Synthesis of Some Novel 2,6-Disubstituted pyridazin-3-ones as TMC120 Analogues

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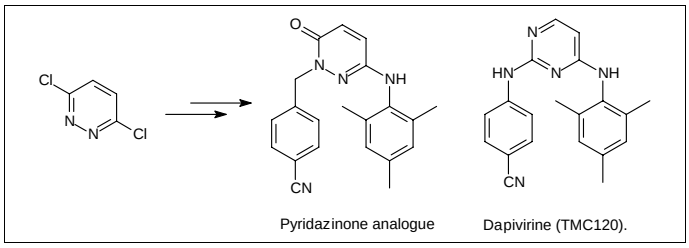

Different analogues of TMC120 derived from pyridazin-3(2*H*)-one rings were synthesized by coupling of 3,6-dichloropyridazine with arylacetonitriles, phenols and/or aniline derivative followed by hydrolysis and alkylation with different benzyl bromide derivatives.

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INTRODUCTION

The non-nucleoside reverse transcriptase inhibitors (NNRTIs) represent a class of structurally diverse, potent and highly-selective anti-HIV agents that were first discovered in the early nineties as a result of large scale compound library screening in antiviral and/or biochemical assays, followed by extensive chemical lead optimization [1–4]. Different chemical classes have been reported to inhibit the reverse transcriptase (RT) enzyme, such as tetrahydroimidazo[4,5,1-*jk*][1,4]-benzodiazepine-2(1*H*)-one (TIBO) [1], 1-[(2-hydroxyethoxy)methyl]-6-(phenylthio)thymine (HEPT) [5,6], α-anilinophenyl-acetamide (α-APA) [7], pyridones [3], imdidazoles [8], imidoyl thiourea (ITU) [9], and finally the diaryltriazine (DATA) and diarylpyrimidine (DAPY) series [10,11] of which dapivirine (TMC120, Figure 1) and etravirine (TMC125, Figure 1) are representatives. TMC125 [12,13], was the first NNRTI demonstrating a beneficial effect on HIV-infected patients with NNRTI-resistant viruses. TMC125 is currently in phase IIB clinical trials. Heeres *et al* [14] have reported novel analogues of etravirine derived from N-methylated pyrazinone rings which are different from the pyrimidine ring of etravirine as shown in Figure 1. Pyrazinone analogues have also shown a significant activity against HIV-1, both wild type and mutants resistant to nucleoside and non-nucleoside RT inhibitors [14].

From this point of view, we report a study on the synthesis and biological evaluation of some pyridazinone derivatives of etravirine and dapivirine.

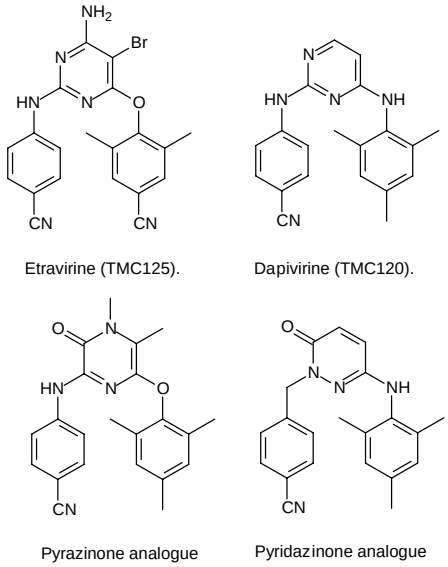


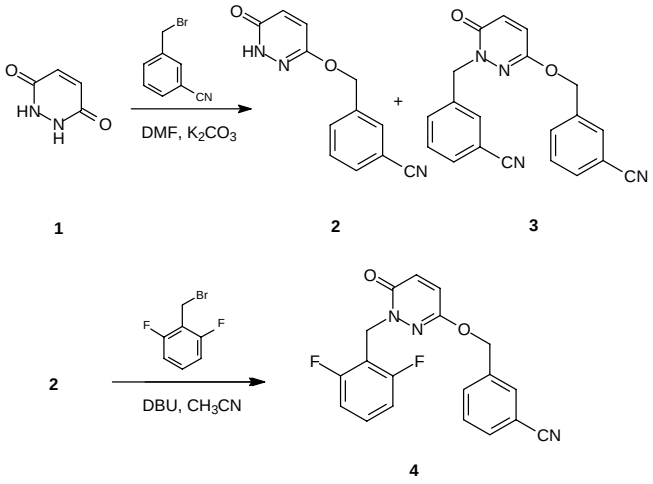
Figure 1

RESULTS AND DISCUSSION

On treatment of the potassium salt of the commercially available pyridazine-3,6-dione (**1**) in dimethylformamide with 3-cyanobenzyl bromide, *O*-monoalkylated product **2** and *O*,*N*-bisalkylated product **3** were isolated from the reaction by column chromatography. Compound **3** was used as a reference to determine *O*- versus *N*-alkylation. The ^{13}C nmr spectrum showed an *N*-substituted benzylic carbon at 52.86 ppm and an *O*-substituted benzylic carbon at 66.96 ppm which is in agreement with the ^{13}C

nmr spectrum of 2-benzyl-4,5-diiodopyridazin-3(2*H*)-one [15]. The DBU salt of compound **2** was coupled with 2,6-diflourobenzyl bromide in dry acetonitrile as a solvent to afford another *O*,*N*-bisalkylated derivative (**4**) (Scheme I).

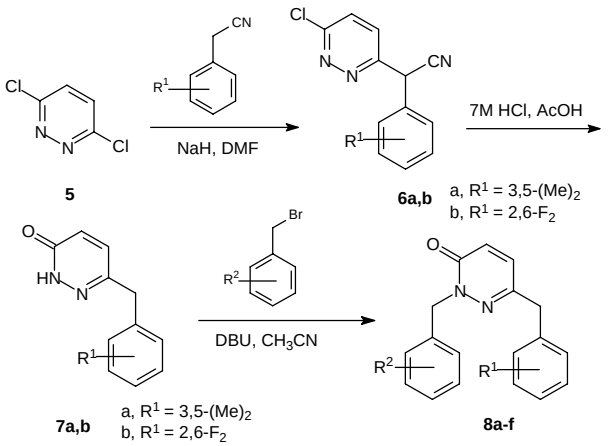
Scheme I



1 2 3 4

3,6-Dichloropyridazine (**5**) was treated with the sodium salt of benzyl cyanide derivatives to afford (aryl)(3-chloropyridazin-6-yl)acetonitriles (**6a,b**) which were hydrolyzed in a mixture of acetic acid and 7 *M* HCl to furnish 6-arylmethylpyridazin-3(2*H*)-ones (**7a,b**). Alkylation of the DBU salt of compounds **7a,b** in acetonitrile with benzyl bromide derivatives afforded 2,6-bis(arylmethyl)pyridzin-3(2*H*)-ones (**8a-f**) (Scheme II).

Scheme II



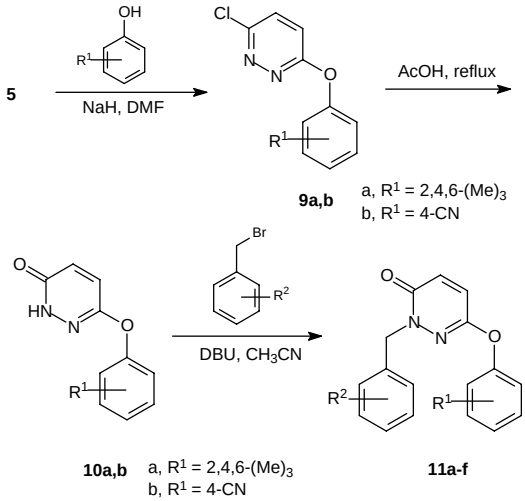
5 **6a,b** a, R^1 = 3,5-$(Me)_2$; b, R^1 = 2,6-F_2

7a,b a, R^1 = 3,5-$(Me)_2$; b, R^1 = 2,6-F_2 **8a-f**

8	R^1	R^2	8	R^1	R^2
a	3,5-$(Me)_2$	3-CN	**d**	2,6-F_2	3-CN
b	3,5-$(Me)_2$	4-CN	**e**	2,6-F_2	4-CN
c	3,5-$(Me)_2$	2,6-F_2	**f**	2,6-F_2	2,4,6-$(Me)_3$

3,6-Dichloropyridazine was reacted with sodium phenoxide derivatives in DMF to afford the 3-aryloxy-6-chloropyridazines (**9a,b**) which were hydrolyzed under mild conditions in refluxing glacial acetic acid to give 6-aryloxypyridazin-3(2*H*)-ones (**10a,b**). Coupling of compounds **10a,b** with benzyl halide derivatives furnished 2-arylmethyl-6-aryloxypyridazin-3(2*H*)-ones (**11a-e**) which are considered to be TMC120 analogues.

Scheme III



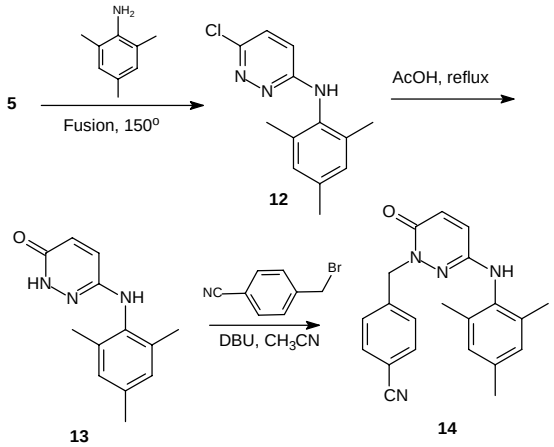
5 **9a,b** a, R^1 = 2,4,6-$(Me)_3$; b, R^1 = 4-CN

10a,b a, R^1 = 2,4,6-$(Me)_3$; b, R^1 = 4-CN **11a-f**

11	R^1	R^2	11	R^1	R^2
a	2,4,6-$(Me)_3$	3-CN	**d**	2,4,6-$(Me)_3$	2,4,6-$(Me)_3$
b	2,4,6-$(Me)_3$	4-CN	**e**	4-CN	2,4,6-$(Me)_3$
c	2,4,6-$(Me)_3$	2,6-F_2			

3,6-Dichloropyridazine was fused under nitrogen with 2,4,6-trimethylaniline to afford 6-chloro-*N*-mesityl-pyridazin-3-amine (**12**) which was hydrolyzed by refluxing in glacial acetic acid to give 6-(mesitylamino)-pyridazin-3(2*H*)-one (**13**). 4-{[3-(Mesitylamino)-6-oxo-pyridazin-

Scheme IV



5 **12**

13 **14**

1(6*H*)-yl]methyl}benzonitrile (**14**, TMC120 analogue) was prepared by reaction of the DBU salt of compound **13** in acetonitrile with 4-cyanobenzyl bromide.

All the compounds were evaluated for their activity against HIV-1 wild type and its NNRTI-resistant mutants. Only compound **4** showed moderate activity against HIV-1 wild type (EC_{50} = 8 µM; CC_{50} = 40 µM). All other compounds were inactive at the maximum concentration tested (100 µM).

EXPERIMENTAL

Nmr Spectra were recorded on a Varian Gemini 2000 nmr 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 were carried out at Chemical Laboratory II at University of Copenhagen, Denmark.

Synthesis of Compounds 2 and 3. Pyridazine-3,6-dione (**1**, 5.6 g, 0.05 mole) was added portionwise to a solution of K_2CO_3 (7 g, 0.05 mole) in dry DMF (50 ml) and the solution was stirred for 0.5 hour. 3-Cyanobenzyl bromide (9.8 g, 0.05 mole) was added to the reaction mixture and stirred for 12 hours. The reaction mixture was poured on ice-cold water (150 ml) with stirring and the solid product formed was filtered off, dried and chromatographed on a column of silica gel using CH_2Cl_2: petroleum ether (1:1, v/v) as an eluent to give **2** and **3**.

3-{[(6-Oxo-1,6-dihydropyridazin-3-yl)oxy]methyl}benzonitrile (**2**). Yield 2.1 g (19%); mp 198-200°; ^{1}H nmr (DMSO-d_6) δ: 5.21 (s, 2H, CH_2), 6.92 (d, 1H, *J* = 9.9 Hz, H5), 7.26 (d, 1H, *J* = 9.9 Hz, H-4), 7.62 (t, 1H, *J* = 7.8 Hz, H_{arom}), 7.79-7.84 (m, 2H, H_{arom}), 7.93 (s, 1H, H_{arom}), 12.26 (s, 1H, NH); ^{13}C nmr (DMSO-d_6) δ: 66.72 (CH_2), 118.54 (CN), 111.33, 127.50, 129.57, 131.25, 132.57, 137.98 (C_{arom}), 131.66 (C4), 133.16 (C5), 152.11 (C3), 159.54 (C6); EI ms: *m/z* 227 (30%, M^+), 116 (100%). *Anal*. Calcd. for $C_{12}H_9N_3O_2$ (227.22): C, 63.43; H, 3.99; N, 18.49. Found: C, 63.12; H, 3.85; N, 18.31.

3-({[1-(3-Cyanobenzyl)-6-oxo-1,6-dihydropyridazin-3-yl]-oxy}methyl)benzonitrile (**3**). Yield 3.6 g (21%); mp 122-124°; ^{1}H nmr (DMSO-d_6) δ: 5.16 (s, 2H, CH_2N), 5.24 (s, 2H, CH_2O), 7.00 (d, 1H, *J* = 9.7 Hz, H5), 7.32 (d, 1H, *J* = 9.7 Hz, H-4), 7.50-7.90 (m, 8H, H_{arom}); ^{13}C nmr (DMSO-d_6) δ: 52.86 (CH_2N), 66.96 (CH_2O), 118.49 (2CN), 111.31, 111.37, 127.28, 129.48, 129.61, 131.18, 131.30, 131.38, 132.62, 132.68, 137.77, 138.17 (C_{arom}), 131.71 (C4), 133.06 (C5), 151.67 (C3), 158.03 (C6); EI ms: *m/z* 342 (36%, M^+), 116 (100%). *Anal*. Calcd. for $C_{20}H_{14}N_4O_2 \cdot 0.8H_2O$ (357.78): C, 67.14; H, 4.68; N, 15.66. Found: C, 67.17; H, 4.23; N, 15.59.

Synthesis of 3-({[1-(2,6-difluorobenzyl)-6-oxo-1,6-dihydropyridazin-3-yl]oxy}methyl) benzonitrile (**4**). Sodium hydride (0.05 g, 1 mmole, 55% susp. in paraffin oil) was added portion wise at room temperature to a stirred solution of compound **2** (0.23 g, 1 mmole) and 2,6-diflourobenzyl bromide (0.21 g, 1 mmole) in dry dimethylformamide (10 ml). The reaction mixture was stirred at room temperature for 1 hour then poured on ice-cold water (50 ml) and the solid product formed was collected by filtration, dried to give 240 mg of the pure compound **4**; yield 68%; mp 133-135°; ^{1}H nmr (DMSO-d_6) δ: 5.09 (s, 2H, CH_2N), 5.21 (s, 2H, CH_2O), 7.01-7.80 (m, 9H, H_{arom}, H-4 and H5); ^{13}C nmr (DMSO-d_6) δ: 40.93 (CH_2N), 66.59 (CH_2O), 111.36 (dd, *J* = 7.9, 16.9 Hz, C_{arom}), 118.49 (CN), 112.82 (t, *J* = 20.1 Hz, C_{arom}), 111.42, 126.91, 129.47, 131.17, 131.67, 137.58 (C_{arom}), 130.57 (t, *J* = 10.3 Hz, C_{arom}), 132.48 (C4), 132.79 (C5), 151.14 (C3), 157.65 (C6), 161.11 (dd, *J* = 7.6, 234.7 Hz, C_{arom}); EI ms: *m/z* 353 (39%, M^+), 127 (100%). *Anal*. Calcd. for $C_{19}H_{13}F_2N_3O_2$ (353.32): C, 64.59; H, 3.71; N, 11.89. Found: C, 64.18; H, 4.10; N, 11.25.

General Procedure for the Synthesis of (aryl)(3-chloropyridazin-6-yl)acetonitriles 6a,b. To a stirred solution of 3,6-dichloropyridazine (**5**, 1.49 g, 0.01 mole) and benzyl cyanide derivatives (3,5-dimethylbenzyl cyanide and/or 2,6-diflourobenzyl cyanide) (0.01 mole) in dry DMF (25 ml) sodium hydride (0.92 g, 0.021 mole, 55% susp. in paraffin oil) was added portionwise at 0º. The reaction mixture is allowed to reach room temperature gradually with stirring, then poured on ice-cold water (100 ml) with stirring and the precipitated material was collected by filtration, dried to give compounds **6a,b**.

(6-Chloropyridazin-3-yl)(3,5-dimethylphenyl)acetonitrile (**6a**). Yield 2.5 g (97%); mp 108-110°; ^{1}H nmr (DMSO-d_6) δ: 2.27 (s, 6H, (CH_3)$_2$Ar), 6.11 (s, 1H, CH-CN), 7.01 (s, 1H, H_{arom}), 7.08 (s, 2H, H_{arom}), 7.81 (d, 1H, *J* = 9.0 Hz, H-4), 7.95 (d, 1H, *J* = 9.0 Hz, H5); ^{13}C nmr (DMSO-d_6) δ: 20.73 (2 x CH_3), 41.27 (*C*H-CN), 118.51 (CN), 125.46 (C4), 129.49, 130.07, 138.73 (C_{arom}), 134.08 (C5), 156.17 (C3), 158.21 (C6). HRms (MALDI, peak matching): *m/z* calcd. for $C_{14}H_{13}ClN_3$ (MH^+) 258.0793, found 258.0789. *Anal*. Calcd. for $C_{14}H_{12}ClN_3 \cdot 0.3\ H_2O$ (263.13): C, 63.91; H, 4.83; N, 15.97. Found: C, 63.81; H 4.47; N, 15.65.

(6-Chloropyridazin-3-yl)(2,6-difluorophenyl)acetonitrile (**6b**). Yield 2.1 g (80%); as red crystals; mp 126-128°; ^{1}H nmr (DMSO-d_6) δ: 6.61 (CH-CN), 7.13-7.65 (m, 3H, H_{arom}), 7.88 (d, 1H, *J* = 9.0 Hz, H5), 8.03 (d, 1H, *J* = 9.0 Hz, H-4); ^{13}C nmr (DMSO-d_6) δ: 31.16 (*C*H-CN), 111.98-112.63 (m, C_{arom}), 119.68 (CN), 128.72 (C4), 130.05 (C5), 132.30 (t, *J* = 10.6 Hz, C_{arom}), 156.12 (C3), 156.29 (C6), 159.92 (dd, *J* = 6.7, 251.3 Hz, C_{arom}); HRms (MALDI, peak matching): *m/z* calcd. for $C_{12}H_7ClF_2N_3$ (MH^+) 266.0297, found 266.0293. *Anal*. Calcd. for $C_{12}H_6ClF_2N_3$ (265.65): C, 54.26; H, 2.28; N, 15.82. Found: C, 54.44; H, 2.06; N, 15.49.

Synthesis of 6-arylmethylpyridazin-3(2*H*)-ones 7a,b. Compound **6a,b** (5 mmole) was refluxed in a solution of conc. HCl (20 ml), acetic acid (10 ml), water (10 ml) for 30 hours. The solvents were evaporated under reduced pressure, and then water (20 ml) was added to the residual material and neutralized with saturated solution of sodium bicarbonate. The solid product formed was collected by filtration and dried to give compounds **7a,b**.

6-(3,5-Dimethylbenzyl)pyridazin-3(2*H*)-one (**7a**). Yield 660 mg (62%); mp 120-122°; ^{1}H nmr (DMSO-d_6) δ: 2.23 (s, 6H, (CH_3)$_2$Ar), 3.78 (s, 2H, CH_2Ar), 6.80 (d, 1H, *J* = 9.6 Hz, H5), 6.85 (s, 3H, H_{arom}), 7.28 (d, 1H, *J* = 9.6 Hz, H-4), 12.83 (s, 1H, NH); ^{13}C nmr (DMSO-d_6) δ: 20.81 ((CH_3)$_2$Ar), 39.61 (CH_2Ar), 126.49, 127.99, 137.58, 137.85 (C_{arom}), 129.95 (C4), 134.05 (C5), 146.87 (C3), 160.17 (C6); HRms (MALDI, peak matching): *m/z* calcd. for $C_{13}H_{14}NaN_2O$

(MNa^+) 237.0998, found 237.0999. *Anal*. Calcd. for $C_{13}H_{14}N_2O \cdot 0.1\ H_2O$ (216.07): C, 72.27; H, 6.62; N, 12.96. Found: C, 72.12; H, 6.71; N, 12.57.

6-(2,6-Difluorobenzyl)pyridazin-3(2*H*)-one (**7b**). Yield 1.1 g (98%); mp 130-132°; ^{1}H nmr (DMSO-d_6) δ: 3.98 (s, 2H, CH_2), 6.87 (d, 1H, *J* = 9.9 Hz, H5), 7.11 (t, 2H, *J* = 8.0 Hz, H_{arom}), 7.34-7.44 (m, 2H, H_{arom} and H-4); ^{13}C nmr (DMSO-d_6) δ: 26.61 (CH_2), 111.47 (dd, *J* = 7.8, 17.4 Hz, C_{arom}), 113.13 (t, *J* = 20.1 Hz, C_{arom}), 129.31 (t, *J* = 10.2 Hz, C_{arom}), 130.09 (C4), 133.63 (C5), 144.44 (C3), 160.80 (d, *J* = 8.5, 246.0 Hz, C_{arom}), 160.04 (C6); HRms (MALDI, peak matching): *m/z* calcd. for $C_{11}H_8F_2N_2NaO$ (MNa^+) 245.0497, found 245.0502.

Synthesis of 2,6-bis(arylmethyl)pyridzin-3(2*H*)-ones 8a-f. Compound **7a,b** (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 benzyl halide derivative (3-cyanobenzyl bromide, 4-cyanobenzyl bromide, 2,6-diflourobenzyl bromide and/or 2,4,6-trimethylbenzyl chloride) (1.1 mmole) was added to the reaction mixture at room temperature and stirred for 5 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 **8a-f**.

3-{[3-(3,5-Dimethylbenzyl)-6-oxopyridazin-1(6*H*)-yl]methyl}-benzonitrile (**8a**). Yield 240 mg (72%); mp 74-76°; ^{1}H nmr (DMSO-d_6) δ: 2.22 (s, 6H, 2 x CH_3), 3.82 (s, 2H, CH_2-C3), 5.29 (s, 2H, CH_2-N), 6.83 (s, 2H, H_{arom}), 6.85 (s, 1H, H_{arom}), 6.93 (d, 1H, *J* = 9.6 Hz, H5), 7.33 (d, 1H, *J* = 9.5 Hz, H-4), 7.57-7.79 (m, 4H, H_{arom}); ^{13}C nmr (DMSO-d_6) δ: 20.76 (2 x CH_3), 39.57 (CH_2-C3), 53.11 (CH_2-N), 118.46 (CN), 130.00 (C4), 132.63 (C5), 111.38, 126.34, 128.02, 129.70, 131.14, 131.30, 133.64, 137.49, 137.56, 138.41 (C_{arom}), 147.48 (C3), 158.69 (C6); HRms (MALDI, peak matching): *m/z* calcd. for $C_{21}H_{20}N_3O$ (MH^+) 330.1601, found 330.1611. *Anal*. Calcd. for $C_{21}H_{19}N_3O \cdot 0.5\ H_2O$ (338.41): C, 74.53; H, 5.96; N, 12.58. Found: C, 74.46; H, 5.70; N, 12.37.

4-{[3-(3,5-Dimethylbenzyl)-6-oxopyridazin-1(6*H*)-yl]methyl}-benzonitrile (**8b**). Yield 220 mg (67%); mp 83-85°; ^{1}H nmr (DMSO-d_6) δ: 2.21 (s, 6H, 2 x CH_3), 3.81 (s, 2H, CH_2-C3), 5.32 (CH_2-N), 6.81 (s, 2H, H_{arom}), 6.85 (s, 1H, H_{arom}), 6.93 (d, 1H, *J* = 9.6 Hz, H5), 7.33 (d, 1H, *J* = 9.6 Hz, H-4), 7.45 (d, 2H, *J* = 8.1 Hz, H_{arom}), 7.82 (d, 2H, *J* = 8.1 Hz, H_{arom}); ^{13}C nmr (DMSO-d_6) δ: 20.76 (2 x CH_3), 39.56 (*C*H_2-C3), 53.50 (CH_2-N), 118.58 (CN), 110.24, 126.36, 128.04, 128.45, 132.37, 137.44, 137.58, 142.46 (C_{arom}), 129.98 (C4), 133.67 (C5), 147.50 (C3), 158.73 (C6); EI ms: *m/z* 329 (100%, M^+). *Anal*. Calcd. for $C_{21}H_{19}N_3O \cdot 0.6\ H_2O$ (340.21): C, 74.14; H, 5.98; H, 12.35. Found: C, 73.93; H, 5.68; H, 12.32.

2-(2,6-Difluorobenzyl)-6-(3,5-dimethylbenzyl)pyridazin-3(2*H*)-one (**8c**). Yield 320 mg (95%); mp 88-90°; ^{1}H nmr ($CDCl_3$) δ: 2.25 (s, 6H, (CH_3)$_2$Ar)), 3.73 (s, 2H, CH_2-C3), 5.42 (s, 2H, CH_2-N), 6.72-7.00 (m, 7H, H_{arom}, H-4 and H5), 7.23-7.33 (m, 1H, H_{arom}); ^{13}C nmr ($CDCl_3$) δ: 21.19 [(CH_3)$_2$Ar], 40.75 (*C*H_2-C3), 43.04 (CH_2-N), 111.20 (dd, *J* = 7.7, 17.9 Hz, C_{arom}), 111.84 (t, *J* = 19.2 Hz, C_{arom}), 126.59, 128.52, 137.15, 138.30 (C_{arom}), 129.95 (C4), 132.57 (C5), 129.78 (t, *J* = 10.1 Hz, C_{arom}), 146.94 (C3), 159.51 (C6), 161.92 (dd, *J* = 7.5, 250.5 Hz, C_{arom}); HRms (MALDI, peak matching): *m/z* calcd. for $C_{20}H_{19}F_2N_2O$ (MH^+) 341.1460, found 341.1449. *Anal*. Calcd. for $C_{20}H_{18}F_2N_2O \cdot 0.6\ H_2O$ (351.19): C, 68.40; H, 5.51; N, 7.98. Found: C, 68.43; H, 5.14; N, 7.93.

3-{[3-(2,6-Difluorobenzyl)-6-oxopyridazin-1(6*H*)-yl]methyl}-benzonitrile (**8d**). Yield 280 mg (83%); mp 108-110°; ^{1}H nmr ($CDCl_3$) δ: 3.99 (s, 2H, CH_2-C3), 5.24 (s, 2H, CH_2-N), 6.86 (d, 1H, *J* = 9.5 Hz, H5), 6.94 (t, 2H, *J* = 7.7 Hz, H_{arom}), 7.14 (d, 1H, *J* = 9.5 Hz, H-4), 7.22-7.65 (m, 5H, H_{arom}); ^{13}C nmr ($CDCl_3$) δ: 27.57 (CH_2-C3), 54.05 (CH_2-N), 118.56 (CN), 112.61, 129.27, 131.52, 132.39, 133.35, 137.48 (C_{arom}), 111.40 (dd, *J* = 7.8, 17.3 Hz, C_{arom}), 112.62 (t, *J* = 19.5 Hz, C_{arom}), 129.13 (t, *J* = 10.3 Hz, C_{arom}), 145.12 (C3), 159.33 (C6), 161.31 (d, *J* = 7.8, 256.6 Hz, C_{arom}); HRms (MALDI, peak matching): *m/z* calcd. for $C_{19}H_{14}F_2N_3O$ (MH^+) 338.1099, found 338.1083. *Anal*. Calcd. for $C_{19}H_{13}F_2N_3O \cdot 0.5\ H_2O$ (346.34): C, 65.89; H, 4.07; N, 12.13. Found: C, 65.44; H, 3.46; N, 11.91.

4-{[3-(2,6-Difluorobenzyl)-6-oxopyridazin-1(6*H*)-yl]methyl}-benzonitrile (**8e**). Yield 300 mg (88%); mp 134-136°; ^{1}H nmr (DMSO-d_6) δ: 4.00 (s, 2H, CH_2-C3), 5.21 (s, 2H, CH_2-N), 6.97 (d, 1H, *J* = 10.2 Hz, H5), 7.12 (t, 2H, *J* = 8.0 Hz, H_{arom}), 7.33-7.43 (m, 4H, H_{arom} and H-4), 7.78 (d, 2H, *J* = 8.1 Hz, H_{arom}); ^{13}C nmr (DMSO-d_6) δ: 26.85 (*C*H_2-C3), 53.57 (CH_2-N), 118.56 (CN), 110.26, 128.47, 133.11, 142.06 (C_{arom}), 111.45 (dd, *J* = 7.5, 17.4 Hz, C_{arom}), 112.81 (t, *J* = 20.1 Hz, C_{arom}), 129.44 (t, *J* = 10.5 Hz, C_{arom}), 145.03 (C3), 158.60 (C6), 160.75 (d, *J* = 8.5, 246.0 Hz, C_{arom}); HRms (MALDI, peak matching): *m/z* calcd. for $C_{19}H_{14}F_2N_3O$ (MH^+) 338.1099, found 338.1091.

6-(2,6-Difluorobenzyl)-2-(mesitylmethyl)pyridazin-3(2*H*)-one (**8f**). Yield 300 mg (85%); mp 110-112°; ^{1}H nmr ($CDCl_3$) δ: 2.19 (s, 6H, 2 x CH_3), 2.26 (s, 3H, CH_3), 3.83 (s, 2H, CH_3-C3), 5.23 (s, 2H, CH_2-N), 6.78-6.87 (m, 5H, H_{arom} and H5), 7.08 (d, 1H, *J* = 9.3 Hz, H-4), 7.15-7.21 (m, 1H, H_{arom}); ^{13}C nmr ($CDCl_3$) δ: 19.94 (2 × CH_3), 20.95 (CH_3), 27.49 (*C*H_2-C3), 47.62 (CH_2-N), 111.06 (dd, *J* = 7.8, 17.3 Hz, C_{arom}), 112.90 (t, *J* = 19.9 Hz, C_{arom}), 128.51 (t, *J* = 10.7 Hz, C_{arom}), 128.68, 129.51, 137.16, 138.39 (C_{arom}), 129.73 (C4), 131.48 (C5), 144.06 (C3), 160.82 (dd, *J* = 8.1, 249.8 Hz, C_{arom}), 159.72 (C6); HRms (MALDI, peak matching): *m/z* calcd. for $C_{21}H_{20}F_2NaN_2O$ (MNa^+) 377.1436, found 377.1420. *Anal*. Calcd. for $C_{21}H_{20}F_2N_2O \cdot 0.7\ H_2O$ (367.01): C, 68.73; H, 5.88; N, 7.63. Found: C, 68.53; H, 5.57; N, 7.73.

Synthesis of 3-aryloxy-6-chloropyridazines 9a,b. Sodium hydride (0.48 g, 11 mmole, 55% susp. in paraffin oil) was added portionwise to a solution of compound **5** (1.49 g, 10 mmole) and phenol derivative (2,4,6-trimethylphenol and/or 4-cyanophenol) (10 mmole) in dry DMF (15 ml). The reaction mixture was stirred at room temperature for 3 hours, poured on ice-cold water (40 ml) and the solid product formed was filtered off, washed with water and dried to give compound **9a,b**.

3-Chloro-6-(mesityloxy)pyridazine (**9a**). Yield 2.4 g (97%); mp 114-116°; ^{1}H nmr ($CDCl_3$) δ: 2.08 (s, 6H, (CH_3)$_2$Ar), 2.29 (s, 3H, C*H*$_3$Ar), 6.90 (s, 2H, H_{arom}), 7.09 (d, 2H, *J* = 9.0 Hz, H5), 7.45 (d, *J* = 9.0 Hz, H4); ^{13}C nmr ($CDCl_3$) δ: 16.34 ((CH_3)$_2$Ar), 20.77 (*C*H$_3$Ar), 118.54 (C4), 129.60, 129.85, 131.45, 151.55 (C_{arom}), 135.44 (C6), 164.30 (C3); HRms (MALDI, peak matching): *m/z* calcd. for $C_{13}H_{14}ClN_2O$ (MH^+) 249.0789, found 249.0788.

4-[(6-Chloropyridazin-3-yl)oxy]benzonitrile (**9b**). Yield 460 mg (20%); mp 138-140°; ^{1}H nmr ($CDCl_3$) δ: 7.26 (s, 1 H, *J* = 9.0 Hz, H5), 7.35 (d, 2H, *J* = 8.7 Hz, H_{arom}), 7.57 (d, *J* = 9.0 Hz, H4), 7.73 (d, 2H, *J* = 8.7 Hz, H_{arom}); ^{13}C nmr ($CDCl_3$) δ: 118.25 (CN), 109.32, 121.92, 132.00, 151.53 (C_{arom}), 130.30 (C4), 134.02 (C5), 153.07 (C6), 156.32 (C3); HRms (MALDI, peak matching): *m/z* calcd. for $C_{11}H_7ClN_3O$ (MH^+) 232.0272, Found 232.0280.

Synthesis of 6-aryloxypyridazin-3(2*H*)-ones 10a,b. Compound **9a,b** (3.3 mmole) was refluxed in acetic acid (20 ml)

for 6 hours. The solvent was removed under reduced pressure, water (30 ml) was added and the solid product formed was collected by filtration, washed with water and dried to give compound **10a,b**.

6-(Mesityloxy)pyridazin-3(2*H*)-one (**10a**). Yield 720 mg (95%); mp 166-168°; ^{1}H nmr (DMSO-d_6) δ: 2.04 (s, 6H, 2 × CH_3), 2.23 (s, 3H, CH_3), 6.91 (s, 2H, H_{arom}), 7.00 (d, 1H, *J* = 9.9 Hz, H5), 7.45 (d, 1H, *J* = 9.9 Hz, H4), 12.03 (bs, 1H, NH); ^{13}C nmr (DMSO-d_6) δ: 15.84 (2 x CH_3), 20.24 (CH_3), 126.89, 129.08, 133.91, 151.80 (C_{arom}), 129.53 (C4), 134.36 (C5), 147.34 (C3), 159.53 (C6); HRms (MALDI, peak matching): *m/z* calcd. for $C_{13}H_{15}N_2O_2$ (MH^+) 231.1128, found 231.1130.

4-[(6-Oxo-1,6-dihydropyridazin-3-yl)oxy]benzonitrile (**10b**). Yield 400 mg (56%); mp 162-164°; ^{1}H nmr (DMSO-d_6) δ: 7.05 (d, 1H, *J* = 9.9 Hz, H5), 7.40 (d, 2H, *J* = 8.7 Hz, H_{arom}), 7.45 (d, 1H, *J* = 9.9 Hz, H4), 7.91 (d, *J* =8.7 Hz, H_{arom}); ^{13}C nmr (DMSO-d_6) δ: 118.38 (CN), 107.24, 120.97, 133.98, 151.45 (C_{arom}), 128.06 (C4), 134.33 (C5), 157.28 (C6), 159.75 (C3); HRms (MALDI, peak matching): *m/z* calcd. for $C_{11}H_7N_3O_2$ (MH^+) 214.0611, found 214.0616.

Synthesis of 2-arylmethyl-6-aryloxypyridazin-3(2*H*)-ones 11a-e. Compound **10a,b** (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 benzyl halide derivative (3-cyanobenzyl bromide, 4-cyanobenzyl bromide, 2,6-diflourobenzyl bromide and/or 2,4,6-trimethylbenzyl chloride) (1.1 mmole) was added to the reaction mixture at room temperature and stirred for 5 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 **11a-e**.

3-{[3-(Mesityloxy)-6-oxopyridazin-1(6*H*)-yl]methyl}benzonitrile (**11a**). Yield 280 mg (80%); mp 90-92°; ^{1}H nmr ($CDCl_3$) δ: 2.01 (s, 6H, 2 x CH_3), 2.33 (s, 3H, CH_3), 5.00 (s, 2H, CH_2), 6.92 (s, 2H, H_{arom}), 6.99 (d, 1H, *J* = 9.9 Hz, H5), 7.14 (d, 1H, *J* = 9.9 Hz, H4), 7.34-7.56 (m, 4H, H_{arom}); ^{13}C nmr ($CDCl_3$) δ: 16.13 ((CH_3)$_2$Ar), 20.76 (CH_3Ar), 52.93 (CH_2), 118.40 (CN), 112.47, 125.93, 129.19, 129.38, 129.70, 132.56, 133.52, 135.29, 137.36, 151.80 (C_{arom}), 131.49 (C4), 133.73 (C5), 147.45 (C3), 158.61 (C6); HRms (MALDI, peak matching): *m/z* calcd. for $C_{21}H_{20}N_3O_2$ (MH^+) 346.1550, found 346.1540.

4-{[3-(Mesityloxy)-6-oxopyridazin-1(6*H*)-yl]methyl}benzonitrile (**11b**). Yield 260 mg (75%); mp 100-102°; ^{1}H nmr ($CDCl_3$) δ: 2.01 (s, 6H, 2 x CH_3), 2.31 (s, 3H, CH_3), 5.03 (CH_2), 6.88 (s, 2H, H_{arom}), 6.99 (d, 1H, *J* = 9.8 Hz, H5), 7.14 (s, 1H, *J* = 9.8 Hz, H4), 7.31 (d, 2H, *J* = 8.1 Hz, H_{arom}), 7.5 (d, 2H, *J* = 8.1 Hz, H_{arom}); ^{13}C nmr ($CDCl_3$) δ: 16.18 (2 x CH_3), 20.74 (CH_3), 53.54 (CH_2), 118.57 (CN), 111.66, 125.92, 129.24, 129.50, 132.10, 135.15, 141.08, 151.77 (C_{arom}), 129.80 (C4), 133.72 (C5), 147.47 (C3), 158.66 (C6); HRms (MALDI, peak matching): *m/z* calcd. for $C_{21}H_{20}N_3O_2$ (MH^+) 346.1550, found 346.1540.

2-(2,6-Difluorobenzyl)-6-(mesityloxy)pyridazin-3(2*H*)-one (**11c**). Yield 280 mg (79%); mp 78-80°; ^{1}H nmr ($CDCl_3$) δ: 1.90 (s, 6H, 2 x CH_3), 2.27 (s, 3H, CH_3), 5.12 (s, 2H, CH_2), 6.68 (t, 2H, *J* = 7.8 Hz, H_{arom}), 6.73 (s, 2H, H_{arom}), 6.98 (d, 1H, *J* = 9.9 Hz, H5), 7.10-7.19 (m, 2H, H4 and H_{arom}); ^{13}C nmr ($CDCl_3$) δ: 15.92 (2CH_3), 20.71 (CH_3), 41.98 (CH_2), 110.75 (dd, *J* = 7.9, 17.5 Hz, C_{arom}), 111.39 (C5), 129.54 (t, *J* = 10.3 Hz, C_{arom}), 125.51, 128.93, 133.25, 151.21, (C_{arom}), 129.80 (C4), 134.55 (C5), 147.49 (C3), 158.77 (C6), 160.07 (d, *J* = 7.4 Hz, C_{arom}), 163.39 (d, *J* = 7.6 Hz, C_{arom}); HRms (MALDI, peak matching): *m/z* calcd. for $C_{20}H_{19}F_2N_2O_2$ (MH^+) 357.1409, found 357.1399. *Anal*. Calcd. for $C_{20}H_{18}F_2N_2O_2 \cdot 0.25H_2O$ (360.88): C, 66.57; H, 5.24; N, 7.76. Found: C, 66.84; H, 5.21; N, 7.39.

2-(2,4,6-Trimethylbenzyl)-6-(2,4,6-trimethylphenoxy)-2*H*-pyridazin-3-one (**11d**). Yield 280 mg (77%); mp 86-88°; ^{1}H nmr ($CDCl_3$) δ: 1.72 (s, 6H, 2 x CH_3), 1.92 (s, 6H, 2 x CH_3), 2.26 (s, 3H, CH_3), 2.30 (s, 3H, CH_3), 5.04 (CH_2), 6.64 (s, 2H, H_{arom}), 6.69 (s, 2H, H_{arom}), 6.98 (d, 1H, *J* = 9.8 Hz, H5), 7.08 (d, 1H, *J* = 9.8 Hz, H4); ^{13}C nmr ($CDCl_3$) δ: 15.77 (2 x CH_3), 19.56 (2 x CH_3), 20.71 (CH_3), 20.90 (CH_3), 46.25 (CH_2), 124.83, 128.40, 128.80, 128.91, 133.18, 137.03, 138.31, 151.02 (C_{arom}), 129.92 (C4), 134.50 (C5), 147.55 (C3), 158.79 (C6); HRms (MALDI, peak matching): *m/z* calcd. for $C_{23}H_{26}NaN_2O_2$ (MNa^+) 385.1887, found 385.1880. *Anal*. Calcd. for $C_{23}H_{26}N_2O_2$ (362.46): C, 73.30; H, 7.38; N, 7.43. Found: C, 73.25; H, 7.41; N, 7.31.

4-{[1-(Mesitylmethyl)-6-oxo-1,6-dihydropyridazin-3-yl]-oxy}benzonitrile (**11e**). Yield 260 mg (75%); mp 108-110°; ^{1}H nmr ($CDCl_3$) δ: 2.10 (s, 6H, 2 x CH_3), 2.32 (s, 3H, CH_3), 5.15 (s, 2H, CH_2), 6.80 (s, 2H, H_{arom}), 6.91 (d, 2H, *J* = 8.7 Hz, H_{arom}), 7.02-7.10 (m, 2H, H4 and H5), 7.46 (d, 2H, *J* = 8.7 Hz, H_{arom}); ^{13}C nmr ($CDCl_3$) δ: 19.81 (2 x CH_3), 20.93 (CH_3), 46.71 (CH_2), 118.36 (CN), 108.16, 121.22, 125.67, 129.39, 133.35, 137.65, 138.22, 150.66 (C_{arom}), 128.76 (C4), 133.44 (C5), 156.13 (C6), 158.75 (C3); HRms (MALDI, peak matching): *m/z* calcd. for $C_{21}H_{19}NaN_3O_2$ (MNa^+) 368.1370, found 368.1373.

Synthesis of 6-chloro-*N*-mesitylpyridazin-3-amine (**12**). Under stream of nitrogen, compound **5** (1.49 g, 10 mmole) was fused with 2,4,6-trimethylaniline (1.48 g, 11 mmole) at 150 °C for 2.5 hours. The reaction mixture was left to reach 70 °C, then ethanol (10 ml) was added and the mixture was stirred with a spatula until obtaining a suspension. The solid product formed was collected by filtration, dried and chromatographed on a column of silica gel using CH_2Cl_2:EtOAc (10:1, v/v) as an eluent to give 2 g of the pure compound **12**; yield 81%; mp 180-182°; ^{1}H nmr ($CDCl_3$) δ: 2.17 (s, 6H, 2 x CH_3), 2.31 (s, 3H, CH_3), 6.25 (d, 1H, *J* = 9.3 Hz, H4), 6.77 (bs, 1H, NH), 7.12 (d, 1H, *J* = 9.3 Hz, H5); ^{13}C nmr ($CDCl_3$) δ: 18.16 (2 x CH_3), 220.91 (CH_3), 113.91 (C4), 129.50, 129.55, 136.57, 137.52 (C_{arom}), 131.88 (C5), 146.97 (C6), 159.00 (C3); EI ms: *m/z* 247 (17%, M^+), 232 (100%). *Anal*. Calcd. for $C_{13}H_{14}ClN_3$ (247.72): C, 63.03; H, 5.70; N, 16.96. Found: C, 63.20; H, 5.46; N, 16.75.

Synthesis of 6-(mesitylamino)pyridazin-3(2*H*)-one (**13**). Compound **12** (5 mmole) was refluxed in acetic acid (20 ml) for 6 hours. The solvent was removed under reduced pressure, water (30 ml) was added and the solid product formed was collected by filtration, washed with water and dried to give compound **13**; yield 460 mg (40%); mp 212-214°; ^{1}H nmr (DMSO-d_6) δ: 2.10 (s, 6H, 2 x CH_3), 2.21 (s, 3H, CH_3), 6.76 (d, 1H, *J* = 9.6 Hz, H5), 6.86 (s, 2H, H_{arom}), 7.12 (d, 1H, *J* = 9.6 Hz, H4), 7.69 (s, 1H, NH), 11.63 (bs, 1H, NH); ^{13}C nmr (DMSO-d_6) δ: 18.03 (2 x CH_3), 20.41 (CH_3), 127.38, 128.40, 134.32, 134.53 (C_{arom}), 131.25 (C4), 134.10 (C5), 146.29 (C3), 158.72 (C6); HRms (MALDI, peak matching): *m/z* calcd. for $C_{13}H_{16}N_3O$ (MH^+) 230.1288, found 230.1286.

Synthesis of 4-{[3-(mesitylamino)-6-oxopyridazin-1(6*H*)-yl]-methyl}benzonitrile (**14**). Compound **13** (0.23 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 4-cyanobenzyl bromide (0.215 g, 1.1 mmole) was added to the reaction mixture at room temperature and stirred for 5 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, dried and purified by column chromatography of silica gel using CH_2Cl_2:EtOAc (10:1, v/v) as an eluent to give 100 mg of compound **14**; yield 30%; mp 154-156°; ^{1}H nmr ($CDCl_3$) δ: 2.12 (s, 6H, 2 x CH_3), 2.30 (s, 3H, CH_3), 5.14 (s, 2H, C*H*$_2$Ar), 5.70 (s, 1H, NH), 6.66 (d, 1H, *J* = 9.9 Hz, H5), 6.81 (d, 1H, *J* = 9.9 Hz, H4), 6.91 (s, 2H, H_{arom}), 7.41 (d, 2H, *J* = 8.4 Hz, H_{arom}), 7.56 (d, 2H, *J* = 8.4 Hz, H_{arom}); ^{13}C nmr ($CDCl_3$) δ: 18.29 (2 x CH_3), 20.86 (CH_3), 53.82 (CH_2), 118.73 (CN), 111.35, 124.68, 129.23, 132.17, 132.71, 136.08, 136.83, 141.95 (C_{arom}), 129.09 (C4), 131.98 (C5), 146.98 (C3), 158.21 (C6); HRms (MALDI, peak matching): *m/z* calcd. for $C_{21}H_{21}N_4O$ (MH^+) 345.1710, found 345.1723.

Antiviral Assay Procedures

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

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 x 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 x 10^7, 1.2 x 10^8, and 2.1 x 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 cytopathicity in MT-4 cells. Briefly, an amount of 50 μl of culture medium containing 1 x 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^o, cell viability was determined by the 3-(4,5-dimethyl-thiazol-1-yl)-2,5-diphenyltetrazolium bromide (MTT) method [16]. 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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