Synthesis of Tricyclic Triazepinones Related to Nevirapine

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Two novel tricyclic triazepinones structurally related to the reverse transcriptase inhibitor nevirapine were prepared from *N*-(2-nitrophenyl)- and *N*-(3-nitro-2-pyridinyl)-1*H*-pyrrol-1-amine. The synthetic sequence includes alkylation, reduction of the nitro group, triphosgene reaction followed by intramolecular cyclization. Activity of the two compounds against the HIV-1 multiplication in acutely infected cells is also reported.

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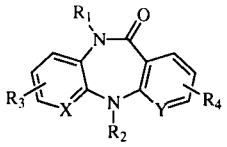
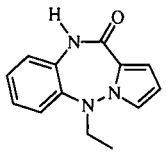
Dipyridodiazepinone nevirapine (**1a**) is a potent and highly specific inhibitor of the reverse transcriptase (RT) of human immunodeficiency virus type 1. It belongs to an important class of non-nucleoside drugs sharing the same binding site on the enzyme.



1: X = N; Y = N
1a: X = N; Y = N; R_1 = H; R_2 = c-C_3H_5; R_3 = 4-CH_3; R_4 = H
1b: X = N; Y = N; R_1 = H; R_2 = C_2H_5; R_3 = 4-CH_3; R_4 = H
2: X = N; Y = CH
3: X = CH; Y = N
4: X = CH; Y = CH

Nevirapine was discovered after screening a number of tricyclic diazepinones **1-4** and was selected for clinical evaluation on the basis of its favorable levels of specificity, bioavailability and metabolic stability, even though it appears to be slightly less potent than corresponding ethyl derivative (**1b**) [1].

In connection with our program on new pyrrole containing heterocycles with potential pharmacological activity [2], we now report the synthesis and preliminary anti HIV-1 activity of 5,10-dihydro-5-ethyl-11*H*-pyrrolo[1,2-*b*][1,2,5]benzotriazepin-11-one (**5**) and 5,11-dihydro-11-ethyl-6*H*-pyrido[3,2-*f*]pyrrolo[1,2-*b*][1,2,5]triazepin-6-one (**6**), a derivative of another novel tricyclic system.



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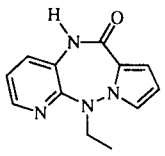

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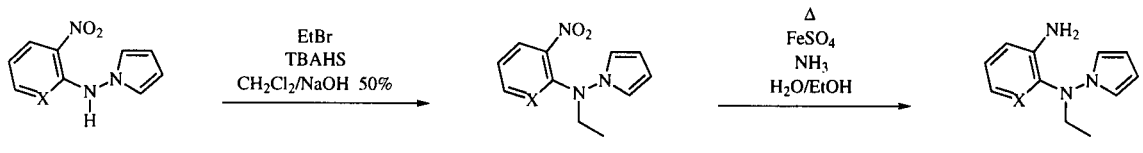
As shown in Scheme I, *N*-(2-nitrophenyl)-1*H*-pyrrol-1-amine (**7a**) [3] was converted to the ethyl derivative **8a** by reaction with ethyl bromide in the presence of tetrabutylammonium hydrogen sulfate. Synthesis of the corresponding pyridine derivative **8b** was accomplished in the same manner, starting from *N*-(3-nitro-2-pyridinyl)-1*H*-pyrrol-1-amine (**7b**), formed in its turn by condensation of 1-aminopyrrole [4] with 2-chloro-3-nitropyridine in dimethylformamide, using sodium carbonate and copper powder. Reduction of the nitro group with ferrous sulfate and ammonium hydroxide gave the amines **9a-b**.

Scheme I



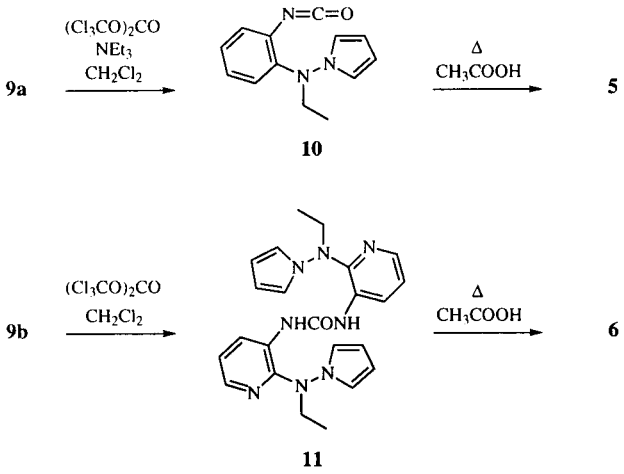
7a: X = CH
7b: X = N

8a: X = CH
8b: X = N

9a: X = CH
9b: X = N

Treatment of **9a** with bis(trichloromethyl)carbonate [5] in the presence of triethylamine gave the corresponding isocyanate **10** (Scheme II), which was cyclized to the lactam **5** in acidic medium. When the same reaction was performed on **9b**, the amine did not react. Treatment of **9b** with bis(trichloromethyl)carbonate in the absence of triethylamine afforded the bis-substituted urea **11**, which was cyclized likewise to lactam **6** in acidic medium.

Scheme II



Activity of compounds **5** and **6** against the HIV-1 multiplication in acutely infected cells was based on inhibition of virus-induced cytopathogenicity in MT-4 cells. It is noteworthy that compound **6** having a pyridine ring is less potent than pyrrolobenzotriazepinone **5** (Table I).

Table I

Compound	CC_{50} [a]	EC_{50} [b]	SI [c]
5	159	48	3.3
6	>200	>200	—
Nevirapine	>200	0.1	>2000

[a] Compound concentration (μ*M*) required to reduce the viability of mock-infected MT-4 cells by 50%; [b] Compound concentration (μ*M*) required to achieve 50% protection of MT-4 cells from the HIV-1 induced cytopathogenicity; [c] Selectivity index: ratio CC_{50}/EC_{50}.

EXPERIMENTAL

Melting points were determined on a Büchi 530 apparatus and are uncorrected. Mass spectral data were determined on a V6-Micromass 7070H spectrometer. The infrared spectra were recorded on a Jasco (FT/IR-200) spectrophotometer. The ^1H and ^{13}C nmr spectra were determined on a Varian 200 instrument using deuteriochloroform as solvent; chemical shifts are given in δ values downfield from tetramethylsilane as internal standard. Chromatographic separations were performed using silica gel 60 (0.015-0.040 mm) or aluminium oxide 90 (0.063-0.200 mm) as solid phase from Merck. Elemental analyses were performed by Dr. E. Cebulec, University of Trieste, Italy.

N-(3-Nitropyridin-2-yl)-1*H*-pyrrol-1-amine (**7b**).

A mixture of 1-aminopyrrole [4] (1.00 g, 12.2 mmoles), 2-chloro-3-nitropyridine (1.93 g, 12.2 mmoles), sodium carbonate (2.50 g, 24.4 mmoles) and powder copper (100 mg) in 40 ml of dimethylformamide was stirred at 130° for 4 hours. After concentration of the solvent under reduced pressure, the residue was dissolved in chloroform and filtered. The organic layer was washed with water followed by a saturated sodium chloride solution, dried over anhydrous sodium sulfate and evaporated. The resultant residue was purified on an alumina column eluting with toluene to afford 1.35 g (54%) of **7b**; mp 148-149° (ligroin/benzene); ^1H nmr (deuteriochloroform): δ 6.23-6.33 (m, 2H, H-3 and H-4 pyrrole), 6.72-6.82 (m, 2H, H-2 and H-5 pyrrole), 6.89-6.99 (m, 1H, H-5 pyridine), 8.42-8.60 (m, 2H, H-4 and H-6 pyridine), 10.22 (s, 1H, NH, deuterium oxide exchangeable); ms: m/z 204 (M^+).

Anal. Calcd. for $C_9H_8N_4O_2$: C, 52.94; H, 3.95; N, 27.44. Found: C, 53.1; H, 3.87; N, 27.4.

N-Ethyl-*N*-(3-nitropyridin-2-yl)-1*H*-pyrrol-1-amine (**8b**).

A solution of **7b** (620 mg, 3.04 mmoles) and tetrabutylammonium hydrogen sulfate (1.04 g, 3.04 mmoles) in dichloromethane (50 ml) was treated with 50% sodium hydroxide solution (30 ml). After cooling the reaction mixture at 0°, an excess of ethyl bromide (0.454 ml, 662 mg, 6.08 mmoles) was added. The mixture was stirred overnight at room temperature, then treated with water (100 ml) and dichloromethane (100 ml) and shaken. Organic extracts were collected, washed with saturated sodium chloride solution, dried over anhydrous sodium sulfate and evaporated. The residue was dissolved in toluene and chromatographed over an alumina column eluting with the same solvent to yield 600 mg (85%) of **8b**, mp 85° (ligroin); ^1H nmr (deuteriochloroform): δ 1.13 (t, 3H, CH_3, J = 7.1 Hz), 3.91 (q, 2H, CH_2, J = 7.1 Hz), 6.05-6.17 (m, 2H, H-3 and H-4 pyrrole), 6.50-6.62 (m, 2H, H-2 and H-5 pyrrole), 6.97 (dd, 1H, H-5 pyridine, J_1 = 7.8 Hz, J_2 = 4.8 Hz), 7.77 (dd, 1H, H-4 pyridine, J_1 = 7.8 Hz, J_2 = 1.7 Hz), 8.41 (dd, 1H, H-6 pyridine, J_1 = 4.8 Hz, J_2 = 1.7 Hz); ms: m/z 232 (M^+).

Anal. Calcd. for $C_{11}H_{12}N_4O_2$: C, 56.89; H, 5.21; N 24.12. Found: C, 56.5; H, 5.13; N, 24.0.

N-Ethyl-*N*-(2-nitrophenyl)-1*H*-pyrrol-1-amine (**8a**).

The compound was obtained as reported for **8b** in 97% yield, starting from **7a** [3] (5.00 g, 24.6 mmoles), mp 40-41° (ligroin); ^1H nmr (deuteriochloroform): δ 1.10 (t, 3H, CH_3, J = 7.0 Hz), 3.50 (q, 2H, CH_2, J = 7.0 Hz), 6.12-6.18 (m, 2H, H-3 and H-4 pyrrole), 6.76-6.82 (m, 2H, H-2 and H-5 pyrrole), 6.85-6.94 (m, 1H, H-6 benzene), 7.05-7.15 (m, 1H, H-4 benzene), 7.35-7.46 (m, 1H, H-5 benzene), 7.60-7.70 (m, 1H, H-3 benzene); ms: m/z 231 (M^+).

Anal. Calcd. for $C_{12}H_{13}N_3O_2$: C, 62.33; H, 5.67; N, 18.17. Found: C, 62.4; H, 5.61; N, 18.0.

N-(3-Aminopyridin-2-yl)-*N*-ethyl-1*H*-pyrrol-1-amine (**9b**).

A solution of **8b** (1.00 g, 4.31 mmoles) in hot ethanol (20 ml) was added to a suspension of iron(II) sulfate heptahydrate (12.0 g,

43.1 mmoles) in water (20 ml) and 32% ammonium hydroxide (2 ml). The mixture was heated under reflux for 1 hour with stirring while 32% ammonium hydroxide (20 ml) was added dropwise. After filtration, the residue was washed with hot ethanol and discarded. The collected solutions were partially evaporated and extracted with chloroform, washed with water followed by a saturated sodium chloride solution and dried over anhydrous sodium sulfate. After evaporation of the solvent, the crude residue was dissolved in toluene and purified by passing through an alumina column, eluting with the same solvent. The elutes were collected and evaporated to give 600 mg (69%) of **9b**, mp 47° (petroleum ether); ^{1}H nmr (deuteriochloroform): δ 1.10 (t, 3H, CH_3, J = 7.1 Hz), 3.60-3.78 (m, 4H, CH_2 and NH_2), 6.05-6.15 (m, 2H, H-3 and H-4 pyrrole), 6.83-6.92 (m, 4H, H-2 and H-5 pyrrole, H-4 and H-5 pyridine), 7.80-7.86 (m, 1H, H-6 pyridine); ms: m/z 202 (M^+).

Anal. Calcd. for $C_{11}H_{14}N_4$: C, 65.32; H, 6.98; N, 27.70. Found: C, 65.3; H, 7.04; N, 27.8.

N-(2-Aminophenyl)-*N*-ethyl-1*H*-pyrrol-1-amine (**9a**).

The compound was obtained as reported for **9b** in 67% yield, starting from **7a** (1.00 g, 4.33 mmoles), oil; ^{1}H nmr (deuteriochloroform): δ 1.12 (t, 3H, CH_3, J = 7.0 Hz), 3.50 (q, 2H, CH_2, J = 7.0 Hz), 3.97 (br s, 2H, NH_2, deuterium oxide exchangeable), 6.06-6.16 (m, 2H, H-3 and H-4 pyrrole), 6.68-7.30 (m, 6H, H benzene, H-2 and H-5 pyrrole).

Compound **9a** was characterized as *N*-(2-acetylaminophenyl)-*N*-ethyl-1*H*-pyrrol-1-amine, mp 82° (ligroin); ir (potassium bromide): ν C=O 1668 cm^{-1}; ^{1}H nmr (deuteriochloroform): δ 1.12 (t, 3H, CH_2CH_3, J = 7.1 Hz), 2.17 (s, 3H, $COCH_3$), 3.49 (q, 2H, CH_2, J = 7.1 Hz), 6.05-6.15 (m, 2H, H-3 and H-4 pyrrole), 6.83-6.93 (m, 2H, H-2 and H-5 pyrrole), 7.07-7.42 (m, 3H, H benzene), 8.02 (s, 1H, NH, deuterium oxide exchangeable), 8.20-8.33 (m, 1H, H benzene); ms: m/z 243 (M^+).

Anal. Calcd. for $C_{14}H_{17}N_3O$: C, 69.11; H, 7.04; N, 17.27. Found: C, 69.2; H, 7.07; N, 17.1.

N-Ethyl-*N*-(2-isocyanatophenyl)-1*H*-pyrrol-1-amine (**10**).

A solution of bis(trichloromethyl)carbonate (214 mg, 0.72 mmole) in anhydrous dichloromethane (15 ml) was dropped slowly into a solution of **9a** (434 mg, 2.16 mmoles) and triethylamine (220 mg, 2.16 mmoles) in the same solvent (30 ml). The solution was stirred under nitrogen at room temperature for 5 hours, then treated with water (50 ml). The organic solution was separated and the aqueous phase was extracted with dichloromethane (4 x 40 ml). The extracts were collected, washed with a saturated sodium chloride solution and dried over anhydrous sodium sulfate. Evaporation of the solvent under reduced pressure afforded 440 mg (90%) of **10** as an oil, which was used for the following reaction without further purification; ir (potassium bromide): ν N=C=O 2238 cm^{-1}; ^{1}H nmr (deuteriochloroform): δ 1.12 (t, 3H, CH_3, J = 7.0 Hz), 3.50 (q, 2H, CH_2, J = 7.0 Hz), 6.08-6.16 (m, 2H, H-3 and H-4 pyrrole), 6.95-7.35 (m, 6H, H benzene, H-2 and H-5 pyrrole); ms: m/z 227 (M^+).

5,10-Dihydro-5-ethyl-11*H*-pyrrolo[1,2-*b*][1,2,5]benzotriazepin-11-one (**5**).

A solution of **10** (500 mg, 2.20 mmoles) in acetic acid (50 ml) was refluxed for 1 hour. After evaporation of the solvent under reduced pressure, the residue was dissolved in chloroform, washed with a sodium hydrogen carbonate solution, dried over anhydrous sodium sulfate and evaporated. The crude residue was dissolved in chloroform and chromatographed over an alumina column eluting with the same solvent. The elutes were collected and evaporated to give 200 mg (40%) of **5**, mp 197-198° (benzene-ligroin); ir (potassium bromide): ν C=O 1643, N-H 2977 cm^{-1}; ^{1}H nmr (deuteriochloroform): δ 0.95 (t, 3H, CH_3, J = 7.0 Hz), 3.25-3.63 (m, 2H, CH_2), 6.09-6.18 (m, 1H), 6.95-7.40 (m, 6H), 9.50 (br s, 1H, NH, deuterium oxide exchangeable); ms: m/z 227 (M^+).

Anal. Calcd. for $C_{13}H_{13}N_3O$: C, 68.71; H, 5.77; N, 18.49. Found: C, 68.6; H, 5.74; N, 18.2.

N-*N*'-bis{[*N*-Ethyl-*N*-(pyrrol-1-yl)]-2-amino-pyridin-3-yl}urea (**11**).

A solution of bis(trichloromethyl)carbonate (831 mg, 2.80 mmoles) in anhydrous dichloromethane (20 ml) was dropped slowly into a solution of **9b** (1.70 g, 8.40 mmoles) in the same solvent (40 ml). The mixture was stirred under nitrogen at room temperature for 4 hours, then treated with water (50 ml) and made neutral by adding sodium hydrogen carbonate. The organic solution was separated and the aqueous phase was extracted with dichloromethane (4 x 40 ml). The extracts were collected, washed with a saturated sodium chloride solution and dried over anhydrous sodium sulfate. Evaporation of the solvent gave 1.79 g (99%) of **11**, mp 136° (toluene-petroleum ether); ir (potassium bromide): ν C=O 1652, N-H 3319 cm^{-1}; ^{1}H nmr (deuteriochloroform): δ 1.13 (t, 6H, CH_3, J = 7.1 Hz), 3.74 (q, 4H, CH_2, J = 7.1 Hz), 6.12-6.19 (m, 4H, H-3 and H-4 pyrrole), 6.31 (s, 2H, NH, deuterium oxide exchangeable), 6.80-6.87 (m, 4H, H-2 and H-5 pyrrole), 7.08 (dd, 2H, H-5 pyridine, J_1 = 8.1 Hz, J_2 = 4.7 Hz) 8.12 (dd, 2H, H-4 or H-6 pyridine, J_1 = 4.7 Hz, J_2 = 1.5 Hz), 8.20 (dd, 2H, H-4 or H-6 pyridine, J_1 = 8.1 Hz, J_2 = 1.5 Hz); ms: m/z 430 (M^+).

Anal. Calcd. for $C_{23}H_{26}N_8O$: C, 64.2; H, 6.09; N, 26.0. Found: C, 64.5; H, 6.10; N, 25.7.

5,11-Dihydro-11-ethyl-6*H*-pyrido[3,2-*f*]pyrrolo[1,2-*b*][1,2,5]triazepin-6-one (**6**).

A solution of **11** (1.00 g, 2.32 mmoles) in acetic acid (50 ml) was refluxed for 14 hours. After evaporation of the solvent under reduced pressure, the residue was dissolved in chloroform, washed with a sodium hydrogen carbonate solution, dried over anhydrous sodium sulfate and evaporated. The crude residue was dissolved in chloroform and chromatographed over an alumina column eluting with the same solvent. The elutes were collected and evaporated to yield 140 mg (26%) of **6**, mp 136° (benzene-ligroin); ir (potassium bromide): ν C=O 1645, N-H 3097 cm^{-1}; ^{1}H nmr (deuteriochloroform): δ 1.08 (t, 3H, CH_3, J = 7.1 Hz), 3.63 (br s, 2H, CH_2), 6.19 (dd, 1H, H-8, J_1 = 4.3 Hz, J_2 = 2.7 Hz), 7.02 (dd, 1H, H-7, J_1 = 4.3 Hz, J_2 = 1.9 Hz), 7.10 (dd, 1H, H-9, J_1 = 2.7 Hz, J_2 = 1.9 Hz), 7.27 (dd, 1H, H-3, J_1 = 7.7 Hz, J_2 = 4.8 Hz), 7.50 (dd, 1H, H-4, J_1 = 7.7 Hz, J_2 = 1.6 Hz), 8.25 (dd, 1H, H-2, J_1 = 4.8 Hz, J_2 = 1.6 Hz), 9.24 (s, 1H, NH, deuterium oxide exchangeable); ^{13}C nmr (deuteriochloroform): δ 13.0 (CH_3), 49.5 (CH_2), 107.5, 116.6, 123.4, 123.9, 126.2, 130.7, 131.6, 144.1, 150.1, 161.8 (CO); ms: m/z 228 (M^+).

Anal. Calcd. for $C_{12}H_{12}N_4O$: C, 63.15; H, 5.30; N, 24.55. Found: C, 63.3; H 5.29; N, 24.4.

Biological Assay.

Compounds.

Test compounds were dissolved in DMSO at an initial concentration of 200 m*M* and then were serially diluted in a culture medium.

Cells.

Cell line were from American Type Culture Collection (ATCC); H9/III_B, MT-4 and C8166 cells [grown in RPMI 1640 containing 10% foetal calf serum (FCS), 100 UI/ml penicillin G and 100 μg/ml streptomycin] were used for anti-HIV-1 assays. Cells culture were checked periodically for the absence of mycoplasma contamination with a Myco Tect Kit (Gibco).

Viruses.

Human immunodeficiency virus type-1 (HIV-1, III_B strain) was obtained from supernatants of persistent infected H9/III_B cells. HIV-1 stock solutions had a titre of 5 x 10^7 cell culture infectious dose fifty ($CCID_{50}$/ml).

Antiviral Assays.

Activity against the HIV-1 multiplication in acutely infected cells was based on inhibition of virus-induced cytopathogenicity in MT-4 cells [6]. Briefly, 50 μL of RPMI 10% FCS containing 1 x 10^4 cells were added to each well of flat-bottomed microtiter trays containing 50 μL of medium and serial dilutions of test compound. Then 20 μL of an HIV-1 suspension containing 100 $CCID_{50}$ were added. After a 4 day incubation at 37°, the number of viable cells was determined by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) method [7,8]. Cytotoxity of compounds, based on the viability of mock-infected cells as monitored by the MTT method, was evaluated in parallel with their antiviral activity.

Linear Regression Analysis.

Viral and cell growth at each drug concentration was expressed as percentage of untreated controls and the concentrations resulting in 50% (EC_{50}, CC_{50}) growth inhibition was determined by linear regression analysis.

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