

Synthesis of the Selective 5-Hydroxytryptamine 4 (5-HT₄) Receptor Agonist (+)-(*S*)-2-Chloro-5-methoxy-4-[5-(2-piperidylmethyl)-1,2,4-oxadiazol-3-yl]aniline

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In a search for novel 5-hydroxytryptamine 4 (5-HT₄) agonists focusing on the linker group of benzamide derivatives, 2-chloro-5-methoxy-4-[5-(2-piperidylmethyl)-1,2,4-oxadiazol-3-yl]aniline (**2**) was prepared and its optical isomers were separated. The *S* isomer **2**(*S*) showed high affinity for the human 5-HT₄ receptor without affinity for the human 5-HT₃ receptor, and potent 5-HT₄ agonistic activity in longitudinal muscle myenteric plexus (LMMP) preparations of guinea pig ileum. The *R* isomer **2**(*R*) showed opposite selectivity. As a result of other receptor binding studies, **2**(*S*) (YM-53389) was shown to be a highly selective 5-HT₄ agonist.

Key words 5-hydroxytryptamine 4 agonist; 1,2,4-oxadiazole; *S* isomer

Serotonin (5-hydroxytryptamine, 5-HT) is widely distributed in the central nervous, gastrointestinal, and cardiovascular systems as a neurotransmitter, neuromodulator and hormone.^{1–6} The 5-hydroxytryptamine 4 (5-HT₄) receptor is distributed from the esophagus to the colon in the gastrointestinal system, and is especially closely related with regulation of gastrointestinal motility.⁷ For these reasons, 5-HT₄ agonists are expected to be effective for treatment of gastrointestinal dysfunctions such as diarrhea, constipation, gastroparesis, ileus, reflux esophagitis, and pseudo-obstructions.

Most of the reported 5-HT₄ agonists, except for 5-HT derivatives,^{8,9} have a benzamide (or other arylcarboxamide) skeleton,⁷ but many possess poor selectivity for the 5-HT₄ receptor due to 5-HT₃ antagonistic activity.

In order to find new 5-HT₄ agonists, we focused on the linker group of the benzamide and synthesized 3-(4-amino-5-chloro-2-methoxyphenyl)-1,2,4-oxadiazole derivatives, derived from the Merck 5-HT₃ antagonist **1** possessing an oxadiazole ring (Chart 1).¹⁰ Consequently, it was found that a 1,2,4-oxadiazole ring could function as a linker group instead of an amide bond for 5-HT₄ agonists as well as for 5-HT₃ antagonists. Among such derivatives, cyclic amine derivatives (quinuclidines, hexahydropyrolizines, piperidines, pyrrolidines) having a basic nitrogen atom at the γ -position from oxadiazole showed potent 5-HT₄ agonistic activity in the longitudinal muscle myenteric plexus (LMMP) preparation of guinea pig ileum, and especially 3-piperidylmethyl derivative (**2**) showed a unique profile. In this paper, we report the synthesis, potency, and receptor selectivity of (*S*)-2-chloro-5-methoxy-4-[5-(2-piperidylmethyl)-1,2,4-oxadiazol-3-yl]aniline [**2**(*S*)] as a 5-HT₄ agonist.

The oxadiazole derivatives were synthesized as follows

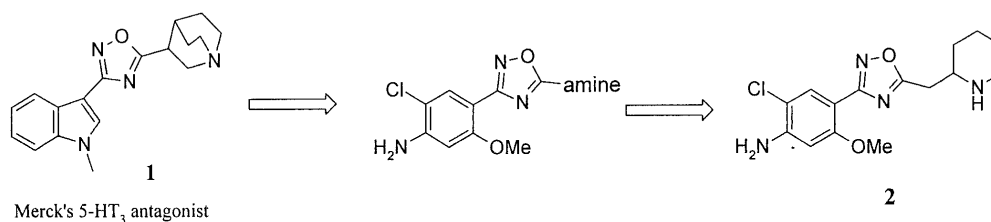
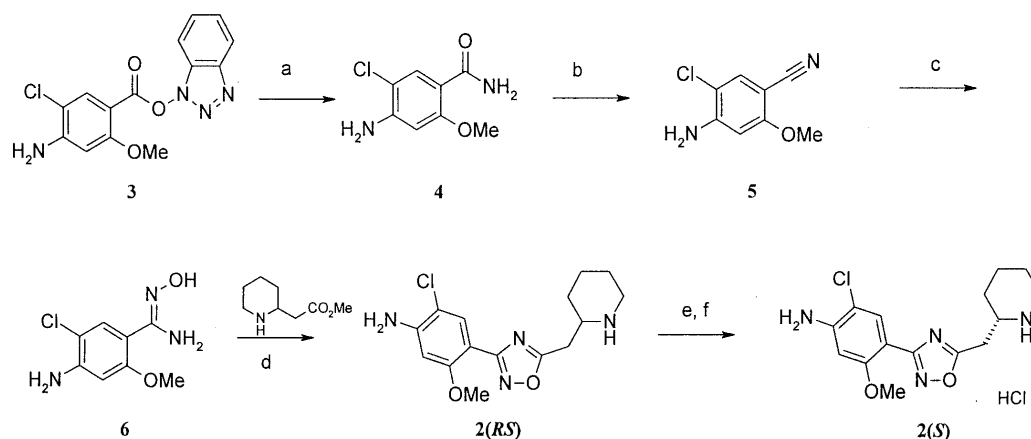


Chart 1

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a) aq.NH₃/DMF b) POCl₃ c) H₂NOH, K₂CO₃/EtOH d) NaH/DMF e) D-DITOTA/DMF-H₂O f) free, HCl

Chart 2

Table 1. Binding Affinities for 5-HT₄ and 5-HT₃ Receptors, and 5-HT₄ Receptor Agonism

Compound	5-HT ₄ receptors K _i (nM) ^{a)}		5-HT ₃ receptors K _i (nM) ^{a)}		5-HT ₄ agonism EC ₅₀ (μM) ^{b)}
	Guinea pig striatum	Human	Rat cortex	Human	
2(S)(YM-53389)	82	54	735	>10000	0.50
2(R)	303	NT	172	NT	NT
Cisapride	96 (33) ^{c)}	42	61 (139) ^{d)}	684	0.15
SC-53116	(21) ^{e)}	—	(51) ^{d)}	—	—

a) [³H]-GR113808 and [³H]-ramosetron were used as 5-HT₄ and 5-HT₃ receptor ligands, respectively. b) 5-HT₄ agonistic activities were measured using LMMP of guinea pig ileum. The results were expressed as EC₅₀ values, which were calculated by subtracting the twitch responses obtained under the 5-methoxytryptamine (5-MOT; 5-HT₄ agonist)-desensitized condition from these obtained with the tested compound alone. c) Pig caudate nucleus, [³H]-GR113808.¹⁸⁾ d) NG 108-15 hybrid cells, [³H]-DAU6215.¹⁸⁾

Table 2. Binding Profiles of 2(RS) for Other Receptors^{a)}

Binding site	Ligand	K _i (nM)	
		2(RS)	Cisapride
Adrenaline α ₁	[³ H]-Prazosin	>10000	79
Adrenaline α ₂	[³ H]-Rauwolscine	>10000	2700
Muscarine M ₁	[³ H]-Pirenzepine	>10000	5400
5-HT _{1A}	[³ H]-8-OH-DPAT ^{b)}	>10000	1600
5-HT ₂	[³ H]-Ketanserin	>10000	8.7
Dopamine D ₂	[³ H]-YM-09151-2	>10000	50
Sigma	[³ H]-DTG ^{b)}	540	110

a) Racemic 2(RS) and cisapride were inactive at the following receptors (K_i, >10000 nM): Adenosine A₁, A₂, adrenaline β₁, β₂, angiotensin II, bombesin, bradykinin B₂, CCK_A, CCK_B, dopamine D₁, histamine H₁, H₃, muscarine M₂, M₃, M₄, neurokinin NK₁, neuropeptide Y₂, opiate δ, κ, μ, and VIP. b) 8-HO-DPAT, 8-hydroxy-2-dipropyl-aminotetralin; DTG, 1,3-di(2-tolyl)guanidine.

ity in the LMMP preparation.

Next, the binding affinities for other receptors were measured to reveal the pharmacological profile of 2(RS) and cisapride (Table 2). All receptor binding studies were performed by standard procedures using standard assay conditions. As a result, 2(RS) showed only weak affinity for the sigma receptor, but no affinity to other 5-HT receptor subtypes and the receptors of other transmitters. These results indicate that YM-53389 is a highly selective 5-HT₄ receptor ligand. This compound is undergoing further evaluation due to the structural novelty, in comparison to benzamide YM-47813, described in the preceding report and to its good pharmacological properties.

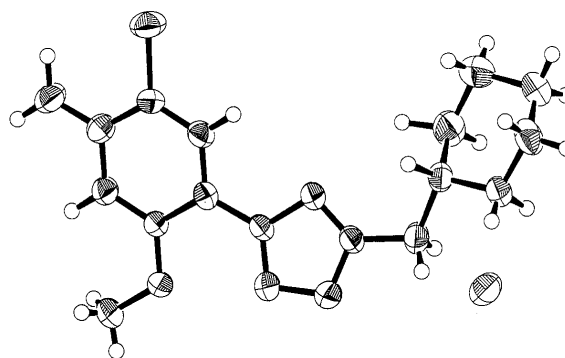


Fig. 1. X-Ray Structure of YM-53389

Experimental

All melting points were determined on a Yanaco MP-500D melting point apparatus and are uncorrected. ¹H-NMR spectra were measured with a JEOL FX90Q, a FX100, a FX270 or FX400 spectrometer; chemical shifts are reported in δ units using tetramethylsilane as internal standard and the following abbreviations are used: s=singlet, d=doublet, t=triplet, q=quartet, m=multiplet, dd=double doublet, dt=double triplet, br=broad. Mass spectra were recorded with a Hitachi M-80 electron impact (EI), JEOL JMS-DX300 (FAB) spectrometer or Hewlett Packard 5970 MSD (GC) spectrometer. Elemental analyses were performed with a Yanaco MT-5. All organic extracts were dried over anhydrous magnesium sulfate and concentrated with a rotary evaporator under reduced pressure.

4-Amino-5-chloro-2-methoxybenzamide (4) Twenty ml of 29% aqueous ammonia was added to a solution of benzotriazolyl 4-amino-5-chloro-2-methoxybenzoate (3) (6.36 g, 20 mmol) in DMF (300 ml) at room temperature and the resulting mixture was stirred for 30 min. The reaction mixture was concentrated and the residue was diluted with AcOEt and aqueous K₂CO₃ solution. The precipitates were collected by filtration and washed

with H₂O, EtOH and AcOEt to afford **4** as a beige solid (3.65 g, 91%). ¹H-NMR (DMSO-*d*₆) δ: 3.82 (3H, s), 5.91 (2H, brs), 6.47 (1H, s), 7.22 (1H, brs), 7.37 (1H, brs), 7.70 (1H, s). EI-MS *m/z*: 200, 202 (M⁺).

4-Amino-5-chloro-2-methoxybenzonitrile (5) A suspension of **4** (11.00 g, 55 mmol) in phosphorus oxychloride (50 ml) was stirred at 50 °C for 30 min. The reaction mixture was concentrated and the residue was diluted with H₂O and made alkaline by K₂CO₃. This mixture was extracted with AcOEt. The extract was washed with brine, dried, and concentrated. The residue was crystallized from hexane–AcOEt and **5** was obtained as a beige solid (7.46 g, 75%). ¹H-NMR (DMSO-*d*₆) δ: 3.81 (3H, s), 6.39 (2H, brs), 6.50 (1H, s), 7.53 (1H, s). EI-MS *m/z*: 182, 184 (M⁺).

4-Amino-5-chloro-N-hydroxy-2-methoxybenzamidine (6) A mixture of **5** (10.32 g, 54 mmol), hydroxylamine hydrochloride (7.93 g, 114 mmol), and anhydrous K₂CO₃ (15.73 g) in EtOH was refluxed for 20 h. The reaction mixture was diluted with H₂O and the precipitate was collected by filtration and washed with H₂O and EtOH to afford **6** as a beige solid (10.72 g, 75%). ¹H-NMR (DMSO-*d*₆) δ: 3.72 (3H, s), 5.48 (2H, brs), 6.30 (2H, brs), 6.48 (1H, s), 7.23 (1H, s), 9.23 (1H, brs). EI-MS *m/z*: 215, 217 (M⁺).

2-Chloro-5-methoxy-4-[5-(2-piperidylmethyl)-1,2,4-oxadiazol-3-yl]aniline (2) A solution of **6** (2.15 g, 10 mmol) in DMF (7 ml) was added dropwise into a suspension of methyl 2-(2-piperidyl)acetate (2.04 g, 13 mmol), and 60% sodium hydride (in oil) (0.50 g, 12.5 mmol) in DMF (15 ml) under water-cooling. The reaction mixture was stirred for 1 h at room temperature. The mixture was then quenched with ice and concentrated. The residue was diluted with water and extracted with AcOEt. The extract was washed with brine, dried, and concentrated. The residue was chromatographed on SiO₂ eluting with CHCl₃–MeOH (10 : 1) to afford **2** as a white solid (2.32 g, 72%). mp 101–103 °C (AcOEt–hexane). ¹H-NMR (CDCl₃) δ: 1.00–2.00 (6H, m), 2.60–3.20 (5H, m), 3.91 (3H, s), 4.36 (2H, brs), 6.38 (1H, s), 7.96 (1H, s). EI-MS *m/z*: 322, 324 (M⁺). *Anal.* Calcd for C₁₅H₁₉ClN₂O₂: C, 55.81; H, 5.93; Cl, 10.98; N, 17.36. Found: C, 55.53; H, 5.89; Cl, 11.14; N, 17.34.

(-)-(R)-2-Chloro-5-methoxy-4-[5-(2-piperidylmethyl)-1,2,4-oxadiazol-3-yl]aniline Monohydrochloride [2(R)] A suspension of racemic **2** (48.0 g, 149 mmol) and *l*-ditoluoyltartaric acid (57.5 g, 149 mmol) in DMF (750 ml) was warmed at 50 °C and dissolved. After removing the oil bath, H₂O (1000 ml) was added to the solution and the resulting mixture was gradually cooled to room temperature and allowed to stand overnight. Precipitate was collected and recrystallized from DMF–H₂O (1 : 1) (total: 3 recrystallizations). The *l*-ditoluoyltartaric acid salt of **2(R)** was added to a mixture of 1 N HCl (80 ml) and AcOEt (100 ml) and stirred for 2 h. The mixture was extracted with diluted HCl. The water layer was made alkaline with K₂CO₃ and extracted with CHCl₃–MeOH (10 : 1). The extract was washed with brine, dried, and concentrated. To the residue in EtOH (100 ml) was added 4 N HCl–AcOEt solution (15 ml) and AcOEt (50 ml), and the mixture allowed to stand overnight at room temperature. The precipitate was collected to afford the HCl salt of **2(R)** (16.53 g, 31%), mp 215–217 °C (EtOH–AcOEt). *Anal.* Calcd for C₁₅H₁₉ClN₂O₂·HCl: C, 50.15; H, 5.61; Cl, 19.74; N, 15.60. Found: C, 50.02; H, 5.60; Cl, 19.55; N, 15.69. [α]_D²⁰ = –14.8 (*c* = 1.00, MeOH).

(+)-(S)-2-Chloro-5-methoxy-4-[5-(2-piperidylmethyl)-1,2,4-oxadiazol-3-yl]aniline Monohydrochloride [2(S)] The filtrate from the first recrystallization was recovered and 22.0 g of crude free base **2(S)** (*S* : *R* = 88 : 12) was obtained. This crude **2(S)** was purified by optical resolution with *D*-ditoluoyltartaric acid and converted into the HCl salt of **2(S)** (18.9 g, 35% from **2**) according to the above procedure, mp 217–218 °C (EtOH–AcOEt). ¹H-NMR (DMSO-*d*₆) δ: 1.45–1.90 (6H, m), 2.93 (1H, dt, *J* = 3, 12 Hz), 3.25–3.40 (3H, m), 3.46 (1H, dd, *J* = 6, 16 Hz), 3.55–3.65 (1H, m), 6.03 (2H, s), 6.59 (1H, s), 7.74 (1H, s), 9.33 (2H, br). FAB-MS *m/z*: 323, 325 (M⁺ + 1). *Anal.* Calcd for C₁₅H₁₉ClN₂O₂·HCl: C, 50.15; H, 5.61; Cl, 19.74; N, 15.60. Found: C, 49.91; H, 5.58; Cl, 19.95; N, 15.51. [α]_D²⁰ = +14.9 (*c* = 1.00, MeOH).

X-Ray Crystallography of YM-53389 Crystals of YM-53389 were grown from AcOEt–EtOH as colorless prisms. Diffraction intensities were collected from a crystal of dimensions 0.52 × 0.38 × 0.15 mm on a Rigaku AFC7R four-circle diffractometer. Of the total of 2602 unique reflections (complete for 2θ < 120°), 1263 satisfied the criterion *F* > 3σ(*F*) and only these were used in the solution and refinement of the structure. Crystal data: C₁₅H₂₀Cl₂N₄O₂, M.W. = 359.25, monoclinic, space group *P*2₁/*c*, *a* = 15.949 (1) Å, *b* = 7.727(3) Å, *c* = 15.203(2) Å, β = 110.631(6)°, *V* = 1753.3(5) Å³, *Z* = 4, *D*_c = 1.36 g/cm³, *F*₀₀₀ = 752.00, CuK_α radiation, graphite-monochromated, λ = 1.54178 Å.

Structure Solution and Refinement: The structure was solved by a direct method using SAPI91, and the final refinement was done by the full-matrix least-squares method with anisotropic thermal parameters for all non-hydrogen atoms and fixed isotropic thermal parameters for all hydrogen atoms. The final *R* value was 0.047. The absolute configuration was determined to be *S* using the method described by Flack [*χ* = 0.01(1)].

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