Synthesis and Pharmacological Activity of 4-Amino-5-chloro-2-methoxy-*N*-[(2*S*,4*S*)-1-ethyl-2-hydroxymethyl-4-pyrrolidinyl]benzamide (TKS159) and Its Optical Isomers

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Of 4-amino-5-chloro-2-methoxy-*N*-(1-ethyl-2-hydroxymethyl-4-pyrrolidinyl)benzamide, four optical isomers, (2*S*,4*S*)-1 (TKS159), (2*S*,4*R*)-25, (2*R*,4*S*)-26 and (2*R*,4*R*)-27, were prepared from optically active 4-amino-1-ethyl-2-hydroxymethylpyrrolidine di-*p*-toluenesulfonate [(2*S*,4*S*)-14, (2*S*,4*R*)-17, (2*R*,4*S*)-20 and (2*R*,4*R*)-23, respectively]. The requisites, (2*S*,4*S*)-14, (2*S*,4*R*)-17, (2*R*,4*S*)-20 and (2*R*,4*R*)-23, were prepared from a commercially available *trans*-4-hydroxy-L-proline. The absolute configurations of (2*S*,4*S*)-1 (TKS159), (2*S*,4*R*)-25, (2*R*,4*S*)-26 and (2*R*,4*R*)-27 were spectroscopically determined. Of the benzamide derivatives, four optical isomers, (2*S*,4*S*)-1, (2*S*,4*R*)-25, (2*R*,4*S*)-26 and (2*R*,4*R*)-27, showed a relatively potent affinity for 5-hydroxytryptamine 4 (5-HT_4) receptors in a radioligand binding assay ([^{3}H]GR113808). The activities of 25—27 were less effective than that of 1 for the gastric emptying of a phenol red semisolid meal in rats. All this suggests that the most potent of the isomers was 4-amino-5-chloro-2-methoxy-*N*-[(2*S*,4*S*)-1-ethyl-2-hydroxymethyl-4-pyrrolidinyl]benzamide (1).

Key words gastroprokinetic activity; 4-amino-5-chloro-2-methoxy-*N*-[(2*S*,4*S*)-1-ethyl-2-hydroxymethyl-4-pyrrolidinyl]benzamide; optically active; serotonin 5-HT_4 receptor; gastric emptying

Haga *et al*. reported the effects of 4-amino-5-chloro-2-methoxy-*N*-[(2*S*,4*S*)-1-ethyl-2-hydroxymethyl-4-pyrrolidinyl]benzamide (**1**, TKS159) as a potential gastroprokinetic agent (Chart 1),[1] whose gastroprokinetic action is caused by the release of acetylcholine by way of stimulating the serotonin 4 (5-hydroxytryptamine 4, 5-HT_4) receptors in the parasympathetic ganglia.[2] On this action clinical studies are ongoing. It is known that there are differences in the serotonin 5-HT_4 receptor agonistic activities among optical isomers as potential gastroprokinetic agents: zacopride (**2**),[3] mosapride (**3**),[4] SC-53116 (**4**)[5] and SK-951 (**5**).[6] Thus, (*S*)-zacopride and (*S*,*S*)-SC-53116 have activities 3 times and 15 times as great, respectively, as the other enantiomers. However, the activities of enantiomers of **3** and **5** have been found to be substantially equipotent. Therefore, it was of interest to compare biological activities of the optical isomers of **1**. In this paper, we shall report the preparation of chiral substituted pyrrolidine derivatives containing two chiral centers at 2- and 4-positions by relevantly convenient methods and the preparation of optically active benzamide derivatives. We shall also report pharmacological activities of four optical isomers.

Chemistry

Chart 2 shows the synthetic routes of preparing the substituted pyrrolidine derivatives with two chiral centers as key intermediates. All chiral pyrrolidine derivatives were prepared from commercially available *trans*-4-hydroxy-L-proline (**6**) as a starting material. Esterification of **6** with thionyl chloride in ethanol and the subsequent *N*-ethylation using diethyl sulfate in dichloromethane in the presence of triethylamine afforded compound (**8**) in a good yield. This compound was converted to the corresponding mesylate (**12**) by treatment with methansulfonyl chloride. The *SN*2 displacement of the methanesulfonyloxy group with sodium azide gave (2*S*,4*S*)-azide ester (**13**). Crystallization as a di-*p*-toluenesulfonic acid (TsOH) salt, followed by the reduction of **13**, afforded (2*S*,4*S*)-aminoalcohol (**14**). Recrystallization of **14** from ethanol gave optically pure **14**.

The preparation of pyrrolidine derivative (**17**) went as follows. Chlorination of the hydroxy group using thionyl chloride was a more effective method for the stereo inversion at 4-position in this case. Chloride (**15**) was obtained from **8** in a good yield. The *SN*2 displacement of the chloride group with sodium azide gave (2*S*,4*R*)-azide ester (**16**). Reduction of **16**, crystallization of the amino alcohol as a di-TsOH salt and recrystallization from ethanol afforded (2*S*,4*R*)-aminoalcohol (**17**). Optically active **20** and **23**, which have the opposite configurations to those of **14** and **17** at 2-position of the pyrrolidine ring, were also prepared as illustrated in Chart 2. Compound (**9**) was prepared by Baker's method.[7] With the assumption that the same overall sequence of the reactions of **14** or **17** was applicable, (2*R*,4*S*)-aminoalcohol (**20**) and

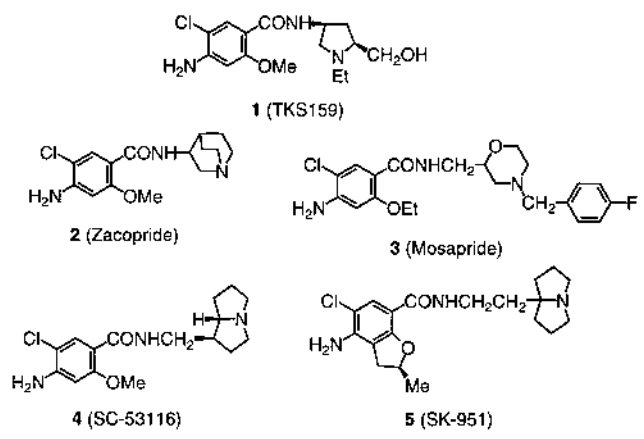

Chart 1

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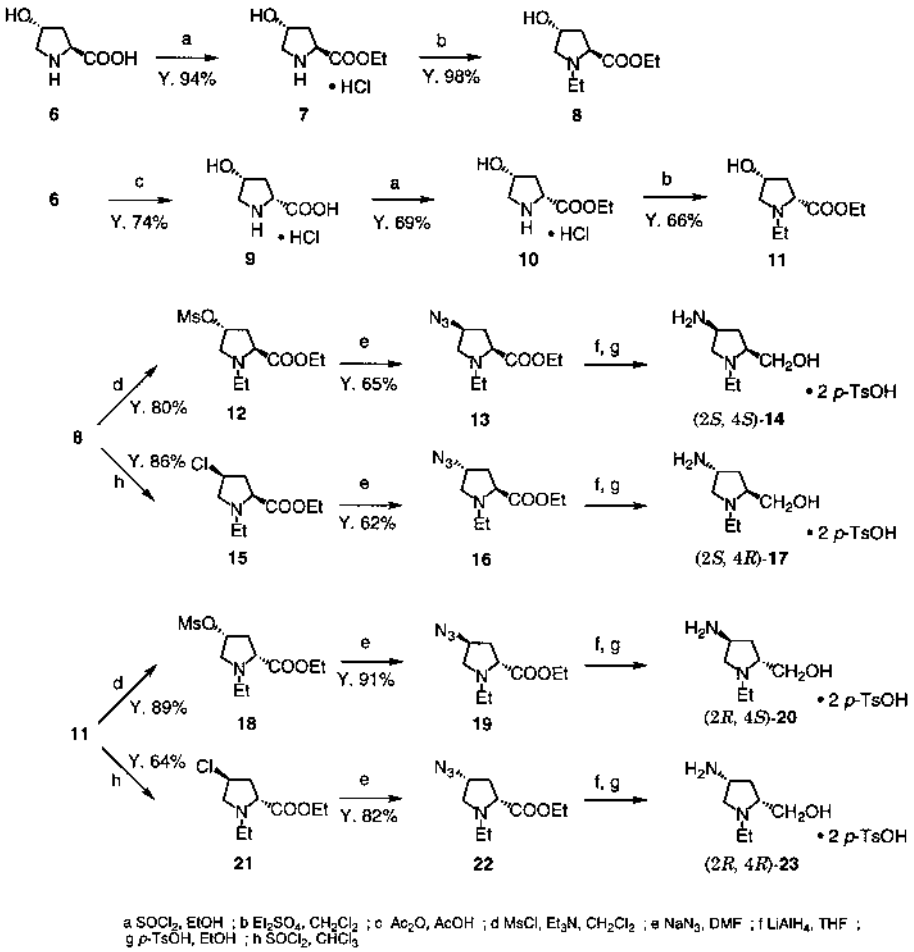


Chart 2

(2*R*,4*R*)-aminoalcohol (**23**) were obtained in similar yields, when the same procedure was applied to **9**.

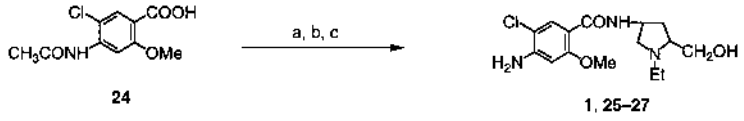
The reaction using chloroformate and triethylamine for coupling **24** and key-intermediates, chiral pyrrolidine derivatives (**14**, **17**, **20** or **23**) and the following hydrolysis with sodium hydroxide gave all optically active benzamide derivatives. The ^{1}H-NMR signals were assigned by homonuclear two dimensional NMR techniques, correlated spectroscopy (COSY), nuclear Overhauser enhancement spectroscopy (NOESY) and homonuclear Hartmann–Harn spectroscopy (HOHAHA). The ^{13}C-NMR signal assignments were carried out using distortionless enhancement by polarization transfer (DEPT), ^{13}C,^{1}H-COSY and ^{13}C,^{1}H-correlated spectroscopy *via* long-range coupling (COLOC) NMR methods on the basis of the results of ^{1}H-NMR signal assignment. The physicochemical data of optically active benzamide derivatives (**1**) and (**25**)—(**27**) are shown in Table 1. The absolute configuration determined by X-ray analysis for **1** was reported in our previous paper,[8] and the comparative study of the NMR spectra and other physicochemical data shows that the absolute configurations of the chiral centers on the pyrrolidine ring of **1**, **25**, **26** and **27** are (2*S*,4*S*), (2*S*,4*R*), (2*R*,4*S*) and (2*R*,4*R*), respectively, as were expected from the synthetic route in Chart 2. Both the diastereomeric and enantiomeric purities were determined for **1**, **25**, **26** and **27** by the HPLC method that uses the ODS column to separate the diastereomers in combination with the chiral column to separate the enantiomers. The diastereomeric and enantiomeric purities of the four isomers were thus determined to be >99% de and >99% ee, respectively.

Pharmacological Results and Discussion

The affinity of the four optical isomers for 5-HT$_4$ receptors was measured by the method described by Grossman *et al.*,[9] and the results are shown in Table 2. All compounds completely inhibited the specific binding of [^{3}H]GR113808 in the guinea pig striatal membrane. Compounds **25** and **27** were less potent than compounds **1** and **26** in that the potency of their affinity toward 5-HT$_4$ receptors seemed to correlate with the chirality of 4-position of the pyrrolidine ring.

The isomers in question were evaluated in terms of their *in vivo* gastroprokinetic activity by way of comparative experiments in which their effects were compared with regard to the gastric emptying of a phenol red semisolid meal in rats. The results are shown in Table 3. Administration *p.o.* 30 min before the test meal showed that compound **1** (TKS159) significantly enhanced the gastric emptying at doses of 1, 3, 10 mg/kg. The rest (**25**—**27**) were less active, and **25** was almost as potent as **26** and **27**. All this suggests that the (2*S*,4*S*)-configuration at the pyrrolidine ring is important for good gastric emptying in rats. The 4-amino-5-chloro-2-methoxybenzamide family is known to have a simple intermolecular hydrogen bonding between the hydrogen of benzamide and the oxygen of 2-methoxy group and to form a

Table 1. Physicochemical Data for Optically Active Benzamide Derivatives [TKS159 (**1**), **25**—**27**]



24 **1**, **25**–**27**

a ClCOOEt, Et_3N, CH_2Cl_2 ; b **14**, **17**, **20** or **23**, Et_3N, CH_2Cl_2 ; c NaOH, H_2O, MeOH

Compd.	Configuration	Note	mp (°C)	$[\alpha]_D^{20a)}$	Formula	Analysis (%) Calcd (Found)			
						C	H	N	Cl
TKS159 (**1**)	(2*S*,4*S*)		137—138	−32.8	$C_{15}H_{22}ClN_3O_3$	54.96 (54.91)	6.76 (6.74)	12.82 (12.92)	10.82 (10.91)
25	(2*S*,4*R*)	$H_2O^{b)}$	150—152	−45.0[c]	$C_{15}H_{22}ClN_3O_3\cdot H_2O$	52.10 (51.93)	7.00 (7.02)	12.15 (12.12)	10.25 (10.11)
26	(2*R*,4*S*)	$H_2O^{b)}$	150—152	+46.7[c]	$C_{15}H_{22}ClN_3O_3\cdot H_2O$	52.10 (51.75)	7.00 (6.97)	12.15 (12.11)	10.25 (10.21)
27	(2*R*,4*R*)		138—139	+29.9	$C_{15}H_{22}ClN_3O_3$	54.96 (54.94)	6.76 (6.75)	12.82 (12.79)	10.82 (10.75)

a) Concentration; 0.10, solvent; 95% ethanol. *b*) Monohydrate. *c*) Calculated on the basis of the anhydrate.

Table 2. 5-HT_4 Receptor Binding Affinity of TKS159 (**1**) and Its Optical Isomers **25**—**27**

Compound	[^{3}H]GR113808 binding affinity IC_{50} (μM)[a)]
TKS159 [(2*S*,4*S*)-**1**]	7.1
(2*S*,4*R*)-**25**	9.5
(2*R*,4*S*)-**26**	5.1
(2*R*,4*R*)-**27**	14.1

a) IC_{50} value was calculated based on duplicate runs of each experiment.

Table 3. Effects of TKS159 (**1**) and Its Optical Isomers **25**—**27** on Gastric Emptying of Phenol Red Semisolid Meal in Rats[a)]

Compound	Dose (mg/kg *p.o.*)	Remaining % of control[b)]
Vehicle	—	100.0
TKS159 [(2*S*,4*S*)-**1**]	1	71.4± 3.5*
	3	49.5± 5.9*
	10	33.5± 5.0*
(2*S*,4*R*)-**25**	1	93.2±12.5
	3	67.0± 6.6*
	10	65.2± 8.3*
(2*R*,4*S*)-**26**	1	82.0±12.4
	3	51.6±10.7*
	10	53.1± 6.6*
(2*R*,4*R*)-**27**	1	98.1±10.1
	3	76.4± 7.8
	10	64.5± 7.6*

a) Each experiment represents the mean±S.E.M. of 6–12 rats. *b*) The asterisks indicate a statistically significant difference from the control group; *, $p<0.05$ (Dunnett's test).

pseudo 6-membered ring.[10] The X-ray analysis of TKS159 showed a three dimensional network of the intra- and intermolecular hydrogen bond formed among the amino, hydroxy and carbonyl groups in the crystals.[8] Therefore, the (2*S*,4*S*)-configuration and the hydroxy group on the side chain of 2-position of the pyrrolidine ring in TKS159 apparently affected the potent gastroprokinetic activity.

We synthesized chiral substituted pyrrolidine derivatives and chiral benzamide derivatives, and evaluated their affinity for 5-HT_4 receptors and their effects on gastric emptying. Among them, TKS159 showed both relatively potent affinity for 5-HT_4 receptors and potent gastroprokinetic activity, emerging as a very promising candidate for a new gastroprokinetic agent.

Experimental

The melting points: determined with a Yanagimoto micromelting point apparatus and remain uncorrected. IR spectra: recorded on a Perkin-Elmer Model 1720X spectrometer. ^{1}H- (400 MHz) and ^{13}C- (100 MHz) NMR spectra: recorded with a Bruker AMX-400 spectrometer. Chemical shifts in δ values (ppm) using tetramethylsilane (TMS) or 3-(trimethylsilyl)-1-propionic acid sodium salt-d_4 (TSP) as the internal standard; coupling constants (*J* values) in hertz (Hz). Optical rotations: measured with a HORIBA SEPA-200 digital polarimeter. Elemental analysis: performed with a Yanako CHN-Corder MT-2. Organic extracts: all dried over anhydrous magnesium sulfate and concentrated with an evaporator under reduced pressure. Procedures of the preparation of compounds **6**—**11**, **12**, **13**, **15**, **16**, **18**, **19**, **21** and **22** were described earlier.[11]

(2*S*,4*S*)-(−)-4-Amino-1-ethyl-2-hydroxymethylpyrrolidine Di-*p*-toluenesulfonate (14) To a slurry of lithium aluminum hydride (LAH) (6.5 g, 0.171 mol) in tetrahydrofuran (THF) (160 ml), a solution of azide ester **13** (26.0 g, 0.122 mol) in THF (30 ml) was added dropwise below 0 °C with an ice-salt bath. The mixture was stirred below 0 °C for 7 h followed by at room temperature for 15 h. It was again stirred to cool down to 0 °C, 54 ml of 1 N NaOH/THF (1 : 3) was added dropwise over 1 h, and celite (40 g) was added and stirred for 1 h. The mixture was filtered and washed well with THF (350 ml). The filtrate solution was concentrated to an oil and distillation of the oil under reduced pressure gave aminoalcohol (15.0 g, 85.3%) in a colorless oil, bp 77.5—80.0 °C (0.4—0.5 mmHg). To the solution of aminoalcohol (15.0 g, 0.104 mol) in 2-propanol (70 ml) *p*-toluensulfonic acid monohydrate (43.5 g, 0.229 mol) was added. Cooled in a refrigerator, it crystallized in white needles, which were then filtered, washed with 2-propanol (20 ml×2) and acetone (20 ml×2), and dried at 40—50 °C, yielding 45.1 g (76%) of crude **14**. Recrystallization of crude **14** (45.1 g, 0.092 mol) from ethanol (225 ml) gave **14** (41.8 g, 70%), mp 205—206.5 °C. $[\alpha]_D^{20}$ −11.2° (c=1.01, H_2O). ^{1}H-NMR (D_2O) δ: 1.35 (3H, t, J=7.3, $NCH_2\underline{CH_3}$), 2.18 (1H, m, pyr-3H), 2.39 (6H, s, arom-CH_3), 2.80 (1H, ddd, J=14.0, 8.0, 8.0, pyr-3H), 3.22 (1H, dq, J=12.9, 7.2, $N\underline{CH_2}CH_3$), 3.51 (1H, dq, J=12.9, 7.2, $N\underline{CH_2}CH_3$), 3.73 (1H, m, pyr-5H), 3.79 (1H, m, pyr-5H), 3.82 (1H, dd, J=12.7, 2.9, $C\underline{H_2}OH$), 3.88 (1H, m, pyr-2H), 4.02 (1H, dd, J=12.7, 2.2, $C\underline{H_2}OH$), 4.26 (1H, ddd, J=12.6, 7.8, 5.9, pyr-4H), 7.37 (4H, d, J=8.2, arom-3H, 5H), 7.69 (4H, d, J=8.2, arom-2H, 6H). ^{13}C-NMR (D_2O) δ: 12.74 ($NCH_2\underline{C}H_3$), 23.35 (arom-CH_3), 34.02 (C-3), 50.11 (C-4), 52.72 ($N\underline{C}H_2CH_3$), 58.45 (C-5), 60.33 ($\underline{C}H_2OH$), 70.02 (C-2), 128.24 (arom-C-2, 6), 132.34 (arom-C-3, 5), 142.43 (arom-C-4), 145.35 (arom-C-1). *Anal*. Calcd for $C_{21}H_{32}N_2O_7S_2$: C, 51.62; H, 6.60; N, 5.73; O, 22.92; S, 13.13. Found: C, 51.41; H, 6.48; N, 5.73; O, 23.18; S, 13.32. IR (nujol) νcm^{-1}: 3209, 1461, 1217, 1180, 1125, 1037, 1012, 684, 568.

(2*S*,4*R*)-(+)-4-Amino-1-ethyl-2-hydroxymethylpyrrolidine Di-*p*-toluenesulfonate (17) For corresponding isomer **14**, **17** was prepared from **16** (22.0 g, 0.104 mol) as described above, yielding 37.0 g (73%), mp 150—152 °C. $[\alpha]_D^{20}$ −13.0° (*c*=1.03, H_2O). ^{1}H-NMR (D_2O) δ: 1.35 (3H, t, *J*=7.3, $NCH_2C\underline{H}_3$), 2.38 (1H, m, pyr-3H), 2.39 (6H, s, arom-CH_3), 2.51 (1H, m, pyr-3H), 3.23 (1H, dq, *J*=12.9, 7.2, $NC\underline{H}_2CH_3$), 3.34 (1H, m, pyr-5H), 3.56 (1H, dq, *J*=12.9, 7.2, $NC\underline{H}_2CH_3$), 3.81 (1H, dd, *J*=12.9, 3.8, $C\underline{H}_2OH$), 3.90(1H, ddd, *J*=12.0, 8.2, 3.6, pyr-4H), 3.97 (1H, dd, *J*=12.9, 3.3, $C\underline{H}_2OH$), 4.1—4.2 (2H, m, pyr-2H and 5H), 7.37 (4H, d, *J*=8.2, arom-3H, 5H), 7.69 (4H, d, *J*=8.2, arom-2H, 6H). ^{13}C-NMR (D_2O) δ: 12.74 ($NCH_2\underline{C}H_3$), 23.35 (arom-CH_3), 33.25 (C-3), 49.40 (C-4), 52.41 ($N\underline{C}H_2CH_3$), 57.96 (C-5), 60.40 ($\underline{C}H_2OH$), 69.31 (C-2), 128.24 (arom-C-2, 6), 132.33 (arom-C-3, 5), 142.41 (arom-C-4), 145.35 (arom-C-1). *Anal*. Calcd for $C_{21}H_{32}N_2O_7S_2$: C, 51.62; H, 6.60; N, 5.73; O, 22.92; S, 13.13. Found: C, 51.61; H, 6.84; N, 5.66; O, 23.16; S, 13.22. IR (nujol) νcm^{-1}: 3203, 1224, 1175, 1124, 1037, 1011, 685, 567.

(2*R*,4*S*)-(+)-4-Amino-1-ethyl-2-hydroxymethylpyrrolidine Di-*p*-toluenesulfonate (20) For corresponding isomer **14**, **20** was prepared from **19** (30.6 g, 0.144 mol) as described above, yielding 52.3 g (74%), mp 149—150.5 °C. $[\alpha]_D^{20}$ +13.0° (*c*=1.02, H_2O). ^{1}H-NMR (D_2O) δ: 1.35 (3H, t, *J*=7.3, $NCH_2C\underline{H}_3$), 2.38 (1H, m, pyr-3H), 2.39 (6H, s, arom-CH_3), 2.51 (1H, m, pyr-3H), 3.23 (1H, dq, *J*=13.0, 7.3, $NC\underline{H}_2CH_3$), 3.34 (1H, m, pyr-5H), 3.56 (1H, dq, *J*=13.0, 7.3, $NC\underline{H}_2CH_3$), 3.81 (1H, dd, *J*=12.9, 3.8, $C\underline{H}_2OH$), 3.90 (1H, ddd, *J*=12.0, 8.2, 3.6, pyr-4H), 3.97 (1H, dd, *J*=12.9, 3.3, $C\underline{H}_2OH$), 4.1—4.2 (2H, m, pyr-2H and 5H), 7.37 (4H, d, *J*=8.2, arom-3H, 5H), 7.70 (4H, d, *J*=8.2, arom-2H, 6H). ^{13}C-NMR (D_2O) δ: 12.74 ($NCH_2\underline{C}H_3$), 23.35 (arom-CH_3), 33.25 (C-3), 49.41 (C-4), 52.41 ($N\underline{C}H_2CH_3$), 57.97 (C-5), 60.40 ($\underline{C}H_2OH$), 69.31 (C-2), 128.24 (arom-C-2, 6), 132.33 (arom-C-3, 5), 142.41 (arom-C-4), 145.35 (arom-C-1). *Anal*. Calcd for $C_{21}H_{32}N_2O_7S_2$: C, 51.62; H, 6.60; N, 5.73; O, 22.92; S, 13.13. Found: C, 51.60; H, 6.69; N, 5.45; O, 23.12; S, 13.18. IR (nujol) νcm^{-1}: 3203, 1224, 1175, 1124, 1037, 1011, 685, 567.

(2*R*,4*R*)-(+)-4-Amino-1-ethyl-2-hydroxymethylpyrrolidine Di-*p*-toluenesulfonate (23) For corresponding isomer **14**, **23** was prepared from **22** (20.0 g, 0.094 mol) as it is described, yielding 30.9 g (67%), mp 205—206 °C. $[\alpha]_D^{20}$ +10.3° (*c*=1.00, H_2O). ^{1}H-NMR (D_2O) δ: 1.34 (3H, t, *J*=7.3, $NCH_2C\underline{H}_3$), 2.18 (1H, m, pyr-3H), 2.39 (6H, s, arom-CH_3), 2.80 (1H, ddd, *J*=14.0, 8.0, 8.0, pyr-3H), 3.22 (1H, dq, *J*=12.8, 7.3, $NC\underline{H}_2CH_3$), 3.50 (1H, dq, *J*=12.8, 7.3, $NC\underline{H}_2CH_3$), 3.74 (1H, m, pyr-5H), 3.79 (1H, m, pyr-5H), 3.82 (1H, dd, *J*=12.8, 2.9, $C\underline{H}_2OH$), 3.88 (1H, m, pyr-2H), 4.02 (1H, dd, *J*= 12.8, 2.5, $C\underline{H}_2OH$), 4.26 (1H, ddd, *J*=12.7, 8.0, 5.8, pyr-4H), 7.37 (4H, d, *J*=8.2, arom-3H, 5H), 7.69 (4H, d, *J*=8.2, arom-2H, 6H). ^{13}C-NMR (D_2O) δ: 12.74 ($NCH_2\underline{C}H_3$), 23.35 (arom-CH_3), 34.02 (C-3), 50.11 (C-4), 52.71 ($N\underline{C}H_2CH_3$), 58.45 (C-5), 60.32 ($\underline{C}H_2OH$), 70.02 (C-2), 128.24 (arom-C-2, 6), 132.34 (arom-C-3, 5), 142.43 (arom-C-4), 145.35 (arom-C-1). *Anal*. Calcd for $C_{21}H_{32}N_2O_7S_2$: C, 51.62; H, 6.60; N, 5.73; O, 22.92; S, 13.13. Found: C, 51.42; H, 6.52; N, 5.58; O, 23.12; S, 13.17. IR (nujol) νcm^{-1}: 3203, 1218, 1178, 1124, 1036, 1012, 683, 567.

4-Amino-5-chloro-2-methoxy-*N*-[(2*S*,4*S*)-1-ethyl-2-hydroxymethyl-4-pyrrolidinyl]benzamide (1) A solution of 4-acetylamino-5-chloro-2-methoxybenzoic acid (9.0 g, 36.9 mmol) and triethylamine (7.9 g, 77.6 mmol) in dichloromethane (45 ml) was cooled to below 10 °C, and ethyl chloroformate (4.4 g, 40.6 mmol) was added dropwise. The solution, kept at the same temperature for 30 min, was given portionwise (2*S*,4*S*)-(−)-4-amino-1-ethyl-2-hydroxymethylpyrrolidine di-*p*-toluenesulfonate (**14**, 18.0 g, 36.9 mmol). Stirring the mixture at below 10 °C, the addition of water (9 ml) and 24% (w/v) NaOH aq. (20.9 g, 126 mmol), and separating the organic layer gave an extract of dichloromethane (10 ml×3). The combined extract was concentrated and methanol (10 ml) , 48% (w/v) NaOH aq. (3.4 g, 40.7 mmol) and water (7 ml) were added to the residue. The mixture was heated to 50—55 °C, stirred for 3 h, then cooled in an ice-bath. The resulting precipitate was filtered, collected, washed with water, and recrystallized from 50% (v/v) aqueous ethanol. It gave 8.5 g (70%) of **1**. ^{1}H-NMR ($CDCl_3$) δ: 1.08 (3H, t, *J*=7.2, $NCH_2C\underline{H}_3$), 1.72 (1H, dddd, *J*=14.0, 5.7, 2.4, 1.5, pyr-3H), 2.30 (1H, dq, *J*=12.1, 7.2, $NC\underline{H}_2CH_3$), 2.44 (1H, ddd, *J*=14.0, 9.9, 8.0, pyr-3H), 2.54 (1H, dd, *J*=9.9, 5.3, pyr-5H), 2.67 (1H, m, pyr-2H), 2.81 (1H, dq, *J*=12.1, 7.2, $NC\underline{H}_2CH_3$), 3.18 (1H, ddd, *J*=9.9, 1.5, 1.5, pyr-5H), 3.39 (1H, dd, *J*=11.0, 1.4, $C\underline{H}_2OH$), 3.70 (1H, dd, *J*=11.0, 3.1, $C\underline{H}_2OH$), 3.89 (3H, s, arom-$OC\underline{H}_3$), 4.36 (2H, s, arom-$N\underline{H}_2$), 4.53 (1H, m, pyr-4H), 6.27 (1H, s, arom-3H), 8.09 (1H, s, arom-6H), 8.13 (1H, d, *J*=6.7, CON$\underline{H}$). ^{13}C-NMR ($CDCl_3$) δ: 13.92 ($NCH_2\underline{C}H_3$), 35.48 (pyr-3C), 47.44 ($N\underline{C}H_2CH_3$), 48.06 (pyr-4C), 55.97 (arom-$O\underline{C}H_3$), 60.61 (pyr-5C), 61.06 ($\underline{C}H_2OH$), 63.65 (pyr-2C), 97.93 (arom-3C), 111.61 (arom-5C), 112.91 (arom-1C), 133.12 (arom-6C), 146.57 (arom-4C), 157.73 (arom-2C), 163.72 (C=ONH). IR (KBr) νcm^{-1}: 3386, 3328, 3214, 2965, 2935, 2875, 2799, 1638, 1583, 1546, 1272, 1250, 1041, 833. Other physicochemical data are shown in Table 1.

4-Amino-5-chloro-2-methoxy-*N*-[(2*S*,4*R*)-1-ethyl-2-hydroxymethyl-4-pyrrolidinyl]benzamide Hydrate (25) For corresponding isomer **1**, **25** was prepared from **17** as described above, yielding 9.2 g (72%). ^{1}H-NMR ($CDCl_3$) δ: 1.10 (3H, t, *J*=7.2, $NCH_2C\underline{H}_3$), 1.66 (2H, br s, H_2O), 1.78 (1H, ddd, *J*=13.1, 8.9, 6.6, pyr-3H), 2.22 (1H, dd, *J*= 9.2, 7.9, pyr-5H), 2.29 (1H, ddd, *J*=13.1, 8.5, 6.8, pyr-3H), 2.35 (1H, dq, *J*=12.1, 7.0, $NC\underline{H}_2CH_3$), 2.62 (1H, br s, $CH_2O\underline{H}$), 2.81 (1H, m, pyr-2H), 2.84 (1H, dq, *J*=12.1, 7.1, $NC\underline{H}_2CH_3$), 3.41 (1H, dd, *J*=10.9, 2.1, $C\underline{H}_2OH$), 3.65 (1H, m, pyr-5H), 3.66 (1H, dd, *J*=10.6, 3.7, $C\underline{H}_2OH$), 3.89 (3H, s, arom-$OC\underline{H}_3$), 4.38 (2H, s, arom-$N\underline{H}_2$), 4.48 (1H, m, pyr-4H), 6.29 (1H, s, arom-3H), 7.64 (1H, d, *J*=6.6, CON$\underline{H}$), 8.09 (1H, s, arom-6H). ^{13}C-NMR ($CDCl_3$) δ: 13.82 ($NCH_2\underline{C}H_3$), 35.28 (pyr-3C), 47.99 ($N\underline{C}H_2CH_3$ or pyr-4C), 48.34 (pyr-4C or $N\underline{C}H_2CH_3$), 56.20 (arom-$O\underline{C}H_3$), 59.68 (pyr-5C), 61.58 ($\underline{C}H_2OH$), 63.41 (pyr-2C), 97.98 (arom-3C), 111.93 (arom-5C), 112.75 (arom-1C), 133.27 (arom-6C), 146.67 (arom-4C), 157.46 (arom-2C), 164.22 (C=ONH). IR (KBr) νcm^{-1}: 3485, 3366, 3220, 2979, 2859, 2803, 1651, 1614, 1587, 1560, 1291, 1271, 1249, 1055, 826. Other physicochemical data are shown in Table 1.

4-Amino-5-chloro-2-methoxy-*N*-[(2*R*,4*S*)-1-ethyl-2-hydroxymethyl-4-pyrrolidinyl]benzamide Hydrate (26) For corresponding isomer **1**, **26** was prepared from **20** as described above, yielding 9.4 g (74%). ^{1}H-NMR ($CDCl_3$) δ: 1.10 (3H, t, *J*=7.2, $NCH_2C\underline{H}_3$), 1.65 (2H, br s, H_2O), 1.78 (1H, ddd, *J*=13.1, 8.9, 6.6, pyr-3H), 2.22 (1H, dd, *J*=9.2, 7.9, pyr-5H), 2.29 (1H, ddd, *J*= 13.1, 8.5, 6.8, pyr-3H), 2.35 (1H, dq, *J*=12.1, 7.0, $NC\underline{H}_2CH_3$), 2.62 (1H, br s, $CH_2O\underline{H}$), 2.82 (1H, m, pyr-2H), 2.84 (1H, dq, *J*=12.1, 7.1, $NC\underline{H}_2CH_3$), 3.41 (1H, dd, *J*=10.9, 2.1, $C\underline{H}_2OH$), 3.65 (1H, m, pyr-5H), 3.66 (1H, dd, *J*= 10.6, 3.7, $C\underline{H}_2OH$), 3.89 (3H, s, arom-$OC\underline{H}_3$), 4.38 (2H, s, arom-$N\underline{H}_2$), 4.48 (1H, m, pyr-4H), 6.29 (1H, s, arom-3H), 7.63 (1H, d, *J*=6.6, CON$\underline{H}$), 8.09 (1H, s, arom-6H). ^{13}C-NMR ($CDCl_3$) δ: 13.81 ($NCH_2\underline{C}H_3$), 35.28 (pyr-3C), 47.99 ($N\underline{C}H_2CH_3$ or pyr-4C), 48.34 (pyr-4C or $N\underline{C}H_2CH_3$), 56.20 (arom-$O\underline{C}H_3$), 59.68 (pyr-5C), 61.58 ($\underline{C}H_2OH$), 63.41 (pyr-2C), 97.99 (arom-3C), 111.93 (arom-5C), 112.75 (arom-1C), 133.27 (arom-6C), 146.67 (arom-4C), 157.46 (arom-2C), 164.22 (C=ONH). IR (KBr) νcm^{-1}: 3485, 3363, 3220, 2979, 2859, 2804, 1651, 1614, 1587, 1561, 1291, 1271, 1249, 1055, 826. Other physicochemical data are shown in Table 1.

4-Amino-5-chloro-2-methoxy-*N*-[(2*R*,4*R*)-1-ethyl-2-hydroxymethyl-4-pyrrolidinyl]benzamide (27) For corresponding isomer **1**, **27** was prepared from **23** as described above, yielding 7.3 g (60%). ^{1}H-NMR ($CDCl_3$) δ: 1.08 (3H, t, *J*=7.2, $NCH_2C\underline{H}_3$), 1.72 (1H, dddd, *J*=14.0, 5.7, 2.4, 1.5, pyr-3H), 2.31 (1H, dq, *J*=12.1, 7.2, $NC\underline{H}_2CH_3$), 2.44 (1H, ddd, *J*=14.0, 9.9, 8.0, pyr-3H), 2.54 (1H, dd, *J*=9.9, 5.3, pyr-5H), 2.67 (1H, m, pyr-2H), 2.81 (1H, dq, *J*=12.1, 7.2, $NC\underline{H}_2CH_3$), 3.18 (1H, ddd, *J*=9.9, 1.5, 1.5, pyr-5H), 3.39 (1H, dd, *J*=11.0, 1.4, $C\underline{H}_2OH$), 3.70 (1H, dd, *J*=11.0, 3.1, $C\underline{H}_2OH$), 3.89 (3H, s, arom-$OC\underline{H}_3$), 4.36 (2H, s, arom-$N\underline{H}_2$), 4.54 (1H, m, pyr-4H), 6.27 (1H, s, arom-3H), 8.09 (1H, s, arom-6H), 8.13 (1H, d, *J*=6.7, CON$\underline{H}$). ^{13}C-NMR ($CDCl_3$) δ: 13.92 ($NCH_2\underline{C}H_3$), 35.48 (pyr-3C), 47.44 ($N\underline{C}H_2CH_3$), 48.06 (pyr-4C), 55.98 (arom-$O\underline{C}H_3$), 60.61 (pyr-5C), 61.06 ($\underline{C}H_2OH$), 63.66 (pyr-2C), 97.93 (arom-3C), 111.62 (arom-5C), 112.92 (arom-1C), 133.12 (arom-6C), 146.57 (arom-4C), 157.73 (arom-2C), 163.73 (C=ONH). IR (KBr) νcm^{-1}: 3386, 3329, 3215, 2966, 2936, 2876, 2800, 1637, 1583, 1546, 1275, 1250, 1042, 834. Other physicochemical data are shown in Table 1.

In Vitro* Study. 5-HT$_4$ Receptor Binding Assay** The 5-HT$_4$ receptor binding assay was employed according to the method of Grossman *et al*.[9] with some modifications. Male Hartley guinea pigs (220—350 g) were killed by cervical dislocation and their brain was removed and dissected. Pooled striatal tissue was placed in the 50 mM HEPES buffer solution (pH 7.4, 4 °C) and homogenized with a glass-teflon homogenizer, and the homogenate was centrifuged at 48000×g*** for 10 min. The pellet was resuspended in the same buffer and centrifugation was repeated. The final pellet was resuspended in the same buffer and the suspension was stored at −80 °C until it was put to use. The receptor binding assay was started by addition of 100 μl of membrane suspension to a tube containing 300 μl of a solution of either a test compound or 5-HT, giving a final concentration of 30 μM (for determination of the non-specific binding) or a buffer (for determination of the total binding), and 100 μl [^{3}H]GR113808 also giving a final concentration of 0.1 nM. The assay tubes were incubated at 37 °C for 30 min. The reaction was terminated by rapid vacuum filtration through a Whatman GF/C glass filter presoaked in 0.1% polyethylenimine. The filter was rinsed twice, each time with 5 ml of an ice-cold HEPES buffer, and placed in the scintillation vial. Then, the ACS-II scintillator was added and the radioactivity on the filter was measured with a liquid scintillation counter. All the determinations were performed in duplicate. The inhibition of binding by a test compound was

analyzed to estimate the IC_{50} (the concentration of a test compound that caused 50% inhibition of the binding) by the probit method.

***In Vivo* Study. Gastric Emptying of Phenol Red Semisolid Meal** Male Wistar rats were fasted overnight with water *ad libitum*. The test compound was administered orally 30 min before administration of the phenol red. The rats (120—160 g) were orally given 1.5 ml/animal of the 0.05% phenol red solution in 2.0% carboxymethylcellulose as a test meal. After 15 min, the animals were sacrificed. The esophagogastric junction and pylorus were immediately clamped and then the stomach was excised. Extraction of the phenol red from the stomach and its contents were measured by the method previously described.[12] The amount of phenol red was measured spectrophotometrically at 560 nm.

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