	SI[c]	EC_{50} (μM)[a]			
			Wild type	EFV^R	Y181C	K103N + Y181C
2a	>100	>16	6 ± 1	>100	>100	>100
2b	>100	5	>20	>20	>20	>20
2c	>100	>11	9 ± 0.9	>100	>100	>100
3a	>100	>16	6 ± 1	>100	>100	>100
3b	>100	5	≥20	>20	>20	>20
3c	>100	>10	10 ± 2	>100	>100	>100
5	>100	>5000	0.02 ±0.001	22 ± 2	3 ± 1	30 ± 8
6	>100	>16666	0.006 ± 0.0005	20 ± 5	0.5 ± 0.2	4 ± 2
7	43 ± 3	717	0.06 ± 0.01	>43	3 ± 1	>43
8	2 ± 0.1	500	0.004 ± 0.0005	>2	0.1 ± 0.04	>2
9	2 ± 0.1	100	0.02 ± 0.005	>2	0.6 ± 0.05	>2
MKC-442	>100	>3333	0.03	100	20	>100
EFV	30	15000	0.002	3	0.008	0.3

[a] Compound dose required to reduce the viability of mock-infected cells by 50%, as determined by the MTT method
[b] Compound dose required to achieve 50% protection of MT-4 cells from HIV-1-induced cytopathogenicity, as determined by the MTT method. The symbol (>) indicates that the CC_{50} was not reached at the highest concentration tested
[c] Selectivity index for wild type HIV-1: Ratio CC_{50}/EC_{50}. EC_{50} and CC_{50} are expressed as the mean values of at least two separate experiments.

activity against HIV-1 mutants than MKC-442, especially against the Y181C mutant. With a terminal fluorine in the ethoxymethyl substituent, compound **6** showed better activity than the corresponding hydroxy compound **5** against HIV-1 wild type and its mutants and it was also the compound with the highest selectivity index (SI > 16666) in the present investigation. Surprisingly the compounds **7–9,** being 1-benzyl derivatives, showed high activity against wild type HIV-1 although they do not have the ether linkage like the lead structure of HEPT (Fig. 1). However, when the hydroxyl group in compound **8** was substituted with fluorine (compound **9**), the efficacy against wild type HIV-1 was decreased by 5 fold and against the Y181 mutant by 6 fold. Unfortunately, the compounds **7–9** showed a higher cytotoxic effect which reduced the selectivity index to the range of 100–717. The influence of hydrophilic properties on the efficacy against HIV-1 of fluoro and hydroxy derivatives follows the same trend as previously predicted by molecular modeling [22].

Experimental

Chemistry

NMR spectra were recorded on a Varian Gemini 2000 spectrometer at 300 MHz for ^{1}H and 75 MHz for ^{13}C with TMS as an internal standard. EI mass spectra were recorded on a Finnigan MAT SSQ 710. MALDI spectra were recorded on a 4.7 T Ultima Fourier transform Mass spectrometer (IonSpec, Irvine, CA). Melting points were determined in a Büchi melting point apparatus. The silica gel (0.040–0.063 mm) used for column chromatography was purchased from Merck. Microanalyses (C, H, N) were carried out at Chemical Laboratory II at University of Copenhagen, Denmark, their results were found to be in good agreement (±0.4%) with the calculated values.

*General procedure for preparation of 1-substituted 6-benzyl-5-hydroxymethyluracil **2a–c***

N,*O*-Bis-(trimethylsilyl)acetamide (BSA, 1.6 mL, 6.6 mmol) was added to a stirred solution of compound **1** (464 mg, 2 mmol) [15] in dry methylene chloride (100 mL) under nitrogen. After 15 min the reaction was cooled to −50°C. TMS-triflate (1.08 mL, 6 mmol) was added to the reaction mixture followed by the addition of the appropriate acetal (4 mmol). The mixture was left overnight with stirring at room temperature. The reaction was quenched by addition of 1 mL saturated aqueous solution of sodium carbonate and water (10 mL). The two layers were separated and the methylene chloride phase was dried over anhydrous magnesium sulfate and evaporated under reduced pressure. The product was chromatographed on a silica gel column using petroleum ether (60–80°C)/EtOAc (v/v, 1:1) as eluent to afford compounds **2a–c**.

*1-[(Allyloxy)methyl]-6-benzyl-5-(hydroxymethyl) pyrimidine-2,4(1H,3H)-dione **2a***

White solid; yield: 49%; m.p.: 96–98°C; ^{1}H-NMR ($CDCl_3$) δ [ppm]: 3.50 (bs, 1H, OH), 4.10 (dt, 2H, J = 5.6, 1.5 Hz, O*C*H_2CH=), 4.28 (s, 2H, CH_2Ph), 4.54 (s, 2H, CH_2OH), 5.18–5.22 (m, 3H, OCH_2N and *HC*H=CH), 5.29 (dq, 1H, J = 17.2, 1.5 Hz, HC*H*=CH), 5.78–5.91 (m, 1H, CH_2=C*H*), 7.14–7.36 (m, 5H, H_{arom}), 10.15 (s, 1H, NH); ^{13}C-NMR ($CDCl_3$) δ [ppm]: 33.52 (CH_2Ph), 56.59 (CH_2OH), 70.58 (O*C*H_2-C=), 72.54 (OCH_2N), 114.25 (C5), 117.93 (*C*H_2=CH), 127.46, 127.55, 129.28, 133.375 (C_{arom}), 134.72 (CH_2=CH), 151.79 (C6), 152.57 (C2), 164.25 (C4); EI-MS: m/z = 302 [M^+] (14%), 41 (100%); HRMS-MALDI: m/z = 325.1164 ($C_{16}H_{18}NaN_2O_4$ [M + Na^+]), requires 325.1159.

6-Benzyl-5-(hydroxymethyl)-1-{[(2-methylprop-2-enyl)oxy]methyl}pyrimidine-2,4(1H,3H)-dione ***2b***

White solid; yield: 51%; m.p.: 110–112°C; ^{1}H-NMR ($CDCl_3$) δ [ppm]: 1.71 (s, 3H, CH_3), 3.52 (bs, 1H, OH), 4.01 (s, 2H, OCH_2-C=), 4.29 (s, 2H, CH_2Ph), 4.53 (s, 2H, CH_2OH), 4.88, 4.96 (2s, 2H, CH_2=), 5.18 (s, 2H, OCH_2N), 7.15–7.36 (m, 5H, H_{arom}), 10.21 (s, 1H, NH); ^{13}C-NMR ($CDCl_3$) δ [ppm]: 19.44 (CH_3), 33.48 (CH_2Ph), 56.55 (CH_2OH), 72.68 (O*C*H_2-C=), 73.54 (OCH_2N), 112.67 (*C*H_2=C), 114.23 (C5), 127.44, 127.55, 129.28, 134.74 (C_{arom}), 141.01 (CH_2=C), 151.77 (C6), 152.59 (C2), 164.28 (C4); EI-MS: m/z = [MH^+] (2%), 227 (100%). Anal. calcd. for $C_{17}H_{20}N_2O_4$ (316.14): C, 64.54; H, 6.37; N, 8.86. Found: C, 64.30; H, 6.43; N, 8.73.

6-Benzyl-5-(hydroxymethyl)-1-[(prop-2-ynyloxy)methyl]pyrimidine-2,4(1H,3H)-dione ***2c***

White solid; yield: 50%; m.p.: 130–132°C; ^{1}H-NMR ($CDCl_3$) δ [ppm]: 2.48 (t, 1H, J = 2.4 Hz, CHC), 3.50 (bs, 1H, OH), 4.26 (s, 2H, CH_2Ph), 4.27 (s, 2H, O*C*H_2-CCH), 4.54 (s, 2H, CH_2OH), 5.23 (s, 2H, OCH_2N), 7.15–7.34 (m, 5H, H_{arom}), 10.14 (s, 1H, NH); ^{13}*C*-NMR ($CDCl_3$) δ [ppm]: 33.50 (CH_2Ph), 56.50 (CH_2OH), 57.26 (OCH_2-C), 72.44 (*C*HC), 75.06 (OCH_2N), 78.86 (CH*C*), 114.39 (C5), 127.50, 127.57, 129.29, 134.61 (C_{arom}), 151.82 (C6), 152.47 (C2), 164.15 (C4); EI-MS: m/z = 300 [M^+] (24%), 39 (100%). Anal. calcd. for $C_{16}H_{16}N_2O_4$ (300.31): C, 63.99; H, 5.37; N, 9.33. Found: C, 63.88; H, 5.44; N, 9.11.

General procedure for the preparation of 1-substituted 6-benzyl-5-fluoromethyluracil ***3a–c***

To a stirred solution of compound **2a–c** (1.3 mmol) in dry methylene chloride (2 mL) was added diethylaminosulfur trifluoride (DAST, 0.26 mL in 0.2 mL methylene chloride, 2 mmol) dropwise at −5°C. The reaction mixture was allowed to reach room temperature gradually and stirred for 3 h and quenched with 5% aqueous sodium bicarbonate (0.2 mL) followed by addition of water (5 mL) and methylene chloride (5 mL). The two layers were separated and the organic layer was dried (sodium sulfate) and evaporated under reduced pressure. The residual oily product was purified by a silica gel column chromatography using ether as eluent to afford compounds **3a–c**.

1-[(Allyloxy)methyl]-6-benzyl-5-(fluoromethyl)pyrimidine-2,4(1H,3H)-dione ***3a***

White solid; yield: 79%; m.p.: 100–102°C; ^{1}H-NMR ($CDCl_3$) δ [ppm]: 4.12 (dt, 2H, J = 5.7, 1.5 Hz, O*C*H_2-CH=), 4.32 (s, 2H, CH_2Ph), 5.19–5.23 (m, 3H, OCH_2N and *HC*H=CH), 5.30 (dq, 2H, J = 17.2, 1.5 Hz, HC*H*=CH), 5.34 (d, 2H, J = 48.9 Hz, CH_2F), 5.80–5.93 (m, 1H, CH_2=C*H*), 7.15–7.39 (m, 5H, H_{arom}), 9.85 (s, 1H, NH); ^{13}C-NMR ($CDCl_3$) δ [ppm]: 33.87 (CH_2Ph), 70.76 (O*C*H_2-CH), 72.53 (OCH_2N), 75.31 (d, J = 165.3 Hz, CH_2-F), 110.20 (d, J = 17.3 Hz, C5), 118.08 (*C*H_2=CH), 127.47, 127.64, 129.37, 134.18 (C_{arom}), 133.27 (CH_2=CH), 151.56 (C6), 156.62 (C2), 162.57 (C4); EI-MS: m/z = 304 [M^+] (11%), 246 (100%). Anal. calcd. for $C_{16}H_{17}FN_2O_3 \cdot 0.25\ H_2O$ (308.83): C, 62.23; H, 5.55; N, 9.07. Found: C, 62.61; H, 5.57; N, 9.03.

6-Benzyl-5-(fluoromethyl)-1-{[(2-methylprop-2-enyl)oxy]methyl}pyrimidine-2,4(1H,3H)-dione ***3b***

White solid; yield: 50%; m.p.: 92–94°C; ^{1}H-NMR ($CDCl_3$) δ [ppm]: 1.72 (s, 3H, CH_3), 4.03 (s, 2H, OCH_2-C=), 4.32 (s, 2H, CH_2Ph), 4.90, 4.97 (2s, 2H, CH_2=C), 5.19 (s, 2H, OCH_2N), 5.34 (d, 2H, $J_{H,F}$ = 48.9 Hz, CH_2-F), 7.14–7.38 (m, 5H, H_{arom}), 9.63 (s, 1H, NH); ^{13}C-NMR ($CDCl_3$) δ [ppm]: 19.41 (CH_3), 33.84 (CH_2Ph), 72.69 (OCH_2N), 73.76 (OCH_2-C=), 75.31 (d, J = 165.0 Hz, CH_2-F), 110.16 (d, J = 17.5 Hz, C5), 112.82 (CH_2=C), 127.47, 127.65, 129.38, 134.17 (C_{arom}), 140.92 (CH_2=C), 151.45 (C6), 156.60 (C2), 162.46 (C4); EI-MS: m/z = 318 [M^+] (6%), 247 (100%). Anal. calcd. for $C_{17}H_{19}FN_2O_3 \cdot 0.25\ H_2O$ (322.85): C, 63.25; H, 5.85; N, 8.56. Found: C, 63.66; H, 5.89; N, 8.60.

6-Benzyl-5-(fluoromethyl)-1-[(prop-2-ynyloxy)methyl]pyrimidine-2,4(1H,3H)-dione ***3c***

White solid; yield: 76%; m.p.: 124–126°C; ^{1}H-NMR ($CDCl_3$) δ [ppm]: 2.48 (t, 1H, J = 2.4 Hz, CHC), 4.29 (d, 2H, J = 2.4 Hz, CHC-*C*H_2), 4.30 (s, 2H, CH_2Ph), 5.25 (s, 2H, OCH_2N), 5.34 (d, 2H, $J_{H,F}$ = 48.9 Hz, CH_2-F), 7.16–7.38 (m, 5H, H_{arom}), 9.85 (s, 1H, NH); ^{13}*C*-NMR ($CDCl_3$) δ [ppm]: 33.86 (CH_2Ph), 57.41 (CHC-*C*H_2), 72.50 (OCH_2N), 75.27 (d, J = 166.3 Hz, CH_2-F), 75.10 (*C*HC), 78.75 (CH*C*), 110.35 (d, J = 17.3 Hz, C5), 127.69, 127.49, 129.38, 134.07 (C_{arom}), 151.61 (C6), 156.45 (C2), 162.51 (C4); EI-MS: m/z = 302 [M^+] (22%), 39 (100%). Anal. calcd. for $C_{16}H_{15}FN_2O_3$ (302.3): C, 63.57; H, 5.00; N, 9.27. Found: C, 63.32; H, 5.11; N, 8.89.

6-(3,5-Dimethylbenzyl)-5-ethyl-1-[(2-hydroxyethoxy)methyl]pyrimidine-2,4(1H,3H)-dione ***5***

Compound **4** (0.51 g, 2 mmol) [23] was stirred in dry acetonitrile (20 mL) under nitrogen and BSA (1.4 mL, 6.6 mmol) was added. The mixture became clear after stirring at room temperature for 10 min. The reaction mixture was cooled to −50°C and TMS-triflate (1.1 mL, 6 mmol) was added followed by dropwise addition of the appropriate acetal (0.3 mL, 4 mmol). The mixture was stirred at room temperature for 3 h, quenched with ice-cold saturated solution of sodium bicarbonate (1 mL), and evaporated under reduced pressure. Water (50 mL) was added to the residual material and the solid product formed was filtered off, washed with water, and dried to afford 0.61 g (92%) compound **5**.

M.p.: 146–148°C; ^{1}H-NMR ($CDCl_3$) δ [ppm]: 1.07 (t, 3H, J = 7.2 Hz, CH_3CH$_2$), 2.28 (s, 6H, $(CH_3)_2$Ar), 2.47 (q, 2H, J = 7.2 Hz, CH$_3$CH_2), 3.68–376 (m, 4H, CH_2CH_2OH), 4.06 (s, 1H, CH_2-C6), 5.18 (s, 1H, NCH_2O), 6.70 (s, 2H, H_{arom}), 6.90 (s, 1H, H_{arom}); ^{13}C-NMR ($CDCl_3$) δ [ppm]: 13.68 (*C*H$_3$CH$_2$), 19.17 (CH$_3$*C*H$_2$), 21.24 ($(CH_3)_2$Ar), 33.33 (*C*H$_2$-C6), 61.16 (CH_2OH), 70.69 (*C*H$_2$CH$_2$OH), 73.13 (NCH_2O), 116.97 (C5), 124.96, 128.99, 134.79, 138.90 (C_{arom}), 149.11 (C6), 152.21 (C2), 163.40 (C4); EI-MS: m/z = 332 [M^+] (5%), 258 (100%). Anal. calcd. for $C_{18}H_{24}N_2O_4 \cdot 0.25\ H_2O$ (336.91): C, 64.17; H, 7.33; N, 8.31. Found: C, 64.10; H, 7.31; N, 8.17.

6-(3,5-Dimethylbenzyl)-5-ethyl-1-[(2-fluoroethoxy)methyl]pyrimidine-2,4(1H,3H)-dione ***6***

To a stirred solution of compound **5** (166 mg, 0.5 mmol) in dry methylene chloride (5 mL) was added DAST (0.1 mL in 1 mL methylene chloride, 0.75 mmol) dropwise at −5°C. The reaction mixture was allowed to reach room temperature and stirred for 3 h and quenched with 5% aqueous sodium bicarbonate (0.5 mL) followed by addition of water (15 mL). The mixture was extracted with methylene chloride (15 mL). The organic phase was dried (sodium sulfate) and the solvent was evaporated under reduced pressure. The residual material was purified by silica gel column chromatography using EtOAc/CH_2Cl_2 (v/v, 1:1) as eluent to afford 117 mg (70%) of compound **6**.

M.p.: 134–136°C; ^{1}H-NMR ($CDCl_3$) δ [ppm]: 1.08 (t, 3H, $J = 7.5$ Hz, CH_3CH_2), 2.29 (s, 6H, $(CH_3)_2Ar$), 2.48 (q, 2H, $J = 7.5$ Hz, CH_3CH_2), 3.86 (dt, 2H, $J = 29.7$, 4.1 Hz, OCH_2CH_2F), 4.08 (s, 2H, CH_2Ar), 4.52 (dt, 2H, $J = 47.7$, 4.1 Hz, CH_2CH_2F), 5.19 (s, 2H, NCH_2O), 6.71 (s, 2H, H_{arom}), 6.90 (s, 1H, H_{arom}), 9.64 (bs, 1H, NH); ^{13}C-NMR ($CDCl_3$) δ [ppm]: 13.71 (CH_3CH_2), 19.17 (CH_3 CH_2), 21.25 ($(CH_3)_2Ar$), 33.23 (CH_2Ar), 68.79 (d, $J = 19.5$ Hz, CH_2CH_2F), 73.12 (NCH_2O), 82.42 (d, $J = 169.9$ Hz, CH_2F), 116.99 (C5), 125.00, 129.00, 134.77, 138.91 (C_{arom}), 149.20 (C6), 152.14 (C2), 163.40 (C4); EI-MS: $m/z = 334$ [M^+] (30%), 255 (100%). Anal. calcd. for $C_{18}H_{23}FN_2O_3$ (334.39): C, 64.65; H, 6.93; N, 8.38. Found: C, 64.78; H, 6.93; N, 8.06.

*6-(3,5-Dimethylbenzyl)-5-ethyl-1-(4-iodobenzyl) pyrimidine-2,4(1H,3H)-dione **7***

Compound **4** (0.58 g, 2 mmol) in dry acetonitrile (20 mL) was silylated under nitrogen by dropwise addition of BSA (1.6 mL, 6.6 mmol) and stirred for 15 min. 4-Iodobenzyl bromide (0.77 g, 2.6 mmol) was added in one portion to the mixture which was refluxed for 90 h. The mixture was cooled to room temperature and the solvent was removed under reduced pressure. The residual material was chromatographed on a silica gel column using $CHCl_3$/MeOH (v/v, 25:1) as eluent to afford 425 mg (45%) of compound **7**.

M.p.: 192–194°C; ^{1}H-NMR ($CDCl_3$) δ [ppm]: 1.05 (t, 3H, $J = 7.4$ Hz, CH_3CH_2), 2.30 (s, 6H, $(CH_3)_2Ar$), 2.46 (q, 2H, $J = 7.4$ Hz, CH_3CH_2), 3.75 (s, 2H, CH_2-C6), 4.85 (s, 2H, CH_2-N), 6.64 (s, 2H, H_{arom}), 6.86 (d, 2H, $J = 8.3$ Hz, H_{arom}), 6.92 (s, 1H, H_{arom}), 7.66 (d, 2H, $J = 8.3$ Hz, H_{arom}), 9.63 (bs, 1H, NH); ^{13}C-NMR ($CDCl_3$) δ [ppm]: 13.90 (CH_3CH_2), 19.35 (CH_3 CH_2), 21.30 ($(CH_3)_2Ar$), 34.18 (CH_2-C6), 46.60 (CH_2-N), 116.74 (C5), 93.01, 124.82, 127.87, 129.17, 134.37, 136.37, 138.03, 139.11 (C_{arom}), 149.38 (C6), 151.84 (C2), 163.27 (C4); EI-MS: $m/z = 474$ [M^+] (55%), 217 (100%). Anal. calcd. for $C_{22}H_{23}IN_2O_2 \cdot 0.5\ H_2O$ (483.35): C, 54.67; H, 5.00; N, 5.80. Found: C, 54.36; H, 4.60; N, 5.53.

*6-(3,5-Dimethylbenzyl)-5-ethyl-1-[4-(3-hydroxyprop-1-ynyl)benzyl]pyrimidine-2,4(1H,3H)-dione **8***

Using a balloon with a long needle immersed in the solution, a stream of argon was flushed through a solution of compound **7** (354 mg, 0.7 mmol) and propargyl alcohol (0.42 mL, 7 mmol) in dry triethylamine (15 mL). The reaction mixture was transferred by a syringe with a long needle to another flask containing copper(I) iodide (8 mg, 6 mol%) and $PdCl_2[P(Ph)_3]_2$ (15 mg, 3 mol%) under argon at room temperature. The mixture was stirred for 3 h, and then coevaporated with chloroform (3×30 mL) to remove triethylamine. Water (20 mL) was added to the residue and the mixture was extracted with chloroform (30 mL). The chloroform phase was dried (sodium sulfate) and evaporated under reduced pressure. The residual material was purified by a silica gel column using $CHCl_3$/MeOH (v/v, 25:1) as eluent to afford 205 mg (73%) of compound **8**.

M.p.: 93–95°C; ^{1}H-NMR ($CDCl_3$) δ [ppm]: 1.05 (t, 3H, $J = 7.4$ Hz, CH_3CH_2), 2.26 (s, 6H, $(CH_3)_2Ar$), 2.46 (q, 2H, $J = 7.4$ Hz, CH_3CH_2), 3.74 (s, 2H, CH_2Ar), 4.50 (s, 2H, CH_2OH), 4.90 (s, 2H, CH_2N), 6.65 (s, 2H, H_{arom}), 6.92 (s, 1H, H_{arom}), 7.04 (d, 2H, $J = 8.3$ Hz, H_{arom}), 7.40 (d, 2H, $J = 8.3$ Hz, H_{arom}), 9.53 (bs, 1H, NH); ^{13}C-NMR ($CDCl_3$) δ [ppm]: 13.89 (CH_3CH_2), 19.36 (CH_3 CH_2), 34.16 (CH_2Ar), 46.82 (CH_2OH), 51.52 (CH_2N), 84.98 (CC-CH_2), 87.82 (CC-CH_2), 116.68 (C5), 122.04, 124.83, 125.83, 129.17, 132.28, 134.40, 136.97, 139.10 (C_{arom}), 149.54 (C6), 151.82 (C2), 163.28 (C4); HRMS-MALDI: $m/z = 425.1822$ ($C_{25}H_{26}N_2NaO_3$ ($M + Na^+$]), requires 425.1836.

*6-(3,5-Dimethylbenzyl)-5-ethyl-1-[4-(3-fluoroprop-1-ynyl) benzyl]pyrimidine-2,4(1H,3H)-dione **9***

To a stirred solution of compound **8** (160 mg, 0.37 mmol) in dry methylene chloride (5 mL) was added DAST (0.075 mL in 1 mL methylene chloride, 0.56 mmol) dropwise at −5°C. The mixture was allowed to reach room temperature gradually and stirred for 3 h. The reaction mixture was quenched with 5% aqueous sodium bicarbonate (0.5 mL) followed by addition of water (15 mL) and extracted with methylene chloride (15 mL). The methylene chloride phase was dried (sodium sulfate) and evaporated under reduced pressure. The residual was purified by a silica gel column chromatography using $CHCl_3$/MeOH (v/v, 25:1) as eluent to afford 112 mg (75%) of compound **9**.

M.p.: 83–85°C; ^{1}H-NMR ($CDCl_3$) δ [ppm]: 1.05 (t, 3H, $J = 7.4$ Hz, CH_3CH_2), 2.30 (s, 6H, $(CH_3)_2Ar$), 2.47 (q, 2H, $J = 7.4$ Hz, CH_3CH_2), 3.74 (s, 2H, CH_2-C6), 4.91 (s, 2H, CH_2-N), 5.18 (d, 2H, $J_{HF} = 47.4$ Hz, CH_2F), 6.65 (s, 2H, H_{arom}), 6.92 (s, 1H, H_{arom}), 7.08 (d, 2H, $J = 8.1$ Hz, H_{arom}), 7.45 (d, 2H, $J = 8.1$ Hz, H_{arom}), 9.57 (bs, 1H, NH); ^{13}C-NMR ($CDCl_3$) δ [ppm]: 13.91 (CH_3CH_2), 19.37 (CH_3 CH_2), 21.31 ($(CH_3)_2Ar$), 34.20 (CH_2-C6), 46.83 (CH_2-N), 71.05 (d, $J = 165.4$ Hz, CH_2F), 83.05 (d, $J = 21.5$ Hz, CC–CH_2F), 88.82 (d, $J = 12.1$ Hz, CC–CH_2F), 116.74 (C5), 121.20, 124.84, 125.91, 129.19, 132.47, 134.39, 137.71, 139.12 (C_{arom}), 149.44 (C6), 151.84 (C2), 163.27 (C4); HRMS-MALDI: $m/z = 405.1968$ ($C_{25}H_{26}FN_2O_2$ [$M + H^+$]), requires 405.1973.

Antiviral assay procedures

Compounds were solubilized in DMSO at 200 mM and then diluted in culture medium.

Cells and viruses

MT-4, C8166, and H9/IIIB cells were grown at 37°C in a 5% CO_2 atmosphere in RPMI 1640 medium supplemented with 10% fetal calf serum (FCS), 100 IU/mL penicillin G, and 100 μg/mL streptomycin. Cell cultures were checked periodically for the absence of mycoplasma contamination with a MycoTect Kit (Gibco). Human immunodeficiency viruses type 1 (HIV-1, IIIB strain) was obtained from supernatants of persistently infected H9/IIIB cells. The HIV-1 stock solutions had titers of 4.5×10^6 50% cell culture infectious dose ($CCID_{50}$)/mL. The K103R + V179D + P225H mutant (EFV^R) was derived from an IIIB strain passage in C8166 cells in the presence of efavirenz (up to 2 μM). The Y181C mutant (NIH N119) was derived from an AZT-sensitive clinical isolate passage initially in CEM and then in MT-4 cells in the presence of nevirapine (10 μM). The K103N + Y181C (NIH A17) was derived from the IIIB strain passaged in H9 cells in the presence of BI-RG 587 (1 μM). K103R + V179D + P225H (EFV^R), Y181C, and K103N + Y181C stock solutions had titers of 3.0×10^5, 1.3×10^6, and 2.5×10^5 $CCID_{50}$/mL, respectively.

HIV titration

Titration of HIV was performed in C8166 cells by the standard limiting dilution method (dilution 1:2, four replica wells per dilution) in 96-well plates. The infectious virus titer was determined by light microscope scoring of syncytia after 4 days of incubation. Virus titers were expressed as $CCID_{50}$/mL.

Anti-HIV assays

The activity of test compounds against multiplication of wild type HIV-1, Y181C, and K103N + Y181C in acutely infected cells was based on inhibition of virus-induced cytopathicity in MT-4 cells. The activity of the compounds against the EFV^R multiplication in acutely infected cells was based on inhibition of p24 antigen in C8166 cells. Briefly, an amount of 50 μL of culture medium containing 1×10^4 cells was added to each well of flat-bottom microtiter trays containing 50 μL of culture medium with or without various concentrations of test compounds. Then an amount of 20 μL of HIV suspensions (containing the appropriate amount of $CCID_{50}$ to cause complete cytopathicity at day 4) was added. After incubation at 37°C, cell viability was determined by the 3-(4,5-dimethylthiazol-1-yl)-2,5-diphenyltetrazolium bromide (MTT) method [24]. Alternatively, p24 levels were determined by an immunoenzymatic kit (Abbott). The cytotoxicity of test compounds was evaluated in parallel with their antiviral activity and was based on the viability of mock-infected cells, as monitored by the MTT method.

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