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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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 in 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 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

Receptor selectivities of 2(S), 2(R), and cisapride were evaluated by the binding affinities using guinea pig 5-HT₄ receptor, rat 5-HT₄ receptor, and cloned human 5-HT₃, and 5-HT₂ receptors, and 5-HT₄ agonistic activities were measured by LMMP preparation (Table 1).¹⁵–¹⁷ Whereas Schiavi et al. previously reported the receptor selectivity of 5-HT₄ agonists,¹⁸ only cisapride and SC-53116 showed higher affinities for the 5-HT₄ receptor (pig caudate nucleus) than the 5-HT₃ receptor (NG 108-15 hybrid cells), and the 5-HT₃/5-HT₄ Kᵢ ratios were 4.2 and 2.5, respectively.¹⁸ In our study, the S isomer 2(S) (YM-53389) showed 9 times higher affinity for the 5-HT₄ receptor than the 5-HT₃ receptor in guinea pig and rat, whereas 2(R) showed the opposite selectivity. YM-53389 retained high affinity for the human 5-HT₄ receptor but had no affinity for the human 5-HT₃ receptor. The receptor selectivity of YM-53389 was vastly superior to that of cisapride in our binding study, and this compound showed a potent stimulatory effect for 5-HT₄ agonistic activ-

Chart 1
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.

**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-d₆) 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

4-Amino-5-chloro-2-methoxybenzonitrile (5) A suspension of 4 (11.00 g, 55 mmol) in phosphorous 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-d₆) 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-methoxynicotinamide (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-d₆) δ: 3.72 (3H, s), 5.48 (2H, brs), 6.30 (2H, brs), 6.48 (1H, s), 7.23 (1H, s). 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-piperidylacetate) (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%). A¹ 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 C₁₅H₂₁ClNO₂: C, 55.81; H, 5.93; Cl, 11.14; 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 1-ditoluoyl tartaric 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 1-ditoluoyltartric acid salt of 2[R] was added to a mixture of 1 N NaOH. The solution was adjusted to pH 7.5 with 6 N HCl, and precipitate was collected and recrystallized from DMF–H₂O (88:12) (16.53 g, 31%), mp 215—217 °C (EtOH–AcOEt).

References

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