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Short communication

Synthesis and biological activity of benzotriazole derivatives structurally related to trazodone†

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Summary — This paper outlines the synthesis and the pharmacological screening of a series of novel 1- and 2-[2-[4-(X)-1-piperazinyl]ethyl]benzotriazoles and 1-[3-[4-(X)-1-piperazinyl]propoxy]benzotriazoles, which are structurally related to trazodone. Antiserotonergic, antiadrenergic and antihistaminic *in vitro* activity and *in vivo* analgesic action are described. Some of the investigated compounds show overall pharmacological profiles similar to that of the antidepressant trazodone.

benzotriazole / antiserotonergic activity / antiadrenergic activity / antihistaminic activity / analgesic activity

Introduction

We have recently reported the synthesis and the preliminary pharmacological screening of a series of 1- and 2-[3-[4-(X)-1-piperazinyl]propyl]benzotriazoles designed as structural analogues of trazodone [1]. The compounds were evaluated for their antiserotonergic, antiadrenergic and antihistaminic *in vitro* activity as well as their *in vivo* analgesic action. The results of these studies showed that 1-benzotriazole derivatives bearing a 4-(X-phenyl)piperazine substituent constitute a set of compounds with a pharmacological profile very similar to that of trazodone. In contrast, among the corresponding 2-substituted isomers, which generally displayed a weaker activity, only 2-[3-[4-(2-chlorophenyl)-1-piperazinyl]propyl]-benzotriazole showed a trazodone-like profile.

Extending our study with the aim of broadening the structure–activity relationships in this class of analogues, we synthesized new compounds in which the distance between the benzotriazole ring and the piperazine nitrogen was varied by replacing the propylene bridge of the above-mentioned compounds with an ethylene or an oxypropylene intermediate moiety. Therefore, we prepared sets of 1- and 2-[2-[4-(X)-1-piperazinyl]ethyl]benzotriazoles **5** and **6** and a set of 1-[3-[4-(X)-1-piperazinyl]propoxy]benzotriazoles **10** to evaluate the possible role of different lengths of the side chain and the presence of an oxygen atom in the side chain in the interaction with various receptors.

Chemistry

All the compounds listed in table I were prepared as illustrated in scheme 1. The procedure involved the coupling of the corresponding 1-(2-chloroethyl)piperazine, which is 4-substituted, with benzotriazole.

The substituted piperazines **1** were usually commercially available. Only the phenethyl derivative was prepared by reaction of piperazine with 2-bromoethylbenzene, according to a procedure described in a previous paper [1].

The 4-substituted 1-(2-chloroethyl)piperazine intermediates **3** were obtained by reaction of 1-bromo-2-chloroethane **2** with the corresponding 4-substituted piperazines. This reaction led to formation of the bis-compound **4** as a by-product. To avoid this, the two reagents were dissolved in toluene as an inert solvent, and the substituted piperazine was added when the reaction mixture, containing a small excess of 1-bromo-2-chloroethane, was already under reflux. Purification of 4-substituted 1-(2-chloroethyl)piperazine **3** was performed by chromatography on a silica-gel column using diethyl ether as eluent.

†Dedicated to the memory of Carlo Silipo.
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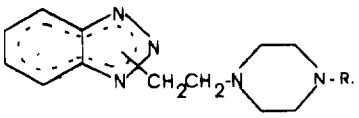
Table I. Physicochemical constants of benzotriazole derivatives **5a–h** and **6a–h**.

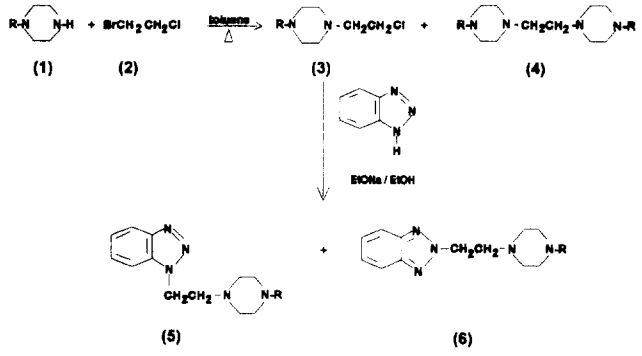


R	*Formula*	*1-Substituted benzotriazoles* ***5a–h***					*2-Substituted benzotriazoles* ***6a–h***				
		Compound	*Yield*[a] *(%)*	*Mp (°C)*	*Recryst solv*	*Log D*[b] *pH = 7.4*	*Compound*	*Yield*[a] *(%)*	*Mp (°C)*	*Recryst solv*	*Log D*[b] *pH = 7.4*
C_6H_5	$C_{18}H_{21}N_5$	**a**	18.6	83–85	a	2.74 (± 0.05)	**a**	41.4	92–94	a	3.04 (± 0.03)
C_6H_4-2-Cl	$C_{18}H_{20}ClN_5$	**b**	22.7	94–96	a	3.35 (± 0.04)	**b**	42.3	115–117	a	3.70 (± 0.05)
C_6H_4-3-Cl	$C_{18}H_{20}ClN_5$	**c**	30.8	84–85	d+c	3.45 (± 0.05)	**c**	39.2	90–92	a	3.80 (± 0.03)
C_6H_4-4-Cl	$C_{18}H_{20}ClN_5$	**d**	21.6	120–122	d+b	3.40 (± 0.04)	**d**	50.4	130–132	d+b	3.75 (± 0.05)
$CH_2C_6H_5$	$C_{19}H_{23}N_5$	**e**	20.7	72–74	a	2.63 (± 0.03)	**e**	24.3	52–54	a	3.00 (± 0.05)
$CH_2CH_2C_6H_5$	$C_{20}H_{25}N_5$•2HCl	**f**	18.7	243–245	e	3.05 (± 0.05)	**f**	36.3	245–246	e	3.42 (± 0.05)
CH_3	$C_{13}H_{19}N_5$•2HCl	**g**	24.0	249–251	e	–0.14 (± 0.05)	**g**	51.0	216–219	e	0.44 (± 0.03)
CH_2CH_2OH	$C_{14}H_{21}N_5O$•2HCl	**h**	31.4	201–203	e	–0.35 (± 0.05)	**h**	41.6	232–234	f	0.13 (± 0.03)

[a]Yield refers to the single structural isomer after separation by chromatography; [b]number in parentheses indicates the 95% confidence interval; a) ethanol; b) diethyl ether; c) hexane; d) chloroform; e) methanol; f) isopropanol.

Reaction of 4-substituted 1-(2-chloroethyl)piperazine **3** with benzotriazole, in anhydrous ethanol and sodium ethoxide [2, 3], gave a mixture of the 1- and 2-substituted isomers **5** and **6** with an overall yield ranging between 48 and 75%; generally the compound bearing the substituent in 2-position was obtained in higher yield. Separation of the obtained benzotriazole derivatives was performed by chromatography on a silica-gel column using diethyl ether/hexane 7:3 v/v as eluent.

Scheme 2 outlines the steps for the preparation of 1-[3-[4-(X)-1-piperazinyl]propoxy]benzotriazoles **10**.

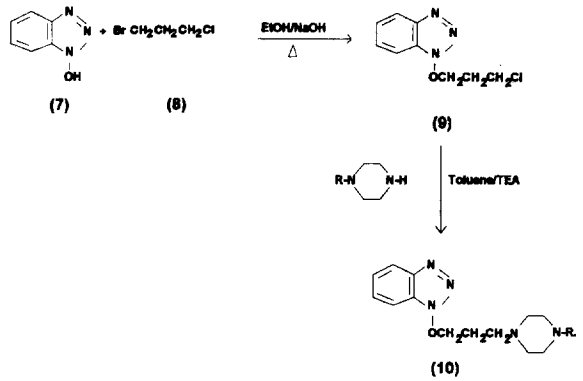


Scheme 1. Synthesis of compounds **5** and **6**. **a**: R = C_6H_5; **b**: R = C_6H_4-2-Cl; **c**: R = C_6H_4-3-Cl; **d**: R = C_6H_4-4-Cl; **e**: R = $CH_2C_6H_5$; **f**: R = $CH_2CH_2C_6H_5$; **g**: R = CH_3; **h**: R = CH_2CH_2OH.

This procedure was found to be more advantageous than scheme 1 with high yields and mild reaction conditions.

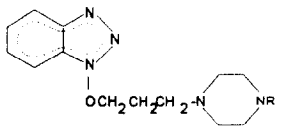
The 1-(3-chloropropoxy)benzotriazole intermediate **9** was prepared by reaction of 1-bromo-3-chloropropane **8** with 1-hydroxybenzotriazole in ethanol and sodium hydroxide. Purification of the crude intermediate **9** was performed by chromatography on a silica-gel column using diethyl/ether hexane 9:1 v/v as eluent.

Condensation of 1-(3-chloropropoxy)benzotriazole **9** with various 4-substituted piperazines, in toluene



Scheme 2. Synthesis of compound **10**. **a**: R = C_6H_5; **b**: R = C_6H_4-2-Cl; **c**: R = C_6H_4-3-Cl; **d**: R = C_6H_4-4-Cl; **e**: R = CH_2-C_6H_5; **f**: R = CH_3; **g**: R = CH_2CH_2OH.

Table II. Physicochemical constants of benzotriazole derivatives **10a–g**.



R	*Formula*	*Compound*	*Yield*[a]	*Mp (°C)*	*Recryst solv*	*Log D*[b] *pH = 7.4*
C_6H_5	$C_{19}H_{23}N_5O$	**a**	65	75–77	a	2.97 (± 0.03)
C_6H_4-2-Cl	$C_{19}H_{22}ClN_5O{\cdot}HCl$	**b**	53	178–180	a	3.60 (± 0.04)
C_6H_4-3-Cl	$C_{19}H_{22}ClN_5O{\cdot}HCl$	**c**	49	170–172	a	3.70 (± 0.05)
C_6H_4-4-Cl	$C_{19}H_{22}ClN_5O$	**d**	56	94–96	b	3.65 (± 0.05)
$CH_2C_6H_5$	$C_{20}H_{25}N_5O$	**e**	63	62–64	a	2.90 (± 0.04)
CH_3	$C_{14}H_{21}N_5O{\cdot}2HCl$	**f**	51	209–211	c	0.23 (± 0.05)
CH_2CH_2OH	$C_{15}H_{23}N_5O_2{\cdot}2HCl$	**g**	58	199–201	c	0.03 (± 0.04)

[a]Yield refers to the single structural isomer after separation by chromatography; [b]number in parentheses indicates the 95% confidence interval; a) ethanol; b) diethyl ether; c) methanol.

and triethylamine solution, gave the expected compounds **10**, in yields ranging between 51 and 63%. The obtained compounds were successively purified by chromatography on a silica-gel column using diethyl ether as eluent.

Analytical purification of each product was obtained by crystallization from the appropriate solvent. To increase the stability of the synthesized products, some of them were transformed into the corresponding hydrochlorides.

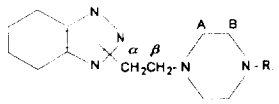
The structures and physicochemical data of the compounds are listed in tables I and II; all were characterized by UV spectroscopy. The UV spectra showed the presence of an aromatic chromophoric system characterized by absorption maxima at λ 255–263 and 279 nm for the 1-substituted derivatives (**5a–h** and **10a–g**), whereas for the 2-substituted derivatives (**6a–h**) a single absorption maximum appeared at λ 277 nm with a poorly defined fine structure at 283 nm [4]. However, 2-substituted isomers with groups bearing an aromatic ring directly bound to piperazine moiety showed UV spectra characterized by two absorption peaks with the same range as the 1-substituted isomers. 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 were characterized by ^{1}H-NMR spectroscopy. It is worth pointing out that the observed differences in the chemical shifts values among the protons of 1- and 2-substituted derivatives confirm the different π-electronic delocalization of the two benzotriazole systems. Indeed, the benzotriazol-2-yl derivatives show a greater molecular symmetry; in fact, in compound **6a**, taken as an example, the aromatic protons of the benzotriazole ring appear as two doublets of doublets at δ 7.85, J = 9.5 and 3.2 Hz (H-4 and H-7) and 7.37, J = 9.5 and 3.2 Hz (H-5 and H-6). Larger differences occur in benzotriazol-1-yl derivatives, such as **5a**, in which the aromatic protons of the benzotriazole moiety appear as two doublets of doublets at δ 8.03 and 7.57, J = 8.5 and 1.1 Hz (H-4 and H-7) and two doublets of triplets at δ 7.45 and 7.32, J = 8.5 and 1.1 Hz (H-6 and H-5, respectively). Similar ^{1}H-NMR data of the aromatic protons of benzotriazole moiety occur in all derivatives **5** and **6**. ^{1}H-NMR data of the side chain of the compounds **5a–h** and **6a–h** are summarized in table III.

Compounds of the general formula **10** showed protonic patterns similar to those of the other 1-substituted analogues **5**. In compound **10a**, as an example, the aromatic protons of benzotriazole moiety appear as two doublets of doublets at δ 7.78 and 7.40, J = 8.5 and 1.1 Hz (H-4 and H-7) and two doublets of triplets at δ 7.30 and 7.16, J = 8.5 and 1.1 Hz (H-6 and H-5). Similar ^{1}H-NMR data of the aromatic protons of benzotriazole ring were found for all derivatives **10**. ^{1}H-NMR data of the side chain of the compounds **10a–g** are reported in table IV.

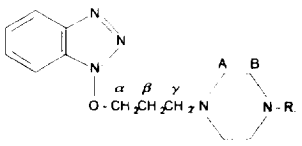
Finally, each new compound was characterized for its physicochemical properties (tables I and II) such as melting point and distribution coefficient (log D) measured in octanol/phosphate buffer at pH 7.4.

Table III. ^{1}H-NMR data of side-chains of benzotriazole derivatives **5a–h** and **6a–h**.



Cpd	*Solvent*	*^{1}H-NMR (δ)*	*Cpd*	*Solvent*	*^{1}H-NMR (δ)*
5a	$CDCl_3$	4.72 (t, 2H, αCH_2; J = 7.5 Hz); 2.92 (t, 2H, βCH_2; J = 7.5 Hz); 2.60 (t, 4H, ACH_2, J = 5.5 Hz); 3.08 (t, 4H, BCH_2; J = 5.5 Hz); 6.70–7.20 (m, 5H, arom)	**6a**	$CDCl_3$	4.82 (t, 2H, αCH_2; J = 7.5 Hz); 3.02 (t, 2H, βCH_2; J = 7.5Hz); 2.62 (t, 4H, ACH_2; J = 5.5 Hz); 3.09 (t, 4H, BCH_2; J = 5.5 Hz); 6.70–7.24 (m, 5H, arom)
5b	$CDCl_3$	4.80 (t, 2H, αCH_2; J = 7.5 Hz); 2 92 (t, 2H, βCH_2; J = 7.5 Hz ; 2.69 (t, 4H, ACH_2; J = 5.5 Hz); 3.18 (t, 4H, BCH_2; J = 5.5 Hz); 6.91–7.25 (m, 4H, arom)	**6b**	$CDCl_3$	4.90 (t, 2H, αCH_2; J = 7.5 Hz), 3.02 (t, 2H, βCH_2; J = 7.5 Hz); 2.73 (t, 4H, ACH_2; J = 5.5 Hz); 3.20 (t, 4H, BCH_2; J = 5.5 Hz); 6.88–7.24 (m, 4H, arom)
5c	$CDCl_3$	4.80 (t, 2H, αCH_2; J = 7.5 Hz); 3.02 (t, 2H, βCH_2, J = 7.5 Hz); 2.67 (t, 4H, ACH_2; J = 5.5 Hz); 3.15 (t, 4H, BCH_2, J = 5.5 Hz); 6.71–7.20 (m, 4H, arom)	**6c**	$CDCl_3$	4.72 (t, 2H, αCH_2; J = 7.5 Hz); 2.94 (t, 2H, βCH_2; J = 7.5 Hz); 2.50 (t, 4H, ACH_2; J = 5.5 Hz); 3.02 (t, 4H, BCH_2; J = 5.5 Hz); 6.52–7.05 (m, 4H, arom)
5d	$CDCl_3$	4.80 (t, 2H, αCH_2; J = 7.5 Hz); 3.02 (t, 2H, βCH_2; J = 7.5 Hz); 2.68 (t, 4H, ACH_2; J = 5.5 Hz); 3.12 (t, 4H, BCH_2; J = 5.5 Hz); 6.77–7.23 (m, 4H, arom)	**6d**	$CDCl_3$	4.90 (t, 2H, αCH_2; J = 7.5 Hz); 3.18 (t, 2H, βCH_2; J = 7.5 Hz); 2.69 (t, 4H, ACH_2; J = 5.5 Hz); 3.11 (t, 4H, BCH_2; J = 5.5 Hz); 6.76–7.24 (m, 4H, arom)
5e	$CDCl_3$	4.75 (t, 2H, αCH_2; J = 7.5 Hz); 2.85 (t, 2H, βCH_2, J = 7.5 Hz); 2.40–2.70 (m, 8H, ACH_2, BCH_2); 3.52 (s, 2H, NCH_2); 7.20–7.40 (m, 5H, arom)	**6e**	$CDCl_3$	4.88 (t, 2H, αCH_2; J = 7.5 Hz); 3.14 (t, 2H, βCH_2; J = 7.5 Hz); 2.35–2.67 (m, 8H, ACH_2, BCH_2); 3.49 (s, 2H, NCH_2); 7.20–7.34 (m, 5H, arom)
5f	D_2O	4.90 (t, 2H, αCH_2; J = 7.5 Hz); 3.50 (t, 2H, βCH_2; J = 7.5 Hz); 2.88 (t, 2H, CH_2-C; J = 7.5 Hz); 3.10–3.40 (m, 10H, ACH_2, BCH_2, NCH_2); 7.05–7.25 (m, 5H, arom)	**6f**	D_2O	5.10 (t, 2H, αCH_2; J = 7.5 Hz); 3.58 (t, 2H, βCH_2; J = 7.5 Hz); 3.00 (t, 2H, CH_2-C; J = 7.5 Hz); 3.20–3.55 (m, 10H, ACH_2, BCH_2, NCH_2); 7.12–7.32 (m, 5H, arom)
5g	D_2O	4.98 (t, 2H, αCH_2; J = 7.5 Hz); 3.69 (t, 2H, βCH_2; J = 7.5 Hz); 3.20–3.58 (m, 8H, ACH_2, BCH_2); 2.78 (s, 3H, NCH_3)	**6g**	$CDCl_3$	4.87 (t, 2H, αCH_2; J = 7.5 Hz); 3.19 (t, 2H, βCH_2; J = 7.5 Hz) 2.50–2.79 (m, 8H, ACH_2, BCH_2); 2.40 (s, 3H, NCH_3)
5h	$CDCl_3$	4.62 (t, 2H, αCH_2; J = 7.5 Hz); 2.80 (t, 2H, βCH_2; J = 7.5 Hz); 2.22–2.50 (m, 10H, ACH_2, BCH_2, NCH_2); 3.48 (t, 2H, CH_2OH, J = 7.5 Hz); 3.30 (bs, 1H, OH)	**6h**	$CDCl_3$	4.85 (t, 2H, αCH_2; J = 7.5 Hz); 3.12 (t, 2H, βCH_2; J = 7.5 Hz); 2.45–2.70 (m, 10H, ACH_2, BCH_2, NCH_2; 2.45–2.70 (m, CH_2OH; J = 7.5 Hz); 3:35 (bs, 1H, OH)

Table IV. ^{1}H-NMR data of side-chains of benzotriazole derivatives **10a–g**.



Compound	*Solvent*	*^{1}H-NMR (δ)*
10a	$CDCl_3$	4.50 (t, 2H, αCH_2; J = 7.5 Hz); 2.12 (m, 2H, βCH_2; J = 7.5 Hz); 3.67 (t, 2H, γCH_2; J = 7.5 Hz); 2.64 (t, 4H, ACH_2; J = 5.5 Hz); 3.21 (t, 4H, BCH_2; J = 5.5 Hz); 6.83–7.34 (m, 5H, arom)
10b	$CDCl_3$	4.67 (t, 2H, αCH_2; J = 7.5 Hz); 2.60 (m, 2H, βCH_2; J = 7.5 Hz); 3.40 (t, 2H, γCH_2; J = 7.5 Hz); 3.05 (t, 4H, ACH_2; J = 5.5 Hz); 3.23 (t, 4H, BCH_2, J = 5.5 Hz); 6.80–7.25 (m, 4H, arom)
10c	$CDCl_3$	4.65 (t, 2H, αCH_2; J = 7.5 Hz); 2.60 (m, 2H, βCH_2; J = 7.5 Hz); 3.20 (t, 2H, γCH_2; J = 7.5 Hz); 3.05 (t, 4H, ACH_2; J = 5.5 Hz); 3.25 (t, 4H, BCH_2; J = 5.5 Hz); 6.80–7.25 (m, 4H arom)
10d	$CDCl_3$	4.66 (t, 2H, αCH_2; J = 7.5 Hz); 2.10 (m, 2H, βCH_2; J = 7.5 Hz); 2.71 (t, 2H, γCH_2; J = 7.5 Hz); 2.63 (t, 4H, ACH_2; J = 5.5 Hz); 3.18 (t, 4H, BCH_2; J = 5.5 Hz); 6.80–7.25 (m, 4H, arom)
10e	$CDCl_3$	4.63 (t, 2H, αCH_2; J = 7.5 Hz); 2.05 (m, 2H, βCH_2; J = 7.5 Hz); 2.63 (t, 2H, γCH_2; J = 7.5 Hz); 2.35–2.60 (m, 8H, ACH_2, BCH_2); 3.52 (s, 2H, NCH_2); 7.21–7.38 (m, 5H, arom)
10f	$CDCl_3$	4.61 (t, 2H, αCH_2; J = 7.5 Hz); 2.05 (m, 2H, βCH_2; J = 7.5 Hz); 2.63 (t, 2H, γCH_2; J = 7.5 Hz); 2.38–2.62 (m, 8H, ACH_2, BCH_2); 2.32 (s, 3H, CH_3)
10g	$CDCl_3$	4.58 (t, 2H, αCH_2; J = 7.5 Hz); 2.00 (m, 2H, βCH_2; J = 7.5 Hz); 2.68 (t, 2H, γCH_2; J = 7.5 Hz); 2.38–2.60 (m, 10H, ACH_2, BCH_2, NCH_2); 3.60 (t, 2H, CH_2OH, J = 7.5 Hz); 3.42 (bs, 1H, OH)

Pharmacology

All compounds were tested *in vitro* for their antiserotonergic (rat stomach fundus), antiadrenergic (rat vas deferens), antihistaminic (guinea-pig ileum) activities and *in vivo* for analgesic activity (writhing test in mice). Trazodone was always used as a reference. Dose–response curves and IC_{50} or ED_{50} values were determined for each compound.

Results and discussion

The IC_{50} values of the antiserotonergic, antiadrenergic and antihistaminic *in vitro* activities and the ED_{50} values of analgesic *in vivo* activity of the tested compounds are reported in table V.

The inhibitory activity against serotonin-induced contractions was evaluated on isolated rat stomach fundus and compared with that of trazodone. Generally, 1-[2-[4-(X)-1-piperazinyl]ethyl]benzotriazole derivatives **5a–h** exhibited a potency superior to or comparable with respect to the corresponding 2-substituted isomers **6a–h**. The three series of compounds **5**, **6** and **10** generally appeared to be less potent than their 1-[3-[4-(X)-1-piperazinyl]propyl]-benzotriazole analogues described in a previous report [1] as well as trazodone. Compounds bearing aliphatic substituents at the piperazine 4-nitrogen were completely devoid of activity (**5g, h** and **6g, h**) or characterized by extremely poor potency (**10f, g**).

In derivatives **5** and **6**, the introduction of an *ortho*-chloro or *meta*-chloro group at the phenyl ring of the side chain increased activity, whereas shifting the chloro substituent to the *para*-position of the same ring caused an about 100- or 10-fold drop in activity. This pattern parallels that found for the previously studied benzotriazole analogues featuring a propylene

bridge [1], thus suggesting that the considered compounds interact with the receptor in a similar fashion.

It seems that the relatively higher conformation flexibility of derivatives **10** has the effect of smoothing differences in potency between the most and less potent compounds.

The introduction of a methylene (**5e**, **6e** and **10e**) or an ethylene bridge (**5f** and **6f**) between the piperazine 4-nitrogen and the phenyl ring led to products with lower activity. By a comparison of the distribution coefficient (log D) values listed in tables I and II, it is clear that such a decrease of activity, rather than being caused by hydrophobic effects, depends on steric and/or electronic factors.

The antiadrenergic activity of benzotriazole derivatives was determined by their ability to block the contractions induced by norepinephrine in rat vas deferens. Similarly to what found for our previously described 1- and 2-[3-[4-(X)-1-piperazinyl]propyl]-benzotriazoles [1], the antiadrenergic activity of 4-(phenyl)- and 4-(chlorophenyl)piperazinyl derivatives (**5a–d**, **6a–d** and **10a–d**) was comparable to that of trazodone. In fact, from the data listed in table V, it can be noted that the above analogues and trazodone itself displayed potencies comprised within a 10-fold range of IC_{50} values (with **5d** and **5b** being the least and the most active compounds, respectively). All the remaining products were characterized by a noradrenaline potentiating effect.

The *in vitro* antihistaminic effects were measured on guinea-pig ileum against histamine-induced contractions and are listed in table V. The highest activity was exhibited by compounds characterized by the simultaneous presence of an oxypropylene bridge and an aromatic moiety in the side chain (the order of potency was **10b** < **10a** < **10c** = **10d** < **10e**). In analogues **10**, when the aryl or benzyl substituent was replaced by a methyl or a β-hydroxyethyl group, an about 100- or 1000-fold drop of activity was observed. Compounds **5** and **6** were generally less potent than the corresponding analogues **10**; the most potent compound was **5d**, with an activity slightly lower than that of trazodone.

Table V. Antiserotonergic (5-HT), antiadrenergic (NE), antihistaminic (Hist) and analgesic activities (writhing test) of 1- and 2-[2-[4-(X)-1-piperazinyl]ethyl]benzotriazoles (**5a–h** and **6a–h**) and of 1-[3-[4-(X)-1-piperazinyl]propoxy]benzotriazoles (**10a–g**).

Compound	*IC_{50} (mol/l)*			*ED_{50} (mol/kg) analgesic activity*
	Anti-5-HT	*Anti-NE*	*Anti-Hist*	
5a	$7.0 (\pm 0.28) \times 10^{-6}$	$1.3 (\pm 0.25) \times 10^{-7}$	$7.0 (\pm 0.28) \times 10^{-7}$	$1.3 (\pm 0.24) \times 10^{-5}$
5b	$7.4 (\pm 0.38) \times 10^{-7}$	$2.0 (\pm 0.30) \times 10^{-8}$	$1.3 (\pm 0.24) \times 10^{-6}$	$1.6 (\pm 0.25) \times 10^{-5}$
5c	$8.8 (\pm 0.37) \times 10^{-7}$	$1.9 (\pm 0.29) \times 10^{-7}$	$4.6 (\pm 0.30) \times 10^{-7}$	$2.0 (\pm 0.31) \times 10^{-5}$
5d	$1.2 (\pm 0.40) \times 10^{-5}$	$2.2 (\pm 0.26) \times 10^{-7}$	$2.0 (\pm 0.28) \times 10^{-7}$	$> 10^{-4}$
5e	$3.5 (\pm 0.36) \times 10^{-6}$	*	$1.6 (\pm 0.34) \times 10^{-6}$	$2.1 (\pm 0.30) \times 10^{-5}$
5f	$4.2 (\pm 0.38) \times 10^{-5}$	*	$2.1 (\pm 0.25) \times 10^{-6}$	$1.4 (\pm 0.25) \times 10^{-5}$
5g	$> 1.0 \times 10^{-4}$	*	$7.0 (\pm 0.32) \times 10^{-6}$	$> 10^{-4}$
5h	$> 1.0 \times 10^{-4}$	*	$9.0 (\pm 0.30) \times 10^{-6}$	$> 10^{-4}$
6a	$8.6 (\pm 0.30) \times 10^{-6}$	$9.0 (\pm 0.27) \times 10^{-8}$	$4.7 (\pm 0.28) \times 10^{-6}$	$2.1 (\pm 0.32) \times 10^{-5}$
6b	$6.2 (\pm 0.26) \times 10^{-6}$	$2.3 (\pm 0.20) \times 10^{-8}$	$5.8 (\pm 0.38) \times 10^{-6}$	$1.6 (\pm 0.26) \times 10^{-5}$
6c	$1.3 (\pm 0.23) \times 10^{-6}$	$1.7 (\pm 0.22) \times 10^{-7}$	$1.7 (\pm 0.24) \times 10^{-6}$	$2.2 (\pm 0.33) \times 10^{-5}$
6d	$2.4 (\pm 0.35) \times 10^{-5}$	$1.2 (\pm 0.23) \times 10^{-7}$	$1.3 (\pm 0.20) \times 10^{-6}$	$1.9 (\pm 0.28) \times 10^{-5}$
6e	$6.4 (\pm 0.38) \times 10^{-5}$	*	$6.0 (\pm 0.26) \times 10^{-7}$	$> 10^{-4}$
6f	$4.6 (\pm 0.40) \times 10^{-5}$	*	$1.0 (\pm 0.30) \times 10^{-6}$	$1.2 (\pm 0.34) \times 10^{-5}$
6g	$> 1.0 \times 10^{-4}$	*	$1.2 (\pm 0.24) \times 10^{-5}$	$> 10^{-4}$
6h	$> 1.0 \times 10^{-4}$	*	$2.0 (\pm 0.26) \times 10^{-5}$	$> 10^{-4}$
10a	$1.0 (\pm 0.30) \times 10^{-6}$	$1.2 (\pm 0.22) \times 10^{-8}$	$2.1 (\pm 0.26) \times 10^{-7}$	$1.5 (\pm 0.20) \times 10^{-5}$
10b	$1.1 (\pm 0.38) \times 10^{-6}$	$2.8 (\pm 0.26) \times 10^{-8}$	$4.2 (\pm 0.28) \times 10^{-7}$	$1.2 (\pm 0.22) \times 10^{-5}$
10c	$7.0 (\pm 0.40) \times 10^{-7}$	$1.2 (\pm 0.28) \times 10^{-7}$	$6.0 (\pm 0.30) \times 10^{-8}$	$1.8 (\pm 0.24) \times 10^{-5}$
10d	$4.7 (\pm 0.28) \times 10^{-6}$	$6.2 (\pm 0.30) \times 10^{-8}$	$6.0 (\pm 0.32) \times 10^{-8}$	$> 10^{-4}$
10e	$6.2 (\pm 0.32) \times 10^{-6}$	*	$4.5 (\pm 0.30) \times 10^{-8}$	$2.0 (\pm 0.35) \times 10^{-5}$
10f	$3.4 (\pm 0.42) \times 10^{-5}$	*	$2.8 (\pm 0.22) \times 10^{-5}$	$> 10^{-4}$
10g	$7.0 (\pm 0.38) \times 10^{-5}$	*	$2.5 (\pm 0.20) \times 10^{-5}$	$> 10^{-4}$
Trazodone	$6.4 (\pm 0.35) \times 10^{-7}$	$5.8 (\pm 0.30) \times 10^{-8}$	$1.4 (\pm 0.22) \times 10^{-7}$	$1.5 (\pm 0.25) \times 10^{-5}$

(*) These compounds strengthened NE agonistic action.

ED_{50} values of the *in vivo* analgesic activity are reported in table V. The writhing test is sensitive and, as a first approximation, predictive of activity for a variety of peripherally acting analgesics. Examination of these data indicates that several of the synthesized compounds have the same order of activity of trazodone. Note that the range spanned by the ED_{50} values is very small. As a general trend, activity is observed when the lipophilicity of the compound is ranging within the interval 2.6 to 3.8 of log D units. Only in compounds **5d**, **6e** and **10d**, as well as in the more hydrophilic compounds bearing the 4-methyl or 4-hydroxyethylpiperazinyl substituent, is the analgesic activity drastically reduced. Although these findings cannot be regarded as definitive in view of the narrow data variance, they nevertheless suggest that any general hypothesis regarding the analgesic activity of the compounds under study must include the effects of hydrophobic interactions.

In conclusion, of the newly synthetized benzotriazole derivatives, it seems that compounds **5c**, **10c** and **5b** display overall pharmacological profiles which best resemble that exhibited by trazodone.

Experimental protocols

Chemisry

Melting points were determined on a Kofler hot-stage apparatus and are uncorrected. All pure compounds gave a satisfactory analysis (C, H, Cl, N) within ±0.3% of the theoretical values.

^{1}H NMR spectra were recorded on a Bruker WM 250 spectrometer using tetramethylsilane as an internal standard when required. Chemical shifts are expressed in units (ppm) and the splitting patterns are designated as follows: s singlet, bs broad singlet, t triplet, dd doublet of doublets, m multiplet. The spectra obtained confirmed the proposed structures.

Analytical TLC was performed on precoated silica-gel (0.2 mm GF 254, 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 (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.

The hydrochloride salts were potentiometrically titrated in glacial acetic acid by adding excess mercuric acetate and using a standard solution of acetous perchloric acid for titration. The equivalent weights of the compounds **5f**, **6f**, **5g**, **6g**, **5h**, **6h**, **10f** and **10g** were consistent with a dihydrochloride salt whereas derivatives **10b** and **10c** resulted as monohydrochloride salt (experimental error ± 1%).

The distribution coefficients (log D) were determined according to the classic shake-flask procedure [5] 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 two phases were therefore adjusted in volume so that satisfactory amounts of compound were present in each phase after partitioning. The concentrations were measured using a Beckman DU40 spectrophotometer. Partitionings were made at four different concentrations to be sure that special interactions were not occurring and to check against other errors. Tables I and II summarize these data.

4-Substituted 1-(2-chloroethyl)piperazine derivatives ***3***

1-Bromo-2-chloroethane (21.5 g, 0.15 mole) was refluxed in 80 ml toluene under stirring and 0.1 mole of the appropriate *N*-substituted piperazine in 30 ml toluene was added dropwise over 1 h. The reaction was monitored using TLC and diethyl ether as eluent. After this time the mixture was cooled and poured into water. The aqueous layer was alkalinized with 2 N NaOH and extracted several times with chloroform. The organic layer was washed with water and dried over anhydrous Na_2SO_4. It was then filtered and the solvent evaporated to dryness *in vacuo*. The resulting crude product was readily purified by passing it through a chromatographic column packed with silica gel using diethyl ether as eluent to obtain pure derivatives **3** (48–60% yield).

1- and 2-[3-[4-(X)-1-Piperazinyl]ethyl]benzotriazoles ***5a–h*** *and* ***6a–h***

In a general procedure, these compounds were prepared by alkylating benzotriazole with sodium ethoxide and the appropriate 4-substituted 1-(2-chloroethyl) piperazine in anhydrous ethanol, according to the reported procedure [1].

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

Characterization of 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 the appropriate solvent. Relevant data for each compound are given in table I.

1-(3-Chloropropoxy)benzotriazole ***9***

To a solution of 1-hydroxybenzotriazole (0.05 mol) and NaOH (0.05 mol) in ethanol (50 ml), was added 1-bromo-3-chloropropane (0.05 mol). The reaction was heated under reflux for 3 h, and successively cooled, filtered and evaporated to dryness. The resulting residue was dissolved in chloroform (150 ml) and extracted with 2 N NaOH. The organic phase was washed with saturated aqueous NaCl, dried over Na_2SO_4 and evaporated to dryness. The resulting crude product was purified by passing it through a chromatographic column packed with silica gel using diethyl ether/hexane 9:1 v/v as eluent; pure derivative **9** was obtained as a pale-yellow oil (5.8 g; 55% yield).

1-[3-[4-(X)-1-Piperazinyl]propoxy]benzotriazoles ***10a–g***

Triethylamine (0.02 mol) and the appropriate *N*-substituted piperazine were gently added to a solution of 1-(3-chloropropoxy)benzotriazole **9** (0.02 mol) in toluene (50 ml). The reaction mixture was vigorously stirred and refluxed for 24–72 h and monitored by TLC. After cooling the reaction mixture was extracted several times with 2 N HCl. The aqueous layer was alkalinized with 2 N NaOH and extracted with chloroform. The combined organic extracts were washed with water, dried over Na_2SO_4 and evaporated *in vacuo*. The resulting crude product was purified by silica-gel column chromatography using diethyl ether as eluent. Further purification of each product was obtained by crystallization from the appropriate solvent. Relevant data for each compound are given in table II.

Pharmacology

In vitro experiments

Rat stomach fundus, guinea-pig ileum and rat vas deferens were employed to measure the *in vitro* pharmacologic activity of compounds under study. Male albino guinea pigs (Arvel, Brusciano, Naples, 250–300 g) and male Wistar rats (Nossan, Correzzana, Milan, 220–250 g) were used. After sacrifice of the animals, the required organs were set up rapidly in the appropriate solution oxygenated with 95% O_2 and 5% CO_2.

The rat stomach fundus was suspended in Krebs bicarbonate solution at 37°C; contractions were induced by serotonin (2×10^{-8} M). The rat vas deferens was suspended in Krebs bicarbonate solution at 35°C; contractions were induced by norepinephrine (3×10^{-7} M). Segments of guinea-pig ileum were suspended in Tyrode's solution at 37°C; contractions were induced by adding histamine (3×10^{-7} M) to the bath.

The tested compounds were added 2 min before the agonists. In order to increase the solubility in water all compounds were used as hydrochlorides. The concentration of each compound inhibiting 50% of responses (IC_{50}) was determined using a curve-fitting program [6]. The data reported are means ± SEM of at least 3 determinations.

Analgesic activity

Analgesic activity was evaluated on male Swiss mice (Nossan, Correzzana, Milan, 18–20 g) using the acetic acid-induced writhing test [7]. The compounds were administered orally at different doses 60 min before intraperitoneal injection of acetic acid (0.25 ml of a 0.5% solution). The number of writhes was counted for 20 min. The ED_{50} was calculated from the percentage of inhibition as compared to the controls.

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