ELSEVIER

www.elsevier.nl/locate/farmac

Il Farmaco 54 (1999) 402–410

IL FARMACO

2-{4-[3-(4-Aryl/heteroaryl-1-piperazinyl)propoxy]phenyl}-2*H*-benzotriazoles and their N-oxides as ligands for serotonin and dopamine receptors☆

Anna Sparatore [a,*], Mara Goegan [b], Alfredo Cagnotto [b], Fabio Sparatore [c]

[a] *Istituto di Chimica Farmaceutica e Tossicologica, Università di Milano, Viale Abruzzi 42, I-20131 Milan, Italy*
[b] *Istituto di Ricerche Farmacologiche 'Mario Negri', Via Eritrea 62, I-20157 Milan, Italy*
[c] *Dipartimento di Scienze Farmaceutiche, Università di Genova, Viale Benedetto XV 3, I-16132 Genoa, Italy*

Received 23 November 1998; accepted 24 March 1999

Abstract

A small set of 2-{4-[3-(4-aryl/heteroaryl-piperazinyl)propoxy]phenyl}-2*H*-benzotriazoles and corresponding N-oxides were prepared. The synthesized compounds were able to bind on some serotonin (5-HT_{1A}, 5-HT_{2A}) and dopamine (D_2, D_3) receptors, while displaying poor or no affinity for 5-HT_{1B}, 5-HT_{2C}, 5-HT_3, and 5-HT_4 subtypes. The strong contribution of the N-oxide function for the binding on 5-HT_{1A}, D_2 and D_3 receptors is noteworthy. For 2-{4-[3-[4-(2-methoxyphenyl)-1-piperazinyl]propoxy]phenyl}-2*H*-benzotriazol-1-oxide (**4b**), the binding constants (K_i) were 11.9 (5-HT_{1A}) and 10.5 nM (D_3). In a general pharmacological screening, the 2-{4-[3-(4-phenyl-1-piperazinyl)propoxy]phenyl}-2*H*-benzotriazole (**3a**) exhibited only very weak activities, with the exception of protecting mice from cyanide-induced hypoxia.

Keywords: Benzotriazoles; Benzotriazole-N-oxides; Serotonin receptor ligands; Dopamine receptor ligands

1. Introduction

In a recent note [1] we described the synthesis and the pharmacological investigation of a set of 2-[4-(dialkylaminoalkoxy)phenyl]benzotriazoles **1** and of the corresponding N-oxides **2**. Among the observed activities, it is worthy to recall the following. At concentrations ranging from 3 to 10 μM, all tested compounds strongly inhibited the guinea pig ileum contractions induced either electrically or by means of several agonists; of particular interest was the antagonism to leukotriene D_4. The 2-[4-(dimethylaminopropoxy)-phenyl]benzotriazole inhibited platelet aggregation induced by tromboxane A_2, PAF and ADP and increased the bleeding time in mice. The same compound and its N-oxide protected mice from potassium cyanide hypoxia and, most importantly, exerted good anti-hypercholesterolemic action (see Scheme 1).

Pursuing the study of basic derivatives of 2-(4-hydroxyphenyl)benzotriazole, we have now prepared a set of 2-{4-[3-(4-aryl/heteroaryl-1-piperazinyl)propoxy]-phenyl}benzotriazoles and corresponding N-oxides of formulas **3** and **4**, which should exhibit affinity for serotonin receptors, beside other possible activities (Scheme 2).

Actually, the aryl/heteroarylpiperazine moiety is a well known template for affinity to serotonin receptors [2], but affinity for dopamine and adrenergic α_1 receptors can also be expected [3–5]. Since the structure–activity relationships are still rather loosely defined, the prepared compounds were now tested as ligands for both serotonin and dopamine receptors. Affinity for α_1 adrenergic receptors will be tested later. However, compound **3a** was subjected to a large pharmacological

☆ Presented at the 'XIII Conv. Naz. -Div. Chim. Farm. S.C.I.', Paestum, Italy, Sept. 23–27, 1996.

* Corresponding author. Fax: +39-022-9514197.
E-mail address: sparator@imiucca.csi.unimi.it (A. Sparatore)

0014-827X/99/$ - see front matter
PII: S0014-827X(99)00046-4

screening (with about 60 in vitro and in vivo assays) in order to explore how the pharmacological profile of compounds of structures **1** and **2** would change just after the replacement of the dialkylamino head with the simplest arylpiperazinyl moiety.

2. Chemistry

Compounds of formulas **3** and **4** were obtained by reacting 2-(4-hydroxyphenyl)benzotriazole (and corresponding N-oxide) with the required 3-(aryl/heteroarylpiperazinyl)propyl chlorides in ethanolic sodium hydroxide solution.

The 2-(4-hydroxyphenyl)benzotriazole and the corresponding N-oxide were prepared as already described [1], while the 3-(*tert*-amino)propylchlorides were obtained by refluxing the dichloromethane solution of 1-bromo-3-chloropropane and aryl/heteroarylpiperazine in a ratio of 1:2.

In the case of 2-methoxyphenylpiperazine, the use of toluene solution and a ratio bromochloropropane/piperazine of 1.5:1 was found to be more satisfactory.

The structures of the final compounds **3a**–**d** and **4a**–**d** were supported by the elemental analytical results and by spectral data.

The ^{1}H NMR spectra did not exhibit any unusual feature, thus only the spectra of compounds **3b** and **4b** are described as examples: **3b** ($CDCl_3$): δ 8.27 and 7.07, $J = 9.1$ Hz (2 pseudo doublets, 2H + 2H, *p*-phenylene), 7.93, $J = 6.59$ and 3.07 Hz and 7.41, $J = 6.57$ and 3.09 Hz (2 pseudo dd, 2H + 2H, benzotriazole), 7.03–6.80 (m, 4H, 2-methoxyphenyl), 4.14, $J = 6.26$ Hz (t, 2H, $-O-CH_2-$), 3.87 (s, 3H, OCH_3), 3.13 and 2.70 (2 pseudo s, 4H + 4H, $4CH_2$ piperazine), 2.64, $J = 7.32$ Hz (t, 2H, $C-CH_2-N$), 2.07, $J = 6.73$ Hz (quint, 2H, $-C-CH_2-C-$). **4b** ($CDCl_3$): δ 8.05 and 7.09, $J = 9.16$ Hz (2 pseudo doublets, 2H + 2H, *p*-phenylene), 7.79, $J = 8.20$ Hz (pseudo t, 2H, H_5 and H_6 benzotriazole N-oxide), 7.52–7.30 (m, 2H, H_4 and H_7 benzotriazole N-oxide), 7.04–6.80 (m, 4H, 2-methoxyphenyl), 4.14, $J = 6.27$ Hz (t, 2H, $-O-CH_2-$), 3.87 (s, 3H, OCH_3), 3.12 and 2.70 (2 pseudo s, 4H + 4H, $4CH_2$ piperazine), 2.63, $J = 7.28$ Hz (t, 2H, $C-CH_2-N$); 2.06, $J = 6.64$ Hz (quint, 2H, $-C-CH_2-C-$).

The UV spectra were quite close to those of other 2-(4-alkoxyphenyl) derivatives of benzotriazole and benzotriazole-1-oxide [1] (λ_{max}: 223sh, 248, 258sh, 317 nm); the presence of the aryl/heteroarylpiperazine residue only produced an intensification of the absorption maximum around 243–248 nm.

3. Experimental

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

The elemental analyses were performed at the Microanalytical Laboratory of the Dipartimento di Scienze Farmaceutiche of Genoa University and the analytical results for the indicated elements were within $\pm 0.3\%$ of the calculated values.

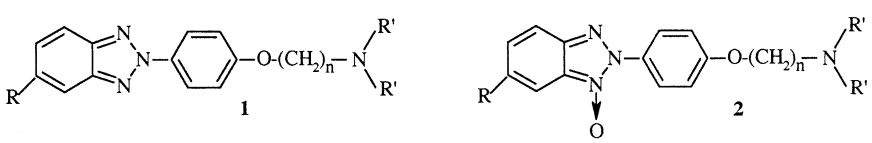
UV spectra were recorded with a Perkin–Elmer Lambda 16 spectrophotometer, ^{1}H NMR spectra were taken on a Bruker AC 200 spectrometer, using $CDCl_3$ as solvent.

3.1. 3-[4-(Aryl/heteroaryl)piperazin-1-yl]propyl chlorides

To a boiling solution of aryl/heteroarylpiperazine (10 mmol) in 7 ml of dichloromethane, 772 mg (5 mmol) of 1-bromo-3-chloropropane was added dropwise. The mixture was further refluxed for about 2 h monitoring the reaction progression through TLC on silica (eluent: dichloromethane, methanol and conc. aqueous ammonia, 20:3:0.5 v/v). After cooling, the solvent was thoroughly removed under reduced pressure and the residue was taken up with dry ether to separate the piperazine hydrobromide.

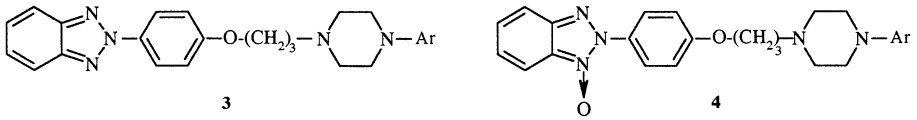
The ether solution was evaporated to dryness and the residue chromatographed on silica using dichloromethane with an increasing amount of methanol (2–10%) as eluent.

The title compound was eluted as first fraction (50–66% yield), followed by 1,3-bis-(4-aryl-1-piperazinyl)propane. Using dichloromethane plus methanol (10%) and conc. aqueous ammonia (1%) the unreacted arylpiperazine was recovered.



R = H; Cl; CF_3; OCH_3 R' = CH_3; C_2H_5 n = 2 ; 3

Scheme 1.



3 4

Ar = Phenyl (**a**); 2-methoxyphenyl (**b**); 2-pyridyl (**c**); 2-pyrimidyl (**d**)

Scheme 2.

3.2. 3-[4-(2-Methoxyphenyl)piperazin-1-yl]propyl chloride

To a boiling solution of 1-bromo-3-chloropropane (3.52 g, 22.4 mmol) in 12 ml of toluene, a solution of 1-(2-methoxyphenyl)piperazine (2.86 g, 14.9 mmol) in 4.5 ml of toluene was added dropwise.

The mixture was refluxed for about 80 min, monitoring the reaction through TLC on silica (dichloromethane, methanol, conc. aqueous ammonia; 20:3:0.3 v/v). The reaction mixture was cooled in ice and the 1-(2-methoxyphenyl)piperazine hydrobromide was filtered and washed with toluene and then with dry ether.

The solution was evaporated to dryness under reduced pressure leaving 2.06 g (calculated 2 g) of an oily product which, on cooling in a freezer, crystallizes (m.p. 47–49°C). The TLC showed the presence of only a very slight amount of the 1,3-bis-(4-substituted-piperazinyl)propane, therefore the compound was used for the next step without further purification.

*3.3. 2-{4-[3-(4-Aryl/heteroaryl-piperazin-1-yl)-propoxy]phenyl}-2H-benzotriazoles and corresponding N-oxides (**3a–d**, **4a–d**)*

To a solution of 10 mmol of 2-(4-hydroxyphenyl)benzotriazole (or corresponding N-oxide) in 11 ml of ethanol, 410 mg ($\sim$ 10 mmol) of sodium hydroxide was added. The solution was boiled under reflux and under a stream of nitrogen and 10.8 mmol of 3-[4-(aryl/heteroaryl)piperazin-1-yl]propyl chloride was added. The mixture was refluxed for about 2 h monitoring the reaction through TLC on silica (dichloromethane, methanol, conc. ammonia; 20:3:0.3 v/v). After cooling, the ethanol was removed under reduced pressure, water was added and the mixture was basified with 2 N NaOH and extracted with ethyl acetate.

After drying with sodium sulfate, the solvent was evaporated and the solid residue was washed with a mixture of dry ether/petroleum ether. The obtained compounds were crystallized as indicated in Table 1.

For the pharmacological assays, the free bases were converted into the hydrochlorides by means of 1 N ethanolic HCl; the solution was evaporated to dryness and the residue washed several times with dry ether.

4. Pharmacology

4.1. Binding assays

Experiments were performed on compounds **3a–d** and **4a–d** to establish their ability to displace specific radioligands from serotonin (5-HT_{1A}, 5-HT_{1B}, 5-HT_{2A}, 5-HT_{2C}, 5-HT_3, 5-HT_4) and dopamine (D_2, D_3) receptors.

Male CRL:CD(SD)BR-COBS rats (125–150 g, Charles River, Calco, Italy) and male Cri:(HA)BR albino guinea pigs (180–200 g, Charles River) were killed by decapitation and their brains were rapidly dissected into the various areas (rat hippocampus for 5-HT_{1A}; rat striatum for 5-HT_{1B} and D_2; rat cortex for 5-HT_{2A}, 5-HT_{2C}, 5-HT_3; rat olfactory tubercle for D_3; guinea pig striatum for 5-HT_4). Tissues were homogenized in about 50 volumes of ice-cold Tris–HCl, 50 mM, pH 7.4 (or Hepes-HCl 50 mM, pH 7.4, for 5-HT_4 and D_3 receptors) using an Ultra Turrax TP-1810 (2×20 s), and homogenates were centrifuged at 50 000 *g* for 10 min (Beckman Avanti J-25 centrifuge). Each pellet was resuspended in the same volume of fresh buffer, incubated at 37°C for 10 min and centrifuged again at 50 000 *g* for 10 min. The pellet was then washed once by resuspension in fresh buffer and centrifuged as before.

Just before the binding assays [6–8] the pellet was resuspended in the appropriate incubation buffer (final volume: 0.5 ml for 5-HT_{1A} and 5-HT_3 receptors; 1 ml for the others): 50 mM Tris–HCl, pH 7.7 for 5-HT_{2A} receptors, with the addition of 10 μM pargyline for the other receptors and further containing 4 mM $CaCl_2$ for 5-HT_{1A}, 4 mM $CaCl_2$ and 0.1% ascorbic acid for 5-HT_{1B}; 4 mM $CaCl_2$, 0.1% ascorbic acid and 0.1 μM spiperone for 5-HT_{2C}; 25 mM Tris–HCl, pH 7.4 + 10 μM pargyline for 5-HT_3 receptors; 50 mM Hepes-HCl, pH 7.4 + 10 μM pargyline for 5-HT_4; 50 mM Tris–HCl, pH 7.4 containing 10 μM pargyline, 120 mM NaCl, 5 mM KCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$ and 0.1% ascorbic acid for D_2 receptors; 50 mM Hepes Na, pH 7.5 containing 1 mM EDTA, 0.1% albumine, 0.005% ascorbic acid and 200 nM eliprodil for D_3 receptors.

The following incubation conditions were used.

5-HT_{1A}: [^{3}H]-8-OH-DPAT (sp. act.127 Ci/mmol), final concentration 1 nM, 30 min, 25°C (non specific binding: 5-HT 1 μM).

5-HT_{1B}: [^{3}H]-serotonin (sp. act.30.0 Ci/mmol), final concentration 2 nM, 30 min, 25°C (non specific binding: 5-HT 10 μM).
5-HT_{2A}: [^{3}H]-ketanserin (sp. act. 80.9 Ci/mmol), final concentration 0.7 nM, 15 min, 37°C (non specific binding: methysergide 1 μM).
5-HT_{2C}: [^{3}H]-mesulergine (sp. act. 82.0 Ci/mmol), final concentration 1 nM, 30 min, 37°C (non specific binding: mesulergine 10 μM).
5-HT_3: [^{3}H]-zacopride (sp. act. 85.0 Ci/mmol), final concentration 0.4 nM, 30 min, 25°C (non specific binding: GR38032 1 μM).
5-HT_4: [^{3}H]-GR113808 (sp. act. 84.0 Ci/mmol), final concentration 0.1 nM, 30 min, 37°C (non specific binding: 5-HT 10 μM).
D_2: [^{3}H]-spiperone (sp. act. 15.0 Ci/mmol), final concentration 0.2 nM, 15 min, 37°C (non specific binding: (−)sulpiride 100 μM).
D_3: [^{3}H]-7-OH-DPAT sp. act. 52 Ci/mmol), final concentration 0.7 nM, 60 min, 25°C (non specific binding: dopamine 1 μM).

Incubation was stopped by rapid filtration under vacuum through GF/B (in 0.5% polyethylenimine for 5-HT_3 receptors) or GF/C (in 0.1% albumine only for D_3 receptors) filters (Schleicher and Schuell), which were then washed with 12 ml (4 × 3 times) of ice-cold Tris–HCl, 50 mM, pH 7.4 or Hepes-HCl, 50 mM, pH 7.4, using a Brandel M-48R cell harvester. Dried filters were immersed in vials containing 4 ml of Filter Count (Packard) for the measurement of trapped radioactivity with a LKB 1214 RACKBETA liquid scintillation spectrometer with a counting efficiency of about 60%. Dose–inhibition curves were analyzed by the 'Allfit' program [9] to obtain the concentration of the unlabeled drug that caused 50% inhibition of ligand binding. The K_i values were derived from the IC_{50} values according the method of Cheng and Prusoff [10].

4.2. General pharmacological screening

A broad pharmacological screening was performed on compound **3a** by MDS Panlabs Inc., Bothell, WA, USA.

This screening consisted of the determination of the maximum tolerated dose (p.os and i.p.), with simultaneous behavioural examination (Irwin test), and in 34 primary in vivo tests (using a suitable m.t.d. fraction, depending on test type) and 26 in vitro tests.

The procedures for these tests have been described already [1,11–16].

5. Results and discussion

The results of radioligand binding experiments are summarized in Tables 2 and 3.

From Table 2 it is evident that the tested compounds displayed quite different degrees of affinity for the six serotonin receptor subtypes investigated. Actually, moderate to good affinity was exhibited for 5-HT_{1A} and 5-HT_{2A} receptors, while only very poor or no affinity was observed for 5-HT_{1B}, 5-HT_{2C}, 5-HT_3, and 5-HT_4 receptors, with IC_{50} ranging from 1.8 to more than 10 μM. Thus, the investigated compounds exhibited, on the whole, a good selectivity for 5-HT_{1A} and 5-HT_{2A} receptors versus the other subtypes. This is particularly interesting for 5-HT_{2A} and 5-HT_{2C} receptors, which share about 80% amino acid homology within the seven transmembrane domains [17]. The most selective compounds in this regard are **4a**, followed by **3b** and **3c**, which inhibit [^{3}H]ketanserin binding to 5-HT_{2A} receptors at about 10^{-7} M while, until the highest concentration tested (10^{-5} M), not affecting [^{3}H]mesulergine binding to 5-HT_{2C} receptors.

The nature of the aryl/heteroaryl moiety on the piperazine nitrogen and the presence or absence of the

Table 1
2-{4-[3-(4-Aryl/heteroarylpiperazin-1-yl)propoxy]phenyl}-2*H*-benzotriazoles (**3a–d**) and 2-{4-[3-(4-aryl/heteroarylpiperazin-1-yl)propoxy]phenyl}-2*H*-benzotriazole-1-oxides (**4a–d**)

Comp	Formula [a]	M.p. (°C) free base	Crystall. solvent [b]	Yield (%)	M.p. (°C) hydrochloride
3a	$C_{25}H_{27}N_5O$	167–168	A	52	252–255
3b	$C_{26}H_{29}N_5O_2$	134–135	B	71	236–238
3c	$C_{24}H_{26}N_6O$	127–128	B	57	238–239
3d	$C_{23}H_{25}N_7O$	152–152.5	B	55	242–244
4a	$C_{25}H_{27}N_7O_2$	140–141	B	65	235–236
4b	$C_{26}H_{29}N_5O_3$	127–128	B	51	203–205
4c	$C_{24}H_{26}N_6O_2$	132–133	B	67	212–213
4d	$C_{23}H_{25}N_7O_2$	160–161	B	47	227–228

[a] Analytical results for C,H,N were within ±0.3% of the calculated values.
[b] A, dry ether; B, ethyl acetate.

Table 2
Affinity of compounds **3a–d**, **4a–d** and reference drugs on serotonin receptors: IC_{50} nM ± S.D. (K_i nM ± S.D.)

Comp. or reference drug	5-HT_{1A} [^{3}H]8OH-DPAT rat hippocampus	5-HT_{1B} [^{3}H]serotonin rat striatum	5-HT_{2A} [^{3}H]ketanserin rat cortex	5-HT_{2C} [^{3}H]mesulergin rat cortex	5-HT_3 [^{3}H]zacopride rat cortex	5-HT_4 [^{3}H]GR 113808 guinea pig striatum	Selectivity K_i5-HT_{2A}/K_i5-HT_{1A}
3a	5728 ± 540 (3431 ± 323)		137 ± 12.6 (84.59 ± 7.6)			>10 000	0.03
3b	73.4 ± 15.2 (40.9 ± 8.4)	2867 ± 811 (1501 ± 424)	193 ± 65 (107 ± 36)	8163 ± 2442 (6540 ± 1956)	>10 000	>10 000	2.62
3c	1992 ± 369 (990 ± 185)	>10 000	557 ± 48 (310 ± 26)	>10 000	>10 000	>10 000	0.31
3d	>10 000	>10 000	1019 ± 198 (568 ± 110)	>10 000	>10 000	>10 000	
4a	320 ± 68.7 (160 ± 34)	6995 ± 1736 (3664 ± 909)	155 ± 26 (86.6 ± 14.6)	>10 000	>10 000	>10 000	0.54
4b	21.5 ± 5.39 (11.9 ± 3.4)	1875 ± 123 (982 ± 64)	756 ± 157 (421 ± 87)	2366 ± 365 (1895 ± 292)	>10 000	9212 ± 2908 (6141 ± 1938)	35.38
4c	1154 ± 313 (643 ± 174)	>10 000	7402 ± 1040 (4126 ± 579)	6603 ± 1539 (5290 ± 1233)	>10 000	>10 000	6.41
4d	156 ± 18.7 (86.9 ± 10.4)	>10 000	3043 ± 697 (1707 ± 391)	>10 000	8657 ± 756 (4222 ± 368)	>10 000	19.64
Serotonin	1.4 ± 0.25 (0.76 ± 0.13)	4.1 ± 0.6 (2.09 ± 0.31)				101 ± 23 (67.3 ± 15.2)	
Methisergide			4.5 ± 0.6 (2.50 ± 0.34)				
Mesulergine				6.4 ± 1.7 (4.03 ± 1.07)			
Quipazine					4.4 ± 0.41 (2.01 ± 0.18)		

Table 3
Affinity of compounds **3a–d**, **4a–d** and reference drugs on dopamine receptors: IC_{50} nM ± S.D. (K_i nM ± S.D.)

Comp. or reference drug	D_2 [^{3}H]spiperone rat striatum	D_3 [^{3}H]7OH-DPAT rat olfactory tubercle	Selectivity K_iD_2/K_iD_3
3a	>10 000	369 ± 78.6 (270.3 ± 57.6)	>27.1[a]
3b	387 ± 60 (172 ± 26)	83.4 ± 5.6 (42.9 ± 2.9)	4.01
3c	4124 ± 177 (1832 ± 78)	1896 ± 220.5 (1310 ± 152)	1.40
3d	>10 000	n.d. [b]	
4a	1161 ± 203 (476 ± 83)	89 ± 18 (47.9 ± 9.7)	9.94
4b	298 ± 55 (132 ± 24)	20 ± 1.4 (10.5 ± 0.7)	12.57
4c	>10 000	262.6 ± 48.5 (181.4 ± 33.5)	>38.1[a]
4d	9631 ± 2410 (4280 ± 1071)	246.9 ± 42.8 (127.1 ± 22.1)	33.67
(−)Sulpiride	287 ± 57 (127 ± 24)		
Dopamine		42.3 ± 5 (20.7 ± 2.4)	

[a] Calculated on IC_{50} ratio.
[b] % Inhibition of specific binding at 10^{-5} and 10^{-7} M, is respectively 61 and 0.

N-oxide function exert an important modulatory role on the affinity of different compounds for 5-HT_{1A} and 5-HT_{2A} receptors.

As already observed by many authors for other kinds of arylpiperazine ligands, the 2-methoxyphenyl residue appeared as the most suitable for binding to 5-HT_{1A} receptors. However, the strong positive influence of the N-oxidation of the benzotriazole ring on the affinity on this receptor must be pointed out, even though it did not homogeneously affect all compounds. Actually, the N-oxidation enhanced the affinity of 2-pyridyl derivative **3c** only 1.5 times, but improved the affinity of the pyrimidyl derivative by more than 100 times.

Compound **4b**, containing the 2-methoxyphenyl residue and the N-oxide function, was definitively the most active one with $K_i = 11.9$ nM.

Concerning the 5-HT_{2A} receptor, the phenyl and, to a minor extent, the 2-methoxyphenyl residues were most suitable for binding.

The N-oxidation of benzotriazole ring exerted a negative influence on the affinity, with a particularly deleterious effect on the 2-pyridyl derivative.

Thus, for this receptor the most active compound was the simple phenyl–piperazinylpropoxyphenylbenzotriazole **3a**, with $K_i = 84.59$ nM.

As a consequence of the positive or negative influence of N-oxidation on the affinity of 5-HT_{1A} and 5-HT_{2A} receptors, respectively, compound **4b** exhibited a good selectivity for 5-HT_{1A} versus 5-HT_{2A} receptor with a ratio 5-HT_{2A}/5-HT_{1A} = 35, while compound **3a** was a very selective 5-HT_{2A} ligand. On the other hand, compounds **3b** and **4a** appear interesting for their balanced and fairly high affinity for both receptors.

In the last few years, a number of 1/2-[ω-(4-arylpiperazin-1-yl)alkyl]benzotriazoles were described [5,18–23] and some of them were found to be good ligands for 5-HT_{1A} and/or 5-HT_{2A} receptors.

These compounds are different from ours because of the absence of the oxyphenyl moiety between the benzotriazole nitrogen and the polymethylene chain.

The binding constants (K_i) relative to 2-[3-(4-phenylpiperazin-1-yl)propyl]benzotriazole and to its 2-methoxyphenyl analogue, reported by Caliendo et al. [21] and by Mokrosz et al. [22,23], were obtained using human recombinant receptors and animal tissue preparations, respectively, and therefore they are not directly comparable.

Nevertheless, they clearly indicate how the phenyl residue is more suitable than the 2-methoxyphenyl one for binding on 5-HT_{2A} receptor and vice-versa for what concerns the 5-HT_{1A} one, as already observed for our compounds.

Even more important is the observation that the absolute values of the binding constants of the two compounds described by Caliendo et al. and by Mokrosz et al. were quite comparable with the K_i of our corresponding compounds **3a** and **3b**, suggesting that the oxyphenyl moiety present in the latter, although increasing the overall size of the molecules and the distance between the benzotriazole ring and the basic head, did not hinder a valid binding to both 5-HT_{1A} and 5-HT_{2A} receptors and actually may contribute to it.

Since such substantial changes introduced in the molecular structure could have affected the importance of the basic arylpiperazine head for the binding on serotonin receptors, the previously described [1] compounds of structures **1** and **2** were subjected to binding experiments on 5-HT_{1A} and 5-HT_3 receptors and were found completely unable to displace the specific radioligands up to the highest concentration tested (10 μM).

The results of Table 3 show that the tested compounds exhibited also variable degrees of affinity for dopamine D_2 and D_3 receptors, with a general higher activity for the D_3 subtype. Apart from the case of 2-pyridyl derivative **3c**, the ratio of affinity D_2/D_3 (K_i ratio) ranged from 4 to more than 38, indicating a moderate to good selectivity for the D_3 receptor.

The phenyl- and 2-methoxyphenyl substituted compounds generally displayed a much higher affinity for

both types of receptors than the 2-pyridyl and 2-pyrimidyl derivatives.

It is worth noting that the N-oxides, with the exception of the 2-pyridyl derivative, always showed a higher affinity for the dopamine receptors considered; once more, the enhancing effect of the N-oxidation did not homogeneously influence all compounds (Table 4).

It should be noted that compounds **3b** and **4b** were comparable to (−)sulpiride ($K_i = 127$ nM) for displacing [^{3}H]spiperone from the D_2 receptor, while compounds **3b** and **4a**–**b** were comparable to dopamine itself ($K_i = 20.7$ nM) for displacing [^{3}H]7-OH-DPAT from the D_3 receptor.

The N-oxide **4b**, containing the 2-methoxyphenyl residue, was rather potent at the D_3 receptor with $K_i = 10.5$ nM, also having a moderate affinity for the D_2 receptor (K_i ratio $D_2/D_3 = 12.6$).

On the whole, the behaviour of these compounds versus D_2 and D_3 receptors recalls that of the isosteric benzimidazole derivatives described by Wright et al. [24].

Among them, 2-{4-[3-(4-phenylpiperazin-1-yl)propoxy]phenyl}benzimidazole was the most potent and selective, exhibiting a K_i for the D_3 receptor of 1.5 nM and a D_2/D_3 ratio of 271. The 2-methoxyphenyl analogue showed a K_i for the D_3 receptor of 1.7 nM and a D_2/D_3 ratio of 5.2. Thus, the benzimidazole ring seems somewhat more suitable for binding to dopamine receptors than the benzotriazole nucleus.

Obviously the comparison of our K_i data with those of Wright et al. should be made with great caution, since the latter were obtained using cloned human D_{2L} and D_3 receptors transfected into CHO-K1 cells, while we used receptor preparation from rat striatum and rat olfactory tubercle; moreover, Wright et al. used [^{3}H]spiperone as a ligand for both D_2 and D_3 receptors, while we used [^{3}H]spiperone for the former receptor and [^{3}H]7OH-DPAT for the latter.

From the global results of the radioligand binding experiments, we may conclude that some of the synthesized compounds appeared endowed with peculiar interesting binding profiles on serotonine 5-HT_{1A} and 5-HT_{2A} and dopamine D_2 and D_3 receptors. Particularly, compounds **3b** and **4b** exhibited affinity for all these receptors, but had higher affinity for the first and last of them. On the other hand, compound **3a** was characterized by a reasonably good affinity for the 5-HT_{2A} and D_3 receptors and good selectivity with respect to the 5-HT_{1A} and D_2 receptors.

Therefore, these compounds deserve further investigation in order to define their agonistic or antagonistic activity and consequently, to study their potential as anxiolytic, antipsychotic or antidepressive agents, possibly devoid of the usual neurological or autonomic side-effects.

Actually, the related dialkylaminoalkoxyphenylbenzotriazoles and their N-oxides (**1** and **2**), previously described [1], displayed a number of biological activities that could be somewhat troublesome for a psychopharmacological agent.

Therefore, the simplest compound **3a**, endowed with only moderate affinities for serotonin and dopamine receptors, was subjected to a general pharmacological screening in order to explore how the exchange of the aliphatic basic head of **1** and **2** with the arylpiperazinyl one would affect the pharmacological profile, besides giving rise to the observed affinity for serotonin and dopamine receptors.

The results of the general pharmacological screening indicated that compound **3a** is well tolerated up to a dose of 300 mg/kg p.os and up to 100 mg/kg i.p. in mice, without any sign of central or autonomic effect during the 72 h of observation after administration.

Results of in vivo tests aimed to show specific activity on CNS (analgesic and anticonvulsive activity, 5-methoxy-*N*,*N*-dimethyltriptamine potentiation, apomorfine and oxotremorine antagonism, tetrabenazine hypothermia antagonism) performed at doses in the range 30–100 mg/kg p.os or 10–30 mg/kg i.p. were completely negative, apart from a slight reduction of phenylquinone-induced writhings (−10%).

Concerning the cardiovascular apparatus, compound **3a**, at the dose of 100 mg/kg p.os, exerted only a modest (−4%) reduction of blood pressure in spontaneously hypertensive rats during the 4 h period of observation, associated with a moderate reduction (−7%) of heart rate. At the same dose, no protection against chloroform induced arrhythmia was seen in mice.

Further negative responses were observed in the following tests for anti-inflammatory, anti-allergic and hypoglicemic activities, protection from ethanol induced gastric ulcers (100 mg/kg; p.os in rats); saluretic and diuretic activities, protection from stress induced gastric ulcers (30 mg/kg; p. os in rats); reduction of pentagastrin induced gastric acidity (10 mg/kg, i.p. in rats); serotonin induced bradycardia = 5-HT_3 antagonism (10 mg/kg; i.p. in mice); antihypercholesterolemic activity (300 mg/kg; p.os in mice). The lack of the hypocholesterolemic activity, so strongly present in the dimethyl-

Table 4
Enhancing or lowering effect of N-oxidation on affinity on different receptors (K_i ratio: benzotriazoles/ benzotriazole N-oxides)

Benzotriazole/benzotriazole N-oxide	5HT_{1A}	5HT_{2A}	D_2	D_3
3a/**4a**	21.4	0.98	>8.6[a]	5.64
3b/**4b**	3.4	0.25	1.3	4.08
3c/**4c**	1.5	0.07	<0.4[a]	7.22
3d/**4d**	>64[a]	0.33	>1[a]	

[a] Calculated on IC_{50} ratio.

aminopropoxyphenylbenzotriazoles **1** is worth noting.

Concerning the in vitro assays on isolated guinea pig left and right atria, ileum and trachea, rat portal vein and vas deferens, compound **3a** failed to show any activity apart from a modest positive inotropic activity (+11%) on guinea pig left atria and a moderate antagonism to calcium (−35%) and neurokinin NK_1 (−29%) induced guinea pig ileum contractions.

Compound **3a** produced these responses at the concentration of 45 μg/ml (100 μM), thus clearly differing from the dialkylaminoalkoxyphenylbenzotriazoles (**1** and **2**) formerly described [1], which, at the same concentration were active in most of the performed assays and gave still significant responses in many of them at 3–10 μM concentrations. Compound **3a** was also lacking any activity on platelet aggregation, thus the activity of **1** (with $R = H$, $R' = CH_3$, $n = 3$) seems rather exclusive.

In spite of these differences, compound **3a** shared with compounds of formulas **1** and **2** the protection of mice from death due to hypoxia produced by an i.v. dose of 2.4 mg/kg (DL_{95}) of potassium cyanide. Compound **3a** resulted the most active, giving an 80% protection at 100 mg/kg p.os and still a 60% protection at 30 mg/kg, being comparable with flunarizine, whose action is commonly connected with its strong calcium antagonism.

Considering jointly the present and past observations [1], no direct correlation can be found for our compounds between protection from cyanide hypoxia and calcium channel blockade; however any connection with antagonism for leukotriene D_4 (as tentatively claimed in [1]) seems also now doubtful, since compound **3a** was completely lacking in any antagonism to the last agent. In any case, having no information about the pharmacokinetic and metabolic features of this compound, no correlation can be attempted at the moment between the observed binding affinities and the so far obtained pharmacological results.

6. Conclusions

The synthesized compounds proved to be able to bind to some serotonin (5-HT_{1A} and 5-HT_{2A}) and dopamine (D_2 and D_3) receptors, while displaying poor or no affinity for 5-HT_{1B}, 5-HT_{2C}, 5-HT_3, and 5-HT_4 subtypes. In one case (compound **4b**) the binding constants (K_i) were as low as 11.9 nM (5-HT_{1A}) and 10.5 nM (D_3).

Noteworthy was the positive contribution of the N-oxide function for the binding to 5-HT_{1A}, D_2 and D_3 receptors, although it was not uniform in all compounds.

The exchange of the aliphatic basic heads of compounds **1** and **2** with the phenylpiperazinyl one, removed most of their pharmacological activities; the only one maintained and somehow improved was the ability to protect mice from cyanide-induced hypoxia.

Acknowledgements

Financial support from the CNR and from the Ministero dell'Università e della Ricerca Scientifica e Tecnologica is gratefully acknowledged. The authors also thank Dr Tiziana Mennini (Istituto di Ricerche Farmacologiche 'Mario Negri', Milan, Italy) for her collaboration.

References

[1] A. Sparatore, F. Sparatore, Synthesis and preliminary pharmacological investigation of some 2-[4-(dialkylaminoalkoxy)-phenyl]benzotriazoles and their N-oxides, Farmaco 53 (1998) 102–112.

[2] R.A. Glennon, R.B. Westkaemper, P. Bartyzel, in: S.J. Peroutka (Ed.), Serotonin Receptor Subtypes, Wiley, New York, 1991, pp. 19–64.

[3] R. Perrone, F. Berardi, N.A. Colabufo, V. Tortorella, M.G. Fornaretto, C. Caccia, R.A. Mc Arthur, Synthesis of arylpiperazines with a terminal nafthothiazole group and their evaluation on 5-HT, DA and α receptors, Eur. J. Med. Chem. 32 (1997) 739–746.

[4] M.L. Lopez-Rodriguez, M.J. Morcillo, E. Fernandez, E. Porras, M. Murcia, A.M. Sanz, L. Orensanz, Synthesis and structure–activity relationships of a new model of arylpiperazines 3. 2-[ω-(4-Arylpiperazin-1-yl)alkyl]perhydropyrrolo[1,2-*c*]imidazoles and -perhydroimidazo[1,5-*a*] pyridines: study of the influence of the terminal amide fragment on 5-HT1A affinity/selectivity, J. Med. Chem. 40 (1997) 2653–2656.

[5] M.J. Mokrosz, M.H. Paluchowska, S. Charakchieva-Minol, A. Bien, Effect of structural modification in 1-arylpiperazine derivatives on α_1-adrenoreceptor affinity, Arch. Pharm. Pharm. Med. Chem. 330 (1997) 177–180.

[6] M. Modica, M. Santagati, F. Russo, L. Parotti, L. De Gioia, C. Selvaggini, M. Salmona, T. Mennini, [[(Arylpiperazinyl)alkyl]thio]thieno[2,3-*d*]pyrimidinone derivatives as high-affinity, selective 5-HT1A receptors ligands, J. Med. Chem. 40 (1997) 574–585.

[7] G. Campiani, V. Nacci, S. Bechelli, S.M. Ciani, A. Garofalo, I. Fiorini, H. Wikstrom, P. De Boer, Y. Liao, P.G. Tepper, A. Cagnotto, T. Mennini, New antipsycotic agents with serotonin and dopamine antagonist properties based on a pyrrolo[2,1-*b*]-[1,3]benzothiazepine structure, J. Med. Chem. 41 (1998) 3763–3772.

[8] G. Campiani, A. Cappelli, V. Nacci, M. Anzini, S. Vomero, M. Hamon, A. Cagnotto, C. Fracasso, C. Uboldi, S. Caccia, S. Consolo, T. Mennini, Novel and highly potent 5-HT3 receptor agonist based on a pyrroloquinoxaline structure, J. Med. Chem. 40 (1997) 3670–3678.

[9] K.W. De Lean, P.J. Munson, D. Rodbard, Simultaneous analysis of families of sigmoidal curves: application to bioassay, radioligand assay and physiological dose-response curves, Am. J. Physiol. 235 (1978) E97–E102.

[10] Y. Cheng, W.H. Prusoff, Relationship between the inhibition constant (K_i) and the concentration of inhibitor which causes 50 per cent inhibition (IC50) of an enzymatic reaction, Biochem. Pharmacol. 22 (1973) 3099–3108.

[11] C. Boido Canu, V. Boido, F. Sparatore, A. Sparatore, Sintesi ed attività farmacologica di 3-chinolizidin-1′-il-5-R-indoli, Farmaco Ed. Sci. 43 (1988) 801–817.
[12] F. Novelli, F. Sparatore, Thiolupinine and some derivatives of pharmacological interest, Farmaco 48 (1993) 1021–1049.
[13] A. Sparatore, F. Sparatore, Preparation and pharmacological activities of 10-homolupinanoyl-2-*R*-phenothiazines, Farmaco 49 (1994) 5–17.
[14] A. Sparatore, F. Sparatore, Preparation and pharmacological activities of homolupinanoyl anilides, Farmaco 50 (1995) 153–166.
[15] G. Iusco, V. Boido, F. Sparatore, Synthesis and preliminary pharmacological investigation of N-lupinyl-2-methoxybenzamides, Farmaco 51 (1996) 159–174.
[16] F. Novelli, F. Sparatore, Preparation and pharmacological activities of spiro[3,4-dihydro-6/7-*R*-1,2,4-benzotriazine-3,4′-(1′-substituted)piperidines], Farmaco 51 (1996) 541–550.
[17] D. Julius, K.N. Huang, T.J. Livelli, R. Axel, T.M. Jessel, The 5-HT2 receptor defines a family of structurally distinct but functionally conserved serotonin receptors, Proc. Natl. Acad. Sci. USA 87 (1990) 928–932.
[18] G. Caliendo, R. Di Carlo, R. Meli, E. Perissutti, V. Santagada, C. Silipo, A. Vittoria, Synthesis and trazodone-like pharmacological profile of 1- and 2-[3-[4-(X)-1-piperazinyl]-propyl]-benzotriazoles, Eur. J. Med. Chem. 28 (1993) 969–974.
[19] G. Caliendo, R. Di Carlo, G. Greco, R. Meli, E. Novellino, E. Perissutti, V. Santagada, Synthesis and biological activity of benzotriazole derivatives structurally related to trazodone, Eur. J. Med. Chem. 30 (1995) 77–84.
[20] A. Boido, F. Sparatore, Aril- e eteroaril-piperazinilalchilbenzotriazoli, XIII Conv. Naz. Div. Chim. Farm. S.C.I., Paestum, 23–27 Sept. 1996, Atti, p. 96.
[21] G. Caliendo, G. Greco, P. Grieco, E. Novellino, E. Perissutti, V. Santagada, D. Barbarulo, E. Esposito, A. De Blasi, Structure–affinity relationship studies on benzotriazole derivatives binding to 5-HT receptor subtypes, Eur. J. Med. Chem. 31 (1996) 207–213.
[22] J.L. Mokrosz, M.H. Paluchowska, E. Chojnacka-Wojcik, M. Filip, S. Charakchieva-Minol, A. Deren-Wesolek, M.J. Mokrosz, Structure–activity relationship studies on central nervous system agents 13. 4-[3-(Benzotriazol-1-yl)propyl]-1-(2-methoxyphenyl)piperazine, a new putative 5-HT1A receptor antagonist and its analogs, J. Med. Chem. 37 (1994) 2754–2760.
[23] J.L. Mokrosz, B. Duszynska, M.H. Paluchowska, S. Charakchieva-Minol, M.J. Mokrosz, A search for new trazodone-like antidepressants: synthesis and preliminary receptor binding studies, Arch. Pharm. 328 (1995) 623–625.
[24] J. Wright, T. Heffner, T. Pugsley, R. Mackenzie, L. Wise, Discovery of selective dopamine D3 ligands: II. 2-[4-[3-(4-aryl-1-piperazinyl)propoxy]phenyl] benzimidazole partial agonists, Bioorg. Med. Chem. Lett. 5 (1995) 2547–2550.