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Short communication

1,5-Benzodiazepines. Part XII. Synthesis and biological evaluation of tricyclic and tetracyclic 1,5-benzodiazepine derivatives as nevirapine analogues☆

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Abstract – A number of properly substituted 5H-pyrimido[4,5-b][1,5]benzodiazepines (**2**) and pyrazolo[3,4-b][1,5]benzodiazepines (**3** and **4**), as well as compounds **5**–**7**, which are derivatives of new tetracyclic systems, were prepared as nevirapine analogues through multistep synthetic routes. The cytotoxic and anti-HIV-1 properties of compounds **2**–**7** were evaluated in cell-based assays, together with their inhibitory activity against the HIV-1 recombinant reverse transcriptase (rRT) in enzyme assays. The modifications introduced into nevirapine heterocyclic skeleton proved to have a negative effect for the anti-HIV-1 activity. It is worth noting that some of the new derivatives proved to be cytotoxic in the low micromolar range. © 2001 Éditions scientifiques et médicales Elsevier SAS

1,5-benzodiazepine fused derivatives / NNRTIs

1. Introduction

Nevirapine (**1a**) [1] is one of the most active non-nucleoside HIV-1 reverse transcriptase inhibitors (NNRTIs) and was the first to reach regulatory approval. It acts as an allosteric inhibitor and is at present used for treatment of HIV-1 infections, in combination with nucleoside reverse transcriptase inhibitors (NRTIs) and HIV-1 protease inhibitors, in order to avoid the appearance of the drug resistance observed in nevirapine monotherapy, resulting from the enzyme mutation. Recently, proper modifications in the substitution pattern of nevirapine resulted in new broad spectrum HIV-1 RT inhibitors, such as compound **1b** and other its 8-[(arylthio)methyl]-2-halo and 8-[(aryloxy)methyl]-2-halo analogues [2].

Therefore, we considered it interesting to investigate whether also suitable changes in the nevirapine heterocyclic skeleton could result in potent anti-HIV-1 activity and, possibly, inhibition of clinically relevant NNRTI resistant mutants.

Thus, as a first step in this investigation, we have synthesised a number of 5H-pyrimido[4,5-b]-[1,5]benzodiazepin-5-ones (**2**) and pyrazolo[3,4-b]-[1,5]benzodiazepin-4-ones (**3** and **4**) as well as tetracyclic derivatives **5**, **6**, and **7b**, as analogues of nevirapine and its very active tetracyclic derivatives [3], respectively (*figure 1*).

Here we report the synthesis of the above compounds and their effect against the HIV-1 multiplication in acutely infected MT-4 cells and the HIV-1 rRT in enzyme assays.

☆ Preliminary results were presented at the Italian–Hungarian–Polish Joint Meeting on Medicinal Chemistry in Giardini Naxos-Taormina (Catania), Italy, 1999 (Abstract PA 36, p. 122).

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2. Chemistry

4-(Dialkylamino)-1,3-dihydro-2H-1,5-benzodiazepin-2-ones **8a**, **c** and the derivative **9a**, previously described by us [4, 5], were used as starting compounds to prepare tricyclic derivatives **2a**–**n**, **3a**–**i** and **4a**, **b**.

Thus, treatment of **8a** [4] with KOH and methyl iodide gave the methyl derivative **8b**. Reaction of compounds **8b** and **8c** [4] with *N*,*N*-dimethylformamide in the presence of PCl_5 afforded then the 3-[(dimethylamino)methylene]derivatives **9b** and **9c**, respectively. Cyclocondensation of compound **9a** [5] or **9b** with proper dinucleophilic reagents (H^+) gave the substituted 6,11-dihydro-5H-pyrimido [4,5-b][1,5]benzodiazepin-5-ones **2a**, **b**, **f**, **h**, **k**. Treatment of these compounds with KOH and suitable alkyl iodides led to the desired derivatives **2c**–**e**, **g**, **i**, **j**, **l**–**n** (see *figure 2* and *table I*).

Analogously, cyclocondensation of phenylhydrazine with **9b** in refluxing butanol (H^+) afforded only the phenyl derivative **3d**, whereas methylhydrazine reacted with **9b** or **9c** under the same conditions to give both compounds **3a** or **3g** and their isomers **4a** (in very low yield) or **4b**, respectively, as we previously observed in a similar case [5]. 10-Alkylderivatives **3b**, **c**, **e**, **f**, **h** and **i** were then easily obtained from the substituted 5,10-dihydropyrazolo[3,4-b][1,5]benzodiazepin-4(1H)-ones **3a**, **d** and **g** (KOH and alkyl iodide) (*figure 2*, *table I*).

Compound **2a** was treated with Lawesson's reagent to give thione **10**, from which the diethyl derivative **11b** was obtained by treatment with KOH and ethyl iodide.

Finally, the reaction of the (ethylthio) derivative **11b** with propargylamine or acetohydrazyde, in the presence of *p*-toluenesulphonic acid afforded the 9-ethyl-3-methyl substituted 9H-imidazo[1,2-a]pyrimido[5,4-c][1,5]benzodiazepine (**5**) or 9H-pyrimido[5,4-c][1,2,4]triazolo[4,3-a][1,5]benzodiazepine (**6**), respectively. On the other hand, the reaction of **11b** with hydroxylamine ($NH_2OH{\cdot}HCl + NaHCO_3$) yielded the (hydroxyamino)derivative **12b** (*figure 3*).

Two different synthetic pathways were tried for compound **7b**. The first one, based on cyclocondensation of the corresponding (hydroxyamino) derivative **12b** with acetaldehyde dimethylacetal to give compound **7b**, was discarded due to the very low yield. A better result was reached through the procedure herein described, starting from the (ethylthio) derivative **11a** which was prepared by treating thione **10** with anhydrous K_2CO_3 and ethyl iodide. By heating a mixture of **11a**, $NH_2OH{\cdot}HCl$ and $NaHCO_3$ in refluxing methanol the (hydroxyamino) derivative **12a** was obtained [6], which was in turn treated with acetaldehyde dimethylacetal in the presence of *p*-toluenesulphonic acid to yield 3-methyl-3H,9H-[1,2,4]-oxadiazolo[4,3-a]pyrimido[5,4-c][1,5]benzodiazepine (**7a**). Finally, treatment of **7a** with KOH and ethyl iodide afforded the desired 9-ethyl derivative **7b** (*figure 3*).

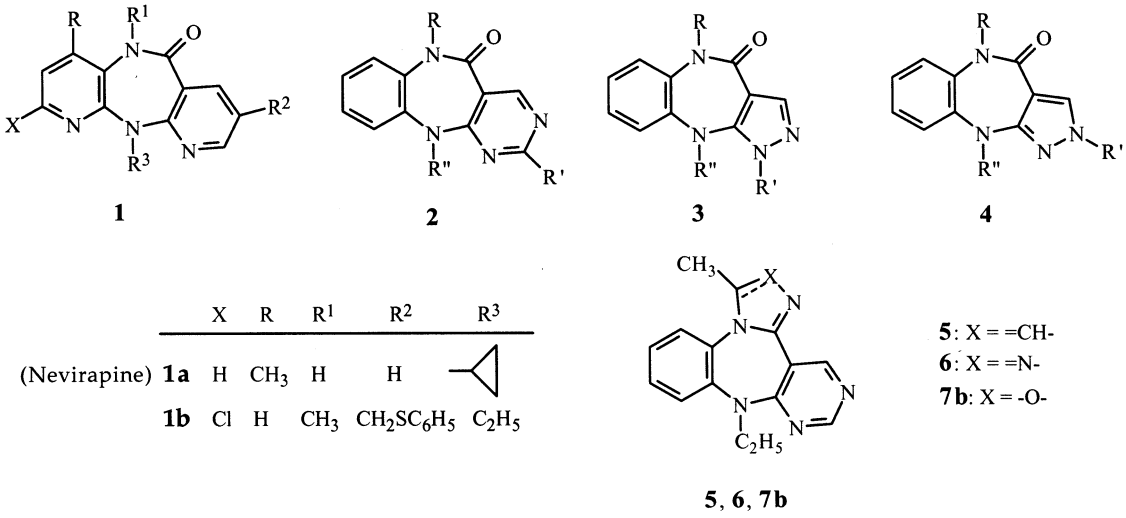


Figure 1. Structures of nevirapine (**1a**), its analogue **1b** and compounds **2**–**7**.

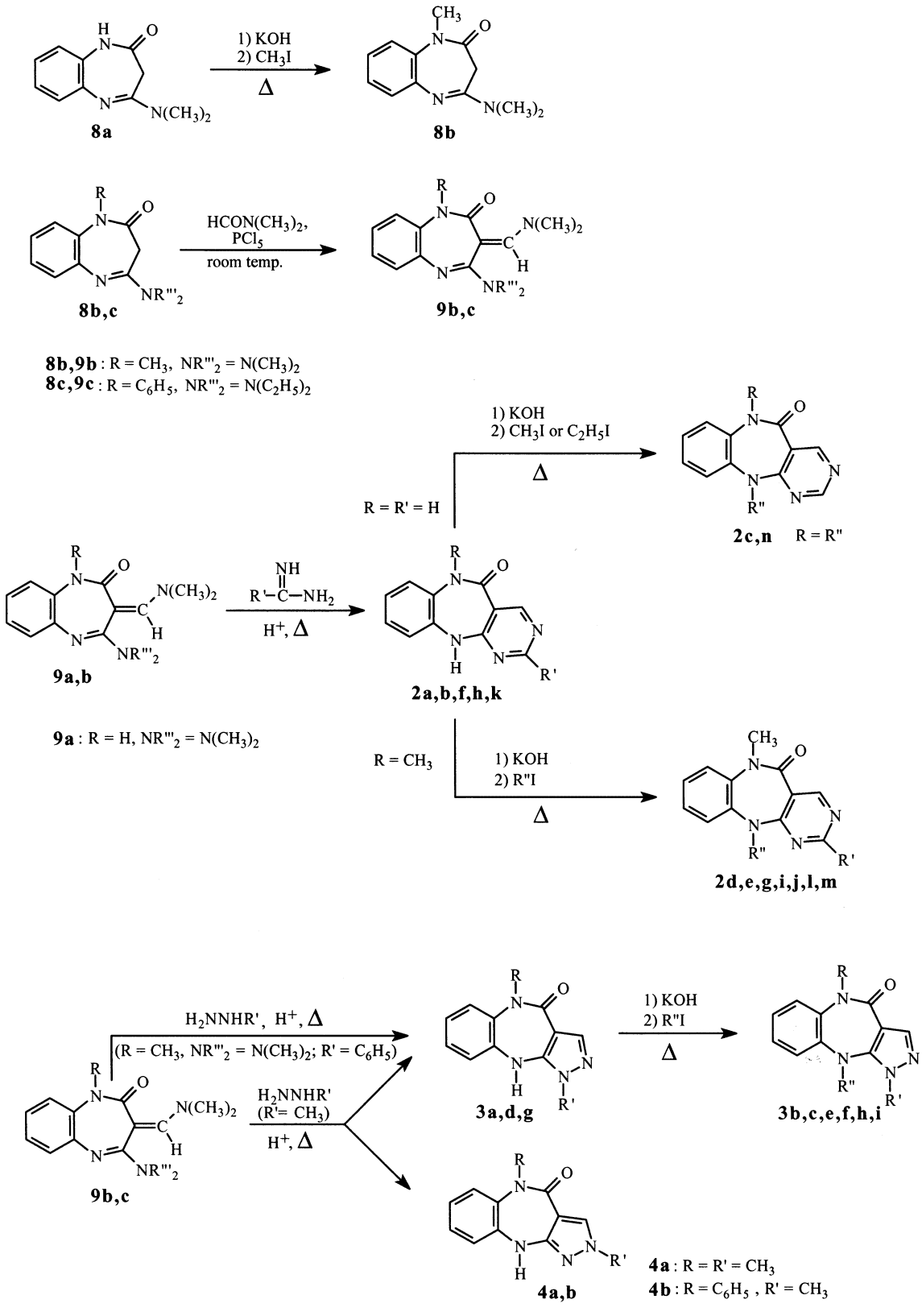
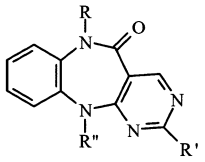
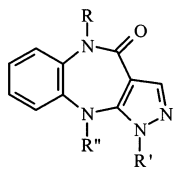


Figure 2. Synthetic route to compounds **2a–n,** and **3a–i**, **4a**, **b**.

Table I. Structures of compounds **2a–n** and **3a–i**.



2a-n



3a-i

Compound	R	R′	R″
2a	H	H	H
2b	CH_3	H	H
2c	CH_3	H	CH_3
2d	CH_3	H	C_2H_5
2e	CH_3	H	i-C_3H_7
2f	CH_3	CH_3	H
2g	CH_3	CH_3	C_2H_5
2h	CH_3	C_6H_5	H
2i	CH_3	C_6H_5	C_2H_5
2j	CH_3	C_6H_5	i-C_3H_7
2k	CH_3	SCH_3	H
2l	CH_3	SCH_3	C_2H_5
2m	CH_3	SCH_3	i-C_3H_7
2n	C_2H_5	H	C_2H_5
3a	CH_3	CH_3	H
3b	CH_3	CH_3	C_2H_5
3c	CH_3	CH_3	i-C_3H_7
3d	CH_3	C_6H_5	H
3e	CH_3	C_6H_5	C_2H_5
3f	CH_3	C_6H_5	i-C_3H_7
3g	C_6H_5	CH_3	H
3h	C_6H_5	CH_3	C_2H_5
3i	C_6H_5	CH_3	i-C_3H_7

The structures attributed to compounds **3a**, **g** and their corresponding isomers **4a**, **b** have been chemically confirmed (see *figure 4*). Actually, in the case of **3a** and **4a**, taking the previously described isomers **3k** and **4c** [5] as reference compounds, treatment of both **3a** and **3k** with KOH and methyl iodide gave the same trimethyl derivative **3j**, thus confirming the structure of **3a**. Furthermore, the methylation of **4c** afforded the trimethyl derivative **4d**, isomer of **3j**, along with the dimethyl derivative **4a**, the isomer of **3a**.

On the other hand, reaction of compound **8d** [4] with *N*-methyl formic hydrazide in the presence of *p*-toluenesulphonic acid afforded compound **4b**, so confirming both its structure and, consequently, the one attributed to its isomer **3g**.

Finally, in order to confirm the structure of compound **3d**, the 4-(2-phenylhydrazino) derivative **13** was reacted with *N*,*N*-dimethylformamide in the presence of PCl_5 to give unambiguously compound **4e** which is the isomer of **3d**. Treatment of compound **4e** with KOH and the proper alkyl iodide gave the 10-alkyl derivatives **4f** or **4g** (*figure 4*).

The structures attributed to the compounds described in this paper are consistent with the results of elemental analyses, IR and ^{1}H-NMR spectral data (see Section 4 and *table III*).

Concerning in particular the ^{1}H-NMR spectra, the multiplicities shown by the 3-CH_2 signal of compound **8b**, the N–CH_2 signals of **2d**, **g**, **i**, **l**, **n**, **3b**, **e**, **h**, **5**, **6**, **7b**, and by the HC(CH_3)$_2$ signals of **2e**, **j**, **m** and **3c**, **f**, **i** depend on non-equivalence of the two geminal hydrogens (CH_2) or methyl groups [HC(CH_3)$_2$], respectively, as we reported for previously described 1,5-benzodiazepine derivatives [7–10].

Furthermore, we can point out that the ^{1}H-NMR spectra of **9b** and **9c** clearly confirm the structure attributed to these compounds, as we previously elucidated for their structural analogues [5, 11]. In this connection, the singlet corresponding to the N(CH_3)$_2$ group of the 3-[(dimethylamino)methylene] substituent is particularly significant, supporting the free rotation of this group.

Compounds **5**–**7** are derivatives of novel heterocyclic systems.

Intermediates **2a** [12] and **2b**, **h** [13] were reported previously in the literature, but obtained through a different procedure.

3. Biological results and conclusions

The title compounds were tested for cytotoxicity and anti-HIV-1 activity in vitro. Most of them were also tested in enzyme assays against HIV-1 recombinant reverse transcriptase (rRT).

Table II summarizes the results of biological evaluations, from which the following conclusions can be drawn.

The skeletal differences between the tricyclic system of nevirapine and the corresponding ones of compounds **2**–**7** proved to have a negative effect for the anti-HIV-1 activity of the latter compounds. In the case of compounds **2** and **5**–**7**, this effect can be reasonably attributed to an unfavourable electronic configuration of their pyrimido[4,5-b][1,5]benzodiazepine ring system (compounds **2**) or ring system moiety (compounds **5**–**7**). Nevertheless, it can be

noted that **2d**, **2e** and **2n**, whose substitution patterns are closely related to that of nevirapine, are the only three compounds showing some anti-HIV-1 activity.

On the other hand, the modifications now introduced into the nevirapine heterocyclic skeleton led to compounds with increased cytotoxic activity. Some of them, in particular **3c**, **3f** and **7b**, proved cytotoxic in the low micromolar range ($CC_{50} = 4–7$ μM).

When tested in vitro against the rRT, the title compounds resulted inactive with the sole exceptions of **2d**, **2n** and, in particular, **3e** which showed some inhibitory effect.

4. Experimental protocols

4.1. Chemistry

Melting points were determined using a Fisher–Johns (electrothermal when above 300 °C) apparatus and are uncorrected. IR spectra were recorded in a Perkin–Elmer 398 spectrophotometer. ^{1}H-NMR spectra were recorded in a Varian Gemini 200 (200 MHz) spectrometer, using $(CH_3)_4Si$ as an internal reference ($\delta = 0$), and chemical shifts (δ) are reported in ppm. Analyses of all new compounds, indicated by the symbols of the elements, were within ±0.4% of the theoretical values and were performed by the Laboratorio di Microanalisi, Dipartimento di Scienze Farmaceutiche, Università di Genova.

Thin layer chromatograms were run on Merck silica gel 60 F_{254} precoated plastic sheets (layer thickness 0.2 mm). Column chromatography was performed using Carlo Erba silica gel (0.05–0.20 mm) or Carlo Erba neutral aluminium oxide (Brockmann activity I).

4.1.1. 4-(Dimethylamino)-1,3-dihydro-1-methyl-2H-1,5-benzodiazepin-2-one (8b)

A mixture of 20.0 mmol (4.06 g) of compound **8a** [4], 80.0 mmol (4.49 g) of finely powdered KOH and 80 mL of dry acetone was refluxed for 10 min, then the solution of 30.0 mmol (4.26 g) of iodomethane in 15 mL of dry acetone was added and the mixture was further refluxed for 30 min, with stirring. The solvent was then removed in vacuo, the residue was partitioned between water and dichloromethane, and the aqueous phase was extracted

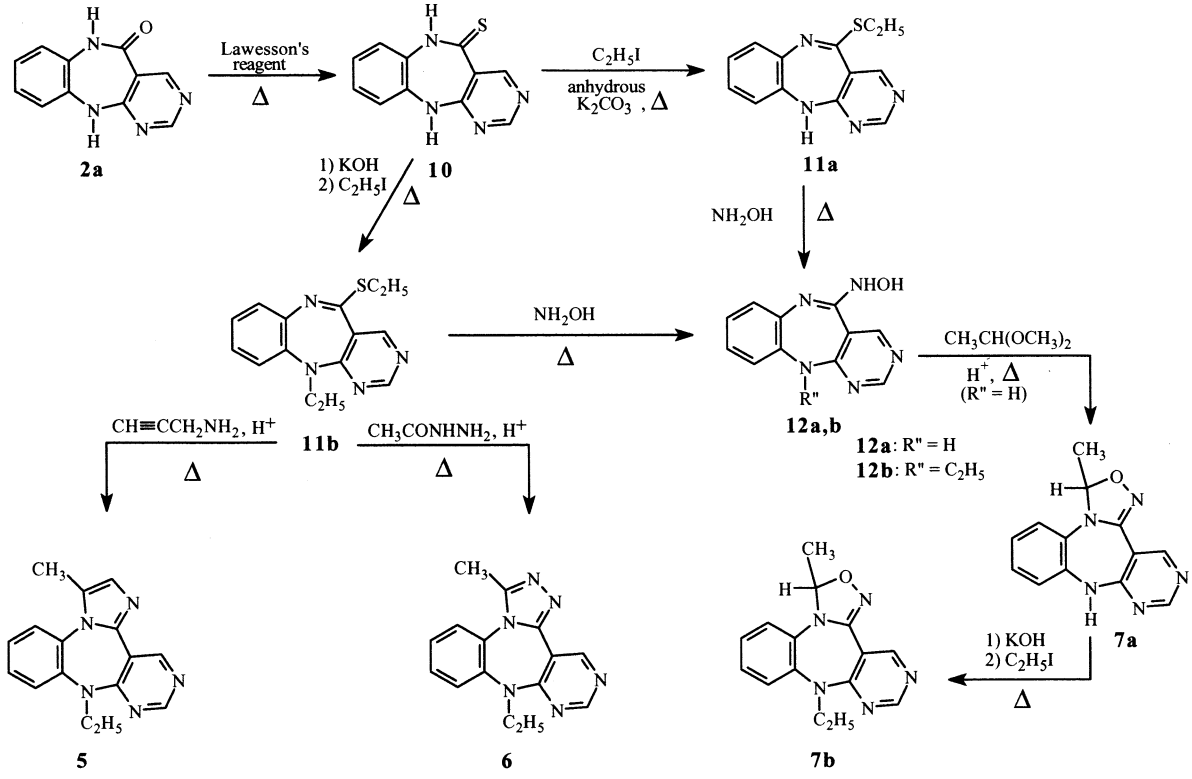


Figure 3. Synthetic route to compounds **5**, **6**, **7a**, **b**.

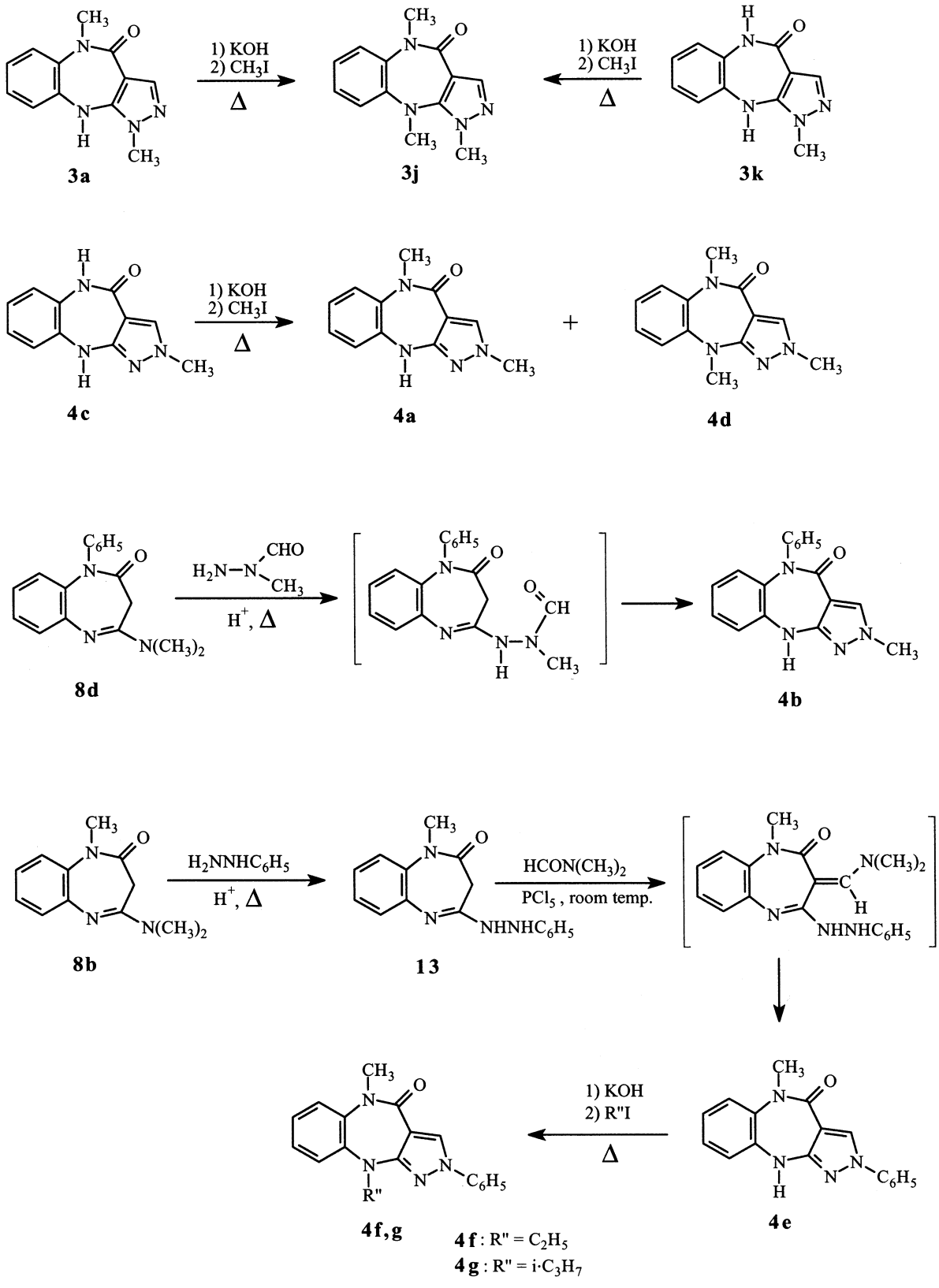


Figure 4. Structure confirmation for compounds **3a**, **d**, **g**, **4a**, **b** and synthesis of compounds **4e**–**g**.

Table II. In vitro biological activities of compounds **2c–e**, **g**, **i**, **j**, **l–n**, **3b**, **c**, **e**, **f**, **h**, **i**, **4f**, **g** and **5**, **6**, **7b**.

Compound	CC_{50} [a] MT-4	EC_{50} [b] HIV-1	SI [c]	IC_{50} [d] HIV-1 rRT
2c	>200	>200	–	>30
2d	>200	51	>4	>30 (26%)
2e	>200	117	>1.7	>30
2g	>200	>200	–	>30
2i	40.4	>40.4	–	>30
2j	>200	>200	–	>30
2l	139	>139	–	>30
2m	61	>61	–	>30
2n	>200	76	>2.6	≥30 (39%)
3b	91	>91	–	>30
3c	4.6	>4.6	–	>30
3e	43	>43	–	=30 (45%)
3f	5	>5	–	>30
3h	50	>50	–	>30
3i	34.4	>34.4	–	>30
4f	≥200	>200	–	–
4g	>200	>200	–	–
5	14.5	>14.5	–	–
6	17.6	>17.6	–	–
7b	6.9	>6.9	–	–
Nevirapine	>200	0.2	>1000	0.6

[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, CC_{50}/EC_{50} ratio.
[d] Compound concentration (μM) required to inhibit the HIV-1 rRT activity by 50%. Data in parentheses represent the percentage of inhibition of enzyme activity at 30 μM.

several more times with the same solvent. The combined extracts were dried (anhydrous Na_2SO_4), then evaporated to dryness under reduced pressure to give a thick oil from which, after addition of some ethyl ether and standing, 3.74 g (86%) of pure **8b** separated out; white crystals, m.p. 122–123 °C, after crystallisation from ethyl acetate–petroleum ether. IR ($CHCl_3$, cm^{-1}): 1660 (CO), 1610, 1581. ^{1}H-NMR ($CDCl_3$): δ 2.89 and 3.71 (AB system, $J = 12$ Hz, 2H, 3-CH_2), 3.18 [s, 6H, $N(CH_3)_2$], 3.36 (s, 3H, 1-CH_3), 6.98–7.22 (m, 4H, H-6,7,8,9). Anal. $C_{12}H_{15}N_3O$ (C, H, N).

*4.1.2. General procedure for 1-substituted 4-(dialkylamino)-3-[(dimethylamino)methylene]-1,3-dihydro-2H-1,5-benzodiazepin-2-ones (**9b**, **c**)*

PCl_5 (3.60 g) was slowly added to the solution of 12.0 mmol of **8b** or **8c** [4] in 42 mL of *N*,*N*-dimethylformamide, with stirring. The resulting solution was stirred at room temperature for 20 h, then poured onto ice-water. After addition of 6 N aqueous NaOH (until alkaline) and 150 mL of dichloromethane, the mixture was vigorously stirred at room temperature for 30 min. The organic solution was collected and the aqueous phase was further extracted several more times with dichloromethane. The combined extracts, after drying and removal of solvent, afforded a thick oil from which, by addition of a little ethyl ether, the nearly pure compounds **9b** or **9c**, respectively, separated out as white solids which were recrystallised from the proper solvent.

*4.1.2.1. Compound **9b***

Yield: 2.87 g (88%) from 2.61 g of **8b**; m.p. 151–152 °C, after crystallisation from ethyl acetate–ethyl ether. IR ($CHCl_3$, cm^{-1}): 1640 (CO), 1605 shoulder, 1590, 1560. ^{1}H-NMR ($CDCl_3$): δ 2.80 [s, 6H, =CH–N(CH_3)$_2$], 3.00 and 3.14 [2s, 3H+3H, 4-N(CH_3)$_2$], 3.27 (s, 3H, 1-CH_3), 6.82 [s, 1H, =CH–N(CH_3)$_2$], 6.95–7.40 (m, 4H, H-6,7,8,9). Anal. $C_{15}H_{20}N_4O$ (C, H, N).

*4.1.2.2. Compound **9c***

Yield: 4.00 g (92%) from 3.69 g of **8c** [4]; m.p. 158–159 °C (from ethyl acetate–petroleum ether). IR ($CHCl_3$, cm^{-1}): 1658 (CO), 1616, 1600, 1560. ^{1}H-NMR ($CDCl_3$): δ 1.10 and 1.29 (2t, 3H+3H, CH_2CH_3), 2.82 [s, 6H, =CH–N(CH_3)$_2$], 3.13–3.53 (m, 3H, 3H of CH_2CH_3), 3.93 (m, 1H, 1H of CH_2CH_3), 6.63–6.85 and 6.97–7.44 (2m, 2H+7H, H-6,7,8,9+phenyl Hs), 6.73 [s, 1H, =CH–N(CH_3)$_2$]. Anal. $C_{22}H_{26}N_4O$ (C, H, N).

*4.1.3. General procedure for compounds **2a**, **b**, **f**, **h** and **k***

A mixture of 6.0 mmol of **9a** [5] (1.55 g) or **9b** (1.63 g), an excess of the proper amidine [12.0 mmol of formamidine acetate (1.25 g) or acetamidine acetate (1.42 g), 9.0 mmol (1.41 g) of benzamidine hydrochloride plus 1.5 mL of triethylamine, or 6.0 mmol (1.67 g) of 2-methylisothiourea sulphate plus 2.5 mL of triethylamine], and 20 mL of the suitable solvent was stirred at the temperature and for the time reported below for each case.

After cooling, compound **2** was recovered as described below for each case.

*4.1.3.1. 6,11-Dihydro-5H-pyrimido[4,5-b][1,5]benzodiazepin-5-one (**2a**)*

The suspension finally obtained from the reaction of **9a** [5] with formamidine acetate (Dowtherm A, 160 °C,

1 h) was diluted with a little acetone and filtered to recover 0.61 g of pure compound **2a** as a white solid.

*4.1.3.2. 6,11-Dihydro-6-methyl-5H-pyrimido[4,5-b][1,5]-benzodiazepin-5-one (**2b**)*

The mixture obtained from the reaction of **9b** with formamidine acetate (Dowtherm A, 160 °C, 1 h) was treated with 10% aqueous Na_2CO_3, then exhaustively extracted with dichloromethane. The combined extracts were dried, then evaporated to give an oil which was subjected to column chromatography (silica gel), eluting first with dichlorometane to remove Dowtherm A and some impurities, then with ethyl acetate to collect, after removal of solvent, 0.54 g of pure compound **2b** as a white solid.

*4.1.3.3. 6,11-Dihydro-2,6-dimethyl-5H-pyrimido[4,5-b]-[1,5]benzodiazepin-5-one (**2f**)*

After the reaction of **9b** with acetamidine acetate (Dowtherm A, 160 °C, 2 h) was carried out, the resulting mixture was subjected to the same procedure described above for the preparation of compound **2b**. The oil ultimately obtained was chromatographed on a silica gel column, eluting first with dichloromethane until Dowtherm A was completely removed, then with dichloromethane–petroleum ether–triethylamine (10:10:1), discarding the first fractions containing impurities. Further exhaustive elution with the same mixture afforded the nearly pure compound **2f** (0.25 g), as a white solid.

*4.1.3.4. 6,11-Dihydro-6-methyl-2-phenyl-5H-pyrimido-[4,5-b][1,5]benzodiazepin-5-one (**2h**)*

The mixture finally obtained from the reaction of **9b** with benzamidine (refluxing butanol, 24 h), was evaporated to dryness under reduced pressure to give an oily residue that was treated, then vigorously stirred (room temperature, 30 min) with 0.5 N aqueous NaOH (100 mL) and (1:1) ethyl ether–petroleum ether (100 mL). This way the nearly pure compound **2h** (0.36 g) separated out as a yellowish solid.

*4.1.3.5. 6,11-Dihydro-6-methyl-2-(methylthio)-5H-pyrimido[4,5-b][1,5]benzodiazepin-5-one (**2k**)*

The solution resulting from the reaction of compound **9b** with 2-methylisothiourea (refluxing dry pyridine, 24 h) was poured into cold water and the mixture was exhaustively extracted with dichloromethane. The combined extracts were dried and the solvents removed in vacuo to give an oily residue which was chromatographed on a silica gel column eluting with dichloromethane–ethyl acetate (4:1) until pure compound **2k** was completely recovered (0.26 g, white solid).

Data for compounds **2a**, **b**, **f**, **h**, **k** are reported in *table III*.

*4.1.4. General procedure for 6,11-dialkyl-6,11-dihydro-5H-pyrimido[4,5-b][1,5]benzodiazepin-5-ones (**2c–e**, **g**, **i**, **j**, **l–n**)*

A mixture of 1.5 mmol of **2b** (0.34 g), **2f** (0.36 g), **2h** (0.45 g), or **2k** (0.41 g), 9.0 mmol (0.50 g) of finely powdered KOH and 50 mL of dry acetone was refluxed for 10 min, with stirring. The solution of 9.0 mmol of iodoethane (1.40 g) or 2-iodopropane (1.53 g) in 15 mL of dry acetone was then added and the mixture was further refluxed for 1 h (compounds **2d**, **g**, **i**, **l**) or 2 h (compounds **2e**, **j**, **m**), while stirring.

In the case of reactions carried out with **2a** (1.5 mmol, 0.32 g), 18.0 mmol (1.0 g) of KOH and 18.0 mmol of iodomethane (2.55 g) (preparation of **2c**) or iodoethane (2.81 g) (preparation of **2n**) were used and the reaction mixture was refluxed for 1 h.

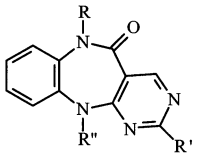
The solvent was then removed in vacuo and the residue was partitioned between water and dichloromethane. The aqueous phase was extracted several more times with dichloromethane. The combined organic phases, after drying and removal of solvent, afforded an oil from which the pure dialkylated compound **2** was recovered as a white solid after a suitable column chromatography [aluminium oxide, ethyl acetate as eluent, in the case of compounds **2c**, **g**, **i**, **j**, **l–n**; silica gel, dichloromethane–petroleum ether–triethylamine (10:10:1) as eluent, in the case of compounds **2d**, **e**].

Data for compounds **2c–e**, **g**, **i**, **j**, **l–n** are reported in *table III*.

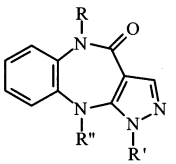
*4.1.5. General procedure for compounds **3a**, **4a**, **3g**, **4b** and **3d***

A mixture of 6.0 mmol of **9b** (1.63 g) or **9c** (2.17 g), 9.0 mmol of methylhydrazine (0.41 g) or phenylhydrazine (0.97 g), 3.0 mL of glacial acetic acid and 30 mL of *n*-butanol was refluxed for 4 h, while stirring. The solvent was then removed in vacuo and the residue was partitioned between 10% aqueous Na_2CO_3 and dichloromethane. The organic layer was collected and the aqueous phase was extracted several more times with dichloromethane. The combined extracts were dried, then evaporated to dryness under reduced pressure to give a residue from which compounds **3a**, **4a**, or **3g**, **4b**, or **3d** were recovered as described below.

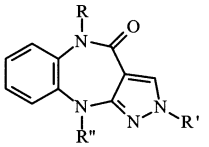
Table III. Data of compounds **2a–n**, **3a–i** and **4a**, **b**.



2a-n



3a-i



4a,b

Compound	Yield (%)	M.p. (°C) (solvent) [a]	Molecular formula [b]	IR [c] (cm^{-1})	^{1}H-NMR [d] (δ, ppm)
2a	48	340 dec. [e] (A)	$C_{11}H_8N_4O$	3260 (NH), 3190 (*NH*CO), 1680s (CO), 1620, 1595, 1570w, 1530w, 1510	6.78–7.32 (m, 4H, H-7,8,9,10), 8.67 and 8.82 (2s, 1H + 1H, H-2, 4), 9.72 [f] (broad s, 1H, NH), 10.00 [f] (broad s, 1H, NH)
2b	40	236–237 [e] (B)	$C_{12}H_{10}N_4O$	3370 (NH), 1640s (CO), 1606w, 1580, 1565sh, 1495br	3.40 (s, 3H, 6-CH_3), 7.08–7.48 (m, 4H, H-7,8,9,10), 8.73 and 8.85 (2s, 1H + 1H, H-2,4), 9.74 [f] (broad s, 1H, NH)
2c	58	145–146 (C)	$C_{13}H_{12}N_4O$	1640s (CO), 1595, 1571, 1540w, 1502	3.49 and 3.51 (2s, 3H + 3H, 6-CH_3 + 11-CH_3), 7.15–7.31 (m, 4H, H-7,8,9,10), 8.81 and 8.94 (2s, 1H + 1H, H-2,4)
2d	58	150–151 (C)	$C_{14}H_{14}N_4O$	1640s (CO), 1596, 1570, 1540w, 1501	1.28 (t, 3H, CH_2CH_3), 3.52 (s, 3H, 6-CH_3), 3.54–3.84 (m, 1H, 1H of CH_2CH_3), 4.20–4.56 (m, 1H, 1H of CH_2CH_3), 7.02–7.37 (m, 4H, H-7,8,9,10), 8.79 and 8.89 (2s, 1H + 1H, H-2,4)
2e	58	142–143 (C)	$C_{15}H_{16}N_4O$	1640s (CO), 1596, 1570, 1533w, 1500	1.49 and 1.56 [2d, 3H + 3H, $CH(CH_3)_2$], 3.52 (s, 3H, 6-CH_3), 4.52 [m, 1H, $CH(CH_3)_2$], 7.15–7.37 (m, 4H, H-7,8,9,10), 8.80 and 8.88 (2s, 1H + 1H, H-2,4)
2f	17	223–224 (D)	$C_{13}H_{12}N_4O$	3240 (NH), 1650s (CO), 1613w, 1591, 1563, 1514br	2.50 (s, 3H, 2-CH_3), 3.38 (s, 3H, 6-CH_3), 7.00–7.47 (m, 4H, H-7,8,9,10), 8.78 (s, 1H, H-4), 9.70 [f] (broad s, 1H, NH)
2g	47	157–158 (C)	$C_{15}H_{16}N_4O$	1640s (CO), 1595, 1575, 1540w, 1501	1.28 (t, 3H, CH_2CH_3), 2.62 (s, 3H, 2-CH_3), 3.51 (s, 3H, 6-CH_3), 3.54–3.84 (m, 1H, 1H of CH_2CH_3), 4.25–4.57 (m, 1H, 1H of CH_2CH_3), 7.12–7.25 (m, 4H, H-7,8,9,10), 8.83 (s, 1H, H-4)
2h	20	233–234 [e] (D)	$C_{18}H_{14}N_4O$	3375 (NH), 1640s (CO), 1608w 1584s, 1548, 1500br	3.52 (s, 3H, 6-CH_3), 6.71–7.70 (m, 8H, H-7,8,9,10 + phenyl H-3′,4′,5′ + NH; 7H after treatment with D_2O), 8.28–8.64 (m, 2H, phenyl H-2′,6′), 9.17 (s, 1H, H-4)
2i	65	128–129 (C)	$C_{20}H_{18}N_4O$	1638s (CO), 1592w, 1566s, 1530w, 1500	1.38 (t, 3H, CH_2CH_3), 3.54 (s, 3H, 6-CH_3), 3.67–4.08 (m, 1H, 1H of CH_2CH_3), 4.40–4.70 (m, 1H, 1H of CH_2CH_3), 7.16–7.33 (m, 4H, H-7,8,9,10), 7.45–7.58 (m, 3H, phenyl H-3′,4′,5′), 8.40–8.53 (m, 2H, phenyl H-2′,6′), 9.02(s, 1H, H-4)
2j	85	162–163 (C)	$C_{21}H_{20}N_4O$	1640s (CO), 1593w, 1566s, 1528w, 1501	1.61 and 1.65 [2d, 3H + 3H, $CH(CH_3)_2$], 3.52 (s, 3H, 6-CH_3), 4.63 [m, 1H, $CH(CH_3)_2$], 7.12–7.41(m, 4H, H-7,8,9,10), 7.46–7.55 (m, 3H, phenyl H-3′,4′,5′), 8.40–8.52 (m, 2H, phenyl H-2′,6′), 8.98 (s, 1H, H-4)
2k	16	206–206.5 (B)	$C_{13}H_{12}N_4OS$	3270 (NH), 1640 (CO), 1600br, 1582, 1540, 1510	2.52 (s, 3H, SCH_3), 3.32 (s, 3H, 6-CH_3), 7.09–7.35 (m, 4H, H-7,8,9,10), 8.65 (s, 1H, H-4), 9.74 [f] (s, 1H, NH)
2l	87	135–136 (E)	$C_{15}H_{16}N_4OS$	1638s (CO), 1594, 1565s, 1525, 1500	1.29 (t, 3H, CH_2CH_3), 2.57 (s, 3H, SCH_3), 3.50 (s, 3H, 6-CH_3), 3.57–3.87 (m, 1H, 1H of CH_2CH_3), 4.22–4.52 (m, 1H, 1H of CH_2CH_3), 7.10–7.25 (m, 4H, H-7,8,9,10), 8.73 (s, 1H, H-4)

Table III. (Continued)

Compound	Yield (%)	M.p. (°C) (solvent) [a]	Molecular formula [b]	IR [c] (cm^{-1})	^{1}H-NMR [d] (δ, ppm)
2m	68	142–142.5 (E)	$C_{16}H_{18}N_4OS$	1640s (CO), 1592w, 1560s, 1520w, 1500	1.48 and 1.57 [2d, 3H+3H, CH(CH_3)$_2$], 2.57 (s, 3H, SCH_3), 3.47 (s, 3H, 6-CH_3), 4.50 [m, 1H, CH(CH_3)$_2$], 7.12–7.37 (m, 4H, H-7,8,9,10), 8.69 (s, 1H, H-4)
2n	38	83–84 (F)	$C_{15}H_{16}N_4O$	1640s (CO), 1597, 1571, 1542w, 1501	1.23 and 1.27 (2t, 3H+3H, 6-CH_2CH_3+11-CH_2CH_3), 3.60–3.89 (m, 2H, 1H of 6-CH_2CH_3+1H of 11-CH_2CH_3), 4.25–4.63(m, 2H, 1H of 6-CH_2CH_3+1H of 11-CH_2CH_3), 7.17–7.33 (m, 4H, H-7,8,9,10), 8.79 and 8.88 (2s, 1H+1H, H-2,4)
3a	61	218–220 (G)	$C_{12}H_{12}N_4O$	3280 (NH), 1620s, br (CO), 1566br, 1502	3.23 (s, 3H, 5-CH_3), 3.74 (s, 3H, 1-CH_3), 7.05–7.28 (m, 4H, H-6,7,8,9), 7.54 (s, 1H, H-3), 8.66 [f] (s, 1H, NH)
3b	70	129–130 (C)	$C_{14}H_{16}N_4O$	1630s, br, (CO), 1600w, 1550w, 1510, 1495	1.13 (t, 3H, CH_2CH_3), 3.46 (q, 2H, CH_2CH_3), 3.47 (s, 3H, 5-CH_3), 3.81 (s, 3H, 1-CH_3), 7.11–7.30 (m, 4H, H-6,7,8,9), 7.82 (s, 1H, H-3)
3c	74	142.5–143 (C)	$C_{15}H_{18}N_4O$	1630s, br, (CO), 1598w, 1546w, 1490br	1.06 and 1.12 [2d, 3H+3H, CH(CH_3)$_2$], 3.47 (s, 3H, 5-CH_3), 3.60 [m, 1H, CH(CH_3)$_2$], 3.81 (s, 3H, 1-CH_3), 7.11–7.30 (m, 4H, H-6,7,8,9), 7.83 (s, 1H, H-3)
3d	82	199–200 (G)	$C_{17}H_{14}N_4O$	3385 (NH), 1632s, br (CO), 1598, 1568w, 1543, 1498	3.28 (s, 3H, 5-CH_3), 7.06–7.33 (m, 4H, H-6,7,8,9), 7.46–7.69 (m, 5H, phenyl Hs), 7.80 (s, 1H, H-3), 8.51 [f] (s, 1H, NH)
3e	75	143–144 (C)	$C_{19}H_{18}N_4O$	1630s, br, (CO), 1597, 1546 w, 1495br	0.97 (t, 3H, CH_2CH_3), 3.03–3.40 (m, 2H, CH_2CH_3), 3.48 (s, 3H, 5-CH_3), 7.15–7.30 (m, 4H, H-6,7,8,9), 7.38–7.66 (m, 5H, phenyl Hs), 7.97 (s, 1H, H-3)
3f	84	182–183 (B)	$C_{20}H_{20}N_4O$	1635s, br, (CO), 1598, 1554w, 1496s, br	0.88 and 0.91 [2d, 3H+3H, CH(CH_3)$_2$], 3.43 [m, 1H, CH(CH_3)$_2$], 3.50 (s, 3H, 5-CH_3), 7.19–7.79 (m, 9H, H-6,7,8,9+phenyl Hs), 8.01 (s, 1H, H-3)
3g	68	267–268.5 (D)	$C_{17}H_{14}N_4O$	3280–3000 br (NH), 1641s (CO), 1607, 1590w, 1566, 1492	3.79 (s, 3H, 1-CH_3), 6.68, 6.91, 7.08 and 7.22–7.48 (4m, 1H+1H+1H+6H, H-6,7,8,9+phenyl Hs), 7.61 (s, 1H, H-3), 8.85 [f] (s, 1H, NH)
3h	59	155–156 (C)	$C_{19}H_{18}N_4O$	1645s, br, (CO), 1595w, 1555w, 1508, 1490	1.31 (t, 3H, CH_2CH_3), 3.62–3.77 (m, 2H, CH_2CH_3), 3.85 (s, 3H, 1-CH_3), 6.81, 6.96–7.17 and 7.22–7.49 (3m, 1H+2H+6H, H-6,7,8,9+phenyl Hs), 7.87 (s, 1H, H-3)
3i	78	124.5–125 (C)	$C_{20}H_{20}N_4O$	1653s, br, (CO), 1598w, 1552w, 1507, 1487	1.17 and 1.26 [2d, 3H+3H, CH(CH_3)$_2$], 3.86 (s, 3H, 1-CH_3), 3.90 [m, 1H, CH(CH_3)$_2$], 6.75–6.86, 7.02–7.14 and 7.19–7.50 (3m, 1H+2H+6H, H-6,7,8,9+phenyl Hs), 7.91 (s, 1H, H-3)
4a	3	185–187 (B)	$C_{12}H_{12}N_4O$	3380 (NH), 1630 s, br (CO), 1608, 1578s, 1500	3.23 (s, 3H, 5-CH_3), 3.68 (s, 3H, 2-CH_3), 6.89–7.27 (m, 4H, H-6,7,8,9), 7.97 (s, 1H, H-3), 8.44 [f] (s, 1H, NH)
4b	16	240–241 (G)	$C_{17}H_{14}N_4O$	3280–3000 br (NH), 1650s (CO), 1610, 1592w, 1574, 1492	3.72 (s, 3H, 2-CH_3), 6.63, 6.71, 7.01, 7.17 and 7.23–7.49 (5m, 1H+1H+1H+1H+5H, H-6,7,8,9+phenyl Hs), 8.06 (s, 1H, H-3), 8.64 [f] (s, 1H, NH)

[a] Crystallisation solvent: A = ethanol–chloroform, B = ethyl acetate–isopropyl ether, C = isopropyl ether, D = ethanol, E = isopropyl ether–petroleum ether, F = petroleum ether, G = ethyl acetate.
[b] Anal. C, H, N.
[c] In $CHCl_3$ solutions, except for **2a**, **f**, **k**, **3a**, **g**, **4b** for which a KBr pellet was used. Abbreviations: s = strong, w = weak, sh = shoulder, br = broad.
[d] In $CDCl_3$ solutions, except for **2a**, **b**, **f**, **k**, **3a**, **d**, **g**, **4a**, **b** for which DMSO-d_6 was used.
[e] Lit. [12]: **2a**, m.p. >300 °C. Lit. [13]: **2b**, m.p. 236–237 °C; **2h**, m.p. 227 °C.
[f] Disappeared with D_2O.

*4.1.5.1. 5,10-Dihydro-1,5-dimethylpyrazolo[3,4-b][1,5]-benzodiazepin-4(1H)-one (**3a**) and 5,10-dihydro-2,5-dimethylpyrazolo[3,4-b][1,5]benzodiazepin-4(2H)-one (**4a**)*

The oily residue obtained from the reaction carried out with **9b** and methylhydrazine was chromatographed on a silica gel column, eluting first with the mixture dichloromethane–petroleum ether–triethylamine (9:3:1). The eluate collected afforded a solid residue which was crystallised from ethyl acetate to give a little amount (0.10 g) of 1-methyl-1H-1,5-benzodiazepine-2,4(3H,5H)-dione (clearly deriving from decomposition of **9b**) which was identified by spectral data and comparison with an authentic sample [14]. After standing, from the mother liquor a white solid separated out which was recrystallised from ethyl acetate–isopropyl ether (1:1) to give a low yield of compound **4a**.

By further eluting the column with acetone, then evaporating the eluate, a white solid residue was recovered, which was treated with a little ethyl ether to give a good yield of **3a**.

*4.1.5.2. 5,10-Dihydro-1-methyl-5-phenylpyrazolo-[3,4-b][1,5]benzodiazepin-4(1H)-one (**3g**) and 5,10-dihydro-2-methyl-5-phenylpyrazolo[3,4-b][1,5]-benzodiazepin-4(2H)-one (**4b**)*

From the reaction of **9c** with methylhydrazine a nearly solid residue was obtained which was treated with a little ethyl acetate to afford a good yield of pure compound **3g**, as a white solid. The oil obtained from evaporation of the mother liquor was chromatographed on a silica gel column, eluting first with dichloromethane–petroleum ether–triethylamine (6:3:1) to recover, after removal of solvents, white compound **4b**. Further elution of the column with ethyl acetate–acetone (1:1) afforded an additional crop of **3g**.

*4.1.5.3. 5,10-Dihydro-5-methyl-1-phenylpyrazolo[3,4-b]-[1,5]benzodiazepin-4(1H)-one (**3d**)*

The thick oily residue obtained from the reaction of **9b** with phenylhydrazine was treated with a little ethyl acetate and allowed to stand until white crystals of pure compound **3d** separated out.

Data for compounds **3a**, **d**, **g** and **4a**, **b** are reported in *table III*.

*4.1.6. General procedure for the 1,5,10-trisubstituted 5,10-dihydropyrazolo[3,4-b][1,5]benzodiazepin-4(1H)-ones (**3b**, **c**, **e**, **f**, **h**, **i**)*

Compound **3a** (1.5 mmol, 0.34 g), **3d** (1.5 mmol, 0.43 g), or **3g** (1.5 mmol, 0.43 g) were 10-alkylated with 9.0 mmol of iodoethane (1.40 g) or 2-iodopropane (1.53 g) under the same conditions above described for the preparation of compounds **2c**–**e**, **g**, **i**, **j**, **l**–**n** (the reaction time was always 1 h). From the final reaction mixture a thick oily residue was then recovered by a procedure identical to that used for compounds **2c**–**e**, **g**, **i**, **j**, **l**–**n**. From this oil the pure 10-alkylated compound **3** was obtained as a white solid after addition of a little ethyl ether (compounds **3e**, **f**), or through a suitable column chromatography [silica gel, ethyl acetate as eluent, in the case of compounds **3b**, **c**; silica gel, dichloromethane–petroleum ether–triethylamine (10:10:1) as eluent for compound **3h**; aluminium oxide, ethyl acetate as eluent for compound **3i**].

Data for compounds **3b**, **c**, **e**, **f**, **h**, **i** are reported in *table III*.

*4.1.7. 6,11-Dihydro-5H-pyrimido[4,5-b][1,5]benzo-diazepine-5-thione (**10**)*

A mixture of 4.0 mmol (0.85 g) of **2a**, 4.0 mmol (1.62 g) of Lawesson's reagent and 6 mL of Dowtherm A was stirred at 150 °C for 30 min. After cooling, the resulting suspension was diluted with a little ethyl acetate and filtered. There was so obtained the nearly pure compound **10** (0.90 g, 99%); orange–yellow solid, m.p. 330–332 °C (dec.) (from methanol). IR (KBr, cm^{-1}): 3260–2880 broad (NH), 1629 weak, 1586 strong, broad, 1504. ^{1}H-NMR (DMSO-d_6): δ 6.97–7.17 (m, 4H, H-7,8,9,10), 8.60 and 9.04 (2s, 1H+1H, H-2,4), 9.45 (s, 1H, NH; disappeared with D_2O), 12.02 (near s, 1H, NH; disappeared with D_2O). Anal. $C_{11}H_8N_4S$ (C, H, N, S).

*4.1.8. Compounds **11a** and **11b***

For the preparation of **11a**, a mixture of 4.0 mmol (0.91 g) of **10**, 1.0 g of anhydrous K_2CO_3, 80.0 mmol (12.48 g) of iodoethane and 100 mL of dry acetone was refluxed for 5 h, with stirring.

In the case of **11b**, the mixture of 4.5 mmol (1.03 g) of **10**, 27.0 mmol (1.51 g) of finely powdered KOH and 100 mL of dry acetone was refluxed for 15 min, then iodoethane (27.0 mmol, 4.21 g) was added and the mixture was further stirred at reflux for 1.5 h.

In both cases the solvent was then removed in vacuo and the residue was partitioned between water and dichloromethane. The organic layer was collected and the aqueous phase was exhaustively extracted with dichloromethane. The combined extracts were dried and solvent removed to give a thick oil which was chromatographed on a silica gel column.

*4.1.8.1. 5-(Ethylthio)-11H-pyrimido[4,5-b][1,5]benzodiazepine (**11a**)*

After eluting with dichloromethane–ethyl acetate (1:1) and evaporating the eluate, the nearly solid residue was treated with a little isopropyl ether to afford orange crystals of pure compound **11a** (0.65 g, 63%); m.p. 192–193 °C, after crystallisation from the same solvent; IR ($CHCl_3$, cm^{-1}): 3375 (NH), 1619, 1596, 1574, 1560 shoulder. ^{1}H-NMR ($CDCl_3$): δ 1.40 (t, 3H, CH_2CH_3), 3.15 (q, 2H, CH_2CH_3), 6.11 (s, 1H, NH; disappeared with D_2O), 6.68 and 6.93–7.17 (2m, 1H+3H, H-7,8,9,10), 8.54 and 8.62 (2s, 1H+1H, H-2,4). Anal. $C_{13}H_{12}N_4S$ (C, H, N, S).

*4.1.8.2. 11-Ethyl-5-(ethylthio)-11H-pyrimido[4,5-b][1,5]benzodiazepine (**11b**)*

The column was eluted with toluene–triethylamine (9:1) and the eluate evaporated to dryness to afford nearly pure compound **11b** (0.96 g, 75%), from which an analytically pure sample (yellow–orange thick oil) was obtained by repeating the same procedure. IR ($CHCl_3$, cm^{-1}): 1611, 1591, 1560, 1540 weak. ^{1}H-NMR ($CDCl_3$): δ 1.28 (t, 3H, SCH_2CH_3), 1.43 (t, 3H, NCH_2CH_3), 3.20 (q, 2H, SCH_2CH_3), 3.90 (q, 2H, NCH_2CH_3), 6.93 and 7.05–7.19 (2m, 1H+3H, H-7,8,9,10), 8.65 and 8.77 (2s, 1H+1H, H-2,4). Anal. $C_{15}H_{16}N_4S$ (C, H, N, S).

*4.1.9. 9-Ethyl-3-methyl-9H-imidazo[1,2-a]pyrimido[5,4-c][1,5]benzodiazepine (**5**)*

A mixture of 2.0 mmol (0.57 g) of **11b**, 12.0 mmol (0.66 g) of propargylamine, 0.10 g of monohydrate *p*-toluenesulphonic acid and 5 mL of Dowtherm A was heated at 180 °C for 2 h, with stirring. After cooling, the mixture was stirred with 10% aqueous Na_2CO_3 and dichloromethane, then allowed to stand until two homogeneous layers were obtained. The organic phase was collected and the aqueous one further extracted several times with dichloromethane. The combined organic phases were dried, then evaporated to afford an oil which was chromatographed on a silica gel column, eluting first with dichloromethane until Dowtherm A was completely removed, then with toluene–triethylamine (9:1). After elimination of some impurities, this eluate was collected and evaporated to dryness to give a thick oily residue from which, by adding a little ethyl ether and petroleum ether, compound **5** separated out as a white solid (0.16 g, 29%); m.p. 187–188 °C (from isopropyl ether). IR ($CHCl_3$, cm^{-1}): 1603 weak, 1580 shoulder, 1568, 1551, 1500 broad. ^{1}H-NMR ($CDCl_3$): δ 1.21 (t, 3H, 9-CH_2CH_3), 2.42 (s, 3H, 3-CH_3), 3.73 and 4.32 (2m, 1H+1H, 9-CH_2CH_3), 7.14 (s, 1H, H-2), 7.20–7.46 (m, 4H, H-5,6,7,8), 8.83 and 8.98 (2s, 1H+1H, H-11,13). Anal. $C_{16}H_{15}N_5$ (C, H, N).

*4.1.10. 9-Ethyl-3-methyl-9H-pyrimido[5,4-c][1,2,4]triazolo[4,3-a][1,5]benzodiazepine (**6**)*

A mixture of 2.0 mmol (0.57 g) of **11b**, 6.0 mmol (0.44 g) of acetohydrazide, 0.10 g of monohydrate *p*-toluenesulphonic acid and 5 mL of Dowtherm A was stirred at 200 °C for 1 h. Following then the same procedure described above for isolation of compound **5**, pure compound **6** (0.26 g, 47%) was obtained; white crystals, m.p. 254–254.5 °C (from ethyl acetate). IR ($CHCl_3$, cm^{-1}): 1600 shoulder, 1586, 1557, 1501. ^{1}H-NMR ($CDCl_3$): δ 1.21 (t, 3H, 9-CH_2CH_3), 2.71 (s, 3H, 3-CH_3), 3.72 and 4.35 (2m, 1H+1H, 9-CH_2CH_3), 7.23–7.51 (m, 4H, H-5,6,7,8), 8.94 and 9.11 (2s, 1H+1H, H-11,13). Anal. $C_{15}H_{14}N_6$ (C, H, N).

*4.1.11. Preparation of compounds **12a** and **12b***

The mixture of 3.0 mmol of **11a** (0.77 g) or **11b** (0.85 g), 3.9 mmol (0.27 g) of hydroxylamine hydrochloride, 3.9 mmol (0.33 g) of $NaHCO_3$, and 25 mL of methanol was refluxed for 2 h, with stirring. Compound **12a** or **12b**, respectively, was isolated from the final reaction mixture as described below.

*4.1.11.1. 5-(Hydroxyamino)-11H-pyrimido[4,5-b][1,5]benzodiazepine (**12a**)*

After cooling, the suspension obtained was filtered and the recovered whitish solid was washed with water, then dried to give 0.48 g (70%) of **12a**; white crystals, m.p. 254–255 °C (from ethanol). IR (KBr, cm^{-1}): 3385, 3300–2700 broad, 1650, 1592, 1572, 1538, 1515. ^{1}H-NMR (DMSO-d_6): δ 6.82–6.98 and 7.15–7.27 (2m, 2H+2H, H-7,8,9,10), 8.26 (s, 1H, NH; disappeared with D_2O), 8.59 and 8.80 (2s, 1H+1H, H-2,4), 9.54 (s, 1H, NH; disappeared with D_2O), 10.97 (s, 1H, OH; disappeared with D_2O). Anal. $C_{11}H_9N_5O$ (C, H, N).

*4.1.11.2. 11-Ethyl-5-(hydroxyamino)-11H-pyrimido[4,5-b][1,5]benzodiazepine (**12b**)*

The residue obtained after removal of solvent was partitioned between water and dichloromethane, then the aqueous phase was exhaustively extracted with dichloromethane. The combined organic phases were dried and evaporated to dryness to give a thick oil from which, after addition of a little ethyl ether and standing, 0.30 g (39%) of nearly pure **12b** separated out as a whitish solid; m.p. 184–184.5 °C (from ethyl acetate–

isopropyl ether). IR (KBr, cm^{-1}): 3390, 3200–2600 broad, 1630, 1600, 1580, 1543 weak, 1510. ^{1}H-NMR (DMSO-d_6): δ 1.17 (t, 3H, CH_2CH_3), 4.01 (q, 2H, CH_2CH_3), 6.94–7.34 (m, 4H, H-7,8,9,10), 8.68 (s, 1H, NH; disappeared with D_2O), 8.70 and 8.82 (2s, 1H+1H, H-2,4), 10.75 (s, 1H, OH; disappeared with D_2O). Anal. $C_{13}H_{13}N_5O$ (C, H, N).

*4.1.12. 3-Methyl-3H,9H-[1,2,4]oxadiazolo[4,3-a]-pyrimido[5,4-c][1,5]benzodiazepine (**7a**)*

A mixture of 2.0 mmol (0.45 g) of **12a**, 4 mL of acetaldehyde dimethylacetal, 0.10 g of monohydrate *p*-toluenesulphonic acid and 10 mL of dimethyl sulphoxide was stirred at 140 °C for 1 h, then cooled and poured into water (200 mL). The resulting mixture was made alkaline with Na_2CO_3 then exhaustively extracted with ethyl ether–ethyl acetate (1:1). The combined extracts were dried and the solvents removed in vacuo to give a thick oil, which was chromatographed on a silica gel column, eluting with toluene–acetone (4:1). The eluate collected gave a solid residue which was taken up in a little ethyl ether and filtered, yielding 0.18 g (36%) of **7a**; white crystals, m.p. 198–199 °C (from ethyl acetate–isopropyl ether). IR ($CHCl_3$, cm^{-1}): 3380 (NH), 1600, 1586, 1512 shoulder, 1500. ^{1}H-NMR ($CDCl_3$): δ 1.63 (d, $J = 5$Hz, 3H, 3-CH_3), 6.52 (q, $J = 5$Hz, 1H, H-3), 6.85–7.16 (m, 5H, H-5,6,7,8+NH; 4H after treatment with D_2O), 8.69 and 9.03 (2s, 1H+1H, H-11,13). Anal. $C_{13}H_{11}N_5O$ (C, H, N).

*4.1.13. 9-Ethyl-3-methyl-3H,9H-[1,2,4]oxadiazolo-[4,3-a]pyrimido[5,4-c][1,5]benzodiazepine (**7b**)*

A mixture of 1.5 mmol (0.38 g) of **7a**, 4.5 mmol (0.50 g) of finely powdered KOH and 80 mL of dry acetone was refluxed for 15 min, then iodoethane (9.0 mmol, 1.40 g) was added and the resulting mixture was refluxed for 30 min, with stirring. The solvent was then removed in vacuo, the residue partitioned between water and dichloromethane, and the aqueous phase exhaustively extracted with dichloromethane. The combined organic phases were dried and solvent was removed to afford a thick oil which was chromatographed on a silica gel column, eluting with toluene–triethylamine (9:1). The eluate, after elimination of solvents, afforded a thick oil which was treated with petroleum ether to give compound **7b** as a white solid (0.11 g, 26%); m.p. 113–113.5 °C (from isopropyl ether–petroleum ether). IR (KBr, cm^{-1}): 1595 strong, 1573, 1528 weak, 1502. ^{1}H-NMR ($CDCl_3$): δ 1.23 (t, 3H, 9-CH_2CH_3), 1.70 (d, $J = 5$ Hz, 3H, 3-CH_3), 3.75 and 4.44 (2m, 1H+1H, 9-CH_2CH_3), 6.44 (q, $J = 5$ Hz, 1H, H-3), 6.92–7.29 (m, 4H, H-5,6,7,8), 8.85 and 8.88 (2s, 1H+1H, H-11,13). Anal. $C_{15}H_{15}N_5O$ (C, H, N).

*4.1.14. 5,10-Dihydro-1,5,10-trimethylpyrazolo[3,4-b]-[1,5]benzodiazepin-4(1H)-one (**3j**)*

*4.1.14.1. Method A (from compound **3k**)*

A mixture of 1.5 mmol (0.32 g) of **3k** [5], 18.0 mmol (1.00 g) of finely powdered KOH and 50 mL of dry acetone was refluxed for 10 min, with stirring. The solution of 18.0 mmol (2.55 g) of iodomethane in 15 mL of dry acetone was then added and the mixture was further refluxed for 1 h. After cooling, the solvent was removed in vacuo, the residue was partitioned between water and dichloromethane, then the aqueous phase was further extracted several times with the same solvent. The combined organic phases were dried and solvent was removed affording an oily residue which was treated with ethyl ether to give 0.26 g (72%) of pure **3j**; white solid, m.p. 155–155.5 °C (from ethyl acetate). IR ($CHCl_3$, cm^{-1}): 1635 strong, broad (CO), 1600 weak, 1545 shoulder, 1515 broad, 1498. ^{1}H-NMR ($CDCl_3$): δ 3.32 (s, 3H, 10-CH_3), 3.45 (s, 3H, 5-CH_3), 3.84 (s, 3H, 1-CH_3), 7.09–7.30 (m, 4H, H-6,7,8,9), 7.78 (s, 1H, H-3). Anal. $C_{13}H_{14}N_4O$ (C, H, N).

*4.1.14.2. Method B (from compound **3a**)*

Starting, as a whole, from a mixture of 1.5 mmol (0.34 g) of **3a**, 9.0 mmol (0.50 g) of KOH, 9.0 mmol (1.28 g) of iodomethane and 65 mL of dry acetone, and following the same procedure described above for the preparation of **3j** from **3k**, a white solid was finally obtained (0.28 g, 77%) which proved to be compound **3j** (m.p., TLC, IR).

*4.1.15. Preparation of 5,10-dihydro-2,5-dimethyl-pyrazolo[3,4-b][1,5]benzodiazepin-4(2H)-one (**4a**) and 5,10-dihydro-2,5,10-trimethylpyrazolo[3,4-b][1,5]-benzodiazepin-4(2H)-one (**4d**) from compound **4c***

Following exactly the same procedure described above for the preparation of **3j** from **3k** (except for a reaction time of 24 h), from the reaction of 1.5 mmol (0.32 g) of **4c** [5] with 18.0 mmol (2.55 g) of iodomethane in the presence of 18.0 mmol (1.00 g) of finely powdered KOH, after the subsequent treatment of the reaction mixture, a final oily residue was obtained and chromatographed on a silica gel column, eluting first with dichloromethane–petroleum ether–triethyl-

amine (10:10:1). The eluate collected, after removal of solvents in vacuo, gave the trimethylderivative **4d** as a white solid (0.12 g, 33%); m.p. 173–174 °C (from isopropyl ether). IR ($CHCl_3$, cm^{-1}): 1630 strong, broad (CO), 1594 weak, 1566 strong, 1491. ^{1}H-NMR ($CDCl_3$): δ 3.31 (s, 3H, 10-CH_3), 3.40 (s, 3H, 5-CH_3), 3.75 (s, 3H, 2-CH_3), 7.04–7.23 (m, 4H, H-6,7,8,9), 7.69 (s, 1H, H-3). Anal. $C_{13}H_{14}N_4O$ (C, H, N).

By subsequent elution with dichloromethane–triethylamine (9:1) a white solid was recovered (0.11 g, 32%) which proved to be (m.p., TLC, IR) compound **4a** (see Section 4.1.5.1 and *table III*).

*4.1.16. Preparation of compound **4b** from **8d***

A mixture of 5.0 mmol (1.40 g) of **8d** [4], 20.0 mmol (1.48 g) of *N*-methyl formic hydrazide [5], 0.10 g of monohydrate *p*-toluenesulphonic acid and 10 mL of dimethyl sulphoxide was stirred at 130 °C for 22 h, then cooled, poured onto ice-water and made alkaline with Na_2CO_3: a solid separated out that was recovered by filtration, washed with water and dried, then chromatographed on an aluminium oxide column. After discarding some impurities eluted with dichloromethane, the elution with dichloromethane–ethyl acetate (1:1) afforded a white solid (0.12 g, 8.3%) which proved to be (m.p., TLC, IR) compound **4b** (see Section 4.1.5.2 and *table III*).

*4.1.17. 1,3-Dihydro-1-methyl-4-(2-phenylhydrazino)-2H-1,5-benzodiazepin-2-one (**13**)*

A mixture of 10.0 mmol (2.17 g) of **8b**, 30.0 mmol (3.24 g) of phenylhydrazine, 0.10 g of monohydrate *p*-toluenesulphonic acid and 20 mL of dimethyl sulphoxide was stirred at 130 °C for 16 h, then cooled and poured onto ice-water. The suspension resulting was made alkaline with Na_2CO_3, then ethyl ether (50 mL) was added and the mixture was stirred at room temperature for 30 min. The solid that separated out was recovered by filtration, washed with water and ethyl ether and dried to give 1.95 g (70%) of compound **13**; whitish crystals, m.p. 242–244 °C (from ethanol). IR (KBr, cm^{-1}): 3310 strong (NH), 1665, 1631 strong, 1600, 1531 weak, 1498. ^{1}H-NMR (DMSO-d_6): δ 3.03 and 3.28 (AB system, $J = 12$ Hz, 2H, 3-CH_2), 3.26 (s, 3H, 1-CH_3), 6.68, 6.98, 7.07–7.32 and 7.43 (4m, 1H+2H+5H+1H, H-6,7,8,9+phenyl Hs), 8.22 (s, 1H, NH; disappeared with D_2O), 8.75 (s, 1H, NH; disappeared with D_2O). Anal. $C_{16}H_{16}N_4O$ (C, H, N).

*4.1.18. 5,10-Dihydro-5-methyl-2-phenylpyrazolo-[3,4-b][1,5]benzodiazepin-4(2H)-one (**4e**)*

PCl_5 (2.40 g) was slowly added to the solution of 8.0 mmol (2.24 g) of **13** in 28 mL of *N*,*N*-dimethylformamide, while stirring. The resulting solution was stirred at room temperature for 60 h, then slowly poured onto ice-water, made alkaline with 6 N aqueous NaOH, then vigorously stirred at room temperature for 30 min. A solid separated out that was recovered by filtration, washed with water and dried, then crystallised from ethyl acetate–dichloromethane (1:1) to yield 0.60 g (26%) of pure **4e**, whitish solid, m.p. 277–278 °C. IR (KBr, cm^{-1}): 3318 and 3115 (NH), 1618 broad (CO), 1598 shoulder, 1573, 1498. ^{1}H-NMR (DMSO-d_6): δ 3.29 (s, 3H, 5-CH_3), 7.04–7.38, 7.50 and 7.83 (3m, 5H+2H+2H, H-6,7,8,9+phenyl Hs), 8.85 (s, 2H, H-3+NH; 1H after treatment with D_2O). Anal. $C_{17}H_{14}N_4O$ (C, H, N).

*4.1.19. Preparation of compounds **4f**, **g***

A mixture of 1.5 mmol (0.43 g) of **4e**, 50 mL of dry 2-butanone and 18.0 mmol (1.00 g) of finely powdered KOH was refluxed for 10 min, with stirring. The solution of 18.0 mmol of iodoethane (2.81 g) (compound **4f**) or 2-iodopropane (3.06 g) (compound **4g**) in 15 mL of 2-butanone was then added and the resulting mixture was further refluxed for 16 h. After removing the solvent in vacuo, the residue obtained was partitioned between water and dichloromethane and the aqueous phase was extracted several more times with the same solvent. The combined organic phases were dried, then evaporated to dryness to give an oily residue, which was chromatographed on a silica gel column, eluting with petroleum ether–dichloromethane–triethylamine (8:2:1). The eluate collected, after removal of solvents, afforded compound **4f** or **4g** as a white solid, which was taken up in a little ethyl ether and recovered by filtration.

*4.1.19.1. 10-Ethyl-5,10-dihydro-5-methyl-2-phenyl-pyrazolo[3,4-b][1,5]benzodiazepin-4(2H)-one (**4f**)*

Yield: 0.39 g (82%); white crystals, m.p. 194–195 °C (from ethyl acetate). IR ($CHCl_3$, cm^{-1}): 1629 (CO), 1596 weak, 1567 strong, 1490. ^{1}H-NMR ($CDCl_3$): δ 1.39 (t, 3H, CH_2CH_3), 3.45 (s, 3H, 5-CH_3), 3.94 (q, 2H, CH_2CH_3), 7.07–7.35, 7.43 and 7.62 (3m, 5H+2H+2H, H-6,7,8,9+phenyl Hs), 8.25 (s, 1H, H-3). Anal. $C_{19}H_{18}N_4O$ (C, H, N).

*4.1.19.2. 5,10-Dihydro-10-isopropyl-5-methyl-2-phenyl-pyrazolo[3,4-b][1,5]benzodiazepin-4(2H)-one (**4g**)*

Yield: 0.41 g (82%); white crystals, m.p. 193–194 °C

(from ethyl acetate). IR ($CHCl_3$, cm^{-1}): 1628 (CO), 1596 weak, 1565 strong, 1488. ^{1}H-NMR ($CDCl_3$): δ 1.40–1.80 [m, 6H, $CH(CH_3)_2$], 3.45 (s,3H, 5-CH_3), 4.20 [m, 1H, $CH(CH_3)_2$], 7.07–7.49 and 7.63 (2m, 7H+2H, H-6,7,8,9+phenyl Hs), 8.25 (s, 1H, H-3). Anal. $C_{20}H_{20}N_4O$ (C, H, N).

4.2. Biological evaluation

4.2.1. Compounds

Test compounds were solubilized in DMSO at 200 mM and then diluted into culture medium.

4.2.2. Cells

MT-4 cells were grown at 37 °C in a 5% CO_2 atmosphere in RPMI 1640 medium, supplemented with 10% foetal calf serum (FCS), 100 UI mL^{-1} penicillin G and 100 μg mL^{-1} streptomycin. Cell cultures were checked periodically for the absence of mycoplasma contamination with the MycoTect Kit (Gibco).

4.2.3. Virus

Human immunodeficiency virus type 1 (HIV-1) was obtained from supernatants of persistently infected H9/III_B cells. The HIV-1 stock solution had a titre of 1.0×10^7 50% cell culture infectious dose ($CCID_{50}$) mL^{-1}.

4.2.4. Antiviral and cytotoxicity assays

Activity of compounds against HIV-1 was based on inhibition of virus-induced cytopathogenicity in MT-4 cells acutely infected at a multiplicity of infection of 0.01. 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 [15].

4.2.5. RT assays

Assays were performed as previously described [16]. Briefly, purified rRT was assayed for its RNA-dependent polymerase activity in a 50 μL volume containing: 50 mM Tris–HCl (pH 7.8), 80 mM KCl, 6 mM $MgCl_2$, 1 mM DTT, 0.1 mg mL^{-1} BSA, 0.5 OD_{260} unit mL^{-1} template: primer [poly(rC)-oligo$(dG)_{12-18}$], and 10 mM [^{3}H]-dGTP (1 Ci mol^{-1}). After incubation for 30 min at 37 °C, samples were spotted on glass fibre filters (Whatman GF/A), and the acid-insoluble radioactivity was determined.

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References

[1] Hargrave K.D., Proudfoot J.R., Grozinger K.G., Cullen E., Kapadia S.R., et al., J. Med. Chem. 34 (1991) 2231–2241.

[2] Cywin C.L., Klunder J.M., Hoermann M.A., Brickwood J.R., David E., et al., J. Med. Chem. 41 (1998) 2972–2984.

[3] Artico M., Farmaco 51 (1996) 305–331.

[4] Roma G., Ermili A., Balbi A., Farm. Ed. Sci. 32 (1977) 81–91.

[5] Roma G., Balbi A., Ermili A., Vigevani E., Farm. Ed. Sci. 38 (1983) 546–558.

[6] Hester J.B. Jr., Rudzik A.D., J. Med. Chem. 17 (1974) 293–295.

[7] Di Braccio M., Roma G., Grossi G.C., Ghia M., Mereto E., Eur. J. Med. Chem. 25 (1990) 681–687.

[8] Roma G., Grossi G.C., Di Braccio M., Ghia M., Mattioli F., Eur. J. Med. Chem. 26 (1991) 489–496.

[9] Di Braccio M., Roma G., Grossi G.C., Leoncini G., Maresca M., Farmaco 47 (1992) 77–90.

[10] Grossi G.C., Di Braccio M., Roma G., Ghia M., Brambilla G., Eur. J. Med. Chem. 28 (1993) 577–584.

[11] Roma G., Di Braccio M., Mazzei M., Ermili A., Farm. Ed. Sci. 39 (1984) 477–486.

[12] Andrews K.J.M., Tong B.P., J. Chem. Soc. C (1968) 1753–1761.

[13] Schaefer H., Riedel R., Klemm K., Senn-Bilfinger J., Eltze M., Eur. Pat. Appl. 24582, 1981; Chem. Abstr. 95 (1981) 132969u.

[14] Rossi S., Pirola O., Maggi R., Chim. Ind. (Milan) 51 (1969) 479–483.

[15] Pauwels R., et al., J. Virol. Methods 20 (1988) 309–321.

[16] Tramontano E., Cheng Y.C., Biochem. Pharmacol. 43 (1992) 1371–1376.