Benzoxazole Derivatives as Novel 5-HT_3 Receptor Partial Agonists in the Gut

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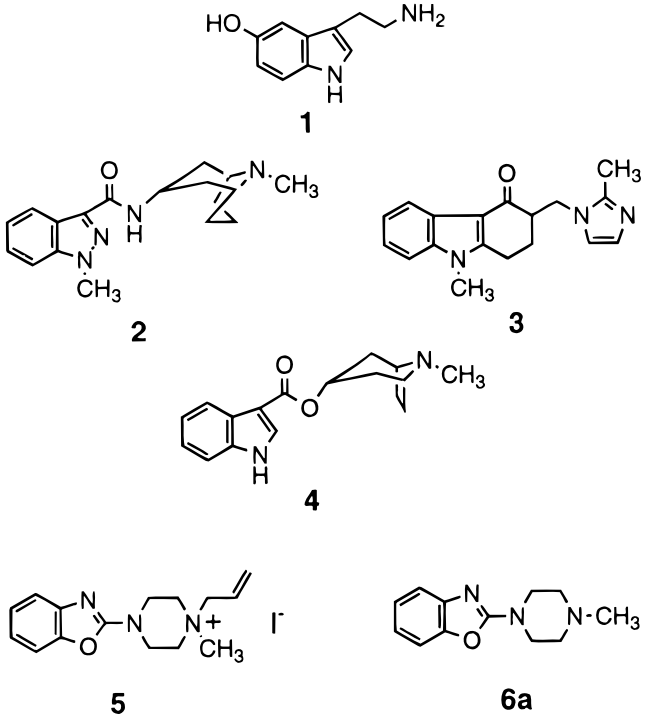
A series of benzoxazoles with a nitrogen-containing heterocyclic substituent at the 2-position was prepared and evaluated for 5-HT_3 partial agonist activity on isolated guinea pig ileum. The nature of the substituent at the 5-position of the benzoxazole ring affected the potency for the 5-HT_3 receptor, and the 5-chloro derivatives showed increased potency and lowered intrinsic activity. 5-Chloro-7-methyl-2-(4-methyl-1-homopiperazinyl)benzoxazole (**6v**) exhibited a high binding affinity in the same range as that of the 5-HT_3 antagonist granisetron, and its intrinsic activity was 12% of that of 5-HT. Compound **6v** inhibited 5-HT-evoked diarrhea but did not prolong the transition time of glass beads in the normal distal colon even at a dose of 100 times the ED_{50} for diarrhea inhibition in mice. Compounds of this type are expected to be effective for the treatment of irritable bowel syndrome without the side effect of constipation.

Introduction

Serotonin (5-hydroxytryptamine, 5-HT, **1**) mediates a wide range of pharmacological effects in the central and peripheral nervous systems.[1] Seven classes of 5-HT receptor subtypes (5-HT_1−5-HT_7) have been characterized by molecular biological methods, as well as by using specific ligands.[2−5] Particular attention has been focused on the 5-HT_3 receptor in medicinal chemistry,[6,7] since 5-HT_3 receptor antagonists prevent the nausea and vomiting that commonly occur during cytotoxic cancer chemotherapy and/or radiation therapy. Selective and high-affinity 5-HT_3 receptor antagonists have been investigated in detail, and several antiemetic drugs, such as granisetron[8] (**2**), ondansetron[9] (**3**), and tropisetron[10] (**4**), are clinically available. These 5-HT_3 receptor antagonists have also been suggested to be useful for the treatment of irritable bowel syndrome (IBS), dyspepsia, pain, anxiety, and psychosis.[11,12]

We have reported that the novel 5-HT_3 receptor ligand 1-allyl-1-methyl-4-(2-benzoxazolyl)piperazinium iodide (CP2289, **5**) is a partial agonist from the viewpoint of gastroenteric motility.[13] Compound **5** had 74 ± 8% intrinsic activity (ia) compared to 5-HT in the in vitro contraction test using isolated guinea pig ileum. Both **5** and granisetron shortened the delay in gastric emptying caused by 5-HT. On administration by itself to mice, **5** did not have the accelerative effect which was shown by granisetron. 5-HT_3 receptor antagonists cause mild constipation in healthy volunteers.[14] Ondansetron improved stool consistency in patients with diarrhea-predominant IBS, but some of them reported discomfort and constipation.[15] In the treatment of diarrhea-predominant IBS, 5-HT_3 receptor antagonists may cause constipation as a side effect by inhibiting normal lower bowel function.[16] However, a 5-HT_3 receptor partial agonist may control gastroenteric motility without complete blocking of 5-HT_3-sensitized nerves. Such a characteristic could make 5-HT_3 receptor partial agonists useful for the treatment of gastroenteric disorders, exemplified by IBS. A transient emesis-inducing effect was observed when **5** was administered to *Suncus murinus*.[13] This is clearly disadvantageous for a gastroenteric drug, but the intrinsic activity of the agonist is expected to correlate with the potency of the emesis-inducing effect.



One of the piperazinyl nitrogen atoms in **5** is in a quaternary alkyl form, and it has been suggested that this amine corresponds to the terminal amine of 5-HT, which is protonated on binding to the 5-HT_3 receptor.[17] We could not find quaternary derivatives of **5** with decreased intrinsic activity, but we found that a small lipophilic substituent at the 5-position of the benzoxazole ring was effective for increasing the receptor affinity.[18] The 2-(4-methyl-1-piperazinyl)benzoxazole

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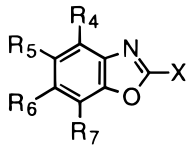
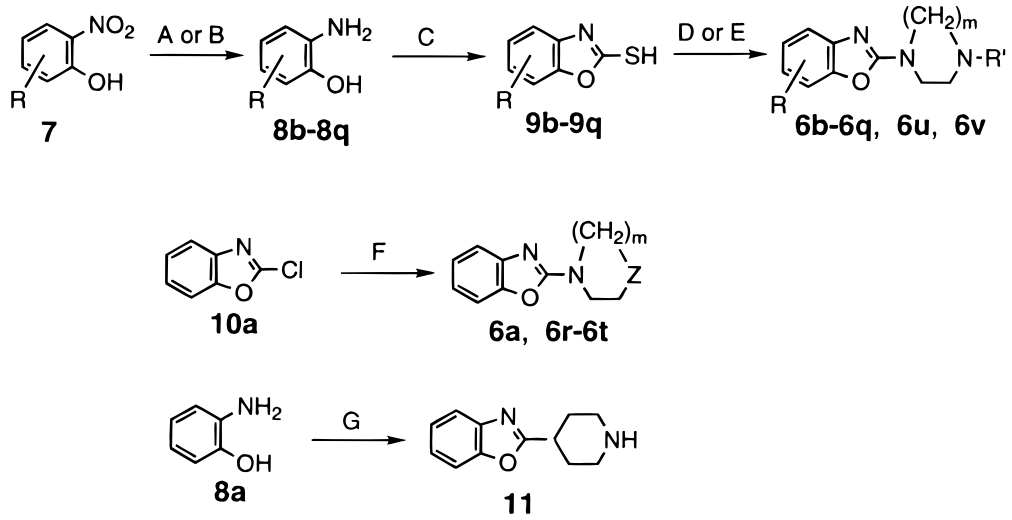
Table 1



compd	R_4	R_5	R_6	R_7	X[a]	formula[b]	method	yield (%)	mp (°C)[c]
6a	H	H	H	H	MP	$C_{12}H_{15}N_3O$	F	85	37−38
6b	CH_3	H	H	H	MP	$C_{13}H_{17}N_3O$	CD	68	oil
6c	H	CH_3	H	H	MP	$C_{13}H_{17}N_3O \cdot {}^1/_8H_2O$	CD	73	63−64
6d	H	H	CH_3	H	MP	$C_{13}H_{17}N_3O$	CD	50	62−63
6e	H	H	H	CH_3	MP	$C_{13}H_{17}N_3O$	ACD	70	oil
6f	H	F	H	H	MP	$C_{12}H_{14}N_3OF$	BCE	70	116−117
6g	H	Cl	H	H	MP	$C_{12}H_{14}N_3OCl$	CD	67	118−119
6h	H	Br	H	H	MP	$C_{12}H_{14}N_3OBr$	BCE	60	100−101
6i	H	CF_3	H	H	MP	$C_{13}H_{14}N_3OF_3$	ACE	21	72−73
6j	H	Et	H	H	MP	$C_{14}H_{19}N_3O$	ACE	80	oil
6k	H	CH_3	CH_3	H	MP	$C_{14}H_{19}N_3O$	ACE	39	130−131
6l	H	CH_3	H	CH_3	MP	$C_{14}H_{19}N_3O$	ACE	73	oil
6m	H	Cl	H	CH_3	MP	$C_{13}H_{16}N_3OCl$	BCE	85	62−64
6n	H	Cl	H	Cl	MP	$C_{12}H_{13}N_3OCl_2$	BCE	37	106−107
6o	H	Cl	CH_3	H	MP	$C_{13}H_{16}N_3OCl$	BCE	65	115−116
6p	H	Cl	CH_3	CH_3	MP	$C_{14}H_{18}N_3OCl$	BCE	60	97−99
6q	H	Cl	CH_3	Cl	MP	$C_{13}H_{15}N_3OCl_2$	BCE	65	123−124
6r	H	H	H	H	1P	$C_{12}H_{14}N_2O$	F	77	67−68
6s	H	H	H	H	NP	$C_{11}H_{13}N_3O \cdot {}^1/_3H_2O$	F	72	68−70
6t	H	H	H	H	MH	$C_{13}H_{17}N_3O$	F	82	58−59
6u	H	Cl	H	H	MH	$C_{13}H_{16}N_3OCl$	CE	49	85−85.5
6v	H	Cl	H	CH_3	MH	$C_{14}H_{18}N_3OCl$	BCE	33	116−117
11	H	H	H	H	4P	$C_{12}H_{14}N_2O \cdot {}^1/_6H_2O$	G	84	amorphous

[a] MP, 1-(4-methylpiperazinyl); 1P, 1-piperidinyl; NP, 1-piperazinyl; MH, 1-(4-methylhomopiperazinyl); 4P, 4-piperidinyl. [b] Elemental analyses for C, H, N. If a compound was obtained as an oil, HRMS analysis was performed. [c] For recrystallization solvent, see the Experimental Section.

Scheme 1. Synthetic Procedures[a]



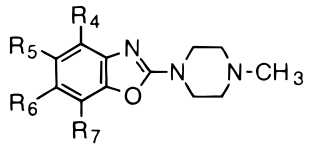
[a] (A) Pd/C, EtOH; (B) Pt on sulfide carbon, EtOH; (C) CS_2, KOH, EtOH, reflux; (D) (i) PCl_5, toluene, reflux, (ii) amine, toluene; (E) amine, chloroform or toluene, reflux; (F) amine, chloroform; (G) 4-piperidinecarboxylic acid, polyphosphoric acid, 180 °C. m = 2 or 3.

(**6a**) exhibited a partial agonist characteristic in the contraction test, having rather low intrinsic activity (62 ± 12%), with weak affinity for the 5-HT_3 receptor.[13] We therefore chose **6a** as a lead compound for 5-HT_3 receptor partial agonists with higher receptor binding affinity and lower intrinsic activity. Herein we report the synthesis of 2-substituted benzoxazole derivatives and their 5-HT_3 receptor partial agonist activities in the gut. Increased affinity and reduced intrinsic activity were achieved in some derivatives by introducing small lipophilic moieties. One of the compounds did not show any adverse effect on the normal lower bowel function in mice.

Chemistry

All the synthesized compounds are shown in Table 1. The synthetic procedures are illustrated in Scheme 1. Compounds substituted at the 4−7-positions of the benzoxazole ring were synthesized starting from *o*-nitrophenols (**7**) or *o*-aminophenols (**8**). Treatment of **8b**−**q** with carbon disulfide and potassium hydroxide in ethanol gave the cyclized thiol compounds **9b**−**q**.[19] Coupling of **9b**−**q** with amine was achieved by means of the following two methods: (i) coupling an amine with crude 2-chlorobenzoxazole obtained by the reaction of 2-mercaptobenzoxazole with phosphorus pentachloride

Table 2



compd	R_4	R_5	R_6	R_7	contraction activity pD_2 ± SEM	contraction activity ia ± SEM	5-HT$_3$ receptor binding[a] (10^{-7} M, %)
6a	H	H	H	H	5.01 ± 0.13	0.62 ± 0.12	55
6b	CH_3	H	H	H	<5.0		25
6c	H	CH_3	H	H	6.00 ± 0.05	0.72 ± 0.04	83
6d	H	H	CH_3	H	5.15 ± 0.02	0.85 ± 0.06	74
6e	H	H	H	CH_3	5.46 ± 0.06	0.58 ± 0.06	77
6f	H	F	H	H	5.57 ± 0.06	0.64 ± 0.04	91
6g	H	Cl	H	H	6.07 ± 0.15	0.50 ± 0.03	94
6h	H	Br	H	H	6.21 ± 0.04	0.48 ± 0.03	92
6i	H	CF_3	H	H	<5.0		22
6j	H	Et	H	H	<5.0		11
6k	H	CH_3	CH_3	H	6.16 ± 0.06	0.66 ± 0.09	32
6l	H	CH_3	H	CH_3	6.32 ± 0.13	0.62 ± 0.12	94
6m	H	Cl	H	CH_3	6.70 ± 0.13	0.24 ± 0.07	85
6n	H	Cl	H	Cl	6.51 ± 0.04	0.14 ± 0.03	62
6o	H	Cl	CH_3	H	6.28 ± 0.04	0.28 ± 0.04	95
6p	H	Cl	CH_3	CH_3	6.67 ± 0.08	0.14 ± 0.04	101
6q	H	Cl	CH_3	Cl	6.64 ± 0.03	0.10 ± 0.01	96

[a] Each compound was tested in duplicate at 10^{-7} M. Values are the means of two experimental results.

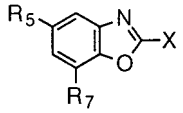
(**6b**−**e**,**g**; procedure D); (ii) heating a mixture of 2-mercaptobenzoxazole and an amine in a nonpolar solvent (**6f**,**h**−**q**,**u**,**v**; procedure E). Compounds **6a**,**r**−**t** were obtained by the treatment of 2-chlorobenzoxazole (**10a**) with an amine. Piperidine-4-carboxylic acid and *o*-aminophenol were heated with polyphosphoric acid to afford the cyclized compound **11**.[20]

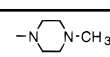
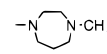
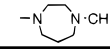
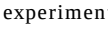
Pharmacological Results and Discussion

To determine the agonistic activity of the derivatives for the 5-HT$_3$ receptor in the gut, contraction tests were carried out using isolated guinea pig ileum.[13,18] Contractions induced by the test compounds were completely blocked by granisetron. Values of potency (pD_2) and intrinsic activity (ia) presented are each the mean ± SEM of five independent determinations. We also examined the affinity of the derivatives for the 5-HT$_3$ receptor[21] at 10^{-7} M to avoid overlooking any compounds showing very low intrinsic activity. The results are summarized in Tables 2 and 3.

A methyl group on the benzoxazole ring showed different effects depending on its position (Table 2, **6b**−**e**). Among them, the 5-substituted compound **6c** had the highest potency for the 5-HT$_3$ receptor. The 6- and 7-methylated compounds **6d**,**e** exhibited improved potency and the 4-methylbenzoxazole derivative **6b** showed weaker potency and binding affinity than **6a**. A large alkyl group (**6j**) or an electron-withdrawing group (**6i**) on the 5-position reduced the binding affinity. When a halogen atom was introduced at the 5-position (**6f**−**h**), the chloro derivative **6g** and the bromo derivative **6h** showed high potency and rather low intrinsic activity. The dimethyl compounds **6l**,**k** showed almost rather similar characteristics to **6c**. On the basis of these results, the 5-chloro or 5-bromo compound seemed to have the desired characteristic of decreased intrinsic activity. We selected the chloro derivative for further examination, for synthetic convenience. Methyl and/or chloro substituents were introduced into **6g**. The 5-chloro-7-methylbenzoxazole **6m** contracted guinea pig ileum at a low dose (pD_2 = 6.70 ± 0.13), and the degree of contraction was almost one-fourth of that of 5-HT (ia = 0.24 ± 0.07). The 5,7-dichloro compound **6n** was similar in potency and intrinsic activity to **6m**. A similar effect of methyl or chloro substitution at the 7-position was observed on the intrinsic activity of 6-methyl derivatives (**6o**−**q**). Although introducing a methyl group at the 6-position tended to decrease the intrinsic activity, it had little effect on the potency for the 5-HT$_3$ receptor.

Table 3



compd	R_5	R_7	X	contraction activity pD2±SEM	contraction activity ia±SEM	5-HT$_3$ receptor binding[a] (10^{-7} M, %)
6a	H	H		5.01±0.13	0.62±0.12	55
6r	H	H		<5.0	-	-7
6s	H	H		5.74±0.10	0.59±0.08	83
11	H	H		5.59±0.07	0.31±0.05	39
6t	H	H		6.03±0.05	0.54±0.07	86
6u	Cl	H		7.00±0.08	0.31±0.06	87
6v	Cl	CH_3		7.76±0.11	0.12±0.02	98

[a] Each compound was tested in duplicate at 10^{-7} M. Values are the means of two experimental results.

Table 3 gives the agonistic activities of compounds in which the 2-piperazine structure of **6a** was replaced with other cyclic amines. Compounds **6s** and **11** retained agonistic activity to the 5-HT$_3$ receptor, though **6r** lost this activity. The strongly basic nitrogen atom at the 4-position of the piperazine ring was essential for the agonistic activity. This finding is consistent with our previous suggestion that the nitrogen atom at the 4-position of the piperazine ring corresponds functionally to the terminal amine of 5-HT.[18] 2-(4-Methylhomopiperazinyl)benzoxazole (**6t**) showed higher potency than the piperazinyl compound **6a**. A 4-methylhomopiperazine ring, therefore, was introduced into the 5-chloro- or 5-chloro-7-methylbenzoxazole structure. The resultant compounds **6u**,**v** had the desired profile of high potency and low intrinsic activity. We choose **6v** (pD_2 = 7.76 ± 0.11, ia = 0.12 ± 0.02) for confirmation of the 5-HT$_3$ receptor partial agonist characteristic in other assays.

Several 2-piperazinylbenzoxazole compounds have been reported as 5-HT$_3$ receptor antagonists.[22] We reported previously that compound **5** also behaved like an antagonist in the inhibition test of 5-HT-evoked reflex bradycardia (Bezold−Jarish reflex, B−J reflex) in rats. But, in contrast to the result in rats, it showed agonistic activity in the gut of guinea pig, mouse, and *Suncus murinus*.[13] In the B−J reflex inhibition test in

Table 4. Receptor Binding Affinity and 5-HT-Evoked Bezold−Jarisch (B−J) Reflex Inhibition Activity of **6v** and Granisetron

compd	5-HT_3 receptor binding K_i (nM ± SEM)	B−J reflex ID_{50} (μg/kg ± SEM)[a]
6v	1.1 ± 0.1	87.6 ± 33.1
granisetron	0.5 ± 0.1	6.8 ± 2.4

[a] Values are the mean ± SEM of six experiments.

Table 5. Binding Assay of **6v** to Receptors

receptor	radioligand	inhibition at 10^{-5} M[a] (%)
adrenergic α_1	[^{3}H]prazosin	22
adrenergic α_2	[^{3}H]rauwolscine	33
adrenergic β (nonselective)	[^{3}H]DHA	24
dopamine D_1	[^{3}H]SCH23390	18
dopamine D_2	[^{3}H]spiperone	5
benzodiazepine (central, nonselective)	[^{3}H]flunitrazepam	12
muscarine (nonselective)	[^{3}H]QNB	59 (5% at 10^{-7} M)
5-HT_1	[^{3}H]-5-HT	14
5-HT_{1A}	[^{3}H]-8-OH-DPAT	−3
5-HT_2	[^{3}H]ketanserine	23
5-HT_4	[^{3}H]GR-113808	88 (8% at 10^{-7} M)

[a] Each compound was tested in duplicate at 10^{-5} or 10^{-7} M. Values are the means of two experiments.

rats,[23] **6v** (ID_{50} = 87.6 ± 33.1 μg/kg) showed a much weaker activity than granisetron (ID_{50} = 6.8 ± 2.4 μg/kg, Table 4). In addition, **6v** alone did not induce the B−J reflex in rats at a concentration that blocked the reflex caused by 5-HT (1 mg/kg, iv, data not shown). In contrast to its weak effect in the cardiovascular system, **6v** bound to the 5-HT_3 receptor isolated from rat brain with high affinity (K_i = 1.1 ± 0.1 nM) comparable to that of granisetron (K_i = 0.5 ± 0.1 nM, Table 4). These results suggest the diversity of 5-HT_3 receptors,[24−26] although it is not clear whether this diversity reflects the existence of further subtypes or species differences of 5-HT_3 receptors[27] without additional studies. Compound **6v** showed very low affinity for other 5-HT receptor subtypes and other monoaminergic and BZD receptors (Table 5).

We examined the inhibition of 5-HT-evoked diarrhea in mice.[28] The effects of **6v** and granisetron on the transition time of glass beads in the distal colon of normal mice were also observed.[29] These results are summarized in Table 6. Both **6v** (ID_{50} = 0.30 mg/kg) and granisetron (ID_{50} = 0.08 mg/kg) inhibited 5-HT-evoked diarrhea. Granisetron significantly elongated the transition time of glass beads at 1 mg/kg, whereas **6v** had no effect even at 30 mg/kg, a dose equivalent to 100 times the ID_{50} for diarrhea inhibition. Thus, **6v** stopped the diarrhea induced by 5-HT but did not inhibit normal bowel function, presumably because of its characteristic 5-HT_3 partial agonist activity. Such a compound should to be applicable to the treatment of bowel hypersensitivity conditions, such as IBS, without the side effect of constipation.

Conclusion

2-Substituted benzoxazole derivatives were synthesized, and their 5-HT_3 receptor partial agonist activities in the gut were examined. Several compounds showed high potency and low intrinsic activity in vitro. Among them, **6v** inhibited 5-HT-induced diarrhea without affecting normal lower bowel function in vivo. Extensive studies are under way to find new drugs for functional bowel disorders based on these derivatives as lead compounds.

Experimental Section

Chemistry. All melting points are uncorrected. IR spectra were recorded on Shimadzu FT-IR 8100 spectrometers. NMR spectra were obtained on JEOL GSX- or GX-400 FT-NMR spectrometers. The following abbreviations are used: s = singlet, d = doublet, t = triplet, q = quartet, quin = quintet, m = multiplet, and br = broad. MS were measured with Hitachi M-80B and JEOL JMS-700 instrument. Serotonin (**1**) and granisetron (**2**) were commercially available.

2-(4-Methyl-1-piperazinyl)benzoxazole (6a). Procedure F. This procedure illustrates the general method of preparation of compounds **6r**−**6t**. 2-Chlorobenzoxazole (**10**; 1.4 g, 9 mmol) was added to the solution of 1-methylpiperazine (1 g, 10 mmol) in chloroform (100 mL) at 0 °C. The reaction mixture was stirred at 0 °C for 30 min and the reaction quenched in ice−water (150 mL). The resulting mixture was extracted with ethyl acetate (200 mL), and the extract was dried over $MgSO_4$. After concentration in vacuo, the residue was chromatographed on silica gel with chloroform−methanol (20:1) and recrystallized from water−acetone to give **6a** (1.8 g, 85%) as a white needle, mp 37−38 °C. NMR ($CDCl_3$) δ: 2.37 (3H, s, CH_3−), 2.54 (4H, t, J = 8 Hz, piperazine $-CH_2-$ × 2), 3.73 (4H, t, J = 8 Hz, piperazine $-CH_2-$ × 2), 7.02 (1H, t, J = 7 Hz, benzoxazole 5-H), 7.18 (1H, t, J = 7 Hz, benzoxazole 6-H), 7.26 (1H, d, J = 7 Hz, benzoxazole 7-H), 7.37 (1H, d, J = 7 Hz, benzoxazole 4-H). MS (EI) m/z: 217 (M^+). IR (KBr, cm^{-1}): 1630, 1575, 1460, 1397. Anal. ($C_{12}H_{15}N_3O$) C, H, N.

2-(1-Piperidinyl)benzoxazole (6r): obtained as a white needle, 140 mg, 77% yield, mp 67−68 °C (ether−hexane). NMR ($CDCl_3$) δ: 1.68 (6H, s), 3.66 (4H, brs), 6.99 (1H, dt, J = 3, 7 Hz), 7.14 (1H, dt, J = 3, 7 Hz), 7.23 (1H, d, J = 8 Hz), 7.34 (1H, d, J = 8 Hz). MS (EI): 202 (M^+). IR (KBr, cm^{-1}): 1781, 1399, 1365, 1291. Anal. ($C_{12}H_{14}N_2O$) C, H, N.

2-(1-Piperazinyl)benzoxazole (6s): obtained as a white needle, 4.8 g, 72% yield, mp 68−70 °C (ether−hexane). NMR ($CDCl_3$) δ: 2.99 (4H, t, J = 5 Hz), 3.48 (1H, s), 3.68 (4H, t, J = 5 Hz), 7.02 (1H, dt, J = 1, 7 Hz), 7.16 (1H, dt, J = 1, 7 Hz), 7.25 (1H, dd, J = 1, 7 Hz), 7.36 (1H, dd, J = 1, 7 Hz). MS (thermospray ionization, TSP) m/z: 204 (M^+ + 1). IR (KBr, cm^{-1}): 3213, 1630, 1578, 1460, 1271, 1240. Anal. ($C_{11}H_{13}N_3O\cdot{}^1/_3H_2O$) C, H, N.

2-(4-Methyl-1-homopiperazinyl)benzoxazole (6t): obtained as a white needle, 1.9 g, 82% yield, mp 58−59 °C (ether−hexane). NMR ($CDCl_3$) δ: 2.00−2.07 (2H, m), 2.39

Table 6. Inhibition of 5-HT-Evoked Diarrhea and Effect on the Transition Time of Glass Beads in the Distal Colon

compd	inhibition of 5-HT-evoked diarrhea[a] ID_{50} (mg/kg sc) (95% CL)	transition time of glass beads (s ± SEM)[b] control	0.3 mg/kg	1 mg/kg	3 mg/kg	30 mg/kg
granisetron	0.08 (0.13−0.05)	301.1 ± 37.5	373.0 ± 41.9	614.4 ± 95.5[c]	827.0 ± 117.0[c]	NT
6v	0.30 (0.45−0.20)	315.2 ± 41.8	262.6 ± 13.8	NT	357.2 ± 41.2	328.8 ± 45.7

[a] 10 animals were used at each dose. CL: confidence limits. [b] Each result represents the mean ± SEM of 8 animals. The test compound was treated on sc. NT: not tested. [c] Significant difference from the control group at $p < 0.05$.

(3H, s), 2.65−2.77 (2H, m), 2.78−2.81 (2H, m), 3.76−3.83 (4H, m), 7.02 (1H, t, J = 8 Hz), 7.16 (1H, t, J = 8 Hz), 7.26 (1H, d, J = 8 Hz), 7.30 (1H, d, J = 8 Hz). MS (EI) m/z: 231 (M^+). IR (KBr, cm^{-1}): 1638, 1578, 1250. Anal. ($C_{13}H_{17}N_3O$) C, H, N.

5-Chloro-2-(4-methyl-1-piperazinyl)benzoxazole (6g). Procedures C[17] and D. This procedure illustrates the general method of preparation of compounds **6b**−**e**. 2-Amino-4-chlorophenol (**8g**; 10 g, 70 mmol) was refluxed for 8 h with potassium hydroxide (4.7 g, 84 mmol) and carbon disulfide (100 mL) in ethanol (150 mL). The reaction mixture was concentrated in vacuo; 1 N aqueous hydrochloric acid (100 mL) and ethyl acetate (200 mL) were added to the residue. The organic layer was washed with water (100 mL), dried over $MgSO_4$, and concentrated in vacuo. The 11.5 g of 5-chloro-2-mercaptobenzoxazole (**9g**) was obtained as a yellow powder and used in the next reaction without further purification.

Phosphorus pentachloride (1.35 g, 6.5 mmol) was added to the solution of 5-chloro-2-mercaptobenzoxazole (**9g**; 1 g, 5.4 mmol) in dry toluene (50 mL) at 20 °C. The reaction mixture was stirred at 120 °C for 1 h and cooled in an ice bath. 1-Methylpiperazine (5.4 g, 54 mmol) was added dropwise to the mixture, and the mixture stirred for 30 min at 0 °C. The reaction mixture was diluted with chloroform (100 mL) and washed with water. The organic layer was dried over $MgSO_4$ and concentrated in vacuo. The residue was chromatographed on silica gel with chloroform−methanol (20:1) to give **6g** (1 g, 67% from **8g**) as a white needle, mp 118−119 °C (acetone−hexane). NMR (CD_3OD) δ: 2.34 (3H, s, CH_3−), 2.57 (4H, t, J = 5 Hz, piperazine −CH_2− × 2), 3.71 (4H, t, J = 5 Hz, piperazine −CH_2− × 2), 7.03 (1H, d, J = 7 Hz, benzoxazole 6-H), 7.22 (1H, s, benzoxazole 4-H), 7.26 (1H, d, J = 7 Hz, benzoxazole 7-H). MS (EI) m/z: 251 (M^+). IR (KBr, cm^{-1}): 1634, 1574, 1449, 1398, 1368, 1356. Anal. ($C_{12}H_{14}N_3OCl$) C, H, N.

4-Methyl-2-(4-methyl-1-piperazinyl)benzoxazole (6b): obtained as a colorless oil, 461 mg, 68% yield from 2-amino-*m*-cresol. NMR ($CDCl_3$) δ: 2.35 (3H, s), 2.49 (3H, s), 2.52 (4H, t, J = 5 Hz), 3.72 (4H, t, J = 5 Hz), 6.92 (1H, t, J = 7 Hz), 6.97 (1H, d, J = 7 Hz), 7.08 (1H, d, J = 7 Hz). MS (EI) m/z: 231 (M^+). IR (neat, cm^{-1}): 1620, 1590, 1489, 1454, 1306, 1277. HR-MS Calcd for $C_{13}H_{18}N_3O$: 232.1450. Found: 232.1443.

5-Methyl-2-(4-methyl-1-piperazinyl)benzoxazole (6c): obtained as a yellow plate, 1.8 g, 73% yield from 2-amino-*p*-cresol, mp 63−64 °C (methanol−ether). NMR ($CDCl_3$) δ: 2.35 (3H, s), 2.39 (3H, s), 2.52 (4H, t, J = 5 Hz), 3.71 (4H, t, J = 5 Hz), 6.82 (1H, d, J = 8 Hz), 7.11 (1H, d, J = 8 Hz), 7.15 (1H, s). MS (EI) m/z: 231 (M^+). IR (KBr, cm^{-1}): 1638, 1586, 1451, 1356. Anal. ($C_{13}H_{17}N_3O \cdot {}^1/_8H_2O$) C, H, N.

6-Methyl-2-(4-methyl-1-piperazinyl)benzoxazole (6d): obtained as a white needle, 166 mg, 50% yield from 6-amino-*m*-cresol, mp 62−63 °C (methanol−ether). NMR ($CDCl_3$) δ: 2.35 (3H, s), 2.40 (3H, s), 2.52 (4H, t, J = 5 Hz), 3.70 (4H, t, J = 5 Hz), 6.97 (1H, d, J = 8 Hz), 7.07 (1H, s), 7.23 (1H, d, J = 8 Hz). MS (EI) m/z: 231 (M^+). IR (KBr, cm^{-1}): 1650, 1578, 1489, 1397. Anal. ($C_{13}H_{17}N_3O$) C, H, N.

7-Methyl-2-(4-methyl-1-piperazinyl)benzoxazole (6e). 2-Methyl-6-nitrophenol[28] (**7e**; 880 mg, 5.75 mmol) was dissolved in ethanol (25 mL), and 10% palladium−carbon (90 mg) was added to the solution. The reaction mixture was stirred under a hydrogen atmosphere for 24 h, and palladium−carbon was removed by filtration. The solution was concentrated in vacuo. The 730 mg of 2-amino-6-methylphenol (**8e**) was obtained as a brown powder and used in the next reaction without further purification.

2-Amino-6-methylphenol (**8e**; 700 mg, 5.69 mmol) was treated as described for the preparation of **6g** to afford **6e** as a yellow oil, 392 mg, 70% yield from **7e**. NMR ($CDCl_3$) δ: 2.36 (3H, s), 2.42 (3H, s), 2.53 (4H, t, J = 5 Hz), 3.73 (4H, t, J = 5 Hz), 6.83 (1H, d, J = 8 Hz), 7.06 (1H, t, J = 8 Hz), 7.19 (1H, d, J = 8 Hz). MS (EI) m/z: 231 (M^+). IR (neat, cm^{-1}): 1650, 1580, 1450, 1270, 1150. HR-MS Calcd for $C_{13}H_{18}N_3O$: 232.1450. Found: 232.1448.

5-Chloro-7-methyl-2-(4-methyl-1-homopiperazinyl)benzoxazole (6v). Procedures B, C, and E. This procedure illustrates the general method of preparation of compounds **6f,h,m**−**q,u.** 4-Chloro-2-methyl-6-nitrophenol[30] (**7v**; 2.0 g, 10.7 mmol) was dissolved in ethyl acetate (60 mL), and 5% platinum on sulfide carbon (60 mg; Aldrich Chemical Co.) was added to the solution. The reaction mixture was stirred under a hydrogen atmosphere for 24 h, and platinum on sulfide carbon was removed by filtration. The solution was concentrated in vacuo. The 1.7 g of 2-amino-4-chloro-6-methylphenol (**8v**) was obtained as a brown powder and used in the next reaction without further purification.

2-Amino-4-chloro-6-methylphenol (**8v**; 1.68 g, 10.7 mmol) was treated as described for the preparation of **9g** to afford 893 mg of 5-chloro-2-mercapto-7-methylbenzoxazole (**9v**) as a light-brown needle, mp 233−234 °C (methanol−ether). NMR ($CDCl_3$) δ: 2.43 (3H, s), 6.98 (1H, s), 7.03 (1H, s). MS (EI) m/z: 199 (M^+). IR (KBr, cm^{-1}): 1609, 1514, 1443, 1339. Anal. Calcd for C_8H_6NOClS: C, 48.13; H, 3.03; N, 7.02. Found: C, 48.02; H, 2.90; N, 6.90.

5-Chloro-2-mercapto-7-methylbenzoxazole **9v** (200 mg, 1.00 mmol) and 1-methylhomopiperazine (0.55 mL, 5.0 mmol) were dissolved by toluene (10 mL). The mixture was refluxed for 16 h and evaporated. The residue was chromatographed on silica gel with dichloromethane−methanol (10:1) to give **6v** (260 mg, 33% from **7v**) as a white needle, mp 116−117 °C (water−methanol). NMR ($CDCl_3$) δ: 2.00−2.07 (2H, m, homopiperazine 6-CH_2), 2.37 (3H, s, homopiperazine 1-CH_3), 2.40 (3H, s, benzoxazole 7-CH_3), 2.63 (2H, t, J = 5 Hz, homopiperazine −CH_2−), 2.74 (2H, t, J = 5 Hz, homopiperazine −CH_2−), 3.79 (2H, t, J = 5 Hz, homopiperazine −CH_2−), 3.85 (2H, t, J = 6 Hz, homopiperazine −CH_2−), 6.78 (1H, d, J = 2 Hz, benzoxazole 6-H), 7.13 (1H, d, J = 2 Hz, benzoxazole 4-H). MS (TSP) m/z: 280 ($M^+ + 1$), 282 ($M^+ + 3$). IR (KBr, cm^{-1}): 1642, 1622, 1572, 1458. Anal. ($C_{14}H_{18}N_3OCl$) C, H, N.

5-Fluoro-2-(4-methyl-1-piperazinyl)benzoxazole (6f): obtained as a white needle, 207 mg, 70% yield from 4-fluoro-2-nitrophenol,[31] mp 116−117 °C (ether−hexane). NMR ($CDCl_3$) δ: 2.35 (3H, s), 2.52 (4H, t, J = 5 Hz), 3.72 (4H, t, J = 5 Hz), 6.71 (1H, dt, J = 2, 9 Hz), 7.04 (1H, dd, J = 2, 9 Hz), 7.14 (1H, dd, J = 4, 9 Hz). MS (EI) m/z: 235 (M^+). IR (KBr, cm^{-1}): 1640, 1578, 1476, 1464. Anal. ($C_{12}H_{14}N_3OF$) C, H, N.

5-Bromo-2-(4-methyl-1-piperazinyl)benzoxazole (6h): obtained as a white needle, 178 mg, 60% yield from 4-bromo-2-nitrophenol,[31] mp 100−101 °C (ether−hexane). NMR ($CDCl_3$) δ: 2.36 (3H, s), 2.47 (3H, s), 2.53 (4H, t, J = 5 Hz), 3.74 (4H, t, J = 5 Hz), 7.26 (1H, s). MS (TSP) m/z: 296 (M^+), 298 ($M^+ + 2$). IR (KBr, cm^{-1}) 1636, 1568, 1460, 1366. Anal. ($C_{12}H_{14}N_3OBr$) C, H, N.

5-Chloro-7-methyl-2-(4-methyl-1-piperazinyl)benzoxazole (6m): obtained as a yellow plate, 270 mg, 85% yield from 4-chloro-2-methyl-6-nitrophenol,[31] mp 62−64 °C (ether−hexane). NMR ($CDCl_3$) δ: 2.36 (3H, s), 2.37 (3H, s), 2.53 (4H, t, J = 5 Hz), 3.72 (4H, t, J = 5 Hz), 6.81 (1H, d, J = 2 Hz), 7.14 (1H, d, J = 2 Hz). MS (TSP) m/z: 266 ($M^+ + 1$), 268 ($M^+ + 3$). IR (KBr, cm^{-1}): 1644, 1626, 1576, 1449, 1410, 1356. Anal. ($C_{13}H_{16}N_3OCl$) C, H, N.

5,7-Dichloro-2-(4-methyl-1-piperazinyl)benzoxazole (6n): obtained as a yellow needle, 90 mg, 37% yield from 2,4-dichloro-6-nitrophenol, mp 106−107 °C (hexane). NMR ($CDCl_3$) δ: 2.36 (3H, s), 2.53 (4H, t, J = 5 Hz), 3.75 (4H, t, J = 5 Hz), 7.00 (1H, d, J = 2 Hz), 7.18 (1H, d, J = 2 Hz). MS (EI) m/z: 285 (M^+), 287 ($M^+ + 2$), 289 ($M^+ + 4$). IR (KBr, cm^{-1}): 1638, 1572, 1447, 1300, 1273, 1143. Anal. ($C_{12}H_{13}N_3OCl_2$) C, H, N.

5-Chloro-6-methyl-2-(4-methyl-1-piperazinyl)benzoxazole (6o): obtained as a yellow needle, 170 mg, 65% yield from 4-chloro-5-methyl-2-nitrophenol,[32] mp 115−116 °C (ether−hexane). NMR ($CDCl_3$) δ: 2.35 (3H, s), 2.39 (3H, s), 2.51 (4H, t, J = 5 Hz), 3.70 (4H, t, J = 5 Hz), 7.10 (1H, s), 7.31 (1H, s). MS (TSP) m/z: 266 ($M^+ + 1$), 268 ($M^+ + 3$). IR (KBr, cm^{-1}): 1630, 1568, 1451, 1267. Anal. ($C_{13}H_{16}N_3OCl$) C, H, N.

4-Chloro-2,3-dimethylphenol.[33] Ether (3.7 g, 50 mmol) was added slowly at 20 °C to a stirred solution of 2,3-xylenol (6.1 g, 50 mmol) and sulfuryl chloride (6.8 g, 50 mmol) in dichloromethane (50 mL). The reaction mixture was stirred for 30 min at room temperature and evaporated. The residue

was dissolved in chloroform (100 mL), washed with water (100 mL), dried over $MgSO_4$, and concentrated in vacuo. The crude compounds were recrystallized from hexane to afford 5.9 g of 4-chloro-2,3-dimethylphenol (white needle, 75% yield, mp 81−82 °C). NMR ($CDCl_3$) δ: 2.20 (3H, s, 2-CH_3), 2.33 (3H, s, 3-CH_3), 6.58 (1H, d, J = 9 Hz, 6-H), 7.07 (1H, d, J = 9 Hz, 5-H). MS (EI) m/z: 156 (M^+), 268 ($M^+ + 2$).

4-Chloro-2,3-dimethyl-6-nitrophenol (7p).[34] Nitric acid (4.0 g, 63.9 mmol) was added slowly at 8 °C to a stirred solution of 4-chloro-2,3-dimethylphenol (5 g, 31.9 mmol) in sulfuric acid (50 mL) and acetic acid (12.5 mL). The reaction mixture was stirred for 2 h at room temperature and the reaction quenched with ice−water. The crude compounds were collected by filtration and recrystallized from ether−hexane to afford 3.5 g of 2,4-dichloro-3-methyl-6-nitrophenol (yellow needle, 54% yield, mp 73−74 °C). NMR ($CDCl_3$) δ: 2.31 (3H, s, 2-CH_3), 2.41 (3H, s, 3-CH_3), 8.01 (1H, s, 5-H), 10.93 (1H, s, phenol OH). MS (EI) m/z: 201 (M^+), 203 ($M^+ + 2$).

5-Chloro-6,7-dimethyl-2-(4-methyl-1-piperazinyl)benzoxazole (6p): obtained as a white needle, 200 mg, 60% yield from 4-chloro-2,3-dimethyl-6-nitrophenol, mp 97−99 °C (ether−hexane). NMR ($CDCl_3$) δ: 2.33 (3H, s), 2.35 (3H, s), 2.36 (3H, s), 2.54 (4H, t, J = 5 Hz), 3.72 (4H, t, J = 5 Hz), 7.20 (1H, s). MS (TSP) m/z: 280 ($M^+ + 1$), 282 ($M^+ + 3$). IR (KBr, cm^{-1}): 1646, 1628, 1576, 1447, 1362. Anal. ($C_8H_8NO_3Cl$) C, H, N.

2,4-Dichloro-3-methyl-6-nitrophenol (7q).[34] 2,4-Dichloro-3-methyphenol (5 g, 28.2 mmol) was nitrated as described for the preparation of **7p** to afford **7q** (4.0 g, 64%) as a yellow needle, mp 77−78 °C (ether−hexane). NMR ($CDCl_3$) δ: 2.57 (3H, s), 8.10 (1H, s), 11.01 (1H, s). MS (EI) m/z: 221 (M^+), 223 ($M^+ + 2$).

5,7-Dichloro-6-methyl-2-(4-methyl-1-piperazinyl)benzoxazole (6q): obtained as a white needle, 250 mg, 65% yield from 2,4-dichloro-3-methyl-6-nitrophenol, mp 123−124 °C (ether−hexane). NMR ($CDCl_3$) δ: 2.36 (3H, s), 2.47 (3H, s), 2.53 (4H, t, J = 5 Hz), 3.74 (4H, t, J = 5 Hz), 7.26 (1H, s). MS (TSP) m/z: 299 ($M^+ - 1$). IR (KBr, cm^{-1}): 1632, 1570, 1439, 1354. Anal. ($C_{13}H_{15}N_3OCl_2$) C, H, N.

5-Chloro-2-(4-methyl-1-homopiperazinyl)benzoxazole (6u): obtained as a yellow needle, 880 mg, 49% yield from 2-amino-4-chlorophenol, mp 85−85.5 °C (ether−hexane). NMR ($CDCl_3$) δ: 2.02−2.10 (2H, m), 2.42 (3H, s), 2.65−2.69 (2H, m), 2.76−2.81 (2H, m), 3.76−3.81 (2H, m), 3.82−3.89 (2H, m), 6.94 (1H, d, J = 8 Hz), 7.13 (1H, d, J = 8 Hz), 7.30 (1H, s). MS (EI) m/z: 265 (M^+). IR (KBr, cm^{-1}): 1632, 1575, 1462, 1350. Anal. ($C_{13}H_{16}N_3OCl$) C, H, N.

5-(Trifluoromethyl)-2-(4-methyl-1-piperazinyl)benzoxazole (6i). Procedure A, C, and E. This procedure illustrates the general method of preparation of compounds **6j−l.** 4-(Trifluoromethyl)-2-nitrophenol (**7e**; 880 mg, 5.75 mmol) was treated as described for the preparation of **8e** to afford 2-amino-4-(trifluoromethyl)phenol (**8i**) as a white powder.

2-Amino-4-(trifluoromethyl)phenol (**8i**) was treated as described for the preparation of **6v** to afford **6i** as a white needle, 47 mg, 21% yield from **7e**, mp 72−73 °C (ether−hexane). NMR ($CDCl_3$) δ: 2.36 (3H, s, piperazine CH_3−), 2.54 (4H, t, J = 5 Hz, piperazine −CH_2−), 3.75 (4H, t, J = 5 Hz, piperazine −CH_2−), 7.30 (2H, m, benzoxazole 5- and 7-H), 7.57 (1H, s, benzoxazole 4-H). MS (TSP) m/z: 286 ($M^+ + 1$). IR (KBr, cm^{-1}): 1657, 1592, 1456, 1443, 1325, 1156. Anal. ($C_{13}H_{14}N_3OF_3$) C, H, N.

5-Ethyl-2-(4-methyl-1-piperazinyl)benzoxazole (6j): obtained as a yellow oil, 286 mg, 80% yield from 4-ethyl-2-nitrophenol.[35] NMR ($CDCl_3$) δ: 1.24 (3H, t), 2.35 (3H, s), 2.52 (4H, t, J = 5 Hz), 2.68 (2H, q, J = 7 Hz), 3.71 (4H, t, J = 5 Hz), 6.85 (1H, dd, J = 1, 8 Hz), 7.14 (1H, d, J = 8 Hz), 7.19 (1H, s). MS (EI) m/z: 245 (M^+). IR (neat, cm^{-1}): 1646, 1586, 1456, 1333. HR-MS Calcd for $C_{14}H_{19}N_3O$: 246.1606. Found: 246.1616.

5,6-Dimethyl-2-(4-methyl-1-piperazinyl)benzoxazole (6k): obtained as a white needle, 260 mg, 39% yield from 4,5-dimethyl-2-nitrophenol,[36] mp 130−131 °C (hexane). NMR ($CDCl_3$) δ: 2.28 (3H, s), 2.29 (3H, s), 2.37 (3H, s), 2.51 (4H, t, J = 5 Hz), 3.69 (4H, t, J = 5 Hz), 7.03 (1H, s), 7.13 (1H, s). MS (TSP) m/z: 246 ($M^+ + 1$). IR (KBr, cm^{-1}): 1626, 1574, 1455, 1269. Anal. ($C_{14}H_{19}N_3O$) C, H, N.

5,7-Dimethyl-2-(4-methyl-1-piperazinyl)benzoxazole (6l): obtained as a yellow oil, 286 mg, 73% yield from 2,4-dimethyl-6-nitrophenol.[37] NMR ($CDCl_3$) δ: 2.34 (6H, s), 2.36 (3H, s), 2.52 (4H, t, J = 5 Hz), 3.71 (4H, t, J = 5 Hz), 6.65 (1H, s), 7.00 (1H, s). MS (EI) m/z: 245 (M^+). IR (neat, cm^{-1}): 1684, 1582, 1458, 1302. HR-MS Calcd for $C_{14}H_{19}N_3O$: 246.1606. Found: 246.1608.

2-(4-Piperidinyl)benzoxazole (11). Procedure G.[20] *o*-Aminophenol (**8a**; 200 mg, 1.83 mmol) and 4-piperidinecarboxylic acid (236 mg, 1.83 mmol) were added to the polyphosphoric acid (1 g). The mixture was heated for 2 h at 180 °C, allowed to cool, poured into water, and filtered off. The filtrate was treated with 50% potassium hydroxide solution until pH = 12 and extracted with dichloromethane (100 mL). The organic layer was dried over $MgSO_4$ and concentrated in vacuo. The residue was chromatographed on silica gel with chloroform−methanol (20:1) to give **11** as a white amorphous solid, 310 mg, 84% yield. NMR ($CDCl_3$) δ: 1.89 (2H, ddd, J = 4, 12, 25 Hz, piperizine −CH_2−), 2.16 (2H, dd, J = 2, 12 Hz, piperizine, −CH_2−), 2.79 (2H, dt, J = 3, 12 Hz, piperizine −CH_2−), 3.05−3.15 (1H, m, piperizine 4-CH), 3.20−3.23 (2H, m, piperizine −CH_2−), 7.27−7.33 (2H, m, benzoxazole 5- and 6-H), 7.46−7.52 (1H, m, benzoxazole 7-H), 7.66−7.72 (1H, m, benzoxazole 4-H). MS (EI) m/z: 202 (M^+). IR (KBr, cm^{-1}): 1611, 1566, 1472, 1456. Anal. ($C_{12}H_{14}N_2O \cdot {}^1/_6H_2O$) C, H, N.

Contraction Test.[13,18] Male Hartley guinea pigs weighing 500−800 g were killed by bleeding from the neck, and the ileum was excised. Pieces (about 20 mm) of ileal longitudinal muscles were placed in a 5-mL organ bath containing Krebs solution aerated with 95% O_2 and 5% CO_2 at 37 °C. The composition of the solution was as follows (mM): NaCl 118, KCl 4.7, KH_2PO_4 1.19, $MgSO_4$ 1.2, $CaCl_2$ 2.54, $NaHCO_3$ 25, and glucose 11. The solution also contained ritanserin (10^{-7} M) to inhibit the 5-HT_2 receptor. The preparations were allowed to equilibrate for at least 30 min under 0.5 g of tension. After equilibration, the preparations were repeatedly exposed to 3×10^{-7} M 5-HT to desensitize the 5-HT_4 receptor. Compounds were added to the bath, and contractions were recorded isometrically. The sensitivities of agonists were expressed as pD_2 values, i.e., the negative logarithm of the molar concentration which produced 50% of the maximum contraction obtained from individual concentration−response curves. The ia of a partial agonist was expressed as the ratio between the maximum response to a test compound and that to 5-HT (10^{-5} M).

5-HT_3 Receptor Binding Assay. The assay was performed according to the method of Kilpatrick et al.[21] Brain cortices were isolated from male Wistar rats (250−300 g), and a membrane fraction was prepared by standard techniques. The membrane fraction (0.04 mg) was incubated with 0.2 nM [^{3}H]GR656630 for 60 min at 22 °C. Membranes were collected by filtration and washed three times. The radioactivity on the filters was counted to determine [^{3}H]GR656630 specifically bound. Nonspecific binding was estimated in the presence of 1 mM ICS205-930. For obtaining K_i values, assays were carried out six times at each dose and displacement curves were fitted by nonlinear regression. IC_{50} values were obtained directly. K_i values were calculated from IC_{50} values by using the equation of Cheng and Prusoff.[38]

Inhibition of 5-HT-Induced Bezold−Jarisch Reflex in Rats. The assay was conducted according to the procedure of Fozard and Host.[23] Male Wistar rats (250−300 g) were anesthetized with urethane (1.25 g/kg ip), and blood pressure and heart rate were recorded. A submaximal dose of 5-HT (10 μg/kg iv) was given repeatedly, and changes in heart rate were observed. Test compounds were given intravenously 5 min prior to administration of 5-HT, and their effect was expressed as percent inhibition of the 5-HT response. ID_{50} values were calculated by nonlinear regression analysis.

Inhibition of 5-HT-Induced Diarrhea in Mice.[28] Male mice were starved for 18 h before the experiments. Test compounds were administered 15 min before 5-HT adminis-

tration. Evaluation of diarrhea was made 35 min after the sc administration of 5-HT (5.0 mg/kg). Diarrhea was defined as wet and unformed stools and was scored as present or absent for each animal. 5-HT (5.0 mg/kg) caused diarrhea in 100% of the mice within 35 min. The dose of a test compound required to reduce incidence of the diarrhea to 50% of the treated animals (ID_{50}) was determined by the probit method, and confidence limits for $p = 0.95$ (95% CL) were calculated.

Measurement of Transition Time in Distal Colon in Mice.[29] Male mice were starved for 4 h before the experiments. A glass bead (3 mm in diameter) was inserted into the distal colon by 3 cm above the anus. Test compounds were administered on sc 20 min before glass bead insertion. The time required to evacuate the bead was measured. The group data were compared by analysis of variance followed by Steel's multiple range test.

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