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Short communication

Synthesis and trazodone-like pharmacological profile of 1- and 2-[3-[4-(*X*)-1-piperazinyl]-propyl]-benzotriazoles

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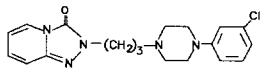
Summary — A series of 1- and 2-[3-[4-(*X*)-1-piperazinyl]-propyl]benzotriazoles were prepared and evaluated for their trazodone-like pharmacological profile; as preliminary pharmacological screening, the compounds were tested for their antiserotonergic, antiadrenergic and antihistaminic *in vitro* activity as well as for their analgesic *in vivo* action. Structure–activity relationships showed that among the synthesized compounds, the analogues bearing on the 4-piperazine nitrogen either an unsubstituted phenyl ring or a 2- or 3-chloro phenyl moiety show a pharmacological profile similar to that of the antidepressant trazodone.

benzotriazoles / antiserotonergic / antiadrenergic / antihistaminic / analgesic

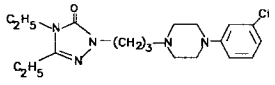
Introduction

Various drugs acting mainly on the central nervous system incorporate an arylpiperazine structure. These include the non-tricyclic antidepressant trazodone and the related triazolo antidepressants nefazodone and etoperidone (chart 1).

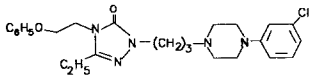
Trazodone is an atypical antidepressant drug that also possesses anxiolytic properties [1]. The mechanism of its antidepressant activity is still a subject of study. It is inactive in standard tests for antidepressive agents; unlike tricyclic antidepressants, trazodone does not have antireserpine activity, does not potentiate methylamphetamine-induced anorexia or the effects on catecholamines of L-DOPA. Furthermore, it is not a monoaminooxidase (MAO) inhibitor nor an anticholinergic agent [2, 3]. Similarly, trazodone does not reduce immobility in the forced swimming test as classical tricyclic antidepressants and MAO inhibitors do [4, 5]. Perhaps the most important action of trazodone is at the level of the serotonin receptors, where it behaves as a potent antagonist, mainly on 5-HT_2 receptors [5–7]. Moreover, trazodone shows a strong affinity for α_1-adrenoceptors and a weaker affinity for α_2-adrenoceptors [8]; its antinoceptive activity which does not involve the opioid receptors [9, 10], is also of interest. The particular selectivity of trazodone has been emphasized in conditions characterized by the activation of mechanisms involving the emotional integration of unpleasant experiences [11].



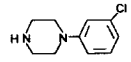
Trazodone **1**



Etoperidone **2**



Nefazodone **3**



1-(*m*-Chlorophenyl)-piperazine **4**

Chart 1.

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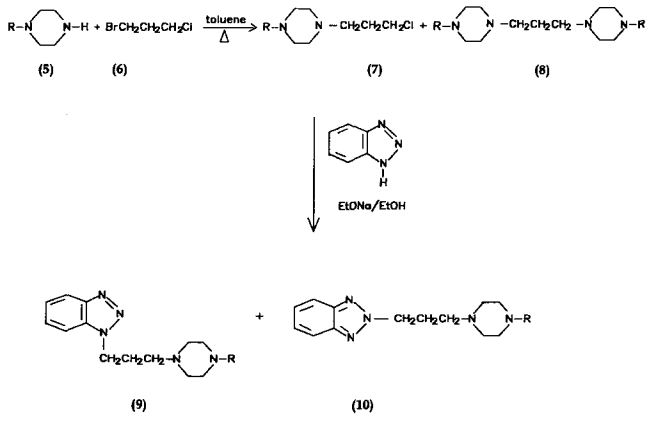
**To whose memory this paper is dedicated.

Nefazodone has been reported to have complex interactions with both 5-HT_1 and 5-HT_2 receptors, moderate effects on adrenoceptors and little anticholinergic action [12]; the analgesic effects of nefazodone alone and in conjuction with opioids has recently been re-examined [13]. The pharmacology of etoperidone is similar to that of trazodone [14–16]; however, it exhibits a number of properties which differ from the latter.

A common metabolic pathway of the drugs shown in chart 1 is cleavage of the side chain with the formation of 1-(*m*-chlorophenyl)-piperazine (*m*CPP). Although earlier work [17–21] suggested that *m*CPP might play an important role in the therapeutic efficacy of these drugs, subsequent studies [22] strongly indicate that the parent compound and not the metabolite *m*CPP mediates the antidepressant-like effects of trazodone.

As part of a program to discover compounds that show a trazodone-like pharmacological profile, in this report we describe the synthesis and characterization of the 1- and 2-substituted derivatives **9** and **10** (scheme 1) obtained by replacing the trazodone 1,2,4-triazolo-[4,3-*a*]pyridin-3(2*H*)-one moiety with the 1,2,3-benzotriazole ring. Our aim was also to study the influence of the position of the Cl group on the piperazine phenyl ring; furthermore, the pharmacological effects when the piperazine phenyl ring was replaced with other aryl moieties as well as the effect of the replacement with an alkyl substituent were studied.

On the basis of the above-reported considerations, in a preliminary pharmacological screening of our compounds we mainly examined their antiserotonergic, antiadrenergic and antihistaminic *in vitro* activity and also their analgesic *in vivo* activity, using trazodone as the reference compound.



(5) (6) (7) (8) (9) (10)

9 or 10	*R*
a	C_6H_5
b	C_6H_4-2-Cl
c	C_6H_4-3-Cl
d	C_6H_4-4-Cl
e	CH_2-C_6H_5
f	CH_2CH_2-C_6H_5
g	CH_3
h	CH_2CH_2OH

Scheme 1.

Chemistry

All target compounds prepared for this study have been listed in table I. They were synthesized as outlined in scheme 1.

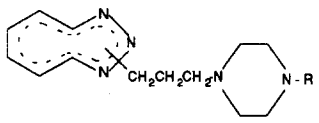
The substituted piperazines **5** were commercially available; only the phenethylpiperazine was not available; it was therefore prepared by reaction of piperazine with (2-bromoethyl)benzene under reflux. The crude product was purified by chromatography on an alumina column using ethanol as eluent.

The 1-(3-chloropropyl)-piperazine-4-substituted intermediates **7** were obtained by reaction of 1-bromo-3-chloropropane **6** with various 4-substituted piperazines. The reaction also yielded the bis compound **8**; to avoid this, the 2 reagents were dissolved in toluene as an inert solvent, using a small excess of 1-bromo-3-chloropropane. It was experimentally observed that to reduce the production of the bis compound the substituted piperazine had to be added when the reaction mixture was already under reflux. The purification of 1-(3-chloropropyl)-piperazine-4-substituted **7** was performed by chromatography on a silica-gel column with diethyl ether as eluent.

Reaction of 1-(3-chloropropyl)-piperazine-4-substituted with benzotriazole in anhydrous ethanol and sodium ethoxide [23, 24] easily provided a mixture of 1- and 2-substituted isomers (**9** and **10**) with an overall yield in the range 50–80%; the compound bearing the substituent in the 2-position was obtained in the highest yield. The separation of benzotriazole derivatives was performed by chromatography on a silica-gel column using diethyl ether/hexane, 7:3 v/v as eluent.

All obtained products were further purified by crystallization from an appropriate solvent. To increase stability, some of these products were transformed into the corresponding chlorides. Compounds synthesized listed in table I were characterized by UV and ^{1}H-NMR spectroscopy. The UV spectra of the

Table I. Physicochemical properties of benzotriazole derivatives **9a–h** and **10a–h**.



		1-Substituted benzotriazoles 9a-h					2-Substituted benzotriazoles 10a-h				
R	Formula*	Compound	Yield** %	m.p. °C	Recryst. solv.	Log D*** pH = 7.4	Compound	Yield** %	m.p. °C	Recryst. solv.	Log D*** pH = 7.4
C_6H_5	$C_{19}H_{23}N_5$	9 a	22.8	120-122	a	3.00 (±0.05)	10 a	42.2	116-117	a	3.35 (±0.04)
C_6H_4-2-Cl	$C_{19}H_{22}ClN_5$	b	19.8	68-69	a	3.65 (±0.05)	b	56.2	95-96	a	4.00 (±0.05)
C_6H_4-3-Cl	$C_{19}H_{22}ClN_5$	c	30.0	90-91	a	3.70 (±0.05)	c	40.0	114-116	a	4.05 (±0.04)
C_6H_4-4-Cl	$C_{19}H_{22}ClN_5$	d	24.4	122-123	b	3.72 (±0.04)	d	49.6	100-101	a	4.03 (±0.05)
CH_2-C_6H_5	$C_{20}H_{25}N_5$	e	22.0	88-89	a	2.95 (±0.05)	e	28.0	52-53	c	3.30 (±0.05)
CH_2CH_2-C_6H_5	$C_{21}H_{27}N_5 \cdot 2HCl$	f	20.8	256-258	d	3.35 (±0.05)	f	37.2	266-267	d	3.72 (±0.05)
CH_3	$C_{14}H_{21}N_5 \cdot 2HCl$	g	37.6	248-250	a	0.25 (±0.05)	g	42.4	216-219	a	0.80 (±0.03)
CH_2CH_2OH	$C_{15}H_{22}N_5O \cdot 2HCl$	h	37.4	214-216	d	0.13 (±0.05)	h	40.6	204-206	e	0.50 (±0.04)

*Satisfactory microanalyses obtained: C, H, Cl, N values are within ± 0.3% of the theoretical values. **Yield refers to the single structural isomer after separation by chromatography. ***Number in parentheses indicates the 95% confidence interval. a) ethyl alcohol; b) diethyl ether; c) hexane; d) methyl alcohol; e) isopropanol.

1-substituted benzotriazoles were characterized by 2 absorption peaks ranging from 250–260 nm and 279–280 nm; the 2-substituted compounds instead, showed a single absorption peak ranging from 276 to 278 nm, with a poorly defined fine structure at 283 nm [25]. However, 2-substituted isomers with groups bearing an aromatic ring directly bound to piperazine moiety showed UV spectra characterized by 2 absorption peaks with the same range as the 1-substituted isomers. However, differentiation *via* UV spectroscopy between 1-substituted and 2-substituted isomers was possible as their spectra had different outlines: the 1-substituted isomers were characterized by very different ε values, while the 2-substituted isomers showed similar ε values to one another. In addition to UV spectra, all compounds reported in this paper were characterized by ^{1}H-NMR spectroscopy.

It should be pointed out that the differences in the chemical shift values among the protons in the series of 1- and 2-substituted benzotriazoles confirm the different π-electron delocalization of the 2 systems. Indeed, benzotriazol-2-yl derivatives showed a greater molecular symmetry; in fact the aromatic protons of benzotriazole ring of compound **10a**, taken as an example, appear as 2 doublets of doublets at δ 7.85, J = 9.5 and 3.2 Hz (H-4 and H-7) and δ 7.37, J = 9.5 and 3.2 Hz (H-5 and H-6); the 3 methylene groups of the side-chain propyl bridge (-αCH_2βCH_2γCH_2-piperazinyl-) are characterized by the following chemical shifts: δ 4.83 (2H, t, J = 7.5 Hz, αCH_2), 2.32 (2H, m, βCH_2) and 2.48 (2H, t, J = 7.5 Hz, γCH_2).

Larger differences occurred in the benzotriazol-1-yl derivatives [*ie* **9a**] in which the aromatic protons of the benzotriazole moiety on the basis of the distributions of the resonance forms of the hybrids, appeared as 2 doublets of doublets at δ 8.03 (H-4) and δ 7.57 (H-7) J = 8.5 and 1.1 Hz and 2 doublets of triplets at 7.45 (H-6) and δ 7.32 (H-5) J = 8.5 and 1.1 Hz. The chemical shifts of the side-chain propyl bridge were the following: δ 4.75 (2H, t, J = 7.5 Hz, αCH_2), 2.23 (2H, m, βCH_2) and 2.39 (2H, t, J = 7.5 Hz, γCH_2).

Similar ^{1}H-NMR data were found in all the other benzotriazole derivatives and were consistent with the described structures.

Finally, the considered substances were characterized for their chemicophysical properties such as melting points and distribution coefficients (log D) in octanol/phosphate buffer at pH 7.4.

Pharmacology

For preliminary pharmacological screening, all of the compounds were evaluated *in vitro* for antiserotonergic (rat stomach fundus), antiadrenergic (rat vas deferens) and antihistaminic (guinea-pig ileum) activity and *in vivo* for analgesic activity (writhing test in mice). Trazodone was used as the reference compound. Dose–response curves and IC_{50} or ED_{50} values of the different compounds were determined.

Results and discussion

The search for compounds showing a trazodone-like pharmacological profile led to the synthesis of 2 series of benzotriazole isomers represented by formulas **9** and **10**. Table II lists the pharmacological tests carried out on the substances under study.

In pharmacological *in vitro* tests on isolated rat stomach fundus all the compounds showed inhibitory activity against 5-hydroxytryptamine (5-HT)-induced contractions. A general trend for the 1-substituted isomers to be more active than the 2-substituted derivatives was noted. The data clearly indicate that the antiserotonergic activity was more pronounced, in the following order, for compounds **9c < 10b = 9a < 9b**; note that the activity of these derivatives bearing a piperazine-phenyl moiety was similar or even higher than that displayed in the same test by trazodone. The effect of Cl-substitution in the *ortho*, *meta*, and *para* positions of the phenyl group is worthy of consideration. The introduction of the *o*-chloro group (**9b** and **10b**) had an appreciable effect on efficacy and yielded the best activity for both series of isomers; the introduction of the *meta*-chloro group (**9c** and **10c**) had no effect in comparison to the unsubstituted derivatives **9a** and **10a** respectively, while shifting the chloro substituent to the *para* position decreased the activity of both isomers (**9d** and **10d**). We may interpret these observations by assuming that if the substituent length from the piperazine nitrogen is increased, its hindrance reduces the overall activity; that is, the decrease in activity seems to depend on steric hindrance at the *para* position rather than on other factors. A similar hypothesis, but in the opposite sense, may be assumed for the Cl-substitution in the *ortho* position; in this case the structure could assume a conformation which would better accomodate the phenyl moiety at the receptor site.

The introduction of a methylene (**9c** and **10e**) or an ethylene bridge (**9f** and **10f**), between the piperazine nitrogen and the phenyl ring led to products which were characterized by a marked decrease in activity. By a comparison of the distribution coefficient (log *D*) values in table I it is clear that such reduced activity cannot solely be caused by hydrophobic effects; hence, its origin must involve electronic and/or steric factors. The hypothesis that the proper balance of bulkiness, hydrophobic and electronic effects plays an important role in modulating the activity was confirmed by the synthesis of compounds in which the phenyl ring was replaced by a methyl group (**9g** and **10g**) or a hydroxyethyl moiety (**9h** and **10h**), respectively; all 4 compounds showed a noticeable decrease in activity.

These results show that antiserotonergic activity is noticeably present in the benzotriazole derivatives in table II when the compounds bear lipophilic and electronic withdrawing groups of appropriate steric hindrance, attached to the nitrogen piperazine moiety.

A comparison between *in vitro* antiadrenergic activity and antiserotonergic data showed that the 2 types of biological data displayed a similar pattern. In fact, clear inhibitory activity against norepinephrine (NE)-induced contractions (rat vas deferens) was displayed by compounds **9d < 10d < 10c < 10a**, and

Table II. Anti-serotonergic (5-HT), anti-adrenergic (NE), anti-histaminic (Hist) and analgesic activities (writhing test) of 1- and 2-substituted benzotriazoles.

Compound		IC_{50} (moles/l)			ED_{50} (moles/Kg)
		Anti-5-HT	Anti-NE	Anti-HIST	Analgesic activity
9	a	$7.4 (\pm 0.35) \times 10^{-7}$	$6.0 (\pm 0.37) \times 10^{-8}$	$2.1 (\pm 0.27) \times 10^{-7}$	$3.1 (\pm 0.24) \times 10^{-5}$
	b	$4.9 (\pm 0.31) \times 10^{-7}$	$2.5 (\pm 0.25) \times 10^{-8}$	$1.8 (\pm 0.20) \times 10^{-7}$	$2.1 (\pm 0.32) \times 10^{-5}$
	c	$8.4 (\pm 0.45) \times 10^{-7}$	$9.4 (\pm 0.49) \times 10^{-8}$	$5.2 (\pm 0.33) \times 10^{-8}$	$1.5 (\pm 0.24) \times 10^{-5}$
	d	$5.2 (\pm 0.32) \times 10^{-6}$	$6.0 (\pm 0.34) \times 10^{-7}$	$2.9 (\pm 0.27) \times 10^{-8}$	$2.5 (\pm 0.31) \times 10^{-5}$
	e	$1.0 (\pm 0.20) \times 10^{-5}$	(*)	$1.5 (\pm 0.22) \times 10^{-7}$	$> 10^{-4}$
	f	$1.8 (\pm 0.22) \times 10^{-5}$	(*)	$1.5 (\pm 0.20) \times 10^{-7}$	$1.8 (\pm 0.29) \times 10^{-5}$
	g	$5.2 (\pm 0.36) \times 10^{-5}$	(*)	$1.7 (\pm 0.25) \times 10^{-5}$	$> 10^{-4}$
	h	$6.1 (\pm 0.46) \times 10^{-5}$	(*)	$2.9 (\pm 0.30) \times 10^{-5}$	$> 10^{-4}$
10	a	$3.7 (\pm 0.29) \times 10^{-6}$	$1.1 (\pm 0.22) \times 10^{-7}$	$2.7 (\pm 0.28) \times 10^{-7}$	$2.0 (\pm 0.31) \times 10^{-5}$
	b	$7.4 (\pm 0.35) \times 10^{-7}$	$5.0 (\pm 0.39) \times 10^{-8}$	$5.2 (\pm 0.38) \times 10^{-7}$	$3.0 (\pm 0.38) \times 10^{-5}$
	c	$2.4 (\pm 0.25) \times 10^{-6}$	$3.4 (\pm 0.30) \times 10^{-7}$	$1.5 (\pm 0.24) \times 10^{-7}$	$2.3 (\pm 0.28) \times 10^{-5}$
	d	$2.5 (\pm 0.25) \times 10^{-5}$	$4.4 (\pm 0.34) \times 10^{-7}$	$2.1 (\pm 0.32) \times 10^{-8}$	$> 10^{-4}$
	e	$1.3 (\pm 0.20) \times 10^{-5}$	(*)	$8.4 (\pm 0.46) \times 10^{-7}$	$5.1 (\pm 0.45) \times 10^{-5}$
	f	$3.1 (\pm 0.23) \times 10^{-5}$	(*)	$3.2 (\pm 0.35) \times 10^{-7}$	$4.5 (\pm 0.42) \times 10^{-5}$
	g	$8.0 (\pm 0.45) \times 10^{-5}$	(*)	$5.4 (\pm 0.40) \times 10^{-5}$	$> 10^{-4}$
	h	$7.4 (\pm 0.40) \times 10^{-5}$	(*)	$1.9 (\pm 0.24) \times 10^{-5}$	$> 10^{-4}$
Trazodone		$6.4 (\pm 0.35) \times 10^{-7}$	$5.8 (\pm 0.30) \times 10^{-8}$	$1.4 (\pm 0.22) \times 10^{-7}$	$1.5 (\pm 0.23) \times 10^{-5}$

(*)These compounds strengthened NE agonistic action.

particularly by the most active compounds **9c** < **9a** < **10b** < **9b**, whereas the other compounds tested were completely devoid of this activity, and further increased the NE agonistic action.

The *in vitro* antihistaminic effects of the compounds listed in table II were compared with that of trazodone. All the compounds inhibited isolated guinea-pig ileum contractions induced by histamine; activity was particularly high in compounds **9c**, **9d** and **10d**, which were found more active than trazodone itself. It is to be noted that the most active compounds in this pharmacological assay were the 2 isomers **9d** and **10d** bearing the Cl group in the *para* position of the piperazine-phenyl ring. In this case, it seems that receptor site had sufficient bulk tolerance at the *para* position for further interaction with the ligand.

Regarding the *in vivo* analgesic activity, the writhing test was used in order to test the analgesic potency of the studied compounds in mice. The most active agents of the series, *ie* compounds **9c**, **9f**, **9b**, **9d**, **10a** and **10c** showed ED_{50} values which were very similar to that of trazodone; however, compounds **9e**, **9g**, **9h**, **10d**, **10g** and **10h** were inactive. A careful examination of the data revealed that the hydrophobic parameter log *D* was not sufficient to account for variations in the biological data that covered a very narrow range.

In conclusion, the data summarized in table II indicate that, besides the 2-substituted isomer **10b**, the 1-benzotriazole derivatives bearing either an unsubstituted phenyl ring (**9a**) or a 2- or 3-chloro phenyl moiety (**9b** and **9c**) as a 4-piperazine substituent constitute a chemical set of potential antidepressant drugs with a pharmacological profile which is very similar to that of trazodone.

Experimental protocols

Chemistry

Melting points were determined on a Kofler hot stage apparatus and are uncorrected. Structures described were supported by UV, ^{1}H-NMR spectra and microanalytical data. UV spectra were taken on a Beckrnan DU-40 spectrophotometer and the product under consideration in 95% ethanol. ^{1}H-NMR spectra were recorded on a Bruker WM 250 spectrometer using $CDCl_3$ as solvent; chemical shifts (δ) are reported relative to tetramethylsilane as internal standard. Signals have been designated as follows: dd, doublet of doublets; t, triplet; m, multiplet. The spectra obtained confirmed the proposed structures. All pure compounds gave a satisfactory analysis (C, H, Cl, N) within ± 0.3% of the theoretical values.

Analytical TLC was performed on precoated silica-gel (0.2 mm GF 254, E Merck) or aluminum oxide glass-backed plates; the spots were located by UV (254 nm) light or by exposure to iodine vapour. Evaporation was performed *in vacuo*. Sodium sulfate was used as the drying agent. Crude products were routinely passed through columns of silica gel (Ø 0.05 ÷ 0.20 mm, Carlo Erba) or basic aluminum oxide (Macherey Nagel) with an appropriate mixture of diethyl ether/hexane 7:3 v/v or ethanol as eluent, respectively.

The distribution coefficients (log *D*) were determined according to the classic shake-flask procedure [26] at room temperature, using octanol as lipophilic phase and phosphate buffer pH = 7.4 as hydrophilic phase. It seemed to be sufficient to determine only the decrease of concentration in the water phase; however, in many cases the concentration of partitioned solute was measured in both the octanol and the buffer phase. The 2 phases were therefore adjusted in volume so that satisfactory amounts of compound were present in each phase. The concentrations were measured using a Beckman DU-40 spectrophotometer. Partitioning was carried out at 4 different concentrations to ensure that special interactions were not occurring and to check against other errors. Table I summarizes these data.

1-(Phenethyl)-piperazine

0.1 mol (18.5 g) (2-bromoethyl)benzene was added dropwise under stirring over a 15-min period to 0.2 mol (17.2 g) piperazine in 100 ml chloroform. After the addition was completed, the hot mixture was then refluxed for 4 h. After cooling to 25°C, the reaction mixture was filtered and the chloroform removed by distillation under reduced pressure. The residue was chromatographed on basic aluminum oxide (activity I, Macherey Nagel) column eluting with ethanol; pure 1-(phenethyl)-piperazine was obtained as a brown oil (6.1 g, 32%); ^{1}H-NMR ($CDCl_3$) δ 2.55 (4H, m, $-N(CH_2)-CH_2$), 2.65 (2H, dd, *J* = 2.5 and 6 Hz, $-CH_2-C$), 2.83 (2H, dd, *J* = 2.5 and 6 Hz, $-CH_2-N$), 2.96 (4H, t, $H-N(CH_2)-CH_2$), 7.20–7.40 (5H, m, arom).

1-(3-Chloropropyl)-piperazine-4-substituted derivatives 7

A solution of the appropriate piperazine-4-substituted (0.1 mol) in toluene (30 ml) was added dropwise over a 1-h period to a boiling solution of 0.15 mol (23.6 g) 1-bromo-3-chloropropane in 80 ml toluene. The reaction mixture was vigorously stirred and refluxed for 5 h and monitored by TLC (with diethyl ether as eluent). After this, the mixture was cooled and poured into water. The aqueous layer was alkalinized with 2 N NaOH and extracted several times with chloroform. The combined organic extracts were washed with water, dried (Na_2SO_4) and evaporated *in vacuo*. The resulting crude product was purified by silica-gel column chromatography using diethyl ether as eluent to obtain pure derivatives **7** (43 + 50% yield referred to virtually pure starting product).

*1- and 2-[3-[4-(X)-1-Piperazinyl]-propyl]-benzotriazole **9a–h** and **10a–h***

In a general procedure, clean sodium (1.15 g, 0.05 mol) was added to anhydrous ice-cooled ethanol and vigorously stirred until complete dissolution. Benzotriazole (5.95 g, 0.05 mol) and then the appropriate 1-(3-chloropropyl)-4-substituted-piperazine were successively added to this solution. The reaction mixture was kept under reflux for 15–24 h and monitored by TLC. After cooling the ethanol was removed under reduced pressure and the residue treated with chloroform (100 ml), separated from the sodium chloride and washed with 2 N sodium hydroxide and water to remove the unreacted benzotriazole. The organic layer was dried over anhydrous sodium sulfate and evaporated to dryness.

TLC examination (diethyl ether/hexane, 7:3 v/v) of the reaction mixture showed the formation of 2 UV absorbing products, one of which appeared in significantly higher proportion. Fractionation was performed on a silica-gel column (3 x 60 cm) using diethyl ether/hexane, 7:3 v/v as eluent.

Characterization of the isolated products by UV and ^{1}H-NMR spectra showed that the first compound to be eluted was the 2-substituted benzotriazole. Further purification of each product was obtained by crystallization from an appropriate solvent. Relevant data for each compound are given in table I.

Pharmacology

In vitro experiments

Guinea-pig ileum, rat stomach fundus and vas deferens were utilized to measure the *in vitro* pharmacologic activity of the test compounds. Male albino guinea pigs (Arvel, Brusciano, Naples, 200–300 g) and male Wistar rats (Nossan, Correzzana, Milan, 200–250 g) were used. The animals were killed, and the required organs set rapidly in the appropriate solution oxygenated with 95% O_2 and 5% CO_2.

Segments of guinea-pig ileum were suspended in Tyrode's solution and maintained at a temperature of 37°C; contractions were induced by histamine (3×10^{-7} M). The rat vas deferens was suspended in Krebs bicarbonate solution and kept at a temperature of 35°C; contractions were induced by norepinephrine (3×10^{-7} M). The rat stomach fundus was suspended in Krebs bicarbonate solution and maintained at a temperature of 37°C; contractions were induced by 5-HT (2×10^{-8} M). The tested compounds were added 2 min before the agonists. IC_{50} was calculated using a curve-fitting program [27]. The data reported are means ± SEM of 3–4 determinations.

Analgesic activity

Analgesic activity was evaluated on male Swiss mice (Nossan, Correzzana, Milan, 18–20 g) using the acetic acid-induced writhing test [28]. The compounds were administered orally at different doses 60 min before ip injection of acetic acid (0.25 ml of a 0.5% solution). The ED_{50} was calculated from the percentage of inhibition as compared to the controls.

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