Synthesis and Anti-HIV-1 Activity of New Delavirdine Analogues Carrying Arylpyrrole Moieties

Gérard Aimè Pinna,*[,a] Giovanni Loriga,[a] Gabriele Murineddu,[a] Giuseppe Grella,[a] Massimo Mura,[b] Laura Vargiu,[b] Chiara Murgioni,[b] and Paolo La Colla[b]

Dipartimento Farmaco Chimico Tossicologico, Università di Sassari,[a] Via F. Muroni 23, 07100 Sassari, Sardinia, Italy and Dipartimento di Biologia Sperimentale, Università di Cagliari,[b] Cittadella Universitaria, 09042 Monserrato, Cagliari, Sardinia, Italy. Received May 11, 2001; accepted August 2, 2001

In our search for novel anti-human immunodeficiency virus (HIV)-1 agents, 14 delavirdine analogues were synthesized and evaluated as potential anti-HIV-1 agents in cell-based assays. Compound 1Aa exhibited potent and selective anti-HIV-1 activity in acutely infected MT4 cells, with effective concentration (EC_{50}) values in the submicromolar range.

Key words delavirdine analogue; arylpyrrole motif synthesis; anti-human immunodeficiency virus (HIV) activity

Acquired immunodeficiency syndrome (AIDS) is the result of an infection by the human immunodeficiency virus (HIV)-1. This retrovirus shows a specific tropism for the helper/inducer T cells, leading to their depletion. The resultant profound immunosuppression predisposes patients to life-threatening opportunistic infections.[1] The current most successful strategy to HIV therapy is treatment with a combination of two types of anti-HIV-1 agents: HIV-1 reverse transcriptase inhibitors (RTIs) and protease inhibitors.[2,3] The RTIs can be further subdivided into nucleoside (NI) and non-nucleoside RT inhibitors (NNRTI).[4] Among NNRTIs,[5] the discovery of the bis(heteroaryl)piperazine (BHAP) class has led to the development of the clinical candidates atevirdine and delavirdine. The latter as mesylate salt is currently marketed under the name Rescriptor®. Unfortunately, clinical evidence has demonstrated limited long-term efficacy for this agent even in combinations, due to the appearance of drug resistant mutant viruses[6] and because of short serum half-life[7] ($t_{1/2}$=5.8 h).

To circumvent these therapeutic difficulties, the discovery of new delavirdine analogues with enhanced activity and/or metabolic stability is actively pursued. Therefore, following molecular simplification as a criterion of rational drug design, we synthesized simple bis(heteroaryl)piperazinyl derivatives of the general structure reported in Chart 1.

Chemistry

Title delavirdine analogues **1** were prepared in a convergent manner as shown in Chart 2.

The acids **2**—**9** were reacted, with a stoichiometric amount of *N*-isopropyl-*N*-(2-piperazine-3-pyridyl)amine (**10**), in the presence of 1,1′-carbonyldiimidazole (CDI) or 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC), to give intermediates **11**—**16** and the target compounds **1Ag** and **1Bg** in low to moderate yields. Anilino-derived compounds **1Ad**—**f** and **1Bd**—**f** were prepared from intermediates **11**—**13** by hydrogenation with H_2 and 10% Pd–C, or from **14**—**16** by transfer hydrogenation with $HCOONH_4$ and 10% Pd–C. Finally, treatment of anilino compounds with methansulfonyl chloride in pyridine gave the mesylates **1Aa**—**c** and **1Ba**—**c**.

Carboxylic acids **2**—**9** were prepared according to Charts 3 and 4. The condensation reaction of β arylacroleins **17**—**20** with methyl azidoacetate and sodium methoxide gave azides **21**—**24**. The pyrroles **25**—**27** and **28**[8] were prepared by thermal cyclization of the azides **21**—**24** using boiling xylene. Alkaline hydrolysis of these pyrroles afforded 5-aryl-1*H*-2-pyrrolcarboxylic acids **2**—**5** (Chart 3).

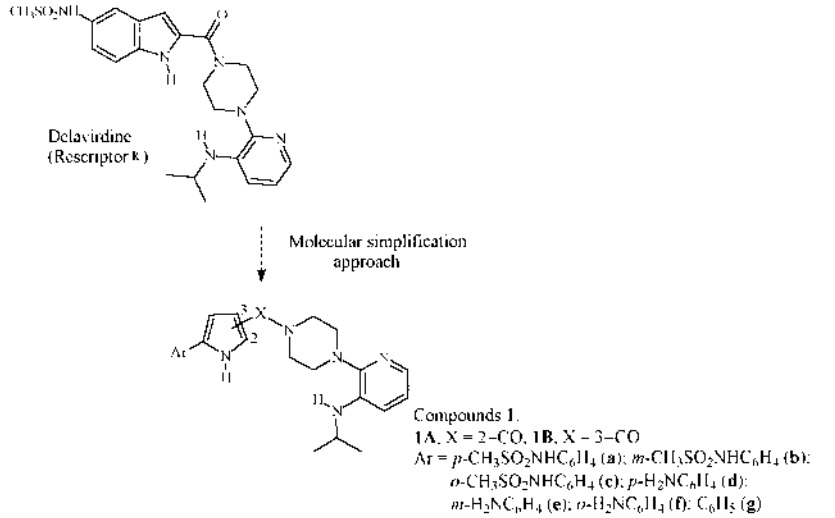
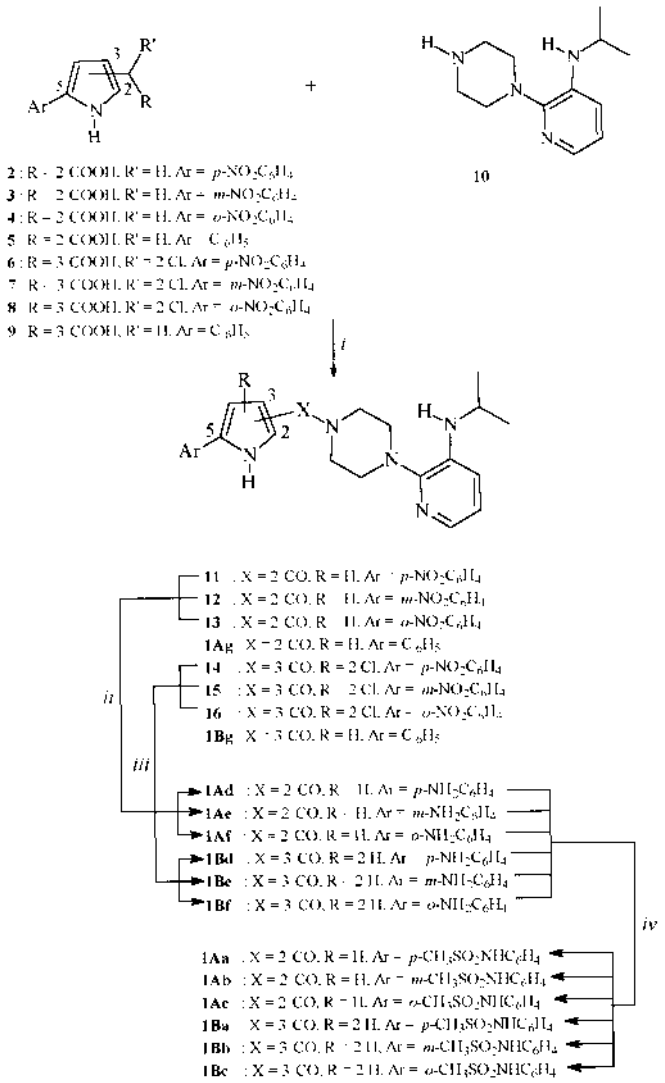


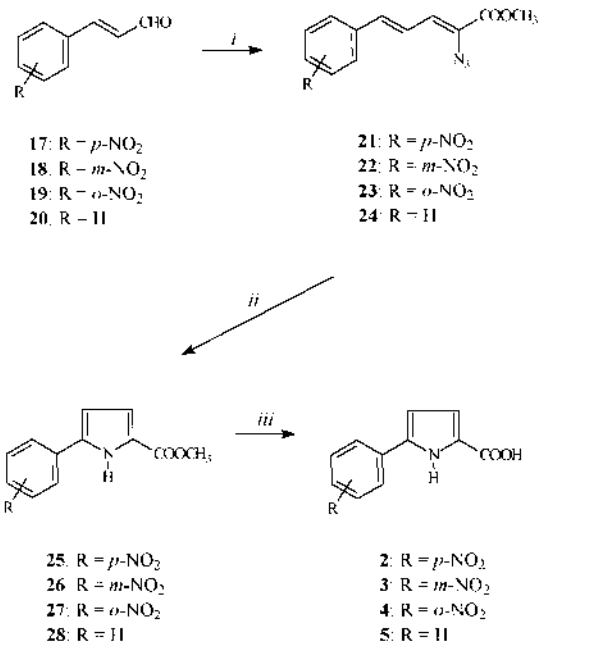
Chart 1

* To whom correspondence should be addressed. e-mail: pinlab@uniss.it



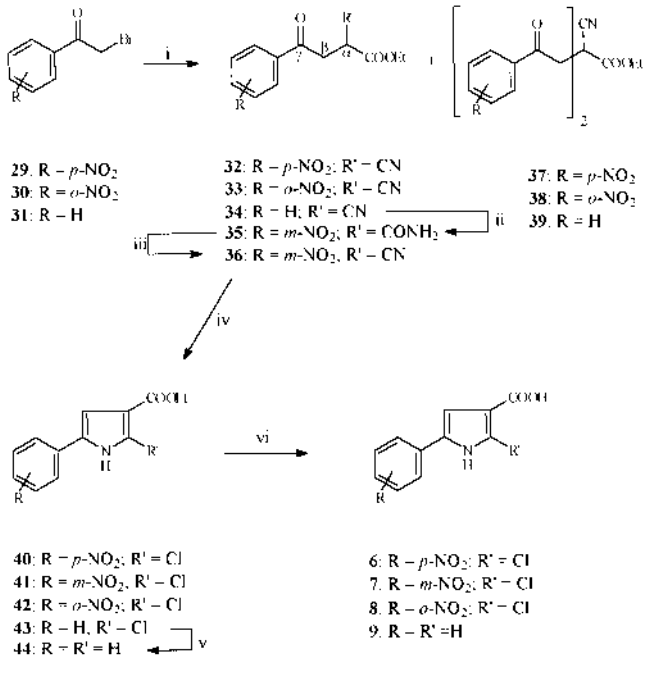
Reagents: (i) CDI or EDC in THF; (ii) 10% Pd/C, EtOH; (iii) HCO_2NH_4, 10% Pd/C, MeOH; (iv) CH_3SO_2Cl, pyridine in CH_2Cl_2.

Chart 2



Reagents: (i) N_3–CH_2COOCH_3, CH_3ONa; (ii) xylene; (iii) KOH/H_2O 12%.

Chart 3



Reagents: (i) NC–CH_2COOEt, K_2CO_3; (ii) H_2SO_4 conc., KNO_3; (iii) $POCl_3$, CH_2Cl_2; (iv) HCl gas, Et_2O; (v) $HCOONH_4$ or H_2, MeOH, 10% Pd/C; (vi) KOH/H_2O 12%.

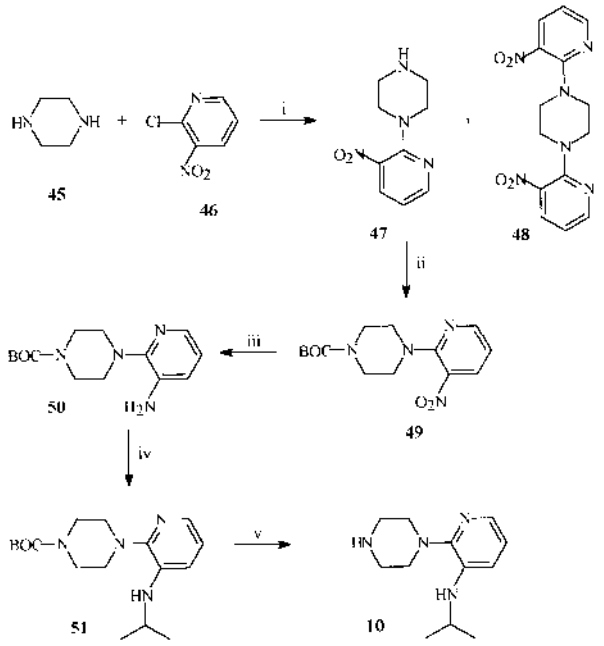
Chart 4

The arylpyrroles **40**—**43** were prepared by 5-*exo*-*trig* cyclization[9] of the α-cyano-γ-keto esters **32**—**34**, **36** with gaseous HCl in Et_2O. Pyrrole **44** was synthesized by hydride hydrogenolysis of **43** using ammonium formate in the presence of palladium on carbon. The α-cyano-γ-keto esters **32**,[10] **33** and **34**[10] were obtained together with the bis alkylating products **37**—**39** by alkylation of ethyl cyanoacetate with known or commercially available bromides **29**,[11] **30**,[11] **31** and K_2CO_3 in acetone. Interestingly, nitration of **34** with potassium nitrate in concentrate sulphuric acid produced the *m*-nitrophenyl-γ-keto-α-carbamoyl-ester which was dehydrated to give the desired cyanoketoester **36**. Final alkaline hydrolysis of **40**—**44** afforded the acids **6**—**9**.

The other key intermediate, the pyridylpiperazine **10** was prepared in a fashion similar to that reported previously[12] and it is shown in Chart 5. However, we optimized the nucleophilic aromatic substitution as follows: the 2-chloro-3-nitropyridine **46** was reacted with 10 eq of piperazine in the presence of diisopropylethylamine furnishing a mixture of 1-(3-nitro-2-pyridyl)piperazine **47**[13] (60%) and of bis-substituted derivative **48**[14] (15%), which were resolved by Al_2O_3 chromatography eluting with $CHCl_3$. Protection of the remaining free nitrogen of **47** as *tert*-butyl-carbamate, and subsequent hydrogenation, reductive alkylation, and deprotection provided the desired *N*-isopropyl-*N*-(2-piperazine-3-pyridyl)amine **10** as already reported.[12]

Results and Discussion

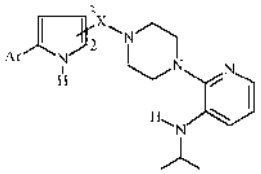
The newly synthesized delavirdine analogues **1** were eval-



Reagents: (i) *N*-Ethyl(iso-Pr)$_2$ amine, CH_2Cl_2; (ii) $(BOC)_2O$, Et_3N, CH_2Cl_2; (iii) H_2, MeOH, 10% Pd/C; (iv) CH_3COCH_3, $NaCNBH_3$, MeOH; (v) CF_3COOH, CH_2Cl_2.

Chart 5

Table 1. Cytotoxicity and Anti-HIV Activity of Compounds **1**



Compd.	Ar	X	CC_{50} [a]	EC_{50} [b]	SI [c]
1Aa	*p*-$CH_3SO_2NHC_6H_4$	2-CO	29	0.25	116
1Ab	*m*-$CH_3SO_2NHC_6H_4$	2-CO	21	6	3.5
1Ac	*o*-$CH_3SO_2NHC_6H_4$	2-CO	>200	>200	
1Ad	*p*-$NH_2C_6H_4$	2-CO	27	2	13.5
1Ae	*m*-$NH_2C_6H_4$	2-CO	42	3	14
1Af	*o*-$NH_2C_6H_4$	2-CO	72	16	4.5
1Ag	C_6H_5	2-CO	26	>26	
1Ba	*p*-$CH_3SO_2NHC_6H_4$	3-CO	24	>24	
1Bb	*m*-$CH_3SO_2NHC_6H_4$	3-CO	35	>35	
1Bc	*o*-$CH_3SO_2NHC_6H_4$	3-CO	22	>22	
1Bd	*p*-$NH_2C_6H_4$	3-CO	>200	>200	
1Be	*m*-$NH_2C_6H_4$	3-CO	21	>21	
1Bf	*o*-$NH_2C_6H_4$	3-CO	98	14	7
1Bg	C_6H_5	3-CO	78	24	3
Delavirdine			>100	0.01	

a) Compound concentration (μM) required to reduce the viability of mock-infected MT-4 cells by 50%, as determined by the MTT method. *b*) Compound concentration (μM) required to achieve 50% protection of MT-4 cells from the HIV-1-induced cytopathogenicity, as determined by the MTT method. *c*) Selectivity Index (ratio: CC_{50}/EC_{50}). Data represent mean values for two separate experiments. Variation among duplicate samples was less than 15%.

uated for their inhibitory effects against the HIV-1 multiplication in acutely infected MT_4 cells (Table 1).

Under our assay conditions, some of the new compounds, elicited significant anti-HIV-1 activities, with effective concentration (EC_{50}) values ranging from 0.25 to 10 μM (**1Aa**, **1Ad**, **1Ae**, **1Ab**).

The most potent compound in this series was compound **1Aa** and it could represent an interesting hit which could further optimized.

Table 2. Antiviral Activity of **1Aa** against HIV Wild-Type and Resistant Mutants

Compd.	CC_{50} [a]	WT_{IIIB}		K103R	Y181C	K103N–Y181C
		EC_{50} [b]	EC_{90} [c]	EC_{90} [c]	EC_{50} [b]	EC_{50} [b]
1Aa	29	0.9	0.8	>20	>20	>20
Efavirenz	35	0.004	0.008	1.8	0.025	0.15

a, *b*) See legend to Table 1. *c*) Compound concentration (μM) required to reduce the amount of p24 by 90% in WT_{IIIB} or K103R resistant mutant. Data represent mean values for two separate experiments. Variation among duplicate samples was less than 15%.

The effect of **1Aa** on the multiplication of NNRTI-resistant mutant viruses possessing the amino acid substitutions K103R, Y181C or the double substitution K103N–Y181C is shown in Table 2. Unfortunately, compound **1Aa** was inactive against the above mutant variants.

On the basis of the results reported in Table 1, several comments can be made. In general the presence of a carbonyl group linked at the α position of the pyrrole ring correlates with significant anti-HIV-1 activity (compounds **1A**). In fact, derivatives bearing the same junction on the pyrrole β carbon (compounds **1B**) were devoid of antiretroviral activity, with the sole exception of compounds **1Bf** and **1Bg**. Among compounds **1A**, high anti-HIV-1 activity is correlated with the presence of a polar group (NH_2 or CH_3SO_2NH) in the *para* position of the phenyl moiety.

Although compound **1Aa** is less potent than delavirdine and, like this latter, is inactive against clinically relevant NNRTIs resistant mutants, we think to introduce further chemical modifications in order to obtain compounds with an enhanced potency against the above mutants.

Experimental

Chemistry. General Unless otherwise noted, all materials were obtained from commercial suppliers and used without purification. Anhydrous solvents were obtained from Aldrich in sure-seal bottles.

All reactions involving air- or moisture-sensitive compounds were performed under an argon atmosphere. Flash chromatography was performed using Merck Silica gel 60 (230—400 mesh ASTM).

Thin layer chromatography (TLC) was performed with Polygram® SIL N-HR-/HV_{254} precoated plastic sheet (0.2 mm). ^{1}H- and ^{13}C-NMR spectra were determined in $CDCl_3$ unless otherwise specified in the Experimental, with superconducting Fourier transform (FT)-NMR using a XL-200 Varian apparatus at 200 MHz.

Chemical shifts are expressed in δ (ppm) downfield from internal tetramethylsilane (TMS) and coupling costants in Hz. Significant ^{1}H-NMR data are reported in the following order: multiplicity (s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; dq, double quartet; dd, double doublet; br s, broad singlet), number of protons, and coupling costants in Hz. IR spectra were recorded as thin films (for oils) or Nujol mulls (for solids) on NaCl plates with a Perkin-Elmer 781 IR spectrophotometer and are expressed in ν (cm^{-1}). UV–Vis spectra were recorded as ethanolic solutions with a Perkin-Elmer Lambda 5 spectrophotometer and the absorption wavelengths are expressed in nm followed by ($\log \varepsilon$). Melting points were determined on a Thomas Hoover capillary melting point apparatus and are uncorrected. Elemental analyses were performed at Laboratory of Microanalysis, Department of Chemistry, University of Sassari (Italy), and are within $\pm$0.4% of the calculated values. The following known materials were prepared as described in the literature: **29**,[11] **30**[11] or obtained from commercial suppliers (**17**, **18**, **19**, **20**, **31**, **45**, **46**).

[4-[3-(Isopropylamino)-2-pyridyl]piperazino]-(5-phenyl-1*H*-2-pyrrolyl)methanone (1Ag) A solution of 5-phenyl-1*H*-2-pyrrolecarboxylic acid[10] (**5**) (0.44 g, 2.37 mmol) and CDI (0.49 g, 2.37 mmol) in

tetrahydrofuran (THF) (6 ml) was stirred for 1 h at room temperature and under argon atmosphere. The reaction mixture was cooled to 0 °C, and *N*-isopropyl-*N*-(2-piperazine-3-pyridyl)amine **10**[12] (0.49 g, 2.22 mmol) in THF (7 ml) was added. The reaction was stirred for 12 h at room temperature, and poured into $NaHCO_3$ solution. The aqueous mixture was extracted with CH_2Cl_2. The organic layer was washed with $NaHCO_3$ solution, dried over Na_2SO_4 and evaporated *in vacuo.* The residue was purified by flash chromatography (petrol ether : EtOAc=8 : 2) to give 0.35 g (40.4%) of **1Ag** as a yellow oil: *Rf* 0.23 (petrol ether : EtOAc=8 : 2); mp 132—135 °C (EtOH) as **1Ag**·3HCl·3H_2O; IR: 3340, 3150, 1620; UV: 206 (4.21), 215 (4.15), 257 (3.96), 308 (4.30); ^{1}H-NMR: 1.25 (d, 6H, *J*=6.2 Hz), 3.05—3.20 (m, 4H), 3.43—3.68 (m, 1H), 3.90—4.10 (m, 4H), 4.11—4.24 (m, 1H, exch. with D_2O), 6.53—7.75 (m, 10H), 10.03 (br s, 1H, exch. with D_2O). *Anal.* Calcd for $C_{23}H_{27}N_5O\cdot 3HCl\cdot 3H_2O$: C, 49.96; H, 6.56; Cl, 19.24; N, 12.67. Found: C, 49.86; H, 6.53; Cl, 19.29; N, 12.69.

The following compounds (**1Bg**, **14**—**16**) were prepared by a manner similar to that used for **1Ag**.

1Bg: Yield 30.8%; *Rf* 0.40 (petrol ether : EtOAc=1 : 1); mp 140—143 °C (EtOH) as **1Bg**·3HCl·3H_2O; IR: 3335, 3150, 1620; UV: 207 (3.89), 216 (3.88), 259 (3.72), 283 (3.75); ^{1}H-NMR: 1.25 (d, 6H, *J*=6.2 Hz), 2.99—3.15 (m, 4H), 3.43—3.68 (m, 1H), 3.80—3.99 (m, 4H), 4.20 (br s, 1H, exch. with D_2O), 6.63—7.69 (m, 10H), 10.48 (br s, 1H, exch. with D_2O). *Anal.* Calcd for $C_{23}H_{27}N_5O\cdot 3HCl\cdot 3H_2O$: C, 49.96; H, 6.56; Cl, 19.24; N, 12.67. Found: C, 49.99; H, 6.54; Cl, 19.21; N, 12.68.

14: Yield 62.5%; *Rf* 0.47 (petrol ether : EtOAc=3 : 7); mp 238—242 °C (triturated with hexane); IR: 3330, 3150, 1620, 1515, 1340; UV: 206 (4.35), 238 (4.20), 318 (3.80), 373 (4.10); ^{1}H-NMR: 1.26 (d, 6H, *J*=6.2 Hz), 3.05—3.22 (m, 4H), 3.48—3.63 (m, 1H), 3.64—3.80 (m, 4H), 3.85—4.02 (br s, 1H, exch. with D_2O), 6.64—8.20 (m, 8H), 12.17 (br s, 1H, exch. with D_2O). *Anal.* Calcd for $C_{23}H_{25}ClN_6O_3$: C, 58.91; H, 5.37; Cl, 7.56; N, 17.92. Found: C, 58.78; H, 5.45; Cl, 7.50; N, 17.88.

15: Yield 59.5%; *Rf* 0.37 (petrol ether : EtOAc=3 : 7); mp 82—85 °C (triturated with hexane); IR: 3325, 3150, 1620, 1510, 1350; UV: 211 (4.40), 256 (4.24), 284 (4.08); ^{1}H-NMR: 1.25 (d, 6H, *J*=6.4 Hz), 3.03—3.20 (m, 4H), 3.48—3.62 (m, 1H), 3.70—3.90 (m, 4H), 4.17 (br s, 1H, exch. with D_2O), 6.52—7.99 (m, 8H), 11.20 (br s, 1H, exch. with D_2O). *Anal.* Calcd for $C_{23}H_{25}ClN_6O_3$: C, 58.91; H, 5.37; Cl, 7.56; N, 17.92. Found: C, 58.95; H, 5.28; Cl, 7.60; N, 17.81.

16: Yield 66.3%; *Rf* 0.45 (petrol ether : EtOAc=3 : 7); mp 108—113 °C (triturated with hexane); IR: 3335, 3140, 1620, 1510, 1350; UV: 210 (4.47), 259 (4.37), 298 (4.35); ^{1}H-NMR: 1.25 (d, 6H, *J*=6.2 Hz), 3.05—3.20 (m, 4H), 3.45—3.62 (m, 1H), 3.65—4.07 (m, 4H), 4.10 (br s, 1H, exch. with D_2O), 6.57—8.38 (m, 8H), 11.20 (br s, 1H, exch. with D_2O). *Anal.* Calcd for $C_{23}H_{25}ClN_6O_3$: C, 58.91; H, 5.37; Cl, 7.56; N, 17.92. Found: C, 59.03; H, 5.41; Cl, 7.49; N, 17.81.

[4-[3-(Isopropylamino)-2-pyridyl]piperazino]-[5-(4-nitrophenyl)-1*H*-2-pyrrolyl]methanone (11) A mixture of 5-(4-nitrophenyl)-1*H*-2-pyrrolecarboxilic acid (**2**) (0.42 g, 1.78 mmol), *N*-isopropyl-*N*-(2-piperazine-3-pyridyl) amine **10**[12] (0.43 g, 1.95 mmol) and EDC (0.41 g, 2.12 mmol) in THF (6 ml) was stirred at room temperature for 12 h under argon atmosphere. The reaction mixture was poured into aqueous solution of $NaHCO_3$ and extracted with $CHCl_3$. The organic layer was washed with brine, dried over $MgSO_4$ and evaporated *in vacuo.* The residue was purified by flash chromatography (petrol ether : EtOAc=6 : 4) to give 0.42 g (53.7%) of **11** as yellow solid: *Rf* 0.42 (petrol ether : EtOAc=1 : 1); mp 224—227 °C (triturated with acetone); IR: 3325, 3180, 1620, 1500, 1320; UV: 208 (4.15), 256 (4.06), sh 320 (3.81), 375 (4.13); ^{1}H-NMR: 1.26 (d, 6H, *J*=6.2 Hz), 3.00—3.20 (m, 4H), 3.55—3.70 (m, 1H), 3.95—4.05 (m, 4H), 6.57—8.24 (m, 9H), 11.32 (br s, 1H, exch. with D_2O). *Anal.* Calcd for $C_{23}H_{26}N_6O_3$: C, 63.58; H, 6.03; N, 19.34. Found: C, 63.52; H, 6.06; N, 19.40.

The following compounds (**12**, **13**) were prepared by a manner similar to that used for **11**.

12: Yield 49.4%; *Rf* 0.46 (petrol ether : EtOAc=1 : 1); mp 78—81 °C; IR: 3330, 3180, 1620, 1510, 1370; UV: 208 (4.30), 256 (4.18), 309 (4.40); ^{1}H-NMR: 1.27 (d, 6H, *J*=6.6 Hz), 3.10—3.20 (m, 4H), 3.50—3.65 (m, 1H), 3.95—4.05 (m, 4H), 6.60—8.10 (m, 8H), 8.46 (s, 1H), 11.11 (br s, 1H, exch. with D_2O). *Anal.* Calcd for $C_{23}H_{26}N_6O_3$: C, 63.58; H, 6.03; N, 19.34. Found: C, 63.52; H, 6.00; N, 19.33.

13: Yield 62.4%; *Rf* 0.20 (petrol ether : EtOAc=1 : 1); mp 150—153 °C; IR: 3325, 3200, 1620, 1530, 1360; UV: 205 (4.34), 255 (4.31), sh 285 (4.10); ^{1}H-NMR: 1.26 (d, 6H, *J*=6.2 Hz), 3.10—3.20 (m, 4H), 3.50—3.65 (m, 1H), 3.85—4.00 (m, 4H), 6.35—7.80 (m, 9H), 10.45 (br s, 1H, exch. with D_2O). *Anal.* Calcd for $C_{23}H_{26}N_6O_3$: C, 63.58; H, 6.03; N, 19.34. Found: C, 63.56; H, 6.08; N, 19.38.

[4-[3-(Isopropylamino)-2-pyridyl]piperazino]-[5-(4-aminophenyl)-1*H*-2-pyrrolyl]-methanone (1Ad) A solution of **11** (0.25 g, 5.75 mmol) in dry EtOH (8 ml) was treated with 10% Pd/C (0.025 g) in a Parr shaker. The apparatus was sealed under hydrogen pressure to 30 psi, heated at 35 °C for 2 h. The apparatus was then cooled to room temperature, vented and the contents filtered. The filtrate was evaporated *in vacuo* to give 0.20 g (86.9%) of **1Ad** as a green solid: *Rf* 0.52 (petrol ether : EtOAc=1 : 1); mp 111—114 °C; IR: 3330, 3190, 1620; UV: 209 (4.20), 256 (4.01), 320 (4.33); ^{1}H-NMR: 1.24 (d, 6H, *J*=7.4 Hz), 1.70 (br s, 2H, exch. with D_2O), 3.08—3.25 (m, 4H), 3.50—3.70 (m, 1H), 3.90—4.08 (m, 4H), 4.20 (br s, 1H, exch. with D_2O), 6.35—7.75 (m, 9H), 9.47 (br s, 1H, exch. with D_2O). *Anal.* Calcd for $C_{23}H_{28}N_6O$: C, 68.29; H, 6.98; N, 20.78. Found: C, 68.36; H, 7.04; N, 20.72.

The following compounds (**1Ae**, **1Af**) were prepared by a manner similar to that used for **1Ad**.

1Ae: Yield 79.8%; *Rf* 0.28 (petrol ether : EtOAc=3 : 7); mp 97—100 °C; IR: 3320, 3200, 1620; UV: 216 (4.37), 252 (4.24), 309 (4.40); ^{1}H-NMR: 1.25 (d, 6H, *J*=6.2 Hz), 1.69 (br s, 2H, exch. with D_2O), 3.02—3.22 (m, 4H), 3.45—3.65 (m, 1H), 3.88—4.08 (m, 4H), 4.20 (br s, 1H, exch. with D_2O), 6.46—7.22 (m, 9H), 9.59 (br s, 1H, exch. with D_2O). *Anal.* Calcd for $C_{23}H_{28}N_6O$: C, 68.29; H, 6.98; N, 20.78. Found: C, 68.33; H, 7.00; N, 20.70.

1Af: Yield 82.6%; *Rf* 0.20 (petrol ether : EtOAc=1 : 1); mp 101—103 °C; IR: 3340, 3210, 1620; UV: 216 (4.36), 253 (4.24), 293 (4.16), 316 (4.21); ^{1}H-NMR: 1.28 (d, 6H, *J*=6.2 Hz), 2.80 (br s, 2H, exch. with D_2O), 3.04—3.22 (m, 4H), 3.45—3.68 (m, 1H), 3.88—4.04 (m, 4H), 4.20 (br s, 1H, exch. with D_2O), 6.40—7.80 (m, 9H), 9.90 (br s, 1H, exch. with D_2O). *Anal.* Calcd for $C_{23}H_{28}N_6O$: C, 68.29; H, 6.98; N, 20.78. Found: C, 68.35; H, 7.02; N, 20.65.

[4-[3-(Isopropylamino)-2-pyridyl]piperazino]-[5-(4-aminophenyl)-1*H*-3-pyrrolyl]-methanone (1Bd) A mixture of **14** (0.20 g, 0.42 mmol), ammonium formate (0.24 g, 3.78 mmol) and 10% Pd/C (0.2 g) in CH_3OH (10 ml) was stirred overnight at room temperature under argon atmosphere. The reaction mixture was filtered and the filtrate was evaporated *in vacuo.* The crude residue was taken with acetone, and the acetone mixture filtered. The filtrate was concentrated *in vacuo* and the resulting residue was submitted to flash silica gel chromatography, eluting with 9.5 : 0.5 $CHCl_3$: MeOH to give 0.13 g (76.4%) of **1Bd** as a cream solid: *Rf* 0.36 ($CHCl_3$: CH_3OH=9.5 : 0.5); mp 99—101 °C; IR: 3355, 3200, 1620; UV: 211 (4.44), 260 (4.20), 292 (4.34); ^{1}H-NMR [$CDCl_3$+2 drops $(CD_3)_2SO$]: 1.25 (d, 6H, *J*=6.2 Hz), 2.98—3.20 (m, 4H), 3.45—3.65 (m, 1H), 3.84—4.00 (m, 4H), 4.20 (br s, 1H, exch. with D_2O), 4.60 (br s, 2H, exch. with D_2O), 6.95—7.90 (m, 9H), 10.05 (br s, 1H, exch. with D_2O). *Anal.* Calcd for $C_{23}H_{28}N_6O$: C, 68.29; H, 6.98; N, 20.78. Found: C, 68.33; H, 7.09; N, 20.71.

The following compounds (**1Be**, **1Bf**) were prepared by a manner similar to that used for **1Bd**.

1Be: Yield 47.6%; *Rf* 0.57 ($CHCl_3$: CH_3OH=9 : 1); mp 96—99 °C; IR: 3350, 3200, 1620; UV: 214 (4.33), 233 (4.31), 281 (4.07), 308 (3.98); ^{1}H-NMR: 1.25 (d, 6H, *J*=6.2 Hz), 3.02—3.20 (m, 4H), 3.42—3.68 (m, 1H), 3.84—4.02 (m, 4H), 4.77 (br s, 3H, exch. with D_2O), 6.50—7.70 (m, 9H), 8.36 (br s, 1H, exch. with D_2O). *Anal.* Calcd for $C_{23}H_{28}N_6O$: C, 68.29; H, 6.98; N, 20.78. Found: C, 68.35; H, 7.01; N, 20.69.

1Bf: Yield 56.5%; *Rf* 0.14 (petrol ether : EtOAc=3 : 7); mp 75—80 °C; IR: 3350, 3180, 1620; UV: 241 (4.46), 279 (4.33), sh 304 (4.14); ^{1}H-NMR: 1.25 (d, 6H, *J*=6.2 Hz), 3.02—3.18 (m, 4H), 3.30—3.70 (m, 4H, 3H exch. with D_2O), 3.82—3.98 (m, 4H), 6.55—7.30 (m, 9H), 9.46 (br s, 1H, exch. with D_2O). *Anal.* Calcd for $C_{23}H_{28}N_6O$: C, 68.29; H, 6.98; N, 20.78. Found: C, 68.23; H, 6.90; N, 20.81.

***N*-[4-[5-([4-[3-(Isopropylamino)-2-pyridyl]piperazino]-carbonyl)-1*H*-2-pyrrolyl]phenyl]methansulfonamide (1Aa)** A mixture of **1Ad** (0.14 g, 0.35 mmol), pyridine (0.056 ml, 0.69 mmol), and methanesulfonyl chloride (0.027 ml, 0.35 mmol) in CH_2Cl_2 (1.5 ml) was stirred for 12 h at room temperature. The mixture was diluted with CH_2Cl_2 (7.5 ml) and water (3 ml), the layers were separated and the organic layer was washed with brine, dried over Na_2SO_4, and evaporated *in vacuo* to give a crude brown oil. The resulting material was submitted to flash silica gel chromatography, eluting with 9 : 1 $CHCl_3$: CH_3OH to afford 0.11 g (64.7%) of **1Aa**: *Rf* 0.16 (petrol ether : EtOAc=4 : 6); mp 221—223 °C; IR: 3340, 3130, 1620, 1390, 1200; UV: 209 (4.29), sh 219 (4.24), 256 (4.10), 314 (4.48); ^{1}H-NMR: 1.29 (d, 6H, *J*=6.2 Hz), 3.01 (s, 3H), 3.30—3.50 (m, 4H), 3.55—3.78 (m, 1H), 3.90—4.10 (m, 4H), 4.15 (br s, 1H, exch. with D_2O), 6.40—7.90 (m, 9H), 8.73 (br s, 1H, exch. with D_2O), 11.02 (br s, 1H, exch. with D_2O). *Anal.* Calcd for $C_{24}H_{30}N_6O_3S$: C, 59.73; H, 6.27; N, 17.41; S, 6.64. Found: C, 59.68; H, 6.18; N, 17.42; S, 6.70.

The following compounds (**1Ab**, **1Ac**, **1Ba**, **1Bb**, **1Bc**) were prepared by a manner similar to that used for **1Aa**.

1Ab: Yield 94.7%; *Rf* 0.42 (petrol ether : EtOAc=3 : 7); mp 214—216 °C (triturated with hexane); IR: 3340, 3150, 1620, 1380, 1210; UV: 211 (4.28), sh 220 (4.08), 253 (4.02), 310 (4.36); ^{1}H-NMR: [$CDCl_3$+2 drops $(CD_3)_2SO$] 1.26 (d, 6H, *J*=6.2 Hz), 2.97 (s, 3H), 3.02—3.25 (m, 4H), 3.46—3.68 (m, 1H), 3.88—4.10 (m, 4H), 4.18 (br s, 1H, exch. with D_2O), 6.50—7.70 (m, 9H), 9.22 (br s, 1H, exch. with D_2O); 10.31 (br s, 1H, exch. with D_2O). *Anal.* Calcd for $C_{24}H_{30}N_6O_3S$: C, 59.73; H, 6.27; N, 17.41; S, 6.64. Found: C, 59.70; H, 6.25; N, 17.36; S, 6.67.

1Ac: Yield 40.0%; *Rf* 0.35 (petrol ether : EtOAc=3 : 7); mp 197—200 °C (triturated with acetone); IR: 3340, 3150, 1620, 1390, 1210; UV: 203 (4.11), sh 226 (3.97), 257 (3.93), 304 (4.04); ^{1}H-NMR: 1.26 (d, 6H, *J*=6.2 Hz), 2.80 (s, 3H), 3.02—3.20 (m, 4H), 3.50—3.70 (m, 1H), 3.80—4.10 (m, 4H), 4.15 (br s, 1H, exch. with D_2O), 6.30—8.61 (m, 10H, 1H exch. with D_2O); 10.50 (br s, 1H, exch. with D_2O). *Anal.* Calcd for $C_{24}H_{30}N_6O_3S$: C, 59.73; H, 6.27; N, 17.41; S, 6.64. Found: C, 59.67; H, 6.31; N, 17.45; S, 6.66.

1Ba: Yield 13.0%; *Rf* 0.42 ($CHCl_3$: CH_3OH=9 : 1); mp 166—168 °C ($EtOH/H_2O$); IR: 3335, 3140, 1620, 1385, 1220; UV: 209 (4.47), 262 (4.25), 293 (4.41); ^{1}H-NMR: 1.25 (d, 6H, *J*=6.2 Hz), 2.98 (s, 3H), 3.00—3.20 (m, 4H), 3.40—3.65 (m, 1H), 3.78—4.00 (m, 4H), 4.20 (br s, 1H, exch. with D_2O), 6.45—7.75 (m, 10H, 1H exch. with D_2O); 9.70 (br s, 1H, exch. with D_2O). *Anal.* Calcd for $C_{24}H_{30}N_6O_3S$: C, 59.73; H, 6.27; N, 17.41; S, 6.64. Found: C, 59.68; H, 6.29; N, 17.44; S, 6.71.

1Bb: Yield 25.8%; *Rf* 0.43 ($CHCl_3$: CH_3OH=9 : 1); mp 108—110 °C (Et_2O); IR: 3340, 3140, 1620, 1390, 1210; UV: 219 (4.47), 259 (4.19), 289 (4.22); ^{1}H-NMR: 1.25 (d, 6H, *J*=6.2 Hz), 3.03 (s, 3H), 3.04—3.22 (m, 4H), 3.50—3.70 (m, 1H), 3.80—4.00 (m, 4H), 4.20 (br s, 1H, exch. with D_2O), 6.60—7.75 (m, 10H, 1H exch. with D_2O); 9.72 (br s, 1H, exch. with D_2O). *Anal.* Calcd for $C_{24}H_{30}N_6O_3S$: C, 59.73; H, 6.27; N, 17.41; S, 6.64. Found: C, 59.80; H, 6.24; N, 17.38; S, 6.71.

1Bc: Yield 8.7%; *Rf* 0.72 ($CHCl_3$: CH_3OH=9 : 1); mp 95—100 °C (triturated with acetone); IR: 3335, 3145, 1620, 1390, 1215; UV: 225 (4.52), 252 (4.50), 284 (4.22), 312 (4.10); ^{1}H-NMR: 1.25 (d, 6H, *J*=6.2 Hz), 2.95 (s, 3H), 3.00—3.20 (m, 4H), 3.40—3.65 (m, 1H), 3.70—3.95 (m, 4H), 4.20 (br s, 1H, exch. with D_2O), 6.45—7.75 (m, 9H), 8.90 (br s, 1H, exch. with D_2O); 11.02 (br s, 1H, exch. with D_2O). *Anal.* Calcd for $C_{24}H_{30}N_6O_3S$: C, 59.73; H, 6.27; N, 17.41; S, 6.64. Found: C, 59.75; H, 6.31; N, 17.44; S, 6.60.

5-(4-Nitrophenyl)-1*H*-2-pyrrole Carboxylic Acid (2) A mixture of **25** (0.33 g, 1.32 mmol) in aqueous 12% KOH solution (18 ml) was refluxed for 1 h. The reaction mixture was poured into 1 N HCl under ice cooling. The precipitated formed was filtered off, washed with water and air dried to give 0.30 g (quant.) of **2** as yellow solid: *Rf* 0.21 (petrol ether : EtOAc=7 : 3); mp 243—245 °C (EtOH); IR: 3400, 1660, 1515, 1350; UV: 206 (3.99), 236 (3.86), 265 (3.94), 390 (4.21); ^{1}H-NMR [$CDCl_3$+2 drops $(CD_3)_2SO$]: 3.28 (br s, 1H, exch. with D_2O); 6.60—6.70 (m, 1H), 6.85—7.08 (m, 1H), 8.1 (ABq, 4H), 11.64 (br s, 1H, exch. with D_2O). *Anal.* Calcd for $C_{11}H_8N_2O_4$: C, 56.90; H, 3.47; N, 12.06. Found: C, 56.87; H, 3.42; N, 12.15.

The following compounds (**3**, **4**, **7**, **8**) were prepared by a manner similar to that used for **2**.

3: Yield (quant), *Rf* 0.50 (petrol ether : EtOAc=1 : 1); mp 228—229 °C (EtOH); IR: 3410, 1660, 1510, 1340; UV: 207 (3.76), 233 (3.50), 286 (3.71), 308 (3.89); ^{1}H-NMR: 3.51 (br s, 1H, exch. with D_2O); 6.60—6.75 (m, 1H), 6.78—7.01 (m, 1H), 7.50—8.85 (m, 4H), 12.35 (br s, 1H, exch. with D_2O). *Anal.* Calcd for $C_{11}H_8N_2O_4$: C, 56.90; H, 3.47; N, 12.06. Found: C, 56.84; H, 3.51; N, 12.03.

4: Yield 98.6%, *Rf* 0.43 ($CHCl_3$: CH_3OH=9 : 1); mp 218—222 °C (EtOH); IR: 3420, 1665, 1525, 1348; UV: 205 (3.99), 279 (4.05); ^{1}H-NMR [$CDCl_3$+2 drops $(CD_3)_2SO$]: 4.50 (br s, 1H, exch. with D_2O); 6.30—6.40 (m, 1H), 6.90—7.00 (m, 1H), 7.40—7.90 (m, 4H), 10.99 (br s, 1H, exch. with D_2O). *Anal.* Calcd for $C_{11}H_8N_2O_4$: C, 56.90; H, 3.47; N, 12.06. Found: C, 56.81; H, 3.49; N, 12.10.

7: Yield 58.0%, *Rf* 0.51 (petrol ether : EtOAc=3 : 7); mp 251—254 °C (EtOH); IR: 3310, 1665, 1525, 1345; UV: 215 (4.25), 273 (4.26), 288 (4.22), 359 (3.08); ^{1}H-NMR [$CDCl_3$+2 drops $(CD_3)_2SO$]: 2.70 (br s, 1H, exch. with D_2O); 7.01 (d, 1H, *J*=3.2 Hz), 7.40—8.70 (m, 4H), 12.30 (br s, 1H, exch. with D_2O). *Anal.* Calcd for $C_{11}H_7ClN_2O_4$: C, 49.55; H, 2.65; N, 10.51; Cl, 13.30. Found: C, 49.57; H, 2.69; N, 10.49; Cl, 13.22.

8: Yield 77.0%, *Rf* 0.13 (petrol ether : EtOAc=6 : 4); mp 213—217 °C (Et_2O); IR: 3410, 1660, 1510, 1350; UV: 210 (4.03), 256 (2.93), 261 (3.78); ^{1}H-NMR [$CDCl_3$+2 drops $(CD_3)_2SO$]: 3.98 (br s, 1H, exch. with D_2O); 6.69 (d, 1H, *J*=3.2 Hz), 7.38—7.98 (m, 4H), 11.89 (br s, 1H, exch. with D_2O). *Anal.* Calcd for $C_{11}H_7ClN_2O_4$: C, 49.55; H, 2.65; N, 10.51; Cl, 13.30. Found: C, 49.49; H, 2.71; N, 10.50; Cl, 13.21.

Methyl 5-(4-Nitrophenyl)-1*H*-2-pyrrole Carboxylate (25) A mixture of azide **21** (0.60 g, 2.19 mmol) in xylene (9.0 ml) was refluxed for 15 min. After cooling the resulting precipitate was filtered to give 0.35 g (64.8%) of **25** as orange prisms: *Rf* 0.57 (petrol ether : EtOAc=7 : 3); mp 245—248 °C; IR: 3320, 1690, 1520, 1348; UV: 207 (4.00), 225 (3.84), 268 (3.91), 367 (4.25); ^{1}H-NMR [$CDCl_3$+2 drops $(CD_3)_2SO$]: 3.89 (s, 3H), 6.66—6.78 (m, 1H), 6.85—7.00 (m, 1H), 8.05 (ABq, 4H), 11.62 (br s, 1H, exch. with D_2O). *Anal.* Calcd for $C_{12}H_{10}N_2O_4$: C, 58.54; H, 4.09; N, 11.38. Found: C, 58.49; H, 4.08; N, 11.40.

The following compounds (**26**, **27**) were prepared by a manner similar to that used for **25**.

26: Yield 83.8%; *Rf* 0.54 (petrol ether : EtOAc=7 : 3); mp 196—198 °C; IR: 3310, 1680, 1525, 1350; UV: 211 (4.06), 235 (3.92), 296 (4.27); ^{1}H-NMR [$CDCl_3$+2 drops $(CD_3)_2SO$]: 3.85 (s, 3H), 6.52—6.70 (m, 1H), 6.88—7.00 (m, 1H), 7.40—8.80 (m,4H), 11.83 (br s, 1H, exch. with D_2O). *Anal.* Calcd for $C_{12}H_{10}N_2O_4$: C, 58.54; H, 4.09; N, 11.38. Found: C, 58.56; H, 4.05; N, 11.35.

27: Yield 76.2%; *Rf* 0.51 (petrol ether : EtOAc=7 : 3); mp 149—150 °C; IR: 3315, 1690, 1528, 1345; UV: 205 (4.03), 233 (3.84), 276 (4.21), 350 (3.23); ^{1}H-NMR: 3.81 (s, 3H), 6.45—6.55 (m, 1H), 6.90—7.02 (m, 1H), 7.40—7.90 (m, 4H), 9.91 (br s, 1H, exch. with D_2O). *Anal.* Calcd for $C_{12}H_{10}N_2O_4$: C, 58.54; H, 4.09; N, 11.38. Found: C, 58.60; H, 4.11; N, 11.33.

Methyl 2-Azido-5-(4-nitrophenyl)-penta-2,4-dienoate (21) To a solution of CH_3ONa, prepared starting from CH_3OH (13.85 ml) and Na (0.38 g), a solution of *p*-NO_2-cynnamaldehyde (**17**) (1.5 g, 8.47 mmol) and methyl azidoacetate (1.95 g, 17 mmol) in CH_3OH (7.5 ml) was added dropwise at −10 °C. The reaction mixture was stirred and allowed to warm to room temperature for 1.5 h, and then poured into water. The aqueous mixture was extracted with Et_2O. The resulting precipitate was filtered to give 0.70 g (30.2%) of **21** as brown solid: *Rf* 0.78 (petrol ether : EtOAc=7 : 3); mp 235—237 °C; IR: 2110, 1680, 1515, 1345; UV: 205 (4.00), 230 (3.86), 292 (3.91), 371 (4.32); ^{1}H-NMR: 3.90 (s, 3H), 6.65—7.40 (m, 3H), 7.90 (ABq, 4H). *Anal.* Calcd for $C_{12}H_{10}N_4O_4$: C, 52.56; H, 3.68; N, 20.43. Found: C, 52.50; H, 3.73; N, 20.35.

The following compounds (**22**, **23**) were prepared by a manner similar to that used for **21**.

22: Yield 23.7%; *Rf* 0.72 (petrol ether : EtOAc=1 : 1); mp 194—195 °C; IR: 2110, 1680, 1520, 1345; UV: 210 (3.99), 240 (4.10), 325 (4.37), 345 (4.31); ^{1}H-NMR: 3.90 (s, 3H), 6.65—7.40 (m, 3H), 7.50—8.35 (m, 4H). *Anal.* Calcd for $C_{12}H_{10}N_4O_4$: C, 52.56; H, 3.68; N, 20.43. Found: C, 52.59; H, 3.62; N, 20.39.

23: Yield 45.8%; *Rf* 0.33 (petrol ether : EtOAc=8 : 2); mp 144—146 °C (MeOH); IR: 2110, 1685, 1550, 1340; UV: 209 (4.23), 255 (4.12), 273 (4.19); ^{1}H-NMR: 3.89 (s, 3H), 6.70—7.38 (m, 3H), 7.40—8.02 (m, 4H). *Anal.* Calcd for $C_{12}H_{10}N_4O_4$: C, 52.56; H, 3.68; N, 20.43. Found: C, 52.48; H, 3.71; N, 20.32.

Ethyl 2-Chloro-5-(3-nitrophenyl)-1*H*-3-pyrrole Carboxylate (41) A solution of **36** (0.50 g, 1.81 mmol) in THF (6 ml) was bubbled with an excess of gaseous HCl (*ca.* 1 g) at 0—5 °C. After the HCl addition was completed the cooling bath was removed and the stirring continued at room temperature for 5 h. Solvent was then evaporated and the resulting residue was subjected to flash silica gel chromatography, eluting with 7 : 3 petrol ether : EtOAc to give 0.25 g (78.6%) of **41** as a yellow prisms: *Rf* 0.66 (petrol ether : EtOAc=7 : 3); mp 211—213 °C (Et_2O); IR: 3220, 1660, 1530, 1340; UV: 209 (4.11), 273 (4.01), 290 (3.97); ^{1}H-NMR [$CDCl_3$+2 drops $(CD_3)_2SO$]: 1.38 (t, 3H, *J*=7.2 Hz), 4.31 (q, 2H, *J*=7.2 Hz), 7.00 (d, 1H, *J*=3 Hz), 6.75—8.30 (m, 4 H), 12.48 (br s, 1H exch. with D_2O). *Anal.* Calcd for $C_{13}H_{11}ClN_2O_4$: C, 52.98; H, 3.76; N, 9.51; Cl, 12.03. Found: C, 52.96; H, 3.79; N, 9.57; Cl, 12.11.

42: This compound was prepared by a manner similar to that used for **41**: yield 76.3%; *Rf* 0.49 (petrol ether : EtOAc=7 : 3); mp 139—142 °C (EtOH); IR: 3190, 1660, 1525, 1342; UV: 211 (4.28), 260 (4.17), 298 (4.15); ^{1}H-NMR: 1.36 (t, 3H, *J*=7.4 Hz), 4.31 (q, 2H, *J*=7.4 Hz), 6.83 (d, 1H, *J*=3.0 Hz), 7.35—7.95 (m, 4H), 9.28 (br s, 1H exch. with D_2O). *Anal.* Calcd for $C_{13}H_{11}ClN_2O_4$: C, 52.98; H, 3.76; N, 9.51; Cl, 12.03. Found: C, 52.85; H, 3.71; N, 9.53; Cl, 12.09.

Ethyl 2-Cyano-4-(4-nitrophenyl)-4-oxobutanoate (32) and Ethyl 2-Cyano-4-(4-nitrophenyl)-2-[4-(nitrophenyl)-2-oxoethyl]-4-oxobutanoate (37) A mixture of ethyl cyanoacetate (23.41 ml, 220 mmol) and finely powdered K_2CO_3 (7.74 g, 56 mmol) was warmed at 40—45 °C and then stirred for 45 min. To the resulting pink suspension a solution of **29** (6.8 g, 28 mmol) in acetone (61 ml) was added dropwise. The mixture was stirred for an additional hour at the same temperature and cooled to room temperature followed by addition of ethyl acetate (50 ml) and water (50 ml). The or-

ganic layer was separated and washed successively with aqueous 10% NaH_2PO_4 solution (35 ml) and brine (25 ml), dried over Na_2SO_4, and evaporated *in vacuo* to afford a oily product. The crude oil was submitted to "bulb to bulb" distillation at 70—80 °C/(0.5 mmHg) to remove the excess of $CNCH_2COOEt$ and the residue subjected to flash silica gel chromatography, eluting with 7 : 3 petrol ether : EtOAc, to give 4.50 g (58.5%) of **32** from the first fraction as a yellow oil which on standing solidified; mp 80—82 °C (Et_2O) [81—83 °C (EtOH)].[10] The second fraction yielded 1.02 g (8.4%) of **37** as a solid, mp 165—166 °C [164—167 °C (Et_2O)].[10]

The following compounds (**33**, **38**, **34**, **39**) were prepared by a manner similar to that used for **32** and **37**.

33: Yield 33.6%; *Rf* 0.47 (petrol ether : EtOAc = 6 : 4); mp 68—70 °C; IR: 2250, 1740, 1710, 1530, 1350; UV: 208 (3.84), 255 (3.52); ^{1}H-NMR: 1.37 (t, 3H, *J* = 7.0 Hz), 3.51 (dq, 2H, *J* = 5.4, 18.4 Hz), 4.21 (dd, 1H, *J* = 5.4, 18.4 Hz), 7.45—8.20 (m, 4H). *Anal.* Calcd for $C_{13}H_{12}N_2O_5$: C, 56.52; H, 4.38; N, 10.14. Found: C, 56.41; H, 4.32; N, 10.17.

38: Yield 10.3%; *Rf* 0.25 (petrol ether : EtOAc = 6 : 4); mp 162—165 °C (Et_2O); IR: 2225, 1740, 1710, 1530, 1350; UV: 206 (4.22), 217 (4.25), 232 (4.09), 368 (4.17); ^{1}H-NMR: 1.36 (t, 3H, *J* = 6.8 Hz), 3.84 (s, 4H), 4.38 (q, 2H, *J* = 6.8 Hz), 7.50—8.20 (m, 8H). *Anal.* Calcd for $C_{21}H_{17}N_3O_8$: C, 57.41; H, 3.90; N, 9.56. Found: C, 57.44; H, 3.87; N, 9.68.

34: Yield 88.6%; mp 52—55 °C [53—55 °C (EtOH)].[10]

39: Yield 5.3%; mp 130—131 °C [130 °C (Et_2O)].[10]

Ethyl 2-(Aminocarbonyl)-4-(3-nitrophenyl)-4-oxobutanoate (35) To a stirred solution of **34** (0.70 g, 3.03 mmol) in 96% sulphuric acid (7 ml) cooled at 0—3 °C, powdered KNO_3 (0.50 g, 4.97 mmol) was added portionwise (30 min). The reaction mixture was poured onto ice to give a brown solid wich was filtered and washed with water. The resulting precipitate was triturated with MeOH to give 0.40 g (73.8%) of **35** as pale yellow prisms: *Rf* 0.41 (petrol ether : EtOAc = 1 : 1); mp 162—165 °C (MeOH); IR: 3380, 3180, 1710, 1690, 1650, 1520, 1350; UV: 205 (3.88), 226 (3.99), 260 (3.69); ^{1}H-NMR [$CDCl_3$ + 2 drops $(CD_3)_2SO$]: 1.28 (t, 3H, *J* = 7.2 Hz), 3.68 (dq, 2H, *J* = 5.4, 7.2 Hz), 4.05 (dd, 1H, *J* = 5.4, 7.2 Hz), 4.23 (q, 2H, *J* = 7.2 Hz), 6.43 (br s, 1H, exch. with D_2O), 7.21 (br s, 1H, exch. with D_2O), 7.70—8.90 (m, 4 H); ^{13}C-NMR: 12.24, 36.15, 45.69, 59.10, 120.60, 125.65, 128.71, 132.24, 135.71, 146.42, 167.62, 194.05. *Anal.* Calcd for $C_{13}H_{14}N_2O_6$: C, 53.06; H, 4.80; N, 9.52. Found: C, 53.16; H, 4.83; N, 9.46.

Ethyl 2-Cyano-4-(3-nitrophenyl)-4-oxobutanoate (36) To a solution of **35** (0.50 g, 1.7 mmol) in CH_2Cl_2 (10 ml), $POCl_3$ (5 ml) was added dropwise at 0 °C. Then the reaction mixture was refluxed for 5 h. After cooling, the mixture was poured onto ice, alkalinized with Na_2CO_3 and extracted with CH_2Cl_2. The organic layer was washed with brine, dried over Na_2SO_4, filtered and evaporated *in vacuo.* The resulting residue was subjected to flash silica gel chromatography, eluting with 6 : 4 petrol ether : EtOAc to afford 0.20 g (50.7%) of **36** as a light yellow solid: *Rf* 0.70 (petrol ether : EtOAc = 1 : 1); mp 78—81 °C (MeOH); IR: 2250, 1730, 1690, 1520, 1370; UV: 206 (3.88), 229 (4.31), 255 (3.85); ^{1}H-NMR: 1.37 (t, 3H, *J* = 7.0 Hz), 3.72 (dq, 2H, *J* = 5.4, 6.8 Hz), 4.19 (dd, 1H, *J* = 5.4, 6.8 Hz), 4.32 (q, 2H, *J* = 7.0 Hz), 7.70—8.85 (m, 4 H). *Anal.* Calcd for $C_{13}H_{12}N_2O_5$: C, 56.62; H, 4.38; N, 10.14. Found: C, 56.57; H, 4.40; N, 10.09.

1-(3-Nitro-2-pyridyl)-piperazine (47) and 1,4-Bis-(3-nitro-2-pyridyl)-piperazine (48) To a solution of 2-chloro-3-nitropyridine (0.50 g, 3.15 mmol), diisopropylethylamine (0.64 ml, 3.67 mmol) in dry CH_2Cl_2 (35 ml) was added piperazine (2.71 g, 31.5 mmol) under ice cooling. The mixture was stirred for 1 h at 0 °C and then allowed to warm to room temperature. After stirring for 16 h, the reaction mixture was poured into aqueous saturated $NaHCO_3$ solution and the whole extracted with CH_2Cl_2. The organic layer was washed with brine, dried over Na_2SO_4 and evaporated *in vacuo.* The resulting residue was subjected to neutral Al_2O_3 column chromatography eluting with $CHCl_3$ to give from the first fraction 0.46 g (70.9%) of **47** as a light yellow solid: mp 83—86 °C ($CHCl_3$/hexane) [82—87 °C].[13] The second fraction yielded 0.08 g (8.0%) of **48** as a yellow solid: mp 201—202 °C (EtOH) [201 °C, pyridine–water].[14]

Antiviral Assay Procedures. Compounds Test compounds were solubilised in dimethyl sulfoxide (DMSO) at 200 mM and then diluted into culture medium.

Cells and Viruses MT-4 and C8166 cells were grown at 37 °C in a 5% CO_2 atmosphere in RPMI 1640 medium, supplemented with 10% fetal calf serum (FCS), 100 UI/ml penicillin G and 100 μg/ml streptomycin. Cell cultures were checked periodically for the absence of mycoplasma contamination with the MycoTect Kit (Gibco). HIV-1 was obtained from supernatants of persistently infected H9/III_B cells. The Y181C mutant (NIH N119) was derived from an AZT-sensitive clinical isolate passaged in CEM cells in the presence of Nevirapine [10 μM] as described by Richman D. *et al.*[15] The K103R virus was derived from a III_B strain passaged in C8166 cells in the presence of Efavirenz [2 μM] and the mutations (K103R, V179D, P225H) were identified by R. Schinazi at the Emory University of Georgia. The double mutant K103N–Y181C (NIH A17) was derived from a IIIB strain passaged in H9 cells in the presence of Nevirapine as described by Nunberg J. H. *et al.*[16] The HIV-1 wt and the mutant Y181C, K103R and K103N–Y181C stock solutions had titres of 1.0×10^7 (wt) 50% cell culture infectious dose ($CCID_{50}$)/ml, 1.3×10^6 (Y181C) $CCID_{50}$/ml, 3.0×10^5 (K103R) $CCID_{50}$/ml and 2.5×10^5 (Y181C-K103N) $CCID_{50}$/ml, respectively.

HIV Titration Virus titration was performed in C8166 cells by the standard limiting dilution method (dilution 1 : 2, four replica wells/dilution) in 96-well plates. The infectious virus titre was determined by light microscope scoring of cytopathicity after 4 d of incubation, and virus titres were expressed as $CCID_{50}$/ml.

Anti-HIV Assays Activity of the compounds against HIV-1 wt, the Y181C mutant and the double mutant K103N–Y181C were based on inhibition of virus-induced cytopathicity in MT-4 cells acutely infected at a multiplicity of infection of 0.01. Activity of compounds against the K103R were based on inhibition of p24 antigen in C8166 cells acutely infected at a m.o.i. = 0.01.[17] Briefly, 50 μl of culture medium containing 1×10^4 cells were added to each well of flat-bottom microtitre trays containing 50 μl of culture medium with or without test compounds. Then 20 μl of an HIV suspension containing 100 (HIV-1) $CCID_{50}$ was added. After a 4-d incubation, cell viability was determined by 3-(4,5-dimethylthiazol-1-yl)-2,5-diphenyltetrazolium bromide (MTT) method. Alternatively, anti-HIV-1 activity was evaluated by reduction of p24 antigen production by ELISA test (Abbott). 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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