Studies on Serotonin (5-HT)$_3$-Receptor Antagonist Effects of Enantiomers of 4,5,6,7-Tetrahydro-1*H*-Benzimidazole Derivatives

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Received June 24, 1994 Accepted November 28, 1994

ABSTRACT—We assessed the 5-HT$_3$-receptor antagonist effects of 4,5,6,7-1*H*-benzimidazole compounds which are derivatives of YM060, a potent and selective 5-HT$_3$-receptor antagonist, in isolated guinea pig colon. YM114 (KAE-393), YM-26103-2, YM-26308-2 (3×10^{-9} to 3×10^{-8} M) produced concentration-dependent shifts to the right of the dose-response curves for both 5-HT and 2-methyl-5-HT (2-Me-5-HT). YM114 ($pA_2 = 9.08$ against 5-HT, $pA_2 = 8.88$ against 2-Me-5-HT), YM-26103-2 ($pA_2 = 8.27$ against 5-HT, $pA_2 = 8.19$ against 2-Me-5-HT), and YM-26308-2 ($pA_2 = 8.58$ against 5-HT, $pA_2 = 8.4$ against 2-Me-5-HT) showed similar pA_2 values irrespective of the agonist used, suggesting that they have 5-HT$_3$-receptor blocking activity irrespective of the *N*-position at the aromatic ring. Since these compounds have an asymmetric center, their enantiomers exist. The *S*-isomers were one to three orders of magnitude less potent than the respective *R*-isomer compounds, indicating that the stereochemical configuration of 4,5,6,7-tetrahydro-1*H*-benzimidazoles is an important determinant of their affinity for 5-HT$_3$ receptors. These results suggest that the highly potent 5-HT$_3$ receptor antagonism and high selectivity for 5-HT$_3$ receptors of 4,5,6,7-tetrahydro-1*H*-benzimidazole derivatives are conserved irrespective of the position of the nitrogen atom in the aromatic ring and that 5-HT$_3$ receptors favor the *R*-isometric conformation of these compounds.

Keywords: 5-HT$_3$ receptor, Colon (guinea pig), Stereoselectivity

Recently, YM060 was reported to be a potent and selective 5-HT$_3$-receptor antagonist (1, 2). Since YM060 has an asymmetric center in its structure and is an *R*-isomer, a stereoisomer (*S*-form) exists. Based on the pA_2 and ED_{50} values, YM060 is approximately 200 and 250 times more potent than its *S*-isomer in 5-HT$_3$-receptor antagonism in isolated guinea pig colon (1) and anesthetized rats (2), respectively.

In general, 5-HT$_3$-receptor antagonists are composed of three fundamental substructures: an aromatic component, a chain containing a carbonyl group and a terminal amine group. The aromatic component of YM060 is an indolyl group. This compound contains 4,5,6,7-tetrahydro-1*H*-benzimidazole as the terminal amine group instead of an imidazole (ondansetron) or an aliphatic and sterically-hindered azabicycloamine (granisetron, zacopride and MDL72222). The present study focused on the aromatic component and its isometric configuration. The compounds used in this study are derivatives of YM060, in which the positions of the nitrogen atom in their aromatic rings differ while the other portions remain the same, including the position of the asymmetric center.

In the present study, we examined the 5-HT$_3$-receptor antagonistic activity of these derivatives of YM060 and the respective *S*-isomers in isolated tissues to investigate whether 5-HT$_3$-receptor antagonistic activity and stereoselectivitiy are affected by changing the *N*-position in the indolyl moiety of YM060.

MATERIALS AND METHODS

General procedures

All tissues were suspended in 10- or 30-ml organ baths containing Krebs-bicarbonate solution warmed to 37℃ and aerated with a gas mixture of 95% O_2 and 5% CO_2. The composition of the solution was: 118.4 mM NaCl, 4.7 mM KCl, 1.2 mM $MgSO_4$, 2.5 mM $CaCl_2$, 11.1 mM dextrose, 25.0 mM $NaHCO_3$ and 1.2 mM KH_2PO_4. These were dissolved in distilled water. The tissues were attached to isometric force-displacement transducers (SB-1T; Nihon Kohden, Tokyo) connected to recorders (SS-100F; Sekonic, Tokyo) through carrier amplifiers (AP-

621G, Nihon Kohden), cardiotachometers (AT-601G, Nihon Kohden) or both. Equilibration was undertaken for 1 or 2 hr before the addition of drugs. After cumulative concentration-response curves for agonists were constructed by increasing the bath concentrations of the agonists approximately 3-fold (3), tissues were exposed to antagonists for 15 to 30 min before rechallenge with agonists in the same preparation in all the experiments except for the experiment on the β_2-adrenoceptor.

Guinea pig colon

5-HT_3-receptor antagonist potencies were determined in the guinea pig colon. The distal portion of the colon was removed from male Hartley guinea pigs (600 to 660 g) and then divided into approximately 20-mm segments. Isometric contraction under a loading tension of 1 g was recorded. Cumulative concentration-response curves were constructed with 5-HT and 2-Me-5-HT.

Dog saphenous vein

5-HT_1-like receptor antagonist potencies were determined in the dog saphenous vein. Mongrel dogs of either sex (9 to 12 kg) were anesthetized with sodium pentobarbital (35 mg/kg, i.v.). The lateral saphenous vein was removed and placed in Krebs solution. Some veins were prepared on the day of use; others were removed from the dog on the day before use and stored in the refrigerator overnight. On the day of use, fat and connective tissues were trimmed, and the vein was cut into equal 5-mm segments. The endothelium of each segment was removed by rubbing. Isometric contraction under a loading tension of 2 g was recorded. The cumulative concentration-response curves was constructed with 5-HT.

Rabbit aorta

Postsynaptic α_1-adrenergic-receptor and 5-HT_2-receptor antagonist potencies were determined in the thoracic aortae of male albino rabbits (3 to 4 kg). Aortas were cut into equal 5-mm ring segments, and the endothelial preparations were removed by rubbing. Isometric contraction under a loading tension of 2 g was recorded. The cumulative concentration-response curves for α_1- and 5-HT_2 receptors were constructed with phenylephrine and 5-HT, respectively.

Guinea pig ileum

Presynaptic α_2-adrenoceptor and histamine H_1-receptor antagonist potencies were determined in the electrically stimulated and nonstimulated longitudinal muscle layer of the guinea pig ileum, respectively (4–7). After washing out the luminal contents, the isolated ileum was divided into approximately 30-mm segments; each segment was stretched over a glass rod, and the longitudinal muscle layer was separated by gentle stroking with a cotton swab at an angle to the mesenteric attachment. Tissues were then vertically suspended in the buffer solution. Cumulative concentration-response curves for histamine H_1-receptors was constructed with histamine. Platinum electrodes were placed near the top and bottom of the tissue in a manner that avoided contact with the tissue. Transmural stimulation was carried out by rectangular pulses of 1-msec duration (40 to 50 V) at a frequency of 1 Hz. Twitch responses to electrical stimulation were isometrically recorded under a resting tension of 0.5 g. Under these conditions, cumulative concentration-inhibitory response curves for presynaptic α_2-receptors were constructed with UK-14,304.

Rat right atrium

Cardiac β_1-adrenoceptor antagonist potencies were determined in the rat right atrium. Atria were isolated from male Wistar rats (300 to 400 g) and suspended in Krebs solution under a loading tension of 1 g. Positive chronotropic responses in the spontaneously beating right atria were recorded with a cardiotachometer (8). Cumulative concentration-response curves were constructed with isoproterenol.

Guinea pig right atrium

Cardiac histamine H_2-receptor antagonist potencies were determined in the guinea pig right atrium. Atria isolated from male Hartley guinea pigs were suspended in Krebs solution under a loading tension of 1 g. Chronotropic responses in the spontaneously beating right atria were recorded with a cardiotachometer. Cumulative concentration-response curves were constructed with histamine.

Guinea pig trachea

Tracheal β_2-adrenoceptor antagonist potencies were determined in the guinea pig trachea. The trachea was cut into 7- to 8-mm segments. These were suspended in Krebs solution under a loading tension of 1 g. After the preparations were contracted by 10^{-6} M methacholine, cumulative concentration-relaxant responses to formoterol, a selective β_2-adrenoceptor agonist (8, 9) were obtained in 7 preparations. In other preparations, responses to formoterol were examined after a 30-min incubation with the antagonist.

Analysis of data

The dose-ratio was obtained from the ratio of the EC_{50} values of an agonist in the presence and absence of an antagonist. Antagonist dissociation constants (K_B) were determined at each antagonist concentration according to the following equation (10):

K_B = [Antagonist (M)]/(Dose ratio − 1)

pA_2 values were then expressed as a negative logarithm of K_B values. In addition, the log (dose ratio − 1) was plotted against the log of the molar concentration of the antagonist, and the regression line and slope of the curve were calculated (11).

Statistical evaluation

Results are expressed as the means ± S.E.M. or the means with 95% confidence limits. Comparison between values from different groups were evaluated by analysis of variance limits. Regression lines were calculated by the least squares method.

Drugs

YM114 (KAE-393, (−)-(*R*)-5-[(2,3-dihydro-1-indolyl) carbonyl]-4,5,6,7-tetrahydro-1*H*-benzimidazole monohydrochloride), YM-26103-2 ((−)-(*R*)-5-[(1-methyl-3-indolizinyl)carbonyl]-4,5,6,7-tetrahydro-1*H*-benzimidazole monohydrochloride), and YM-26308-2 ((−)-(*R*)-5-[(3-methyl-1-indolizinyl)carbonyl]-4,5,6,7-tetrahydro-1*H*-benzimidazole monohydrochloride) (Fig. 1), 2-Me-5-HT, cimetidine, and formoterol fumarate were prepared by Yamanouchi Pharmaceutical Co., Ltd. UK-14,304 tartrate (UK14,304-18) was a gift from Pfizer, Inc. (New York, NY, USA). Serotonin creatinine sulphate was purchased from E. Merck (Darmstadt, FRG); and methacholine chloride and (−)-isoproterenol hydrochloride, prazosin, idazoxan hydrochloride and pyrilamine maleate (mepyramine) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Histamine hydrochloride and phenylephrine hydrochloride were purchased from Wako Pure Chemical Industries (Osaka), and ketanserin tartrate was purchased from Funakoshi (Tokyo). All drugs were dissolved in either distilled water or 0.9% w/v sodium chloride solution (saline).

RESULTS

5-HT$_3$ receptors

5-HT (10^{-7} to 10^{-5} M) and 2-Me-5-HT (3×10^{-7} to 3×10^{-5} M) caused concentration-dependent contractions in the guinea pig colon, with EC_{50} values of $(1.9\pm0.4)\times10^{-6}$ and $(4.8\pm0.2)\times10^{-6}$ M and E_{max} values of 8.3 ± 0.5

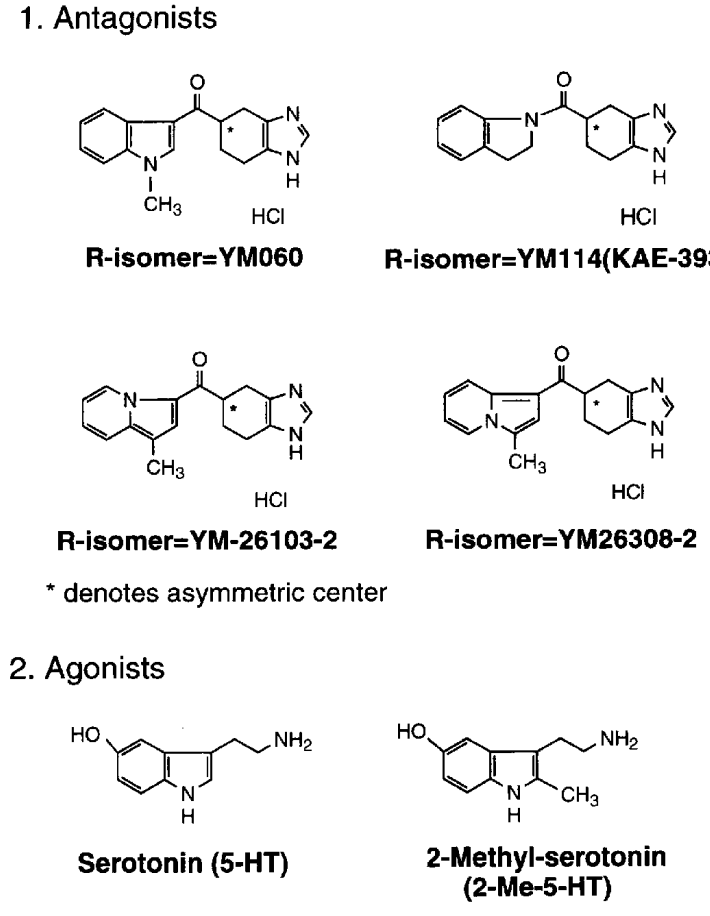

Fig. 1. Chemical structures of YM060, YM114, YM-26103-2, YM-26308-2 and the 5-HT$_3$ receptor agonists used.

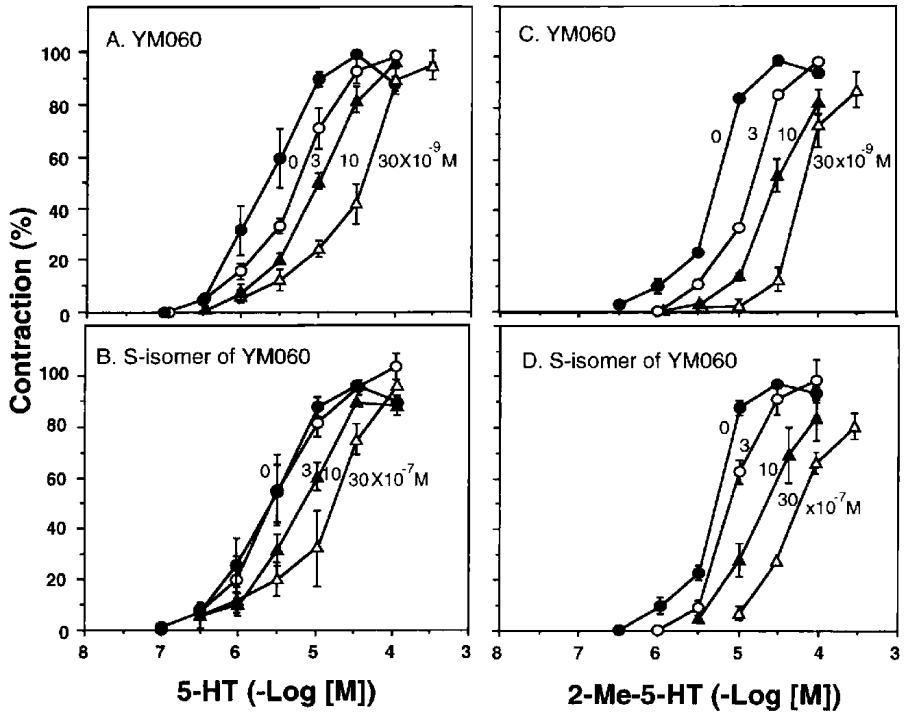

Fig. 2. Antagonism by YM060 and its *S*-isomer of the contractile effects of 5-HT and 2-Me-5-HT in isolated guinea pig colon (data from K. Miyata et al., ref. 1, with permission from J. Pharmacol. Exp. Ther.). The results are the mean±S.E.M. of three or four experiments.

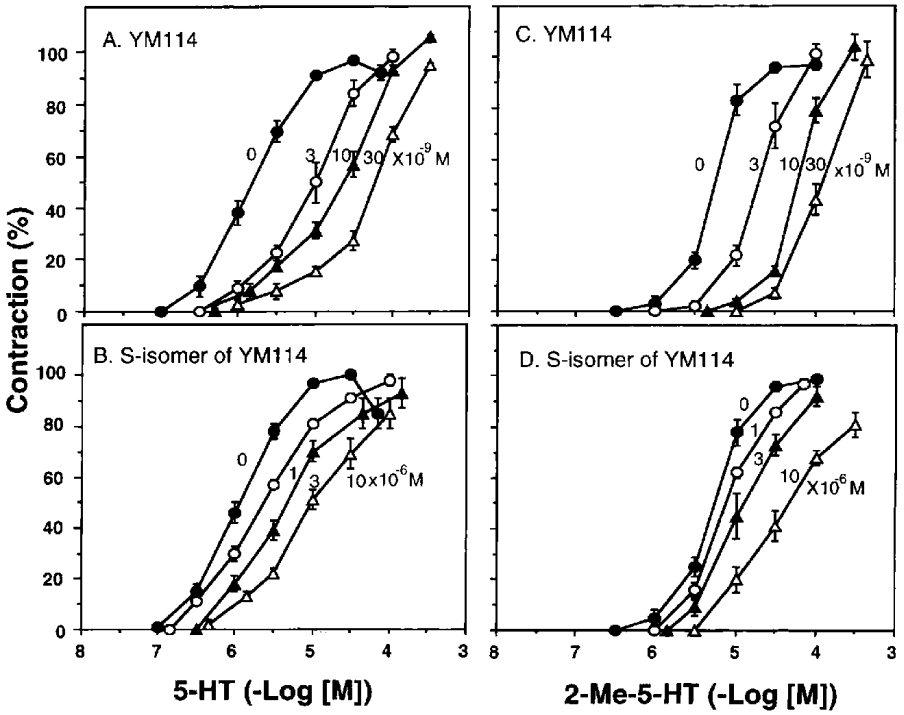

Fig. 3. Antagonism by YM114 and its *S*-isomer of the contractile effects of 5-HT and 2-Me-5-HT in isolated guinea pig colon. The results are the mean±S.E.M. of three or four experiments.

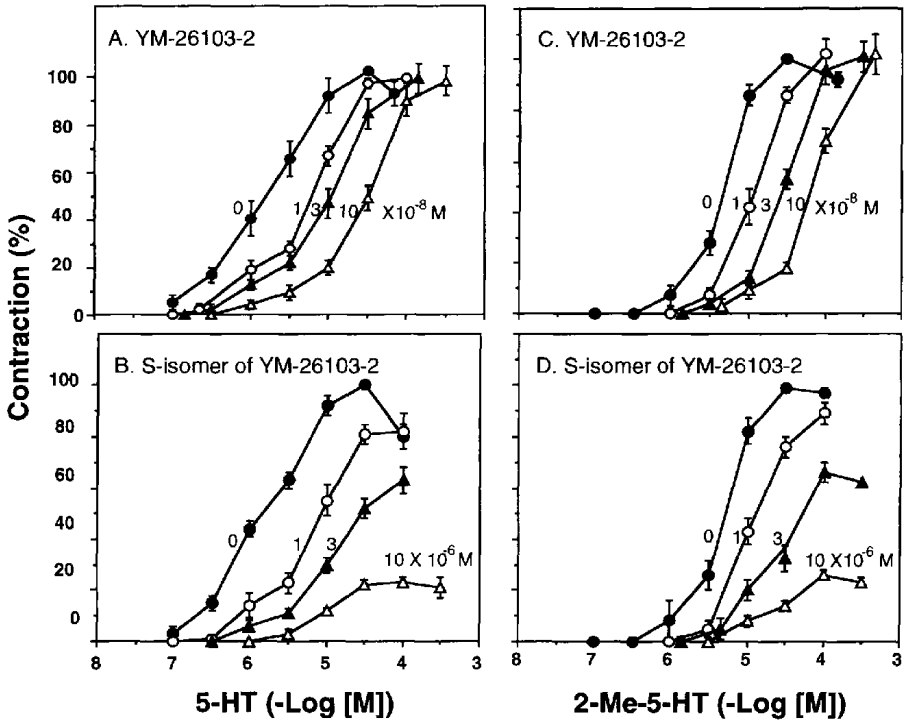

Fig. 4. Antagonism by YM-26103-2 and its *S*-isomer of the contractile effects of 5-HT and 2-Me-5-HT in isolated guinea pig colon. The results are the mean±S.E.M. of three or four experiments.

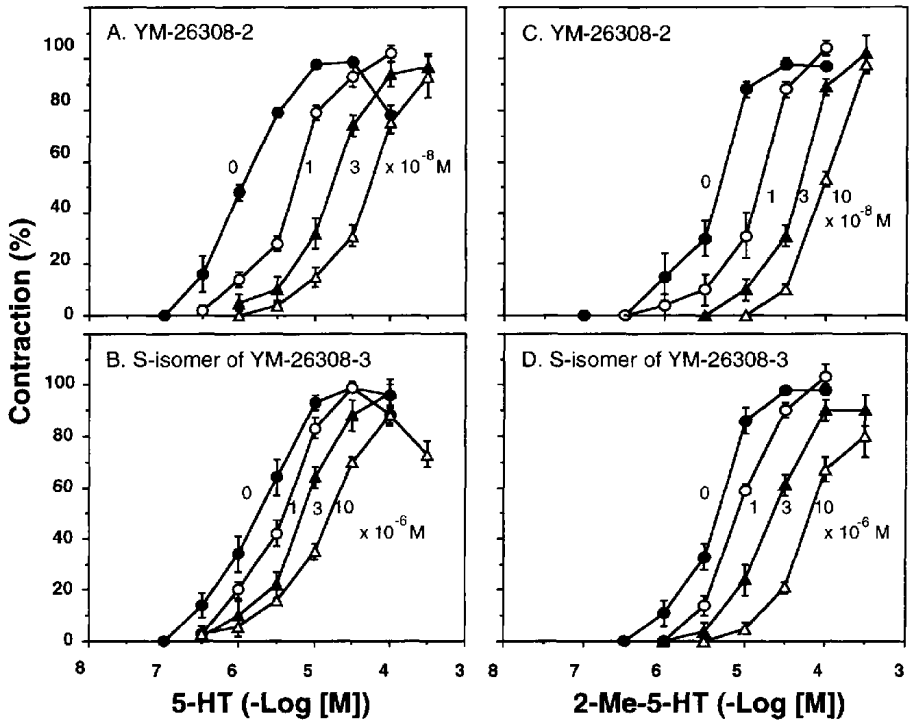

Fig. 5. Antagonism by YM-26308-2 and its *S*-isomer of the contractile effects of 5-HT and 2-Me-5-HT in isolated guinea pig colon. The results are the mean±S.E.M. of three or four experiments.

Table 1. Serotonin (5-HT_3)-receptor blocking activities of enantiomers of 4,5,6,7-tetrahydro-1*H*-benzimidazole derivatives in isolated guinea pig colon

Agonist	YM060	*S*-isomer	R/S ratio	YM114	*S*-isomer	R/S ratio
5-HT	8.71±0.09 (12)[a] 0.92 [0.40–1.44][a]	6.33±0.06 (9)[a] 0.85 [0.52–1.18][a]	240	9.08±0.04 (12) 0.85 [0.64–1.05]	5.97±0.06 (12) 0.94 [0.58–1.29]	1288
2-Me-5-HT	8.69±0.06 (12)[a] 0.94 [0.60–1.28][a]	6.47±0.10 (9)[a] 1.43 [0.72–2.11][a]	166	8.88±0.04 (12) 0.85 [0.62–1.08]	5.73±0.05 (12) 1.11 [0.82–1.39]	1413
Agonist	YM26103-2	*S*-isomer	R/S ratio	YM206308-2	*S*-isomer	R/S ratio
5-HT	8.71±0.09 (12) 0.86 [0.61–1.11]	6.61±0.09 (4)[b] ND	46	8.58±0.04 (12) 0.85 [0.64–1.05]	5.99±0.05 (12) 0.89 [0.64–1.14]	389
2-Me-5-HT	8.69±0.06 (12) 0.94 [0.60–1.28]	6.26±0.05 (4)[b] ND	85	8.40±0.05 (12) 0.97 [0.69–1.26]	6.14±0.05 (12) 1.23 [1.00–1.46]	182

Upper data are the means±S.E.M. of pA_2 and the 95% CL (number of experiments). Lower data are the slopes of the Schild plots. [a] Data from K. Miyata et al. (ref. 1), with permission from J. Pharmacol. Exp. Ther. [b] Data determined from one concentration (1×10^{-6} M), ND: Not determined.

and 8.3±0.5 g, respectively, in 14 paired preparations from 7 animals. The 5-HT_3-receptor blocking potencies of the antagonists were evaluated in the guinea pig colon using 5-HT and 2-Me-5-HT as agonists. Similar to YM060 (Fig. 2), YM114 (Fig. 3), YM-26103-2 (Fig. 4) and YM-26308-2 (Fig. 5) produced parallel and concentration-dependent shifts to the right of the 5-HT- and 2-Me-5-HT-concentration-response curves without any decrease in the maximal responses. The slope of the Schild plot of each antagonist did not differ from unity (Table 1). None of the antagonists affected the baseline tension of the preparations at the concentrations used. Based on the pA_2 values, YM114 showed 1.5 to 2.3, 4.9 to 6.5 and 3.0 to 3.2-fold more potent 5-HT_3-receptor antagonism than YM060, YM-26103-2 and YM-26308-2, respectively (Table 1). The respective *S*-isomers of these compounds (10^{-6} to 10^{-5} M) also caused concentration-dependent shifts to the right of the concentration-response curves (Figs. 2 to 4). Neither the *S*-isomer of YM114 nor that of YM-26308-2 affected the maximal response to 5-HT or 2-Me-5-HT. In contrast, the *S*-isomer of YM-26103-2 (3×10^{-6} and 10^{-5} M) did cause a decrease in the responses. The pA_2 values for the *S*-isomer of YM-26103-2 were therefore calculated from the data at a concentration of 10^{-6} M. The *S*-isomers were 2 to 4 orders of magnitude less potent than the respective *R*-isomers (Table 1).

5-HT_1-like receptors

At 3×10^{-9} to 3×10^{-5} M, 5-HT induced concentration-dependent tension in the dog saphenous vein with an EC_{50} value of $(4.4 \pm 0.9) \times 10^{-8}$ M and an E_{max} value of 10.4±0.7 g (n=6). YM114, YM-26103-2 and YM-26308-2 had no effect on 5-HT_1-like receptors (Table 2). None of

Table 2. Receptor (without 5-HT_3 receptor) blocking activities of *R*-isomers of 4,5,6,7-tetrahydro-1*H*-benzimidazole derivatives in in vitro studies

Receptor	Preparation	Agonist	YM114	YM-26103-2	YM-26308-2
5-HT_1-like	Dog saphenous vein	5-HT	<5.0 (4)	<5.0 (4)	<5.0 (4)
5-HT_2	Rabbit aorta	5-HT	<5.0 (4)	<5.0 (4)	<5.0 (4)
Alpha-1	Rabbit aorta	Phenylephrine	<5.0 (4)	<5.0 (4)	<5.0 (4)
Alpha-2	Guinea pig ileum	UK-14,304	<5.0 (4)	5.68±0.2 (4)	<5.50±0.2 (4)
Beta-1	Rat right atrium	Isoproterenol	<5.0 (4)	<5.0 (4)	<5.0 (4)
Beta-2	Guinea pig trachea	Formoterol	<5.0 (4)	<5.0 (4)	<5.0 (4)
Histamine-1	Guinea pig ileum	Histamine	<5.0 (4)	<5.0 (4)	<5.0 (4)
Histamine-2	Guinea pig atrium	Histamine	<5.0 (4)	<5.0 (4)	<5.0 (4)

Data are the means±S.E.M. of pA_2 and the 95% CL (the number of experiments).

the three compounds affected baseline tension of the preparation at the concentrations used.

5-HT$_2$ receptors

At 10^{-7} to 10^{-5} M, 5-HT produced a concentration-dependent contraction in the rabbit aorta, with an EC_{50} value of $(2.4\pm0.5)\times10^{-7}$ M and an E_{max} value of 4.6 ± 0.4 g (n=6). The specific 5-HT$_2$-receptor antagonist ketanserin (3×10^{-9} to 3×10^{-8} M) caused a parallel and concentration-dependent rightward shift of the 5-HT-concentration-response curve, without decreasing the maximal response. The slope of the Schild plot for ketanserin did not significantly differ from unity (1.31[0.57−2.04]). The pA_2 value for ketanserin was 8.65 ± 0.1 (n=10), which was consistent with the data reported by Peroutka (12). Neither YM114, YM-26103-2 nor YM-26308-2 antagonized the 5-HT-induced contractions (Table 2). These three antagonists did not affect the baseline tension of the preparation at the concentrations used.

α_1-Adrenoceptors

Phenylephrine 3×10^{-8} to 10^{-5} M, a selective α_1-adrenoceptor agonist, caused a concentration-dependent contraction in the rabbit aorta, with an EC_{50} value of $(2.2\pm0.3)\times10^{-7}$ M and an E_{max} value of 8.3 ± 0.7 g (n=6). Prazosin (10^{-8} to 10^{-7} M), a selective α-adrenoceptor antagonist, and idazoxan (10^{-6} to 10^{-5} M) produced a parallel displacement to the right of the phenylephrine-induced concentration-response curve without decreasing the maximal response. The pA_2 values for prazosin and idazoxan were 9.01 ± 0.1 (n=9) and 6.11 ± 0.06 (n=9), respectively. These values were consistent with the results of our previous study (7). The slopes of the Schild plot of prazosin (0.89[0.25−1.51]) and idazoxan (0.83[0.49−1.18]) did not differ from unity. Neither YM114, YM-26103-2, nor YM-26308-2 antagonized phenylephrine-induced contractions (Table 2).

α_2-Adrenoceptors

At 10^{-9} to 10^{-6} M, UK-14,304 induced concentration-dependent inhibition of the electrically stimulated contraction of the guinea pig ileum with an EC_{50} value of $(4.8\pm2.5)\times10^{-8}$ M, which was consistent with the results of Ruffolo (6) and those of our previous study (7). Idazoxan (10^{-8} to 10^{-7} M) and prazosin (10^{-6} to 10^{-5} M) antagonized the action of UK-14,304. The pA_2 values for idazoxan and prazosin were 8.42 ± 0.1 (n=10) and 5.95 ± 0.08 (n=8), respectively, being consistent with the results of our previous study (7). YM114 had no effect on the action of UK-14, 304. YM-26103-2 and YM-26308-2 showed weak inhibition against UK-14,304, with pA_2 values of 5.68 ± 0.2 and 5.50 ± 0.2, respectively (Table 2). These three antagonists did not affect the baseline tension of the preparations at the concentrations used.

β_1-Adrenoceptors

The resting rate of the rat right atria was 275 ± 14 beats/min Isoproterend (10^{-11} to 10^{-8} M) produced a Concentration- dependent chronotropic effect on the atrium with an EC_{50} value of $(7.0\pm0.7)\times10^{-10}$ M and an E_{max} value of 167 ± 5 beats/min (n=19). Neither YM114, YM26103-2, nor YM-26308-2 at up to 10^{-5} M had any effect on the isoproterenol-induced contractile response (Table 2).

β_2-Adrenoceptors

At 10^{-6} M, methacholine induced a tension of 2.9 ± 0.2 g (n=19) in the tracheal ring preparation of guinea pigs. Formoterol (10^{-10} to 10^{-8} M) caused a concentration-dependent relaxation of this contraction with an EC_{50} value of $(3.9\pm0.6)\times10^{-10}$ M (n=7). YM114, YM-26103-2 and YM-26308-2 had no effect on formoterol-induced relaxant responses at up to 10^{-5} M (Table 2).

Histamine H_1-receptors

At 10^{-8} to 10^{-5} M, histamine caused a concentration-dependent increase in the tension of the longitudinal muscle preparation of the guinea pig ileum with an EC_{50} value of $(3.5\pm0.6)\times10^{-10}$ M and an E_{max} value of 6.1 ± 0.5 g (n=6). Mepyramine (3×10^{-9} to 3×10^{-8} M) caused a parallel rightward shift of the concentration-response curve for histamine, with a pA_2 value of 9.27 ± 0.08 (n=8). The slope of the Schild plot for mepyramine was 0.86 (0.36−1.36), which was not significantly different from unity. Neither YM114, YM26103-2, nor YM-26308-2 at up to 10^{-5} M had any effect on the histamine-induced contractile response (Table 2).

Histamine H_2-receptors

The resting rate of the guinea pig right atria was 206 ± 3 beats/min (n=21). Histamine caused a positive chronotropic response in the atrium with an EC_{50} value of $(1.1\pm0.1)\times10^{-6}$ M and an E_{max} of 145 ± 3 beats/min (n=21). Cimetidine (3×10^{-6} to 3×10^{-5} M) produced a parallel rightward shift of the concentration-response curve for histamine without depressing the maximal response. The pA_2 value for cimetidine was 6.03 ± 0.03 (n=9), this being consistent with the results of Black et al. (13). The slope of the Schild plot, 0.93 (0.78−1.13), was not significantly different from unity. Neither YM114, YM-26103-2 nor YM-26308-2 at up to 10^{-5} M had any effect on the action of histamine (Table 2).

DISCUSSION

Generally, selective 5-HT_3-receptor antagonists are composed of three components, namely an aromatic group, a chain with a carbonyl group, and a terminal amine group (14–19). Previous investigations of the chain and the amine portions have suggested that a carbonyl oxygen atom in the chain and a basic nitrogen atom in the terminal amine group interact with the 5-HT_3 receptor. The aromatic component of YM060, a potent and selective 5-HT_3-receptor antagonist (1, 2), is an indolyl moiety. Changing the position of the nitrogen atoms in this moiety yielded YM114, YM-26103-2 and YM-26308-2.

In the present study, YM114, YM-26103-2 and YM-26308-2 showed potent antagonistic activities against 5-HT and 2-Me-5-HT, a 5-HT_3-receptor agonist, in the isolated guinea pig colon. The pA_2 values of these antagonists were constant irrespective of the agonist used. In a previous study, we used ketanserin, methysergide, atropine and tetrodotoxin to show that 5-HT- and 2-Me-5-HT-induced contractions of the guinea pig colon is mediated by neuronal 5-HT_3 receptors (1). The high potencies of the YM-compounds in 5-HT_3-receptor antagonism were also seen in anesthetized rats (20). Our present results showed that all three YM-compounds used in the present study have no or only weak effects on serotonin 5-HT_1-recepror-like and 5-HT_2, adrenergic α_1-, α_2-, β_1- and β_2- and histamine H_1- and H_2- receptors in the isolated tissue. In the guinea pig colon, in addition to the contractile response via 5-HT_3 receptors, that via 5-HT_4 receptors was also reported (21, 22). However, the sensitivity of 5-HT_4 receptors in the guinea pig colon depends on the size of the animals used. Compared to the colon of very young animals (150–200 g), that of larger animals (450–550 g) showed much lower sensitivity (approximately 100-fold less sensitive) to 5-HT_4-receptor stimulation (22). In our study, the body weight of the animals used (600–660 g) was much heavier than those used in the above study (21, 22). In fact, in the present and the previous study (1), the responses of the colonic preparations to 5-HT was almost completely blocked by YM-compounds, ondansetron and granisetron. Furthermore, in the preliminary experiments, YM060 showed neither 5-HT_4-receptor agonist activity in the isolated guinea pig ileum at up to 10^{-4} M nor 5-HT_4 receptor antagonist activity in the isolated rat esophageal musclaris mucosa at up to 3×10^{-5} M. Taken together, these results indicate that the YM-compounds are 5-HT_3-receptor antagonists, with high potencies and selectivities for 5-HT_3 receptors comparable to those of YM060.

Because 5-HT and 2-Me-5-HT do not have stereoisomers, stereochemical requirements for the activation of 5-HT_3 receptors have not been studied. On the other hand, stereochemical requirements for several 5-HT_3 receptor antagonists have been reported. Both ondansetron and zacopride have an asymmetric carbon in their structure, resulting in the presence of *R*- and *S*-isomers. In 5-HT_3-receptor antagonism against 5-HT- and 2-Me-5-HT-induced contraction of the isolated guinea pig ileum, the pA_2 values for the *R*- and *S*-isomers of ondansetron were 7.02 to 7.14 and 6.30 to 6.42, respectively (23). These pA_2 values give an isometric activity ratio (*R*-isomer/*S*-isomer) of 5 to 7. For zacopride, the pA values for the *S*- and *R*-isomers in the isolated guinea pig ileum were 8.11 and 7.27, respectively, and the isometric activity ratio was 7 (24).

The YM-compounds tested in the present study all have a benzimidazole ring and a chain containing a carbonyl group. They differ in the position of the nitrogen atom in their aromatic rings. All three compounds have an asymmetric carbon in their structures and are the *R*-isomer. The *S*-isomers of YM114, YM-26103-2 and YM-26308-2 also have 5-HT_3 receptor antagonistic activities, but these were much less potent compared with the respective *R*-isomers. The ratio of potencies in 5-HT_3-receptor antagonism of YM114, YM-26103-2 and YM-26308-2 over their respective *S*-isomers were 1288, 46 and 389 against the 5-HT-responses and 1413, 85 and 182 against the 2-Me-5-HT-responses. A similar relationship for 5-HT_3-receptor antagonism was obtained for YM060 and its *S*-isomer, which are also 4,5,6,7-tetrahydro-1*H*-benzimidazole derivatives, in isolated guinea pig colon (1), anesthetized rats (2) and ferrets (25). Our present results suggest that 5-HT_3 receptors favor the *R*-isometric comformation of 4,5,6,7-tetrahydro-1*H*-benzimidazole derivatives. The isometric activity ratio of YM114 over its *S*-isomer was one to two orders of magnitude greater than those for the other YM-compounds. These results suggest that changing the position of the nitrogen atom in the aromatic ring affects the degree of stereoselectivity without affecting the superiority of *R*-isomers to *S*-isomers.

In the present and our previous studies (1), all *R*-isomers of YM-compounds have potent 5-HT_3 receptor antagonistic activities. Therefore, it seems that the position of the nitrogen atom in the aromatic ring is not an essential determinant for YM-compounds to be 5-HT_3-receptor antagonists. It is possible that a planar configuration of the aromatic ring is important as suggested by Schmidt and Proutka (26). According to Schmidt, atropine is homologous to ICS205-930, a 5-HT_3-receptor antagonist, in its 3-dimentional structure, except for the steric characteristics of the single atom in the equivalent portion to the aromatic ring of ICS205-930; and therefore, this part of atropine cannot adopt a planar steric structure and has no affinity to 5-HT_3 receptors. In fact, to our

knowledge, all reported 5-HT_3 receptor antagonists have planar configurations in this part, and this planar configuration may be important for the interaction with 5-HT_3 receptors.

The steric configuration of the terminal amine group in the compounds is also important, because the 5-HT_3 receptor antagonistic activities of the *S*-isomers were very markedly lower than those of the *R*-isomers in all the YM-compounds. Therefore, the steric relationship of the terminal amine group and the other portion of the molecule is one of the important factors to determine the 5-HT_3 receptor antagonism. Because there was a difference in the *R*-isomer/*S*-isomer activity ratios among the YM-compounds, there is a possibility that a subtle change in the aromatic portion affects the steric configuration of the terminal amine groups. Furthermore, the structure of the terminal amine groups of YM-compounds are different from those of ondansetron and zacopride. These factors may contribute to the great difference in the isometric ratios in 5-HT_3 receptor antagonism of YM-compounds from those of ondansetron and zacopride.

Since the isometric activity ratios of the YM-compounds used herein were much greater than those of ondansetron and zacopride, and since these YM-compounds showed highly potent 5-HT_3 receptor antagonism, these compounds may be useful pharmacological tools for investigating the interactions between 5-HT_3 receptors and ligands.

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