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1,5-Benzodiazepines IX. A new route to substituted 4*H*-[1,2,4]triazolo[4,3-*a*][1,5]benzodiazepin-5-amines with analgesic and/or anti-inflammatory activities

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Summary — A new two-step synthetic pathway to substituted 4*H*-[1,2,4]triazolo[4,3-*a*][1,5]benzodiazepin-5-amines **2a–p** is described. The cyclocondensation of (dimethylamino)benzodiazepinone **1a** with hydrazides afforded triazolobenzodiazepinones **5** which in turn reacted with suitable primary or secondary amines, in the presence of titanium tetrachloride, to give the desired 5-amino-derivatives **2a–p**. When compounds **5** were treated with the Lawesson's reagent thiolactams **6** were obtained, which then reacted with sodium hydride and proper alkyl halides to yield 5-(alkylthio)derivatives **7a–d**. Compounds **2a–h, j–p**, and **7a–d** were tested for their analgesic and anti-inflammatory activities, as well as for their acute toxicity and gross behavioral effects. The analgesic activity appeared noteworthy in the writhing test, where fifteen compounds were more effective than both the reference drugs acetylsalicylic acid and dipyrone, but was less evident in the hot plate test. An anti-inflammatory activity, lower than that of indomethacin but reaching the level of statistical significance, was displayed in the carrageenin-induced edema assay by five of the nineteen test compounds.

Résumé — Benzo-1,5-diazépines IX. Une nouvelle méthode de synthèse de 4*H*-[1,2,4]triazolo[4,3-*a*]benzo-1,5-diazépineamines-5 substituées à activités analgésique et/ou anti-inflammatoire. *On décrit une nouvelle méthode de synthèse en deux étapes des 4H-[1,2,4]triazolo[4,3-a]benzo-1,5-diazépine 5-amines substituées **2a–p**. La cyclocondensation du (diméthylamino)benzodiazépinone **1a** avec des hydrazides a donné les triazolobenzodiazépinones **5**, qui à leur tour, en réagissant avec des amines primaires ou secondaires convenables en présence de tétrachlorure de titane, ont conduit aux 5-aminodérivés correspondants **2a–p**. Les composés **5** traités avec le réactif de Lawesson ont produit les thiolactames **6**, qui avec des halogénures d'alkyle et de l'hydrure de sodium ont permis de préparer les 5-(alkylthio)dérivés **7a–d**. Les composés **2a–h, j–p** et **7a–d** ont été testés pour l'évaluation des activités analgésique et anti-inflammatoire ainsi que pour leur toxicité aiguë et leurs effets comportementaux. On a constaté que l'activité analgésique évaluée avec le 'writhing test' est supérieure à celle de l'acide acétylsalicylique et du dipyrone pour quinze composés, ce qui est moins évident avec le test de la plaque chaude. L'activité anti-inflammatoire de cinq dérivés est statistiquement significative dans le test à la carragéenine, mais elle est inférieure en tout cas à celle de l'indométhacine.*

substituted 4*H*-[1,2,4]triazolo[4,3-*a*][1,5]benzodiazepin-5-amines / toxicity / analgesic activity / anti-inflammatory activity

Starting from the 1,5-benzodiazepine derivatives **1** [1, 2], we have recently prepared the 4*H*-[1,2,4]triazolo[4,3-*a*][1,5]benzodiazepine derivatives **2** through a three-step synthetic pathway [2]. Compounds **2** were obtained together with lower amounts of tetracyclic derivatives **3**. No example of [1,2,4]triazolo[4,3-*a*][1,5]benzodiazepines amino substituted on the diazepine ring was previously reported in the literature.

Some compounds **2** showed statistically significant analgesic or anti-inflammatory properties, depending on the structure [2]. Also some of the analogous triazolobenzodiazepines **4** were previously reported to exhibit appreciable analgesic and anti-inflammatory activities [3].

The above mentioned results prompted us to continue our chemical and pharmacological study of compounds of structure **2**. Thus, in the present paper we describe the pharmacological properties of several novel compounds **2** (R = H: **2a–p**) which have been prepared through a new more efficient two-step synthetic route, starting from 4-(dimethylamino)-1,3-dihydro-2H-1,5-benzodiazepin-2-one **1a** [**1**: R = H, $N<^{R'}_{R''}$) = $N(CH_3)_2$] [1] (scheme 1).

In this connection we considered it interesting to prepare also some analogous (alkylthio)derivatives **7** (scheme 1), in order to evaluate their pharmacological behaviour. Also compounds **7** represent a new group of 4*H*-[1,2,4]triazolo[4,3-*a*][1,5]benzodiazepine derivatives.

Chemistry

The cyclocondensation of the starting compound **1a** with excess hydrazides, in the presence of glacial acetic acid (Dowtherm A, 200°C, 2 h) afforded the 4*H*-[1,2,4]triazolo[4,3-*a*][1,5]benzodiazepin-5(6*H*)-ones **5**, which were the intermediates in the synthesis both of the amino derivatives **2a–p** and of the (alkylthio)derivatives **7a–d**.

Actually, from the reaction of compounds **5** with excess amines, in the presence of titanium tetrachloride and anisole (refluxing anhydrous toluene, 6 h) [4] the desired substituted 4*H*-[1,2,4]triazolo[4,3-*a*][1,5]-benzodiazepin-5-amines **2a–p** were obtained (scheme 1).

Treatment of compounds **5** with the Lawesson's reagent (Dowtherm A, 160°C, 30 min) afforded the corresponding triazolobenzodiazepinethiones **6**, from which, by reaction with sodium hydride (45 min) and alkyl halides (1 h) in refluxing anhydrous tetrahydrofuran, 5-(alkylthio)-4*H*-[1,2,4]triazolo[4,3-*a*][1,5]benzodiazepines **7a–d** were obtained (scheme 1).

The results of elemental analyses and the IR and ^1H NMR spectral data confirm the structures attributed to the compounds described in this paper (see *Experimental protocols* and table II).

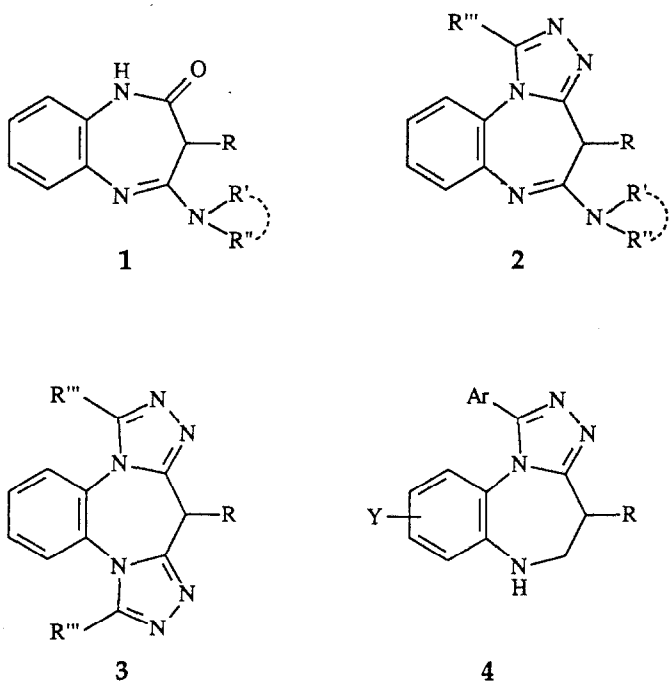
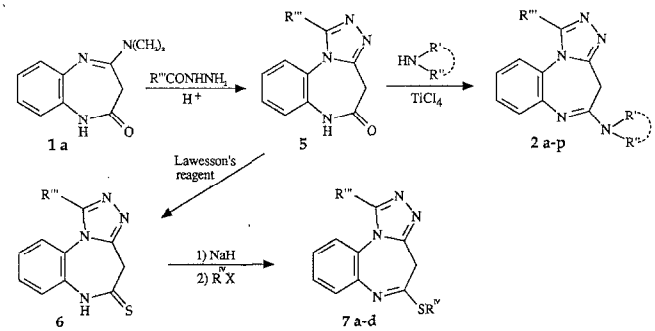


Fig 1.



Compound	R'''	$N\begin{smallmatrix}R'\\R''\end{smallmatrix}$	R^{IV}
5a	H	—	—
5b	CH_3	—	—
5c	C_6H_5	—	—
2a	H	$N(C_3H_7)_2$	—
2b	H	$N(C_4H_9)_2$	—
2c	H	$N(CH_2\text{-}CH{=}CH_2)_2$	—
2d	H		—
2e	H		—
2f	H		—
2g	H		—
2h	H		—
2i	CH_3		—
2j	CH_3		—
2k	CH_3		—
2l	C_6H_5	$NHCH_3$	—
2m	C_6H_5	NHC_2H_5	—
2n	C_6H_5		—
2o	C_6H_5		—
2p	C_6H_5		—
6a	H	—	—
6b	C_6H_5	—	—
7a	H	—	CH_3
7b	H	—	C_3H_7
7c	H	—	$CH_2COOC_2H_5$
7d	C_6H_5	—	CH_3

Scheme 1.

Thus, IR and ^1H NMR spectra of 4*H*-[1,2,4]triazolo[4,3-*a*][1,5]benzodiazepin-5-amines **2a–p** agree with those of the first group of compounds **2** recently described by us [2].

It can be noted that the ^1H NMR 4-CH_2 signals of triazolobenzodiazepine derivatives **2**, **5**, **6**, **7** appear as AB quartets or as sharp singlets: the AB quartet evidently originates from the presence of one only of the two boat conformations of the benzodiazepine ring, no interconversion occurring at the registration temperature [5]. Furthermore, as we already observed for the first group of compounds **2** [2], the ^1H NMR 4-CH_2 signal of amines **2a–e**, **g–p** and also of the analogous (alkylthio)derivatives **7a–d** is an AB quartet or a singlet according to whether the C-1 of the compound is substituted or not, most likely due to steric hindrance of the 1-substituent [2]; this signal is absent in the spectrum [$(CD_3)_2SO$ + CF_3 COOD] of **2f**, because of replacement of the methylene protons with deuterium.

The ^1H NMR spectrum of compound **6a** was not run due to the insufficient solubility of this compound in all the normally used solvents.

Pharmacological results

Compounds **2a–h**, **j–p** and **7a–d** were screened for their analgesic and anti-inflammatory activities, as well as for their gross behavioral effects and their acute toxicity. All the drugs were administered orally; the dose, except for the Irwin-Morpurgo's test, was equal to 1/4 po LD_{50} in mice. The results of the pharmacological evaluation are given in table I.

Seventeen of the nineteen triazolobenzodiazepines produced a statistically significant antinociceptive effect in the acetic acid writhing test in the mouse; the degree of protection ranged from 55 to 99%, and for most of them was greater than that afforded by equitoxic doses of acetylsalicylic acid and dipyrone; only compounds **7a**, **c** were inactive. However, when the hot plate test was used to verify the analgesic activity of the nine triazolobenzodiazepines that produced a greater than 90% protection in the writhing test, only compounds **2b**, **m** exerted a statistically significant effect, the degree of protection afforded being equal to 70% and 40%, respectively. The discrepancy observed between the results of the two tests might be attributed to their different sensitivity; as a matter of fact, it is known that ED_{50} values provided by the writhing test are usually markedly lower than those obtained with other experimental procedures, such as the tailflick or the hot plate test [6]. The antinociceptive effect of the three compounds that were found to be the most active in both analgesic assays were partially reversed by naloxone in the writhing test: the protection was reduced to 49% for **2b**, to 17% for **2m**, and to 74% for **2o**. Taking into account this behaviour and the fact that the hot plate test chiefly detects central analgesic drugs [7], the hypothesis could be advanced that the observed antinociceptive effect might be, at least in part, attributed to an opioid type of activity. In any case, our results further suggest that the analgesic activity of new drugs should be assessed by using at least two different methods.

The triazolobenzodiazepines **2d**, **e**, **l**, **o** and **7c** exhibited statistically significant but variably pronounced anti-inflammatory effects in the carrageenin-induced edema assay in the rat. The most active was **2l** which afforded a 43% protection; with the equitoxic dose of indomethacin the protection was equal to 60%. The remaining compounds were only weakly active or completely inactive.

Concerning the behavioral effects, evaluated in mice with the Irwin-Morpurgo's screening procedure, the highest toxic doses of compounds **2a–e**, **l**, **n** and **7d** produced marked depression, and death generally occurred for respiratory failure between 6 and 24 h after treatment; with subtoxic doses slight and inconstant signs, such as sedation, moderate decrease of spontaneous motor activity, passivity and ptosis were observed. In contrast, for toxic doses of compounds **2f**, **g**, **j**, **o** and **7c** the death was preceded by generalized tonic-clonic convulsions, and at lower subtoxic dosages the same compounds induced slight and variable symptoms of central nervous system stimulation, such as hyperactivity, tremors, and Straub tail effect. Finally, mice receiving compounds **2h**, **k**, **m**, **p** and **7a**, **b** displayed mild signs of central nervous system depression (hypoactivity, passivity and ptosis) at lower doses, and tonic-clonic convulsions often followed by death at toxic dosages. With all the triazolobenzodiazepines tested, the surviving animals appeared normal 24 h after treatment, and remained so during the 7-day observation period.

Conclusions

Pharmacological results show that some of the amino-derivatives **2a–h**, **j–p** possess anti-inflammatory and/or analgesic properties associated with low acute toxicity. The analgesic action, presumably related to both an opioid type of activity and a peripheral mechanism, appears especially noteworthy.

Thus, compounds **2d**, **e**, **l**, **o** exhibited a statistically significant anti-inflammatory activity in the carrageenin-induced edema assay, the highest activity being shown by compound **2l** (R''' = C_6H_5, $N\langle^{R'}_{R''}$ = $NHCH_3$).

On the other hand, all compounds **2a–h**, **j–p** produced statistically significant and often marked anti-

Table I. Pharmacological data of compounds **2a–h, j–p** and **7a–d.**

Compd	*Approximate oral LD_{50} in mice*	*Analgesic activity in mice*		*Anti-inflammatory activity in rats*[b]	
	(mg/kg)	*Writing test*[a]	*Hot plate test*[a]	*Edema (µl) (mean ± SD)*	*Inhibition (%)*
2a	1600	91***[c]	20	289 ± 31	15
2b	1130	99***	70**[c]	313 ± 45	8
2c	950	80**	nd[d]	350 ± 64	0
2d	1600	80**	nd	254 ± 57*c	25
2e	800	80**	nd	222 ± 58**	34
2f	670	95***	30	264 ± 54	22
2g	300	55**	nd	302 ± 39	11
2h	800	91***	20	287 ± 78	15
2j	800	62**	nd	314 ± 30	7
2k	800	88**	nd	276 ± 80	19
2l	1600	88**	nd	194 ± 29**	43
2m	480	98***	40*	292 ± 61	14
2n	800	82**	nd	337 ± 122	0
2o	310	99***	30	240 ± 71*	29
2p	620	95***	20	309 ± 54	9
7a	280	26	nd	319 ± 125	6
7b	540	93***	30	303 ± 100	11
7c	1600	23	nd	239 ± 72*	29
7d	520	96***	30	275 ± 73	19
Aspirin	800	53**	nd	nd	nd
Dipyrone	3120	73**	70**	nd	nd
Indomethacin	25	nd	nd	134 ± 54***	60

[a]% Protection produced by oral administration of 1/4 LD_{50} in mice (see *Experimental protocols*). [b]Carrageenin paw edema test (control value 339 ± 80 µl); effect produced by oral administration of 1/4 LD_{50} in mice (see *Experimental protocols*). [c]Statistical significance *versus* controls was evaluated by the Wilcoxon two sample test for the writhing test, by the Fisher exact test for the hot plate test, and by the Student's *t*-test for anti-inflammatory activity: *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$. [d]nd not determined.

nociceptive effects in the writhing test, but when the seven ones which resulted the most active were submitted to the hot plate test, only compounds **2b**, **m** were statistically effective. Anyway, considering both tests, compound **2b** [R''' = H, $N<^{R'}_{R''}$ = $N(C_4H_9)_2$] seems to be the most active as an analgesic.

If we consider the results obtained in the carrageenin-induced edema assay and in the hot plate test for both the first group of aminoderivatives **2** previously synthesized by us [2] and the compounds **2a–h**, **j–p** herein described, it appears reasonable to suggest the following conclusions: in this class of [1,2,4]triazolo[4,3-*a*][1,5]benzodiazepine derivatives the presence of the phenyl substituent in position 1 and of a small alkylsubstituted amino group in position 5 are structural features favourable for anti-inflammatory activity, whereas the lack of the 1-phenyl substituent and the presence of a bulky dialkylamino group in position 5 are favourable for analgesic activity.

Concerning the (alkylthio)derivatives **7a–d**, compound **7c** showed a statistically significant anti-inflammatory activity, whereas compounds **7b**, **d** resulted very effective as analgesics in the writhing test.

Experimental protocols

Chemistry

Melting points were determined using a Fisher-Johns (electrothermal when above 300°C) apparatus and are uncorrected. IR spectra were recorded on a Perkin-Elmer 398 spectrophotometer. ^{1}H NMR spectra were recorded on a Hitachi Perkin-Elmer R 600 (60 MHz) spectrometer, using $(CH_3)_4Si$ as an internal reference (δ = 0). Analyses of all new compounds, indicated by the symbols of the elements, were within ± 0.4% of the theoretical values and were performed by Laboratorio di Microanalisi, Istituto di Scienze Farmaceutiche, Università di Genova.

Thin layer chromatograms were run on Merck silica gel 60 F_{254} pre-coated plastic sheets (layer thickness 0.2 mm). Column chromatography was performed using Carlo Erba silica gel (0.05–0.20 mm).

4H-[1,2,4]Triazolo[4,3-a][1,5]benzodiazepin-5(6H)-ones ***5a–c***
General procedure. A mixture of 0.010 mol (2.03 g) of compound **1a** [1], 0.020 mol of formylhydrazine (1.20 g), acetylhydrazine (1.48 g) or benzoylhydrazine (2.72 g), 10 ml of Dowtherm A and 0.20 ml of glacial acetic acid (0.05 ml in the case of compound **5c**) was heated at 200°C for 2 h, while stirring. After cooling, compounds **5a–c** were isolated from the final reaction mixture as below described.

4H-[1,2,4]Triazolo[4,3-a][1,5]benzodiazepin-5(6H)-one ***5a.*** The suspension obtained from the reaction of **1a** with formylhydrazine was filtered. The whitish amorphous solid recovered was washed with a little methanol, then crystallized from the same solvent, affording 1.08 g (54%) of white crystalline compound **5a**, mp = 317–318°C dec (a small amount of solid impurity insoluble in boiling methanol was eliminated by filtration during the crystallization of the crude product). Anal $C_{10}H_8N_4O$ (C, H, N). IR (KBr) cm^{-1}: 3205 (NH), 3125, 1686 (CO), 1605 weak, 1597 weak, 1537, 1510. ^{1}H NMR [$(CD_3)_2SO$], δ: 3.87 (s, 2H, 4-CH_2), 7.04–7.98 (m, 4H, H-7,8,9,10), 9.12 (s, 1H, H-1), 10.42 (s, 1H, H-6; disappeared with D_2O).

1-Methyl-4H-[1,2,4]triazolo[4,3-a][1,5]benzodiazepin-5(6H)-one ***5b.*** The mixture obtained from the reaction of **1a** with acetylhydrazine was treated with a little chloroform. The resulting solution was chromatographed on a silica gel column. The column was first eluted with the mixture chloroform-ethyl acetate (1:1) until Dowtherm A and impurities were completely removed, then with chloroform-methanol (9:1). This eluate was evaporated and a little ethyl acetate was added to the oily residue. After standing, compound **5b** (white solid, 1.05 g, 49%) separated out. By crystallizing from ethanol, **5b·0.5 H_2O** was obtained, mp = 235–236°C. Anal $C_{11}H_{10}N_4O{\cdot}0.5\ H_2O$ (C, H, N). IR (KBr), cm^{-1}: 3520 broad (water of crystallization), 3200 (NH), 1682 (CO), 1606 weak, 1597 weak, 1543, 1500. ^{1}H NMR [$(CD_3)_2SO$], δ: 2.51 (s, 3H, 1-CH_3), 3.74 (s, 2H, 4-CH_2), 7.18–7.90 (m, 4H, H-7,8,9,10), 10.43 (s, 1H, H-6; disappeared with D_2O).

1-Phenyl-4H-[1,2,4]triazolo[4,3-a][1,5]benzodiazepin-5(6H)-one ***5c.*** Starting from **1a** and benzoylhydrazine and following the procedure above described for the preparation of compound **5b**, crude compound **5c** ultimately separated out. After crystallization from ethanol, white crystals of pure **5c** (1.71 g, 62%) were obtained, mp = 308–310°C. Anal $C_{16}H_{12}N_4O$ (C, H, N). IR (KBr), cm^{-1}: 3190 (NH), 1682 (CO), 1603, 1537, 1503. ^{1}H NMR [$(CD_3)_2SO$], δ: 3.82 (s, 2H, 4-CH_2), 6.75–7.64 (m, 9H, H-7,8,9,10 + phenyl H's), 10.55 (s, 1H, H-6; disappeared with D_2O).

4H-[1,2,4]Triazolo[4,3-a][1,5]benzodiazepine-5(6H)-thiones ***6a, b***
A mixture of 0.010 mol of compound **5a** (2.00 g) or **5c** (2.76 g), 0.0055 mol (2.22 g) of Lawesson's reagent and 15 ml of Dowtherm A was heated at 160°C for 30 min (compound **6a**) or for 1 h (compound **6b**), while stirring. After cooling, the resulting suspension was diluted with ethyl acetate, then filtered. The yellowish solid recovered was washed with ethyl acetate and dried. There was obtained the nearly pure compound **6a** or **6b** which was crystallized from ethanol.

4H-[1,2,4]Triazolo[4,3-a][1,5]benzodiazepine-5(6H)-thione ***6a.*** 1.82 g (84%), pale yellow crystals melting at 303–305°C dec after crystallization. Anal $C_{10}H_8N_4S$ (C, H, N, S). IR (KBr) cm^{-1}: 3170, 2980–2660 broad, 1605, 1552, 1529, 1500.

1-Phenyl-4H-[1,2,4]triazolo[4,3-a][1,5]benzodiazepine-5(6H)-thione ***6b.*** 2.40 g (82%), pale yellow crystals melting at 262–263°C after crystallization. Anal $C_{16}H_{12}N_4S$ (C, H, N, S). IR (KBr), cm^{-1}: 3160, 2990–2660 broad, 1603, 1546, 1536, 1493. ^{1}H NMR [$(CD_3)_2SO$], δ: 4.11 and 4.37 (AB q, J = 14.4 Hz, 2H, 4-CH_2), 6.76–7.69 (m, 9H, H-7,8,9,10 + phenyl H's), 8.46–9.29 (broad signal, 1H, H-6; disappeared with D_2O).

5-(Alkylthio)-4H-[1,2,4]triazolo[4,3-a][1,5]benzodiazepines ***7a–d***
General procedure. To a suspension of 6.25 mmol of compound **6a** (1.35 g) or **6b** (1.83 g) in anhydrous tetrahydrofuran (350 ml for **6a**, and 120 ml for **6b**), 0.19 g of 80% NaH in white oil (6.25 mmol) was added, heating then at reflux for 45 min, while stirring. After adding 7.25 mmol of the suitable alkyl halide, the mixture was further refluxed for 90 min. The solvent was then removed *in vacuo* and the residue partitioned between dichloromethane and water. The aqueous phase was extracted several times with dichloromethane. The combined organic phases were dried (anhydrous sodium sulfate) and the

solvent removed to afford a solid or thick oily residue from which compound **7** was recovered according to the following procedures.

5-(Methylthio)-4H-[1,2,4]triazolo[4,3-a][1,5]benzodiazepine ***7a***. The solid obtained from the reaction of **6a** with methyl iodide (1.03 g) was treated with a little ethyl acetate, then filtered, to give 0.92 g (64%) of compound **7a**, whitish crystals melting at 194–195°C after crystallization from the same solvent. Anal $C_{11}H_{10}N_4S$ (C, H, N, S). IR ($CHCl_3$), cm^{-1}: 1611, 1591, 1529. ^1H NMR ($CDCl_3$), δ: 2.44 (s, 3H, CH_3), 3.80 (s, 2H, 4-CH_2), 7.20–7.67 (m, 4H, H-7,8,9,10), 8.56 (s, 1H, H-1).

5-(Propylthio)-4H-[1,2,4]triazolo[4,3-a][1,5]benzodiazepine ***7b***. By adding some ethyl ether to the oil obtained from **6a** and propyl iodide (1.23 g) a solid separated out which was crystallized from cyclohexane to give 1.07 g (66%) of white crystalline compound **7b**, melting at 151–152°C. Anal $C_{13}H_{14}N_4S$ (C, H, N, S). IR ($CHCl_3$), cm^{-1}: 1612, 1593, 1530. ^1H NMR ($CDCl_3$), δ: 1.00 (t, 3H, CH_3), 1.63 (mc, 2H, CH_2CH_3), 3.04 (t, 2H, S-CH_2), 3.79 (s, 2H, 4-CH_2), 7.13–7.72 (m, 4H, H-7,8,9,10), 8.58 (s, 1H, H-1).

5-[[(Ethoxycarbonyl)methyl]thio]-4H-[1,2,4]triazolo[4,3-a][1,5]benzodiazepine ***7c***. The oil obtained from the reaction of **6a** with ethyl bromoacetate (1.21 g) was dissolved in a little chloroform and chromatographed on a silica gel column. The column was first eluted with chloroform-ethyl acetate (1:1) to remove the impurities, then with chloroform-ethyl acetate-methanol (9:9:1). After removing the solvents from this eluate, a little ethyl ether was added to the oily residue to give compound **7c** (1.04 g, 55%) as white crystals melting at 136–137°C after recrystallization from ethyl acetate-petroleum ether 40–70°C. Anal $C_{14}H_{14}N_4O_2S$ (C, H, N, S). IR ($CHCl_3$), cm^{-1}: 1730 (CO), 1617, 1595, 1528. ^1H NMR ($CDCl_3$), δ: 1.23 (t, 3H, CH_3), 3.84 (s, 4H, S-CH_2 + 4-CH_2), 4.21 (q, 2H, CH_2CH_3), 7.15–7.70 (m, 4H, H-7,8,9,10), 8.57 (s, 1H, H-1).

5-(Methylthio)-1-phenyl-4H-[1,2,4]triazolo[4,3-a][1,5]benzodiazepine ***7d***. The oil obtained from **6b** and methyl iodide (1.03 g) was dissolved in a little chloroform and chromatographed on a silica gel column. The column was eluted with ethyl acetate, the solvent removed from the eluate, and a little ethyl ether added to the oily residue. After standing, the white crystalline compound **7d** (1.71 g, 89%) separated out, mp = 167–168°C after recrystallization from ethyl acetate-petroleum ether 40–70°C. Anal $C_{17}H_{14}N_4S$ (C, H, N, S). IR ($CHCl_3$), cm^{-1}: 1610, 1590, 1530. ^1H NMR ($CDCl_3$), δ: 2.49 (s, 3H, CH_3), 3.46 and 4.11 (AB q, J = 15 Hz, 2H, 4-CH_2), 6.74–7.70 (m, 9H, H-7,8,9,10 + phenyl H's).

Substituted 4H-[1,2,4]triazolo[4,3-a][1,5]benzodiazepin-5-amines ***2a–p***

General procedure. To an ice bath cooled solution of 0.0109 mol (1.2 ml) of titanium tetrachloride and 2 ml of anisole in 40 ml of dry toluene, a solution of 5 ml of the suitable amine in 5 ml of dry toluene was added, while stirring. 0.010 mol of compound **5** (2.00 g of **5a**, 2.14 g of **5b**, or 2.76 g of **5c**) and a solution of 3 ml of the amine in 10 ml of dry toluene were then added to the mixture. In the case of 4-phenylpiperidine, two toluene solutions of 4.5 g and 2.7 g of this solid amine were added, respectively. In the reactions with methylamine or ethylamine, only the first addition was carried out using 8 ml of the amine previously cooled to – 20°C. Freshly distilled dipropylamine, dibutylamine and diallylamine were used. The resulting mixture was then refluxed with stirring for 6 h.

After cooling, 3 ml of 2-propanol, 2 g of diatomaceous earth and 3 ml of concentrated ammonium hydroxide were added to the mixture. After stirring, the resulting suspension was filtered, the solid collected was thoroughly washed with toluene (with dichloromethane in the preparations of compounds **2l–n**), and the filtrate and washings were dried (anhydrous sodium sulfate), then evaporated under reduced pressure. From the resulting residue compound **2** was obtained following the below described procedures.

Compounds ***2d, f–i, l–n***. The solid or thick oily residue was treated with a little ethyl acetate, then filtered to collect compound **2**, white or whitish solid, which was crystallized from the suitable solvent.

Compounds ***2a–c, e, j, k, o, p***. The thick oily residue was dissolved in a little chloroform and chromatographed on a silica gel column. The column was eluted with the mixture benzene-triethylamine (9:1), then the solvents were removed from the eluate to give an oily residue which was treated with some ethyl ether-petroleum ether 40–70°C (1:1). After standing, white or whitish crystalline compound **2** separated out, which was recrystallized from the suitable solvent.

The data of compounds **2a–p** are reported in table II.

Pharmacology

Male albino Swiss mice (18–22 g) and male Sprague-Dawley rats (180–220 g) were used. The animals were starved for about 12 h before treatment. All the test compounds were administered orally in a 1% carboxymethylcellulose suspension. Except for Irwin-Morpurgo's test and concomitant toxicity evaluation, the dose constantly employed in pharmacological assays was 1/4 po LD_{50} in mice.

Gross behavioral effects and acute toxicity in mice

The Morpurgo's modification [8] of the Irwin's multidimensional screening procedure was used on groups of 4 mice to evaluate drug-induced behavioral alteration. The test compounds were administered at log-spaced doses, and detailed observation of mice was performed 1, 3, and 24 h after treatment. Perphenazine (50 mg/kg ip) and methyl phenidate (50 mg/kg ip) were used for comparison. The approximate LD_{50} was obtained from the mortality observed during a 7-day period.

Analgesic activity

Writhing test [9]. Groups of 6 mice were injected intraperitoneally with a 0.6% acetic acid solution (0.01 ml/g) 1 h after administration of the test compound. The writhing movements of each animal were counted for 10 min (between the 5th and 15th minute after injection of the irritant). The analgesic effect was expressed as the percentage of protection compared with the control group. Equitoxic doses of acetylsalicylic acid (200 mg/kg) and dipyrone (780 mg/kg) were used as reference standards. To test for naloxone reversibility of the antinociceptive effect observed in the writhing test [10], groups of 6 mice were given a subcutaneous injection of naloxone (1 mg/kg) 40 min after the oral administration of the test compound, and the antinociceptive effect was subsequently determined as described above.

Hot plate test [11]. Groups of 10 mice were used. They were placed individually on a hot copper plate (48 ± 0.5°C), and the time of a reaction to pain, licking of the hind paws or jumping, was recorded before and 30, 60, and 90 min after administration of the test compound. The mice were removed as soon as they reacted or, if they failed to react, after 60 s. An equitoxic dose of dipyrone (780 mg/kg) was used for comparison.

Anti-inflammatory activity

The carrageenin-induced paw edema test [12] was used on groups of 6 rats. Sixty minutes after drug administration,

Table II. Data of compounds **2a–p**.

Compd.	Molecular formula [a]	Yield (%)	mp °C (solv.) [b]	IR [c] (cm^{-1})	^1H NMR [d] (δ, ppm)
2a	$C_{16}H_{21}N_5$	29	129-130 (A)	1610,1579s,1530.	0.92(t,6H,CH_3), 1.62(mc,4H,CH_2-CH_3), 3.45(t,4H,N-CH_2), 3.74(s,2H,4-CH_2), 6.76-7.56(m,4H,H-7,8,9,10), 8.53(s,1H,H-1).
2b	$C_{18}H_{25}N_5$	42	113-114 (B)	1609,1578s,1528.	0.70-1.98(m,14H,$CH_2CH_2CH_3$), 3.48(t,4H,N-CH_2), 3.74(s,2H,4-CH_2), 6.82-7.56 (m,4H,H-7,8,9,10), 8.54(s,1H,H-1).
2c	$C_{16}H_{17}N_5$	29	113-113.5 (B)	1641w,1611,1584s, 1529.	3.74(s,2H,4-CH_2), 4.13(d,4H,N-CH_2), 4.91-5.42(m,4H,CH=CH_2),5.53-6.26(m, 2H,CH=CH_2), 6.86-7.58(m,4H,H-7,8,9,10), 8.52(s,1H,H-1).
2d	$C_{15}H_{17}N_5$	81	201 (C)	1606,1574s,1527.	1.61(mc,6H,β-CH_2 + γ-CH_2), 3.67(mc,4H,α-CH_2), 3.77(s,2H,4-CH_2), 6.90-7.59 (m,4H,H-7,8,9,10), 8.55(s,1H,H-1).
2e	$C_{21}H_{21}N_5$	68	140-141 (D)	1608,1578s,1529.	1.24-2.14(m,4H,β-CH_2), 2.44-3.35(m,3H,α-CH_2 + CH), 3.80(s,2H,4-CH_2), 4.37--4.86(m,2H,α-CH_2), 6.66-7.60(m,9H,H-7,8,9,10 + phenyl H's), 8.54(s,1H,H-1).
2f	$C_{14}H_{15}N_5O$	78	262-262.5 (C)	1611,1585s,1530.	3.68-4.21(m,8H,morpholine CH_2's), 7.38-8.00(m,4H,H-7,8,9,10), 9.28(s,1H,H-1).[e]
2g	$C_{15}H_{18}N_6$	55	207-207.5 (C)	1608,1579s,1529.	2.13-2.69[m,4H,$CH_3N(CH_2)_2$], 2.28(s,3H,CH_3), 3.49-4.03(m,4H,N-CH_2), 3.78(s, 2H,4-CH_2), 6.91-7.59(m,4H,H-7,8,9,10), 8.57(s,1H,H-1).
2h	$C_{17}H_{20}N_6O_2$	81	206-207 (C)	1687(CO),1609, 1582s,1528.	1.25(t,3H,CH_3), 3.30-3.93(m,8H,N-CH_2), 3.79(s,2H,4-CH_2), 4.15(q,2H,CH_2-CH_3), 6.92-7.57(m,4H,H-7,8,9,10), 8.56(s,1H,H-1).
2i	$C_{16}H_{19}N_5$	56	210-211 (C)	1605,1572s,1534.	1.61(mc,6H,β-CH_2 + γ-CH_2), 2.58(s,3H,1-CH_3), 2.99 and 4.40(AB q,J=14.4 Hz,2H, 4-CH_2), 3.66(mc,4H,α-CH_2), 6.92-7.48(m,4H,H-7,8,9,10).
2j	$C_{16}H_{20}N_6$	70	170-171 (E)	1608,1579s,1537.	2.04-2.73[m,4H,$CH_3N(CH_2)_2$],2.29(s,3H,N-CH_3), 2.58(s,3H,1-CH_3), 3.02 and 4.39 (AB q,J=13.8 Hz,2H,4-CH_2), 3.39-3.92(m,4H,N-CH_2), 6.90-7.56(m,4H,H-7,8,9,10).
2k	$C_{18}H_{22}N_6O_2$	72	164.5-165 (B)	1688(CO),1610,1583s, 1536w.	1.26(t,3H,CH_2-CH_3), 2.59(s,3H,1-CH_3), 3.08 and 4.41(AB q,J=13.8 Hz,2H,4-CH_2), 3.35-3.91(m,8H,N-CH_2), 4.16(q,2H,CH_2-CH_3), 6.98-7.61(m,4H,H-7,8,9,10).
2l	$C_{17}H_{15}N_5$	67	283-284 (F)	3248 and 3070 (NH), 1622,1590,1570,1529.	2.82(d,3H,CH_3; s after treatment with D_2O), 3.39 and 3.98(AB q,J=14.4 Hz,2H, 4-CH_2), 6.67-7.67(m,9H,H-7,8,9,10 + phenyl H's), 7.84 [f](mc,1H,NH).
2m	$C_{18}H_{17}N_5$	90	278-279 (F)	3247 and 3070 (NH), 1617,1589,1566,1530.	1.25(t,3H,CH_3), 3.10-3.81(m,2H,N-CH_2), 3.37 and 4.34(AB q,J=14.4 Hz,2H,4-CH_2), 6.63-7.76(m,9H,H-7,8,9,10 + phenyl H's), 8.00 [f](mc,1H,NH).
2n	$C_{19}H_{17}N_5$	63	257-258 (C)	3248 and 3063 (NH), 1618,1589,1560,1534.	0.26-0.86(m,4H,cyclopropyl CH_2),2.87(mc,1H,N-CH), 3.36 and 3.93(AB q,J=13.8 Hz,2H,4-CH_2), 6.63-7.64(m,9H,H-7,8,9,10 + phenyl H's), 7.92 [f](near d,1H,NH).
2o	$C_{21}H_{22}N_6$	73	195-195.5 (E)	1609,1581s,1529w.	2.14-2.67[m,4H,$CH_3N(CH_2)_2$], 2.30(s,3H,CH_3), 3.11 and 4.43(AB q,J=14.4 Hz,2H, 4-CH_2), 3.58-3.96(m,4H,N-CH_2), 6.71-7.74(m,9H,H-7,8,9,10 + phenyl H's).
2p	$C_{23}H_{24}N_6O_2$	90	200-200.5 (G)	1688(CO),1610,1585s, 1528w.	1.26(t,3H,CH_3), 3.16 and 4.45(AB q,J=14.4 Hz,2H,4-CH_2), 3.37-3.94(m,8H,N-CH_2), 4.17(q,2H,CH_2-CH_3), 6.62-7.76(m,9H,H-7,8,9,10 + phenyl H's).

[a]Anal C, H, N. [b]Solvent of crystallization: A = ethyl ether/petroleum ether 40–70°C, B = isopropyl ether, C = ethyl acetate, D = ethyl acetate/cyclohexane, E = cyclohexane, F = ethanol, G = ethyl acetate/isopropyl ether. [c]In $CHCl_3$ solutions for **2a–k**, **o**, **p**; in KBr pellets for **2l–n**. Abbreviations: s = strong, w = weak. [d]Solvents: $CDCl_3$ for **2a–e**, **g–k**, **m**, **o**, **p** $(CD_3)_2SO$ for **2l**, **n**, $(CD_3)_2SO$ + CF_3COOD for **2f**. [e]The 4-CH_2 signal of **2f** is absent due to replacement of methylene protons with deuterium. [f]Disappeared with D_2O.

0.1 ml of a 1% carrageenin solution in saline was injected into the plantar surface of the right hind paw of each rat. Paw volume, determined by measuring the amount of water displaced after immersing the paw to the level of the lateral malleolus, was recorded immediately after the carrageenin injection, and again 3 h later; the difference between these two values was taken as edema volume. The percent inhibition of the edema of treated rats with respect to controls was calculated and compared with that produced by an equitoxic dose of indomethacin (6 mg/kg).

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