Synthesis and Anti-HIV-1 Evaluation of 1,5-Disubstituted Pyrimidine-2,4-diones

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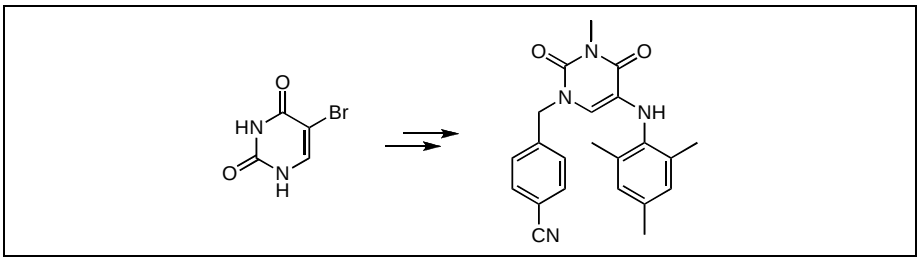

1,5-Disubstituted pyrimidine-2,4-diones were synthesized by fusion of 5-bromouracil with an aniline derivative followed by coupling with benzyl halide whereas the opposite synthesis sequence failed. Also 4,6-dichloro-*N,N*,5-trimethylpyrimidin-2-amine was treated with phenols. The products were tested for their activity against HIV-1.

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INTRODUCTION

The discovery of novel anti-HIV-1 agents with long-lasting suppression of viral replication is one of the most important goals in the field of AIDS research. In addition to nucleoside analogues, a number of different chemical classes of compounds have been described that show potent and selective inhibition of HIV-1 replication by acting as non-nucleoside reverse transcriptase inhibitors (NNRTIs). There are at the present time 21 FDA approved anti-HIV drugs for therapeutic use. Among these, 11 compounds are RT inhibitors comprising seven nucleosides (NRTIs), one nucleotide (NtRTI), three non-nucleoside RT inhibitors (NNRTIs), eight protease inhibitors, one viral fusion inhibitor, enfuvirtide (Fuzeon®) [1-4] and one entry inhibitor, Selzentry®, FDA approved August 2007. The three NNRTIs, nevirapine (Viramune®) [5,6], delavirdine (Rescriptor®) [7] and efavirenz (Sustiva®) [8,9] were approved for the treatment of HIV infection in 1996, 1997 and 1998, respectively. They became cornerstones of HIV therapy because of their full potential as a component of the highly-active anti-retroviral therapy (HAART) [10]. However, one of the major drawbacks in using NNRTIs in HIV therapy is due to the rapidly introduction of RT mutations. Especially the loss of activity against the Y181C and K103N mutated HIV-1 strains is problematic.

Recently, Das *et al.* [11] reported that *N*-methylated pyrazinone analogues (Figure 1) showed activity against both wild-type and NNRTI- and NRTI-resistant virus. Nair *et al.* reported that bis-benzylated nucleobase with a β-diketo acids are potent HIV integrase inhibitors [12].

In the present work, we found it of interest to synthesize new analogues of 1,5-disubstituted pyrimidine-2,4-diones such as target compound **10b** with structural resemblances to the pyrazinone skeleton (Figure 1) for biological evaluation against HIV-1. Also 1,3-disubstituted and 4,6-disubstituted pyrimidine derivatives were considered interesting.

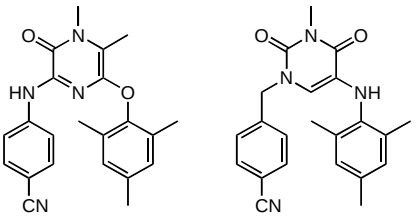


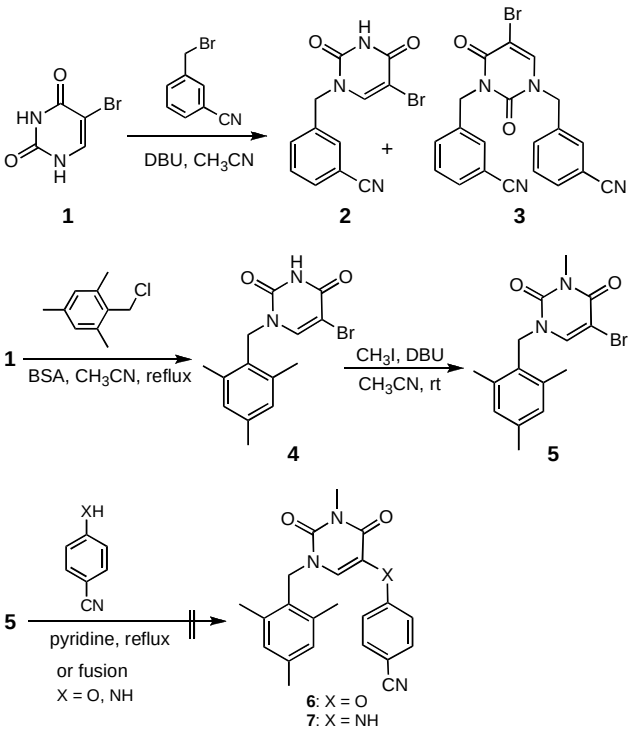
Figure 1

RESULTS AND DISCUSSION

Commercially available 5-bromouracil (**1**) was alkylated with 3-cyanobenzyl bromide in acetonitrile using 1,8-diazabicyclo[5.4.0]-undec-7-ene (DBU) as a sterically hindered base. Two products were isolated from the reaction by column chromatography. The major product is the desired N1-monoalkylated 3-[(5-bromo-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl)methyl]benzo-

nitrile (**2**) and the other one is the N1,N3-bislakylated product **3** as the minor product. Although compound **2** is the major product, the yield is low (40%). To increase the yield of the mono-alkylated product we introduced a silylation reaction prior to the alkylation step [13]. 5-Bromouracil (**1**) was silylated by *N,O*-bis(trimethylsilyl)acetamide (BSA) followed by refluxing with 2,4,6-trimethylbenzyl chloride and only 5-bromo-1-(mesitylmethyl)pyrimidine-2,4(1*H*,3*H*)-dione (**4**) was isolated as the sole product without using column chromatography. Compound **4** was methylated by methyl iodide to afford 5-bromo-1-(mesitylmethyl)-3-methylpyrimidine-2,4(1*H*, 3*H*)-dione (**5**). However, fusion of **5** with 4-cyanophenol or 4-cyanoaniline could not be acomplished to afford the desired compounds **6** and **7**, respectively (Scheme I).

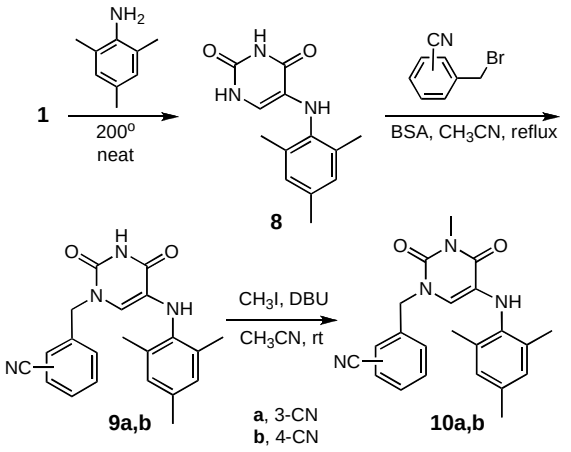
Scheme I



Instead, 5-bromouracil was fused with 2,4,6-trimethylaniline at 200° to afford 5-(mesitylamino)-pyrimidine-2,4(1*H*,3*H*)-dione (**8**) which was silylated with BSA followed by refluxing with 3-cyano or 4-cyano benzyl bromides to furnish 3- or 4-{[5-(mesitylamino)-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl]methyl} benzo-nitrile (**9a,b**). Compounds **9a,b** were methylated with methyl iodide to afford the 3- or 4-{[5-(mesitylamino)-3-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2*H*)-yl]methyl}benzo-nitrile **10a,b** (Scheme II).

On irradiation of CH_3-N in compound **10a** at 3.47 ppm, no NOE effect was detected with any proton while H6 at 5.73 ppm showed 6.5 % NOE when N-CH_2 at 4.80 ppm was irradiated. Also on irradiation of CH_3-N in compound **10b** at 3.47 ppm, no NOE effect was detected with any proton while H6 at 5.72 ppm showed 6.5 % NOE when N-CH_2 at 4.82 ppm was irradiated.

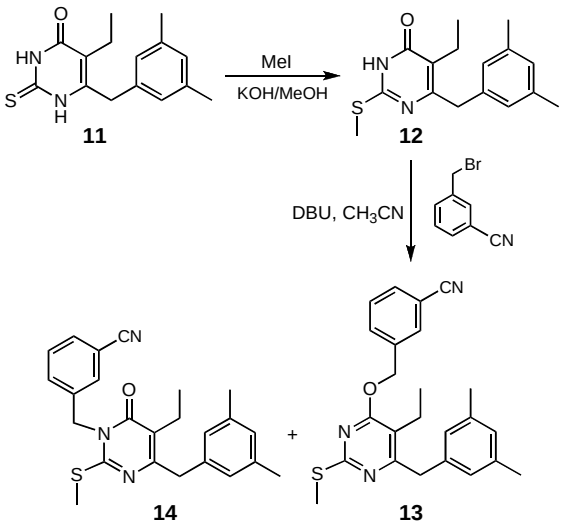
Scheme II



With the 6-(3,5-dimethylbenzyl)-5-ethyl-2,3-dihydro-2-thioxopyrimidin-4(1*H*)-one (**11**) and 4,6-dichloro-*N,N*-dimethylpyrimidin-2-amine (**15**) in our hands from a previous HIV project we find it of interest to expand the pyrimidine series using these compounds.

Methylation of 6-(3,5-dimethylbenzyl)-5-ethyl-2,3-dihydro-2-thioxopyrimidin-4(1*H*)-one (**11**) [14] with methyl iodide gave the *S*-methylated derivative **12** which by treatment with 3-cyanobenzyl bromide afforded the *O*-alkylated derivative (**13**) and the *N3*-alkylated derivative (**14**) (Scheme III). The ^{13}C nmr spectrum of compound **13** showed the existence of CH_2-O at 65.96 ppm and of compound **14** CH_2-N at 46.81 ppm.

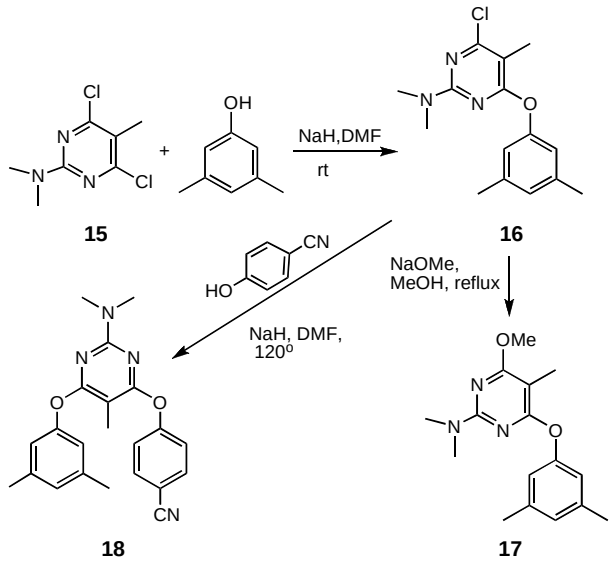
Scheme III



The ^{13}C nmr spectrum of compound **14** clearly showed alkylation on *N3* and not on the *N1* nitrogen atom when the characteristic chemical shift (157.52 (C6), 159.14 (C2), and 162.74 (C4)) are compared with the corresponding values of 2,3-dihydro-7*H*-thiazolo[3,2-*a*]-pyrimidin-7-one (144.4, 165.5, and 169.2 corresponding to C6, C4, and C2, respectively) for the *N1*-alkylated derivative versus 2,3-dihydro-5*H*-thiazolo[3,2-*a*]pyrimidin-5-one (160.3, 161.2, and 161.8 corresponding to C6, C4, and C2, respectively) for the *N3*-alkylated derivative [15].

4-(3,5-Dimethylphenoxy)-6-chloro-*N,N*,5-trimethyl-pyrimidin-2-amine (**16**) was prepared by coupling of 4,6-dichloro-*N,N,5*-trimethylpyrimidin-2-amine (**15**) [16] with the sodium salt of 3,5-dimethylphenol in dimethylformamide. Refluxing of compound **16** with sodium methoxide in methanol afforded the methoxy derivative **17**. Heating of compound **16** with the sodium salt of 4-cyanophenol in dimethylformamide at 120° furnished 4-(2-(dimethylamino)-6-(3,5-dimethyl-phenoxy)-5-methylpyrimidin-4-yloxy)benzonitrile (**18**) (Scheme IV).

Scheme IV



Antiviral activity. All compounds were evaluated for their activity against HIV-1 wild-type and its NNRTI-resistant mutants. Only compound **12** showed moderate activity against HIV-1 wild-type (EC_{50} = 2 μM and CC_{50} >100 μM). All other compounds were inactive at the maximum concentration tested (100 μM).

EXPERIMENTAL

Nmr Spectra were recorded on a Varian Gemini 2000 spectrometer at 300 MHz for ^1H 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 were carried out at Chemical Laboratory II at University of Copenhagen, Denmark.

Synthesis of compounds 2 and 3. DBU (1.8 mL, 12 mmole) was added to a stirred solution of 5-bromouracil (1.9 g, 10 mmole) in dry acetonitrile (30 mL). 3-Cyanobenzyl bromide (1.96 g, 10 mmole) was added to the reaction mixture and stirred for 12 hours, acetic acid (1 mL) was added to the reaction mixture and the solvents were removed under reduced pressure. Methylene chloride (50 mL) was added to the residual material and the solid product formed was collected by filtration, washed with methylene chloride followed by water and dried to afford compound **2**. The two layers of the filtrate were separated and the organic layer was evaporated under reduced pressure, ether (15 mL) was added to the residual material and stirred for 15 min. The solid product formed was collected by filtration and dried to afford compound **3**.

3-[(5-Bromo-2,4-dioxo-3,4-dihydropyrimidine-1(2*H*)-yl)-methyl]benzonitrile (2). Yield 1.2 g (40%); mp 238-240°; ^1H nmr (DMSO-d_6) δ: 4.93 (s, 2H, CH_2Ar), 7.58 (t, 1H, J = 7.8 Hz, H_{arom}), 7.68 (d, 1H, J = 7.8 Hz, H_{arom}), 7.79 (d, 1H, J = 7.8 Hz, H_{arom}), 7.85 (s, 1H, H_{arom}), 8.38 (s, 1H, H6), 11.84 ppm (s, 1H, NH); ^{13}C nmr (DMSO-d_6) δ: 50.22 (*C*H_2Ar), 95.44 (C5), 118.54 (CN), 111.45, 129.72, 131.09, 131.43, 132.38, 138.01, (C_{arom}), 142.13 (C6), 150.36 (C2), 159.62 ppm (C4); EI MS: *m/z* 305 (17%, M^+, ^{79}Br), 307 (18%, M^+, ^{81}Br). *Anal.* Calcd. for $C_{12}H_8BrN_3O_2$ (306.11): C, 47.08; H, 2.63; N, 13.73. Found: C, 47.84; H, 2.54; N, 13.46.

3-{[5-Bromo-3-(3-cyanobenzyl)-2,4-dioxo-3,4-dihydropyrimidine-1(2*H*)-yl]methyl}benzonitrile (3). Yield 600 mg (14%); mp 133-135°; ^1H nmr ($CDCl_3$) δ: 4.98 (s, 2H, CH_2), 5.17 (s, 2H, CH_2), 7.40-7.76 ppm (m, 8H, H_{arom}); ^{13}C nmr ($CDCl_3$) δ: 45.18 (CH_2), 52.22 (CH_2), 97.06 (C5), 117.99 (CN), 118.49 (CN), 112.55, 113.34, 129.34, 130.10, 131.46, 131.66, 132.32, 132.40, 132.75, 133.85, 136.18, 137.24 (C_{arom}), 141.49 (C6), 150.71 (C2), 158.82 ppm (C4); EI MS: *m/z* 420 (37%, M^+, ^{79}Br), 422 (34%, M^+, ^{81}Br). *Anal.* Calcd. for $C_{20}H_{13}BrN_4O_2$ (421.25): C, 57.02; H, 3.11; N, 13.30. Found: C, 57.28; H, 2.99; N, 13.26.

Synthesis of 5-bromo-1-(mesitylmethyl)pyrimidine-2,4(1*H*,3*H*)-dione (4). Compound **1** (1.9 g, 10 mmole) in dry acetonitrile (40 mL) was silylated under nitrogen by dropwise addition of *N,O*-bis(trimethylsilyl)acetamide (BSA) (8 mL, 33 mmole) and stirring for 15 min. 2,4,6-Trimethylbenzyl chloride (2.2 g, 13 mmole) was added in one portion to the reaction mixture and refluxed for 20 hours. The reaction mixture was cooled to room temperature and the solvent was removed under reduced pressure, water (50 mL) was added to the residual material and the solid product formed was collected by filtration, washed with water and dried to afford compound **4**. Yield 3.0 g (93%); mp 212-214°; ^1H nmr (DMSO-d_6) δ: 2.23 (s, 6H, (CH_3)$_2$Ar), 2.30 (s, 3H, CH_3Ar), 4.84 (s, 2H, CH_2Ar), 6.92 (s, 2H, H_{arom}), 7.24 (s, 1H, H6), 11.84 ppm (s, 1H, NH); ^{13}C nmr (DMSO-d_6) δ: 19.37 (2 × CH_3), 20.48 (CH_3), 44.99 (CH_2), 94.85 (C5), 127.60, 128.35, 129.26, 137.89 (C_{arom}), 142.24 (C6), 150.36 (C2), 159.28 ppm (C4); EI MS: *m/z* 322 (4%, M^+, ^{79}Br), 324 (3%, M^+, ^{81}Br).

Synthesis of 5-bromo-1-(mesitylmethyl)-3-methylpyrimidine-2,4(1*H*,3*H*)-dione (5). Compound **4** (2.26 g, 7 mmole) was

added to a stirred solution of potassium hydroxide (0.5 g, 9 mmole) in methanol (30 mL). The solution was stirred for 15 min., then methyl iodide (0.53 mL, 8.4 mmole) was added and the reaction mixture was stirred for 2 hours. Water (60 mL) was added to the reaction mixture and the solid product formed was collected by filtration, washed with water and dried to afford compound **5**. Yield 1.9 g (81%); mp 176-178°; ^{1}H nmr ($CDCl_3$) δ: 2.24 (s, 6H, (CH_3)$_2$Ar), 2.32 (s, 3H, CH_3Ar), 3.46 (s, 3H, NCH_3), 4.94 (s, 2H, N-CH_2), 6.93 (s, 1H, H6), 6.96 (s, 2H, H_{arom}); ^{13}C nmr ($CDCl_3$) δ: 19.68 ((CH_3)$_2$Ar), 21.01 (CH_3Ar), 29.27 (NCH_3), 46.21 (N-CH_2), 95.95 (C5), 125.68, 129.94, 138.25, 138.53 (C_{arom}), 139.47 (C6), 151.28 (C2), 159.16 (C4); HRms (MALDI, peak matching): *m/z* calcd. for $C_{15}H_{17}BrNaN_2O_2$ (MNa^+) 359.0366, found 359.0375. *Anal.* Calcd. for $C_{15}H_{17}BrN_2O_2$ (337.21): C, 53.43; H, 5.08; N, 8.31. Found: C, 53.69; H, 4.94; N, 8.25.

5-(Mesitylamino)pyrimidine-2,4(1*H*,3*H*)-dione (8). Under a stream of nitrogen, compound **1** (1.9 g, 10 mmole) was fused with 2,4,6-trimethylaniline (1.48 g, 11 mmole) at 200° for 3 hours. The reaction mixture was left to reach 70°, then ethanol (10 mL) was added and the mixture was stirred with a spatula until getting a suspension. The solid product formed was collected by filtration, dried and chromatographed on a column of silica gel using CH_2Cl_2:EtOAc (1:1, v/v) as an eluent to give compound **12**.

Yield 910 mg (37%); mp > 250°; ^{1}H nmr (DMSO-d_6) δ: 2.08 (s, 6H, (CH_3)$_2$Ar), 2.21 (s, 3H, CH_3Ar), 5.54 (s, 1H, NH), 5.89 (s, 1H, H6), 6.89 (s, 2H, H_{arom}), 10.00 (bs, 1H, NH), 11.27 (bs, 1H, NH); ^{13}C nmr (DMSO-d_6) δ: 17.46 (2 × CH_3), 20.44 (CH_3), 113.05 (C6), 122.03, 128.94, 134.81, 135.50 (C_{arom}), 134.50 (C5), 149.43 (C2), 161.05 (C4); HRms (MALDI, peak matching): *m/z* calcd. for $C_{13}H_{15}NaN_3O_2$ (MNa^+) 268.1057, found 268.1057.

Synthesis of compound 9a,b. Compound **8** (300 mg, 1.2 mmole) in dry acetonitrile (15 mL) was silylated under nitrogen by dropwise addition of *N,O*-bis(trimethylsilyl)acetamide (BSA) (1.3 mL, 5.3 mmole) and stirring for 15 min. 3-Cyano or 4-cyanobenzyl bromide (1.6 mmole) was added in one portion to the reaction mixture and refluxed for 14 hours. The reaction mixture was cooled to room temperature and the solvent was removed under reduced pressure, water (20 mL) was added to the residual material and the solid product formed was collected by filtration, washed with water and dried to afford compounds **9a,b**.

3-{[5-(Mesitylamino)-2,4-dioxo-3,4-dihydropyrimidine-1(2*H*)-yl]methyl}benzonitrile (9a). Yield 350 mg (81%); mp 184-186°; ^{1}H nmr (DMSO-d_6) δ: 2.05 (s, 6H, (CH_3)$_2$Ar), 2.20 (s, 3H, CH_3Ar), 4.82 (s, 2H, CH_2), 5.94 (s, 1H, H6), 6.10 (bs, 1H, NH), 6.86 (s, 2H, H_{arom}), 7.50-7.57 (m, 2H, H_{arom}), 7.62 (s, 1H, H_{arom}), 7.74 (dt, 1H, *J* = 7.5, 1.5 Hz, H_{arom}), 11.51 (s, 1H, NH); ^{13}C nmr (DMSO-d_6) δ: 17.46 (2 × CH_3), 20.40 (CH_3), 48.93 (CH_2), 116.15 (C6), 118.48 (CN), 122.96 (C5), 111.34, 128.92, 129.75, 130.65, 131.10, 131.94, 134.35, 134.62, 134.89, 138.98 (C_{arom}), 149.00 (C2), 160.46 (C4); HRms (MALDI, peak matching): *m/z* calcd. for $C_{21}H_{21}N_4O_2$ (MH^+) 361.1665, found 361.1649. *Anal.* Calcd. for $C_{21}H_{20}N_4O_2$ (360.41): C, 69.98; H, 5.59; N, 15.55. Found: C, 70.11; H, 5.61; N, 15.36.

4-{[5-(Mesitylamino)-2,4-dioxo-3,4-dihydropyrimidine-1(2*H*)-yl]methyl}benzonitrile (9b). Yield 300 mg (70%); mp 202-204°; ^{1}H nmr (DMSO-d_6) δ: 2.05 (s, 6H, (CH_3)$_2$Ar), 2.20 (s, 3H, CH_3Ar), 4.86 (s, 2H, CH_2), 5.93 (s, 1H, H6), 6.86 (s, 2H, H_{arom}), 7.32 (d, 2H, *J* = 8.3 Hz, H_{arom}), 7.79 (d, 2H, *J* = 8.3 Hz, H_{arom}), 11.64 ppm (s, 1H, NH); ^{13}C nmr (DMSO-d_6) δ: 17.50 (2 × CH_3), 20.39 (CH_3), 49.48 (CH_2), 110.00, 122.92, 127.70, 128.91, 132.39, 134.36, 134.65, 143.12 (C_{arom}), 134.85 (C5), 148.99 (C2), 160.46 (C4); HRms (MALDI, peak matching): *m/z* calcd. for $C_{21}H_{21}N_4O_2$ (MH^+) 361.1651, found 361.1665.

Synthesis of compound 10a,b. Compound **9a,b** (0.36 g, 1 mmole) was added to a solution of DBU (0.2 mL, 1.3 mmole) in dry acetonitrile (10 mL) and stirred for 15 min., then methyl iodide was added to the reaction mixture at room temperature and stirred for 3 hours. The solvents were removed under reduced pressure, water (15 mL) was added to the residual material and the solid product formed was collected by filtration, washed with water, and dried to give compounds **10a,b**.

3-{[5-(Mesitylamino)-3-methyl-2,4-dioxo-3,4-dihydro-pyrimidine-1(2*H*)-yl]methyl}benzonitrile (10a). Yield 320 mg (86%); mp 124-126°; ^{1}H nmr ($CDCl_3$) δ: 2.11 (s, 6H, (CH_3)$_2$Ar), 2.28 (s, 3H, CH_3Ar), 3.47 (s, 3H, N-CH_3); 4.80 (s, 2H, NCH_2), 5.40 (s, 1H, NH), 5.73 (s, 1H, H6), 6.91 (s, 2H, H_{arom}), 7.44-7.61 (m, 4H, H_{arom}); ^{13}C nmr ($CDCl_3$) δ: 17.75 ((CH_3)$_2$Ar), 20.81 (CH_3Ar), 28.48 (NCH_3), 51.56 (NCH_2), 113.82 (C6), 118.20 (CN), 130.89 (C5), 112.95, 122.93, 129.45, 129.66, 131.67, 131.90, 133.78, 134.85, 136.04, 137.60 (C_{arom}), 149.68 (C2), 160.21 (C4); HRms (MALDI, peak matching): *m/z* calcd. for $C_{22}H_{21}N_4O_2$ (MH^+) 375.1821, found 375.1785. *Anal.* Calcd. for $C_{22}H_{22}N_4O_2$·0.9 H_2O (390.66): C, 67.64; H, 6.14; N, 14.34. Found: C, 67.62; H, 5.95; N, 14.37.

4-{[5-(Mesitylamino)-3-methyl-2,4-dioxo-3,4-dihydropyr-imidine-1(2*H*)-yl]methyl}benzonitrile (10b). Yield 230 mg (61%); mp 140-142°; ^{1}H nmr ($CDCl_3$) δ: 2.10 (s, 6H, (CH_3)$_2$Ar), 2.28 (s, 3H, CH_3Ar), 3.47 (s, 3H, NCH_3), 4.82 (s, 2H, NCH_2), 5.40 (s, 1H, NH), 5.72 (s, 1H, H6), 6.90 (s, 2H, H_{arom}), 7.28 (d, 2H, *J* = 8.7 Hz, H_{arom}), 7.62 (d, 2H, *J* = 8.7 Hz, H_{arom}); ^{13}C nmr ($CDCl_3$) δ: 17.79 (2 × CH_3), 20.83 (CH_3), 28.52 (NCH_3), 51.93 (CH_2), 113.92 (C6), 118.33 (CN), 122.94 (C5), 111.98, 128.01, 129.45, 132.59, 133.82, 134.90, 136.05, 141.30 (C_{arom}), 149.71 (C2), 160.25 (C4); HRms (MALDI, peak matching): *m/z* calcd. for $C_{22}H_{23}N_4O_2$ (MH^+) 375.1821, found 375.1772. *Anal.* Calcd. for $C_{22}H_{22}N_4O_2$ (1162.3): C, 69.23; H, 5.46; N, 15.67. Found: C, 69.53; H, 5.85; N, 14.57.

6-(3,5-Dimethylbenzyl)-5-ethyl-2-(methylthio)-pyrimidine-4(1*H*)-one (12). Compound **11** (1.1 g, 4 mmole) was added to a stirred solution of potassium hydroxide (0.25 g, 4.4 mmole) in methanol (15 mL). The solution was stirred for 15 min., then methyl iodide (0.27 mL, 4.4 mmole) was added and the reaction mixture was stirred for 1 hour. Water (30 mL) was added to the reaction mixture and the solid product formed was collected by filtration, washed with water and dried to afford compound **12**. Yield 750 mg (65%); mp 213-215°; ^{1}H nmr ($CDCl_3$) δ: 1.09 (t, 3H, *J* = 7.5 Hz, CH_3CH_2), 2.27 (s, 6H, (CH_3)$_2$Ar), 2.50 (s, 3H, CH_3S), 2.58 (q, 2H, *J* = 7.5 Hz, CH_3CH_2), 3.84 (s, 2H, CH_2Ar); ^{13}C nmr ($CDCl_3$) δ: 13.17 (CH_3CH_2), 18.80 (CH_3CH_2), 21.28 (CH_3S), 40.32 (CH_2Ar), 122.08 (C5), 126.77, 127.99, 137.77, 138.04 (C_{arom}), 156.85 (C2), 161.81 (C4), 165.03 (165.03); EI MS: *m/z* 288 (100%, M^+). *Anal.* Calcd.. for $C_{16}H_{20}N_2OS$ (288.41): C, 66.63; H, 6.99; N, 9.71. Found: C, 66.26; H, 6.91; N, 9.59.

Synthesis of compounds 13 and 14. Compound **12** (0.29 g, 1 mmole) was added to a stirred solution of DBU (0.2 mL, 1.3 mmole) in dry acetonitrile (10 mL) and stirred for 15 min., then 3-cyanobenzyl bromide (0.22 g, 1.1 mmole) was added to the reaction mixture at room temperature, stirred for 3 hours and then refluxed for 5 hours. The solvent was removed under

reduced pressure, water (15 mL) was added to the residual material and the solid product formed was collected by filtration, dried and chromatographed on a column of silica gel using petroleum ether:EtOAc (11:1, v/v) as an eluent to give compounds **13** and **14**.

3-{[6-(3,5-Dimethylbenzyl)-5-ethyl-2-(methylthio)-pyrimidine-4-yloxy]methyl}benzonitrile (13). Yield 73 mg (18 %); mp 95-97°; ^{1}H nmr ($CDCl_3$) δ: 0.94 (t, 3H, J = 7.4 Hz, CH_3CH$_2$), 2.18 (s, 6H, (CH_3)$_2$Ar), 2.43 (s, 3H, CH_3S), 2.52 (q, 2H, J = 7.4 Hz, CH_3CH_2), 3.88 (s, 2H, CH_2Ar), 5.38 (s, 2H, CH_2O), 6.74, 6.77 (2 × s, 3H, H_{arom}), 7.41-7.63 ppm (m, 4H, H_{arom}); ^{13}C nmr ($CDCl_3$) δ: 12.77 (*C*H$_3$CH$_2$), 13.45 (CH$_3$*C*H$_2$), 17.60 (CH_3S), 20.63 ((*C*H$_3$)$_2$Ar), 39.45 (*C*H$_2$Ar), 65.96 (CH_2O), 115.80 (CN), 117.98 (C5), 111.83, 125.94, 127.39, 128.85, 130.32, 130.91, 131.37, 131.12, 137.36, 137.84 (C_{arom}), 165.89 (C4), 166.00 (C2), 166.69 (C6); HRms (MALDI, peak matching): *m/z* calcd. for $C_{24}H_{25}NaN_3OS$ (MNa^+) 426.1611, found 426.1612.

3-{[4-(3,5-Dimethylbenzyl)-5-ethyl-2-(methylthio)-6-oxopyrimidine-1(6*H*)-yl]methyl}benzonitrile (14). Yield 80 mg (20%); mp 128-130°; ^{1}H nmr ($CDCl_3$) δ: 1.04 (t, 3H, J = 7.4 Hz, CH_3CH$_2$), 2.21 (s, 6H, (CH_3)$_2$Ar), 2.41 (s, 3H, CH_3S), 2.56 (q, 2H, J = 7.4 Hz, CH_3CH_2), 3.75 (s, 2H, CH_2Ar), 5.14 (s, 2H, CH_2N), 6.78, 6.85 (2 × s, 3H, H_{arom}), 7.31-7.56 ppm (m, 4H, H_{arom}); ^{13}C nmr ($CDCl_3$) δ: 13.16 (*C*H$_3$CH$_2$), 14.98 (CH$_3$*C*H$_2$), 19.52 (CH_3S), 21.26 ((*C*H$_3$)$_2$Ar), 40.15 (*C*H$_2$Ar), 46.81 (CH_2N), 118.55 (CN), 121.258 (C5), 112.62, 127.04, 128.06, 129.32, 131.41, 131.53, 132.60, 136.95, 137.74, 137.86 (C_{arom}), 157.52 (C6), 159.14 (C2), 162.74 (C4); HRms (MALDI, peak matching): *m/z* calcd. for $C_{24}H_{25}NaN_3OS$ (MH^+) 426.1611, found 426.1597.

4-Chloro-6-(3,5-dimethylphenoxy)-*N*,*N*,5-trimethylpyrimidine-2-amine (16). To a solution of 4,6-dichloro-*N*,*N*,5-trimethylpyrimidin-2-amine (**15**) (2.06 g, 10 mmole) and 3,5-dimethylphenol (1.34 g, 11 mmole) in dry dimethylformamide (20 mL) was added sodium hydride (55% suspension in paraffin oil, 0.52 g, 12 mmole) portionwise. After stirring for 2 hours under dry conditions at room temperature, the reaction mixture was quenched by addition of water (2 mL) dropwise followed by 40 mL water in one portion, pure compound **16** was precipitated then collected by filtration and dried. Yield: 2.85 g (98%), as a white solid; mp 111-113°; ^{1}H nmr ($CDCl_3$): δ 2.19 (s, 3H, CH_3), 2.31 (s, 6H, (CH_3)$_2$Ar)), 2.95 (s, 6H, (CH$_3$)$_2$N), 6.77 (s, 2H, H_{arom}), 6.82 (s, 1H, H_{arom}); ^{13}C nmr ($CDCl_3$): δ 10.97 (CH_3), 21.20 ((*C*H$_3$)$_2$Ar), 36.63 ((CH$_3$)$_2$N), 101.95 (C5), 119.21, 126.36, 138.68, 153.00 (C_{arom}), 159.09 (C6), 160.58 (C2), 168.02 (C4); HRms (MALDI, peak matching): *m/z* calcd. for $C_{15}H_{19}N_3OCl$ (MH^+) 292.1211, found 292.1213.

4-(3,5-Dimethylphenoxy)-6-methoxy-*N*,*N*,5-trimethyl-pyrimidin-2-amine (17). Sodium (0.93 g, 40.4 mmole) was dissolved in dry methanol (80 mL), followed by addition of 4-chloro-6-(3,5-dimethylphenoxy)-*N*,*N*,5-trimethylpyrimidin-2-amine (**16**) (1.4 g, 4.8 mmole). After refluxing at 80° for 65 hours, the solvent was removed under reduced pressure and water (40 mL) was added to the residue, followed by neutralization by 4 *M* hydrochloric acid. Ether (30 mL) was added and the two layers were separated. The water layer was washed two times with ether (15 mL) and the ether extracts were collected, dried over magnesium sulfate, filtered and evaporated under reduced pressure. The residual material was chromatographed on a column of silica gel using petroleum ether/ether (16:1, v/v) as an eluent to afford compound **17**. Yield: 0.33 g (24%) as a white solid; mp 66-68°; ^{1}H nmr ($CDCl_3$): δ 1.98 (s, 3H, CH_3), 2.29 (s, 6H, (CH_3)$_2$Ar)), 2.97 (s, 6H, (CH$_3$)$_2$N)), 3.92 (s, 3H, OCH_3), 6.75 (s, 3H, H_{arom}); ^{13}C nmr ($CDCl_3$): δ 6.96 (CH_3), 21.23 ((*C*H$_3$)$_2$Ar), 36.47 ((CH$_3$)$_2$N), 53.41 (OCH_3), 88.48 (C5), 118.65, 125.38, 138.45, 154.10 (C_{arom}), 159.21 (C2), 167.69 (C4), 169.52 (C6), HRms (MALDI, peak matching): *m/z* calcd. for $C_{16}H_{22}N_3O_2$ (MH^+) 288.1707, found 288.1713.

4-(2-(Dimethylamino)-6-(3,5-dimethylphenoxy)-5-methylpyrimidine-4-yloxy)benzonitrile (18). To a solution of compound **16** (0.29 g, 1 mmole) and 4-hydroxybenzonitrile (0.13 g, 1.1 mmole) in dry dimethylformamide (9 mL) was added sodium hydride (55% suspension in paraffin oil, 0.052 g, 1.2 mmole) portionwise. After refluxing at 120° for 70 hours, the reaction mixture was quenched by dropwise addition of water (2 mL). After addition of water (35 mL) and ether (25 mL), the two layers were separated and the water layer was extracted two times with ether (25 mL). The ether extracts were collected, dried over magnesium sulfate, filtered and evaporated under reduced pressure. The residual material was chromatographed on a silica gel column using petroleum ether/ether (5:1, v/v) as an eluent to afford compound **18**. Yield: 0.11 g (29%) as a white solid; mp 105-107°; ^{1}H nmr ($CDCl_3$): δ 2.13 (s, 3H, CH_3), 2.32 (s, 6H, (CH_3)$_2$Ar), 2.80 (s, 6H, (CH$_3$)$_2$N), 6.80 (s, 3H, H_{arom}), 7.27 (d, 2H, J = 8.9 Hz, H_{arom}), 7.65 (d, 2H, J = 8.9 Hz, H_{arom}); ^{13}C nmr ($CDCl_3$): δ 7.22 (CH_3), 21.22 ((*C*H$_3$)$_2$Ar), 36.31 ((CH$_3$)$_2$N), 89.69 (C5), 107.54 (C-CN), 118.80 (CN), 119.09, 122.10, 126.05, 133.23, 138.60, 153.40, 157.53 (C_{arom}), 158.76 (C2), 167.45 (C4), 169.15 (C6); HRms (MALDI, peak matching): *m/z* calcd. for $C_{22}H_{23}N_4O_2$ (MH^+), 375.1816, found 375.1805.

Cells and Viruses. MT-4, C8166, and H9/IIIB cells were grown at 37° 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 passaged in MT-4 cells in the presence of efavirenz (up to 2 μM). The Y181C mutant (NIH N119) was derived from an AZT-sensitive clinical isolate passaged initially in CEM and then in MT-4 cells in the presence of nevirapine (10 μM). The double mutant K103N+Y181C (NIH A17) was derived from the IIIB strain passaged in H9 cells in the presence of BI-RG 587 (1 μM). EFV^R, N119, and A17 stock solutions had titers of 4.0×10^7, 1.2×10^8, and 2.1×10^7 $CCID_{50}$/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, EFV^R, N119, and A17 in acutely infected cells was based on inhibition of virus-induced cytopathogenicity in MT-4 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 cytopathogenicity at day 4) was added. After incubation at 37°, cell viability was determined by the 3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) method [17]. 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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REFERENCES AND NOTES

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