Synthesis and Biological Activities of the Optical Isomers of (±)-4-Amino-5-chloro-2-ethoxy-*N*-[[4-(4-fluorobenzyl)-2-morpholinyl]methyl]benzamide (Mosapride)

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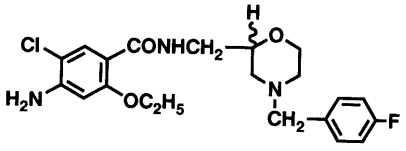
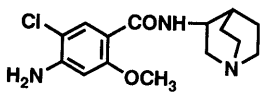
The enantiomers, (*S*)-(−)-1 and (*R*)-(+)-1, of (±)-4-amino-5-chloro-2-ethoxy-*N*-[[4-(4-fluorobenzyl)-2-morpholinyl]methyl]benzamide (mosapride) [(±)-1], a new and selective gastroprokinetic agent, were prepared from optically active [4-(4-fluorobenzyl)-2-morpholinyl]methylamines (*S*)-(−)-4 and (*R*)-(+)-4, respectively. The requisite (*S*)-(−)-4 and (*R*)-(+)-4 were prepared by optical resolution of [4-(4-fluorobenzyl)-2-morpholinyl]methyl *p*-toluenesulfonate [(±)-5] using (−)- and (+)-*N*-(*p*-toluenesulfonyl)glutamic acids, followed by amination of the tosyloxy groups of (*R*)-(−)-5 and (*S*)-(+)-5, respectively. The absolute configurations of (*R*)-(−)-5 and (*S*)-(+)-5 were determined on the basis of an asymmetric synthesis of (*R*)-(−)-5 from (*S*)-(+)-benzyl glycidyl ether [(*S*)-(+)-11]. Mosapride and its enantiomers, (*S*)-(−)-1 and (*R*)-(+)-1, were essentially equipotent in serotonin 5-HT$_4$ receptor agonistic activity on the electrically evoked contractions in isolated guinea pig ileum.

Keywords gastroprokinetic activity; mosapride; optical resolution; absolute configuration; enantiomer; serotonin 5-HT$_4$ receptor agonistic activity

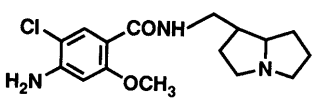
In our previous papers,[1,2] we reported the synthesis of racemic 4-amino-5-chloro-2-ethoxy-*N*-[[4-(4-fluorobenzyl)-2-morpholinyl]methyl]benzamide [mosapride, (±)-**1**] citrate (AS-4370), which showed potent gastroprokinetic activity without dopamine D_2 receptor antagonistic activity. Clinical studies of mosapride citrate as a gastroprokinetic agent are ongoing. It is known that there are differences in serotonin 5-HT$_4$ receptor agonistic activity between the optical isomers of zacopride (**2**)[3] and SC-49518 (**3**)[4] as potential gastroprokinetic agents; thus (*S*)-zacopride and (*R*,*R*)-SC-49518 have 3 times and 15 times greater activity, respectively, than the other enantiomers. It was therefore of interest to us to compare the biological activities of the enantiomers of mosapride.



1 (mosapride)



2 (zacopride)



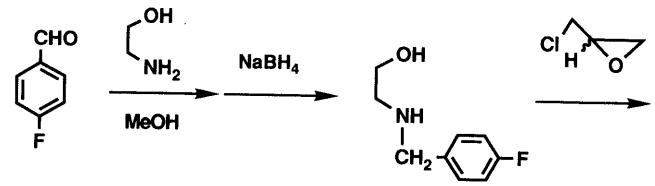
3 (SC-49518)

Chart 1

The present paper describes the preparation of the enantiomers (*S*)-(−)-**1** and (*R*)-(+)-**1** by optical resolution and the determination of their absolute configurations by asymmetric synthesis. The comparative biological activity of mosapride and its enantiomers is also reported.

Optical Resolution Initial attempts at resolution of mosapride itself into its enantiomers using various resolving agents were unsuccessful. Then a key intermediate, (±)-[4-(4-fluorobenzyl)-2-morpholinyl]methylamine [(±)-**4**], was derived into its diastereomeric amides with an optically active amino acid. However, the diastereomeric amides thus prepared could not be resolved by fractional recrystallization and/or silica gel column chromatography.

Hence we focused our efforts on the resolution of racemic [4-(4-fluorobenzyl)-2-morpholinyl]methyl *p*-toluenesulfonate [(±)-**5**]. Howe *et al.*[5] have reported that



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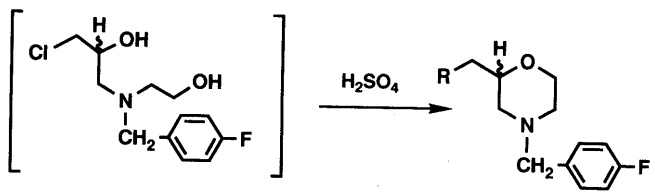
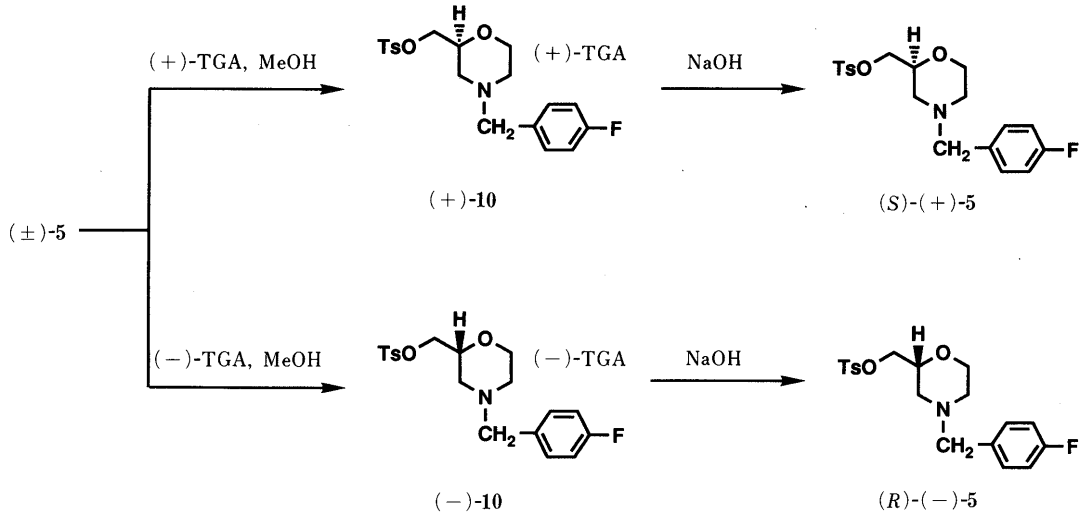


(±)-7

(±)-**8**: R=Cl
(±)-**9**: R=OH
(±)-**5**: R=TsO

Ts: 4-$(CH_3)C_6H_4SO_2$

Chart 2



TGA: 4-$(CH_3)C_6H_4SO_2NHCH(COOH)CH_2CH_2COOH$

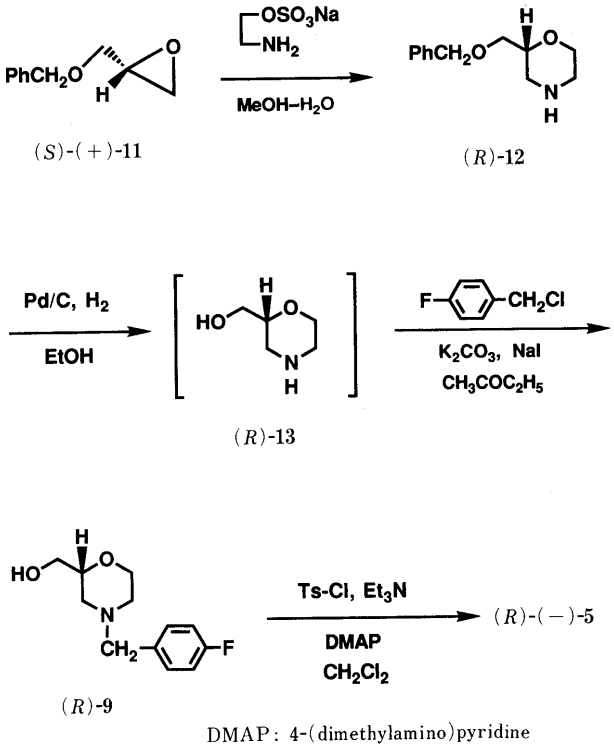
Chart 3

an analogue of (±)-**5**, (±)-(4-benzyl-2-morpholinyl)-methyl *p*-toluenesulfonate, was successfully resolved by use of (+)-*N*-(*p*-toluenesulfonyl)-L-glutamic acid [(+)-TGA]. We accordingly applied their method using (+)-TGA to the optical resolution of (±)-**5**.

The requisite (±)-**5** was synthesized by a modification of the Loftus method[6] (Chart 2). Thus, 2-[(4-fluorobenzyl)amino]ethanol (**6**) was prepared by the reaction of 4-fluorobenzaldehyde with 2-aminoethanol, followed by reduction with sodium borohydride. The reaction of **6** with (±)-epichlorohydrin gave the intermediate diol (±)-**7**. The crude (±)-**7** was successively treated with concentrated sulfuric acid to afford (±)-**2**-(chloromethyl)-4-(4-fluorobenzyl)morpholine [(±)-**8**] in 76% yield. Hydrolysis of (±)-**8** with water in formamide, followed by tosylation of the resultant 4-(4-fluorobenzyl)-2-morpholinylmethanol [(±)-**9**] in the presence of 4-(dimethylamino)pyridine, gave (±)-**5**, which was then converted to its TGA salt (Chart 3). Four fractional recrystallizations of the (+)-TGA salt of (+)-**5** from MeOH provided the enantiomerically pure salt (+)-**10**. This salt (+)-**10** was converted to the free base (+)-**5**, $[\alpha]_D^{25}$ +20.8° (c=1.0, MeOH), by treatment with 10% NaOH solution. The mother liquor, including mainly the (+)-TGA salt of (−)-**5**, was worked up with 10% NaOH to recover (−)-**5**. Treatment of the recovered (−)-**5** with (−)-TGA, followed by three fractional recrystallizations from MeOH, gave the optically pure salt (−)-**10**, which was finally converted to the free base (−)-**5**, $[\alpha]_D^{25}$ −21.4° (c=1.0, MeOH). The chiral morpholines (+)-**5** and (−)-**5** were thus obtained in an approximately 1:1 ratio and served later as intermediates for the synthesis of optically active mosapride.

On the acid treatment of the diol **7**, however, its chiral center might racemize through dehydration of the relevant hydroxyl group prior to the cyclization to the morpholine ring. Consequently the route shown in Chart 2 seemed to us to be inherently unsuited to a stereoselective synthesis of the chiral morpholine ring.

Asymmetric Synthesis In order to establish the absolute configurations of the enantiomers of (±)-**5**, we next planned to develop an alternative and efficient method for the stereoselective synthesis of the chiral morpholine (+)-**5** and (−)-**5**.



DMAP: 4-(dimethylamino)pyridine

Chart 4

Kojima *et al.*[7] reported that the reaction of (±)-1-[inden-7(or 4)-yloxy]-2,3-epoxypropane with excess 2-aminoethyl hydrogen sulfate in 70% NaOH gave (±)-2-[(inden-7(or 4)-yloxy)methyl]morpholine. In this reaction, the configuration of the epoxide chiral carbon must be retained, judging from the product which unambiguously arose from attack of the amino group of 2-aminoethyl hydrogen sulfate on the less substituted epoxide carbon. We considered that if one were to treat (*R*)- or

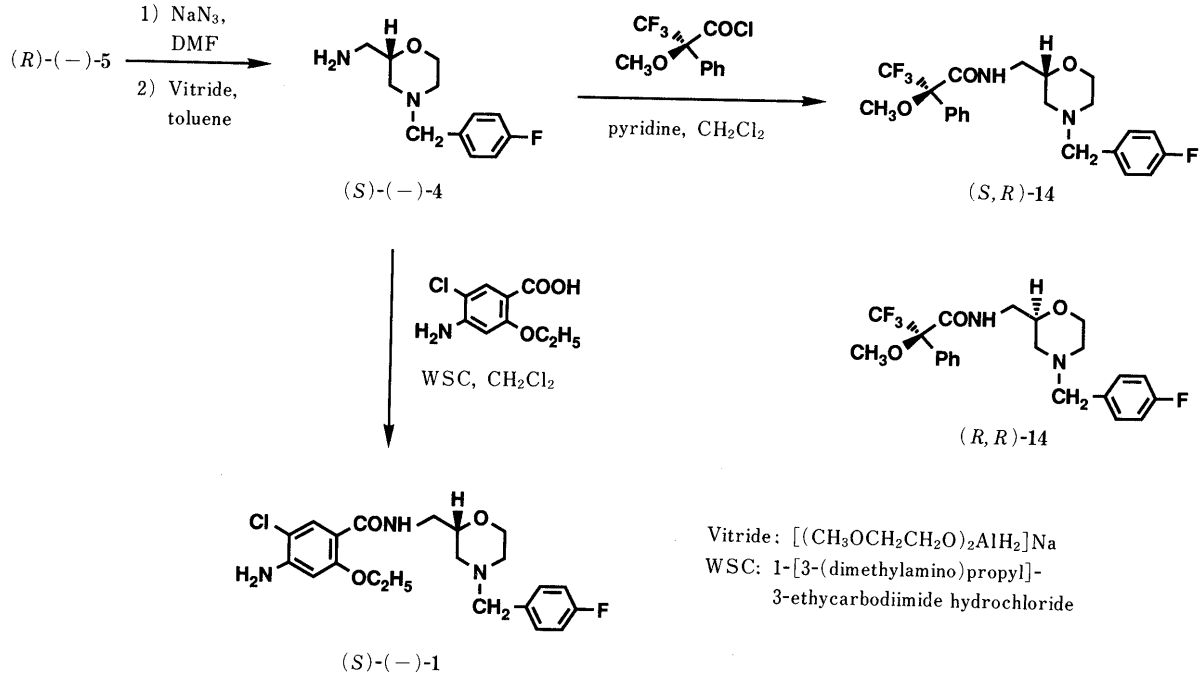


Chart 5

(*S*)-benzyl glycidyl ether (**11**) with sodium 2-aminoethyl sulfate, optically active 2-(benzyloxymethyl)morpholine (**12**) should be formed (Chart 4). In fact, the reaction of (*S*)-**11** with 2-aminoethyl hydrogen sulfate in a mixture of 40% NaOH and MeOH proceeded stereoselectively to produce (*R*)-**12** as an oil, in over 98% enantiomeric excess (ee).[8]

Hydrogenolysis of (*R*)-**12** over a palladium catalyst gave (*R*)-2-morpholinylmethanol [(*R*)-**13**], which was treated, without purification, with 4-fluorobenzyl chloride to afford (*R*)-**9**. The subsequent treatment of (*R*)-**9** with *p*-toluenesulfonyl chloride afforded optically active (*R*)-(−)-**5**, $[\alpha]_D^{27}$ −21.3° (c=1.0, MeOH), whose ^{1}H-NMR spectrum was identical to that of the sample obtained by the foregoing optical resolution method. The morpholine (−)-**5** was thereby proved to have *R*-configuration.

Synthesis of Optically Active Mosapride The synthesis of the enantiomers of mosapride [(±)-**1**] is shown in Chart 5, for the case of the *S* enantiomer. Treatment of (*R*)-(−)-**5** with sodium azide followed by reduction with sodium bis(2-methoxyethoxy)aluminum hydride, Vitride, gave oily [4-(4-fluorobenzyl)-2-morpholinyl]methylamine [(*S*)-(−)-**4**] in 81% yield. Its enantiomer (*R*)-(+)-**4** was obtained in an analogous manner from (*S*)-(+)-**5** in 89% yield. The amines (*S*)-(−)-**4** and (*R*)-(+)-**4** were converted to Mosher's amide with a view to determining their optical purities. Thus, the reaction of (*S*)-(−)-**4** and (*R*)-(+)-**4** with (*S*)-(+)-3,3,3-trifluoro-2-methoxy-2-phenylpropionyl chloride[9] gave the diastereomers of (*R*)-3,3,3-trifluoro-2-methoxy-2-phenylpropionamide [(*S*,*R*)-**14** and (*R*,*R*)-**14**], respectively. In the ^{19}F-NMR spectra of (*S*,*R*)-**14** and (*R*,*R*)-**14**, the ^{19}F-signal due to the trifluoromethyl group appeared as a sharp singlet at δ −69.97 and −70.02, respectively, with no signal attributable to the trifluoromethyl group of the respective diastereomer; this finding proved (*S*,*R*)-**14** and (*R*,*R*)-**14** to be optically pure at the spectroscopic level.

Finally, (*S*)-(−)- and (*R*)-(+)-4-amino-5-chloro-2-ethoxy-*N*-[[4-(4-fluorobenzyl)-2-morpholinyl]methyl]-benzamides [(*S*)-(−)-**1** and (*R*)-(+)-**1**] were prepared by the reaction of the amines (*S*)-(−)-**4** and (*R*)-(+)-**4**, respectively, with 4-amino-5-chloro-2-ethoxybenzoic acid by the same method as described previously.[2] The enantiomeric purities of (*S*)-(−)-**1** and (*R*)-(+)-**1** thus obtained were determined to be practically 100% ee on the basis of the chiral HPLC method.

Biological Activity Benzamide gastroprokinetic agents with a 4-amino-5-chloro-2-methoxybenzoyl moiety such as metoclopramide,[10] cisapride,[11] renzapride,[12] zacopride,[3] and SC-49518[4] are believed to act *via* a serotonergic mechanism in the enteric nervous system. However, the activity does not correlate with the interaction at serotonin 5-HT$_1$, 5-HT$_2$, or 5-HT$_3$ receptor. Dumuis *et al.*[13] recently reported that the activities of gastroprokinetic agents could be correlated with agonistic activity at a new serotonin receptor subtype (5-HT$_4$). Regarding the mechanism of action of mosapride, Yoshida *et al.*[14] of our laboratories reported recently that it acts as a partial agonist for serotonin 5-HT$_4$ receptor and facilitates cholinergic transmission, like metoclopramide and cisapride.

The serotonin 5-HT$_4$ receptor agonistic activity of mosapride and its enantiomers [(*S*)-(−)-**1** and (*R*)-(+)-**1**] was measured in isolated guinea pig ileum as reported by Craig and Clarke.[15] Mosapride and its enantiomers dose-relatedly increased the electrically evoked contraction, and the dose-response curve obtained with (*S*)-(−)-**1** or (*R*)-(+)-**1** was very similar to that observed with mosapride. The values of 50% effective concentration (EC_{50}) for these compounds are shown in Table I. The potencies of (*S*)-(−)-**1** and (*R*)-(+)-**1** were almost the same as that of mosapride. Further comparative bio-

TABLE I. Serotonin 5-HT_4 Receptor Agonistic Activity of Mosapride [(±)-**1**] and Its Enantiomers [(*S*)-(−)-**1** and (*R*)-(+)-**1**]

Compound[a]	5-HT_4 agonistic activity[b] (EC_{50}, nM)
Mosapride (±)-**1**	74.2 (53.2—104.4)
(*S*)-(−)-**1**	58.5 (38.9— 96.2)
(*R*)-(+)-**1**	85.9 (58.2—139.0)

a) Each compound was used as the citric acid salt. *b*) Potency of compounds was estimated on the basis of enhancement of the electrically evoked contractions in isolated guinea pig ileum. The EC_{50} value for each compound represents the concentration causing 50% enhancement. The 95% confidence limit is indicated in parentheses.

logical studies of mosapride and its enantiomers will be described elsewhere.

In conclusion, the enantiomers of mosapride, (*S*)-(−)-**1** and (*R*)-(+)-**1**, were synthesized in approximately 25% overall yield with high optical purity *via* optical resolution of the intermediate (±)-**5** using (+)- and (−)-*N*-(*p*-toluenesulfonyl)glutamic acids. Mosapride and its enantiomers were found to be substantially equipotent in serotonin 5-HT_4 receptor agonistic activity *in vitro*, unlike zacopride and SC-49518.

Experimental

Chemistry All melting points were determined on a Yanagimoto micromelting point apparatus and are uncorrected. IR spectra were recorded on a Hitachi 260-10 spectrometer. Electron ionization (EI) and chemical ionization (CI) mass spectra (abbreviated as EIMS and CIMS, respectively) were obtained on a JEOL JMS D-300 spectrometer or a Hitachi M-80B spectrometer. ^{1}H- (200 MHz) and ^{19}F- (282 MHz) NMR spectra were recorded on a Varian Gemini-200 spectrometer and a Varian XL-300 spectrometer, respectively, in $CDCl_3$. Chemical shifts are expressed as δ values (ppm) with tetramethylsilane (^{1}H-NMR) or hexafluorobenzene (^{19}F-NMR, $\delta = -162.9$) as an internal standard, and coupling constants (J values) are given in hertz (Hz). Optical rotations were measured at 589 nm with a Jasco DIP-4 digital polarimeter. Analytical HPLC was performed with Shimadzu LC-6A and SPD-6A instruments. Organic extracts were dried over anhydrous $MgSO_4$ unless otherwise specified. The solvent was evaporated under reduced pressure. Merck Silica gel 60 (70—230 mesh) was used for column chromatography.

The (*S*)-(+)-benzyl glycidyl ether [(*S*)-(+)-**11**] was purchased from Daiso Co., Ltd. (Osaka, Japan); optical purity, >98% ee.

2-[(4-Fluorobenzyl)amino]ethanol (6) The method of Picciola[16] was applied. A mixture of 2-aminoethanol (18.3 g, 0.30 mol), 4-fluorobenzaldehyde (31.0 g, 0.25 mol), $NaHCO_3$ (31.5 g, 0.38 mol), and MeOH (300 ml) was heated to reflux for 4 h and cooled to 5 °C. Sodium borohydride (11.4 g, 0.30 mol) was added portionwise to the stirred reaction mixture during a period of 2 h at *ca.* 10 °C. The stirred mixture was kept at the same temperature for 0.5 h and then at room temperature for 2 h. The insoluble materials were removed by filtration, and the filtrate was concentrated to dryness. The residue was dissolved in $CHCl_3$, and the solution was washed successively with water and brine. The solvent was evaporated to leave a crude product, which was distilled to give 37.8 g (89%) of **6** as a colorless oil, bp 144—145 °C/(6 mmHg) (lit.[17] bp 95—98 °C/(3 mmHg)). ^{1}H-NMR δ: 2.77 (2H, t, J=2.0, NCH_2), 3.62 (2H, t, J=2.0, OCH_2), 3.76 (2H, s, $C\underline{H}_2C_6H_4F$), 6.96—7.08 (2H, m, arom H), 7.22—7.35 (2H, m, arom H). IR (neat) ν cm^{-1}: 3290, 1590, 1500.

(±)-2-(Chloromethyl)-4-(4-fluorobenzyl)morpholine [(±)-8] A mixture of **6** (30.0 g, 0.18 mol) and (±)-epichlorohydrin (17.2 g, 0.19 mol) was stirred at room temperature for 4 h. To the resulting viscous oil (including the resultant diol **7**), concentrated H_2SO_4 (128 g, 1.3 mol) was gradually added. The mixture was rapidly heated to 150 °C, kept at the same temperature for 0.5 h, and then cooled to room temperature. The resulting brown solution was diluted with ice-water. Then the solution was basified with 48% NaOH and extracted with toluene. The extract was washed successively with water and brine and evaporated to give a pale brown oil, which was chromatographed on silica gel with AcOEt–*n*-hexane (1 : 1) to afford 32.8 g (76%) of (±)-**8** as a pale yellow oil. ^{1}H-NMR δ: 2.02 (1H, t like, J=11.0, 3-H_{ax}), 2.19 (1H, td, J=11.0, 3.0, 5-H_{ax}), 2.65 (1H, m), 2.8 (1H, m), 3.4—3.6 (2H, m), 3.50 (2H, s, $C\underline{H}_2C_6H_4F$), 3.62—3.96 (3H, m), 6.96—7.08 (2H, m, arom H), 7.21—7.35 (2H, m, arom H). IR (neat) ν cm^{-1}: 1495, 1215, 1110. EIMS m/z: 243 (M^+).

A portion of the oily (±)-**8** was converted to its fumarate in the usual manner. mp 154—156 °C (EtOH). *Anal.* Calcd for $C_{12}H_{15}ClFNO \cdot C_4H_4O_4$: C, 53.41; H, 5.32; Cl, 9.85; F, 5.28; N, 3.89. Found: C, 53.56; H, 5.51; Cl, 10.13; F, 5.37; N, 3.90.

(±)-4-(4-Fluorobenzyl)-2-morpholinylmethanol [(±)-9] A mixture of (±)-**8** (35.0 g, 0.14 mol), H_2O (7.8 ml, 0.43 mol), and formamide (72 ml) was heated to reflux for 6 h and then cooled to room temperature. The reaction mixture was diluted with water (350 ml), basified with 10% NaOH, and extracted with toluene. The extract was washed successively with water and brine. The solvent was evaporated to give a crude oil, which was chromatographed on silica gel with $CHCl_3$–MeOH (20 : 1) to afford 31.2 g (96%) of (±)-**9** as a pale yellow oil. ^{1}H-NMR δ: 1.98 (1H, dd, J=10.0, 12.0, 3-H_{ax}), 2.27 (1H, td, J=11.0, 3.7, 5-H_{ax}), 2.5—2.9 (3H, m), 3.4—3.8 (4H, m), 3.46 (2H, s, $C\underline{H}_2C_6H_4F$), 3.89 (1H, ddd, J=10.0, 3.7, 1.8, 6-H_{eq}), 6.95—7.05 (2H, m, arom H), 7.20—7.35 (2H, m, arom H). IR (neat) ν cm^{-1}: 3380, 2920, 2855, 2805, 1595, 1500, 1215, 1105, 1040. EIMS m/z: 225 (M^+).

A portion of the oily (±)-**9** was converted to its fumarate in the usual manner. mp 141—142 °C (EtOH–*n*-hexane). *Anal.* Calcd for $C_{12}H_{16}FNO_2 \cdot C_4H_4O_4$: C, 56.30; H, 5.91; F, 5.57; N, 4.10. Found: C, 56.15; H, 5.88; F, 5.40; N, 4.31.

(±)-[4-(4-Fluorobenzyl)-2-morpholinyl]methyl *p*-Toluenesulfonate [(±)-5] Compound (±)-**5** was prepared by a modification of the Loftus method.[6] *p*-Toluenesulfonyl chloride (62.0 g, 0.33 mol) was added portionwise to a mixture of (±)-**9** (49.0 g, 0.22 mol), triethylamine (44.0 g, 0.44 mol), and 4-(dimethylamino)pyridine (2.7 g, 21 mmol) in CH_2Cl_2 (490 ml) at 5 °C. The mixture was stirred at room temperature for 24 h and then washed successively with water, 1 N HCl, and brine, then dried, and concentrated to dryness. The residue was recrystallized from Et_2O–petroleum ether to give 53.0 g (64%) of (±)-**5**, mp 73—75 °C. ^{1}H-NMR δ: 1.93 (1H, t like, J=11.0, 3-H_{ax}), 2.13 (1H, td, J=11.0, 3.5, 5-H_{ax}), 2.43 (3H, s, $C\underline{H}_3C_6H_4SO_2$), 2.5—2.75 (2H, m), 3.43 (2H, s, $C\underline{H}_2C_6H_4F$), 3.59 (1H, td, J=2.5, 11.0, 6-H_{ax}), 3.75 (1H, m), 3.80 (1H, ddd, J=2.0, 3.5, 11.0, 6-H_{eq}), 3.95 (1H, dd, J=4.5, 10.5, $C\underline{H}_2OSO_2$), 4.02 (1H, dd, J=5.5, 10.5, $C\underline{H}_2OSO_2$), 6.95—7.05 (2H, m, arom H), 7.2—7.4 (4H, m, arom H), 7.75—7.85 (2H, m, arom H). *Anal.* Calcd for $C_{19}H_{22}FNO_4S$: C, 60.14; H, 5.84; F, 5.01; N, 3.69; S, 8.45. Found: C, 60.25; H, 5.89; F, 5.15; N, 3.84; S, 8.68. IR (KBr) ν cm^{-1}: 1590, 1500, 1350, 1165. EIMS m/z: 379 (M^+).

Optical Resolution of (±)-5. (A) TGA Salts (10) A mixture of (±)-**5** (37.6 g, 99 mmol) and (+)-*N*-(*p*-toluenesulfonyl)-L-glutamic acid[18] [(+)-TGA, 30.0 g, 99 mol] was completely dissolved in MeOH (350 ml), and the solution was kept at room temperature for 15 h. The resulting crystals were collected by filtration. Four fractional recrystallizations of the crystals from MeOH provided 21.0 g of (+)-4-(4-fluorobenzyl)-2-[(*p*-toluenesulfonyloxy)methyl]morpholinium *N*-(*p*-toluenesulfonyl)-L-glutamate [(+)-**10**], mp 163—165 °C. $[\alpha]_D^{26}$ +21.4° (c=0.5, MeOH). ^{1}H-NMR δ: 1.6—1.9 (2H, m), 1.80 (1H, t like, J=11.0, 3-H_{ax}), 2.02 (1H, td, J=11.0, 3.5, 5-H_{ax}), 2.20 (1H, t, J=7.5, $CH_2C\underline{H}(COOH)NH$), 2.37 (3H, s, $C\underline{H}_3C_6H_4$), 2.42 (3H, s, $C\underline{H}_3C_6H_4$), 2.5—2.6 (2H, m), 3.38, 3.47 (each 1H, d, J=13.5, $C\underline{H}_2C_6H_4F$), 3.45 (1H, m), 3.6 (1H, m), 3.68—3.8 (3H, m), 3.95 (1H, dd, J=5.5, 11.0, $C\underline{H}_2O$), 4.01 (1H, dd, J=4.0, 11.0, $C\underline{H}_2O$), 7.05—7.8 (12H, m, arom H), 8.05 (1H, br s, $NHSO_2$), 12.38 (2H, br s, COOH). *Anal.* Calcd for $C_{19}H_{22}FNO_4S \cdot C_{12}H_{15}NO_6S$: C, 54.69; H, 5.48; F, 2.79; N, 4.12; S, 9.42. Found: C, 54.72; H, 5.41; F, 2.85; N, 4.02; S, 9.40. IR (KBr) ν cm^{-1}: 3150, 1680, 1615, 1145.

The initial filtrate [obtained after separation of (+)-**10**] was concentrated to leave additional glutamate salt, which was then treated with 10% NaOH, giving the free base including mainly (−)-**5**. The recovered crude (−)-**5** (20.6 g, 54 mmol) was treated with (−)-*N*-(*p*-toluenesulfonyl)-D-glutamic acid[19] [(−)-TGA, 16.0 g, 54 mmol] in a similar manner to that used for the preparation of (+)-**10**, thus giving 26.0 g of pure (−)-4-(4-fluorobenzyl)-2-[(*p*-toluenesulfonyloxy)methyl]morpholinium *N*-(*p*-toluenesulfonyl)-D-glutamate [(−)-**10**], mp 163—165 °C (MeOH). $[\alpha]_D^{26}$ −22.0° (c=0.5, MeOH). *Anal.* Calcd for

$C_{19}H_{22}FNO_4S \cdot C_{12}H_{15}NO_6S$: C, 54.69; H, 5.48; F, 2.79; N, 4.12; S, 9.42. Found: C, 54.74; H, 5.59; F, 2.85; N, 4.09; S, 9.73. IR (KBr) ν cm^{-1}: 3150, 1680, 1615, 1145.

(B) Optical Isomers (5) The salt (+)-**10** (21.0 g, 31 mmol) was added to excess 2 N NaOH (30 ml) and extracted with Et_2O (200 ml). The extract was washed with brine and dried over anhydrous Na_2SO_4. The solvent was evaporated to leave a crystalline residue, which was recrystallized from Et_2O–petroleum ether to give 11.5 g [31% yield from (±)-**5**] of (+)-**5**, mp 45—46 °C. $[\alpha]_D^{25}$ +20.8° (c=1.0, MeOH). *Anal.* Calcd for $C_{19}H_{22}FNO_4S$: C, 60.14; H, 5.84; F, 5.01; N, 3.69; S, 8.45. Found: C, 60.28; H, 6.01; F, 5.02; N, 3.69; S, 8.63.

The salt (−)-**10** (26.0 g) was treated in a similar manner to that employed for the preparation of (+)-**5** to give (−)-**5** [14.0 g, 37% yield from (±)-**5**], mp 45—46 °C (Et_2O–petroleum ether). $[\alpha]_D^{25}$ −21.4° (c=1.0, MeOH). *Anal.* Calcd for $C_{19}H_{22}FNO_4S$: C, 60.14; H, 5.84; F, 5.01; N, 3.69; S, 8.45. Found: C, 60.43; H, 5.88; F, 5.09; N, 3.92; S, 8.52.

(*R*)-2-(Benzyloxymethyl)morpholine [(*R*)-12] The method of Kojima *et al.*[7] was applied. A solution of (*S*)-(+)-benzyl glycidyl ether [(*S*)-(+)-**11**, 8.2 g, 50 mmol] in MeOH (50 ml) was added to a solution of 2-aminoethyl hydrogen sulfate (35.2 g, 0.25 mol) in 40% NaOH (60 ml), with stirring at *ca.* 50 °C. The mixture was stirred at the same temperature for 1 h and then an additional 40% NaOH (100 ml) was added. The reaction mixture was heated at 50—55 °C for 16 h, diluted with H_2O, and extracted with toluene. The extract was washed successively with water and brine. The solvent was evaporated to leave a crude product, which was chromatographed on silica gel with $CHCl_3$–MeOH (9:1 to 4:1) to give 3.8 g (37%) of (*R*)-**12** as an oil. ^{1}H-NMR δ: 2.40 (1H, s, NH), 2.66 (1H, dd, J=10.5, 12.0, 3-H$_{ax}$), 2.75—2.85 (2H, m), 2.92 (1H, dd, J=2.5, 12.0, 3-H$_{eq}$), 3.35—3.8 (4H, m), 3.9 (1H, d like, J=11, 6-H$_{eq}$), 4.56 (2H, s, C$\underline{H}_2$C$_6$H$_5$), 7.2—7.4 (5H, m, arom H). CIMS m/z: 208 (MH$^+$). IR (neat) ν cm^{-1}: 3300, 1445, 1090.

A portion of the oily (*R*)-**12** was converted to its fumarate in the usual manner. mp 123—124 °C (EtOH–*n*-hexane). $[\alpha]_D^{27}$ −0.6° (c=1.0, MeOH). *Anal.* Calcd for $C_{12}H_{17}NO_2 \cdot C_4H_4O_4$: C, 59.43; H, 6.55; N, 4.33. Found: C, 59.43; H, 6.58; N, 4.27.

In order to determine the optical purity of (−)-**12**, the racemate (±)-**12**, prepared from (±)-**11** by the same procedure as that used for the preparation of (*R*)-**12**, was firstly analyzed by chiral HPLC [column, Ultron ES-OVM (Shinwa Chemical Industries, Ltd., Japan), 6.0 mm i.d. × 150 mm; eluent, 20 mM KH_2PO_4 (pH 7.2)/CH_3CN=9/1; flow rate, 1.0 ml/min; column temperature, 25 °C; detection, 258 nm]. The racemate showed peaks at 10.8 min and 13.3 min due to the enantiomers; the peak at 10.8 min was identified as that of (*R*)-**12**. By chiral HPLC analysis under the same conditions, the optical purity of (*R*)-**12** was then determined to be more than >98% ee.

(*R*)-4-(Fluorobenzyl)-2-morpholinylmethanol [(*R*)-9] A solution of (*R*)-**12** (5.0 g, 24 mmol) in EtOH (10 ml) was hydrogenated over 10% palladium-on-carbon (1.0 g) at 50 °C. After the theoretical amount of hydrogen had been absorbed, the catalyst was removed by filtration. The filtrate was concentrated to dryness to give *ca.* 3 g of crude (*R*)-2-morpholinylmethanol [(*R*)-**13**]. CIMS m/z: 118 (MH$^+$).

A mixture of the crude (*R*)-**13** (3 g), 4-fluorobenzyl chloride (5.2 g, 36 mmol), NaI (0.8 g), anhydrous K_2CO_3 (8.4 g, 61 mmol), and methyl ethyl ketone (200 ml) was heated to reflux for 18 h and cooled to room temperature. The insoluble materials were removed by filtration, and the filtrate was concentrated to dryness. The residue was diluted with water and extracted with $CHCl_3$. The extract was washed with brine. The solvent was evaporated to give an oil, which was chromatographed on silica gel with AcOEt to afford 3.6 g [66% overall yield from (*R*)-**12**] of (*R*)-**9** as an oil. This compound was confirmed to be identical with (±)-**9** obtained from (±)-**8**, on the basis of TLC, CIMS, and ^{1}H-NMR comparisons.

(*R*)-(−)-[4-(4-Fluorobenzyl)-2-morpholinyl]methyl *p*-Toluenesulfonate [(*R*)-(−)-5] Compound (*R*)-**9** was treated according to the same method as employed for the preparation of (±)-**5** to give (*R*)-(−)-**5**, mp 42—43 °C (Et_2O–petroleum ether). $[\alpha]_D^{27}$ −21.3° (c=1.0, MeOH). *Anal.* Calcd for $C_{19}H_{22}FNO_4S$: C, 60.14; H, 5.84; F, 5.01; N, 3.69; S, 8.45. Found: C, 60.18; H, 5.85; F, 4.94; N, 3.64; S, 8.63.

(*S*)-(−)- and (*R*)-(+)-[4-(4-Fluorobenzyl)-2-morpholinyl]methylamines [(*S*)-(−)-4 and (*R*)-(+)-4] A stirred mixture of (*R*)-(−)-**5** (3.2 g, 8.4 mmol), sodium azide (0.8 g, 12 mmol), and *N*,*N*-dimethylformamide (30 ml) was heated at 120 °C for 2 h. The reaction mixture was diluted with water and extracted with ether. The extract was washed successively with water and brine and dried over anhydrous Na_2SO_4. The solvent was evaporated to give 2.1 g of the optically active (*S*)-2-(azidomethyl)-4-(4-fluorobenzyl)morpholine as an oil. A solution of this compound in toluene (100 ml) was added dropwise at −5 °C to a stirred solution of 70% sodium bis(2-methoxyethoxy)aluminum hydride in toluene (40 ml). The reaction mixture was stirred at room temperature for 1.5 h and cooled to 5 °C, and the excess of the reducing agent was decomposed by addition of 10% NaOH. The organic layer was separated and washed successively with water and brine. The solvent was evaporated to give 1.5 g [81% overall yield from (*R*)-(−)-**5**] of (*S*)-(−)-**4** as a pale yellow oil. ^{1}H-NMR δ: 1.47 (2H, s, NH$_2$), 1.87 (1H, dd, J=10.0, 11.5, 3-H$_{ax}$), 2.14 (1H, td, J=11.5, 3.5, 5-H$_{ax}$), 2.5—2.8 (4H, m), 3.45 (1H, m), 3.42 (2H, s, C$\underline{H}_2$C$_6$H$_4$F), 3.66 (1H, td, J=11.5, 2.5, 6-H$_{ax}$), 3.87 (1H, ddd, J=1.5, 3.5, 11.5, 6-H$_{eq}$), 6.9—7.1 (2H, m, arom H), 7.2—7.4 (2H, m, arom H). IR (neat) ν cm^{-1}: 3355, 1595, 1500, 1225, 1105.

In a similar manner to that described above, (*R*)-(+)-**4** was prepared in 89% overall yield from (*S*)-(+)-**5**.

Each portion of (*S*)-(−)-**4** and (*R*)-(+)-**4** was converted in the usual manner to the corresponding dimaleate:

(*S*)-(−)-**4**·dimaleate: mp 153—155 °C (EtOH). $[\alpha]_D^{25}$ −14.5° (c=1.0, MeOH). *Anal.* Calcd for $C_{12}H_{17}FN_2O \cdot 2C_4H_4O_4$: C, 52.63; H, 5.52; F, 4.16; N, 6.14. Found: C, 52.69; H, 5.58; F, 4.13; N, 6.02.

(*R*)-(+)-**4**·dimaleate: mp 153—155 °C (EtOH). $[\alpha]_D^{25}$ +14.5° (c=1.0, MeOH). *Anal.* Calcd for $C_{12}H_{17}FN_2O \cdot 2C_4H_4O_4$: C, 52.63; H, 5.52; F, 4.16; N, 6.14. Found: C, 52.77; H, 5.63; F, 4.14; N, 6.06.

(*S*)-(−)- and (*R*)-(+)-4-Amino-5-chloro-2-ethoxy-*N*-[[4-(4-fluorobenzyl)-2-morpholinyl]methyl]benzamides [(*S*)-(−)-1 and (*R*)-(+)-1] A mixture of (*S*)-(−)-**4** (3.3 g, 28 mmol), 4-amino-5-chloro-2-ethoxybenzoic acid[2] (6.0 g, 28 mmol), 1-[3-(dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (5.4 g, 28 mmol), and CH_2Cl_2 (180 ml) was stirred at room temperature for 3 h. The reaction mixture was washed successively with water, 10% NaOH, water, and brine and concentrated to dryness. The crude product was chromatographed on silica gel with $CHCl_3$–MeOH (20:1) to give a solid, which was recrystallized from EtOH–Et_2O to give 10.1 g (86%) of (*S*)-(−)-**1**, mp 122—124 °C. $[\alpha]_D^{26}$ −29.8° (c=1.0, MeOH). ^{1}H-NMR δ: 1.48 (3H, t, J=7.0, OCH$_2$C$\underline{H}_3$), 2.00 (1H, t like, J=11.0, 3-H$_{ax}$), 2.17 (1H, td, J=11.0, 3.0, 5-H$_{ax}$), 2.66 (1H, d, J=11.0, CONC$\underline{H}_2$), 2.77 (1H, d, J=11.0, CONC$\underline{H}_2$), 3.3 (1H, m), 3.47 (2H, s, C$\underline{H}_2$C$_6$H$_4$F), 3.58—3.79 (3H, m), 3.9 (1H, m), 4.06 (2H, q, J=7.0, OC$\underline{H}_2$CH$_3$), 4.35 (2H, s, NH$_2$), 6.26 (1H, s, arom 3-H), 6.93—7.06 (2H, m, arom H), 7.2—7.34 (2H, m, arom H), 8.10 (1H, s, arom 6-H), 8.21 (1H, br t, CONH). *Anal.* Calcd for $C_{21}H_{25}ClFN_3O_3$: C, 59.78; H, 5.97; Cl, 8.40; F, 4.50; N, 9.96. Found: C, 59.49; H, 6.01; Cl, 8.30; F, 4.54; N, 9.79. IR (KBr) ν cm^{-1}: 3475, 3380, 3310, 3200, 1630, 1525. EIMS m/z: 421 (M$^+$).

Compound (*R*)-(+)-**4** was treated in a similar manner to that described above to give (*R*)-(+)-**1** in 85% yield. mp 122—124 °C (EtOH–Et_2O). $[\alpha]_D^{26}$ +27.8° (c=1.0, MeOH). *Anal.* Calcd for $C_{21}H_{25}ClFN_3O_3$: C, 59.78; H, 5.97; Cl, 8.40; F, 4.50; N, 9.96. Found: C, 59.64; H, 6.05; Cl, 8.21; F, 4.37; N, 9.63.

Each compound was converted in the usual manner to the corresponding citrate:

(*S*)-(−)-**1**·citrate: mp 123—126 °C (EtOH). $[\alpha]_D^{26}$ −22.8° (c=1.0, MeOH). *Anal.* Calcd for $C_{21}H_{25}ClFN_3O_3 \cdot C_6H_8O_7$: C, 52.82; H, 5.42; Cl, 5.77; F, 3.09; N, 6.84. Found: C, 52.55; H, 5.22; Cl, 5.78; F, 3.01; N, 6.76. IR (KBr) ν cm^{-1}: 3450, 3360, 1715, 1610.

(*R*)-(+)-**1**·citrate: mp 123—126 °C (EtOH). $[\alpha]_D^{26}$ +22.8° (c=1.0, MeOH). *Anal.* Calcd for $C_{21}H_{25}ClFN_3O_3 \cdot C_6H_8O_7$: C, 52.82; H, 5.42; Cl, 5.77; F, 3.09; N, 6.84. Found: C, 52.56; H, 5.51; Cl, 5.72; F, 3.13; N, 6.73.

The optical purities of the products were analyzed by chiral HPLC [column, Ultron ES-OVM; 4.6 mm i.d. × 150 mm; eluent, 20 mM KH_2PO_4 (pH 4.6)/CH_3CN=10/1; flow rate, 1.0 ml/min; column temperature, 25 °C; detection, 220 nm]. The retention times for (*S*)-(−)-**1** and (*R*)-(+)-**1** were 10.7 min and 19.6 min, respectively.

***N*-(*S*)-[[4-(4-Fluorobenzyl)-2-morpholinyl]methyl]- and *N*-(*R*)-[[4-(4-Fluorobenzyl)-2-morpholinyl]methyl]-(*R*)-3,3,3-trifluoro-2-methoxy-2-phenylpropionamides [(*S*,*R*)-14 and (*R*,*R*)-14]** A mixture of (*R*)-(+)-3,3,3-trifluoro-2-methoxy-2-phenylpropionic acid (328 mg, 1.4 mmol) and thionyl chloride (3.5 ml) was heated to reflux for 6 h. After removal of the excess thionyl chloride, the residue was dissolved in a mixture of CH_2Cl_2 (5 ml) and pyridine (2 ml). To this solution was added a solution of (*S*)-(−)-**4** (314 mg, 1.4 mmol) in CH_2Cl_2 (5 ml) at 0 °C. The reaction mixture was stirred at room temperature for 2 h and then washed

successively with 10% HCl and water. The solvent was evaporated to leave a crude product, which was chromatographed on silica gel with AcOEt to give 340 mg (55%) of (*S*,*R*)-**14** as an oil. ^{1}H-NMR δ: 1.93 (1H, dd, J=10.0, 11.0, 3-H$_{ax}$), 2.09 (1H, ddd, J=13.0, 11.0, 3.0, 5-H$_{ax}$), 2.67 (2H, m), 3.29 (1H, ddd, J=14.0, 7.0, 5.0, CH$_2$NHCO), 3.46 (2H, s, CH$_2$C$_6$H$_4$F), 3.50 (1H, m, CH$_2$NHCO), 3.64 (2H, m), 3.85 (1H, ddd, J=11.0, 3.0, 2.0, 6-H$_{eq}$), 6.94—7.35 (5H, m, CH$_2$C$_6$H$_4$F, CONH), 7.38—7.58 (5H, m, C$_6$H$_5$). ^{19}F-NMR δ: −69.97 (3F, s, CF$_3$). IR (neat) ν cm^{-1}: 1690. EIMS m/z: 440 (M$^+$).

In a similar manner, (*R*)-(+)-**4** was converted to (*R*,*R*)-**14** as an oil in 58% yield. ^{1}H-NMR δ: 1.81 (1H, dd, J=10.0, 11.0, 3-H$_{ax}$), 2.09 (1H, ddd, J=13.0, 11.0, 3.0, 5-H$_{ax}$), 2.64 (2H, m), 3.19 (1H, ddd, J=14.0, 7.0, 5.0, CH$_2$NHCO), 3.41 (2H, s, CH$_2$C$_6$H$_4$F), 3.57 (1H, ddd, J=14.0, 7.0, 5.0, CH$_2$NHCO), 3.64 (2H, m), 3.85 (1H, ddd, J=11.0, 3.0, 2.0, 6-H$_{eq}$), 6.94—7.33 (5H, m, CH$_2$C$_6$H$_4$F, CONH), 7.38—7.62 (5H, m, C$_6$H$_5$). ^{19}F-NMR δ: −70.02 (3F, s, CF$_3$). IR (neat) ν cm^{-1}: 1690. EIMS m/z: 440 (M$^+$).

Serotonin 5-HT$_4$ Receptor Agonistic Activity Male guinea pigs of the Hartley strain (Nihon Animals Co.) weighing 300—550 g were used. The guinea pigs were killed by a blow on the head. The distal ileum was removed at least 10 cm proximal to the cecum, and the longitudinal muscles with the myenteric plexus (2—3 cm) were prepared according to the method of Craig and Clarke.[15] Each preparation was mounted between two parallel platinum wire electrodes in a 10 ml organ bath containing Krebs–Henseleit solution (NaCl 118 mM, KCl 4.75 mM, $CaCl_2$ 2.5 mM, KH_2PO_4 1.2 mM, $MgSO_4$ 1.2 mM, $NaHCO_3$ 2.5 mM, and glucose 10 mM). The solution was maintained at 37 °C and saturated with 95% O_2 and 5% CO_2. A resting tension of 1 g was added, and the response was recorded isometrically through a force displacement transducer. The strips were equilibrated for 60 min. The strips were then stimulated through two parallel platinum electrodes with square-wave pulses (1 msec duration, supramaximal voltage, 0.2 Hz frequency) from an electrical stimulator. Thereafter, the strips were treated with phenoxybenzamine (3×10^{-7} M) for 30 min. After washing out of the phenoxybenzamine several times, the stimulus voltage was adjusted to the submaximal voltage inducing approximately 50% of the maximal contractile response. Mosapride [(±)-**1**] and its enantiomers [(*S*)-(−)-**1** and (*R*)-(+)-**1**] (as their citrates), dissolved in deionized water containing 1% lactic acid, were cumulatively applied.

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