Synthesis of 2-Piperazinylbenzothiazole and 2-Piperazinylbenzoxazole Derivatives with 5-HT_3 Antagonist and 5-HT_4 Agonist Properties

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New 2-piperazinylbenzothiazole and 2-piperazinylbenzoxazole derivatives were prepared and tested as 5-HT_3 receptor antagonists. Some of the new compounds antagonized the effect of 5-HT at the longitudinal muscle myenteric plexus (LMMP) preparation of the guinea pig ileum, and two benzothiazole derivatives, compounds **2e** and **2f**, were more potent than ondansetron in this regard. However, these two compounds were much weaker than the typical 5-HT_3 receptor antagonist as displacers of [^{3}H]BRL-43694 binding to rat cerebral cortex homogenates or as antagonists of the bradycardia response to 5-HT in the anaesthetized rat. Like the prokinetic agent cisapride, some of the new compounds enhanced gastric emptying in rats. Compound **2f** not only markedly enhanced gastric emptying but was also a potent agonist at the isolated rat oesophageal tunica muscularis mucosae, a preparation sensitive to 5-HT_4 receptor stimulation, and enhanced the twitch response in the LMMP preparation. The latter effect was blocked by a high concentration of tropisetron or by previous desensitization with 5-methoxytryptamine. Compound **2f** appears to show a promising pharmacological profile as a potential gastrokinetic agent.

Introduction

Multiple serotonin (5-HT) receptor subtypes have been described in recent years. Among the growing number of 5-HT receptor subtypes, it has been suggested that both 5-HT_3 and 5-HT_4 receptors are involved in the control of gastrointestinal motility. Antagonists at 5-HT_3 receptors enhance gastric emptying in the rat and guinea pig.[1,2] However, the role of 5-HT_3 antagonists as prokinetic agents is doubtful because some potent 5-HT_3 antagonists are devoid of this effect,[3,4] and moreover, this class of compounds does not enhance gastric emptying in the dog.[5] Activation of 5-HT_4 receptors stimulates peristaltic activity in the isolated guinea pig ileum,[6,7] and this observation may provide an explanation for the gastrokinetic activity of benzamides such as metoclopramide and cisapride (see Chart 1). These compounds facilitate cholinergic neurotransmission, thus enhancing gastrointestinal motility and gastric emptying.[8,9] It is yet possible that cisapride may facilitate cholinergic transmission in the dog through pathways not involving 5-HT_4 receptors.[10]

The key pharmacophoric elements of 5-HT_3 antagonists generally include an aromatic moiety, a linking acyl group, and a basic amine,[11] as is the case for drugs such as ondansetron, granisetron, or tropisetron. These structural requirements are also present in some recently developed 5-HT_4 antagonists such as SDZ 205-557[12] and DAU 6285,[13] and also in some structurally related 5-HT_4 agonists[14] (see Chart 1). Other 5-HT_3 antagonists include a thiazole ring between the aromatic and basic moieties, and it has been suggested that the nitrogen atom in the thiazole ring may represent a bioisostere equivalent to the carbonyl group.[15,16] In addition, compounds such as quipazine and other arylpiperazines (see Chart 1), which bind with high affinity to 5-HT_3 receptors,[17] do not share the above mentioned common structural requirements. Moreover, some recently described piperazinylcyanoquinoxaline derivatives also behave as highly potent 5-HT_3 antagonists in the guinea pig ileum.[18]

In the present study we report the synthesis and initial pharmacological evaluation of new 2-piperazinylbenzothiazole and 2-piperazinylbenzoxazole derivatives. These compounds are endowed with 5-HT_3 antagonist and 5-HT_4 agonist properties.

Chemistry

The general synthetic procedures used in this study are illustrated in Scheme 1.

2-(1-Piperazinyl)benzothiazole derivatives (**2**) were obtained from 2-chlorobenzothiazole (**1**) through nucleophilic substitution of the chlorine atom by the appropriate piperazine in the presence of sodium bicarbonate in 2-PrOH/H_2O media (procedure A).[19] **2e** and **2f** were also synthesized through and an alternative pathway from 2-mercaptobenzothiazole (**3**) by methylation of the mercapto group with CH_3I in acetone to give **4**, followed by oxidation to 2-(methylsulfonyl)benzothiazole (**5**) with 3-chloroperbenzoic acid in CH_2Cl_2, and further substitution of this group by the desired piperazine by fusion in the presence of sodium bicarbonate (procedure B).

2-(1-Piperazinyl)benzoxazole derivatives (**7**) were obtained from 2-chlorobenzoxazole (**6**) and the appropriate piperazine using procedure A.

Biological Results and Discussion

Serotonin (5-HT) produces a contraction of the longitudinal muscle myenteric plexus (LMMP) preparation of the guinea pig ileum through activation of different 5-HT receptor subtypes. Low 5-HT concentrations should preferentially stimulate 5-HT_4 receptors, and higher concentrations would rather stimulate 5-HT_3 receptors.[20] A preferential stimulation of 5-HT_3 receptors should be consequently expected after the high 5-HT concentration (10 μM) which was selected for screening purposes. The 5-HT response was inhibited by about 62% with the typical

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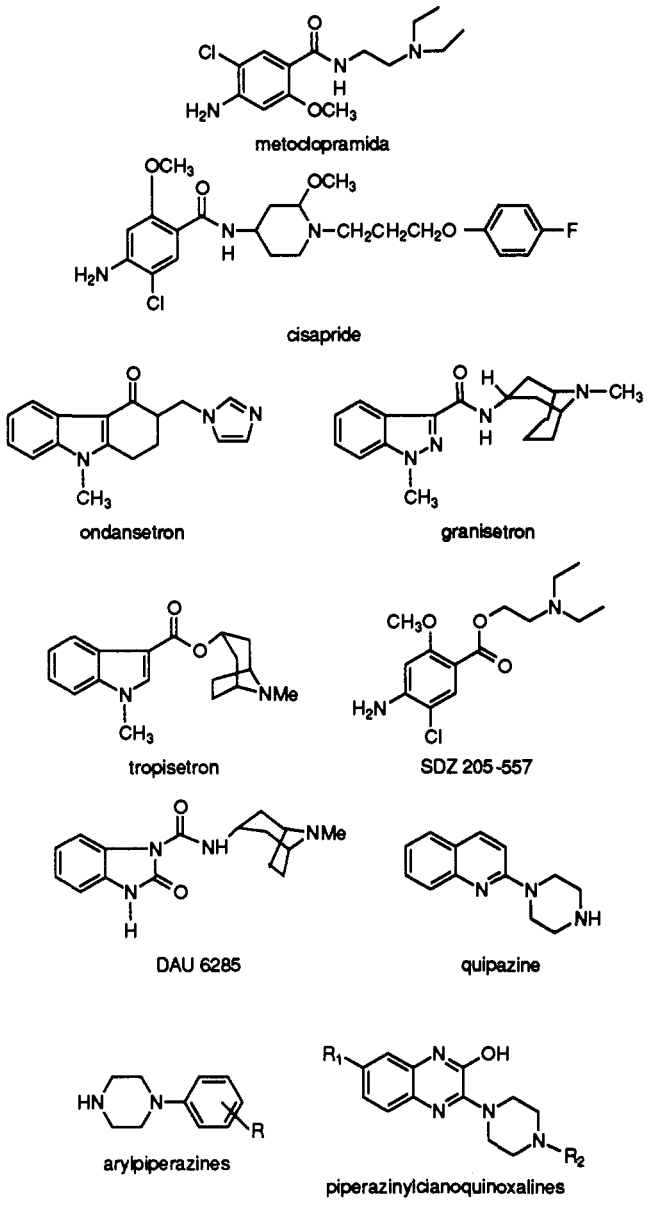
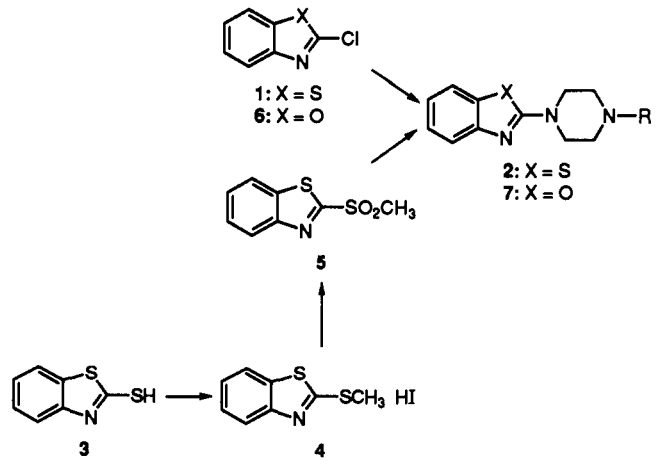
Chart 1



Scheme 1



5-HT_3 antagonist ondansetron, 10 μM. A higher percentage inhibition was obtained with identical concentrations of the benzothiazole derivatives **2e** and **2f** and especially with the prokinetic agent cisapride (Table 2). The pA_2 values, which were determined using the 5-HT_3 receptor agonist 2-methyl-5-HT, were in the same range for compounds **2e**, **2f**, and ondansetron (Table 2). The corresponding benzoxazole analogues of **2e** and **2f** (i.e., compounds **7e** and **7f**) were weaker 5-HT_3 antagonists. Since acetylcholine release seems to be involved in the contractile response to 5-HT_3 receptor agonists in the guinea pig ileum,[21] it was of interest to discard any muscarinic antagonist property of test compounds. This possibility was analyzed using compound **2f**, the more potent 5-HT_3 antagonist. The pEC_{50} of acetylcholine in the LMMP preparation was 5.79 ± 0.10 (mean ± SEM, n = 5). In the presence of a 10 μM concentration of compound **2f**, the pEC_{50} of acetylcholine, 5.81 ± 0.15, was substantially identical.

Using the same concentration of the test compounds, only a very weak to moderate antagonism was observed at 5-HT_2 receptors of the isolated rat thoracic aorta; this was in marked contrast with the almost complete inhibition of the 5-HT response observed after the 5-HT_2 antagonist ketanserin at a 100-fold lower concentration (Table 2).

Only the two more potent 5-HT_3 antagonists in the LMMP preparation, i.e., compounds **2e** and **2f**, were able to displace, at concentrations below 10 μM, the binding of [^{3}H]BRL-43694 to rat 5-HT_3 receptors from cerebral cortex homogenates. The displacing potency of compound

Table 1. Physical Properties of 2 and 7

no.	R	% yield[a]	crystn solvent	mp, °C	formula[b]
2a	2-HOC_6H_4	77	EtOH	178.1	$C_{17}H_{17}N_3OS$
2b	2-$MeOC_6H_4$	75	EtOH/H_2O	108.4	$C_{18}H_{19}N_3OS$
2c	2-$EtOC_6H_4$	93	EtOH/H_2O	111.8	$C_{18}H_{21}N_3OS$
2d	3-ClC_6H_4	72	EtOH	165.4	$C_{17}H_{19}N_3SCl$
2e[c]	benzyl	92[d]	EtOH	133.3	$C_{18}H_{19}N_3S$
2f	piperonyl	77[e]	EtOH/DMF	170.4	$C_{19}H_{19}N_3O_2S$
7a	2-HOC_6H_4	83	EtOH	164.6	$C_{17}H_{17}N_3O_2$
7b	2-$MeOC_6H_4$	82	EtOH/H_2O	123.8	$C_{18}H_{19}N_3O_2$
7e	benzyl	79	H_2O	96.5	$C_{18}H_{19}N_3O$
7f	piperonyl	73	2-PrOH/H_2O	127.5	$C_{19}H_{19}N_3O_3$

[a] All by procedure A. [b] All compounds showed satisfactory elemental analysis. [c] Previously reported.[19] [d] 90% from **5**. [e] 75% from **5**.

Table 2. Antagonism by Piperazinylbenzothiazole (2) and Piperazinylbenzoxazole (7) Derivatives of the Response to 5-HT or 2-Methyl-5-HT in the Isolated Guinea Pig Ileum (Longitudinal Muscle–Myenteric Plexus Preparation) and in the Isolated Rat Thoracic Aorta[a]

	guinea pig ileum			rat aorta	
	% inhibition		pA_2	% inhibition	
compd	mean ± SEM	(n)	(95% CL)	mean ± SEM	(n)
2a	40.8 ± 6.1**	(8)		49.3 ± 5.0*	(4)
2b	43.0 ± 4.8*	(4)		7.1 ± 3.1	(4)
2c	-22.2 ± 7.0	(4)		10.3 ± 5.3	(3)
2d	-21.9 ± 11.2	(4)			
2e	74.4 ± 4.3**	(4)	6.3 (5.9–6.6)	14.8 ± 4.3**	(4)
2f	74.5 ± 7.5**	(6)	6.5 (6.1–6.8)	21.2 ± 0.7**	(4)
7a	46.1 ± 11.5*	(4)		29.1 ± 6.1	(4)
7b	25.6 ± 4.2*	(4)		14.7 ± 2.0**	(4)
7e	48.8 ± 5.5**	(4)		21.9 ± 6.4*	(5)
7f	55.8 ± 5.1*	(4)		19.4 ± 2.8*	(5)
ketanserin				90.0 ± 1.2**	(5)
methysergide				78.9 ± 6.2**	(12)
ondansetron	62.3 ± 5.0**	(5)	6.9 (6.6–7.3)		
cisapride	98.1 ± 1.1**	(4)	9.0 (84.–9.7)		

[a] The guinea pig ileum was stimulated with 10 μM 5-HT (first column) and antagonists tested at the fixed 10 μM concentration. pA_2 values were estimated using 2-methyl-5-HT in five to seven different tissues. The rat aorta was stimulated with 30 μM 5-HT and the antagonists tested at the fixed concentration of 10μM, except ketanserin and methysergide which were tested at 0.1 μM. Significant inhibition: *$p < 0.05$, **$p < 0.01$.

Table 3. Displacement of Binding to 5-HT_3 and 5-HT_1 Serotonin Receptors and to D_2 Dopamine Receptors by Piperazinylbenzothiazole (2) and Piperazinylbenzoxazole (7) Derivatives[a]

compd	IC_{50}, M		
	5-HT_3	5-HT_1	D_2
2a	$>10^{-5}$	$>10^{-5}$	$>10^{-5}$
2b	$>10^{-5}$	$>10^{-5}$	$>10^{-5}$
2c	$>10^{-5}$	$>10^{-5}$	$>10^{-5}$
2d	$>10^{-5}$	$>10^{-5}$	$>10^{-5}$
2e	3.3×10^{-8}	$>1-^{5}$	$>10^{-5}$
2f	9.5×10^{-7}	$>10^{-5}$	$>10^{-5}$
7a	$>10^{-5}$	$>10^{-5}$	$>10^{-5}$
7b	$>10^{-5}$	$>10^{-5}$	$>10^{-5}$
7e	$>10^{-5}$	$>10^{-5}$	$>10^{-5}$
7f	$>10^{-5}$	$>10^{-5}$	$>10^{-5}$
5-HT		5.9×10^{-8}	
ondansetron	4.6×10^{-8}		
cisapride	9.0×10^{-7}		6.8×10^{-7}
haloperidol			2.5×10^{-8}

[a] Tissues and tritiated radioligands used for the binding assays were the following: 5-HT_3 recetors, rat cortex and [^{3}H]BRL-43694; 5-HT_1 receptors, rat cortex and [^{3}H]-5-HT; D_2 receptors, rat striatum and [^{3}H]spiroperidol. Values are the means from four to six separate experiments. SEM for IC_{50} alues were less than 10% of the mean. When a discrete value was not generated, IC_{50} is reported as $>10^{-5}$.

2f was approximately identical to that of cisapride, whereas ondansetron was a more potent displacer by 1 order of magnitude (Table 3). There was no correlation between these binding studies and 5-HT antagonism in the guinea pig ileum. The present results are probably indicative of multiple tissue- and species-dependent 5-HT_3 receptors subtypes.[22-24] It is also of interest to mention that cisapride was a displacer of binding of [^{3}H]spiroperidol to D_2 dopamine receptors, although much weaker than haloperidol (Table 3). The IC_{50} for cisapride was approximately the same at 5-HT_3 and D_2 receptors whereas the new benzothiazole derivatives did not show any affinity at dopamine D_2 receptors. None of the tested compounds modified the binding of [^{3}H]-5-HT to rat cortical homogenates. This last finding is probably indicative of a lack of effect of the new compounds on the different 5-HT_1 receptor subtypes which should be preferentially labeled by [^{3}H]-5-HT.

An additional test for *in vivo* 5-HT_3 antagonism, the von Bezold–Jarisch reflex in rats, was included in the present study. While compounds **2e** and **2f** were slightly more potent than cisapride as antagonists of 5-HT-induced bradycardia, they were much weaker than ondansetron. The approximate ID_{50}'s for compounds **2e** and **2f** in this test were 0.16 and 0.18 mg/kg, respectively, whereas the ID_{50} for ondansetron was below 1 μg/kg. Cisapride produced a maximum 30% blockade of the bradycardia at 0.1 mg/kg.

The effect on gastric emptying in rats was studied after fixed doses of test compounds (10 mg/kg po), which were compared with the same doses of the prokinetic agents metoclopramide and cisapride. Ondansetron was also included in this study at a 10-fold lower dose because of the marked acute toxicity of this drug. Using this fixed dose, both cisapride and compound **2f** increased gastric emptying by more than 60%. A lower percentage increase in gastric emptying was found with all other tested compounds (Table 4).

The effects on gastric emptying do not obviously correlate with 5-HT_3 antagonist activity. Another experiment was conducted in which the more potent gastrokinetic compound **2f** was compared with cisapride, 5-HT, and the

Table 4. Effect on Gastric Emptying in the Rat of Piperazinylbenzothiazole (2) and Piperazinylbenzoxazole (7) Derivatives

compd	dose, mg/kg po	n	gastric emptying % increase[a]
2a	10	10	-14.2
2b	10	10	-27.5
2e	10	9	44.6**
2f	5	6	45.0*
	7.5	9	43.3**
	10	9	62.8**
7a	10	10	-30.2
7e	10	10	15.1
7f	10	10	23.1*
ondansetron	1	7	44.2*
metoclopramide	10	9	37.1**
cisapride	5	10	47.2*
	7.5	10	59.5**
	10	10	67.0**

[a] Significant increase vs controls: $*p < 0.05$, $**p < 0.01$.

Table 5. Agonist Potency in the Rat Isolated Oesophageal Tunica Muscularis Mucosae[a]

agonist	pEC_{50} (95% CL)	ECR[b]	intrinsic activity
2f	6.58 (6.28–6.89)	22	0.91
cisapride	7.23 (6.86–7.61)	7	0.92
5-HT	7.91 (7.85–7.96)	1	1.00
2-methyl-5-HT	4.90 (4.78–5.04)	643	1.00

[a] Results are the mean of 24 experiments (5-HT) or 8 experiments (all other drugs). [b] ECR (equipotent concentration ratio) = IC_{40} test agonist/IC_{40} 5-HT. These values were limited to experiments in which concentration–effect curves for both agonists were obtained in the same preparation.

5-HT_3 agonist 2-methyl-5-HT in the isolated rat oesophageal tunica muscularis mucosae, a preparation sensitive to 5-HT_4 receptor agonists.[14] As can be seen in Table 5, compound **2f**, as well as cisapride and 5-HT, relaxed the preparation with EC_{50}'s below 1 μM whereas 2-methyl-5-HT was a weaker agonist by 2–3 orders of magnitude. In order to further characterize compound **2f** as a 5-HT_4 receptor agonist, it was also studied in the electrically-stimulated LMMP of guinea pig ileum. It is known that 5-HT and 5-HT_4 receptor stimulants enhance the twitch response in this preparation.[25,26] Compound **2f** (1–50 μM) also enhanced the twitch response in a concentration-dependent manner. After the higher concentration, the percentage increase in force development was 79.5 ± 10.9 (mean ± SEM of five experiments). In the presence of tropisetron (1 μM), which is not only a 5-HT_3 but also a weak 5-HT_4 receptor antagonist,[26] the effect of compound **2f** was blocked by 52% (n = 4). Moreover, previous desensitization of the preparation by incubation for 15 min with 5-methoxytryptamine (0.5 μM) produced a percentage inhibition of 75.0 ± 6.4 (n = 4) in the response to compound **2f**, 50 μM. Conversely, no desensitizing effect was found after previous incubation with the 5-HT_3 receptor agonist 2-methyl-5-HT. Preliminary studies (not shown) indicate that, like cisapride, compound **2f** was able to stimulate cAMP formation in the tunica muscularis mucosae of the rat oesophagus at concentrations of 0.01–10 μM, and this effect was again blocked by a high concentration, 10 μM, of tropisetron.

In conclusion, some of the new piperazinylbenzothiazole and piperazinylbenzoxazole derivatives show a moderate activity as 5-HT_3 receptor antagonists along with a gastric motility stimulant effect in rats. The latter effect is of particular interest with compound **2f** and seems to be a consequence, at least in part, of its 5-HT_4 receptor agonist

action. This compound is now under investigation as a potential gastrokinetic agent.

Experimental Section

Chemistry. Every compound was characterized by elemental analysis, IR spectra, and ^{1}H-NMR spectra. IR spectra were recorded on a Perkin-Elmer 681 apparatus, using KBr tablets; the frequencies are expressed in cm^{-1}. The ^{1}H-NMR spectra were obtained on a Brüker AC-200E (200 MHz) instrument, with Me_4Si as the internal standard at a concentration of about 0.1 g/mL and with DMSO-d_6 as the solvent; the chemical shifts are reported in ppm of Me_4Si in δ units, the coupling constants in hertzs. The ^{13}C-NMR spectra were obtained on a Brüker AC-200E (50.5 MHz) instrument, with Me_4Si as the internal standard at a concentration of about 0.2 g/mL; the chemical shifts are reported in ppm of Me_4Si in δ units. The mass spectra were recorded on a Hewlett-Packard 5988-A instrument at 70 eV. Melting points were determined using a Mettler FP82 + FP80 apparatus and are uncorrected. Elemental analyses were obtained from vacuum-dried samples (over phosphorus pentoxide at 3–4 mmHg, 24 h at about 60–80 °C). Thin-layer chromatography (TLC) was carried out on silica gel (DSF-5 Cammaga 0.3-mm thickness) with CH_2Cl_2/MeOH (9:1) as solvent, and the plates were revealed with I_2.

The IR and ^{1}H-NMR spectra were consistent with assigned structures. Elementary analyses were within 0.4% of the theoretical values.

2-(1-Piperazinyl)benzothiazoles (2). Procedure A. A mixture of sodium bicarbonate (2 g, 24 mmol) and the corresponding piperazine (12 mmol) were dissolved in 2-PrOH (70 mL) and water (10 mL) and refluxed during 30 min followed by a dropwise addition of a solution containing **1** (0.73 mL, 6 mmol) in 2-PrOH (5 mL). The new mixture was refluxed for 2–3 h, cooled to room temperature, poured over ice–water (100 mL), and stirred for 1 h. The obtained solid was filtered, washed with abundant water, and recrystallized from the appropriate solvent. This gave **2** (**2a–f**): 72–93% yield. **Procedure B.** A mixture of **5** (0.5 g, 2.35 mmol), sodium bicarbonate (0.79 g, 9.4 mmol), and the corresponding piperazine (4.7 mmol) were heated up to melting, and the temperature was maintained between 60 and 80 °C during 30 min. After cooling, water was added, and the resulting solid was collected through vacuum filtration, washed with abundant hot water, and recrystallized from the appropriate solvent. This gave **2e** and **2f**: 75–90% yield.

2-[1-(4-(2-Hydroxyphenyl)piperazinyl)]benzothiazole (2a) was obtained as pinkish crystals: 1.40 g, 77% yield. ^{1}H-NMR (DMSO-d_6, 200 MHz): δ 3.10 (bs, 4H, H_{ax} piperazine), 3.77 (bs, 4H, H_{eq} piperazine), 6.82 (m, 1H, $H_{4'}$), 6.91 (m, 3H, $H_{3'}$ + $H_{5'}$ + $H_{6'}$), 7.12 (t, 1H, H_6), 7.33 (t, 1H, H_5, $J_{5,6}$ = 7.5 Hz), 7.55 (d, 1H, H_7, $J_{6,7}$ = 7.9 Hz), 7.80 (d, 1H, H_4, $J_{4,5}$ = 7.7 Hz), 9.16 (sa, 1H, OH). IR: 1587 (m, C=N), 1231 (s, CO), 759 (vs, 1,2-disubst) cm^{-1}. Anal. ($C_{19}H_{19}N_3O_2S$) C, H, N. MS (EI): *m/z* (rel intensity, 70 eV) 311 (M^+, 5.98), 285 (2.35), 189 (1.97), 161 (100), 148 (68.69), 120 (68.15), 108 (11.50), 69 (15.64).

2-[1-(4-Piperonyl)piperazinyl]benzothiazole (2f) was obtained as a white powder: 1.6 g, 77% yield, following procedure A and 1.56 g, 75% yield, following procedure B. ^{1}H-NMR (DMSO-d_6, 200 MHz): δ 2.46 (bs, 4H, H_{ax} piperazine), 3.42 (bs, 2H, NCH_2), 3.54 (bs, 4H, H_{eq} piperazine), 5.99 (s, 2H, OCH_2O), 6.76 (d, 1H, $H_{6'}$, $J_{5',6'}$ = 7.9 Hz), 6.85 (m, 2H, $H_{2'}$ + $H_{5'}$), 7.05 (t, 1H, H_6), 7.27 (t, 1H, H_5, $J_{5,6}$ = 7.5 Hz), 7.45 (d, 1H, H_7, $J_{6,7}$ = 7.9 Hz), 7.74 (d, 1H, H_4, $J_{4,5}$ = 7.7 Hz). ^{13}C-NMR (DMSO-d_6, 50.5 MHz): δ 47.98 (2C piperazine), 51.53 (2C piperazine), 61.43 (1C, NCH_2), 100.70 (1C, OCO), 107.73–130.31 (8C aromatic ring), 131.51 (1C, C_g), 146.19 (1C, $C_{d'}$), 147.19 (1C, $C_{c'}$), 152.39 (1C, C_b), 167.93 (1C, C_a). IR: 1595 (m, C=N), 1436 (vs, CN), 1240 (s, CO), 811 (s, 1,3,4-trisubst) 754 (vs, 1,2-disubst) cm^{-1}. Anal. ($C_{19}H_{19}N_3O_2S$) C, H, N. MS (EI): *m/z* (rel intensity, 70 eV) 353 (M^+, 14.93), 338 (1.04), 283 (1.26), 203 (14.14), 135 (100), 77 (10.09).

2-Methylthiobenzothiazole Hydroiodide (4). Iodomethane (0.75 mL, 12 mmol) was added dropwise to a solution of 2-mercaptobenzothiazole (**3**) (2 g, 12 mmol) in acetone (30 mL) for 10 min at room temperature. The mixture was stirred for 45 more minutes. The product, a yellow solid, was recovered through filtration and recrystallized from ethanol: 1.8 g, 48.7% yield. Mp: 128.3 °C. ^{1}H-NMR (DMSO-d_6, 200 MHz): δ 2.84 (s, 3H, CH_3), 7.40 (t, 1H, H_6), 7.51 (t, 1H, H_5, $J_{5,6}$ = 7.5 Hz), 7.89 (d, 1H, H_7, $J_{6,7}$ = 7.8 Hz), 8.06 (d, 1H, H_4, $J_{4,5}$ = 7.7 Hz), 8.83 (bs, 1H, IH). IR: 2667 (vs, IH), 1597 (s, C=N), 1443 (vs, CN), 1249 (s, CS), 752 (vs, 1,2-disubst) cm^{-1}. Anal. ($C_8H_8NS_2I$) C, H, N.

2-(Methylsulfonyl)benzothiazole (5). Compound **4** (1 g, 3.2 mmol) was suspended in dichloromethane (30 mL) by stirring at 0 °C. 3-Chloroperbenzoic acid (1.38 g, 8 mmol) was then added in small portions for 5 min, and the new mixture was stirred under anhydrous conditions at room temperature for 17 h. The solvent was removed by rotatory evaporation yielding **5** as a red solid: 0.5 g, 72.4% yield. Mp: 90 °C (EtOH). ^{1}H-NMR (DMSO-d_6, 200 MHz): δ 3.60 (s, 3H, CH_3), 7.62 (m, 2H, H_6 + H_5), 8.27 (d, 1H, H_7, $J_{6,7}$ = 7.0 Hz), 8.35 (d, 1H, H_4, $J_{4,5}$ = 7.8 Hz). IR: 1625 (s, C=N), 1467 (vs, SO_2), 1415 (vs, CN), 1231 (s, CS), 762 (vs, 1,2-disubst) cm^{-1}. Anal. ($C_8H_7NO_2S_2$) C, H, N.

2-(1-Piperazinyl)benzoxazoles (7). A solution containing **6** (0.74 mL, 6.5 mmol) in 2-PrOH (5 mL) was added dropwise for 15 min to a mixture of sodium bicarbonate (2.2 g, 26 mmol) and the corresponding piperazine (13 mmol) dissolved in 2-PrOH (70 mL) and water (10 mL) previously refluxed for 30 min. After the addition the new mixture was refluxed for 2 h and left to cool. Then it was poured over ice–water (100 mL) and stirred for another hour. The solid was collected, washed with water, and recrystallized from the appropriate solvent. This gave **7** (**7a–f**): 71–90% yield.

2-[1-(4-(2-Hydroxyphenyl)piperazinyl)]benzoxazole (7a) was obtained as a pink–white powder: 1.6 g, 83% yield. ^{1}H-NMR (DMSO-d_6, 200 MHz): δ 3.05 (bs, 4H, H_{ax} piperazine), 3.78 (bs, 4H, H_{eq} piperazine), 6.85 (m, 4H, $H_{3'}$ + $H_{4'}$ + $H_{5'}$ + $H_{6'}$), 7.03 (t, 1H, H_6), 7.16 (t, 1H, H_5, $J_{5,6}$ = 7.4 Hz), 7.32 (d, 1H, H_7, $J_{6,7}$ = 7.6 Hz), 7.41 (d, 1H, H_4, $J_{4,5}$ = 7.7 Hz), 9.11 (sa, 1H, OH). IR: 1629 (m, C=N), 1242 (s, CO), 744 (s, 1,2-disubst) cm^{-1}. Anal. ($C_{19}H_{19}N_3O_2S$) C, H, N. MS (EI): *m/z* (rel intensity, 70 eV) 295 (M^+, 20.23), 176 (2.05), 161 (94.47), 120 (100), 93 (16.55), 65 (14.15).

2-[1-(4-Piperonyl)piperazinyl]benzoxazole (7f) was obtained as nacred crystals: 1.6 g, 73% yield. ^{1}H-NMR (DMSO-d_6, 200 MHz): δ 2.46 (t, 4H, H_{ax} piperazine), 3.42 (s, 2H, NCH_2), 3.59 (t, 4H, H_{eq} piperazine, J = 4.5 Hz), 6.00 (s, 2H, OCH_2O), 6.76 (d, 1H, $H_{6'}$, $J_{5',6'}$ = 7.9 Hz), 6.86 (m, 2H, $H_{2'}$ + $H_{5'}$), 7.01 (t, 1H, H_6), 7.14 (t, 1H, H_5, $J_{5,6}$ = 7.4 Hz), 7.29 (d, 1H, H_7, $J_{6,7}$ = 7.6 Hz), 7.48 (d, 1H, H_4, $J_{4,5}$ = 7.8 Hz). ^{13}C-NMR (DMSO-d_6, 50.5 MHz): δ 45.25 (2C piperazine), 51.50 (2C piperazine), 61.55 (1C, NCH_2), 100.76 (1C, OCO), 107.82-131.58 (8C aromatic ring), 142.92 (1C, C_b), 146.23 (1C, $C_{d'}$), 147.24 (1C, $C_{c'}$), 148.26 (1C, C_g), 161.76 (1C, C_a). IR: 1647 (vs, C=N), 1460 (vs, CN), 1251 (s, CO), 743 (vs, 1,2-disubst), 695 (s, monosubst) cm^{-1}. Anal. $C_{19}H_{19}N_3O_3$) C, H, N. MS (EI): *m/z* (rel intensity, 70 eV) 337 (M^+, 21.33), 267 (1.40), 203 (8.03), 176 (8.81), 135 (100), 105 (3.80), 77 (12.35).

Pharmacology. 1. Isolated Longitudinal Muscle–Myenteric Plexus Preparation from Guinea Pig Ileum. Guinea pigs of either sex weighing 300–400 g were stunned by a blow to the head and bled. The ileum was excised, approximately 10 cm from the ileo–caecal junction, and longitudinal muscle strips with the myenteric plexus attached (LMMP) were prepared as previously described.[27] LMMP strips were suspended in a 10-mL organ bath containing Tyrode's solution (composition in mM: NaCl, 136; KCl, 2.7; $CaCl_2$, 1.8; $MgCl_2$, 1.05; NaH_2PO_4, 0.42; $NaHCO_3$, 11.9; glucose, 5.5), aerated with 95% O_2/5% CO_2, and maintained at 37 °C. Methysergide (1 μM) was always present in the solution. Contractile responses were isometrically recorded with a resting tension of 0.5 g. Following a 30-min equilibration period, tissues were stimulated with increasing concentrations of 5-HT, from 10^{-8} to 10^{-4} M. A fixed 5-HT concentration (10^{-5}), approximately the ED_{80}, was used for the subsequent antagonism studies. The response to 10 μM 5-HT was expressed as 100%. After 30 min of incubation with the test compounds, serotonin was added into the bath and the subsequent response was measured. The antagonist effect of the compounds was expressed as a percentage of the previous response to 5-HT. pA_2 values were calculated, using the 5-HT_3 receptor agonist 2-methyl-5-HT and the following equation:[27] pA_2 = –log[antagonist concentration] + log(DR – 1), where DR represents the agonist dose ratio.

2. Field-Stimulated LMMP Preparation from Guinea Pig Ileum. Longitudinal muscle strips were suspended in a 10-mL

tissue bath containing a physiological solution (pH 7.4, 37 °C) [composition (in mM): NaCl, 127; $CaCl_2$, 2.6; KCl, 3.8; $NaHCO_3$, 25; NaH_2PO_4, 1.1; $MgCl_2$, 1.2; glucose, 10.8; and methysergide 0.001] and aerated continuously with 95% O_2/5% CO_2 under a 500-mg tension. Electrical field stimulation (0.2 Hz, 1 ms, 60% maximal voltage) was delivered by platinum electrodes to evoke the cholinergically-mediated twitch response.[28] When responses were stabilized, the effect of compounds was tested. The response was referred to changes in the basal tone and in the amplitude of the twitch response.

3. Isolated Rat Thoracic Aorta. Male Wistar rats (200–250 g) were sacrificed by decapitation and exsanguinated. The descending thoracic aorta was removed and cut into rings of approximately 2–3-mm width. These rings were suspended in 10-mL organ baths containing a Krebs–Henseleit solution [composition (in mM): NaCl, 118.4; KCl, 4.7; $CaCl_2$, 2.5; $MgSO_4$, 1.2; $NaHCO_3$, 25; and glucose, 11]. The rings were prepared as previously described.[29] The solutions were kept at 37 °C and gassed continously with 95% O_2/5% CO_2. The tissues were stretched to an initial tension of 1.5 g and equilibrated for 60–90 min. After the initial equilibration period, the aortic rings were contracted with a submaximal concentration of 5-HT (3×10^{-5} M). Control contractile responses to 5-HT were obtained and considered as 100%. After a 30-min incubation with the new compounds, 5-HT was added into the bath, and the subsequent response was measured. The antagonist effect was expressed as the percentage of the previous response to 5-HT.

4. Binding to Several Neurotransmitter Receptors. Binding of [^{3}H]BRL-43694 to 5-HT_3 receptors from rat cerebral cortex homogenates was performed according to a previously described method.[30] Whole cerebral cortex tissue was obtained from male Wistar rats (150–200 g) and homogenized in 10 volumes of ice-cold HEPES buffer (50 mM, pH 7.5). The homogenate was centrifuged at 50000*g* for 10 min, and the pellet was washed and centrifuged two additional times. The final pellet was suspended in 10 volumes of HEPES buffer and stored on ice until required. Displacement studies were performed with 1 nM [^{3}H]BRL-43694 (final concentration) and eight different concentrations of the new compounds. The incubation (23 °C for 30 min) was terminated by rapidly filtering through GB/B filters using a Brandel Cell Harvester. The filters were rinsed immediately and measured by liquid scintillation counting.

Binding to [^{3}H]-5-HT-labeled 5-HT_1 receptors of rat cortical membranes was performed using a previously described procedure.[31] The tissue was homogenized in 20 volumes of 50 mM Tris-HCl and centrifuged at 45000*g* for 10 min. The supernatant was discarded, and the pellet was rehomogenized in the same buffer. The tissue homogenate was then incubated at 37 °C for 10 min before being subjected to a second 10-min centrifugation. The final pellet was resuspended in 100 volumes of buffer containing 0.1% ascorbic acid and 10 μM pargyline. The final mixture consisted of 0.1 mL of 0.3 nM [^{3}H]-5-HT (30 Ci/mmol, NEN), 0.1 mL of buffer or varying concentrations of test compounds, and 0.8 mL of tissue suspension. Following incubation at 37 °C for 10 min, the tubes were rapidly filtered and washed twice with 5 mL of buffer. Radioactivity was determined by liquid scintillation counting.

The binding of [^{3}H]spiroperidol (Amersham) to dopamine D_2 receptors of rat striatum was performed as described[32] with small modifications. Striata were homogenized in 50 mM Tris-HCl buffer (pH 7.7) and centrifuged at 40000*g* for 15 min. The pellet was washed twice and resuspended in 100 volumes of the same buffer containing 120 mM NaCl and 5 mM KCl. The incubation tubes contained 0.2 mL of the tissue suspension, 25 μL of the labeled ligand (0.1 nM), and 25 μL of varying concentrations of test compounds. Samples, run in triplicate, were incubated at 37 °C for 10 min, filtered, and rinsed four times with the same buffer.

5. von Bezold–Jarisch reflex. The antagonism to the bradycardic effect of 5-HT in anaesthetized rats was evaluated as described.[33] Male Sprague–Dawley rats (500–550 g) were anaesthetized with uretane (1 g/kg ip). The carotid artery was cannulated and connected to a TRA-021 Letica pressure transducer. Heart rate was derived from the arterial blood pressure signal using a cardiotachometer (CAR-306 Letica). A jugular vein was exposed, cannulated, and used for iv administration of compounds. Bolus intravenous injection of 4 μg/kg of serotonin repeated every 15 min reproducibly elicited the von Bezold–Jarisch reflex. Antagonists were injected iv 2 min before serotonin, and their effect was expressed as percent inhibition of the serotonin response. ID_{50}'s were calculated by the method of Litchfield and Wilcoxon.[34]

6. Gastric Emptying in Rats. Wistar rats weighing 160 ± 10 g were fasted overnight with water *ad libitum*. Vehicle or test compounds were administered orally. After 60 min, rats received 40 steel spheroids (1-mm diameter) in 2 mL of a 3% solution of carboxymethylcellulose by gavage. The animals were killed by CO_2, 60 min later, and the spheroids remaining inside the stomach were counted and the number of spheroids emptied calculated. Gastric emptying induced by test compounds were expressed as the percentage of the number of spheroids emptied by paired control rats according to the formula: (no. spheroids test − no. spheroids control) × 100/40.

7. Isolated Rat Oesophageal Tunica Muscularis Mucosae. Male Wistar rats (200–300 g) were stunned by decapitation, and a 2-cm segment of intrathoracic oesophagus, proximal to the diafragma, was excised and placed in Tyrode's solution of the following composition (mmol/L): NaCl, 136; KCl, 2.7; $MgCl_2 \cdot 6H_2O$, 1.0; $CaCl_2 \cdot 2H_2O$, 1.8; $NaH_2PO_4 \cdot H_2O$, 0.4; $NaHCO_3$, 11.9; glucose, 5.6, pH 7.4. The external muscularis propia, containing the outer longitudinal and circular muscle layers of the oesophagus, was carefully removed in order to isolate the inner smooth muscle tube of the tunica muscularis mucosae as described.[35] The strips were suspended in a 10-mL tissue bath containing Tyrode's solution at 37 °C aerated with 95% O_2/5% CO_2, under 250 mg of tension and equilibrated for 60 min. Pargyline (100 μmol/L), cocaine (30 μmol/L), corticosterone (30 μmol/L), and methysergide (1 μmol/L) were included in the Tyrode's solution. Responses were recorded isometrically using UF-1 transducers coupled to a Panlab Polygraph. Concentration–effect curves were obtained after contracting the rat oesophagus with carbachol (3 μmol/L). Responses to the cumulative addition of agonists are expressed as percentage relaxation of the carbachol-induced tone. A complete concentration–effect curve to 5-HT was obtained with a maximum relaxation of approximately 80% of the initial contraction. The ability of compounds to relax oesophagus was expressed both in absolute terms, as EC_{50} relative to their individual maxima, and in terms of their relative potency versus 5-HT. Potency relative to 5-HT was calculated from experiments in which two concentration–effect curves were constructed in the same preparation: the first to 5-HT itself and the second to either 5-HT or to a test compound. The relative potency of agonist was expressed as equipotent concentration ratio (ECR) measured at the 40% inhibition point (IC_{40}) of the carbachol-induced contraction.

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Supplementary Material Available: Information on 28 other new compounds, structurally related to the compounds herein described, that have not been tested on 5-HT receptors because of their lack of solubility in the adequate solutions will be provided by the authors upon request.

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