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Il Farmaco 60 (2005) 113–125
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1,5-Benzodiazepines XIV. Synthesis of new substituted 9*H*-bis-[1,2,4]triazolo[4,3-*a*:3′,4′-*d*] [1,5]benzodiazepines and relate compounds endowed with in vitro cytotoxic properties

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Received 20 July 2004; received in revised form 10 December 2004; accepted 20 December 2004

Abstract

A series of 11 new 9*H*-bis-[1,2,4]triazolo[4,3-*a*:3′,4′-*d*] [1,5]benzodiazepine derivatives **8e–o** was synthesized. Ten of these compounds (**8e–m,o**), along with four analogues (**8a–d**) (previously synthesized by us) were tested in vitro in order to evaluate their cytotoxic and anti-HIV-1 properties. In this connection other six original compounds, i.e., five 9-substituted compounds prepared starting from the 6,12-diphenylderivative **8c** (compounds **10**, **11**, **12**, **13a,b**) and the bis-triazolone derivative **14**, were synthesized and tested for the same purpose. While none of the 20 compounds tested exhibited any appreciable anti-HIV-1 activity, some of them exhibited interesting cytotoxic properties, the best results being shown by compounds **8c,d,k** and **11** (CC_{50} range = 3–12 μM). Therefore, these four compounds were further evaluated for their antiproliferative activity against a panel of human tumor cell lines; actually, compounds **8d**, **8k** and **11** showed antiproliferative properties against either or both leukemia- and lymphoma-derived cell lines in the low micromolar range.

Keywords: 1,5-Benzodiazepine fused derivatives; Cytotoxic activity; Antiproliferative activity

1. Introduction

We previously described the synthesis and the biological evaluation of a series of 5*H*-pyrimido[4,5-*b*] [1,5]benzodiazepines **2** and pyrazolo[3,4-*b*] [1,5]benzodiazepines **3**, **4** designed as novel analogues of the well known non-nucleoside HIV-1 reverse transcriptase inhibitor Nevirapine (**1**) in order to test their in vitro anti-HIV-1 activity [1]. Among all the synthesized compounds, some pyrimidine derivatives **2**, which were more closely related to the Nevirapine structure, exhibited a slight anti-HIV-1 activity, whereas the pyrazole derivatives **3**, **4** showed mainly cytotoxic properties [1].

Since the literature reported a high anti-HIV-1 activity for some tetracyclic derivatives of Nevirapine, in which the lactam group had been replaced by an imidazole or triazole ring [2], with the aim to improve the anti-HIV-1 activity of the best 5*H*-pyrimido[4,5-*b*] [1,5]benzodiazepine derivative **2d**, we also synthesized the tetracyclic derivatives **5**, **6**, **7** structurally related to **2d** (Fig. 1). Actually, compounds **5**, **6** and **7** resulted inactive against HIV-1 and proved to be cytotoxic in the low micromolar range [1].

This result prompted us to extend our study on other tetracyclic 1,5-benzodiazepine derivatives analogues of the compounds **5–7** for their potential cytotoxic properties.

As a first step in this study, we have now synthesized a number of substituted 9*H*-bis-[1,2,4]triazolo[4,3-*a*:3′,4′-*d*] [1,5]benzodiazepines **8**. Four examples of these latter (compounds **8a–d**) were previously obtained by us as by-products in the synthesis of the analgesic/anti-inflammatory agents *N,N*-disubstituted 4*H*-[1,2,4]triazolo[4,3-*a*] [1,5]benzodiazepin-5-amines **9** [3] (Fig. 2).

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doi:10.1016/j.farmac.2004.12.005

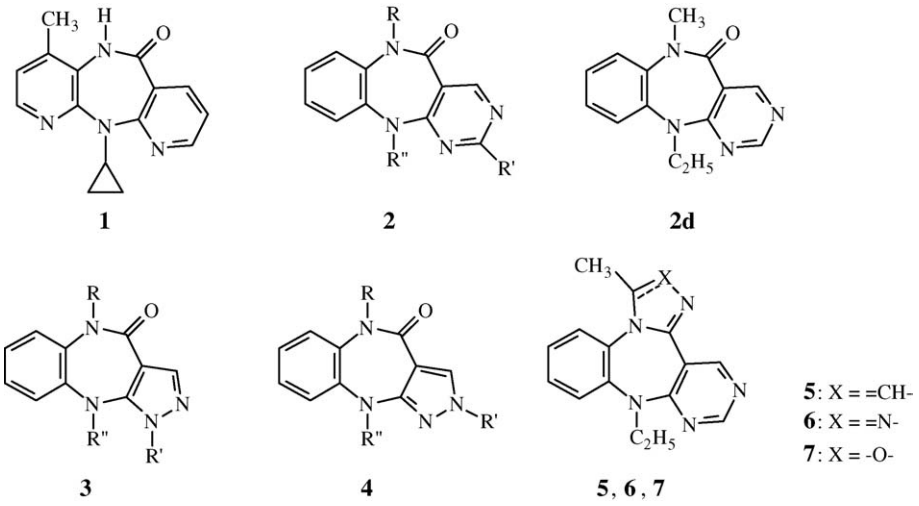


Fig. 1. Structures of compounds 1–7.

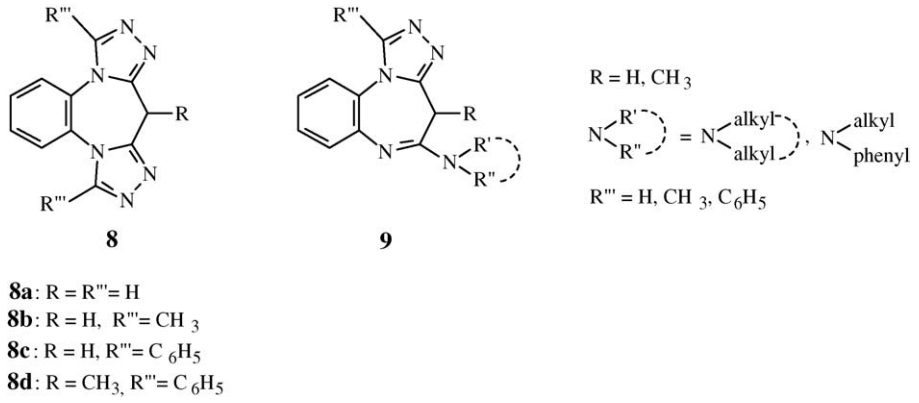


Fig. 2. Structures of compounds **8a-d** previously obtained by us [3] as by-products in the synthesis of the analgesic/anti-inflammatory agents **9**.

For the preparation of the novel compounds **8e–o** (Scheme 1) we planned a synthesis aimed at obtaining high yields of the tetracyclic derivatives **8** as unique reaction products: on the basis of the results of a preliminary screening for the cytotoxicity, we have developed chiefly the 9-methyl-substituted compounds (**8h–o**).

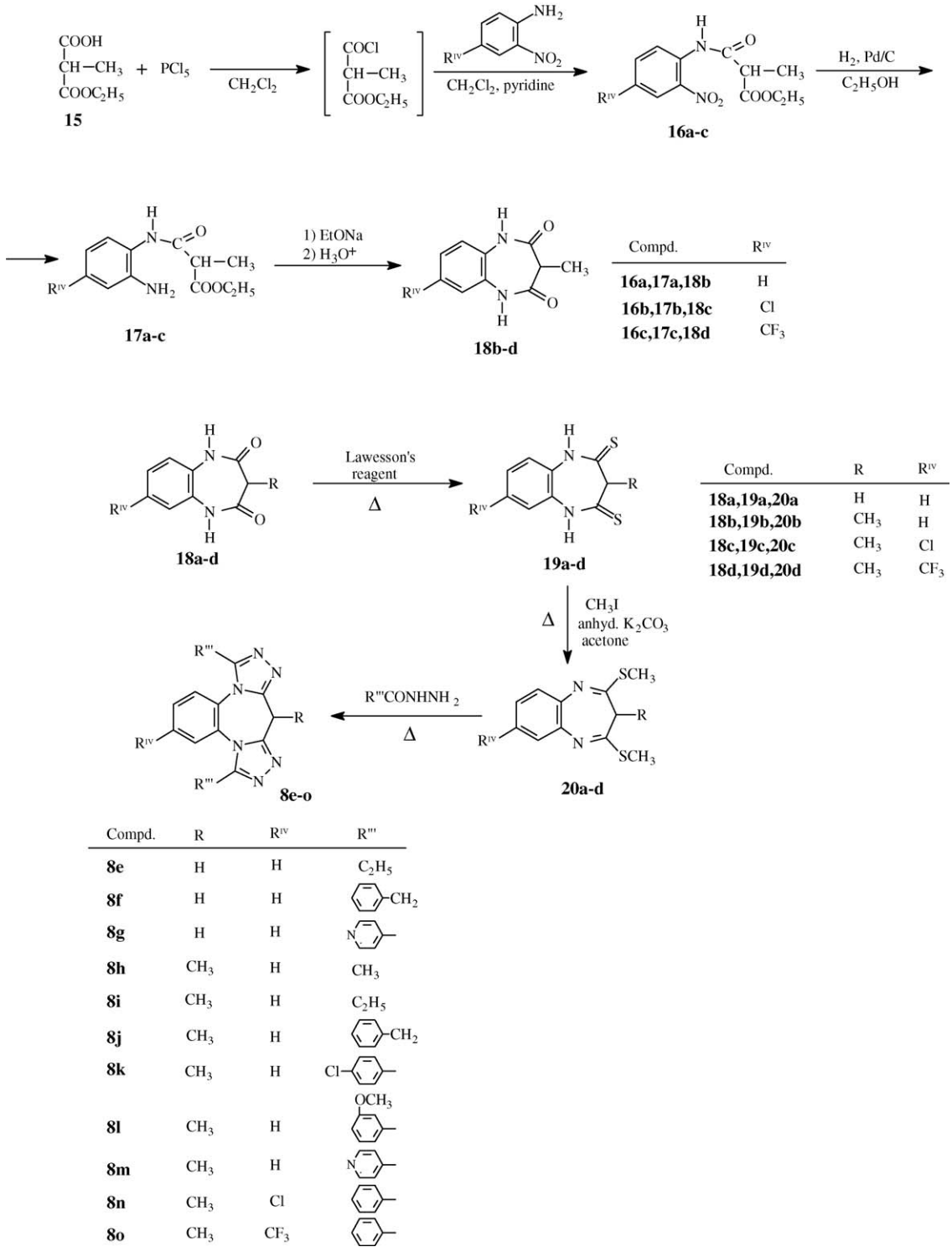
In this connection, we have also prepared other 9-substituted compounds starting from the most active 9-unsubstituted compound **8c**: the 9-[(dimethylamino)-methylene]derivative **10**, the 9-chloro- and the 9,9-dichloroderivatives **11** and **12**, respectively, and the 9-(dialkyl-amino)derivatives **13a,b**.

Moreover, we also introduced a change in the triazole moiety by synthesizing the bis-triazolone derivative **14** (Fig. 3).

2. Chemistry

The 3-methylsubstituted 1*H*-1,5-benzodiazepine-2,4(3*H*,5*H*)diones **18b–f** were prepared by a method analogous to that described by Weber et al. [4] for the preparation of similar compounds. Thus, the monoethylester of 2-methylmalonic acid (**15**) was treated with excess PCl_5 (CH_2Cl_2, room temperature, 3 h), then with the suitable 2-nitroaniline, in the presence of dry pyridine (room temperature, 1 h) to give the *N*-(2-nitrophenyl)malonamates **16a–c** which in turn were transformed into the corresponding *N*-(2-aminophenyl)malonamates **17a–c** by hydrogenation at 5–15 psi in a Parr apparatus in the presence of 5% palladium on charcoal. The intermediates **17a–c** underwent to cyclization to desired 3-methyl-1*H*-1,5-benzodiazepine-2,4(3*H*,5*H*)-diones **18b–d** by treatment with an ethanolic solution of sodium ethoxide (30 °C, overnight) followed by acidification with 2 N aqueous HCl. The diones **18a** [5] and **18b–d** were heated with Lawesson's reagent (Dowtherm A, 150 °C, 2 h) to give the corresponding dithiones **19a–d** from which the 2,4-bis(methylthio)derivatives **20a–d** were obtained by reaction with iodomethane (dry acetone at reflux, in the presence of anhydrous K_2CO_3, 2 h). Finally, the reaction of compounds **20a–d** with suitable hydrazides in excess (Dowtherm A, 200 °C, 2 h) in the presence of *p*-toluenesulfonic acid gave usually good yields of the desired 9-alkyl-6,12-dialkyl(diaryl)-9*H*-bis-[1,2,4]triazolo[4,3-*a*:3′,4′-*d*] [1,5]benzodiazepines **8e–o** (Scheme 1).

The reaction of **8c** [3] with a large excess of *N,N*-dimethylformamide dimethyl acetal in pyridine at reflux (24 h), afforded the 9-[(dimethylamino)methylene]derivative **10**.



Compd.	R^{IV}
16a,17a,18b	H
16b,17b,18c	Cl
16c,17c,18d	CF_3

Compd.	R	R^{IV}
18a,19a,20a	H	H
18b,19b,20b	CH_3	H
18c,19c,20c	CH_3	Cl
18d,19d,20d	CH_3	CF_3

Compd.	R	R^{IV}	R'''
8e	H	H	C_2H_5
8f	H	H	CH_2-Ph
8g	H	H	4-pyridyl
8h	CH_3	H	CH_3
8i	CH_3	H	C_2H_5
8j	CH_3	H	CH_2-Ph
8k	CH_3	H	4-Cl-Ph
8l	CH_3	H	4-OCH_3-Ph
8m	CH_3	H	4-pyridyl
8n	CH_3	Cl	Ph
8o	CH_3	CF_3	Ph

Scheme 1. Synthesis of substituted 9*H*-bis-[1,2,4]triazolo[4,3-*a*:3′,4′-*d*] [1,5]benzodiazepines **8e–o**.

On the other hand, the reaction of **8c** with excess *N*-chlorosuccinimide [CCl_4–$CHCl_3$ (1:1) at reflux, 24 h] yielded a mixture of 9-chloroderivative **11** and 9,9-dichloroderivative **12**.

The reaction of **11** with a large excess of morpholine or 1-methylpiperazine (dimethyl sulfoxide, 120 °C, 1–2 h) afforded then the 9-(dialkylamino)derivatives **13a,b**, respectively.

Finally, the bis-triazolone derivative **14** was obtained from the 2,4-bis(methylthio)derivative **20b** through the reaction with excess ethyl carbazate (Dowtherm A, 200 °C, 2 h) (Scheme 2).

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 Tables 2–4).

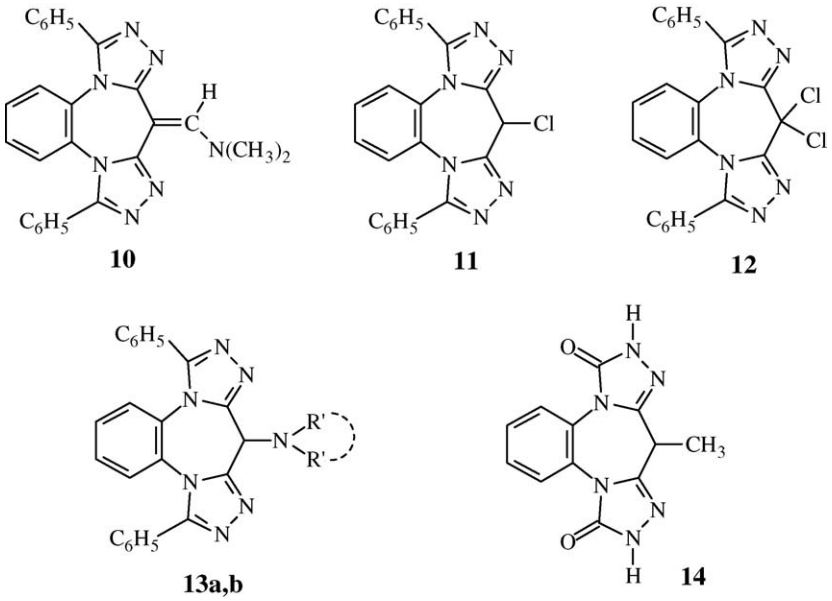


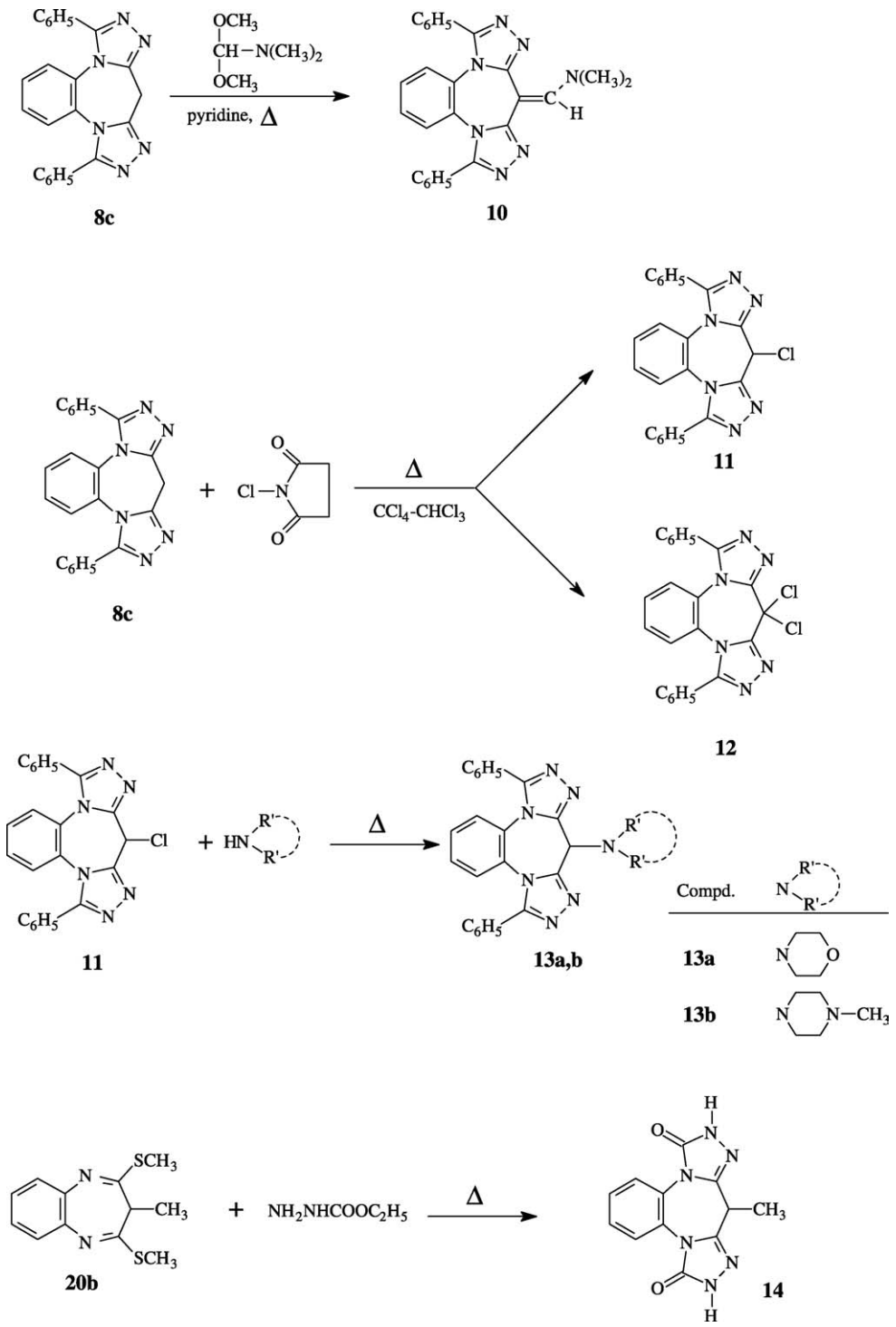
Fig. 3. 9-Substituted derivatives of **8c** (compounds **10**, **11**, **12**, and **13a,b**) and 9-methyl-9*H*-bis[1,2,4]triazolo[4,3-*a*:3′,4′-*d*] [1,5]benzodiazepine-6,12(7*H*,11*H*)-dione (**14**).

The ^{1}H-NMR spectra were particularly useful in order to study the conformational features of compounds **8e–o** and their analogues **11**, **12**, **13a,b** and **14**. It is worth noting that the tetracyclic system of compounds **8** is not planar (as shown by the structures drawn by MacroModel software, version 8.0 [6]) and its seven membered diazepine ring, in the solid state and in solution (e.g., in $CDCl_3$) at room temperature, is blocked in only one boat conformation (Fig. 4), no conformational inversion occurring, very likely due to the restricting effect of the R‴ substituents of the triazole rings. Therefore, in the 9-unsubstituted derivatives the two protons at C-9 are diastereotopic and, in the ^{1}H-NMR spectra, the quasi-equatorial proton is clearly more deshielded than the quasi-axial one, because this latter projects into the shielding cone of the benzene ring; similar remarks have been previously reported for 1,4-benzodiazepine derivatives [7]. This situation is indeed confirmed by the ^{1}H-NMR spectra (in $CDCl_3$ solutions) of the 9-unsubstituted compounds **8e–g** that show for the two protons at C-9 two distinct signals [doublets (J = 16 Hz) as a half of AB quartet] at δ ~3.8 ppm (quasi-axial proton) and at δ ~5 ppm (quasi-equatorial proton), respectively (See Section 4, Table 4). When C-9 is substituted with an alkyl group, the interconversion barrier of the seven membered ring is further increased and the R substituent is very likely located in the thermodynamically more favoured quasi-equatorial position, according to the literature suggestions for analogous cases [8,9] (Fig. 4).

Actually, we found only one conformer in all the 9-methylsubstituted compounds **8** (**8h–o**), and in these compounds H-9 appears to be in quasi-axial position (^{1}H-NMR signal: quartet at δ ~4.1 ppm) and, therefore, the CH_3 group is in quasi-equatorial position (^{1}H-NMR signal: doublet at δ ~2.2 ppm).

Also the 9-methylsubstituted bis-triazolone derivative **14** appears to be in only one conformation, i.e., the same of 9-methylsubstituted compounds **8h–o**, as confirmed by its ^{1}H-NMR spectral data (DMSO-d_6 solution, H-9: quartet at δ 4.07 ppm; 9-CH_3: doublet at δ 1.54 ppm). It is reasonable to think that, also in the case of the other 9-monosubstituted compounds (**11**, **13a,b**), the only conformer obtained is that one with the bulky 9-substituent located in the more stable quasi-equatorial position, even though the ^{1}H-NMR data regarding the H-9 chemical shifts of these compounds does not allow any comparison with those of compounds **8h–o**, due the electronic effect of 9-substituent (chloro or dialkylamino group).

The first examples of the 9*H*-bis-[1,2,4]triazolo[4,3-a:3′,4′-d] [1,5]benzodiazepine system were described by us [3] in 1990; 1 year later the synthesis of a group of compounds belonging to the general structure **8** (six compounds, among which two had been already described by us [3]) has been reported [10]. Even though the last steps of the synthetic route followed by these authors are very similar to those now used by us, we can point out that in that paper [10] no 9-substituted bistriazolobenzodiazepine derivative has been reported, whereas we now describe mainly 9-substituted compounds (**8h–o**, **10**, **11**, **12**, **13a,b**, **14**). Furthermore, in the above mentioned paper no detailed melting point (both for intermediates and final compounds) was reported but all melting points were given as > 250 °C, or > 300 °C, or > 350 °C; actually, if these data are likely for the intermediate benzodiazepinediones, -dithiones, and for the bistriazolobenzodiazepine derivatives, they appear quite unlikely for the 2,4-bis(methylthio)-3*H*-1,5-benzodiazepine derivatives. For instance, the 2,4-bis(methylthio)-3*H*-1,5-benzodiazepine (**20a**, Scheme 1) was there described as a crystalline solid



Scheme 2. Synthetic routes to the compounds **10**, **11**, **12**, **13a,b** and **14**.

melting over 300 °C [10], whereas we found that this compound is a low-melting solid (53.5–55 °C, Table 3).

3. Biological results and conclusions

Compounds **8a–d** [3] and the novel **8e–m,o**, **10**, **11**, **12**, **13a,b** and **14** were tested in vitro for cytotoxicity and anti-HIV-1 activity and the results of biological evaluation are reported in Table 1.

None of the 20 compounds tested exhibited any anti-HIV-1 activity; on the other hand, six compounds showed a clear cytotoxic activity (CC_{50} < 50 μM), while further four compounds afforded only a moderate cytotoxic effect (CC_{50} ranging from 50 to 137 μM).

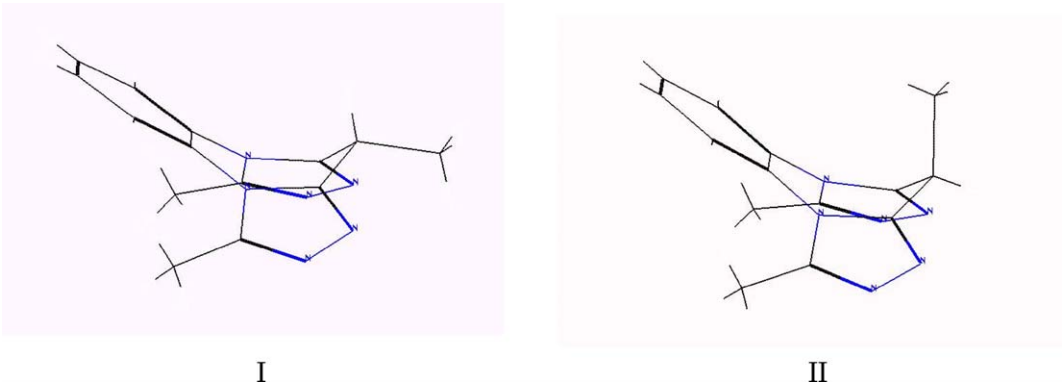


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Fig. 4. Conformations of 6,9,12-trimethyl-9*H*-bis-[1,2,4]triazolo[4,3-*a*:3′,4′-*d*] [1,5]benzodiazepine **8h** (chosen as an example) drawn with MacroModel version 8.0 [6]: the conformer with 9-methyl group in quasi-equatorial position (I) is more likely than that one with the 9-methyl group in the quasi-axial position (II).

As regards the compounds **8**, phenyl groups as R‴ substituents afforded the highest cytotoxicity (compounds **8c** and **8d**). Compounds devoid of cytotoxicity (CC_{50} > 200 μM) were obtained when R‴ were alkyl groups (compounds **8a,b,e,h,i**) or 4-pyridyl groups (**8g,m**), while when R‴ were benzyl groups (compounds **8f,j**), in only one case a slight cytotoxic effect was observed (**8f**, CC_{50} = 58 μM).

As the 9-methylsubstituted derivative **8d** was clearly more cytotoxic (CC_{50} = 4 μM) than the 9-unsubstituted analogue **8c** (CC_{50} = 12 μM), further seven 9-methylderivatives (**8 h–m,o**) were prepared and tested. However, the biological results were quite disappointing, with the sole exception of **8k** which displayed fairly good cytotoxic properties (CC_{50} = 12 μM). Furthermore, the 9-methyl substitution was negative in the case of 6,12-dibenzylderivatives, since the 9-unsubstituted compound **8f** was more cytotoxic than the 9-methyl analogue **8j** (CC_{50} = 58 and > 200 μM, respectively).

Compounds bearing substituents on the benzene ring (**8o**) or on the phenyl R‴ groups (**8k,l**) showed a decreased cytotoxicity compared to unsubstituted compounds. In particular, the substitution with 3-methoxyphenyl as R‴ groups resulted very unfavourable (compound **8l**).

Among the compounds derived from the cytotoxic compound **8c**, a very good result was afforded by the 9-chloroderivative **11** (CC_{50} = 3 μM), while the 9,9-dichloroderivative **12** displayed lower cytotoxicity (CC_{50} = 30 μM) similar to that of the 9-morpholino derivative **13a** (CC_{50} = 32 μM). More modest results were given by the 9-(4-methyl-1-piperazinyl)derivative **13b** and by the 9-[(dimethylamino)-methylene]derivative **10**. On the other hand, the bis-triazolone derivative **14** did not result cytotoxic (CC_{50} >200 μM).

In an effort to more completely define the biological profile of title compounds, those resulted most cytotoxic against MT-4 cells (Table 1), i.e., **8c**, **8d**, **8k** and **11**, were evaluated for antiproliferative activity against a panel of cell lines derived from either haematological (CCRF-CEM, WIL-2NS, and CCRF-SB) or solid (SKMEL28, MCF7, SKMES-1, HepG2, and DU145) human tumors. They were also evaluated against cell lines derived from normal human tissues (MRC-5, CRL7065).

In general, test compounds specifically inhibited the proliferation of cells derived from haematological tumours (Table 2) at concentrations (IC_{50} range = 1.9–25 μM, Table 2) significantly lower than those inhibitory for solid tumors (Table 3). Exceptions were compound **8k,** which was fairly active also against skin melanoma, breast adenocarcinoma and lung squamous carcinoma (IC_{50} range = 8.4–8.6 μM, Table 3), and compound **11**, which was also active against breast adenocarcinoma (IC_{50} = 10 μM, Table 3). It is worth noting that the test compounds did not show significant antiproliferative properties against the normal tissue-derived cells [IC_{50} = 100 or >100 μM, except for **8k** against CRL7065 cells (25 μM), Table 3].

In conclusion, among the 20 9*H*-bis-[1,2,4]triazolo[4,3-a:3′,4′-d] [1,5]benzodiazepine derivatives tested, only compounds **8d**, **8k** and **11**, showed antiproliferative activity against either or both leukaemia- and lymphoma-derived cell lines in the low micromolar range. Therefore, it can be concluded that, in this molecular class, the substitution with phenyl or *p*-chlorophenyl groups at C-6 and C-12, and with a methyl or chloro at C-9, are the main structural features favouring the antiproliferative activity.

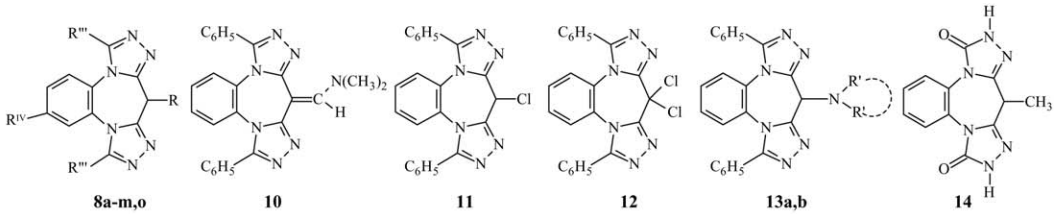
Further experiments aimed at defining target and mechanism of action of title compounds are in progress.

4. Experimental

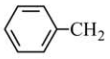
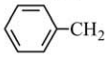
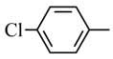
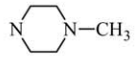
4.1. Chemistry

Melting points were determined using a Fisher–Johns apparatus (Electrothermal when above 300 °C) and are uncorrected. IR spectra were recorded on a Perkin-Elmer "Spectrum One" spectrophotometer (abbreviations relative to IR bands: br = broad, s = strong, w = weak, sh = shoulder). ^{1}H-NMR spectra were recorded on a Varian Gemini 200 (200 MHz) spectrometer and chemical shifts (δ) are reported in ppm using $(CH_3)_4Si$ as an internal reference (δ = 0). Spin multiplicities are given as follows: s (singlet), d (doublet), dd (double doublet), t (triplet), q (quartet), m (multiplet). Analy-

Table 1
In vitro biological activities of compounds **8a–m,o**, **10**, **11**, **12**, **13a,b** and **14**



8a-m,o **10** **11** **12** **13a,b** **14**

Compd.	R	R‴	R^{IV}	N(R')(R'')	CC_{50}[a] MT-4	EC_{50}[b] HIV-1
8a [3]	H	H	H	-	>200	>200
8b [3]	H	CH_3	H	-	>200	>200
8c [3]	H		H	-	12	>12
8d [3]	CH_3		H	-	4	>4
8e	H	C_2H_5	H	-	>200	>200
8f	H		H	-	58	>58
8g	H		H	-	>200	>200
8h	CH_3	CH_3	H	-	>200	>200
8i	CH_3	C_2H_5	H	-	≥200	>200
8j	CH_3		H	-	>200	>200
8k	CH_3		H	-	12	>12
8l	CH_3		H	-	>200	>200
8m	CH_3		H	-	>200	>200
8o	CH_3		CF_3	-	137	>137
10	-	-	-	-	133	>133
11	-	-	-	-	3	>3
12	-	-	-	-	30	>30
13a	-	-	-		32	>32
13b	-	-	-		126	>126
14	-	-	-	-	>200	>200
Nevirapine					>200	0.05

[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.

Table 2
In vitro antiproliferative activity of compounds **8c,d,k** and **11** against human leukemia/lymphoma-derived cell lines

Compound	IC_{50} (μM) [a]			
	MT-4 [b]	CCRF-CEM [c]	WIL-2NS [d]	CCRF-SB [e]
8c	12 ± 0.2	13 ± 1	25.5 ± 0.9	15 ± 1
8d	4 ± 0.3	6.6 ± 0.2	15 ± 2	10 ± 0.1
8k	12 ± 1	7 ± 2	10 ± 2	7.5 ± 2
11	3 ± 0.1	4.2 ± 1.5	3.6 ± 0.4	1.9 ± 0.5

[a] Compound concentration required to reduce cell proliferation by 50%, as determined by the MTT method, under conditions allowing untreated controls to undergo at least three consecutive rounds of multiplication. Data represent mean values (± SD) for three independent determinations.
[b] $CD4^+$ human T-cells containing an integrated HTLV-1 genome.
[c] $CD4^+$ human acute T-lymphoblastic leukemia.
[d] Human splenic B-lymphoblastoid cells.
[e] Human acute B-lymphoblastic leukemia.

ses 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, University of Genoa.

Thin-layer chromatograms were run on Merck silica gel $60F_{254}$ 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. General procedure for the preparation of ethyl 2-methyl-N-(2-nitrophenyl)malonamates **16a–c***

In a two-neck flask, protected by the moisture with a calcium chloride tube, phosphorus pentachloride (11.44 g, 55.0 mmol) was carefully added, in little amounts, to the solution of 50.0 mmol (7.30 g) of 2-methylmalonic acid monoethyl ester **15** in 150 ml of dichloromethane. The resulting mixture was then stirred at room temperature for 3 h, then the solution of the 35.0 mmol of the proper aromatic amine [4.83 g of 2-nitroaniline, or 6.04 g of 4-chloro-2-nitroaniline, or 7.21 g of 4-(trifluoromethyl)-2-nitroaniline] in 50 ml of dichloromethane and 15 ml of dry pyridine was slowly added by a dropping funnel (an exothermic reaction with emission of white fumes occurred during the addition). The resulting warm mixture was stirred at room temperature for 30 min, then poured into 800 ml of cold water and the mixture was vigorously stirred at room temperature for 1 h The organic layer was collected and the aqueous one was thoroughly extracted with dichloromethane. The combined extracts were washed with 5% aqueous $NaHCO_3$, then with water, dried (anhydrous sodium sulphate) and finally evaporated to dryness in vacuo. By treating the oily residue with a little petroleum ether and standing compounds **16a–c** separated out as pale yellow solids, which were then crystallized from the suitable solvents. Data of compounds **16a–c** are reported in Table 4.

*4.1.2. General procedure for the preparation of ethyl N-(2-aminophenyl)-2-methylmalonamates **17a–c***

To a solution of 25.00 mmol of the proper compound **16** (6.66 g of **16a,** or 7.52 g of **16b**, or 8.36 g of **16c**) in 150 ml of ethanol, 5% palladium on charcoal (0.50 g) was added, and the mixture was subjected to hydrogenation at 15 psi (5 psi, in the case of chloro derivative **16b**) in a Parr apparatus at room temperature. When the hydrogen absorption ceased, the mixture was filtered and the resulting colourless solution was evaporated to dryness in vacuo to give solid or oily residues from which, after treatment with little ethyl ether, compounds **17a–c** separated out as whitish solids, which were then crystallized from the proper solvent. Data of compounds **17a–c** are reported in Table 4.

*4.1.3. General procedure for the preparation of 3-methyl-1H-1,5-benzodiazepine-2,4(3H,5H)-diones **18b–d***

Sodium (30.0 mmol, 0.69 g) was dissolved in 150 ml of anhydrous ethanol: to this solution 20.0 mmol of the proper compound **17** (4.73 g of **17a**, or 5.41 g of **17b**, or 6.09 g of **17c**) were added and the mixture was stirred at 30 °C for 8 h. The solvent was then removed in vacuo and the residue was treated with aqueous 2 N HCl: the crude compound **18** that separated out as a whitish solid was collected by filtration, washed with water, dried, and crystallized from the suitable solvent. Data of compounds **18b–d** are reported in Table 5.

*4.1.4. General procedure for the preparation of 1H-1,5-benzodiazepine-2,4(3H,5H)-dithiones **19a–d***

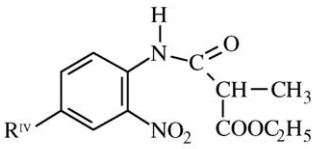
A mixture of 5.0 mmol of the proper compound **18** (0.88 g of **18a** [5]; 0.95 g of **18b**; 1.12 g of **18c**; 1.21 g of **18d**),

Table 3
In vitro antiproliferative activity of compounds **8c,d,k** and **11** against human solid tumor-derived cell lines and normal tissue-derived cell lines

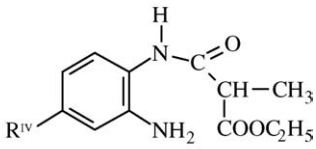
Compound	IC_{50} (μM) [a]						
	SK-MEL-28 [b]	MCF7 [c]	SK-MES-1 [d]	HepG2 [e]	DU145 [f]	MRC-5 [g]	CRL7065 [h]
8c	74 ± 14	44 ± 0.2	89 ± 1	75 ± 8	>100	>100	>100
8d	>100	67 ± 0.4	>100	>100	>100	>100	>100
8k	8.4 ± 0.4	8.5 ± 0.5	8.6 ± 0.4	35 ± 8	20 ± 2	100	25 ± 2
11	35 ± 2	10 ± 1	64 ± 13	36 ± 3	>100	>100	100

[a] Compound concentration required to reduce cell proliferation by 50%, as determined by the MTT method, under conditions allowing untreated controls to undergo at least three consecutive rounds of multiplication. Data represent mean values (± SD) for three independent determinations.
[b] Human skin melanoma.
[c] Human breast adenocarcinoma.
[d] Human lung squamous carcinoma.
[e] Human hepatocellular carcinoma.
[f] Human prostate carcinoma.
[g] Human lung fibroblasts.
[h] Human foreskin fibroblasts.

Table 4
Data of compounds **16a–c** and **17a–c**



16a-c



17a-c

Compound	R^{IV}	Yield (%)	m.p. (°C) (solvent) [a]	Molecular formula[b]	IR[c] (cm^{-1})	^{1}H-NMR[d] (δ, ppm)
16a	H	92	59.5–60.5 (A)	$C_{12}H_{14}N_2O_5$	3330 (NH), 1740 s, br (ester CO), 1702 s, br (amide CO), 1609, 1588.	1.30 (t, 3H, CH_2CH_3), 1.55 (d, 3H, $CHCH_3$), 3.50 (q, 1H, $CHCH_3$), 4.29 (q, 2H, CH_2CH_3), 7.23 (near t, 1H, phenyl H-4), 7.70 (near t, 1H, phenyl H-5), 8.27 (near d, 1H, phenyl H-3), 8.76 (near d, 1H, phenyl H-6), 10.95 [e] (broad s, 1H, *NH*CO).
16b	Cl	92	67.5–68.5 (B)	$C_{12}H_{13}ClN_2O_5$	3330 (NH), 1740 s, br (ester CO), 1708 s, br (amide CO), 1610, 1580.	1.34 (t, 3H, CH_2CH_3), 1.55 (d, 3H, $CHCH_3$), 3.60 (q, 1H, $CHCH_3$), 4.35 (q, 2H, CH_2CH_3), 7.67 (dd, $J_{5,6}$ = 9 Hz, $J_{5,3}$ = 3 Hz, 1H, phenyl H-5), 8.27 (d, $J_{3,5}$ = 3 Hz, 1H, phenyl H-3), 8.82 (d, $J_{6,5}$ = 9 Hz,1H, phenyl H-6), 10.84 [e] (broad s, 1H, *NH*CO).
16c	CF_3	65	53–54 (B)	$C_{12}H_{13}F_3N_2O_5$	3345 (NH), 1748 s, br (ester CO), 1719 s, br (amide CO), 1633, 1588, 1523.	1.34 (t, 3H, CH_2CH_3), 1.58 (d, 3H, $CHCH_3$), 3.62 (q, 1H, $CHCH_3$), 4.31 (q, 2H, CH_2CH_3), 7.90 (near d, 1H, phenyl H-5), 8.53 (near s, 1H, phenyl H-3), 8.98 (d, $J_{6,5}$ = 9 Hz, 1H, phenyl H-6), 11.21 [e] (broad s, 1H, *NH*CO).
17a	H	94	97–98 (C)	$C_{12}H_{16}N_2O_3$	3416 and 3340 sh (NH_2), 3251 (NH), 1749 s (ester CO), 1642 s (amide CO), 1604 w, 1592 w, 1543.	1.29 (t, 3H, CH_2CH_3), 1.50 (d, 3H, $CHCH_3$), 3.49 (q, 1H, $CHCH_3$), 3.80 [e] (s, 2H, NH_2), 4.25 (q, 2H, CH_2CH_3), 6.60–7.40 (m, 4H, phenyl H-3,4,5,6), 8.44 [e] (broad s, 1H, *NH*CO).
17b	Cl	79	140–141 (D)	$C_{12}H_{15}ClN_2O_3$	3446 and 3387 (NH_2), 3294 (NH), 1729 s (ester CO), 1667 s (amide CO), 1629, 1600 w, 1546.	1.26 (t, 3H, CH_2CH_3), 1.50 (d, 3H, $CHCH_3$), 3.45 (q, 1H, $CHCH_3$), 3.86[e] (broad s, 2H, NH_2), 4.23 (q, 2H, CH_2CH_3), 6.50–7.37 (m, 3H, phenyl H-3,5,6), 8.26 [e] (broad s, 1H, *NH*CO).
17c	CF_3	71	172–172.5 (D)	$C_{13}H_{15}F_3N_2O_3$	3444 and 3370 (NH_2), 3272 (NH), 1740 s (ester CO), 1654 s (amide CO), 1605 w, 1537.	1.30 (t, 3H, CH_2CH_3), 1.57 (d, 3H, $CHCH_3$), 3.51 (q, 1H, $CHCH_3$), 4.00 [e] (broad s, 2H, NH_2), 4.30 (q, 2H, CH_2CH_3), 6.90–7.70 (m, 3H, phenyl H-3,5,6), 8.60 [e] (broad s, 1H, *NH*CO).

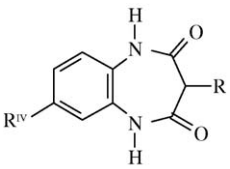
[a] Crystallization solvent: A = ethanol/petroleum ether, B = petroleum ether, C = ethyl acetate/isopropyl ether, D = ethanol.
[b] Anal. C,H,N. (C, H, N, Cl for **16b**, **17b**).
[c] In $CHCl_3$ solutions (compounds **16a–c**); in Kbr pellets (compounds **17a–c**). Abbreviations: s = strong, br = broad, w = weak, sh = shoulder.
[d] In $CDCl_3$ solutions. Abbreviations: s = singlet, d = doublet, dd = double doublet, t = triplet, q = quartet, m = multiplet.
[e] Disappeared with D_2O.

7.5 mmol (3.03 g) of the Lawesson's reagent and 10 ml of Dowtherm A was heated at 150 °C for 2 h with stirring. In the case of the reactions carried out with compounds **18a–c**, the suspension obtained was diluted with a little acetone and the whitish insoluble solid was collected by filtration, washed with the same solvent and dried to yield the nearly pure compounds **19a–c**. Due the very low solubility of these compounds in most solvents, only a little amount of them was crystallized from the proper solvent in order to prepare the analytical sample; the remaining amount was used rough in the subsequent synthetic step. On the contrary, compound **19d** was very soluble in many solvents: therefore the final mixture of the reaction carried out with **18d** was chromatographed on a aluminium oxide column eluting first with dichloromethane until Dowtherm A was removed, then with tetrahydrofuran in order to recover **19d**. The eluate collected, after removal of solvent afforded a whitish solid, which was taken up in a little petroleum ether, filtered and crystallized from the suitable solvent. Data of compounds **19a–d** are reported in Table 5.

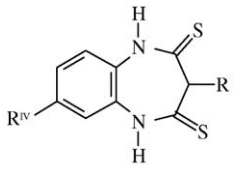
*4.1.5. General procedure for the preparation of 2,4-bis(methylthio)-3H-1,5-benzodiazepines **20a–d***

A mixture of 5.0 mmol of the proper dithione **19** (1.04 g of **19a**; 1.11 g of **19b**; 1.28 g of **19c**; 1.45 g of **19d**), 1.40 g of anhydrous potassium carbonate, 1.60 ml (~25 mmol) of iodomethane and 50 ml of dry acetone was refluxed for 2 h. The solvent was then removed in vacuo and the residue partitioned between water and dichloromethane. The aqueous phase was extracted several more times with dichloromethane.

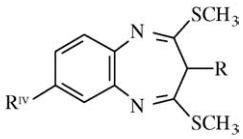
Table 5
Data of compounds **18b–d, 19a–d** and **20a–d**



18b-d



19a-d



20a-d

Compound	R	R^{IV}	Yield (%)	m.p. (°C) (solvent) [a]	Molecular formula[b]	IR[c] (cm^{-1})	^{1}H-NMR[d] (δ, ppm)
18b	CH_3	H	63	>360 (A)	$C_{10}H_{10}N_2O_2$	3170 and 3062 (NH), 1703 s (CO), 1650 s, 1604 w, 1503.	1.11 (d, 3H, 3-CH_3), 3.11 (q, 1H, H-3), 7.03–7.30 (m, 4H, H-6,7,8,9), 10.41 [e] (s, 2H, *NH*CO).
18c	CH_3	Cl	40	359–360 (A)	$C_{10}H_9ClN_2O_2$	3193 and 3065 (NH), 1716 s (CO), 1658 s, 1598 w, 1497.	1.12 (d, 3H, 3-CH_3), 3.20 (q, 1H, H-3), 7.09–7.30 (m, 3H, H-6,8,9), 10.51 [e] (s, 2H, *NH*CO).
18d	CH_3	CF_3	54	361–363 (A)	$C_{11}H_9F_3N_2O_2$	3190 and 3065 (NH), 1720 s (CO), 1657 s, 1619, 1500 w.	1.23 (d, 3H, 3-CH_3), 3.37 (q, 1H, H-3), 7.30–7.80 (m, 3H, H-6,8,9), 10.50 [e] (broad s, 2H, *NH*CO).
19a	H	H	95	315–317 (B)	$C_9H_8N_2S_2$	3102 (NH), 1662, 1603, 1532 s, 1487, 1170 s (CS).	4.06 (s, 2H, CH_2), 7.30 (s, 4H, H-6,7,8,9), 12.47 [e] (s, 2H, *NH*CS).
19b	CH_3	H	98	319–320 (C)	$C_{10}H_{10}N_2S_2$	3159 and 3110 (NH), 1661, 1605, 1537 s, 1491 , 1168 s (CS).	1.43 (d, 3H, 3-CH_3), 3.67 (q, 1H, H-3), 7.14–7.62 (m, 4H, H-6,7,8,9), 12.51 [e] (s, 2H, *NH*CS).
19c	CH_3	Cl	87	303–304 (C)	$C_{10}H_9ClN_2S_2$	3153 and 3105 (NH), 1664, 1599, 1537 s, 1485, 1167 s (CS).	1.48 (d, 3H, 3-CH_3), 3.42 (q, 1H, H-3), 6.70–7.60 (m, 3H, H-6,8,9), 10.90 [e] (s, 2H, *NH*CS).
19d	CH_3	CF_3	98	280–281 (D)	$C_{11}H_9F_3N_2S_2$	3162 and 3115 (NH), 1660 w, 1621, 1601 w, 1533 s, 1505 , 1167 s (CS).	1.60 (d, 3H, 3-CH_3), 3.50 (q, 1H, H-3), 7.38–7.78 (m, 3H, H-6,8,9), 12.48 [e] (s, 2H, *NH*CS).
20a	H	H	83	53.5–55 (E)	$C_{11}H_{12}N_2S_2$	1597 s, 1570, 1545 sh, 1460.	2.50 (s, 6H, SCH_3), 3.09 (s, 2H, CH_2), 7.09–7.49 (m, 4H, H-6,7,8,9).
20b	CH_3	H	62	150–151 (F)	$C_{12}H_{14}N_2S_2$	1602, 1583 s, 1545 sh, 1460.	1.52-1.83 (m, 3H, 3-CH_3), 2.47 (s, 6H, SCH_3), 2.68–2.93 (m, 1H, H-3), 7.13–7.42 (m, 4H, H-6,7,8,9).
20c	CH_3	Cl	76	54–55 (G)	$C_{12}H_{13}ClN_2S_2$	1607, 1587 s, 1550 w, 1460.	1.20–1.86 (m, 3H, 3-CH_3), 2.45 (s, 6H, SCH_3), 2.60–2.95 (m, 1H, H-3), 7.08–7.50 (m, 3H, H-6,8,9).
20d	CH_3	CF_3	64	99–100 (G)	$C_{13}H_{13}F_3N_2S_2$	1605, 1582 s, 1550 w, 1450 w, 1410.	1.48–1.80 (m, 3H, 3-CH_3), 2.47 (s, 6H, SCH_3), 2.65–2.86 (m, 1H, H-3), 7.33–7.51 (m, 2H, H-8,9), 7.65 (near s, 1H, H-6).

[a] Crystallization solvent: A = ethanol, B = methanol/dichloromethane, C = ethanol/tetrahydrofuran, D = ethyl acetate/isopropyl ether, E = petroleum ether, F = isopropyl ether, G = methanol.
[b] Anal. C,H,N (compounds **18b,d**); C,H,N,Cl (compound **18c**); C,H,N,S (compounds **19a,b,d, 20 a,b,d**); C,H,N,S,Cl (compounds **19c, 20 c**).
[c] In Kbr pellets (compounds **18b–d, 19a–d**); in $CHCl_3$ solutions (compounds **20a–d**). Abbreviations: s = strong, w = weak, sh = shoulder.
[d] In DMSO-d_6 solutions (**18b–d, 19a–d**); in $CDCl_3$ solutions (compounds **20a–d**). Abbreviations: s = singlet, d = doublet, q = quartet, m = multiplet.
[e] Disappeared with D_2O.

The combined organic extracts were dried (anhydrous sodium sulphate), then evaporated to dryness to give an oily residue, which was purified by chromatography on a silica gel column, eluting with the mixture dichloromethane–petroleum ether (1:1). The fraction collected was evaporated to dryness in vacuo and the resulting white oil, after treatment with a little petroleum ether and standing at 4 °C, afforded compounds **20a–d** as white solids, which were then recrystallized from the suitable solvents. The low-melting compounds **20a,c**, due their very high solubility in nearly all solvents, were used as oils in the subsequent synthetic step, only a small amount of them being recrystallized from the proper solvent in order to prepare the analytical sample.

Data for compounds **20a–d** are reported in Table 5.

*4.1.6. General procedure for the preparation of the substituted 9H-bis-[1,2,4]triazolo[4,3-a:3′,4′-d][1,5]benzodiazepines **8e–o***

A mixture of 2.5 mmol of the proper 2,4-bis(methylthio)derivative **20** (0.59 g of **20a**; 0.63 g of **20b**; 0.71 g of **20c**; 0.80 g of **20d**), 7.5 mmol of the suitable hydrazide (0.56 g of acetylhydrazine; 0.66 g of propionylhydrazine; 1.08 g of (phenylacetyl)hydrazine; 1.02 g of benzoylhydrazine; 1.28 g of (4-chlorobenzoyl)hydrazine; 1.13 g of (3-methoxybenzoyl)hydrazine; 1.03 g of isonicotinoylhydrazine), 0.15 g of *p*-toluenesulfonic acid and 10 ml of Dowtherm A was

stirred at 200 °C for 2 h. After cooling, compounds **8e–o** were recovered from the final mixture as follows.

*Compounds **8e,g,i,l,m***: the white solid that separated out was taken up in a little ethanol and filtered to give the nearly pure compound **8**.

*Compounds **8f,j***: the solid that separated out (0.30 g) was recovered by filtration and washed with a little ethyl ether. It was shown to be (elemental analysis, IR, and ^{1}H-NMR spectra) 1,2-diphenylacetylhydrazine, white needles, m.p.: 231–233 °C, after crystallization from ethanol (Ref. [11]: m.p.: 231 °C). The filtrate and washings, after removal of solvent gave an oil which was chromatographed on a silica gel column, eluting first with dichloromethane until Dowtherm A was removed, then with ethyl acetate in order to remove some impurities and finally with the mixture dichloromethane/methanol (9:1). This last eluate, after removal of solvents gave the nearly pure compound **8**.

*Compounds **8 h,k**:* the reaction mixture was directly chromatographed on a silica gel column; the pure compound **8** was recovered proceeding exactly as above described for the chromatography of compounds **8f,j**.

*Compounds **8n,o***: the solid that separated out (0.25 g) was recovered by filtration and washed with a little acetone. It was shown (elemental analysis, IR, and ^{1}H-NMR spectra) to be 1,2-dibenzoylhydrazine, white needles, m.p.: 239–240 °C, after crystallization from ethanol (Ref. [12]: m.p.: 238 °C). The filtrate and washings, after removing of acetone afforded an oily residue which was subjected to a chromatography on a silica gel column carried out as in the two last cases, to yield the pure compound **8**.

Compounds **8e–o** were then crystallized from the suitable solvents. Their data are reported in Table 6.

*4.1.7. 9-[(Dimethylamino)methylene]-6,12-diphenyl-9H-bis [1,2,4]triazolo[4,3-a:3′,4′-d] [1,5]benzodiazepine (**10**)*

A mixture of 2 mmol (0.75 g) of **8c** [3], 7.0 ml of *N,N*-dimethylformamide dimethyl acetal, and 5.0 ml of anhydrous pyridine was refluxed (120 °C), with stirring, for 24 h. The mixture was then evaporated to dryness in vacuo and the solid residue was taken up in a little acetone and filtered to give the nearly pure compound **10** · 0.5 H_2O (0.55 g, 62%), pale orange crystals, m.p. 355–357 °C after crystallization from ethanol. IR (KBr): 3440 br (H_2O), 1630 s, 1505, 1472, 1440, 1419 cm^{-1}. ^{1}H-NMR (DMSO-d_6): δ 3.10 [s, 6H, $N(CH_3)_2$], 6.85–6.98 and 7.08–7.22 (2 m, 2H + 2H, H-1,2,3,4), 7.37–7.62 (m, 11H, = CH- + phenyl H's). Anal. $C_{26}H_{21}N_7 \cdot 0.5\ H_2O$ (C, H, N).

*4.1.8. 9-Chloro-6,12-diphenyl-9H-bis [1,2,4]triazolo[4,3-a:3′,4′-d] [1,5]benzodiazepine (**11**) and 9,9-dichloro-6,12-diphenyl-9H-bis [1,2,4]triazolo[4,3-a:3′,4′-d] [1,5]benzodiazepine (**12**)*

A mixture of 2.0 mmol (0.75 g) of **8c** [3], 10.0 mmol (1.33 g) of *N*-chlorosuccinimide and 100 ml of chloroform–carbon tetrachloride (1:1) was refluxed with stirring for 24 h. After cooling 2 N aqueous NaOH (50 ml) was added and the mixture was shaken in a separatory funnel: the organic layer was collected and the aqueous one was further extracted twice with chloroform. The combined organic phase was washed with water, then dried (anhydrous sodium sulphate), concentrated in vacuo to a little volume and finally subjected to chromatography on a silica gel column, first eluting with the mixture chloroform–ethyl acetate (1:1). The first eluate collected gave, after removal of solvents the pure dichloro derivative **12** as a white solid (0.14 g, 16%), which decomposes without melting after 330 °C after crystallization from acetone/ethyl acetate. IR (KBr): 1602 w, 1579 w, 1530 w, 1508, 1470, 1447, 1416, 1390 cm^{-1}. ^{1}H-NMR ($CDCl_3$): δ 6.98–7.13 and 7.24–7.38 (2 m, 2H + 2H, H-1,2,3,4), 7.42–7.65 (m, 10H, phenyl H's). Anal. $C_{23}H_{14}Cl_2N_6$ (C, H, N, Cl).

The elution was completed with ethyl acetate: the fraction collected gave, after removal of solvent, the pure compound **11** as a white solid (0.57 g, 69%) which decomposes without melting after 320 °C after crystallization from acetone/ethyl acetate. IR (KBr): 2947 (9-*CH*Cl), 1610 w, 1600 sh, 1581 w, 1530 sh, 1505, 1472, 1449, 1422, 1410 cm^{-1}. ^{1}H-NMR ($CDCl_3$): δ 6.96 (s, 1H, H-9), 7.03–7.17 and 7.24–7.38 (2 m, 2H + 2H, H-1,2,3,4), 7.43–7.66 (m, 10H, phenyl H's). Anal. $C_{23}H_{15}ClN_6$ (C, H, N, Cl).

*4.1.9. General procedure for the preparation of 9-(dialkylamino)derivatives **13a,b***

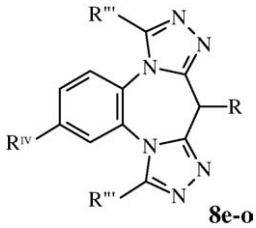
A mixture of 1.5 mmol (0.62 g) of chloro derivative **11**, 5 ml of the suitable dialkylamine, and 7 ml of dimethyl sulphoxide was heated at 120 °C for 1 h (compound **13b**) or for 2 h (compound **13a**), while stirring. After cooling, the mixture was poured into ice-cooled water (200 ml) and the resulting emulsion was exhaustively extracted with dichloromethane.

From combined extracts (washed with water and dried over anhydrous Na_2SO_4 then evaporated to dryness in vacuo), an oily residue was obtained from which compound **13** was recovered as below described for each case.

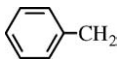
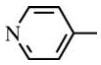
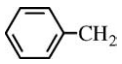
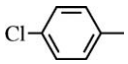
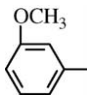
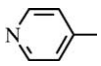
*4.1.9.1. 9-Morpholino-6,12-diphenyl-9H-bis [1,2,4]triazolo-[4,3-a:3′,4′-d] [1,5]benzodiazepine (**13a**).* The oil obtained from the reaction carried out with morpholine was subjected to chromatography on a silica gel column first eluting with the mixture dichloromethane–petroleum ether–triethylamine (5:5:1) in order to remove some impurities, then with the mixture dichloromethane–triethylamine (9:1): the eluate collected, after removal of solvents afforded the nearly pure compound **13a** · H_2O (0.47 g; 65%) as a white solid, m.p. 332–334°C, after crystallization from ethanol. IR (KBr): 3420 br (H_2O), 1596 w, 1578 w, 1522, 1502, 1473, 1448, 1421, 1402 cm^{-1}. ^{1}H-NMR ($CDCl_3$): δ 2.36 (near t, 4H, morpholine N–CH_2s), 3.18 (near t, 4H, morpholine O–CH_2s), 5.53 (s, 1H, H-9), 6.93–7.06 and 7.21–7.35 (2 m, 2H + 2H, H-1,2,3,4), 7.38–7.65 (m, 10H, phenyl H's). *Anal.* $C_{27}H_{23}N_7O \cdot H_2O$ (C, H, N).

*4.1.9.2. 9-(4-Methyl-1-piperazinyl)-6,12-diphenyl-9H-bis [1,2,4]triazolo[4,3-a:3′,4′-d] [1,5]benzodiazepine (**13b**).* The oil resulting from the reaction carried out with 1-methyl-

Table 6
Data of compounds **8e–o**



8e-o

Compound	R	R‴	R^{IV}	Yield (%)	m.p. (°C) (solvent) [a]	Molecular formula[b]	IR[c] (cm^{-1})	^{1}H-NMR[d] (δ, ppm)
8e	H	C_2H_5	H	62	345–347 (A)	$C_{15}H_{16}N_6$	1545, 1523, 1500, 1460, 1427, 1410.	1.34 (t, 6H, CH_2CH_3), 2.69–2.92 and 2.96–3.18 (2 m, 2H + 2H, CH_2CH_3), 3.74 and 4.90 (AB q, J = 16 Hz, 1H + 1H, 9-CH_2), 7.52–7.76 (m, 4H, H-1,2,3,4).
8f	H		H	30	281–281.5 (B)	$C_{25}H_{20}N_6$	1595w, 1580w, 1532, 1499, 1468, 1452, 1430w, 1412.	3.70 and 4.92 (AB q, J = 16 Hz, 1H + 1H, 9-CH_2), 3.97 and 4.26 (AB q, J = 16 Hz, 2H + 2H, $CH_2C_6H_5$), 6.94–7.56 (m, 14H, H-1,2,3,4 + phenyl H's).
8g	H		H	81	333–334 (A)	$C_{21}H_{14}N_8 \cdot 0.33\ H_2O$	3395br (H_2O), 1603, 1542, 1501, 1465, 1418br.	3.94 and 5.14 (AB q, J = 16 Hz, 1H + 1H, 9-CH_2), 7.10–7.20 and 7.42–7.51 (2 m, 2H + 6H, H-1,2,3,4 + pyridyl H-3′,5′), 8.79 (near d, 4H, pyridyl H-2′,6′).
8h	CH_3	CH_3	H	48	358–359 (A)	$C_{14}H_{14}N_6$	1595w, 1527s,br 1509s,br, 1460 , 1420, 1402.	2.16 (d, 3H, 9-CH_3), 2.59 and 2.61 (over-lapped singlets, 6H, 6,12-CH_3), 3.93 (q, 1H, H-9), 7.47–7.60 and 7.63–7.75 (2 m, 2H + 2H, H-1,2,3,4).
8i	CH_3	C_2H_5	H	73	336–338 (A)	$C_{16}H_{18}N_6$	1530, 1501, 1468, 1430, 1405.	1.17 (t, 6H, CH_2CH_3), 1.86 (d, 3H, 9-CH_3), 2.65–2.86 and 2.94–3.17 (2 m, 2H + 2H, CH_2CH_3), 4.20 (q, 1H, H-9) 7.65–7.78 and 7.85–7.96 (m, 2H + 2H, H-1,2,3,4).
8j	CH_3		H	20	284–286 (B)	$C_{26}H_{22}N_6$	1602w, 1590w, 1528, 1502, 1468, 1458, 1431, 1412.	2.17 (d, 3H, 9-CH_3), 3.87 (q, 1H, H-9), 3.93 and 4.27 (AB q, J = 16 Hz,2H + 2H, $CH_2C_6H_5$), 7.02–7.53 (m, 14H, H-1,2,3,4 + phenyl H's).
8k	CH_3		H	72	367–369 (C)	$C_{24}H_{16}Cl_2N_6$	1600, 1570w, 1530, 1518sh, 1501, 1468, 1430, 1417.	2.26 (d, 3H, 9-CH_3), 4.09 (q, 1H, H-9), 7.04–7.15 and 7.32–7.52 (2 m, 2H+10H, H-1,2,3,4+p-chlorophenyl H's).
8l	CH_3		H	75	255.5–257 (A)	$C_{26}H_{22}N_6O_2$	1615sh, 1602, 1583, 1522, 1501, 1488, 1460br, 1438, 1420.	2.27 (d, 3H, 9-CH_3), 3.79 (s, 6H, OCH_3), 4.11 (q, 1H, H-9), 6.97–7.16 and 7.24–7.40 (2 m, 8H + 4H, H-1,2,3,4 + *m*-methoxyphenyl H's).
8m	CH_3		H	82	354–355.5 (A)	$C_{22}H_{16}N_8 \cdot 1.75\ H_2O$	3405br (H_2O), 1608, 1560, 1530, 1501, 1470, 1455 sh, 1427.	2.28 (d, 3H, 9-CH_3), 4.11 (q, 1H, H-9), 7.09–7.20 and 7.37–7.57 (2 m, 2H + 6H, H-1,2,3,4 + pyridyl H-3′,5′), 8.77 (near d, 4H, pyridyl H-2′,6′).
8n	CH_3		Cl	15	315–317 (D)	$C_{24}H_{17}ClN_6$	1602w, 1585w, 1525, 1501, 1473, 1445, 1420.	2.27 (d, 3H, 9-CH_3), 4.10 (q, 1H, H-9), 6.98–7.06 and 7.22–7.32 (2 m, 1H + 2H, H-1,3,4), 7.44–7.60 (m, 10H, phenyl H's).
8o	CH_3		CF_3	47	331–333 (A)	$C_{25}H_{17}F_3N_6$	1628w, 1600w, 1582w, 1528, 1481, 1450, 1423.	2.27 (d, 3H, 9-CH_3), 4.12 (q, 1H, H-9), 7.13–7.31 (m, 3H, H-1,3,4), 7.40–7.64 (m, 10H, phenyl H's).

[a] Crystallization solvent: A = ethanol, B = methanol, C = ethanol/dichloromethane, D = ethyl acetate/isopropyl ether.
[b] Anal. C,H,N. (C, H, N, Cl for **8k,n**).
[c] In KBr pellets. Abbreviations: w = weak, br = broad, s = strong, sh = shoulder.
[d] In $CDCl_3$ solutions, except for **8i** (DMSO-d_6). Abbreviations: s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet.

piperazine was chromatographed on a silica gel column, first eluting with ethyl acetate in order to remove a little amount (0.04 g) of the starting compound **11**, then with the mixture dichloromethane–triethylamine (9:1): this eluate afforded 0.24 g (42%) of compound **8c** (deriving from a dehalogenation of **11**) and which was identified by comparison (m.p., TLC and IR) with an authentical sample [3]. Finally, the fraction eluted with the mixture chloroform–methanol (9:1) gave, after removal of solvents, the pure compound **13b** · H_2O (0.17 g, 23%) as white crystals melting at 320–322 °C, after crystallization from ethanol. IR (KBr): 3420 br (H_2O), 1600 w, 1580 w, 1522, 1501, 1470, 1448, 1420, 1403 cm^{-1}. ^{1}H-NMR ($CDCl_3$): δ 1.60–2.15 and 2.30–2.47 (2 m, 4H + 4H, piperazine CH_2s), 2.04 (s, 3H, NCH_3), 5.53 (s, 1H, H-9), 6.92–7.05 and 7.21–7.33 (2 m, 2H + 2H, H-1,2,3,4), 7.40–7.67 (m, 10H, phenyl H's). *Anal.* $C_{28}H_{26}N_8 \cdot H_2O$ (C, H, N).

*4.1.10. 9-Methyl-9H-bis [1,2,4]triazolo[4,3-a:3′,4′-d] [1,5]benzodiazepine-6,12(7H,11H)-dione (**14**)*

A mixture of 4 mmol (1.00 g) of **20d**, 16.0 mmol (1.67 g) of ethyl carbazate and 10 ml of Dowtherm A was stirred at 200 °C for 2 h. After cooling a white solid separated out: it was taken up in a little ethyl acetate and recovered by filtration, then subjected to chromatography on a silica gel column, eluting first with the mixture dichloromethane–ethyl acetate (1:1) in order to remove some impurities. The subsequent elution with the mixture acetone–ethyl acetate (2:1) afforded the pure compound **14** · 0.75 H_2O (0.50 g, 44%); white crystals (from ethanol) which decompose without melting after 380 °C. IR (KBr): 3420 br (H_2O), 3277 and 3069 (NH), 1695 s, br (CO), 1604, 1586, 1513, 1465, 1429 cm^{-1}. ^{1}H-NMR (DMSO-d_6): δ 1.54 (d, 3H, 9-CH_3), 4.07 (q, 1H, H-9), 7.42–7.67 and 7.77–8.02 (2 m, 2H + 2H, H-1,2,3,4), 12.10 (broad s, 2H, *NH*CO; disappeared with D_2O). Anal. $C_{12}H_{10}N_6O_2 \cdot 0.75\ H_2O$ (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

Cell lines were purchased from American Type Culture Collection (ATCC). Haematological tumor-derived cells were grown in RPMI-1640 medium supplemented with 10% foetal calf serum (FCS), 100 UI/ml penicillin G and 100 μg/ml streptomycin. Solid tumor-derived cells were grown in their specific media supplemented with 10% FCS and antibiotics. Cells cultures were incubated at 37 °C in a humidified, 5% CO_2 atmosphere. The absence of mycoplasma contamination was checked periodically 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.

4.2.4. Antiviral 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 [13].

Acknowledgements

Authors wish to thank Prof. Paola Fossa (Dipartimento di Scienze Farmaceutiche, Università di Genova) for her valuable help in conformational studies; financial support from Italian MIUR is gratefully acknowledged.

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