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Synthesis and pharmacological evaluation of aryl/heteroaryl piperazinyl alkyl benzotriazoles as ligands for some serotonin and dopamine receptor subtypes

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Abstract

Thirteen [(aryl/heteroaryl-piperazinyl)alkyl]benzotriazoles were prepared as potential trazodone- and buspirone-like drugs. The synthesized compounds displayed from moderate to good affinity to the serotonin 5-HT_{1A} receptor and only modest or poor affinity to the dopamine D_2 receptor, similar to buspirone. The introduction of substituents on the benzotriazole ring did not improve the affinity to the 5-HT_{1A} receptor, compared to the previously described unsubstituted derivatives. In a general pharmacological screening, which concerned only three of these compounds so far (**5**, **7** and **13**), several in vitro and in vivo activities were observed.

The guinea pig ileum contractions, induced either electrically or by several agonists, were strongly inhibited; at higher concentrations also the spontaneous tone of the guinea pig trachea was reduced. Compound **13** exhibited good analgesic activity in mice in the formalin-induced algesia and in the writhing test. The same at 30 mg kg^{-1} p.o. also displayed antihypertensive activity probably related to calcium channel blockade and adrenergic α_1 antagonism. In binding assays, **13** showed a $IC_{50} = 580$ nM for displacing [^{3}H]prazosin from α_1 receptor.

Finally, compound **5** (and, to a minor extent, compound **13**) protected mice against potassium cyanide induced hypoxia.

Keywords: Benzotriazole derivatives; Serotonin 5-HT_{1A} receptor; Dopamine D_2 receptor; Analgesics; Antihypertensives; Antihypoxia agents

1. Introduction

For a long time we have been engaged in the study of benzotriazole derivatives of potential pharmacological interest. From 1962 to 1965 we described sets of 1/2-(aminoalkyl)benzotriazoles including some (piperazinyl)alkyl derivatives [1,2]. At the same time, similar research was undertaken by other authors [3–9] and our interest shifted to other types of benzotriazole derivatives (see Refs. [10–15] and references cited therein).

Of particular concern were the 5-[(arylpiperazinyl)alkyl]benzotriazoles described in 1964 by Ash et al. [9]. Contrary to all expectations, they were not followed by analogous compounds bearing such basic side chains in positions 1 or 2 of the heterocyclic ring.

Thus, resuming our investigation on basic benzotriazoles, we thought it would be interesting to prepare 1/2-[(arylpiperazinyl)alkyl]benzotriazoles in order to obtain trazodone-like antidepressant agents or buspirone-like anxiolytics.

The psychotropic activity of trazodone and buspirone are mostly related to their action at the serotonin receptor level, where the former behaves as potent antagonist (mainly on 5-HT_{2A} receptors), while the latter is a 5-HT_{1A} partial agonist. Both drugs exhibit affinity for D_2 dopaminergic and α_1/α_2-adrenergic receptors, though of quite different levels. Trazodone is also an inhibitor of the serotonin uptake and displays antinociceptive activity, which, however, does not involve the opioid receptors.

As part of the present research program, Caliendo et al. [16–18] have already described several groups of 1/2-[(4-substituted-1-piperazinyl)alkyl]benzotriazoles,

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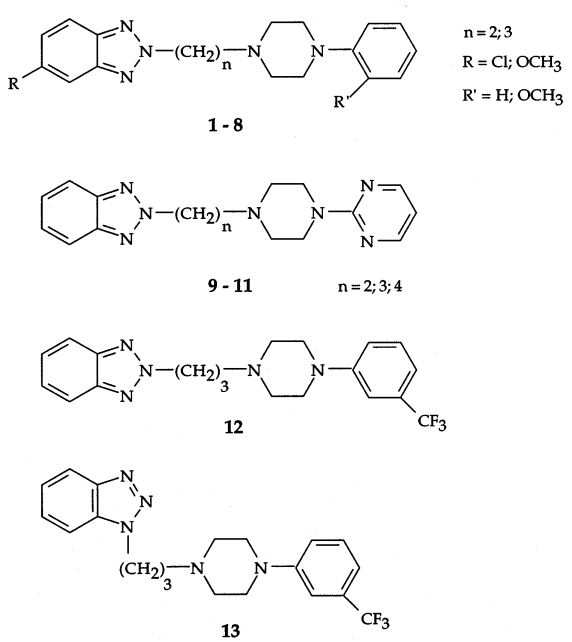


Scheme 1.

while Sparatore et al. [19–21] described some *O*-(dialkylaminoalkyl)- and *O*-[3-(4-aryl/heteroaryl-1-piperazinyl)propyl] - derivatives of 2 - (4 - hydroxyphenyl)-benzotriazole and corresponding N-oxides.

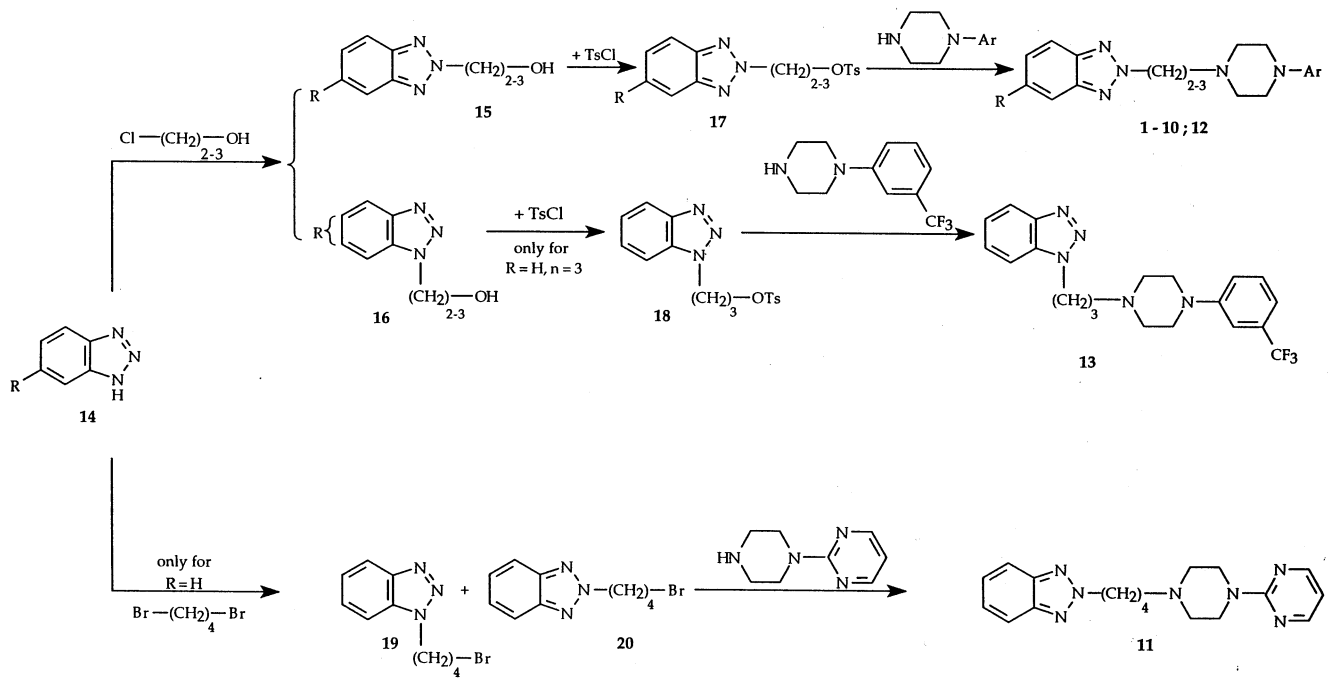
While observing the growing interest of other research groups on arylpiperazinylalkylbenzotriazoles [22–25], we deemed it convenient to describe the results of the binding assays on some serotonin (5-HT_{1A}, 5-HT_{2A}) and dopamine (D_2) receptors of 13 new [(aryl/heteroarylpiperazinyl)alkyl]benzotriazoles **1–13** [26], even though the collateral investigation of their general pharmacological profile is still under way and the data which are available concern only three of the prepared compounds.

The new compounds (Scheme 1) are characterized by the presence of substituents (Cl, OCH_3) on the benzotriazole ring or by the presence of aryl/heteroaryl moieties (2-pyrimidinyl; 3-trifluoromethylphenyl), which have not yet been taken into account. The former is present in buspirone, ipsapirone, lesopitron and other anxiolytics; the latter is present in trifluoromethylphenylpiperazine (TFMPP) which is a well known ligand for 5-HT_1 (more specifically 5-HT_{1B}) and 5-HT_2 receptors [27,28].

2. Chemistry

Compounds **1–13** were synthesized as outlined in Scheme 2.

The required benzotriazoles with R = H and Cl are commercially available, while the 5-methoxyderivative was prepared through modification [29] of the method used by Fel'dman et al. [30]. The suitable benzotriazole in alkaline solution was alkylated by means of 2-chloroethanol or 3-chloropropanol as previously described [10]. Starting from unsubstituted benzotriazole, two isomers were obtained, while from 5-substituted



Scheme 2.

Table 1
Characteristics of unknown intermediates **15** and **17** (Scheme 2)

Compound	R	*n*	Formula [a]	M.p. (°C)	Solvent [b]	Yield (%)
15	Cl	2	$C_8H_8ClN_3O$	86.5–87	A	36
15	OCH_3	2	$C_9H_{11}N_3O_2$	90–91	A	33
15	Cl	3	$C_9H_{10}ClN_3O$	Oil		40
15	OCH_3	3	$C_{10}H_{13}N_3O_2$	Oil		41
17	H	2	$C_{15}H_{15}N_3O_3S$	108–109	A	77
17	Cl	2	$C_{15}H_{14}ClN_3O_3S$	120–122	B	83
17	OCH_3	2	$C_{16}H_{17}N_3O_4S$	116–117	B	88
17	Cl	3	$C_{16}H_{16}ClN_3O_3S$	69–70	B	68
17	OCH_3	3	$C_{17}H_{19}N_3O_4S$	57–58	B	77

[a] All unknown intermediates were analysed for C, H, N and the results were within $\pm 0.3\%$ of the calculated values.
[b] A, dry ether; B, ethanol.

Table 2
Analytical results of intermediates **15** and **17**

Compound	R	*n*	Formula	%C		%H		%N	
				Found	Calc.	Found	Calc.	Found	Calc.
15	Cl	2	$C_8H_8ClN_3O$	48.37	48.61	3.99	4.08	21.21	21.26
15	OCH_3	2	$C_9H_{11}N_3O_2$	55.65	55.95	5.67	5.74	21.63	21.75
15	Cl	3	$C_9H_{10}ClN_3O$	50.83	51.07	4.94	4.76	20.13	19.85
15	OCH_3	3	$C_{10}H_{13}N_3O_2$	58.24	57.96	6.02	6.32	20.14	20.28
17	H	2	$C_{15}H_{15}N_3O_3S$	56.54	56.76	4.66	4.76	13.27	13.26
17	Cl	2	$C_{15}H_{14}ClN_3O_3S$	51.00	51.21	3.95	4.01	11.90	11.94
17	OCH_3	2	$C_{16}H_{17}N_3O_4S$	55.06	55.32	4.82	4.93	11.98	12.10
17	Cl	3	$C_{16}H_{16}ClN_3O_3S$	52.37	52.53	4.37	4.41	11.40	11.49
17	OCH_3	3	$C_{17}H_{19}N_3O_4S$	56.39	56.50	5.28	5.30	11.54	11.63

Table 3
Characteristics of compounds **1**–**13** (Scheme 1)

Compound	R	*n*	R′	M.p. (°C) free base	M.p. (°C) hydrochloride	Yield (%)	Formula [a]
1	Cl	2	H	108–110	218–219	81 [b]	$C_{18}H_{20}ClN_5 \cdot HCl$
2	OCH_3	2	H	128–130	227–228	84 [b]	$C_{19}H_{23}N_5O \cdot HCl$
3	Cl	2	OCH_3	Oil	251–253 dec.	74 [c]	$C_{19}H_{22}ClN_5O \cdot HCl$
4	OCH_3	2	OCH_3	Oil	237–238	85 [b]	$C_{20}H_{25}N_5O_2 \cdot HCl$
5	Cl	3	H	148–149	205–207	81 [b]	$C_{19}H_{22}ClN_5 \cdot HCl$
6	OCH_3	3	H	Oil	200–202	77 [b]	$C_{20}H_{25}N_5O \cdot HCl$
7	Cl	3	OCH_3	Oil	205–207	79 [c]	$C_{20}H_{24}ClN_5O \cdot HCl$
8	OCH_3	3	OCH_3	Oil	245–247	77 [c]	$C_{21}H_{27}N_5O_2 \cdot HCl \cdot 0.5\ H_2O$
9		2		Oil	225–227	69 [c]	$C_{16}H_{19}N_7 \cdot 2HCl$
10		3		Oil	238–240	84 [c]	$C_{17}H_{21}N_7 \cdot 2HCl \cdot 0.5H_2O$
11		4		Low melting solid	242–244	60 [b]	$C_{18}H_{23}N_7 \cdot 2HCl$
12				Oil	190–192	77 [b]	$C_{20}H_{22}F_3N_5 \cdot HCl \cdot 0.5H_2O$
13				Oil	214–216	77 [b]	$C_{20}H_{22}F_3N_5 \cdot HCl$

[a] All compounds were analysed as hydrochlorides for C, H, N and the results were within $\pm 0.3\%$ of the calculated values.
[b] Yield calculated on the free base purified by chromatography.
[c] Yield calculated after conversion of free base to hydrochloride.

benzotriazoles, three isomers were formed. The thorough separation of the isomeric derivatives is very important in order to define the meaning of the bearing of the final basic chain on position 1 or 2. 1- and 2-(ω-hydroxyalkyl)benzotriazoles were separated taking advantage of the basicity of the 1-substituted isomer, which was precipitated as the hydrochloride from the ether solution, while the non-basic 2-substituted isomer remained in the solution. The two isomers were further purified by chromatography on silica using dichloromethane as eluent. The separation of the non-basic 2,5-disubstituted derivatives from the other iso-

mers was performed as usual, but the separation of each of the two basic (1,5- and 1,6-disubstituted derivatives) isomers is very tedious and has not been pursued further at the moment.

The hydroxyalkylbenzotriazoles were converted to tosylates, which were then reacted with the appropriate *N*-aryl/heteroarylpiperazine in a 1:2 molar ratio.

The above procedure was not suitable for the preparation of 2-(4-hydroxybutyl)benzotriazole, which was formed in only low yields when benzotriazole was reacted with 4-chlorobutanol as already observed in the past [10]. In fact a large quantity of 1,4-bis(benzotriazol-1-yl)-butane was isolated and its presence made the separation of the 1- and 2-substituted isomers still more difficult.

Though 2-(4-hydroxybutyl)benzotriazole could be prepared by reduction of ethyl benzotriazolylbutirate, we resorted to 2-(4-bromobutyl)benzotriazole, which was reacted with pyrimidinylpiperazine to prepare compound **11**. The bromobutyl derivative was obtained as suggested by Mokrosz et al. [22] from benzotriazole and a large excess of 1,4-dibromobutane in the presence of the catalyst (KF/Al_2O_3) introduced by Texier-Boullet et al.

Table 4
Analytical results of compounds **1–13** hydrochlorides

Compound	Formula	%C		%H		%N	
		Found	Calc.	Found	Calc.	Found	Calc.
1	$C_{18}H_{20}ClN_5 \cdot HCl$	57.15	57.42	5.60	5.67	18.51	18.62
2	$C_{19}H_{23}N_5O \cdot HCl$	61.04	61.30	6.47	6.43	18.73	18.74
3	$C_{19}H_{22}ClN_5O \cdot HCl$	55.89	55.93	5.68	5.75	17.15	17.23
4	$C_{20}H_{25}N_5O_2 \cdot HCl$	59.47	59.18	6.49	6.49	17.34	17.37
5	$C_{19}H_{22}ClN_5 \cdot HCl$	57.92	58.17	5.90	5.91	17.82	17.85
6	$C_{20}H_{25}N_5O \cdot HCl$	61.79	61.93	6.77	6.76	18.17	18.05
7	$C_{20}H_{24}ClN_5O \cdot HCl$	56.88	56.58	5.97	5.88	16.58	16.83
8	$C_{21}H_{27}N_5O_2 \cdot HCl \cdot 0.5\ H_2O$	59.08	58.96	6.85	6.66	16.40	16.54
9	$C_{16}H_{19}N_7 \cdot 2\ HCl$	50.51	50.26	5.74	5.54	25.48	25.65
10	$C_{17}H_{21}N_7 \cdot 2\ HCl \cdot 0.5\ H_2O$	50.61	50.38	6.15	5.97	23.98	24.19
11	$C_{18}H_{23}N_7 \cdot 2\ HCl$	52.41	52.69	6.31	6.14	24.03	23.89
12	$C_{20}H_{22}F_3N_5 \cdot HCl \cdot 0.5\ H_2O$	55.01	55.24	5.40	5.56	16.28	16.10
13	$C_{20}H_{22}F_3N_5 \cdot HCl$	56.19	56.40	5.71	5.44	16.40	16.44

Table 5
Results of binding assays on serotonin (5-HT_{1A}, 5-HT_2) and dopamine D_2 receptors

Compound	*n*	R	R′	IC_{50} (nM) [a]			Ratio IC_{50}	
				5-HT_{1A}	5-HT_{2A}	D_2	D_2/5-HT_{1A}	5-HT_{2A}/5-HT_{1A}
1	2	Cl	H	950		6500	6.84	
2	2	OCH_3	H	800		6000	7.50	
3	2	Cl	OCH_3	45		700	15.56	
4	2	OCH_3	OCH_3	50		800	16.0	
5	3	Cl	H	850	300	3000	3.53	0.35
6	3	OCH_3	H	200	150	2500	12.50	0.75
7	3	Cl	OCH_3	90		400	4.45	
8	3	OCH_3	OCH_3	45		400	8.89	
9	2			2500		>10000	>4	
10	3			350		7500	21.43	
11	4			100		900	9.0	
12	3			600	450	3000	5.0	0.75
13	3			250	150	2500	10	0.6
8-OH-DPAT				0.88				
Ketanserin					3.0			
(+)Butaclamol						14		
Trazodone [23]				244 [b]	38 [b]			0.16 [b]
Buspirone [42]				30	>10000	280	9.33	>333

[a] IC_{50} values are the mean of duplicate experiments.
[b] K_i values and ratio K_i values.

Table 6
Maximum tolerated dose in mice [37] for compounds **5**, **7** and **13**

Administr. route	Dose (mg kg^{-1})	No of animals dead (and effects) after treatment with compounds		
		5	**7**	**13**
Oral	300	0/3 Sl. decrease spont. act.and limb tone	0/3 Sl. decrease spont. act. and explor. behav. sl. ataxia	0/3 Sl. ataxia and salivation and muscle relax.
	100		Sl. decrease abdom. tone and limb tone	Sl. decrease limb tone
Intraperit.	100	0/3 Sl. decrease spont. act. sl. ataxia and muscle relax.	0/3 Sl. decrease abdom. tone and limb tone	0/3 Sl. decrease abdom. tone and limb tone
	30	No effect	No effect	No effect

Table 7
Some effects on CNS and analgesic activity in mice (nt, not tested)

Test	Route; dose (mg kg^{-1})	Effect	Effects produced by compound			Reference drug	Route; dose (mg kg^{-1})	Effect
			5	**7**	**13**			
Behaviour depress. [37]	p.o. 300	[a]	58	50	55			
Phenylquin. writhing [37]	p.o. 100	[b]	36	38	75	Aspirin	p.o. 100	68
	p.o. 30		nt	nt	33	Iibuprofen	p.o. 30	65
Formalin algesia [40]	p.o. 100	[c]	nt	nt	96	Aspirin	p.o. 300	52
	p.o. 30		nt	nt	78	Ibuprofen	p.o. 30	66
	p.o. 10		nt	nt	47	Morphine	p.o. 10	62
Tail flick [37]	i.p. 30	[d]	nt	nt	16	Tifluadom	i.p. 10	65
Dopamine antag. [38]	i.p. 30	[e]	17	17	17			

[a] Scores for treated animals (normal animals score 60 points; scores below 40 denote significant depression).
[b] % Inhibition of number of writhes.
[c] % Reduction of induced paw licking time recorded between 20 and 30 min after formalin injection.
[d] % Prolongation of the time to elicit pain response.
[e] % Reduction of apomorphine induced climbing behaviour.

Table 8
Antihypertensive activity and heart rate modification [36,37] in spontaneously hypertensive rats of compounds **5**, **7** and **13**

Comp.	Dose p.o. (mg kg^{-1})	% variation [a] of blood pressure after			% variation [a] of heart rate after		
		1 h	2 h	4 h	1 h	2 h	4 h
5	100	−8	−8	−4	−4	0	2
7	100	−3	−2	−1	−5	−3	−6
13	100	−10	−14	−14	−14	−20	−15
	30	−12	−15	−10	−6	−8	−7
	10	−8	−2	−2	−10	−4	−4
Prazosin	1	−12	−14	−14	1	3	3
Clonidine	0.1	−22	−22	−20	−22	−25	−22
Nifedipine	5	−18		−14	−7		−10

[a] Variation of blood pressure and heart rate higher than 10 and 20%, respectively, are considered highly significant.

[31], followed by the usual separation of the isomeric 1- and 2-(4-bromobutyl)benzotriazoles.

Usually Caliendo et al. [16–18] and Mokrosz et al. [22] effected the separation of the 1- and 2-substituted benzotriazoles through the chromatography on silica of the ''final'' piperazinyl derivative. Such a procedure

proved to be not as effective as the one we used due to the levelling effect of the piperazine moiety on the basicity of the isomeric compounds.

The structures of all the prepared compounds were supported by elemental analyses and spectral (UV and NMR) data.

The NMR spectra conformed to the assigned structures and did not exhibit any peculiar features, thus only three spectra are described in Section 4 as examples.

3. Pharmacology

Compounds **1**–**13** were assayed for their ability to displace specific radioligands from serotonin 5-HT_{1A} and dopamine D_2 receptors.

As a preliminary investigation four of these compounds (**5**, **6**, **12** and **13**), containing the phenyl- or 3-trifluoromethylphenylpiperazinylpropyl moieties, were also tested for affinity to 5-HT_{2A} receptor. The

Table 9
In vitro assays related to cardiocirculatory function (nt, not tested)

Test	Conc. (μM)	Effect	Response to compound			Reference drug	Conc. (μM)	Response
			5	**7**	**13**			
Cardiac inotropy (guinea pig left atria) [37]	100	[a]	7	−45	−33			
Cardiac chronotropy (guinea pig right atria) [37]	100	[a]	−33	−45	−21	Clonidine	3	−18
	30		−26	−40	−17			
	10		−12	−24	−13			
	3		−3	−8	−8			
Adrenergic β_1 antag. (guinea pig left atria) [39]	100	[b]	26	76	10	Propranolol	0.3	82
	30		nt	53	nt			
	10		nt	28	nt			
Ca^{2+} antag. (guinea pig left atria) [37]	100	[c]	0	2	53	Nifedipine	1	68
	30		nt	nt	19			
Rat portal vein spont. activated [37]	100	[d]	35	59	67	Cromakalim	0.3	81
	30		nt	29	59			
	10		nt	nt	16			
Rat portal vein K^+ depolarized [37]	100	[e]	34	64	73	Nifedipine	0.1	76
	30		nt	30	36			
Rat vas deferens contractility [19]	100	[f]	31	[g]	71	2-Chloroadenosine	1	73
	30		nt	[g]	62			
	10		nt	[g]	0			
Adrenergic α_1 antag. (rat vas deferens) [40]	100	[h]	100	100	98	Prazosin	0.08	70
	30		80	100	93			
	10		59	92	82			
	3		14	89	79			
	1		nt	82	70			
	0.3		nt	70	36			
	0.1		nt	20	nt			
Adrenergic α_2 antag. (rat vas deferens) [41]	100	[i]	89	100	0	Yohimbine	0.85	68
	30		73	84	nt			
	10		30	72	nt			
	3		nt	0	nt			

[a] % Variation in contractile force or rate.
[b] % Reduction of isoproterenol induced positive inotropic effect.
[c] % Reduction of Ca^{2+} induced increase in contractile force.
[d] % Inhibition of spontaneous movements.
[e] % Inhibition of K^+ induced contractions.
[f] % Reduction of neurogenic twitch response relative to 10 nM clonidine.
[g] Compound **7** strongly increases the electrically stimulated responses of rat vas deferens (at 10 μM concentration, the increase was still of 87%).
[h] % Reduction of phenylephrine induced contractile response.
[i] % Inhibition of clonidine-induced reduction in twitch response.

Table 10
In vivo assays related to cardiocirculatory function (nt, not tested)

Test	Dose (mg kg^{-1}); route	Effect	Response to compound			Reference drug	Dose (mg kg^{-1}); route	Response
			5	**7**	**13**			
Adrenergic α_1 antag. (in mice) [39]	30 i.p.	[a]	15	12	5			
Ganglionic blockade (mydriasis; in mice) [39]	30 i.p.	[b]	nt	nt	0			
Adrenergic α_2 antag. (in rat) [40]	30 i.p. 10 i.p. 3 i.p.	[c]	61 54 30	0 nt nt	nt nt nt	Yohimbine	10 i.p.	70
Hypoxia, cyanide induced (in mice) [39]	100 p.o. 30 p.o. 10 p.o.	[d]	80 60 0	0 nt nt	40 nt nt	Flunarizine	30 p.o.	60

[a] % Reduction of norepinephrine induced mydriasis.
[b] Pupil diameter exceeding 1 mm (>1) indicates significant ganglionic blockade.
[c] % Reversal of clonidine induced bradycardia.
[d] % Surviving mice 60 min after i.v. administration of submaximal lethal dose of KCN.

selection was made taking into account the results of Caliendo et al. [18] indicating the highest potency on 5-HT_{2A} receptor in compounds featuring a propylene bridge and a phenyl (or 3/4-chlorophenyl) group attached to the N_4-position of the piperazine ring.

Finally, compounds **5**, **7** and **13** were subjected to a large pharmacological screening which included 60 in vivo and in vitro assays so as to explore their toxicological and pharmacological profiles.

4. Experimental

Melting points were determined by the capillary method on a Büchi apparatus and are uncorrected.

Elemental analyses were performed with CE EA 1110 CHNS-O instruments and the results obtained for the indicated elements were within $\pm 0.3\%$ of the calculated values (Tables 2 and 4).

UV and IR spectra were recorded, respectively, on Perkin–Elmer model 550 S and Paragon 1000 PC spectrophotometers; ^{1}H NMR were taken on a Varian Gemini 200 spectrometer, using $CDCl_3$ as solvent with TMS as internal standard.

*4.1. (2-Hydroxyethyl)- and (3-hydroxypropyl)benzotriazoles (**15** and **16**)*

Benzotriazole or the suitable substituted benzotriazoles (65–84 mmol) were dissolved in 2 N NaOH (molar ratio 1:1.2) and treated with 65–84 mmol of 2-chloroethanol or 3-chloropropanol. The mixture was heated at 100°C for 90 min and, after cooling, extracted with dichloromethane. The solvent was removed and the residue was dissolved in dry ether and treated by stirring and cooling, drop by drop, with a solution of dry hydrogen chloride in ether, until the precipitate no longer increased. The ether solution, containing the 2-substituted isomer, was decanted, shaken with diluted ammonia water and evaporated to dryness.

The pasty precipitate (containing the hydrochlorides of unreacted benzotriazole and of 1-substituted isomer and minor amount of 2-substituted isomer) was taken up with diluted ammonia water and dichloromethane. The organic phase was evaporated to dryness. The two fractions were further purified by chromatography on silica gel, using dichloromethane as eluent in both cases.

Characteristics of new alcohols are collected in Table 1.

4.2. 1,4-Bis(benzotriazol-1-yl)butane

A similar reaction of benzotriazole with 4-chlorobutanol gave only a minor amount of 2-(4-hydroxybutyl)benzotriazole and a mixture of unreacted benzotriazole, 1-(4-hydroxybutyl)benzotriazole and, as main product, 1,4-bis(benzotriazol-1-yl)butane. After crystallization from ethanol the latter melted at 149°C. Analysis (C, H, N) for $C_{16}H_{16}N_6$. UV (ethanol) λ_{max} (log ε): 257 (4.02), 263 (4.02), 280 (3.86). ^{1}H NMR: δ 1.95–2.15 (m, 4H, C–CH_2–CH_2–C), 4.60–4.80 (m, 4H, 2 N–CH_2), 7.32–7.5 (m, 6H, arom), 8.03–8.1 (m, 2H, arom).

*4.3. (Tosyloxyalkyl)benzotriazoles (**17** and **18**)*

The suitable hydroxyalkyl benzotriazole (14–18 mmol) was dissolved in anhydrous benzene (13–15 ml), and triethylamine (excess 40%) and the equivalent

Table 11
In vitro assays for activity on guinea pig ileum contractions induced by several agents and for tracheal relaxation

Test	Conc. (μM)	Effect	Response to compound			Reference drug	Conc. (μM)	Response
			5	**7**	**13**			
Ca^{2+} antag; (channel type L) [37]	100	[a]	100	100	100	Nifedipine	0.01	78
	30		94	94	97			
	10		87	85	79			
	3		38	68	57			
	1		nt	23	20			
Angiotensin I inhibition [37]	100	[a]	100	100	100	Captopril	10	67
	30		53	80	100			
	10		18	21	81			
	3		nt	nt	5			
Angiotensin II (AT_1) antagon. [37]	100	[a]	90	100	100	[Sar^1, Thr^8] angiotensin II	0.1	94
	30		60	93	95			
	10		41	43	32			
Bradykinin B_2 antagon. [37]	100		100	100	100	Nifedipine	0.03	60
	30		94	75	100			
	10		67	56	50			
	3		6	23	19			
Cholecystokinin CCK_A antag. [37]	100	[a]	100	100	100	Devazepide	0.03	70
	30		92	76	96			
	10		53	54	68			
	3		34	12	6			
Leukotriene LTD_4 antagon. [38]	100	[a]	100	100	100	LY-17883 [b]	1	74
	30		93	99	95			
	10		38	81	83			
	3		nt	19	53			
	1		nt	nt	15			
Acetylcholine antagon. [37] [c]	100	a	100	100	100	Atropine	0.03	70
	30		93	88	92			
	10		36	71	72			
	3		nt	57	42			
	1		nt	22	nt			
Neurokinin NK_1 antagon. [37]	100	[a]	98	100	100	Spantide	1	65
	30		85	99	100			
	10		29	56	81			
	3		nt	13	18			
Inhibition elec-trically stimulated contractions [37]	100	[d]	+	+	+	Atropine	0.1	+
	30		+	+	+			
	10		+	+	+			
	3		−	−	−			
Naloxone antagon. (opiate agonism) [19]	10	[e]	−	−	−			
Tracheal contrac. (guinea pig) [37]	100	[f]	80; 68	71; 64	54; 57	Epinephrine	0.3	79
	30		25	25	13	Theophilline	166	60

[a] % Reduction of contractile response to the various agents.
[b] 5-[4-(4-Acetyl-3-hydroxy-2-propylphenoxy)butyl]tetrazole.
[c] The previously used methacoline chloride [37] was replaced by 0.55 μM acetylcholine chloride in the present assay.
[d] % Inhibition of contractions by more than 50% is recorded as significant (+).Inhibition less than 50% is recorded as (−).
[e] Reversal by naloxone of the reduction of contractile response elicited in the foregoing assay by the minimum inhibitory concentration, may indicate an opioid mechanism (+).
[f] % Inhibition of guinea-pig tracheal tone relative to relaxation induced by 0.3 μg ml^{-1} (1.6 μM) epinephrine.

amount of tosylchloride, dissolved in benzene (10 ml), were then added. The mixture was stirred for two days at room temperature. The benzene was removed under reduced pressure; the residue was dissolved in dichloromethane and treated three times with 5% sodium carbonate solution and, finally, with water.

After removing the solvent, the residue was either treated with a little dry ether giving a crystalline product, or chromatographed on silica gel eluting with dichloromethane.

Characteristics of new tosyl esters are collected in Table 1.

4.4. 1|2–(4-Bromobutyl)benzotriazole (**19** and **20**)

Compounds **19** and **20** were prepared according to literature [22] with only minor modifications. The reaction mixture was refluxed for 2 h and the mixture of isomers was separated by chromatography on silica gel using dichloromethane–*n*-hexane (1:1) as eluent. Each crude isomer was rechromatographed in the same manner. Yields and purity of both the isomers were improved by working on a larger scale than that indicated in Ref. [22].

4.5. 2-[(Aryl|heteroarylpiperazinyl)alkyl]benzotriazoles

The 2-(tosyloxyalkyl)benzotriazole (5-6 mmol) was dissolved in anhydrous benzene (15–40 ml) and suitable aryl/heteroarylpiperazine (10–12 mmol) dissolved in the same solvent (5–10 ml) was added. The mixture was stirred either (a) for four days at room temperature (**5**, **6**, **12** and **13**) or (b) refluxed for two (**3**, **4**, **7** and **10**) or three (**1** and **2**) days. The precipitated aryl/heteroarylpiperazine tosylate was filtered and the benzene solution evaporated to dryness. The residue was partitioned between dichloromethane and 1 N hydrochloric acid that removed the residual unreacted aryl/heteroarylpiperazine.

After evaporation of dichloromethane, the residue was washed with dry ether to remove the unreacted tosyl ester, leaving the tosylate of the final product as white crystals. This salt was dissolved in absolute ethanol and treated with an equivalent amount of 2 N ethanolic sodium hydroxide solution. The solvent was evaporated under reduced pressure and the residue was treated with water and ether, which extracted the title compound.

It was at times necessary to chromatography the final product on alumina (CH_2Cl_2) so as to remove traces of the tosyl ester which had not been eliminated by the ethereal washing.

In some cases (**1** and **2**) during the partitioning of the reaction mixture between dichloromethane and 1 N hydrochloric acid, the separation of a substantial amount of hydrochloride of the final product was observed.

Sometimes, the 1 N hydrochloric acid solution extracted a small amount of the final product besides the unreacted arylpiperazine. In such cases the acid solution was basified with strong ammonia water and extracted with dichloromethane. The extract was, finally, chromatographed on alumina eluting with dichloromethane.

In all cases, the free bases were converted to hydrochlorides by means of the stoichiometric volume of 1 N ethanolic HCl, evaporation to dryness and repeated washing with dry ether. If necessary the salt was recrystallized from ethanol.

Characteristics of title compounds are collected in Table 3.

Since the NMR spectra of all the prepared compounds were in conformity with the assigned structures, without any peculiar features, only the following are described as examples.

^1H NMR spectra. Compound **3**: 2.68–2.82 (m, 4H, $2CH_2$–N–CH_2), 2.97–3.13 (m, 4H, $2CH_2$–N–Ph), 3.13–3.29 (m, 2H, CH_2–N), 3.87 (s, 3H, OCH_3), 4.9 (t, $J = 6$ Hz, 2H, CH_2–Bzt), 6.83–7.04 (m, 4H, arom), 7.30–7.43 (m, 1H, arom), 7.80–7.92 (m, 2H, arom). Compound **7**: 2.20–2.45 (m, 2H, CH_2–CH_2–CH_2), 2.45–2.60 (m, 2H, CH_2–N), 2.60–2.70 (m, 4H, $2CH_2$–N–CH_2), 3.02–3.07 (m, 4H, $2CH_2$–N–Ph), 3.87 (s, 3H, OCH_3), 4.82 (t, $J = 6$ Hz, 2H, CH_2–Btz), 6.83–7.04 (m, 4H, arom), 7.30–7.38 (m, 1H arom), 7.78–7.88 (m, 2H arom). Compound **10**: 2.26–2.43 (m, 2H, CH_2–CH_2–CH2), 2.43–2.62 (m, 6H, $2CH_2$–N–$CH_2 + CH_2$–N), 3.80 (t, $J = 6$ Hz, 4H, $2CH_2$–N–pyrim), 4.85 (t, $J = 6$ Hz, 2H, CH_2–Btz), 6.46 (t, $J = 5$ Hz, 1H, arom), 7.37–7.42 (m, 2H, arom), 7.83–7.91 (m, 2H, arom), 8.30 (d, $J = 8$ Hz, 2H, arom).

4.6. 2-{4-[1-(2-Pyrimidinyl)piperazin-4-yl]butyl}-benzotriazole (**11**)

2-(4-Bromobutyl)benzotriazole (3.43 g, 13 mmol) and 2-pyrimidinylpiperazine (2.2 g, 13 mmol) were dissolved in *n*-butanol (95 ml) and 3.6 g of anhydrous potassium carbonate was added. The mixture was heated at reflux with stirring for 3 h and further stirred overnight at room temperature. The inorganic precipitate was filtered and most butanol was removed under reduced pressure; the residual butanol was eliminated through the formation of an azeotropic mixture with *n*-hexane. The residue was chromatographed on silica gel, eluting with dichloromethane plus 2% methanol. The first fractions were rinsed with dry ether to obtain 2.75 g (60% yield) of light greenish crystals. The corresponding dihydrochloride was colourless.

Characteristics of compound **11** are collected in Table 3.

4.7. Binding assays

Binding assays were performed at the Receptology Department of CEREP, Celle L'Evescault, France.

4.7.1. Serotonin 5-HT_{1A} binding [32]

Membranes were prepared by standard techniques from rat brain cortices and incubated with 0.5 nM [^{3}H]8-hydroxy-2-(di-*n*-propylamino)-tetraline (8-OH-DPAT) for 30 min at 22°C. Non-specific binding was determined in the presence of 10 μM 8-OH-DPAT. Membranes were rapidly filtered under vacuum through glass fibre filters. Filters were washed three times with ice-cold buffer and bound radioactivity was measured with a scintillation counter. Compounds are initially screened at 10 μM.

4.7.2. Serotonin 5-HT_{2A} binding [33]

Membranes from rat brain cortices were incubated with 0.5 nM [^{3}H]ketanserin for 15 min at 37°C. Non-specific binding was determined in the presence of 1 μM ketanserin.

4.7.3. Dopamine D_2 binding [34]

Membranes from rat striatum were incubated with 0.1 nM [^{3}H]-nemonapride (YM-09151-2) for 60 min at 22°C. Non-specific binding was determined in the presence of 10 μM (+)butaclamol.

4.7.4. Non-selective α_1-adrenergic binding [35]

Membranes from rat brain cortices were incubated with 0.25 nM [^{3}H]prazosin for 60 min at 22°C. Non-specific binding was determined in the presence of 0.5 μM prazosin.

4.8. General pharmacological screening

A general pharmacological screening (Pharma-Screen®) was performed by MDS-Panlabs, Bothell, WA, USA, on compounds **5**, **7** and **13** in the form of hydrochlorides. This screening consisted in the determination of the maximum tolerated dose in mice (MTD, p.o. and i.p.) with simultaneous behavioural examination (Irwin test) and in 34 primary in vivo tests (using a suitable MTD fraction, depending on the test type) and in 20 in vitro tests, concerning CNS, cardiovascular and gastrointestinal apparatuses, intermediary metabolism, allergy and inflammation.

For in vivo tests, compounds were generally administered orally using a gastric tube in the form of aqueous solutions or finely homogenized suspensions in Tween 80 (2%), when the highest dose (300 mg kg^{-1}) was used. In a few cases compounds were introduced intraperitoneally as aqueous solutions (10 ml kg^{-1}). Groups of three or five animals (rat or mice) were used. For in vitro assays, the dissolution of a test compound in a buffer or saline solution was speeded up by means of DMSO; the final concentration of DMSO, not interfering with the tests, was 0.1% for platelet aggregation and 0.5% for all others.

The procedures for these assays have already been described [19,36–41]; a pre-established level of response which is high enough to suggest significant activity is indicated for each assay. Doses (mg kg^{-1}) or concentrations (μg ml^{-1} or μM) indicated in the methods were the highest utilized routinely, depending on toxicity; when significant activity was detected, lower doses or concentrations were tested in order to define the minimal effective ones, and secondary tests were performed to provide some insight for the possible mechanisms of action.

In Tables 6–11 only results of the more significant assays are collected; the pertinent reference for the method employed is indicated near each assay name.

5. Results and discussion

Results of the binding assays are summarized in Table 5.

All tested compounds exhibited affinity to serotonin 5-HT_{1A} receptor from moderate to good. For compounds **3**, **4**, **7**, **8**, and **11** the affinity was higher than that of trazodone and often comparable with that of buspirone.

On the other hand, affinity for D_2 receptor was rather modest or definitely poor and, in any case, lower than that of buspirone. Therefore, some selectivity for 5-HT_{1A} receptor versus D_2 receptor was observed, which was maximal for compound **4** with a ratio of IC_{50} values of 16, while buspirone showed a ratio of 9.3.

The increasing length of the chain linking benzotriazole to piperazine increased the affinity for both 5-HT_{1A} and D_2 receptors; however, this observation does not hold for the affinity of compound **7** to 5-HT_{1A} receptor.

Concerning the affinity for 5-HT_{1A} receptor, a similar trend was observed for the corresponding unsubstituted benzotriazole derivatives, while the affinity for D_2 receptor was not investigated for the latter compounds.

The influence of the connective chain length on the affinity to receptor was, on the whole, inferior to that of the nature of the aromatic moiety joined to the other piperazine nitrogen. The 2-methoxyphenyl residue exerted the strongest enhancing effect, followed by the pyrimidinyl.

The enhancing effect of 2-methoxyphenyl group for the affinity to 5-HT_{1A} receptor is well known, while such an effect on the binding to D_2 receptor has also been recently observed by Perrone et al. [42] for other kinds of arylpiperazinyl derivatives.

Compound **11**, which shares the pyrimidinylpiperazinylbutyl moiety with buspirone, exhibited affinity for 5-HT_{1A} and D_2 receptors just a little inferior to those of the reference drug, but selectivity ratios (D_2/5–HT_{1A}) were practically identical. Thus the benzotriazole ring seems to be a suitable substitute for 8-azaspiro-[4,5]decan-7,9-dione moiety.

The effect on the affinity to the 5-HT_{1A} receptor of introducing substituents (Cl and OCH_3) on the benzotriazole was tentatively evaluated by comparing our binding results with data concerning the corresponding unsubstituted derivatives obtained by Caliendo et al. [18] and by Mokrosz et al. [22,23] using different experimental models. However, the former authors used recombinant human receptor and the latter hippocampus of rat brain preparation, while we used rat brain cortices. Nevertheless, it seems reasonable to assert that the presence of substituents on the benzotriazole nucleus does not improve the affinity to the 5-HT_{1A} receptor and may occasionally be deleterious.

One last comment is deserved for compounds **12** and **13**, which are derivatives of 3-trifluoromethylphenylpiperazine (TFMPP).

TFMPP is a potent ligand for 5-HT_{1B} receptor subtype ($K_i = 30$ nM), but it is a rather poor one for 5-HT_{1A} subtype ($K_i = 1950$ nM) [28], thus differing from *N*-(2-methoxyphenyl)piperazine (2-MPP) which exhibits a $K_i = 170$ nM for the latter receptor subtype [43].

The two ligands differ even further because of their affinity to the 5-HT_2 receptor, which was good for the former and poor for the latter with a consequent inverted selectivity to 5-HT_{1A} versus 5-HT_2 receptor [27].

The insertion of the 3-(1/2-benzotriazolyl)propyl residue on the piperazine nitrogen of TFMPP produced a levelling effect on the affinity to the 5-HT_{1A} and 5-HT_2 receptor, increasing the former but leaving the latter almost unchanged. The effect of this structural modification on the affinity to the 5-HT_{1B} subtype was not investigated.

On the contrary, the analogous insertion of the benzotriazolylpropyl residue on 2-MPP, operated by Mokrosz et al. [22], increased the affinity to both 5-HT_{1A} and 5-HT_2 receptor yet improving the selectivity to 5-HT_{1A} versus 5-HT_2 receptor subtype.

Results of the general pharmacological screening of compounds **5**, **7** and **13** are collected in Tables 6–11.

These compounds were well tolerated up to the dose of 300 mg kg^{-1} p.o. and 100 mg kg^{-1} i.p., with only a slight decrease of spontaneous activity, explorative behaviour and limb tone during the 72 h observation period.

The search for specific activities on CNS (anticonvulsive, 5-methoxy-*N*,*N*-dimethyltryptamine potentiation, oxotremorine antagonism, tetrabenazine hypothermia antagonism, modification of apomorphine-induced climbing behaviour) gave negative or subsignificant results.

Moderate analgesic activity in the writhing test was seen with compounds **5** and **7**, while compound **13** exhibited an activity comparable to that of aspirin at the same dose (100 mg kg^{-1}, p.o.). However, in the formalin algesia test, **13** was much more active than aspirin and almost comparable to morphine. It is worth noting that there is a structural similarity between compound **13** and 3-{3-[4-(3-trifluoromethylphenyl)-1-piperazinyl]-propyl}benzoxazolinone, which was found to be endowed with important analgesic activity [44]. The two compounds differ only because one contains a benzotriazole and the other a benzoxazolinone nucleus. Thus, the similar overall molecular geometry could be responsible for their analgesic activity, which deserves further investigation.

Concerning the cardio circulatory function (Tables 8–10), significant antihypertensive activity and the reduction of heart rate was observed with compound **13** at 30 mg kg^{-1} p.o. in spontaneous hypertensive rats, while compounds **5** and **7** showed, respectively, moderate or only minimal activity at 100 mg kg^{-1} p.o. The antihypertensive activity of **13** may relate, at least in part, to α_1-adrenoceptor antagonism, which was observed in vitro but not in vivo (inhibition of norepinephrine-induced mydriasis in mice), and also to calcium antagonism, which was observed in the guinea pig ileum and atria and in the rat portal vein. In addition, negative chronotropic activity was observed in spontaneously beating guinea pig right atria. Compound **13**, at concentrations of 3–10 μM, antagonized guinea pig ileum contractile responses induced by angiotensin I and II, LTD_4, bradykinin, substance P, methacholine, CCK and electrical stimulation, suggesting a general strong relaxant activity on the smooth muscle. On the other hand, ganglionic blockade (pupil dilation test) was not seen in mice.

The interaction of compound **13** with α_1-receptor was further supported by binding experiments, where a IC_{50} value of 580 nM was observed for the displacement of [^{3}H]prazosin from rat brain membrane preparation.

For compound **13**, adrenergic α_2-antagonism was not observed either in vitro (inhibition of clonidine-induced reduction of twitch responses of rat vas deferens) or in vivo (reverse of clonidine induced bradycardia in rats), while it was observed for compounds **5** and **7**.

The α_2-antagonism of these compounds could contribute to an explanation for their lack of antihypertensive activity in spite of being able to antagonize the guinea pig ileum contractions induced by many agonists and to exhibit calcium antagonism in some assays.

The in vivo α_2-antagonism of compound **5** was comparable to that of yohimbine at the same dose (10 mg kg^{-1} i.p.).

Compounds **5** and **7** (contrary to **13**) failed to reduce the electrically stimulated responses of rat vas deferens; moreover and quite unusually, compound **7** strongly increased these responses (even at 10 μM concentration, the increase was of 87%). At the moment it is not possible to make any inference on the meaning of this very uncommon response.

The three compounds, at 100 μM concentration, reduced the spontaneous tone in the guinea pig trachea. The observed activity was superior to or comparable with that of theophylline at 166 μM concentration (30 $\mu g\,ml^{-1}$). The relaxant action was unrelated to β_2-adrenoceptor agonism or cyclooxygenase inhibition (lack of any inhibition of arachidonic acid-induced platelet aggregation). Moreover these compounds failed to exhibit positive inotropic activity, while negative chronotropic activity was noted in guinea pig atria. Thus a theophylline-like mechanism of action was also excluded. A possible connection of this relaxant activity with the observed antagonism to leukotriene D_4 deserves further investigation.

Finally, it is worth noting that compound **5**, at the oral dose of 100 $mg\,kg^{-1}$, protects 80% of mice from mortality induced by e.v. injection of LD_{95} of potassium cyanide. Such antihypoxia action could be related to the observed calcium channel blockade and/or antagonism to leukotriene D_4 (Table 11) but compounds **7** and **13**, which were endowed with stronger calcium and leukotriene D_4 antagonism, failed to protect mice or exhibited only moderate protection.

A similar situation has already been met with other benzotriazole derivatives [19,20]; thus calcium channel blockade and leukotriene D_4 antagonism could be useful factors, but not sufficient to produce the antihypoxia effect.

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