Full Paper

Synthesis and Anti-HIV-1 Activity of 1-Substiuted 6-(3-Cyanobenzoyl) and [(3-Cyanophenyl)fluoromethyl]-5-ethyl-uracils

Yasser M. Loksha[1,2], Erik B. Pedersen[1], Roberta Loddo[3], and Paolo La Colla[3]

[1] Nucleic Acid Centre*, Department of Physics and Chemistry, University of Southern Denmark, Odense M, Denmark
[2] Department of Pharmaceutical Chemistry, Faculty of Pharmacy and Pharmaceutical Industries, Sinai University, North Sinai, Egypt
[3] Dipartimento di Scienze e Tecnologie Biomediche, Sezione di Microbiologia e Virologia Generale e Biotecnologie Microbiche, Università di Cagliari, Monserrato, Italy

1-Substiuted 6-(3-cyanobenzoyl) and [(3-cyanophenyl)fluoromethyl]-5-ethyl-uracils were synthesized and evaluated in cell-based assays against HIV-1 wild-type and its clinically relevant non-nucleoside reverse transcriptase inhibitor (NNRTI)-resistant mutants. Some of the synthesized compounds showed activity against HIV-1 wild-type in the same range as Emivirine (MKC-442). 3-{[3-(Allyloxymethyl)-5-ethyl-2,6-dioxo-1,2,3,6-tetrahydropyrimidin-4-yl]fluoromethyl}-benzonitrile **11b** showed moderate activity against the Y181C HIV-1 mutant strain.

Keywords: 5-Cyanobenzoyluracils / Diethylaminosulfur trifluoride (DAST) / Human immune deficiency virus (HIV) / Non-nucleoside reverse transcriptase inhibitors (NNRTIs) / Trimethylsilyl iodide (TMSI)

Received: March 17, 2009; accepted: April 29, 2009

DOI 10.1002/ardp.200900058

Introduction

Although many drugs are currently available on the market [1 – 4], the search for new HIV inhibitors is still going on [5 – 8], mainly because of the induced resistance most of these drugs cause. The reverse transcriptase enzyme (RT) is responsible for making a copy of the viral RNA into a complementary DNA as soon as the virus enters the host cell, a process named reverse transcription. 6-Benzyl-1-(ethoxymethyl)-5-isopropyluracil (Emivirine, formerly MKC-442) [9 – 11] is one of the non-nucleoside reverse transcriptase inhibitors (NNRTIs) that are allosteric inhibitors of HIV-1 RT. It binds to a hydrophobic pocket close to but distinct from the active site. Binding of the inhibitor alters the conformation of the active site and inactivates the enzyme. Since the synthesis of Emivirine and the discovery of its activity against the wild-type of HIV-1, many research projects have been published reporting improvement of the activity of its analogues against HIV-1 wild-type and mutant strains [12 – 16]. Recent publications about NNRTI diaryl ethers described highly active compounds against mutant HIV-1 strains when the diaryl ethers comprised a *m*-cyano group [17 – 21]. Therefore, we now focus on 3-cyanobenzoyl and (3-

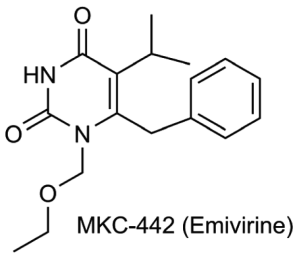
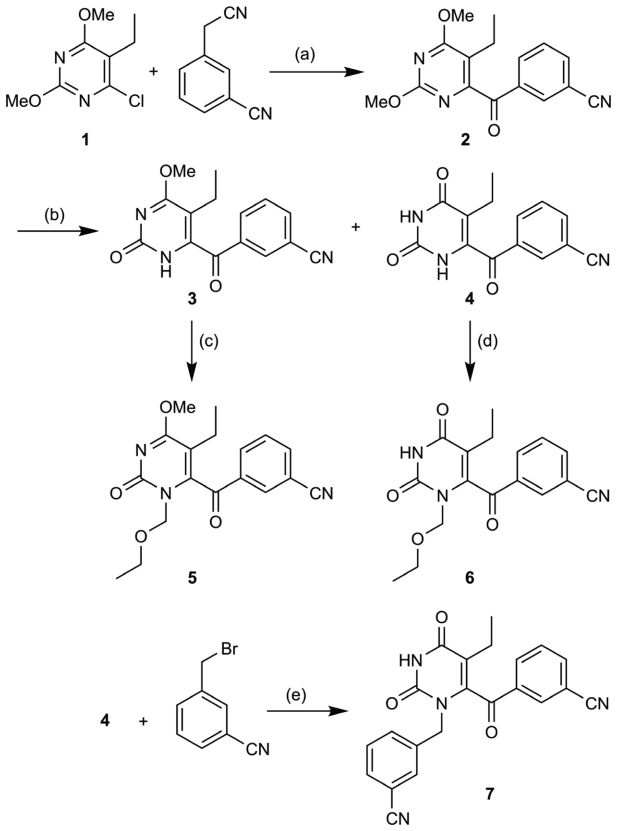


Figure 1. Structure of MKC-442.

Correspondence: Erik B. Pedersen, Nucleic Acid Center, Department of Physics and Chemistry, University of Southern Denmark, Campusvej 55, DK-5230 Odense M, Denmark.
E-mail: EBP@ifk.sdu.dk
Fax: +45 6615-8780

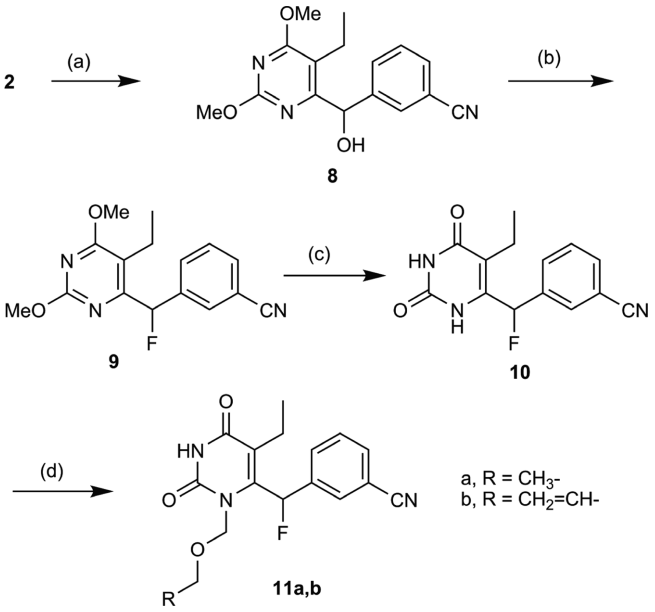
Abbreviations: **DAST**, Diethylaminosulfur trifluoride; **NNRTIs**, Non-nucleoside reverse transcriptase inhibitors;**TMSI**, Trimethylsilyl iodide

* A research center funded by The Danish National Research Foundation for studies on nucleic acid chemical biology.



Reagents and conditions: (a) i) NaH, DMF, 0°C; ii) O_2, r.t.; (b) TMSI, $CHCl_3$, reflux; (c) Et_3N, $EtOCH_2Cl$, CH_2Cl_2, r.t.; (d) BSA, $EtOCH_2Cl$, CH_2Cl_2, r.t.; (e) BSA, $Et(i\text{-}Pr)_2N$, CH_2Cl_2.

Scheme 1. Synthesis of compounds **2**–**7**.



Reagents and conditions: (a) $NaBH_4$, MeOH; (b) DAST, CH_2Cl_2; (c) TMSI, $CHCl_3$, reflux; (d) BSA, $EtOCH_2Cl$ for synthesis of **11a**; i) BSA, CH_3CN; ii) TMS-triflate, -50°C, $(CH_2{=}CH\text{-}CH_2\text{-}O)_2CH_2$ for synthesis of **11b**.

Scheme 2. Synthesis of compounds **8**–**11a**,**b**.

cyanobenzyl)fluoromethyl as 6-substituents in MKC-442 derivatives in order to improve their activity against HIV-1 mutants.

Results and discussion

Chemistry

Treatment of 4-chloro-5-ethyl-2,6-dimethoxy-pyrimidine with the sodium salt of 3-cyanobenzyl cyanide in dry dimethylformamide at 0°C followed by oxidation of the resultant product at room temperature afforded 3-[(5-ethyl-2,6-dimethoxypyrimidin-4-yl)-carbonyl]benzonitrile **2**, which was deprotected by treatment with trimethylsilyl iodide (TMSI) in chloroform at room temperature to give the partially deprotected compound **3**, 3-[(5-ethyl-6-methoxy-2-oxo-2,3-dihydropyrimidin-4-yl)-carbonyl]-benzonitrile, and the completely deprotected compound **4**, 6-(3-cyanobenzoyl)-5-ethyluracil in overall yields of 33 and 31%, respectively. The simple alkylation reaction of compound **3** with ethoxymethyl chloride in the presence of triethylamine afforded compound **5**. Compound **4** was silylated by *N*,*O*-bis(trimethylsilyl)acetamide (BSA) followed by treatment with ethoxymethyl chloride to give only the N1-alkylated product **6** in 61% yield. Also, the silylated derivative of compound **4** was treated with 3-cyanobenzyl bromide in the presence of triethylamine to afford 3-{[6-(3-cyanobenzoyl)-5-ethyl-2,4-dioxo-3,4-dihydro-pyrimidin-1(2*H*)-yl]methyl}-benzonitrile **7** (Scheme 1).

Compound **2** was reduced by sodium borohydride in methanol at 0°C to give 3-[(5-ethyl-2,6-dimethoxypyrimidin-4-yl)(hydroxy)methyl]benzonitrile **8**, which was fluorinated by diethylaminosulfur trifluoride (DAST) in methylene chloride to furnish the fluoro derivative **9** in 40% overall yield. [(3-Cyanophenyl)fluoromethyl]-5-ethyluracil **10** was prepared by deprotection of compound **9** through refluxing with TMSI in dry chloroform. MKC-442 analogues **11a**,**b** were synthesized by silylation of compound **10** with *N*,*O*-bis(trimethylsilyl)acetamide (BSA) and alkylated either by treatment with ethoxymethyl chloride in methylene chloride at room temperature or by bis(allyloxy)methane [13] in the presence of trimethylsilyl trifluoromethanesulfonate (TMS triflate) as the Lewis-acid catalyst [22] at −50°C (Scheme 2).

Compounds **6** and **7** showed steric hinderence for rotation around the carbonyl group. The slow rotation in case of compound **7** was clear from ^{1}H-NMR. Each diastereotopic proton of CH_2CH_3 in the 5-position appeared as broad singlet. Compound **6** showed even slower rotation

Table 1. Cytotoxicity and anti-HIV-1 activity of compounds **5**–**7**, **11a**,**b**, and the reference compounds MKC-442 and efavirenz (EFV).

Compound	CC_{50} (μM)[a]	SI[c]	EC_{50} (μM)[b]			
			Wild type	EFV^R	Y181C	K103N + Y181C
5	>100		10 ± 0.5	>100	>100	>100
6	>100		0.1 ± 0.01	>100	8 ± 3	67
7	>100		0.2 ± 0.1	>100	46	44
11a	>100		0.07 ± 0.005	>100	3 ± 1	14 ± 1
11b	>100		0.05 ± 0.03	>100	1.5 ± 0.05	20 ± 10
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: ratio: CC_{50}/EC_{50}. EC_{50} and CC_{50} are expressed as the mean values of at least two separate experiments. EFV^R is the triple mutant (K103R + V179D + P225H).

so that each diastereotopic proton of CH_2CH_3 appeared as multiplet. For compound **5**, the rotation was fast and the CH_2CH_3 appeared as normal quartet in ^{1}H-NMR.

Antiviral activity

Human immunodeficiency viruses type 1 (HIV-1, IIIB strain) 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.

Conclusion

It is interesting to note that all the new cyano derivatives, except for compound **5**, show activity against both HIV-1 variants carrying single and double mutations. Although the activity was low against double mutated HIV-1, one should notice that the N1-benzyl substituted derivative **7** also showed activity against that strain. The carbonyl group at the 6-position of the uracil ring (compounds **5** and **7**) decreases the activity against wild type HIV-1, while the fluoromethylene group at 6-position of the uracil ring in compounds **11a**,**b** resulted in activity comparable to MKC-442 and in increased activity against HIV-1 mutant strains.

This work received funding from the European Community's Sixth Framework Programme under contract number LSHP-CT-2004-503162 (Selection and development of microbicides for mucosal use to prevent sexual HIV transmission/acquisition) [24].

The authors have declared no conflict of interest.

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 (Varian, Palo Alto, CA, USA). EI mass spectra were recorded on a Finnigan MAT SSQ 710 (Thermo Electron Corporation, Bremen, Germany). MALDI spectra were recorded on a 4.7 T Ultima Fourier transform Mass spectrometer (IonSpec, Irvine, CA, USA). Melting points were determined in a Büchi melting point apparatus (Büchi Labortechnik, Flawil, Switzerland). The silica gel (0.040 – 0.063 mm) used for column chromatography was purchased from Merck (Merck, Germany). Microanalyses were carried out at Chemical Laboratory II at the University of Copenhagen, Denmark.

*Synthesis of 3-[(5-ethyl-2,6-dimethoxypyrimidin-4-yl)carbonyl]benzonitrile **2***

Sodium hydride (2.9 g, 66 mmol, 55% suspension in paraffin oil) was added portionwise to a stirred solution of compound **1** (4.1 g, 20 mmol) and 3-cyanobenzyl cyanide (4 g, 28 mmol) in dry dimethylformamide (40 mL) at 0°C. The mixture was allowed to reach room temperature gradually and left to be stirred for 30 h in an open flask (using air for oxidation). The reaction mixture was poured into ice-cold water (200 g) and the solid product formed was filtered off, washed with water and dried to afford 5.23 g of compound **2** as a pure solid, yield: 88%; m.p.: 103 – 105°C; ^{1}H-NMR ($CDCl_3$) δ [ppm]: 1.10 (t, *J* = 7.5 Hz, 3H, CH_3CH_2), 2.50 (q, *J* = 7.5 Hz, 2H, CH_3CH_2), 3.94, 4.09 (2s, 6H, $2OCH_3$), 7.62 (t, *J* = 8.1 Hz, 1H, H_{arom}), 7.87 (dt, *J* = 7.8, 1.4 Hz, 1H, H_{arom}), 8.14 – 8.17 (m, 2H, H_{arom}); ^{13}C-NMR ($CDCl_3$) δ [ppm]: 14.14 (CH_3CH_2), 18.28 (CH_3CH_2), 54.49, 54.93 ($2OCH_3$), 113.00, 129.50, 133.93, 133.99, 136.43, 136.26 (C_{arom}), 116.17 (CN), 117.76 (C5), 160.34 (C2), 162.75 (C4), 170.71 (C6), 191.26 (C=O); EI MS *m/z*: 297 [M + H$^+$] (48%), 268 (100%). Anal. calcd. for $C_{16}H_{15}N_3O_3$ (297.31): C, 64.64; H, 5.09; N, 14.13. Found: C, 64.11; H, 5.03; N, 13.72.

*Synthesis of compounds **3** and **4***

Under nitrogen, trimethylsilyl iodide (TMSI) (0.62 mL, 4.4 mmol) was added dropwise at r.t. to a stirred solution of compound **2**

(0.6 g, 2 mmol) in dry chloroform (20 mL) and the solution was refluxed for 3 h. The reaction mixture was cooled to r.t. and quenched with 5% aq. sodium bicarbonate solution (2 mL). Water (10 mL) was added and the two layers were separated. The chloroform layer was dried using sodium sulfate and evaporated under reduced pressure. The residual material was chromatographed on a silica gel column using petroleum ether / ether (2 : 1, v/v) as eluent to give two compounds **3** and **4**.

*3-[(5-Ethyl-6-methoxy-2-oxo-2,3-dihydropyrimidin-4-yl)carbonyl]benzonitrile **3***

Yield: 35%; m.p.: 223 – 225°C; ^{1}H-NMR ($CDCl_3$) δ [ppm]: 0.91 (t, *J* = 7.1 Hz, 3H, C*H*$_3$CH$_2$), 2.15 (q, *J* = 7.1 Hz, 2H, CH$_3$C*H*$_2$), 3.86 (s, 3H, OCH_3), 5.31 (bs, 1H, NH), 7.78 (t, *J* = 8.0 Hz, 1H, H_{arom}), 8.16-8.19 (m, 2H, H_{arom}), 8.28 (s, 1H, H_{arom}); ^{13}C-NMR ($CDCl_3$) δ [ppm]: 14.57 (*C*H$_3$CH$_2$), 17.68 (CH$_3$CH$_2$), 53.31 (OCH_3), 105.07 (C5), 117.83 (CN), 112.12, 130.28, 133.32, 133.84, 135.82, 137.37 (C_{arom}), 155.87 (C4), 161.42 (C2), 169.50 (C6), 190.91 (C=O); EI MS *m/z*: 283 [M$^+$] (59%), 254 (100%); HRMS (MALDI) *m/z*: 284.1029 ($C_{15}H_{14}N_3O_3$ [M + H$^+$]), requires: 284.1030.

*3-[(5-Ethyl-2,6-dioxo-1,2,3,6-tetrahydropyrimidin-4-yl)carbonyl]benzonitrile **4***

Yield: 37%; m.p.: 226 – 228°C; ^{1}H-NMR (DMSO-*d*$_6$) δ [ppm]: 0.87 (t, *J* = 7.2 Hz, 3H, C*H*$_3$CH$_2$), 2.01 (q, *J* = 7.2 Hz, 2H, CH$_3$C*H*$_2$), 7.84 (t, *J* = 7.8 Hz, 1H, H_{arom}), 8.24 (dt, *J* = 7.8, 1.4 Hz, 2H, H_{arom}), 8.30 (dt, *J* = 7.8, 1.4 Hz, 1H, H_{arom}), 8.56 (t, *J* = 1.5 Hz, 1H, H_{arom}), 11.08, 11.32 (2s, 2H, 2 NH); ^{13}C-NMR (DMSO-*d*$_6$) δ [ppm]: 13.58 (*C*H$_3$CH$_2$), 17.96 (CH$_3$CH$_2$), 112.60 (C5), 111.76, 130.57, 133.57, 133.67, 134.99, 138.26 (C_{arom}), 144.05 (C4), 150.39 (C2), 164.39 (C6), 188.73 (C=O); HRMS (MALDI) *m/z*: 292.0688 ($C_{14}H_{11}N_3O_3$ [M + H$^+$]), requires: 292.0693.

*Synthesis of 3-{[3-(ethoxymethyl)-5-ethyl-6-methoxy-2-oxo-2,3-dihydropyrimidin-4-yl]carbonyl}-benzonitrile **5***

Triethylamine (0.06 mL, 0.42 mmol) was added to a stirred solution of compound **3** (100 mg, 0.35 mmol) and ethoxymethyl chloride (0.04 mL, 0.42 mmol) in dry methylene chloride (10 mL) at r.t. The reaction mixture was stirred for 3 h, the solvent was evaporated under reduced pressure and the residual material was purified by silica gel column chromatography using CH_2Cl_2 as eluent to furnish 66 mg of compound **5**, yield 55%; m.p.: 70 – 72°C; ^{1}H-NMR ($CDCl_3$) δ [ppm]: 1.12 (t, *J* = 7.4 Hz, 3H, C*H*$_3$CH$_2$), 1.25 (t, *J* = 7.2 Hz, 3H, C*H*$_3$CH$_2$), 2.52 (q, *J* = 7.4 Hz, 2H, CH$_3$C*H*$_2$), 3.80 (q, *J* = 7.2 Hz, 2H, CH$_3$C*H*$_2$), 4.09 (s, 3H, OCH_3), 5.54 (s, 2H, NCH_2O), 7.61 (t, *J* = 7.5 Hz, 1H, H_{arom}), 7.87 (dt, *J* = 7.8, 1.5 Hz, H_{arom}), 8.17-8.21 (m, 2H, H_{arom}); ^{13}C-NMR ($CDCl_3$) δ [ppm]: 14.08 (*C*H$_3$CH$_2$), 15.02 (*C*H$_3$CH$_2$O), 18.28 (CH$_3$CH$_2$), 54.60 (OCH_3), 65.70 (CH$_3$*C*H$_2$O), 91.79 (NCH_2O), 116.98 (C5), 117.75 (CN), 112.95, 129.46, 133.97, 134.15, 136.19, 136.43 (C_{arom}), 160.18 (C2), 161.25 (C4), 170.84 (C6), 190.93 (C=O); HRMS (MALDI) *m/z*: 364.1258 ($C_{18}H_{19}NaN_3O_4$ [M + Na$^+$])], requires: 364.1268.

*Synthesis of 3-{[3-(ethoxymethyl)-5-ethyl-2,6-dioxo-1,2,3,6-tetrahydropyrimidin-4-yl]carbonyl}-benzonitrile **6***

Compound **4** (0.29 g, 1 mmol) was stirred in dry methylene chloride (15 mL) under nitrogen atmosphere, and *N,O*-bis(trimethylsilyl)acetamide (BSA) (0.87 mL, 3.5 mmol) was added. After complete silylation (clear solution, after 10 min), ethoxymethyl chloride (0.14 mL, 1.5 mmol) was added dropwise to the reaction mixture at r.t. and stirred for 3 h. The reaction was quenched by addition of 5% aq. sodium bicarbonate solution (3 mL). Water (10 mL) and methylene chloride (10 mL) were added to the reaction mixture. The two layers were separated and the organic layer was dried using sodium sulfate. The solvent was removed under reduced pressure and the residual material was purified by silica gel column chromatography using ethyl acetate / methylene chloride (1 : 1, v / v) as eluent to afford 200 mg of compound **6**, yield: 61%; m.p.: 123 – 125°C; ^{1}H-NMR ($CDCl_3$) δ [ppm]: 0.79 (t, *J* = 7.1 Hz, 3H, C*H*$_3$CH$_2$), 0.97 (t, *J* = 7.5 Hz, 3H, C*H*$_3$CH$_2$O), 1.94 – 2.01 (m, 1H, CH$_3$-*H*CH), 2.24 – 2.31 (m, 1H, CH$_3$-HC*H*), 3.29-3.45 (m, *J* = 7.5 Hz, 2H, CH$_3$-C*H*$_2$-O), 4.82 (d, *J* = 10.1 Hz, 1H, N-*H*CH-O), 5.56 (d, *J* = 10.1 Hz, 1H, N-HC*H*-O), 7.72 (t, *J* = 7.8 Hz, 1H, H_{arom}), 8.0 (dt, *J* = 7.8, 1.4 Hz, 1H, H_{arom}), 8.16 (dt, *J* = 8.1, 1.2 Hz, 1H, H_{arom}), 8.25 (t, *J* = 1.4 Hz, 1H, H_{arom}), 9.57 (s, 1H, NH); ^{13}C-NMR ($CDCl_3$) δ [ppm]: 13.22 (*C*H$_3$CH$_2$), 14.14 (*C*H$_3$CH$_2$O), 19.15 (CH$_3$CH$_2$), 64.33 (CH$_3$*C*H$_2$O), 72.97 (NCH_2O), 117.21 (C5), 115.45 (CN), 113.70, 129.98, 132.90, 133.21, 136.17, 137.46 (C_{arom}), 144.34 (C4), 150.42 (C2), 162.54 (C6), 187.87 (C=O); EI MS *m/z*: 327 [M$^+$] (11%), 59 (100%). Anal. calcd. for $C_{17}H_{17}N_3O_4$ (327.33): C, 62.38; H, 5.23; N, 12.84. Found: C, 62.46; H, 5.28; N, 12.65.

*Synthesis of 3-{[6-(3-cyanobenzoyl)-5-ethyl-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl]methyl}-benzonitrile **7***

To a stirred solution of compound **4** (80 mg, 0.3 mmol) dry methylene chloride (10 mL) under nitrogen atmosphere, *N,O*-bis(trimethylsilyl)acetamide (BSA) (0.25 mL, 1 mmol) was added. After complete silylation (clear solution, after 10 min), 3-cyanobenzyl bromide (86 mg, 0.44 mmol) was added to the mixture and it was refluxed for 16 h. The reaction mixture was cooled to r.t. and the solvent was removed under reduced pressure. The residual material was purified by silica gel column chromatography using EtOAc / CH_2Cl_2 (1 : 1, v / v) as eluent to afford 60 mg of compound **7**, yield: 52%; m.p.: 250-252°C; ^{1}H-NMR (DMSO-*d*$_6$) δ [ppm]: 0.83 (t, *J* = 7.2 Hz, 3H, CH$_3$CH$_2$), 1.86, 2.00 (2bs, 2H, CH$_3$C*H*$_2$), 4.64, 4.87 (2bs, 2H, C*H*$_2$Ar), 7.30 – 7.39 (m, 2H, H_{arom}), 7.54 – 760 (m, 2H, H_{arom}), 7.68 (t, *J* = 7.8 Hz, 1H, H_{arom}), 8.10 (d, *J* = 7.8 Hz, 1H, H_{arom}), 8.23 (d, *J* = 7.8 Hz, 1H, H_{arom}), 8.44 (s, 1H, H_{arom}); ^{13}C-NMR (DMSO-*d*$_6$) δ [ppm]: 13.09 (*C*H$_3$CH$_2$), 18.68 (CH$_3$CH$_2$), 47.01 (*C*H$_2$Ar), 113.32 (C5), 117.25 (CN), 118.31 (CN), 111.10, 112.38, 129.12, 130.31, 130.65, 130.92, 131.72, 133.09, 134.27, 134.59, 137.59, 138.46 (C_{arom}), 145.15 (C6), 151.01 (C2), 163.065 (C4), 188.59 (*C*OAr); EI MS *m/z*: 384 [M$^+$] (27%), 116 (100%). Anal. calcd. for $C_{22}H_{16}N_4O_3$. 0.2 H_2O (388.00): C, 68.10; H, 4.26; N, 14.44. Found: C, 68.10; H, 4.17; N, 14.08.

*Synthesis of 3-[(5-ethyl-2,6-dimethoxypyrimidin-4-yl)(hydroxy)methyl]benzonitrile **8***

Under ice cooling bath, sodium borohydride (170 mg, 4.5 mmol) was added portionwise to a stirred solution of compound **2** (1.2 g, 4 mmol) in methanol (20 mL). The reaction mixture was left to reach r.t. gradually with stirring for 1 h, then acetic acid (1 mL) was added followed by addition of water (40 mL) and ether (50 mL). The two layers were separated and the ether layer was dried ($MgSO_4$) and evaporated under reduced pressure. The oily residual material was 0.8 g of the pure compound **8**, yield 67%; yellow oil; ^{1}H-NMR ($CDCl_3$) δ [ppm]: 0.83 (t, *J* = 7.5 Hz, 3H, C*H*$_3$CH$_2$), 2.31-2.50 (m, 2H, CH$_3$C*H*$_2$), 4.02, 4.05 (2s, 6H, 2OCH_3), 5.15 (d, *J* = 7.5 Hz, 1H, OH), 5.76 (d, *J* = 7.5 Hz, 1H, CH), 7.41 – 7.60 (m, 4H, H_{arom}); ^{13}C-NMR ($CDCl_3$) δ [ppm]: 12.61 (*C*H$_3$CH$_2$), 17.43 (CH$_{3C}$H$_2$), 54.38, 54.79 (2 OCH_3), 70.47 (CH), 118.47 (CN), 113.18

(C5), 112.61, 129.37, 130.87, 131.50, 131.77, 143.79 (C_{arom}), 162.69 (C4), 164.53 (C2), 170.41 (C6); HRMS (MALDI) *m/z*: 322.1160 ($C_{16}H_{17}NaN_3O_3$ [M + Na^+]), requires: 322.1162.

*Synthesis of 3-[(5-ethyl-2,6-dimethoxypyrimidin-4-yl)(fluoro)methyl]benzonitrile **9***

To a stirred solution of compound **8** (0.6 g, 3 mmol) in dry methylene chloride (10 mL) was added DAST (0.6 mL, 4.5 mmol) in CH_2Cl_2 (1 mL) dropwise at −5°C. The reaction mixture was allowed to reach r.t. and stirred for 3 h. 5% Aqueous sodium bicarbonate (0.5 mL) was added to the reaction mixture followed by addition of water (15 mL) and methylene chloride (15 mL). The two layers were separated and the organic layer was dried using sodium sulfate (10 g) and evaporated under reduced pressure. The residual oily product was purified by silica gel column chromatography using ether as eluent to afford 0.54 g of compound **9**, yield: 60%; as a yellow oil; ^{1}H-NMR ($CDCl_3$) δ [ppm]: 1.04 (t, *J* = 7.4 Hz, 3H, C*H*$_3$CH$_2$), 2.57 (q, *J* = 7.4 Hz, 2H, CH$_3$C*H*$_2$), 3.94, 4.01 (2s, 6H, 2OCH$_3$), 6.54 (d, $J_{H,F}$ = 47.1 Hz, 1H, CH-F), 7.49 (t, *J* = 7.7 Hz, 1H, H_{arom}), 7.61 – 7.74 (m, 3H, H_{arom}); ^{13}C-NMR ($CDCl_3$) δ [ppm]: 13.91 (*C*H$_3$CH$_2$), 17.60 (d, *J* = 2.3 Hz, CH$_3$*C*H$_2$), 54.33, 54.66 (2OCH$_3$), 91.30 (d, *J* = 178.3 Hz, CH-F), 115.51 (C5), 118.37 (CN), 112.53, 129.15 (C_{arom}), 129.61 (d, *J* = 6.9 Hz, C_{arom}), 130.33 (d, *J* = 6.4 Hz, C_{arom}), 131.96 (d, *J* = 1.3 Hz, C_{arom}), 139.41 (d, *J* = 22.6 Hz, C_{arom}), 161.40 (d, *J* = 20.4 Hz, C4), 163.01 (C2), 170.79 (C6); HRMS (MALDI) *m/z*: 324.117 ($C_{16}H_{16}FNaN_3O_2$ [M + Na^+]), requires: 324.119.

*Synthesis of 3-[(5-ethyl-2,6-dioxo-1,2,3,6-tetrahydropyrimidin-4-yl)(fluoro)methyl] benzonitrile **10***

Under nitrogen, trimethyl silyl iodide (TMSI) (0.4 mL, 3 mmol) was added dropwise at r.t. to a stirred solution of compound **9** (0.39 g, 1.3 mmol) in dry chloroform (20 mL) and the solution was refluxed for 3 h. The reaction mixture was cooled to r.t. and quenched with 5% aq. sodium bicarbonate solution (1.5 mL), water (10 mL) was added and the two layers were separated. The chloroform layer was dried using sodium sulfate and evaporated under reduced pressure. The residual material was chromatographed on a silica gel column using petroleum EtOAc / CH_2Cl_2 (4 : 1, v / v) as eluent to give 0.46 g of compound **10**, yield: 57%; m.p.: 168 – 170°C; ^{1}H-NMR (DMSO-d_6) δ [ppm]: 0.83 (t, *J* = 7.4 Hz, 3H, C*H*$_3$CH$_2$), 2.31 (q, *J* = 7.4 Hz, 2H, CH$_3$C*H*$_2$), 6.80 (d, *J* = 45.0 Hz, 1H, CH-F), 7.67 – 8.05 (m, 4H, H_{arom}), 10.81, 11.22 (2s, 2H, 2NH); ^{13}C-NMR (DMSO-d_6) δ [ppm]: 13.53 (*C*H$_3$CH$_2$), 17.08 (CH$_3$*C*H$_2$), 86.86 (d, *J* = 175.4 Hz, CH-F), 112.46 (C5), 118.27 (CN), 111.76, 130.04 (C_{arom}), 130.16 (d, *J* = 6.0 Hz, C_{arom}), 131.28 (d, *J* = 5.2 Hz, C_{arom}), 137.10 (d, *J* = 21.9 Hz, C_{arom}), 144.12 (d, *J* = 20.0 Hz, C4), 150.67 (C2), 164.09 (C6); EI MS *m/z*: 273 [M^+] (20%), 91 (100%). Anal. calcd. for $C_{14}H_{12}FN_3O_2$: C, 61.53; H, 4.43; N, 15.38. Found: C, 61.50; H, 4.26; N, 15.20.

*Synthesis of 3-[(3-(ethoxymethyl)-5-ethyl-1,2,3,6-tetrahydro-2,6-dioxopyrimidin-4-yl)fluoromethyl]-benzonitrile **11a***

Compound **10** (137 mg, 0.5 mmol) was stirred in dry CH_2Cl_2 (10 mL) under nitrogen atmosphere, and *N,O*-bis(trimethylsilyl)-acetamide (BSA) (0.43 mL, 1.75 mmol) was added. After complete silylation (clear solution, after 10 min), ethoxymethyl chloride (0.1 mL, 1 mmol) was added dropwise to the reaction mixture at r.t. and stirred for 3 h. The reaction was quenched by addition of 5% aq sodium bicarbonate solution (3 mL). Water (10 mL) and methylene chloride (15 mL) were added to the reaction mixture. The two layers were separated and the organic layer was dried using sodium sulfate. The solvent was removed under reduced pressure and the residual material was purified by silica gel column chromatography using EtOAc / CH_2Cl_2 (1 : 4, v / v) as eluent to afford 75 mg of compound **11a**, yield: 45%; m.p.: 90 – 92°C; ^{1}H-NMR ($CDCl_3$) δ [ppm]: 0.97 (t, *J* = 7.4 Hz, 3H, C*H*$_3$CH$_2$-C5), 1.04 (t, *J* = 6.9 Hz, 3H, C*H*$_3$CH$_2$O), 2.27 – 2.47 (m, 2H, CH$_3$C*H*$_2$-C5), 3.45 – 362 (m, 2H, CH$_3$C*H*$_2$O), 5.22 (d, *J* = 11.2 Hz, 2H, N-*H*CH-O), 5.34 (d, *J* = 11.2 Hz, 2H, N-HC*H*-O), 6.86 (d, *J* = 46.5 Hz, 1H, CH-F), 7.56 – 7.72 (m, 4H, H_{arom}), 9.73 (s, 1H, NH); ^{13}C-NMR ($CDCl_3$) δ [ppm]: 13.50 (*C*H$_3$CH$_2$), 14.69 (*C*H$_3$CH$_2$), 19.13 (CH$_3$*C*H$_2$C5), 64.99 (CH$_3$*C*H$_2$O), 73.12 (NCH$_2$O), 87.18 (d, *J* = 179.5 Hz, CH-F), 117.91 (CN), 113.26, 129.65, 132.65 (C_{arom}), 119.84 (d, *J* = 2.9 Hz, C5), 129.12 (d, *J* = 6.0 Hz, C_{arom}), 129.65 (d, *J* = 5.2 Hz, C_{arom}), 137.63 (d, *J* = 21.7 Hz, C_{arom}), 145.17 (d, *J* = 18.6 Hz, C6), 151.28 (C2), 162.99 (C4); HRMS (MALDI) *m/z*: 354.1220 ($C_{17}H_{18}FN_3NaO_3$ [M + Na^+]), requires: 354.1224.

*Synthesis of 3-[{3-(allyloxymethyl)-5-ethyl-2,6-dioxo-1,2,3,6-tetrahydropyrimidin-4-yl}fluoro-methyl]benzonitrile **11b***

Compound **10** (95 mg, 0.35 mmol) was stirred in dry acetonitrile (10 mL) under nitrogen atmosphere, and *N,O*-bis(trimethylsilyl)acetamide (BSA) (0.3 mL, 1.2 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 (0.06 mL, 0.35 mmol) was added followed by dropwise addition of bis(allyloxy)methane (90 mg, 0.7 mmol) in CH_2Cl_2 (1 mL). The reaction mixture was stirred at room temperature for 3 h, quenched with ice-cold saturated solution of $NaHCO_3$ (1 mL), and evaporated under reduced pressure. The residue was extracted with Et_2O (3 × 50 mL), and the combined organic fractions were dried ($MgSO_4$) and evaporated under reduced pressure. The residue was chromatographed on a silica gel column using EtOAc / CH_2Cl_2 (1 : 4) as eluent to furnish 80 mg of compound **11b**, yield: 67%; m.p.: 98 – 100°C; ^{1}H-NMR ($CDCl_3$) δ [ppm]: 0.98 (t, *J* = 7.4 Hz, 3H, C*H*$_3$CH$_2$), 2.33 – 2.41 (m, 2H, CH$_3$C*H*$_2$), 4.02 (dd, *J* = 1.7, 4.1 Hz, 2H, C*H*$_2$-CH), 5.13 – 5.17 (m, 2H, C*H*$_2$=CH), 5.23 (d, *J* = 10.8 Hz, 1H, N*H*CHO), 5.35 (d, *J* = 11.36 Hz, 1H, NHC*H*O), 5.64 – 5.77 (m, 1H, C*H*=CH$_2$), 6.86 (d, *J* = 46.5 Hz, 1H, CH-F), 7.54 – 7.71 (m, 4H, H_{arom}), 9.51 (s, 1H, NH); ^{13}C-NMR ($CDCl_3$) δ [ppm]: 13.57 (*C*H$_3$CH$_2$), 19.16 (CH$_3$*C*H$_2$), 70.45 (O*C*H$_2$CH), 72.87 (NCH$_2$O), 87.19 (d, *J* = 178.8 Hz, CH-F), 117.90 (CN), 118.14 (*C*H$_2$=CH), 119.92 (d, *J* = 3.2 Hz, C5), 113.34 (C_{arom}), 129.04 (d, *J* = 6.1 Hz, C_{arom}), 129.62 (d, *J* = 5.6 Hz, C_{arom}), 129.83 (C_{arom}), 132.70 (CH$_2$=*C*H), 132.75 (d, *J* = 9.5 Hz, C_{arom}), 137.49 (d, *J* = 21.5 Hz, C_{arom}), 145.04 (d, *J* = 18.5 Hz, C6), 151.19 (C2), 162.81 (C4); HRMS (MALDI) *m/z*: 366.1225 ($C_{18}H_{18}FNaN_3O_3$ [M + Na^+]), requires: 366.1224.

Cell-based assays

Compounds, cells, and viruses

Compounds were dissolved in DMSO at 100 mM and then diluted in culture medium.

Cell lines were purchased from American Type Culture Collection (ATCC). The absence of mycoplasma contamination was checked periodically by the Hoechst staining method. Cell lines supporting the multiplication of HIV-1 were the $CD4^+$ human T-cells containing an integrated HTLV-1 genome (MT-4).

Cytotoxicity assays

For cytotoxicity evaluations, exponentially growing cells derived from human haematological tumors [$CD4^+$ human T-cells containing an integrated HTLV-1 genome (MT-4)] were seeded at an initial density of 1×10^5 cells/mL in 96-well plates in RPMI-1640 medium supplemented with 10% fetal calf serum (FCS), 100 units/mL penicillin G and 100 μg/mL streptomycin. Cell cultures were then incubated at 37°C in a humidified, 5% CO_2 atmosphere in the absence or presence of serial dilutions of test compounds. Cell viability was determined after 96 h at 37°C by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) method [23].

Antiviral assays

Activity of compounds against Human Immunodeficiency virus type-1 (HIV-1) was based on the inhibition of virus-induced cytopathogenicity in MT-4 cells acutely infected with a multiplicity of infection (m. o. i.) of 0.01. Briefly, 50 mL of RPMI containing 1×10^4 MT-4 were added to each well of flat-bottom microtitre trays containing 50 mL of RPMI, without or with serial dilutions of the test compounds. Then, 20 mL of an HIV-1 suspension containing 100 $CCID_{50}$ were added. After a 4-day incubation, cell viability was determined by the MTT method.

References

[1] J. Ren, R. Esnouf, E. Garman, D. Somers, *et al.*, *Nat. Struct. Biol.* **1995**, *2*, 293 – 302.

[2] R. Esnouf, J. Ren, A. L. Hopkins, C. K. Ross, *et al.*, *Proc. Natl. Acad. Sci. U. S. A.* **1997**, *94*, 3984 – 3989.

[3] J. Ren, J. Milton, K. L. Weaver, S. A. Short, *et al.*, *Struct. Fold Des.* **2000**, *8*, 1089 – 1094.

[4] J. Lindberg, S. Sigurdsson, S. Lowgren, H. O. Andersson, *et al.*, *Eur. J. Biochem.* **2002**, *269*, 1670 – 1677.

[5] S. Cesarini, A. Spallarossa, A. Ranise, S. Schenone, *et al.*, *Bioorg. Med. Chem.* **2008**, *16*, 6353 – 6363.

[6] T. J. Tucker, J. T. Sisko, R. M. Tynebor, T. M. Williams, *et al.*, *J. Med. Chem.* **2008**, *51*, 6503 – 6511.

[7] A. Mai, M. Artico, D. Rotili, D. Tarantino, *et al.*, *J. Med. Chem.* **2007**, *50*, 5412 – 5424.

[8] S. Hannongbua, *Top. Heterocycl. Chem.* **2006**, *4*, 55 – 84.

[9] H. Tanaka, H. Takashima, M. Ubasawa, K. Sekiya, *et al.*, *J. Med. Chem.* **1995**, *38*, 2860 – 2865.

[10] S. Yuasa, Y. Sadakata, H. Takashima, K. Sekiya, *et al.*, *Mol. Pharmacol.* **1993**, *44*, 895 – 900.

[11] M. Baba, S. Shigeta, S. Yuasa, H. Takashima, *et al.*, *Antimicrob. Agents Chemother.* **1994**, *38*, 688 – 692.

[12] M. Wamberg, E. B. Pedersen, N. R. El-Brollosy, C. Nielsen, *Bioorg. Med. Chem.* **2004**, *12*, 1141 – 1149.

[13] N. R. El – Brollosy, P. T. Jørgensen, B. Dahan, A. M. Boel, *et al.*, *J. Med. Chem.* **2002**, *45*, 5721 – 5726.

[14] L. Petersen, T. H. Hansen, N. M. Khalifa, P. T. Jørgensen, *et al.*, *Monatsh. Chem.* **2002**, *133*, 1031 – 143.

[15] X. Lu, Y. Chen, Y. Guo, Z. Liu, *et al.*, *Bioorg. Med. Chem.* **2007**, *15*, 7399 – 7407.

[16] N. R. El – Brollosy, E. R. Sørensen, E. B. Pedersen, G. Sanna, *et al.*, *Arch. Pharm.* **2008**, *341*, 9 – 19.

[17] T. J. Tucker, S. Saggar, J. T. Sisko, R. M. Tynebor, *et al.*, *Bioorg. Med. Chem. Lett.* **2008**, *18*, 2959 – 2966.

[18] T. J. Tucker, J. T. Sisko, R. M. Tynebor, T. M. Williams, *et al.*, *J. Med. Chem.* **2008**, *51*, 6503 – 6511.

[19] Z. K. Sweeney, S. F. Harris, N. Arora, H. Javanbakht, *et al.*, *J. Med. Chem.* **2008**, *51*, 7449 – 7458.

[20] Z. K. Sweeney, J. P. Dunn, Y. Li, G. Heilek, *et al.*, *Bioorg. Med. Chem. Lett.*, **2008**, *18*, 4352 – 4354.

[21] Z. K. Sweeney, S. Acharya, A. Briggs, J. P. Dunn, *et al.*, *Bioorg. Med. Chem. Lett.*, **2008**, *18*, 4348 – 4351.

[22] H. Vorbrüggen, K. Krolikiewiecz, B. Bennua, *Chem. Ber.* **1981**, *114*, 1234 – 1255.

[23] R. Pawels, J. Balzarini, M. Baba, R. Snoeck, *et al.*, *J. Virol. Methods* **1998**, *20*, 309 – 321.

[24] The information in this document is provided as it is and no guarantee or warranty is given that the information is fit for any particular purpose. The user thereof uses the information at its sole risk and liability.

