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Original article

1,5-Benzodiazepines Part XIII. Substituted 4*H*-[1,2,4]triazolo[4,3-*a*][1,5]benzodiazepin-5-amines and 4*H*-imidazo[1,2-*a*][1,5]benzodiazepin-5-amines as analgesic, anti-inflammatory and/or antipyretic agents with low acute toxicity☆

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Abstract

The reaction of proper *N*,*N*-dialkyl-4*H*-[1,2,4]triazolo[4,3-*a*][1,5]benzodiazepin-5-amines (**1**) with N-chlorosuccinimide afforded their 4-chloroderivatives **3** which in turn were treated with cyclic amines to give the corresponding 4,5-diaminoderivatives **4**. The *N*,*N*-dialkyl-4*H*-imidazo[1,2-*a*][1,5]benzodiazepin-5-amines (**5**) were prepared starting from suitable 4-(dialkylamino)-1,3-dihydro-2*H*-1,5-benzodiazepin-2-ones (**8**), through multistep synthetic routes. At the 200 mg kg^{-1} os dose, some compounds **3** and **4** showed notable analgesic or anti-inflammatory activity but no antipyretic properties, whereas the 5-(dibutylamino) derivatives **5b** and **5f** proved to be significantly endowed with all these activities. Almost all the compounds **3**, **4** and **5** did not show acute toxicity in mice up to 800 mg kg^{-1} os dose.

Keywords: 1,5-Benzodiazepine tricyclic derivatives; Analgesic; Anti-inflammatory; Antipyretic; Gastrolesivity; Acute toxicity

1. Introduction

In previous papers of this series we described the preparation and pharmacological properties of the substituted 4*H*- and 6*H*-[1,2,4]triazolo[4,3-*a*][1,5]benzodiazepin-5-amines **1** [1,2] and **2** [3], respectively (Fig. 1). Compounds **1** proved to be completely devoid of anticonvulsant activity whereas some of them showed statistically significant analgesic and/or anti-inflammatory properties, depending on the structure [1,2]. On the other hand, the presence of the 6-phenyl and 8-chloro substituents endowed compounds **2** with a clear-cut central nervous system (anticonvulsant) activity, as expected [3]. Both triazolobenzodiazepines **1** and **2** usually exhibited rather low acute toxicity [1–3].

No example of [1,2,4]triazolo[4,3-*a*][1,5]benzodiazepines amino substituted on the diazepine ring had been previously reported in the literature.

The novelty of the structure and the interesting safety profile of the analgesic and/or anti-inflammatory agents **1** [1,2] have now prompted us to design and synthesise a number of their analogues with modified substitution pattern or different pentatomic heterocycle, in order to evaluate their analgesic, anti-inflammatory, antipyretic and toxicological properties. In this paper we just describe the synthesis and pharmacological properties of compounds **3**, **4** and **5** (Fig. 2).

It must be noted that the pharmacological properties of some significant examples of compounds **1** have been

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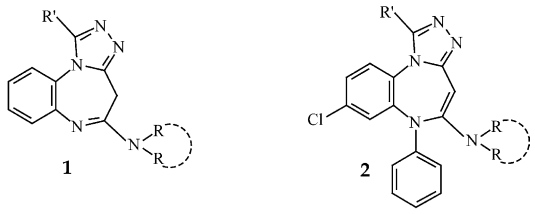


Fig. 1. Structures of the substituted 4*H*- and 6*H*-[1,2,4]triazolo[4,3-*a*][1,5]benzodiazepin-5-amines **1** and **2**.

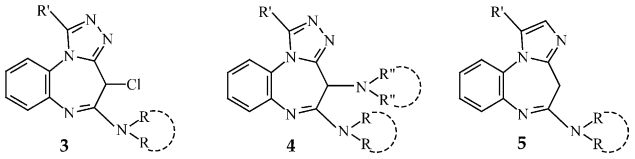


Fig. 2. Structures of compounds **3** and **4** (4-substituted derivatives of compounds **1**) and of compounds **5** (isosteric analogues of compounds **1**).

recently further investigated. In summary, they showed no affinity for the central and peripheral 1,4- and 1,5-benzodiazepine receptors and proved to affect the inflammatory response in mice by inhibiting interleukin-6 and prostaglandin E_2 production [4].

2. Chemistry

The reaction of the substituted 4*H*-[1,2,4]triazolo[4,3-*a*][1,5]benzodiazepin-5-amines **1a**–**d** with *N*-chlorosuccinimide in refluxing carbon tetrachloride afforded the corresponding 4-chloroderivatives **3a**–**d** (Fig. 3, Table 1). In the case of compound **3c** also a small amount of 4,4-dichloroderivative **7** was obtained.

The starting compounds **1a**,**c** [1] and **1b** [2] were previously described by us, whereas **1d** was prepared by the reaction of compound **6** [2] with excess morpholine (refluxing anhydrous toluene), in the presence of titanium tetrachloride and anisole (Fig. 3).

The substituted 4*H*-[1,2,4]triazolo[4,3-*a*][1,5]benzodiazepin-4,5-diamines **4a**–**g** were obtained from the treatment of 4-chloroderivatives **3a**–**c** with proper excess amines (ethylene glycol, 160 °C for **4a**,**b**; dimethyl sulphoxide, 120 °C for **4c**–**g**) (Fig. 3, Table 1).

The substituted 4*H*-imidazo[1,2-*a*][1,5]benzodiazepin-5-amines **5a**–**f** were synthesised through literature procedures [5] properly modified, starting from the substituted 4-(methylthio)-3*H*-1,5-benzodiazepin-2-amines **11a** [6], **11b**, or the 4-(dimethylamino)-1,3-dihydro-2*H*-1,5-benzodiazepin-2-one (**8a**) [7] (Fig. 4, Table 1).

The starting compound **11b** was prepared through the following three-step synthetic route. The reaction of *o*-phenylenediamine with ethyl *N*,*N*-dibutylmalonamate in the presence of phosphorus oxychloride (110 °C) afforded a mixture of the desired (dibutylamino)benzodiazepinone **8b** and the 2-(2-benzimidazolyl)-*N*,*N*-dibutylacetamide (**9**). Compound **8b** was then treated with Lawesson's reagent in refluxing anhydrous toluene to give the corresponding 1,5-benzodiazepine-2-thione **10**, which in turn was reacted with methyl iodide, in the presence of anhydrous K_2CO_3 (acetone at reflux), affording **11b** (Fig. 4).

The raw thick oil obtained from the reaction of (methylthio)derivative **11a** or **11b** with aminoacetaldehyde dimethyl acetal (ethylene glycol, 160 °C) was then dissolved and heated in refluxing 99% formic acid to obtain the cyclocondensation of the intermediate aminoderivative to the tricyclic compound **5a** or **5b**, respectively.

When compound **11a** or **11b** was treated with propargylamine in the presence of *p*-toluenesulphonic acid (Dowtherm A, 180 °C) the *N*,*N*-dialkyl-1-methyl-4*H*-imidazo[1,2-*a*][1,5]benzodiazepin-5-amine **5e** or **5f** was obtained, respectively.

On the other hand, the reaction of compound **8a** with aminoacetaldehyde dimethyl acetal, using *p*-toluenesulphonic acid as the catalyst (Dowtherm A, 180 °C), directly afforded a small amount of the tricyclic compound **12** along with its intermediate aminoderivative **8c**, which in turn gave **12** by treatment with 99% formic acid at reflux. The treatment of 4*H*-imidazo[1,2-*a*][1,5]benzodiazepin-5(6*H*)-one (**12**) with an excess of piperidine or morpholine, in the presence of titanium tetrachloride and anisole (refluxing anhydrous toluene), yielded the desired compound **5c** or **5d**, respectively (Fig. 4).

Compounds **5a**–**f** are the first examples of imidazo[1,2-*a*][1,5]benzodiazepines amino substituted on the diazepine ring.

The structures attributed to the compounds described in this paper are supported by the results of elemental analyses and IR and ^{1}H-NMR spectral data (see Section 5 and Table 2).

As we reported for previously described compounds **1** [1,2], also for the tricyclic compounds **1d** ($CDCl_3$), **5a**–**f** ($CDCl_3$) and **12** (DMSO-d_6) the 4-CH_2 ^{1}H-NMR signal appears as an AB quartet (**1d**, **5e**, **5f**), or as a sharp (**12**) or broad (**5a**–**d**) singlet, depending on the presence or lack, respectively, of 1-substituent and its steric hindrance. Actually, the AB quartet signal for 4-CH_2 is evidently due to the geminal coupling of the two non-equivalent protons, as a result of the presence of only one of the two boat conformations of the diazepine ring, no interconversion occurring in the solution at the registration temperature (21 °C). On the contrary, when a high or slow rate interconversion occurs, the 4-CH_2 signal appears as a sharp or broad singlet, respectively [8].

Concerning then the ^{1}H-NMR spectra of the 4,5-disubstituted tricyclic derivatives **3** and **4**, it can be observed that the signal (a sharp singlet) corresponding

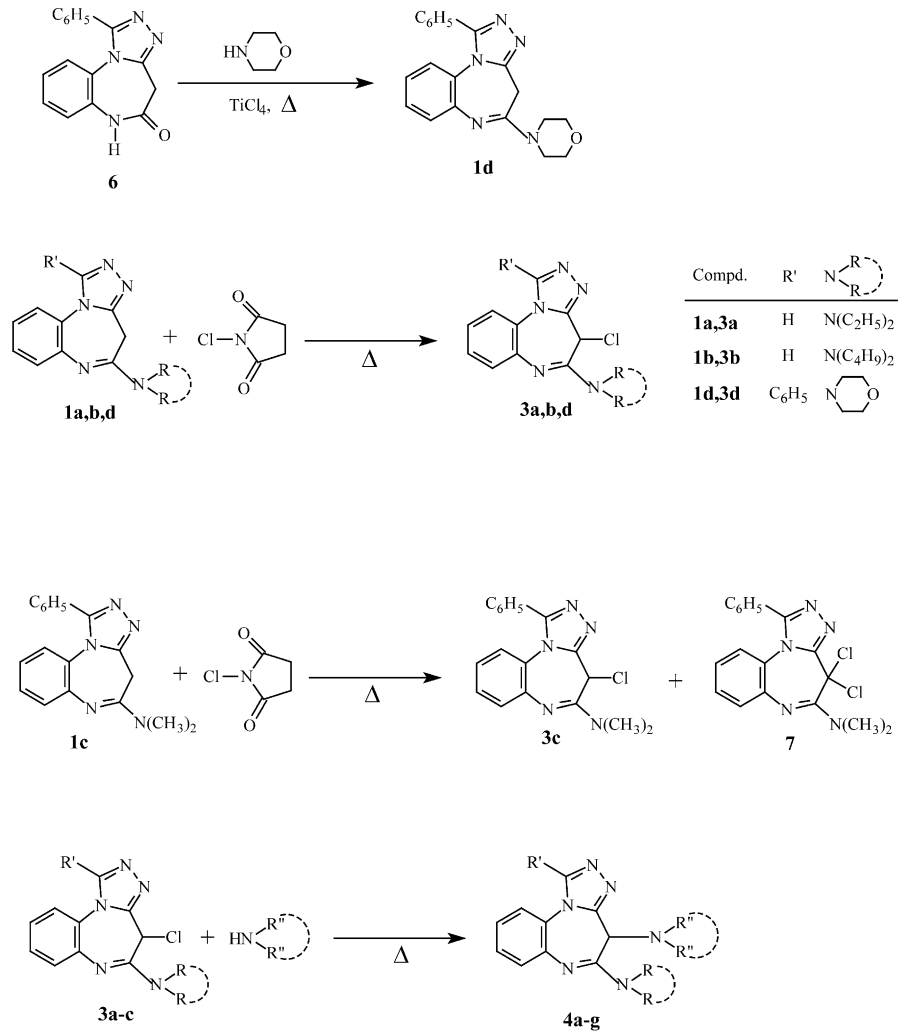


Fig. 3. Synthesis of compounds **3a**–**d** and **4a**–**g**.

to the unsymmetrically substituted 4-CH is in accordance with the presence (previously reported in the literature for similar compounds [8]) of only one conformation (the most stable) of the diazepine ring in the solution at the registration temperature ($CDCl_3$, 21 °C). Besides, the multiplicities of the $H_2C–N–CH_2$ signals of compounds **3b** and **4f**,**g** seem to be due only to the chirality of the molecules, whereas the more complex multiplicities of the $H_2C–N–CH_2$ signals of the 4,5-bis(dialkylamino) derivatives **4a**–**e** are most likely attributable to both the molecule chirality and the reciprocally hindered free rotation of the two bulky (diakylamino) substituents.

3. Pharmacological results and discussion

Compounds **3a**–**d**, **4a**–**g** and **5a**–**f** were tested in vivo for their antinociceptive, anti-inflammatory and antipyretic activities. They were administered orally and assayed at the initial dose of 200 mg kg^{-1}. Compounds which exhibited a statistically significant activity at this dose were further tested at doses decreasing by a factor of two, until statistically significant activity was observed. Only for compounds **5b** and **5f**, in the case of LPS induced fever test in rats, the highest dose used was 100 mg kg^{-1}. Gastric tolerability and acute toxicity were evaluated in mice after oral administration of compounds at the 400 and 800 mg kg^{-1} doses, respectively (see Section 5).

The pharmacological results obtained for the above compounds are reported in Table 1, as well as those of the 'lead compounds' **1a**–**c** [1,2] and the unpublished data concerning the antipyretic activity and gastrolesivity of compound **1b**, in order to compare it with its isosteric analogue **5b**.

Among the novel compounds tested, a statistically significant activity at the 200 mg kg^{-1} dose was shown by **3d**, **4c**, **5b** and **5f** (inhibition range 61–90%) in the acetic acid writhing test in mice (analgesic activity), by **3c**, **4b**, **4f**, **5b**, **5f** (inhibition range 46–80%) in the carrageenan induced rat paw edema test (anti-inflam-

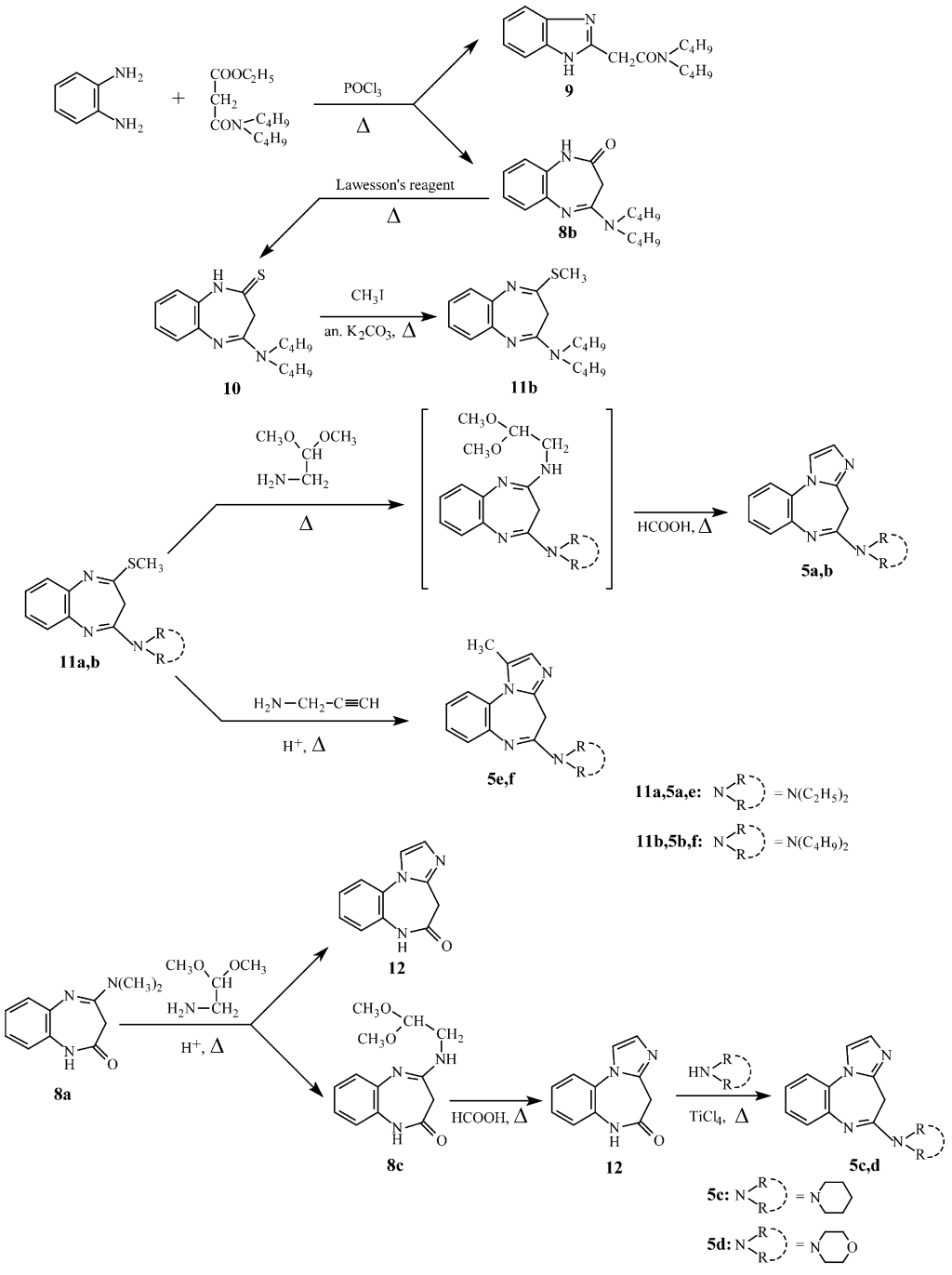
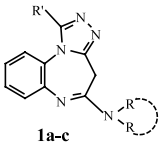


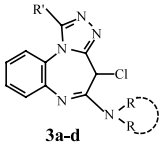
Fig. 4. Synthetic routes to compounds **5a**–**f**.

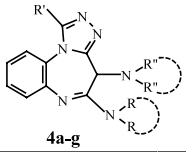
matory activity), and by the 4*H*-imidazo[1,2-*a*][1,5]benzodiazepine derivatives **5b**,**f** (100 mg kg^{-1}) and **5d** (200 mg kg^{-1}) (inhibition range 80–92%) in the LPS induced fever test in rats (antipyretic activity). In this connection, it is interesting to point out that all the 4,5-disubstituted 4*H*-[1,2,4]triazolo[4,3-*a*][1,5]benzodiazepine derivatives **3** and **4** reported herein proved to be completely devoid of antipyretic activity, as well as the 4-unsubstituted precursor **1b** [2].

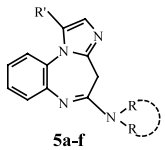
On the whole, the highest activities were afforded by **5f** as analgesic agent (90% protection at the 200 mg kg^{-1} dose) and **5b** as anti-inflammatory (80% edema inhibition at the 200 mg kg^{-1} dose) and antipyretic agent (92% protection at the 100 mg kg^{-1} dose). However, compounds **4c** and **5f** still exhibited at the 100 mg kg^{-1} dose a statistically significant analgesic (45% protection) or anti-inflammatory (35% edema inhibition) activity, respectively, whereas **5b** afforded

Table 1
Structures and pharmacological data of compounds **3a–d**, **4a–g**, **5a–f** and **1a–c**


1a-c


3a-d


4a-g


5a-f

Compd.	R'			Dose (mg/kg os)	Analgesic activity [a]	Anti-inflammatory activity [b]	Antipyretic activity [c]	Acute gastrolesivity in mice [d]	Acute toxicity in mice [e]
					% Inhibition	% Inhibition	% Inhibition	(400 mg/kg os)	(800 mg/kg os)
3a	H	$N(C_2H_5)_2$	-	200	0	24	0	0/8	0/8
3b	H	$N(C_4H_9)_2$	-	200	0	0	0	0/8	0/8
3c	C_6H_5	$N(CH_3)_2$	-	200 100	24 -	70** [f] 22	0 -	0/8	0/8
3d	C_6H_5		-	200 100	84** 19	0 -	0 -	0/8	0/8
4a	H	$N(C_2H_5)_2$		200	11	0	0	0/8	0/8
4b	H	$N(C_2H_5)_2$		200 100	15 -	71** 35	0 -	0/8	0/8
4c	H	$N(C_2H_5)_2$		200 100 50	62** 45* 9	0 - -	0 - -	0/8	0/8
4d	H	$N(C_4H_9)_2$		200	0	6	0	0/8	0/8
4e	H	$N(C_4H_9)_2$		200	15	21	0	0/8	0/8
4f	C_6H_5	$N(CH_3)_2$		200 100	0 -	46** 11	0 -	0/8	0/8
4g	C_6H_5	$N(CH_3)_2$		200	21	21	0	0/8	0/8
5a	H	$N(C_2H_5)_2$	-	200	11	13	0	0/8	0/8
5b	H	$N(C_4H_9)_2$	-	200 100 50 25	61** 0 - -	80** 0 - -	- 92** 73* 0	0/8	0/8
5c	H		-	200	0	20	0	0/8	0/8
5d	H		-	200 100	0 -	24 -	80* 0	0/8	4/8
5e	CH_3	$N(C_2H_5)_2$	-	200	22	21	0	0/8	0/8
5f	CH_3	$N(C_4H_9)_2$	-	200 100 50	90** 21 -	69** 35* 0	- 80** 0	0/8	0/8
Indomethacin [g]				10	84**	51**	93**	-	-
1a	H	$N(C_2H_5)_2$	-	207.5 [h, j]	60** [h,k]	8 [h]	-	-	-
1b	H	$N(C_4H_9)_2$	-	282.5 [i, j]	99** [i] 70** [i,k]	8 [i]	0 [l]	0/8	-
1c	C_6H_5	$N(CH_3)_2$	-	127.5 [h, j]	20 [h,k]	49** [h]	-	-	-

[a] Acetic acid induced writhing in mice.
[b] Carrageenan induced rat paw edema.
[c] LPS induced fever in rats.
[d] Number of animals showing gastric lesions.
[e] Frequency of lethal events.
[f] * P<0.05, ** P<0.01 significance as compared to controls (Student's *t*-test).
[g] For indomethacin gastrolesivity see Ref. [9].
[h] Ref. [1].
[i] Ref. [2].
[j] 1/4 LD_{50} in mice.
[k] Hot plate test in mice.
[l] At the 200 mg/kg os dose.

an interesting statistically significant antipyretic effect (73% protection) at the 50 mg kg^{-1} dose.

Besides, the data for acute gastrolesivity and toxicity in mice clearly provided preliminary satisfactory indications about the safety profile of both the tricyclic 1,2,4-triazole derivatives **3**, **4** and imidazole derivatives **5**, being only compound **5d** endowed with toxicity at the 800 mg kg^{-1} dose. In this connection, indomethacin was previously reported to produce gastric lesions in mice at anti-inflammatory doses (10 mg kg^{-1} os) [9].

Finally, we must remark that the steric hindrance of the 5-(dialkylamino) substituent of starting compounds **1a,b** and **1c** (i.e. the most effective compounds **1** as analgesic or anti-inflammatory agents, respectively [1,2]) only allowed the introduction of low hindering cyclic amino groups as 4-substituents of compounds **4** (compounds **4a**–**g**).

4. Conclusions

Concerning the structure–activity relationships (SAR), pharmacological data listed in Table 1 seem to suggest the following conclusions:

a) The 4-chloro substituent of compounds **3** can produce (compounds **3a** and **3b**) the loss of the analgesic activity shown by the corresponding starting compounds **1** (**1a** [1] and **1b** [2], respectively).
b) The introduction of a cyclic 4-amino substituent into analgesic (**1a**,**b**) or anti-inflammatory (**1c**) compounds **1** generally lowers the activity of these compounds (partial exceptions are here the 4-amino substituted compounds **4c** and **4f**).
c) In the cases of both 4-chloroderivatives **3** and 4-aminoderivatives **4**, the presence of a morpholino substituent in proper position can yield notable activity modifications with respect to the reference compounds devoid of this group: compare the high anti-inflammatory and low analgesic activities of 5-(dimethylamino)derivative **3c** with the high analgesic and null anti-inflammatory activities of 5-morpholinoderivative **3d**, as well as the remarkable anti-inflammatory properties of 4-morpholinoderivative **4b** with the analgesic ones of the corresponding 4-unsubstituted compound **1a** [1].
d) The replacement of the 1,2,4-triazole of compounds **1** with the imidazole fused ring affords compounds **5**, some of which exhibited not only appreciable analgesic and anti-inflammatory, but also, depending on the substitution pattern, notable antipyretic properties which were not shown by the 1,2,4-triazole derivatives **1b**, **3** and **4**.

Actually, on the whole, the 4*H*-imidazo[1,2-*a*][1,5]-benzodiazepine derivatives **5b** and **5f** appear to be the most interesting of all compounds synthesised and tested in this study, combining remarkable analgesic, anti-inflammatory and antipyretic activities at doses devoid of acute adverse effects.

5. Experimental

5.1. Chemistry

M.p.s were determined using a Fisher–Johns apparatus and are uncorrected. IR spectra were recorded on a Perkin–Elmer 398 spectrophotometer. ^{1}H-NMR spectra were recorded on 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 $\pm 0.4\%$ 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).

5.1.1. 5-Morpholino-1-phenyl-4H-[1,2,4]triazolo[4,3-a][1,5]benzodiazepine (**1d**)

To an ice bath cooled solution of 10.9 mmol (1.2 mL) of $TiCl_4$ and 2 mL of anisole in 40 mL of dry $C_6H_5CH_3$, a solution of 5 mL of morpholine in 5 mL of dry $C_6H_5CH_3$ was added, while stirring. 1-Phenyl-4*H*-[1,2,4]triazolo[4,3-*a*][1,5]benzodiazepin-5(6*H*)-one (**6**) (10.0 mmol, 2.76 g) [2] and the solution of 3 mL of morpholine in 10 mL of dry $C_6H_5CH_3$ were then added and the resulting mixture was refluxed with stirring for 6 h.

After cooling, 3 mL of 2-propanol, 2 g of diatomaceous earth and 3 mL of 28% aq. NH_3 were added to the mixture. After stirring, the resulting suspension was filtered, the solid collected was thoroughly washed with CH_2Cl_2, and the filtrate and washings were dried (anhydrous Na_2SO_4), then evaporated to dryness under reduced pressure. By adding a little EtOAc to the oily residue, the nearly pure whitish compound **1d** separated out (2.75 g, 80%); white crystals, m.p. 182–183.5 °C, after crystallisation from the same solvent with charcoal. IR ($CHCl_3$, cm^{-1}): 1610, 1586 strong, 1530 weak, 1476. ^{1}H-NMR ($CDCl_3$): δ 3.16 and 4.42 (AB system, $J = 14$ Hz, 1H + 1H, 4-CH_2), 3.58–3.90 (m, 8H, morpholine CH_2's), 6.81–6.94 and 7.23–7.63 (2m, 2H + 7H, H-7,8,9,10 + phenyl H's). Anal. $C_{20}H_{19}N_5O$ (C, H, N).

*5.1.2. General procedure for N,N-dialkyl-4-chloro-4H-[1,2,4]triazolo[4,3-a][1,5]benzodiazepin-5-amines **3a–d***

A mixture of 5.0 mmol of **1a** (1.28 g) [1], **1b** (1.56 g) [2], **1c** (1.52 g), or **1d** (1.73 g), 5.0 mmol (0.67 g) of N-chlorosuccinimide [6 mmol (0.80 g) in the case of reaction with **1c**] and 50 mL of CCl_4 was refluxed for 30 min (**1a**,**c**), 1 h (**1b**), or 3 h (**1d**), while stirring. After cooling, 100 mL of CH_2Cl_2 and 100 mL of aq. 0.5 N NaOH were added to the mixture which was then vigorously stirred at room temperature (r.t.) for 30 min. The organic phase was collected and the aqueous one was extracted several more times with CH_2Cl_2. The combined extracts were washed with water, then dried (anhydrous Na_2SO_4) and evaporated to dryness in vacuo to give an oily residue.

Compounds **3a**,**b**,**d**: By adding a proper solvent (isopropyl ether for **3a**,**b** and Et_2O for **3d**) to the oils obtained starting from **1a**, **1b** or **1d**, compounds **3a**, **3b** or **3d**, respectively, separated out as white solids, which were crystallised from the suitable solvent.

Compounds **3c** *and* **7**: The oily residue obtained starting from **1c** was subjected to column chromatography (silica gel), eluting first with CH_2Cl_2–EtOAc (1:1) to recover dichloroderivative **7**, then with EtOAc to collect the desired 4-chloroderivative **3c**. Each compound separated out as a white solid by adding a little isopropyl ether to the oil recovered from the respective eluate.

Data for compounds **3a**–**d** are reported in Table 2.

*5.1.2.1. 4,4-Dichloro-N,N-dimethyl-1-phenyl-4H-[1,2,4]triazolo[4,3-a][1,5]benzodiazepin-5-amine (**7**).* White crystals (7.5%); m.p. 179–181 °C (from isopropyl ether). IR ($CHCl_3$, cm^{-1}): 1616, 1596, 1495 weak, 1478. ^{1}H-NMR ($CDCl_3$): δ 3.10 [s, 6H, $N(CH_3)_2$], 6.75–7.10 and 7.28–7.75 (2m, 2H+7H, H-7,8,9,10+phenyl H's). Anal. $C_{18}H_{15}Cl_2N_5$ (C, H, N, Cl).

*5.1.3. General procedure for N,N,N′,N′-tetralkyl-4H-[1,2,4]triazolo[4,3-a][1,5]benzodiazepin-4,5-diamines **4a–g***

A mixture of chloroderivative **3a**, **3b**, or **3c** (8.0 mmol), 7 mL of the suitable dialkylamine, and 7 mL of DMSO (compounds **4c**–**g**) or ethylene glycol (compounds **4a**,**b**) was heated at 120 °C for 1 h (**4d**–**g**) or 2 h (**4c**), or at 160 °C for 1 h (**4a**,**b**), while stirring. After cooling, the mixture was poured into ice-cooled water (200 mL) and the resulting emulsion or suspension was exhaustively extracted with CH_2Cl_2. From combined extracts (washed with water and dried, then evaporated to dryness in vacuo) an oily residue was obtained from which compound **4** was recovered (white solid) as described below for each case.

*5.1.3.1. N,N-Diethyl-4-(1-piperidinyl)-4H-[1,2,4]triazolo[4,3-a][1,5]benzodiazepin-5-amine (**4a**).* The thick oil deriving from reaction of **3a** (2.32 g) with piperidine was subjected to column chromatography (silica gel) eluting with EtOAc: by adding a little Et_2O to the residue obtained from the eluate, 1.58 g of **4a** separated out.

*5.1.3.2. N,N-Diethyl-4-morpholino-4H-[1,2,4]triazolo[4,3-a][1,5]benzodiazepin-5-amine (**4b**).* The oil obtained from reaction of **3a** (2.32 g) with morpholine was chromatographed on a silica gel column eluting with CH_2Cl_2–petroleum ether–Et_3N (5:5:1). The eluate collected, after removal of solvents and treatment of the residue with a little isopropyl ether, afforded 1.77 g of **4b**.

*5.1.3.3. N,N-Diethyl-4-(4-methyl-1-piperazinyl)-4H-[1,2,4]triazolo[4,3-a][1,5]benzodiazepin-5-amine (**4c**).* The oily residue resulting from reaction of **3a** (2.32 g) with 4-methylpiperazine was chromatographed on a silica gel column eluting first with EtOAc–Me_2CO (1:1) to remove some impurities, then with CH_2Cl_2–Et_3N (9:1) to recover **4c**. The thick oil finally obtained from this eluate was treated with a little petroleum ether to give 1.79 g of **4c**.

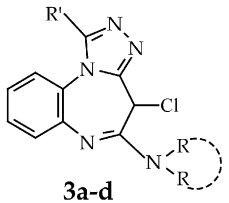
*5.1.3.4. N,N-Dibutyl-4-(1-piperidinyl)-4H-[1,2,4]triazolo[4,3-a][1,5]benzodiazepin-5-amine (**4d**).* The reaction of 2.77 g of **3b** with piperidine gave an oil which was chromatographed on a silica gel column eluting with petroleum ether–EtOAc (1:1); after discarding the first fractions containing a little amount of starting compound **3b**, the eluate subsequently collected, after a treatment identical to that above described for the recovery of **4b**, yielded 1.59 g of **4d**.

*5.1.3.5. N,N-Dibutyl-4-morpholino-4H-[1,2,4]triazolo[4,3-a][1,5]benzodiazepin-5-amine (**4e**).* The residue resulting from the reaction of 2.77 g of **3b** with morpholine was subjected to column chromatography [Al_2O_3, CH_2Cl_2–EtOAc (1:1)]. The eluate collected afforded a thick oil from which, after the same treatment described above for compounds **4b**,**d**, 1.71 g of **4e** separated out.

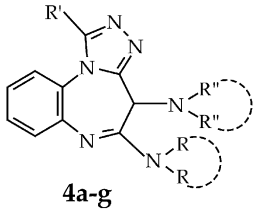
*5.1.3.6. N,N-Dimethyl-1-phenyl-4-(1-pyrrolidinyl)-4H-[1,2,4]triazolo[4,3-a][1,5]benzodiazepin-5-amine (**4f**).* The oil obtained from reaction of 2.70 g of **3c** with pyrrolidine was chromatographed on a silica gel column, eluting first with CH_2Cl_2–EtOAc (1:1) to recover 0.40 g of starting compound **3c**, then with EtOAc to collect **4f**. This eluate, after removal of solvent, afforded sticky oil from which, after treatment with a little Et_2O and standing, 1.21 g of **4f** separated out.

*5.1.3.7. N,N-Dimethyl-1-phenyl-4-(1-piperidinyl)-4H-[1,2,4]triazolo[4,3-a][1,5]benzodiazepin-5-amine (**4g**).*

Table 2
Physical and chemical data of compounds **3a**–**d** and **4a**–**g**



3a-d



4a-g

Compound	Yield (%)	M.p.(°C) (solvent) [a]	Molecular formula [b]	IR [c] (cm^{-1})	^{1}H-NMR [d] (δ, ppm)
3a	90	128–129 (A)	$C_{14}H_{16}ClN_5$	1612, 1588s, 1512 br, w.	1.30 [t, 6H, N(CH_2CH_3)$_2$], 3.56 [q, 4H, N(CH_2CH_3)$_2$], 6.40 (s, 1H, H-4), 7.09–7.50 (m, 4H, H-7,8,9,10), 8.64 (s, 1H, H-1).
3b	82	120–122 (A)	$C_{18}H_{24}ClN_5$	1610, 1585s, 1510 br, w.	0.97 [t, 6H, N($CH_2CH_2CH_2CH_3$)$_2$], 1.38 [m, 4H, N($CH_2CH_2CH_2CH_3$)$_2$], 1.50–1.90 [m, 4H, N($CH_2CH_2CH_2CH_3$)$_2$], 3.35–3.64 [m, 4H, N($CH_2CH_2CH_2CH_3$)$_2$], 6.39 (s, 1H, H-4), 7.08–7.50 (m, 4H, H-7,8,9,10), 8.63 (s, 1H, H-1).
3c	71	205–206 (B)	$C_{18}H_{16}ClN_5$	1613, 1588s, 1510w, 1481.	3.23 [s, 6H, N(CH_3)$_2$], 6.48 (s, 1H, H-4), 6.70–6.88 and 7.20–7.60 (2m, 2H + 7H, H-7,8,9,10 + phenyl H's).
3d	72	224–224.5 (C)	$C_{20}H_{18}ClN_5O$	1615, 1590, 1512w, 1480.	3.50–3.94 (m, 8H, morpholine CH_2's), 6.35 (s, 1H, H-4), 6.80–6.98 and 7.23–7.63 (2m, 2H + 7H, H-7,8,9,10 + phenyl H's).
4a	59	193–194 (D)	$C_{19}H_{26}N_6$	1608, 1578s, 1521w, 1500w.	0.90–1.50 [m, 12H, N(CH_2CH_3)$_2$ + piperidine β-CH_2's + γ-CH_2], 1.83–2.09 and 2.17–2.40 (2m, 2H + 2H, piperidine α-CH_2's), 3.19–3.73 [m, 4H, N(CH_2CH_3)$_2$], 5.04 (s, 1H, H-4), 6.83–7.33 (m, 4H, H-7,8,9,10), 8.55 (s, 1H, H-1).
4b	65	184–185 (A)	$C_{18}H_{24}N_6O$	1610, 1581s, 1523w, 1496w.	1.04–1.48 [m, 6H, N(CH_2CH_3)$_2$], 2.00–2.19 and 2.32–2.51 (2m, 2H + 2H, morpholine NCH_2's), 3.16 (near t, 4H, morpholine OCH_2's), 3.25–3.75 [m, 4H, N(CH_2CH_3)$_2$], 5.11 (s, 1H, H-4), 6.95–7.40 (m, 4H, H-7,8,9,10), 8.57 (s, 1H, H-1).
4c	63	129–130 (E)	$C_{19}H_{27}N_7$	1612, 1582s, 1525, 1498w.	1.07–1.42 [m, 6H, N(CH_2CH_3)$_2$], 1.70–2.19 [m, 6H, piperazine $CH_3N(CH_2)_2$ + 2H of piperazine NCH_2's], 2.05 (s, 3H, NCH_3), 2.32–2.52 (m, 2H, 2H of piperazine NCH_2's), 3.27–3.70 [m, 4H, N(CH_2CH_3)$_2$], 5.09 (s, 1H, H-4), 6.94–7.35 (m, 4H, H-7,8,9,10), 8.56 (s, 1H, H-1).
4d	51	113.5–114 (F)	$C_{23}H_{34}N_6$	1610, 1582s, 1522w, 1495w.	0.80–1.88 [m, 20H, N($CH_2CH_2CH_2CH_3$)$_2$ + piperidine β-CH_2's + γ-CH_2], 1.90–2.12 and 2.22–2.43 (2m, 2H + 2H, piperidine α-CH_2's), 3.10–3.68 [m, 4H, N($CH_2CH_2CH_2CH_3$)$_2$], 5.03 (s, 1H, H-4), 6.85–7.33 (m, 4H, H-7,8,9,10), 8,54 (s, 1H, H-1).
4e	54	142–143 (A)	$C_{22}H_{32}N_6O$	1610, 1583s, 1523w, 1498w.	0.74–1.11 [m, 6H, N($CH_2CH_2CH_2CH_3$)$_2$], 1.16–1.92 [m, 8H, N($CH_2CH_2CH_2CH_3$)$_2$], 1.97–2.20 and 2.30–2.52 (2m, 2H + 2H, morpholine NCH_2's), 2.95–3.70 [m, 4H, N($CH_2CH_2CH_2CH_3$)$_2$], 3.17 (m, 4H, morpholine OCH_2's), 5.09 (s, 1H, H-4), 6.93–7.42 (m, 4H, H-7,8,9,10), 8.57 (s, 1H, H-1).
4f	41	187–188 (A)	$C_{22}H_{24}N_6$	1611, 1587s, 1521br,w, 1480.	1.32–1.51 (m, 4H, pyrrolidine β-CH_2's), 2.08–2.17 and 2.33–2.52 (2m, 2H + 2H, pyrrolidine α-CH_2's), 3.21 [s, 6H, N(CH_3)$_2$], 5.23 (s, 1H, H-4), 6.62–6.76 and 7.12–7.64 (2m, 2H + 7H, H-7,8,9,10 + phenyl H's).
4g	40	192.5–193.5 (A)	$C_{23}H_{26}N_6$	1614, 1590s, 1520br,w, 1480.	0.89–1.28 (m, 6H, piperidine β-CH_2's + γ-CH_2), 2.00–2.17 and 2.23–2.40 (2m, 2H + 2H, piperidine α-CH_2's), 3.20 [s, 6H, N(CH_3)$_2$], 5.14 (s, 1H, H-4), 6.65–6.76 and 7.12–7.63 (2m, 2H + 7H, H-7,8,9,10 + phenyl H's).

[a] Crystallization solvent: A = isopropyl ether, B = ethyl acetate/petroleum ether, C = ethyl acetate, D = ethyl acetate/isopropyl ether, E = ethyl ether/petroleum ether, F = petroleum ether.
[b] Anal. C, H, N (C, H, N, Cl for **3a**–**d**).
[c] In $CHCl_3$ solutions. Abbreviations: s = strong, br = broad, w = weak.
[d] In $CDCl_3$ solutions.

By proceeding exactly as above described for the preparation of **4f**, from the reaction of **3c** (2.70 g) with piperidine, 0.70 g of starting compound **3c** and 1.24 g of the desired **4g** were finally recovered.

Data for compounds **4a**–**g** are reported in Table 2.

5.1.4. 4-(Dibutylamino)-1,3-dihydro-2H-1,5-benzodiazepin-2-one (*8b*) and 2-(2-benzimidazolyl)-N,N-dibutylacetamide (*9*)

Phosphorus oxychloride (0.10 mol, 15.33 g) was added dropwise with stirring to an ice-cooled suspension of 0.10 mol (10.81 g) of 1,2-phenylenediamine in 0.20 mol (48.67 g) of ethyl *N*,*N*-dibutylmalonamate [10], and the resulting mixture was then heated at 110 °C for 4 h, while stirring. Water (400 mL) was then added to the dark viscous slurry obtained and the mixture was vigorously stirred at 100 °C until a fluid emulsion was obtained. After cooling, 200 mL of Et_2O–EtOAc (1:1) were added and the mixture was further stirred at r.t. for 30 min. After discarding some insoluble tars, the acidic aqueous layer was collected and the organic phase was exhaustively extracted with 2 N aq. HCl. The combined aqueous phases were decolourised with charcoal, then made alkaline with 28% aq. NH_3, and the resulting emulsion was thoroughly extracted with CH_2Cl_2. The combined extracts were dried, then evaporated to dryness in vacuo to afford a thick oil, which was dissolved in a little 2-propanol and treated with a saturated HCl Et_2O solution. This way a whitish solid separated out which was filtered, washed with anhydrous Et_2O, then treated with 100 mL of 10% aq. Na_2CO_3. The resulting emulsion was extracted several times with CH_2Cl_2, then the solvent was removed from the combined extracts (dried over anhydrous Na_2SO_4) to give an oil (mixture of **8b** and **9**), which was subjected to column chromatography (silica gel), eluting first with $C_6H_5CH_3$–Me_2CO (6:1).

The eluate, after removal of solvents in vacuo, gave a thick oil which was treated with a little petroleum ether to yield 4.55 g (16%) of pure **8b**; white crystals, m.p. 109.5–110 °C after crystallisation from the same solvent. IR ($CHCl_3$, cm^{-1}): 3395 (NH), 1674 (CO), 1609, 1573. ^{1}H-NMR ($CDCl_3$): δ 0.95 [t, 6H, $N(CH_2CH_2CH_2CH_3)_2$], 1.35 [m, 4H, $N(CH_2CH_2CH_2CH_3)_2$], 1.62 [m, 4H, $N(CH_2CH_2CH_2CH_3)_2$], 3.21 (s, 2H, 3-CH_2), 3.47 [t, 4H, $N(CH_2CH_2CH_2CH_3)_2$], 6.90–7.21 (m, 4H, H-6,7,8,9), 8.30 (s, 1H, NH; disappeared with D_2O). Anal. $C_{17}H_{25}N_3O$ (C, H, N).

From the eluate subsequently collected (EtOAc), a thick oil was finally obtained from which, after addition of a little isopropyl ether and standing, 2.75 g (10%) of pure compound **9** separated out; white crystals, m.p. 84–85 °C after crystallisation from the same solvent. IR ($CHCl_3$, cm^{-1}): 3380 broad (NH), 1630 broad, strong (CO), 1524. ^{1}H-NMR ($CDCl_3$): δ 0.90 and 0.93 [2t, 6H, $N(CH_2CH_2CH_2CH_3)_2$], 1.31 [m, 4H, $N(CH_2CH_2CH_2CH_3)_2$], 1.40–1.66 [m, 4H, $N(CH_2CH_2CH_2CH_3)_2$], 3.35 [m, 4H, $N(CH_2CH_2CH_2CH_3)_2$], 4.05 (s, 2H, CH_2CO), 7.15–7.26 and 7.48–7.62 (2m, 4H, H-4,5,6,7). Anal. $C_{17}H_{25}N_3O$ (C, H, N).

5.1.5. 4-(Dibutylamino)-1,3-dihydro-2H-1,5-benzodiazepine-2-thione (*10*)

A mixture of 10.0 mmol (2.87 g) of **8b**, 5.5 mmol (2.22 g) of Lawesson's reagent and 20 mL of dry $C_6H_5CH_3$ was stirred at reflux for 30 min. The solvent was then removed in vacuo and the residue taken up in CH_2Cl_2 and subjected to column chromatography [Al_2O_3, CH_2Cl_2–EtOAc (1:1)]. The eluate collected afforded a thick oil from which, after a treatment with a little petroleum ether, pure compound **10** separated out (2.79 g, 92%); ivory–white crystals, m.p. 114.5–115 °C, after crystallisation from the same solvent. IR ($CHCl_3$, cm^{-1}): 3360 (NH), 1609, 1570 strong, 1510 weak. ^{1}H-NMR ($CDCl_3$): δ 0.95 [t, 6H, $N(CH_2CH_2CH_2CH_3)_2$], 1.36 [m, 4H, $N(CH_2CH_2CH_2CH_3)_2$], 1.50–1.75 [m, 4H, $N(CH_2CH_2CH_2CH_3)_2$], 3.57 [m, 6H, $N(CH_2CH_2CH_2CH_3)_2$+3-CH_2], 6.90–7.22 (m, 4H, H-6,7,8,9), 10.06 (s, 1H, NH; disappeared with D_2O). Anal. $C_{17}H_{25}N_3S$ (C, H, N, S).

5.1.6. N,N-Dibutyl-4-(methylthio)-3H-1,5-benzodiazepin-2-amine (*11b*)

A mixture of 5.0 mmol (1.51 g) of **10**, 0.70 g of anhydrous K_2CO_3, 1.0 mL of MeI and 40 mL of dry Me_2CO was refluxed for 2 h, while stirring. The solvent was then removed, the residue partitioned between water and CH_2Cl_2, then the aqueous phase extracted several more times with the same solvent. The oil obtained from the combined organic phases (after drying and removal of solvent) was chromatographed on a silica gel column eluting with CH_2Cl_2. The eluate collected was evaporated to dryness under reduced pressure to give a thick oil from which, after addition of a little MeOH and standing at 4 °C, pure compound **11b** crystallised as a white solid (1.45 g, 91%); m.p. 65–67 °C after recrystallisation from the same solvent. IR ($CHCl_3$, cm^{-1}): 1590, 1560. ^{1}H-NMR ($CDCl_3$): δ 0.94 [t, 6H, $N(CH_2CH_2CH_2CH_3)_2$], 1.34 [m, 4H, $N(CH_2CH_2CH_2CH_3)_2$], 1.50–1.75 [m, 4H, $N(CH_2CH_2CH_2CH_3)_2$], 2.50 (s, 3H, SCH_3), 3.09 (s, 2H, 3-CH_2), 3.46 [t, 4H, $N(CH_2CH_2CH_2CH_3)_2$], 6.92–7.34 (m, 4H, H-6,7,8,9). Anal. $C_{18}H_{27}N_3S$ (C, H, N, S).

5.1.7. N,N-Dialkyl-4H-imidazo[1,2-a][1,5]benzodiazepin-5-amines *5a,b*

A mixture of 8.0 mmol of **11a** (2.09 g) [6] or **11b** (2.54 g), 24.0 mmol (2.52 g) of aminoacetaldheyde dimethyl acetal and 10 mL of ethylene glycol was stirred at 160 °C for 1 h. After cooling, the resulting solution was poured into water (300 mL) and the mixture was exhaustively extracted with CH_2Cl_2. The combined

extracts were dried and solvent was removed to give an oil which was dissolved in 10 mL of 99% HCOOH: this solution was then refluxed (100 °C) for 1.5 h, while stirring. The final reaction mixture was poured onto ice-water and the resulting solution was carefully treated with Na_2CO_3 until alkaline and extracted several times with CH_2Cl_2. From the combined extracts, after drying and removal of solvent, an oily residue was obtained which was chromatographed on an Al_2O_3 column [CH_2Cl_2–EtOAc (9:1)]. The eluate collected was evaporated to dryness under vacuo to give a thick oil from which compound **5a** or **5b** was recovered as reported below.

*5.1.7.1. N,N-Diethyl substituted derivative **5a**.* By adding a little petroleum ether to the oil obtained from **11a**, pure compound **5a** (1.04 g, 51%) separated out as a white solid, m.p. 106 °C after crystallisation from isopropyl ether. IR ($CHCl_3$, cm^{-1}): 1608, 1573 strong, 1525 weak, 1500. ^{1}H-NMR ($CDCl_3$): δ 1.25 [t, 6H, $N(CH_2CH_3)_2$], 3.34–3.83 [q+broad s, 6H, $N(CH_2CH_3)_2$+4-CH_2], 6.97–7.13 and 7.21–7.43 (2m, 2H+4H, H-1,2,7,8,9,10). Anal. $C_{15}H_{18}N_4$ (C, H, N).

*5.1.7.2. N,N-Dibutyl substituted derivative **5b**.* The oil deriving from the reaction carried out with **11b** was treated with a solution of 16.0 mmol (1.86 g) of maleic acid in 10 mL of anhydrous EtOH. The resulting solution was stirred for few minutes at r.t., then diluted with anhydrous Et_2O and petroleum ether until it became cloudy. After standing, the **5b** dimaleate (**5b**·$2H_4C_4O_4$) separated out as a white solid which was recovered by filtration and dried (2.23 g, 51%), then recristallysed from anhydrous EtOH/Et_2O to give white crystals, m.p. 114–115 °C. Anal. $C_{27}H_{34}N_4O_8$ (C, H, N).

An analytical sample of this salt was treated with 10% aq. Na_2CO_3, the mixture was extracted with CH_2Cl_2 and the extract was dried then evaporated in vacuo. A thick colourless oil was obtained which was thoroughly dried in vacuo to afford the pure free base **5b**. IR ($CHCl_3$, cm^{-1}): 1610, 1575 strong, 1525 weak, 1501. ^{1}H-NMR ($CDCl_3$): δ 0.94 [t, 6H, $N(CH_2CH_2CH_2CH_3)_2$], 1.14–1.48 [m, 4H, $N(CH_2CH_2CH_2CH_3)_2$], 1.50–1.78 [m, 4H, $N(CH_2CH_2CH_2CH_3)_2$], 3.30–3.76 [t+broad s, 6H, $N(CH_2\ CH_2CH_2CH_3)_2$+4-CH_2], 6.98–7.15 and 7.20–7.44 (2m, 2H+4H, H-1,2,7,8,9,10).

*5.1.8. N,N-Dialkyl-1-methyl-4H-imidazo[1,2-a][1,5]benzodiazepin-5-amines **5e,f***

A mixture of 8.0 mmol of **11a** (2.09 g) [6] or **11b** (2.54 g), 16.0 mmol (0.88 g) of propargylamine, 0.15 g of monohydrate *p*-toluenesulphonic acid and 10 mL of Dowtherm A was stirred at 180 °C for 1 h. After cooling, Et_2O (100 mL) and 2 N aq. HCl (100 mL) were added to the mixture. After stirring, the aqueous phase was collected and the organic one was further extracted twice with 2 N aq. HCl. The combined aqueous phases were treated with 28% aq. NH_3 until alkaline and the mixture was exhaustively extracted with CH_2Cl_2. The combined extracts were dried and evaporated to dryness under reduced pressure to afford an oily residue, which was chromatographed on Al_2O_3 column, eluting with CH_2Cl_2–EtOAc (9:1). The eluate collected, after removal of solvents, gave a thick oil from which compound **5e** or **5f** was recovered as reported below.

*5.1.8.1. N,N-Diethyl substituted derivative **5e**.* By adding a little isopropyl ether to the oil obtained from **11a**, pure compound **5e** (1.14 g, 53%) separated out as a white solid, m.p. 129–130 °C after crystallisation from the same solvent. IR ($CHCl_3$, cm^{-1}): 1608, 1574 strong, 1514. ^{1}H-NMR ($CDCl_3$): δ 1.24 [t, 6H, $N(CH_2CH_3)_2$], 2.32 (s, 3H, 1-CH_3), 2.95 and 4.11 (AB system, $J=14$ Hz, 1H+1H, 4-CH_2), 3.53 [near q, 4H, $N(CH_2CH_3)_2$], 6.84 (s, 1H, H-2), 6.98–7.10 and 7.22–7.30 (2m, 1H+3H, H-7,8,9,10). Anal. $C_{16}H_{20}N_4$ (C, H, N).

*5.1.8.2. N,N-Dibutyl substituted derivative **5f**.* By using exactly the same procedure that was employed to obtain **5b** dimaleate and the free base **5b** (see above), **5f** dimaleate (**5f**·$2H_4C_4O_4$) (2.47 g, 56%) and the free base **5f** were obtained from the oil afforded by the reaction of **11b**.

5f *Dimaleate:* White solid, m.p. 124 °C after crystallisation from anhydrous EtOH/Et_2O. Anal. $C_{28}H_{36}N_4O_8$ (C, H, N).

Compound **5f**: Colourless thick oil. IR ($CHCl_3$, cm^{-1}): 1609, 1572 strong, 1515. ^{1}H-NMR ($CDCl_3$): δ 0.94 [t, 6H, $N(CH_2CH_2CH_2CH_3)_2$], 1.18–1.82 [m, 8H, $N(CH_2CH_2CH_2CH_3)_2$], 2.31 (s, 3H, 1-CH_3), 2.94 and 4.12 (AB system, $J=14$ Hz, 1H+1H, 4-CH_2), 3.18–3.69 [m, 4H, $N(CH_2CH_2CH_2CH_3)_2$], 6.84 (s, 1H, H-2), 6.98–7.12 and 7.20–7.35 (2m, 1H+3H, H-7,8,9,10).

*5.1.9. 4-[(2,2-Dimethoxyethyl)amino]-1,3-dihydro-2H-1,5-benzodiazepin-2-one (**8c**) and 4H-imidazo[1,2-a][1,5]benzodiazepin-5(6H)-one (**12**)*

A mixture of 10.0 mmol (2.03 g) of **8a** [7], 30.0 mmol (3.15 g) of aminoacetaldheyde dimethyl acetal, 0.30 g of monohydrate *p*-toluenesulphonic acid and 10 mL of Dowtherm A was heated at 180 °C for 3 h, while stirring. After cooling, the reaction mixture was dissolved in CH_2Cl_2 (200 mL) and this solution was washed with 5% aq. $NaHCO_3$, then with water and dried over anhydrous Na_2SO_4. After removal of solvent in vacuo, the liquid residue obtained was chromatographed on a silica gel column, eluting first with CH_2Cl_2 to remove Dowtherm A, then with EtOAc to recover **8c**. The eluate collected afforded a solid residue, which was taken up in a little Et_2O and filtered. There was so obtained pure **8c** (1.07 g, 41%); white crystals, m.p. 199.5–200 °C after

crystallisation from EtOAc with charcoal. IR (KBr, cm^{-1}): 3350 (NH), 3120 broad (*NH*CO), 1676 (CO), 1615, 1585, 1562, 1534. ^{1}H-NMR ($CDCl_3$): δ 3.08 (s, 2H, 3-CH_2), 3.42 (s, 6H, OCH_3's), 3.58 (t, 2H, NH*CH*$_2$; d after treatment with D_2O), 4.57 [t, 1H, $CH_2CH(OCH_3)_2$], 5.26 (near t, 1H, *NH*CH_2; disappeared with D_2O), 6.94–7.29 (m, 4H, H-6,7,8,9), 8.25 (s, 1H, 1-NH; disappeared with D_2O). Anal. $C_{13}H_{17}N_3O_3$ (C, H, N).

The column was further eluted with Me_2CO and the fraction collected, after removal of solvent, afforded compound **12** as a pale brown solid (0.15 g, 7.5%); whitish crystals, m.p. 282–284 °C (dec.), after crystallisation from EtOAc with charcoal. IR (KBr, cm^{-1}): 3190 broad (NH), 1685 strong (CO), 1600 weak, 1532 weak, 1510. ^{1}H-NMR (DMSO-d_6): δ 3.63 (s, 2H, 4-CH_2), 7.09 (d, $J = 1.4$ Hz, 1H, H-2), 7.28–7.50 and 7.62–7.69 (2m, 3H+1H, H-7,8,9,10), 7.71 (d, $J = 1.4$ Hz, 1H, H-1), 10.36 (s, 1H, NH; disappeared with D_2O). Anal. $C_{11}H_9N_3O$ (C, H, N).

5.1.10. Cyclocondensation of *8c* to *12*

HCOOH (99%, 10 mL) was added to 4.0 mmol (1.05 g) of **8c** and the resulting solution was refluxed for 1.5 h, while stirring. The reaction mixture was then poured onto ice-water and the solution obtained was carefully treated with Na_2CO_3 until alkaline. The resulting suspension was stirred for 30 min at r.t., and then the solid was recovered by filtration, washed with water and dried. There was so obtained 0.54 g of nearly pure compound **12**. By further extraction with CH_2Cl_2 of the aqueous filtrate and washings, an additional crop (0.11 g) of **12** was recovered (total yield 0.65 g, 82%).

5.1.11. N,N-Dialkyl-4H-imidazo[1,2-a][1,5]benzodiazepin-5-amines *5c,d*

A solution of 5 mL of the proper amine in 5 mL of dry $C_6H_5CH_3$ was added to an ice bath cooled solution of 1.2 mL (10.9 mmol) of $TiCl_4$ and 2 mL of anisole in 40 mL of dry $C_6H_5CH_3$, while stirring, then 10.0 mmol (1.99 g) of compound **12** and the solution of 3 mL of the amine in 10 mL of dry $C_6H_5CH_3$ were further added and the resulting mixture was stirred at reflux for 6 h.

After cooling, 3 mL of 2-propanol, 2 g of diatomaceous earth and 3 mL of 28% aq. NH_3 were added to the mixture. After stirring, the resulting suspension was filtered, the solid collected was thoroughly washed with CH_2Cl_2, and the filtrate and washings were dried (anhydrous Na_2SO_4), then evaporated to dryness under reduced pressure. The oily residue was chromatographed on a silica gel column eluting with EtOAc (compound **5c**) or EtOAc–Me_2CO (1:1) (compound **5d**); the eluate collected, after removal of solvents, gave a residue from which compound **5c** or **5d** was recovered as reported below.

5.1.11.1. 5-(1-Piperidinyl)-4H-imidazo[1,2-a][1,5]benzodiazepine (*5c*).

The oily residue obtained from the reaction carried out with piperidine was treated with a little isopropyl ether to give pure compound **5c** (1.64 g, 62%), white crystalline solid, m.p. 131–132 °C after recrystallisation from the same solvent. IR ($CHCl_3$, cm^{-1}): 1605, 1568 strong, 1530 shoulder, 1500 weak. ^{1}H-NMR ($CDCl_3$): δ 1.62 (m, 6H, piperidine β-CH_2's+γ-CH_2), 3.55–3.80 (m+broad s, 6H, piperidine α-CH_2's+4-CH_2), 7.02–7.14 and 7.23–7.44 (2m, 2H+4H, H-1,2,7,8,9,10). Anal. $C_{16}H_{18}N_4$ (C, H, N).

5.1.11.2. 5-Morpholino-4H-imidazo[1,2-a][1,5]benzodiazepine (*5d*).

The nearly solid residue deriving from the reaction carried out with morpholine was taken up in a little Et_2O and filtered. There was so obtained 1.74 g (65%) of pure **5d**; white crystals, m.p. 154–155 °C after crystallisation from EtOAc with charcoal. IR ($CHCl_3$, cm^{-1}): 1608, 1580 strong, 1525 weak, 1498. ^{1}H-NMR ($CDCl_3$): δ 3.40–4.05 (m+broad s, 10H, morpholine CH_2's+4-CH_2), 7.04–7.17 and 7.21–7.48 (2m, 2H+4H, H-1,2,7,8,9,10). Anal. $C_{15}H_{16}N_4O$ (C, H, N).

5.2. Pharmacology

Male Wistar rats (250–300 g) and male Swiss mice (25–35 g) were used. The test compounds were suspended in 0.5% methylcellulose and administered by oral gavage in groups of eight animals fasted overnight.

Indomethacin (10 mg kg^{-1} os) was used as reference drug in all the experimental tests while control animals received an equivalent volume of vehicle alone.

The ethical guidelines for investigation of experimental pain in conscious animals were followed and all of the tests were carried out according to the EEC ethical regulation (EEC Council 86/609; D.L.27/01/1992, No. 116).

5.2.1. Anti-inflammatory activity

Anti-inflammatory activity was studied by inducing paw edema according to Winter's method [11]. Carrageenan 1% (0.1 mL) was injected into the plantar surface of the rat hind paw simultaneously with the oral administration of the test compounds. Paw volume was determined immediately after the injection of phlogogen agent and again 3 h later by means of a plethysmometer.

5.2.2. Analgesic activity

Antinociceptive activity was studied by writhing test [12] through the intraperitoneal injection of 0.2 mL/mouse of AcOH solution (0.6%) 1 h after oral treatment with the test drugs. Complete extension of either hind limb was regarded as a writhing response. The number

of writhings was recorded for 5 min between 10 and 15 min after the injection of the noxious agent.

5.2.3. Antipyretic activity

Antipyretic activity was determined in rats in which fever was induced by intraperitoneal injection of 100 μg kg^{-1} *Escherichia coli* lipopolysaccharides (LPS) (Serotype 0111:B4 Sigma, Milan, Italy) according to Romanovsky et al. [13]. The test compounds were administered orally 1 h before the injection and rectal temperature was recorded in an air-conditioned room (23 °C) immediately before and 5 h after pyretogen injection.

5.2.4. Ulcerogenicity

The acute gastric mucosal damage of the test compounds was evaluated by examining the stomachs excised 5 h after oral administration of the drugs in mice. The stomachs, fixed in 2% formalin, were opened and examined with a stereomicroscope by an observer unaware of the treatment the mice received. The number and the length of the gastric lesions are measured by means of an image analyser.

5.2.5. Acute toxicity

After oral administration of the compounds under study in groups of eight mice, the frequency of lethal events was taken in a 24 h period of observation.

5.2.6. Data analysis

Results were calculated as mean ± S.E.M. Differences between treated and control groups were determined by Student's *t*-test. $*P < 0.05$ was reported when such differences were statistically significant and $**P < 0.01$ was reported when the differences were highly significant. Then, the pharmacological activities of the compounds were expressed as the percentages of inhibition calculated from the aforementioned differences in the response between the treated and the control groups.

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