Serotonin 5-HT$_4$ Receptor Agonistic Activity of the Optical Isomers of (±)-4-Amino-*N*-[2-(1-azabicyclo[3.3.0]octan-5-yl)ethyl]-5-chloro-2,3-dihydro-2-methylbenzo[*b*]furan-7-carboxamide

Takuji KAKIGAMI,*[,a] Toshinao USUI,[a,1)] Takao IKAMI,[a] Katsura TSUKAMOTO,[a] Yoshihisa MIWA,[b] Tooru TAGA,[b] and Tadashi KATAOKA[c]

General Research Park, Sanwa Kagaku Kenkyusho Co., Ltd.,[a] 363, Shiosaki, Hokusei-cho, Inabe-gun, Mie 511–0406, Japan, Faculty of Pharmaceutical Sciences, Kyoto University,[b] Yoshida Shimoadachi-cho, Sakyo-ku, Kyoto, 606–8403, Japan and Gifu Pharmaceutical University,[c] 5–6–1 Mitahora-higashi, Gifu, 502–8585, Japan.
Received November 25, 1997; accepted March 3, 1998

The enantiomers, (*R*)-(−)-1 and (*S*)-(+)-1, of (±)-4-amino-*N*-[2-(1-azabicyclo[3.3.0]octan-5-yl)ethyl]-5-chloro-2,3-dihydro-2-methylbenzo[*b*]furan-7-carboxamide [(±)-1] were prepared from optically active benzyl 4-acetylamino-2,3-dihydro-2-methylbenzo[*b*]furan-7-carboxylate [(*R*)-(+)-6, (*S*)-(−)-6], respectively. The requisite (*R*)-(+)-6 and (*S*)-(−)-6 were prepared by large-scale preparative HPLC on chiral stationary phases (CSPs). The absolute configuration of (*S*)-(+)-1 was determined by single crystal X-ray analysis. The serotonin 5-HT$_4$ receptor agonistic activity of (*S*)-(−)-1 hemifumarate (SK-951) which was hemifumarate of (*S*)-(+)-1 was about twice that of the other enantiomer (*R*)-(+)-1 hemifumarate which was hemifumarate of (*R*)-(−)-1.

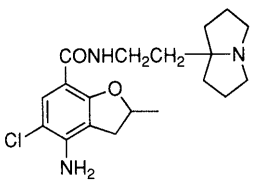
Key words SK-951; optical resolution; large-scale preparative HPLC; absolute configuration; enantiomer; 5-HT$_4$ receptor agonistic activity

In our previous paper,[2)] we reported the synthesis of racemic 4-amino-*N*-[2-(1-azabicyclo[3.3.0]octan-5-yl)-ethyl]-5-chloro-2,3-dihydro-2-methylbenzo[*b*]furan-7-carboxamide [(±)-**1**] hemifumarate, which showed potent serotonin 5-HT$_4$ receptor agonistic activity without dopamine D$_2$ receptor antagonistic activity. 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 (±)-**1**. On the other hand, the importance of optically active compounds for pharmaceutical usage has increased. The HPLC method was convinced for the analysis of optically active compounds, however, in the past it was thought that HPLC was not a general method for sample preparation. Recently, HPLC resolution on chiral stationary phases (CSPs) has been useful for sample preparations for estimation in the developing stages as well as for analysis of the compounds; this is a result of the development of various CSPs and the remarkable progress in HPLC instruments. The present paper describes the preparation of the enantiomers (*R*)-(+)-**6** and (*S*)-(−)-**6** which are the key intermediates of (*R*)-**1** and (*S*)-**1**, respectively. The large-scale preparative HPLC study and X-ray crystallographic determination of the absolute configuration of (*S*)-**1** are reported. The synthesis of the optically active compounds (*R*)-(−)-**1** and (*S*)-(+)-**1**, and the comparative biological activity of (±)-**1** and its enantiomers are also reported.

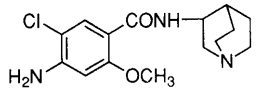
Chemistry

Optical Resolution Initial attempts at the resolution of (±)-**1** itself into its enantiomers using various resolving agents were unsuccessful. A key intermediate, (±)-4-acetylamino-2,3-dihydro-2-methylbenzo[*b*]-furan-7-carboxylic acid (**5**), was then derived into its diastereomeric amides with an optically active amine. The diastereomeric amides thus prepared were resolved by preparative HPLC column (Develosil 30-7, Nomura Chemical Co., Ltd.), however, the hydrolysis of the amide was unsuccessful.

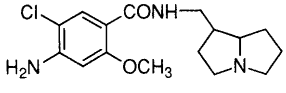
Hence, we focused our efforts on the resolution of racemic (±)-benzyl 4-acetylamino-2,3-dihydro-2-methylbenzo[*b*]furan-7-carboxylate [(±)-**6**] by the HPLC method on CSPs. Oguni *et al.*[5)] reported that the HPLC resolution on CSPs consisting of cellulosic derivatives was a successful method for the preparation of various optically active compounds. We investigated the HPLC resolution of (±)-**6** on CSPs consisting of cellulosic derivatives with an analytical scale. The requisite (±)-**6** was synthesized from (±)-methyl 4-acetylamino-2,3-dihydro-2-methylbenzo[*b*]furan-7-carboxylate [(±)-**4**] which was synthe-



(±)-**1**



2 (zacopride)



3 (SC-49518)

Chart 1

* To whom correspondence should be addressed.

sized by the method in our previous paper (Chart 2).[2]

The analytical HPLC separation of (±)-**6** was successfully carried out on a Chiralcel OJ (Daicel Chemical Industries, Ltd., Japan) (Fig. 1). On the basis of this result, the large-scale HPLC separation of (±)-**6** was performed by Daicel Chemical Industries, Ltd. (*R*)-(+)-**6** and (*S*)-(−)-**6** were then prepared with high yield (85%) and high enantiomeric purity (100%).

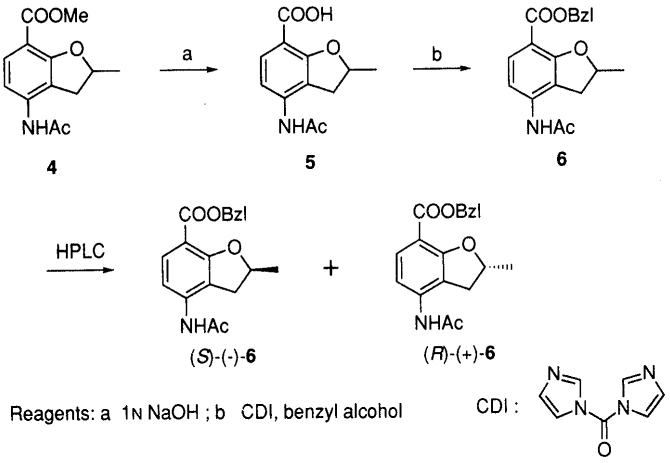


Chart 2

Synthesis of Optically Active Compound 1 The enantiomers of (±)-**1** were prepared by the method described previously (Chart 3).[2] Compound **9** was prepared using the method described by Suzuki *et al.*[6] The enantiomeric purities of (*R*)-(−)-**1** and (*S*)-(+)-**1** thus obtained were determined to be practically 100% e.e. on the basis of chiral HPLC (Fig. 2). (*R*)-(−)-**1** and (*S*)-(+)-**1** were converted to the hemifumarates [(*R*)-(+)-**1** hemifumarates and (*S*)-(−)-**1** hemifumarates, respectively] for the biological tests.

X-Ray Crystallographic Study The absolute configuration of (+)-**1** was determined by the single crystal X-ray analysis (Table 1). The crystal structure consists of (+)-**1** molecule including a chlorine atom. The analysis of the dispersion intensities from the crystal revealed that the absolute configuration at the 2-position of (+)-**1** was *S*

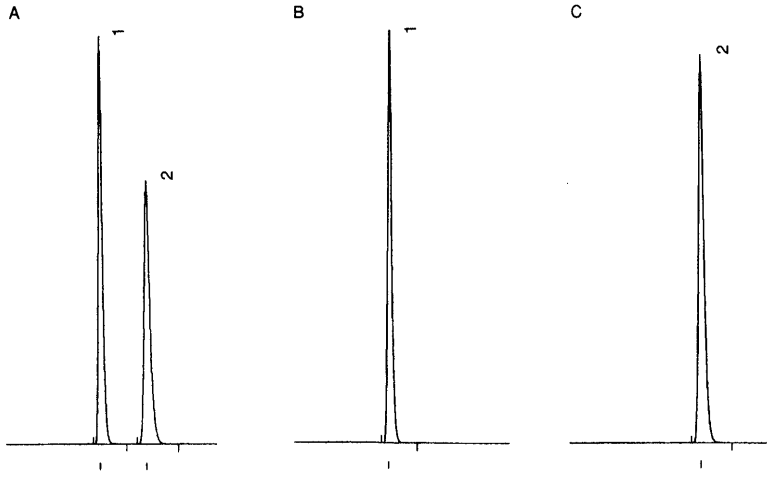
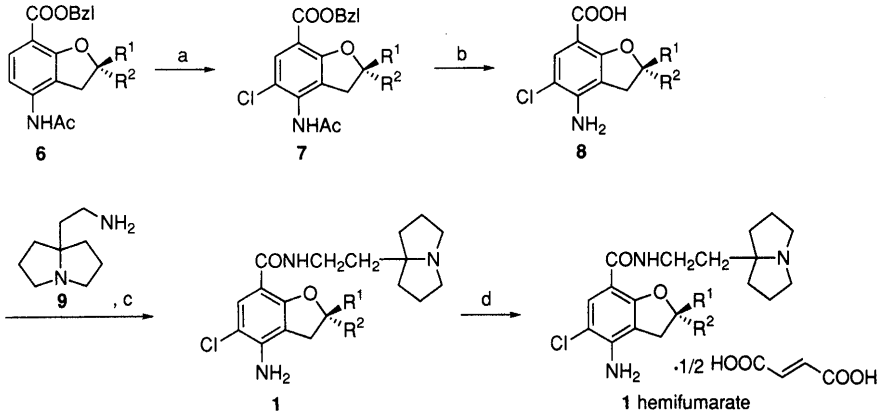


Fig. 1. (A) Analytical HPLC Separation of (±)-**6**, t_R: 8.70 min, 13.1 min; (B) Chromatogram of Separated (*S*)-(−)-**6**, t_R: 8.70 min; (C) Chromatogram of Separated (*R*)-(+)-**6**, t_R: 13.1 min; on a Chiralcel OJ (250 mm × 4.6 mm i.d.) with a Mobile Phase of *n*-Hexane : ethanol (80 : 20)
A flow rate of 1.0 ml/min and detection at 300 nm.



(*S*)-isomer : R^1 = Me ; R^2 = H
(*R*)-isomer : R^1 = H ; R^2 = Me

Reagents: a *N*-chlorosuccinimide(NCS); b 2N NaOH; c 1,1-carbonyldiimidazole(CDI) ; d fumaric acid, EtOH

Chart 3

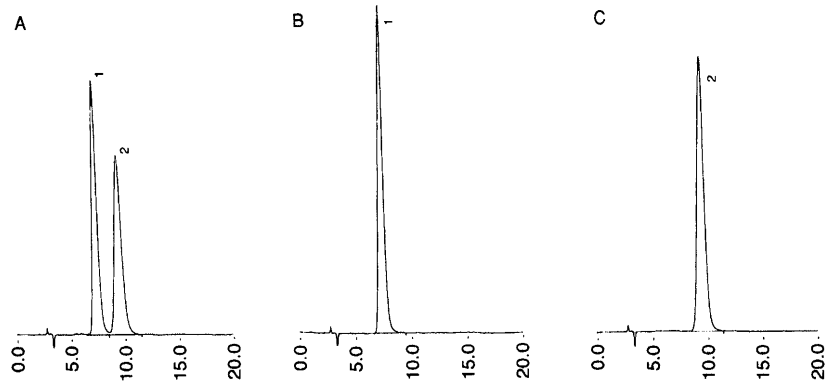


Fig. 2. (A) Analytical HPLC Separation of (±)-**1**; (B) Chromatogram of (*S*)-(+)-**1**; (C) Chromatogram of (*R*)-(−)-**1**; on a Chiralcel OD (250 mm × 4.6 mm i.d.) with a Mobile Phase of *n*-Hexane : ethanol : diethylamine (80 : 20 : 0.1)

A flow rate of 1.0 ml/min and detection at 227 nm.

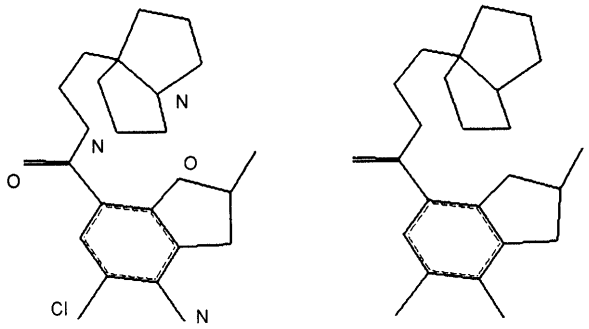


Fig. 3. Stereoview of (*S*)-(+)-**1**

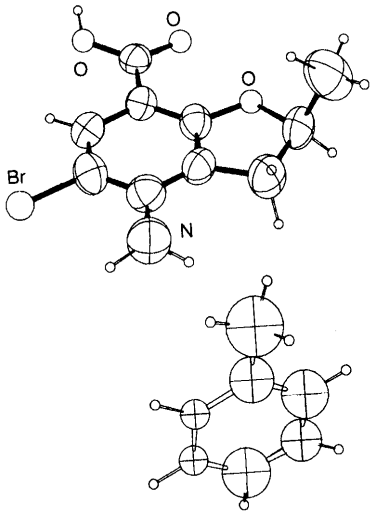
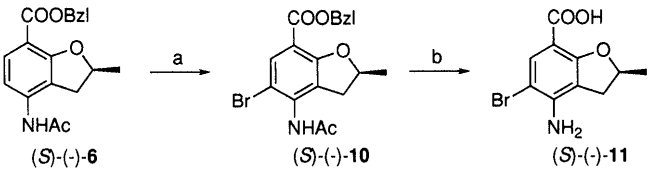


Fig. 4. ORTEP-Type Drawing of the Complex of (*S*)-(−)-**11** and Toluene

(Fig. 3). This absolute configuration was confirmed by the single crystal X-ray analysis of another compound (−)-**11** which had a bromine atom instead of a chlorine atom at the 5-position of (−)-**6** (Fig. 4). The absolute configuration at the 2-position of (−)-**11** was *S* also. It must be the same as (+)-**1**, because both (*S*)-(+)-**1** and (*S*)-(−)-**11** were synthesized from the same compound [(−)-**6**], and the procedures generally do not cause racemization (Chart 4).

Pharmacological Results and Discussion

The serotonin 5-HT_4 receptor agonistic activity of (±)-**1** hemifumarate and its enantiomers [(*R*)-(+)-**1** hemifumarate and (*S*)-(−)-**1** hemifumarate] was measured in accordance with the method described by Baxter *et al.*[7] The values of the 50% effective concentration (ED_{50}) of these compounds are shown in Table 2. The serotonin 5-HT_4 receptor agonistic activity of (*S*)-(−)-**1** hemifumarate was only twice that of the other enantiomer, (*R*)-(+)-**1** hemifumarate, while (*S*)-zacopride and SC-49518, which have an asymmetric carbon in an azabicyclo moiety, had 3 times and 15 times greater activity than the



Reagents: a *N*-bromosuccinimide; b 2N NaOH

Chart 4

Table 1. Crystal Data and Structure Refinements

	(*S*)-**1**	(*S*)-**11**
Formula	$C_{19}H_{26}ClN_3O_2$	$C_{17}H_{18}BrNO$
Formula weight	363.87	332.24
Space group	$P2_12_12_1$	$P4_22_12$
Crystal system	Orthorhombic	Tetragonal
a, Å	12.651(2)	15.200(5)
b, Å	14.439(2)	15.200(5)
c, Å	10.568(2)	10.407(1)
Cell volume, $Å^3$	1930.4	2404.4
Formula units	4	8
D_{calc}, $g\,cm^{-3}$	1.252	1.83
Number of reflections	2705	1902
R	0.078	0.063
wR	0.053	0.048

Table 2. Serotonin 5-HT_4 Receptor Agonistic Activity of (±)-**1** and Its Enantiomers [(*R*)-(+)-**1** Hemifumarate and (*S*)-(−)-**1** Hemifumarate]

Compound	5-HT_4 agonistic activity (ED_{50}, nM)
(±)-**1** hemifumarate	30
(*R*)-(+)-**1** hemifumarate	32
(*S*)-(−)-**1** hemifumarate	14

Table 3. ^{3}H-Labeled Ligand Binding Profile of (±)-**1** Hemifumarate, Its Enantiomers [(*S*)-(−)-**1** Hemifumarate and (*R*)-(+)-**1** Hemifumarate], and Cisapride

Binding site	IC_{50} (μM)			
	(±)-**1** hemifumarate	(*S*)-(−)-**1** hemifumarate	(*R*)-(+)-**1** hemifumarate	Cisapride
5-HT_1	>100	>100	>100	11.5
5-HT_2	88	18	60	0.0027
5-HT_3	0.34	0.42	0.37	0.81
D_1	>100	>100	>100	5.3
D_2	>100	>100	>100	0.63
M_1	1.2	1.3	1.5	>10
M_2	19	19	83	>10

other enantiomer, respectively. It is thought that the construction of 2,3-dihydro-2-methylbenzo[*b*]furan skeleton causes an increase in agonistic activity,[2] although the direction of the substituent at the 3-position does not show a remarkable effect.

In the radioligand binding assays (Table 3), (±)-**1** hemifumarate and its enantiomers [(*R*)-(+)-**1** hemifumarate and (*S*)-(−)-**1** hemifumarate] showed no affinity at a concentration of 100 μM for the dopamine D_1, D_2, serotonin 5-HT_1, and, 5-HT_2 binding sites in the rat brain synaptic membranes and weak muscarin M_1, and M_2 binding affinity with IC_{50}'s of 1.2 and 19.8 μM, respectively. However, (±)-**1** hemifumarate and its enantiomers [(*R*)-(−)-**1** and (*S*)-(+)-**1**] had a high serotonin 5-HT_3 binding affinity with an IC_{50} of 0.34—0.42 μM. The affinity of (*R*)-(−)-**1** and (*S*)-(+)-**1** was almost the same as that of (±)-**1** hemifumarate and about twice that of cisapride.

In conclusion, the enantiomers of (*R*)-(+)-**6** and (*S*)-(−)-**6** were prepared in approximately recoverable yield with high optical purity by large-scale HPLC separation of the racemate (±)-**6**. The enantiomers (*R*)-(−)-**1** and (*S*)-(+)-**1** were then synthesized from (*R*)-(+)-**6** and (*S*)-(−)-**6**, respectively. The serotonin 5-HT_4 receptor agonistic activity of (*S*)-(−)-**1** hemifumarate was only twice that of the other enantiomer, (*R*)-(+)-**1** hemifumarate. Therefore, considering the toxic data for both,[8] (*S*)-(−)-**1** hemifumarate (SK-951) was selected as a promising candidate as a selective gastroprokinetic agent.

Experimental

Chemistry All melting points were determined on a Yanagimoto micromelting point apparatus and are uncorrected. IR spectra were recorded on a Perkin Elmer 1600 spectrometer. The mass spectra were obtained on a JEOL JMS-SX 120A spectrometer. ^{1}H-(270 MHz) NMR spectra were recorded on a JEOL JNM-GSX 270, respectively, in $CDCl_3$ or DMSO-d_6. Chemical shifts are expressed as δ values (ppm) with tetramethylsilane as an internal standard, and coupling constants (*J* values) are given in hertz (Hz). $[\alpha]_D$ were recorded on a Horiba SEPA-300. Analytical HPLC were performed with Waters 625LC and 486 instruments [column, Chiralcel OD or OJ (Daicel Chemical Industries, Ltd.), 250 mm × 4.6 mm i.d.; eluent, solvent A: *n*-hexane : ethanol : diethylamine = 80 : 20 : 0.1; solvent B: *n*-hexane : ethanol = 80 : 20; flow rate, 1.0 ml/min; column temperature, 25 °C; detection, 227 nm].

(±)-4-Acetylamino-2,3-dihydro-2-methylbenzo[*b*]furan-7-carboxylic Acid [(±)-5] A slurry of (±)-methyl 4-acetylamino-2,3-dihydro-2-methylbenzo[*b*]furan-7-carboxylate [(±)-**4**][2] (61.0 g, 245 mmol) in 1 N NaOH was stirred for 30 min at 80—90 °C, cooled, and filtered. The filtrate was acidified to pH 4 with 6 N HCl, and the resultant precipitate was collected by filtration to give 51.3 g (89.1%) of (±)-**5** as prisms, mp 238 °C (dec.). ^{1}H-NMR ($CDCl_3$) δ: 1.59 (3H, d, *J* = 6.8 Hz, CH_3), 2.22 (3H, s, CH_3CO), 2.84 (1H, dd, *J* = 15.1, 7.4 Hz, CH_2), 3.24 (1H, dd, *J* = 15.1, 9.3 Hz, CH_2), 5.2—5.3 (1H, m, CH), 6.97 (1H, br s, NH), 7.44 (1H, br s, C5-H), 7.85 (1H, d, *J* = 8.8 Hz, C6-H). IR (KBr) cm^{-1}: (C=O). High-resolution MS *m/z*: Calcd for $C_{12}H_{13}NO_4$: 235.0844. Found: 235.0858.

(±)-Benzyl 4-Acetylamino-2,3-dihydro-2-methylbenzo[*b*]furan-7-carboxylate [(±)-6] To a slurry of (±)-4-acetylamino-2,3-dihydro-2-methylbenzo[*b*]furan-7-carboxylic acid [(±)-**5**] (61.0 g, 245 mmol) in dry THF (400 ml), 1,1-carbonyldiimidazole (CDI) (71.2 g, 1.32 mol)) was added and stirred for 1 h at 20 °C. Benzyl alcohol (34.5 g, 289 mmol) was added to the solution, and the whole was stirred for 1 h at 20 °C and refluxed for 20 h. The reaction mixture was concentrated *in vacuo*, dissolved in chloroform, washed with 0.1 N HCl, saturated $NaHCO_3$ and then water, and thereafter, the solvent was concentrated *in vacuo*. The residue was refined by silica gel column chromatography (AcOEt : hexane = 3 : 1) to give 30.1 g (54.4%) of (±)-**6** as a powder, mp 104—106 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.54 (3H, d, *J* = 6.3 Hz, CH_3), 2.20 (3H, s, CH_3CO), 2.73 (1H, dd, *J* = 15.1, 7.3 Hz, CH_2), 3.24 (1H, dd, *J* = 15.1, 8.8 Hz, CH_2), 5.1—5.2 (1H, m, CH), 5.21, 5.47 (2H, each d, *J* = 12.7 Hz, CH_2-Ph), 6.97 (1H, br s, NH), 7.3—7.5 (5H, m, phenyl), 7.47 (1H, br s, C5-H), 7.77 (1H, d, *J* = 8.8 Hz, C6-H). IR (KBr) cm^{-1}: 3265 (NH), 1724, 1712 (C=O). High-resolution MS *m/z*: Calcd for $C_{19}H_{19}NO_4$: 325.1314. Found: 325.1302.

(*S*)-(−)- and (*R*)-(+)-Benzyl 4-Acetylamino-2,3-dihydro-2-methylbenzo[*b*]furan-7-carboxylate [(*S*)-(−)-6 and (*R*)-(+)-6] Compound (±)-**6** (30.0 g) was resolved by the large-scale HPLC separation. Compound (*S*)-(−)-**6** (13.6 g) was obtained from the first peak as prisms, mp 107—108 °C. $[\alpha]_D$ (28 °C): −10.6° (*c* = 4.03, CH_3OH). ^{1}H-NMR ($CDCl_3$) δ: 1.52 (3H, d, *J* = 5.9 Hz, CH_3), 2.19 (3H, s, CH_3CO), 2.72 (1H, dd, *J* = 15.1, 7.3 Hz, CH_2), 3.23 (1H, dd, *J* = 15.1, 9.2 Hz, CH_2), 5.0—5.2 (1H, m, CH), 5.30, 5.37 (2H, each d, *J* = 12.7 Hz, CH_2-Ph), 7.05 (1H, br s, NH), 7.2—7.5 (6H, m, phenyl, NH), 7.77 (1H, d, *J* = 8.8 Hz, C6-H). HPLC t_R: 8.70 min (Chiralcel OJ, solvent B), purity: 100%. IR (KBr) cm^{-1}: 3265 (NH), 1724, 1712 (C=O). High-resolution MS *m/z*: Calcd for $C_{19}H_{19}NO_4$: 325.1314. Found: 325.1330. (*R*)-(+)-**6** (12.7 g) was obtained from the second peak as prisms, mp 106—107 °C. $[\alpha]_D$ (28 °C): +10.6° (*c* = 4.02, CH_3OH). ^{1}H-NMR ($CDCl_3$) δ: 1.52 (3H, d, *J* = 5.9 Hz, CH_3), 2.19 (3H, s, CH_3CO), 2.72 (1H, dd, *J* = 15.1, 7.3 Hz, CH_2), 3.23 (1H, dd, *J* = 15.1, 9.2 Hz, CH_2), 5.0—5.2 (1H, m, CH), 5.30, 5.37 (2H, each d, *J* = 12.7 Hz, CH_2-Ph), 7.05 (1H, br s, NH), 7.2—7.5 (6H, m, phenyl, C5-H), 7.77 (1H, d, *J* = 8.8 Hz, C6-H). HPLC t_R: 13.1 min (Chiralcel OJ, solvent B), purity: 100%. IR (KBr) cm^{-1}: 3265 (NH), 1724, 1712 (C=O). High-resolution MS *m/z*: Calcd for $C_{19}H_{19}NO_4$: 325.1314. Found: 325.1318.

Preparation of the Enantiomers [(*S*)-(−)-1 Hemifumarate and (*R*)-(+)-1 Hemifumarate] The enantiomers [(*S*)-(−)-**1** hemifumarate and (*R*)-(+)-**1** hemifumarate] were prepared in a similar procedure as employed in the synthesis of (±)-**1** hemifumarate[2] from (*S*)-(−)-**6** and (*R*)-(+)-**6**, respectively.

(*S*)-(−)-Benzyl 4-Acetylamino-5-chloro-2,3-dihydro-2-methylbenzo-[*b*]furan-7-carboxylate [(*S*)-(−)-**7**]: mp 171—172 °C. $[\alpha]_D$ (28 °C): −21.6° (*c* = 4.03, $CHCl_3$). ^{1}H-NMR ($CDCl_3$) δ: 1.53 (3H, d, *J* = 5.9 Hz, CH_3), 2.20 (3H, s, CH_3CO), 2.85 (1H, dd, *J* = 16.6, 7.8 Hz, CH_2), 3.31 (1H, dd, *J* = 16.6, 8.8 Hz, CH_2), 5.0—5.2 (1H, m, CH), 5.31, 5.37 (2H, each d, *J* = 12.7 Hz, CH_2-Ph), 7.05 (1H, br s, NH), 7.2—7.5 (6H, m, phenyl, C5-H), 7.79 (1H, s, C6-H). IR (KBr) cm^{-1}: 3255 (NH), 1696, 1667 (C=O). High-resolution MS *m/z*: Calcd for $C_{19}H_{18}ClNO_4$: 359.0924. Found: 359.0902.

(*R*)-(+)-Benzyl 4-Acetylamino-5-chloro-2,3-dihydro-2-methylbenzo-[*b*]furan-7-carboxylate [(*R*)-(+)-**7**]: mp 171—172 °C. $[\alpha]_D$ (28 °C): +21.5° (*c* = 4.03, $CHCl_3$). ^{1}H-NMR ($CDCl_3$) δ: 1.53 (3H, d, *J* = 5.9 Hz, CH_3), 2.20 (3H, s, CH_3CO), 2.85 (1H, dd, *J* = 16.6, 7.8 Hz, CH_2), 3.31 (1H, dd, *J* = 16.6, 8.8 Hz, CH_2), 5.0—5.2 (1H, m, CH), 5.31, 5.37 (2H, each d, *J* = 12.7 Hz, CH_2-Ph), 7.05 (1H, br s, NH), 7.2—7.5 (6H, m, phenyl, C5-H), 7.79 (1H, s, C6-H). IR (KBr) cm^{-1}: 3255 (NH), 1696, 1667 (C=O). High-resolution MS *m/z*: Calcd for $C_{19}H_{18}ClNO_4$: 359.0924. Found: 359.0911.

(*S*)-(−)-4-Amino-5-chloro-2,3-dihydro-2-methylbenzo[*b*]furan-7-carboxylic Acid [(*S*)-(−)-**8**]: mp 176—177 °C. $[\alpha]_D$ (24 °C): −10.3° (*c* = 5.00, CH_3OH). ^{1}H-NMR ($CDCl_3$) δ: 1.58 (3H, d, *J* = 6.3 Hz, CH_3), 2.68 (1H, dd, *J* = 7.3, 14.6 Hz, C3-H), 3.20 (1H, dd, *J* = 9.8, 14.6 Hz, C3-H), 4.43 (2H, br s, NH_2), 5.1—5.3 (1H, m, C2-H), 7.78 (1H, s, C6-H). IR (KBr) cm^{-1}: 3358 (NH), 1714, 1626 (C=O). High-resolution MS *m/z*: Calcd for $C_{10}H_{10}ClNO_3$: 227.0349. Found: 227.0352.

(*R*)-(+)-4-Amino-5-chloro-2,3-dihydro-2-methylbenzo[*b*]furan-7-carboxylic Acid [(*R*)-(+)-**8**]: mp 176—177 °C. $[\alpha]_D$ (24 °C): +11.1° (*c*=5.00, CH_3OH). ^{1}H-NMR ($CDCl_3$) δ: 1.57 (3H, d, *J*=6.3 Hz, CH_3), 2.68 (1H, dd, *J*=7.3, 14.6 Hz, C3-H), 3.20 (1H, dd, *J*=8.3, 14.6 Hz, C3-H), 4.43 (2H, br s, NH_2), 5.1—5.3 (1H, m, C2-H), 7.78 (1H, s, C6-H). IR (KBr) cm^{-1}: 3358 (NH), 1714, 1626 (C=O). High-resolution MS *m/z*: Calcd for $C_{10}H_{10}ClNO_3$: 227.0349. Found: 227.0333.

(*S*)-(−)-4-Amino-*N*-[2-(1-azabicyclo[3.3.0]octan-5-yl)ethyl]-5-chloro-2,3-dihydro-2-methylbenzo[*b*]furan-7-carboxamide Hemifumarate [(*S*)-(−)-**1** Hemifumarate, SK-951] (*S*)-(+)-**1**: mp 142—143 °C. $[\alpha]_D$ (20 °C): +0.77° (*c*=5.00, CH_3OH). (*S*)-(−)-**1** hemifumarate: mp 236 °C (dec.). $[\alpha]_D$ (20 °C): −3.2° (*c*=5.00, CH_3OH : H_2O=1 : 1). HPLC t_R: 7.34 min (Chiralcel OD, solvent A), purity: 100%. *Anal.* Calcd for $C_{19}H_{26}ClN_3O_2 \cdot 1/2C_4H_4O_4$: C, 59.78; H, 6.69; N, 9.96. Found: C, 59.50; H, 6.72; N, 9.86.

(*R*)-(+)-4-Amino-*N*-[2-(1-azabicyclo[3.3.0]octan-5-yl)ethyl]-5-chloro-2,3-dihydro-2-methylbenzo[*b*]furan-7-carboxamide Hemifumarate [(*R*)-(+)-**1** Hemifumarate] (*R*)-(−)-**1**: mp 142—143 °C. $[\alpha]_D$ (20 °C): −0.72° (*c*=5.00, CH_3OH). (*R*)-(+)-**1** hemifumarate: mp 238 °C (dec. EtOH). $[\alpha]_D$ (20 °C): +3.5° (*c*=5.00, CH_3OH : H_2O=1 : 1). HPLC t_R: 9.43 min (Chiralcel OD, solvent A), purity: 100%. *Anal.* Calcd for $C_{19}H_{26}ClN_3O_2 \cdot 1/2C_4H_4O_4$: C, 59.78; H, 6.69; N, 9.96. Found: C, 59.53; H, 6.71; N, 9.88.

(*S*)-(−)-Benzyl 4-Acetylamino-5-bromo-2,3-dihydro-2-methylbenzo-[*b*]furan-7-carboxylate [(*S*)-(−)-10] A solution of (*S*)-(−)-**6** (1.00 g, 3.07 mmol) and *N*-bromosuccinimide (0.602 g, 3.38 mmol) in *N*,*N*-dimethylformamide (7.0 ml) was stirred for 6 h at 20—25 °C. The reaction mixture was poured into ice-water (200 ml). The resultant precipitate was collected by filtration to give 1.22 g (98.2%) of (*S*)-(−)-**10** as a powder, mp 170—172 °C. $[\alpha]_D$ (28 °C): −19.3° (*c*=5.00, $CHCl_3$). ^{1}H-NMR ($CDCl_3$) δ: 1.52 (3H, d, *J*=5.9 Hz, CH_3), 2.23 (3H, s, CH_3CO), 2.86 (1H, dd, *J*=16.6, 7.8 Hz, CH_2), 3.31 (1H, dd, *J*=16.6, 8.8 Hz, CH_2), 5.0—5.2 (1H, m, CH), 5.31, 5.37 (2H, each d, *J*=12.7 Hz, CH_2-Ph), 7.23 (1H, br s, NH), 7.2—7.5 (6H, m, phenyl, C5-H), 7.95 (1H, s, C6-H). IR (KBr) cm^{-1}: 3262 (NH), 1698, 1671 (C=O). High-resolution MS *m/z*: Calcd for $C_{19}H_{18}BrNO_4$: 403.0419. Found: 403.0402.

(*S*)-(−)-4-Amino-5-bromo-2,3-dihydro-2-methylbenzo[*b*]furan-7-carboxylic Acid [(*S*)-(−)-11] Compound (*S*)-(−)-**10** (800 mg, 1.98 mmol) was similarly alkali hydrolyzed to give 426 mg (79.1%) of (*S*)-(−)-**11** as a powder, mp 190—191 °C. $[\alpha]_D$ (24 °C): −9.20° (*c*=5.00, CH_3OH). ^{1}H-NMR ($CDCl_3$) δ: 1.57 (3H, d, *J*=6.3 Hz, CH_3), 2.69 (1H, dd, *J*=7.3, 14.6 Hz, C3-H), 3.21 (1H, dd, *J*=9.3, 14.6 Hz, C3-H), 4.46 (2H, br s, NH_2), 5.1—5.3 (1H, m, C2-H), 7.94 (1H, s, C6-H). IR (KBr) cm^{-1}: 3369 (NH), 1676, 1620 (C=O). High-resolution MS *m/z*: Calcd for $C_{10}H_{10}BrNO_3$: 270.9844. Found: 270.9853.

X-Ray Crystal Analysis of (*S*)-1 and (*S*)-11 Crystals were grown from toluene as colorless prisms. Crystal data are summarized in Table 1. The diffraction intensities were collected at 22 °C on a Rigaku AFC-5 diffractometer using graphite-monochromated Cu*K*α radiation. The structure was solved by a direct method using MULTAN88[9] and refined using KPPXRAY ORFLS[10] with anisotropic thermal parameters for non-hydrogen atoms. The absolute structure was confirmed by comparison of the observed intensity ratios of the Bijvoet pairs with the calculated values.

Serotonin 5-HT$_4$ Receptor Agonistic Activity The agonistic activity of the 5-HT$_4$ receptor was tested using the methodology of Baxter *et al.*[7] Briefly, the tunica muscularis mucosae (TMM) preparation was obtained from rat esophagus, and the responses to the cumulative addition of the compounds were expressed as percentage relaxation of the carbachol-induced tone. The potency of agonistic activity was estimated by calculating the concentration of 50% relaxation (EC_{50}).

Radioligand Binding Assay The test compounds at concentrations 1, 10, and 100 μM were tested in binding assays using rat brain synaptic membranes in competition with the following ligands at their respective binding sites: 5-HT$_1$,[11] [^{3}H]5-HT in the rat forebrain; 5-HT$_2$,[12] [^{3}H]ketanserin in the rat frontal cortex; 5-HT$_3$,[13] [^{3}H]GR65630 in the guinea pig ileum; dopamine D$_1$,[14] [^{3}H]SCH23390 in the rat striatum; dopamine D$_2$,[12] [^{3}H]spiperon in the rat striatum; muscarine M$_1$,[15] [^{3}H]pirenzapine in the rat frontal cortex; muscarine M$_2$,[14] [^{3}H]quinuclidinyl benzylate (QNB) in the rat frontal cortex. Each assay was started by addition of tissue preparations and terminated by rapid filtration through Whatman GF/B glass-fiber filters under reduced pressure. The filters were transferred to scintillation vials, scintillator ACS II was added, the radioactivity in the filters was then counted. The IC_{50} values of the test compounds (the concentrations causing 50% inhibition of ^{3}H-labeled ligand specific binding) were determined by probit analysis.

Acknowledgments The authors thank Dr. Takahiko Mitani for his suport and Mr. Hitoshi Hamajima of the Drug Discovery Research Department for elemental analyses and mass spectral measurements.

References and Notes

1) Present address: *Licensing Department, Sanwa Kagaku Kenkyusyo Co., Ltd., 35, Higashisotobori-cho, Higashi-ku, Nagoya, Aichi, 461–8631, Japan.*
2) Kakigami T., Usui T., Tsukamoto K., Kataoka T., *Chem. Pharm. Bull.*, **46**, 42—52 (1998).
3) Gullikson G. W., Virina M. A., Loeffler R. F., Yang D. C., Goldstin B., Flynn D. L., Moormann A. E., *Drug Dev. Res.*, **26**, 405—417 (1992).
4) Flynn D. L., Zabrowski D. L., Becker D. P., Nosal R., Villamil C. I., Gullikson G. W., Moummi C., Yang D. C., *J. Med. Chem.*, **35**, 1486—1489 (1992).
5) Oguni K., Oda H., Ichida A., *J. Chromatogr.*, **A 694**, 91—100 (1995).
6) Suzuki T., Oka M., Maeda K., Furusawa K., Mitani T., Kataoka T., *Chem. Pharm. Bull.*, **45**, 1218—1220 (1997).
7) Baxter G. S., Craig D. A., Clarke D. E., *Naunyn-Schmiedeberg's Arch Pharmacol.*, **343**, 439—446 (1991).
8) Unpublished.
9) Main P., Hull S. E., Lessinger L., Germain G., Declercq J. P., Woolfson M. M., (1988). MULTAN88. A System of Computer Programs for the Automatic Solution of Crystal Structure from X-ray Diffraction Data. Universities of York, England, and Louvain, Belgium.
10) Taga T., Masuda K., Higashi T., Iizuka H., (1991). KPPXRAY. Kyoto Program Package for X-ray Crystal Structure Analysis. Kyoto University, Japan.
11) Nowak H. P., Mahle C. D., Yocca F. D., *Br. J. Pharmacol.*, **109**, 1206—1211 (1993).
12) Kawanami T., Morinobu S., Totsuka S., Endoh M., *Eur. J. Pharmacol.*, **216**, 385—392 (1992).
13) Pinkus L. M., Sarbin N. S., Barefoot D. S., Gordon J. C., *Eur. J. Pharmacol.*, **168**, 355—362 (1989).
14) May T., *Eur. J. Pharmacol.*, **215**, 313—316 (1992).
15) Giraldo E., Hammer R., Ladinsky H., *Life Science*, **40**, 833—840 (1985).