Synthesis and Biological Activity of 4-Amino-5-chloro-2-ethoxy-3-hydroxybenzamides, Metabolites of a New Gastroprokinetic Agent, Mosapride

Shiro KATO,* Toshiya MORIE, and Naoyuki YOSHIDA

Discovery Research Laboratories I, Dainippon Pharmaceutical Co., Ltd., Enoki 33–94, Suita, Osaka 564, Japan.
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To confirm the proposed structures of the minor metabolites of a potential gastroprokinetic agent, mosapride, 4-amino-5-chloro-2-ethoxy-3-hydroxy-*N*-(2-morpholinylmethyl)benzamide (3) and the *N*-(5-oxo-2-morpholinyl)-methyl analogue 4 were prepared. As the common intermediate, 2-ethoxy-3-hydroxy-4-nitrobenzoic acid (15) was prepared *via* the regioselective ethylation of 2,3-dihydroxybenzaldehyde (10) and subsequent nitration of the resultant 2-ethoxy-3-hydroxybenzaldehyde (11). The key intermediate 15 was converted into the benzamides 3 and 4. After enzymatic treatment of the isolated metabolites, their structures were identified by comparison with the synthetic compounds. Serotonin-4 receptor binding affinity of these metabolites was found to be lower than that of mosapride.

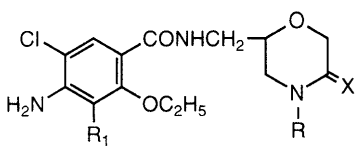
Key words mosapride; gastroprokinetic activity; serotonin-4 receptor agonist; 4-amino-5-chloro-2-ethoxy-*N*-{[4-(4-fluorobenzyl)-2-morpholinyl]methyl}benzamide

We have previously reported the synthesis of 4-amino-*N*-[(4-benzyl-2-morpholinyl)methyl]-5-chloro-2-methoxybenzamide and its derivatives as gastroprokinetic agents without dopamine D_2 receptor antagonistic activity.[1] These compounds have the clear therapeutic advantages of reducing the central nervous system depression and extrapyramidal symptoms which are observed with the standard gastroprokinetic agent metoclopramide and other dopamine D_2 receptor antagonists. Among the compounds prepared, 4-amino-5-chloro-2-ethoxy-*N*-{[4-(4-fluorobenzyl)-2-morpholinyl]methyl}benzamide (mosapride) was found to show the most potent gastroprokinetic activity and is presently under clinical study as a potential gastroprokinetic agent.[2] The gastroprokinetic action is accepted to be due to agonistic activity at a serotonin-4 receptor.[3] During the course of metabolic studies of mosapride, four metabolites **1**—**4** were isolated from rat urine (Chart 1). The metabolites **1** and **2** were proposed on the basis of MS and ^{1}H-NMR spectral analyses to be 4-amino-5-chloro-2-ethoxy-*N*-(2-morpholinylmethyl)benzamide and the corresponding 5-oxo analogue, respectively.[4] Their structures were confirmed by comparison with the synthetic compounds.[5] The structures of the metabolites **3** and **4** were proposed to be 4-amino-5-chloro-2-ethoxy-3-hydroxy-*N*-(2-morpholinylmethyl)benzamide and the corresponding 5-oxo analogue, respectively; ^{1}H-NMR spectral data obtained for **3** and **4** indicated the absence of the hydrogen at the 3-position of a 4-amino-5-chloro-2-ethoxybenzoyl moiety. Furthermore, the molecular weights of **3** and **4** suggested by MH^+ peaks of **3** at *m/z* 330 and of **4** at *m/z* 344 indicated that these molecules might be the 3-hydroxylated derivatives of **1** and **2**, respectively.[4] Aromatic hydroxylation at the 3-position of a 4-amino-5-chloro-2-methoxybenzamide had previously been reported as a metabolic route of the structurally analogous gastroprokinetic agent cisapride.[6] However, the corresponding metabolites have not yet been synthesized.

The present study was undertaken to confirm the structures of the metabolites **3** and **4** by chemical synthesis and to compare their serotonin-4 receptor binding affinity.

Synthesis of 4-(Acetylamino)-5-chloro-2-ethoxy-3-hydroxybenzoic Acid (22) First, we examined the synthesis of 5-chloro-2-ethoxy-3-hydroxy-4-nitrobenzoic acid (**9**), the precursor of 4-amino-5-chloro-2-ethoxy-3-hydroxybenzoic acid which is the common benzoyl moiety of **3** and **4**, *via* chlorination of 3-(benzoyloxy)-2-hydroxybenzoic acid[7] (**5**) and subsequent regioselective nitration (Chart 2). Chlorination at the 5-position of **5** was performed with *N*-chlorosuccinimide (NCS) in acetic acid (AcOH) at 80 °C to give **6** in a high yield. Treatment of **6** with diethyl sulfate furnished ethyl 3-(benzoyloxy)-5-chloro-2-ethoxybenzoate (**7**), which was hydrolyzed to afford the corresponding 3-hydroxybenzoic acid **8**. Attempted nitrations of **7** or **8** were unsuccessful.

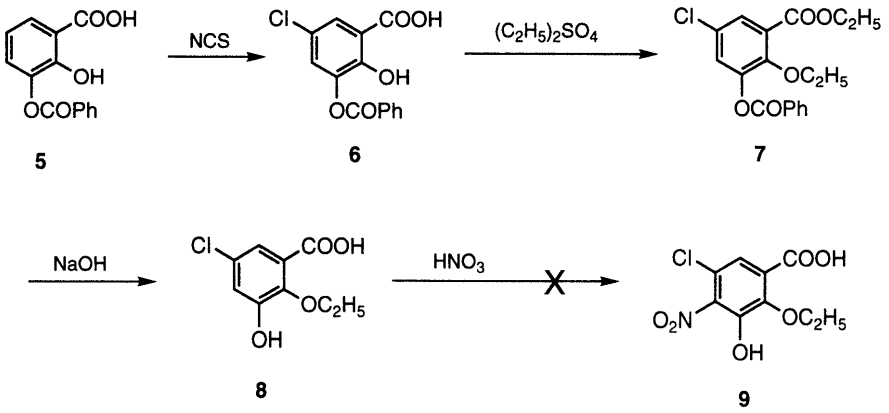
We next examined on a new method of nitration of 2-ethoxy-3-hydroxybenzaldehyde (**11**), followed by reduction of the 4-nitro group and subsequent chlorination (Chart 3). Kessar *et al.*[8] have reported that reaction of 2,3-dihydroxybenzaldehyde (**10**) with one molar equivalent each of NaH and methyl iodide in dimethyl sulfoxide (DMSO) regioselectively gave 3-hydroxy-2-methoxybenzaldehyde in 52% yield. We accordingly applied their method to the preparation of **11**. Treatment of **10** with one mol each of ethyl iodide (instead of methyl iodide) and NaH in DMSO at room temperature afforded 2-



R = 4-$FC_6H_4CH_2$, R_1 = H, X = H_2 (Mosapride)
1; R = R_1 = H, X = H_2
2; R = R_1 = H, X = O
3; R = H, R_1 = OH, X = H_2
4; R = H, R_1 = OH, X = O

Chart 1

* To whom correspondence should be addressed.



NCS; *N*-chlorosuccinimide

Chart 2

ethoxy-3-hydroxybenzaldehyde (**11**) in 55% yield. The structure was confirmed by comparison of the ^{1}H-NMR spectra ($CDCl_3$) of the available 3-ethoxysalicylaldehyde[9] and **11**. The signal of the 3-hydroxy proton of **11** appeared as a singlet at δ 5.85. On the other hand, the signal for the 2-hydroxy proton of 3-ethoxysalicylaldehyde showed a peak at δ 11.04, because the carbonyl group in the *ortho* position shifts the phenolic proton absorption downfield to the range of about δ 10—12 due to intramolecular hydrogen bonding. Aimi *et al.*[10] have reported that nitration of 3-hydroxy-2-methoxybenzaldehyde using concentrated HNO_3 in toluene at $-78\,^\circ$C to $-20\,^\circ$C gave 4-nitro- and 6-nitro-3-hydroxy-2-methoxybenzaldehydes with the same yields of 36%. Analogously, the reaction of **11** with concentrated HNO_3 in benzene– or toluene–AcOH at 5—10 °C produced the desired 4-nitro derivative **12** in 22% yield, along with the 6-nitro regioisomer **13** (25% yield) and 2,3-dihydroxy-6-nitrobenzaldehyde (**14**, 28% yield). The structures of compounds **12** and **13** were proposed on the basis of ^{1}H-NMR spectral studies in $CDCl_3$ solution; the signals of the 3-hydroxy protons of **12** and **13** are observed at δ 10.82 and δ 6.40, respectively. The lower chemical shift of **12** can be explained by the formation of an intramolecular hydrogen bond between the phenol hydrogen and the nitro oxygen. Furthermore, the fact that **12** was the desired 4-nitro derivative was confirmed through its conversion to the known 2,3-dihydroxy-4-nitrobenzoic acid[11] *via* the oxidation product **15**, which was obtained by treatment of **12** with $AgNO_3$ in an alkaline solution. The 6-position of the nitro group of **13** was determined by nuclear Overhauser effect (NOE) experiments; in the differential NOE spectra of **13**, irradiation at δ 11.40 (OH) enhanced the signal intensity of the aromatic 4-position (δ 7.13). However, NOEs were not observed at the aromatic protons on irradiation at δ 11.30 (CHO). The ^{1}H-NMR spectrum of **14** was identical with that of the compound described in the literature.[12]

Hydrogenation of the nitro group of **15**, obtained above, afforded the highly unstable 4-amino-2-ethoxy-3-hydroxybenzaldehyde (**16**), which could not be isolated. Thus, **15** was converted to the methyl ester **17**, and the 4-nitro group was reduced with palladium on carbon under hydrogen to produce the methyl 4-aminobenzoate **18** as

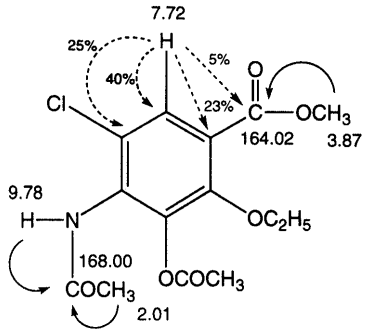


Fig. 1. Long-Range Correlations in the HMBC and Selective ^{13}C–^{1}H NOE Spectra of **21**

Chemical shifts (δ) are given in ppm. Solid and dotted arrows indicate HMBC and selective ^{13}C–^{1}H NOE, respectively.

pale brown crystals in 65% yield. Reaction of **18** with acetic anhydride (Ac_2O) gave the 4-(acetylamino)ester **19**, and hydrogenation of **17** in the presence of Ac_2O directly afforded **19** in a good yield. Treatment of **18** or **19** with Ac_2O in the presence of 4-dimethylaminopyridine (DMAP) in toluene at 110 °C gave methyl 3-acetoxy-4-(acetylamino)-2-ethoxybenzoate (**20**) in a good yield. Compound **20** was chlorinated with NCS to furnish the corresponding 5-chloro derivative **21**, whose structure was confirmed by using the bidimensional heteronuclear multiple bond correlation (HMBC) spectroscopic technique and by a selective ^{13}C–^{1}H NOE experiment (Fig. 1). Thus, the carbonyl carbon signal of the *N*-acetyl group at δ_C 168.00 and the ester carbonyl carbon signal at δ_C 164.02 showed a long-range correlation to the amino-hydrogen (δ_H 9.78) and the acetylmethyl (δ_H 2.01) signals and to the ester-methyl signal (δ_H 3.87), respectively, in the HMBC spectrum. In selective ^{13}C–^{1}H NOE, irradiation of the aromatic proton signal at δ_H 7.72 induced a 10% increment of the ester carbonyl signal; the hydrogen of **21** is therefore located at the 6-position. Reaction of **21** with aqueous NaOH solution gave 4-(acetylamino)-2-ethoxy-3-hydroxybenzoic acid (**22**) in a good yield. Acid hydrolysis of **22**, however, unexpectedly produced less polar materials instead of the target 4-amino-5-chloro-2-ethoxy-3-hydroxybenzoic acid.

Synthesis of the Debenzylated Metabolite 3 and the 5-Oxomorpholine Metabolite 4 We selected 4-acetyl-2-

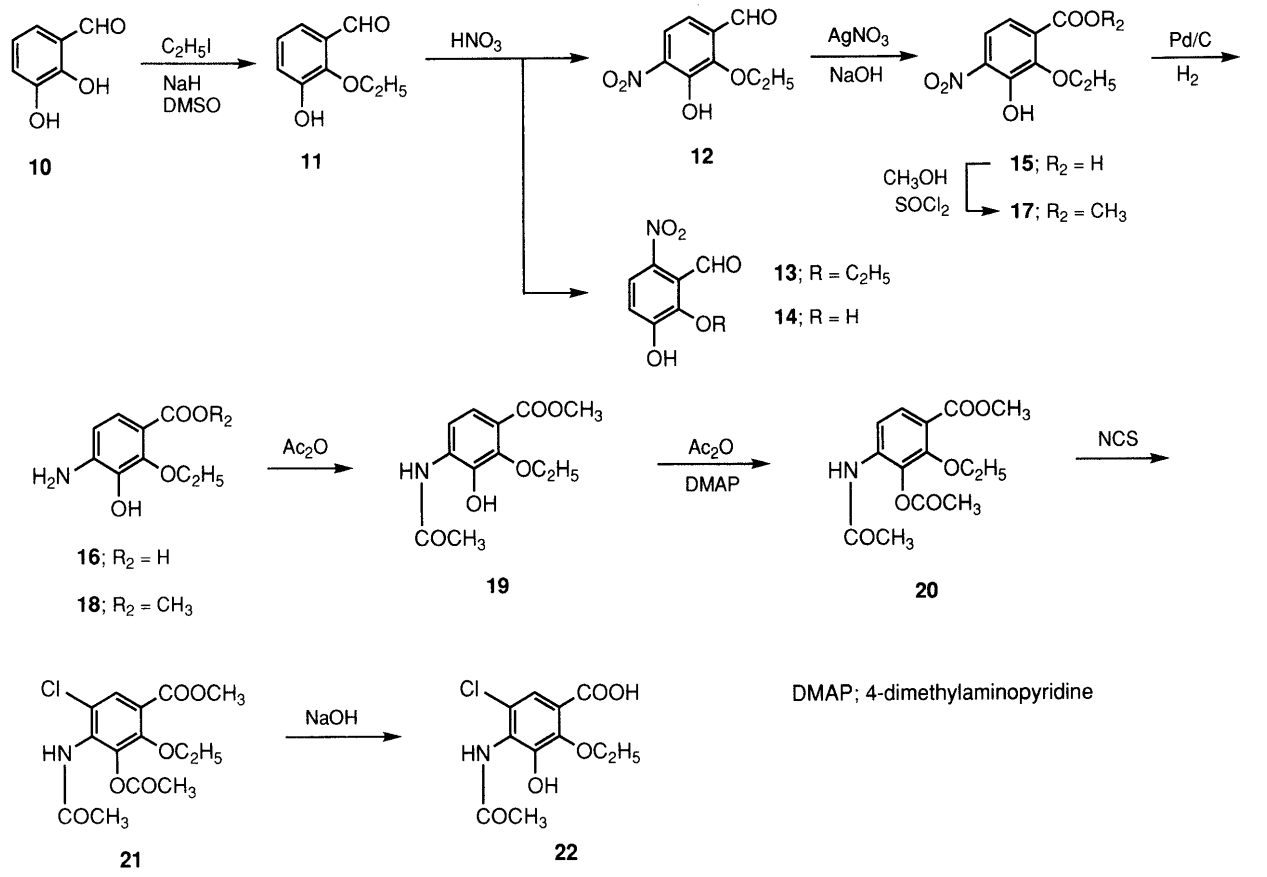


Chart 3

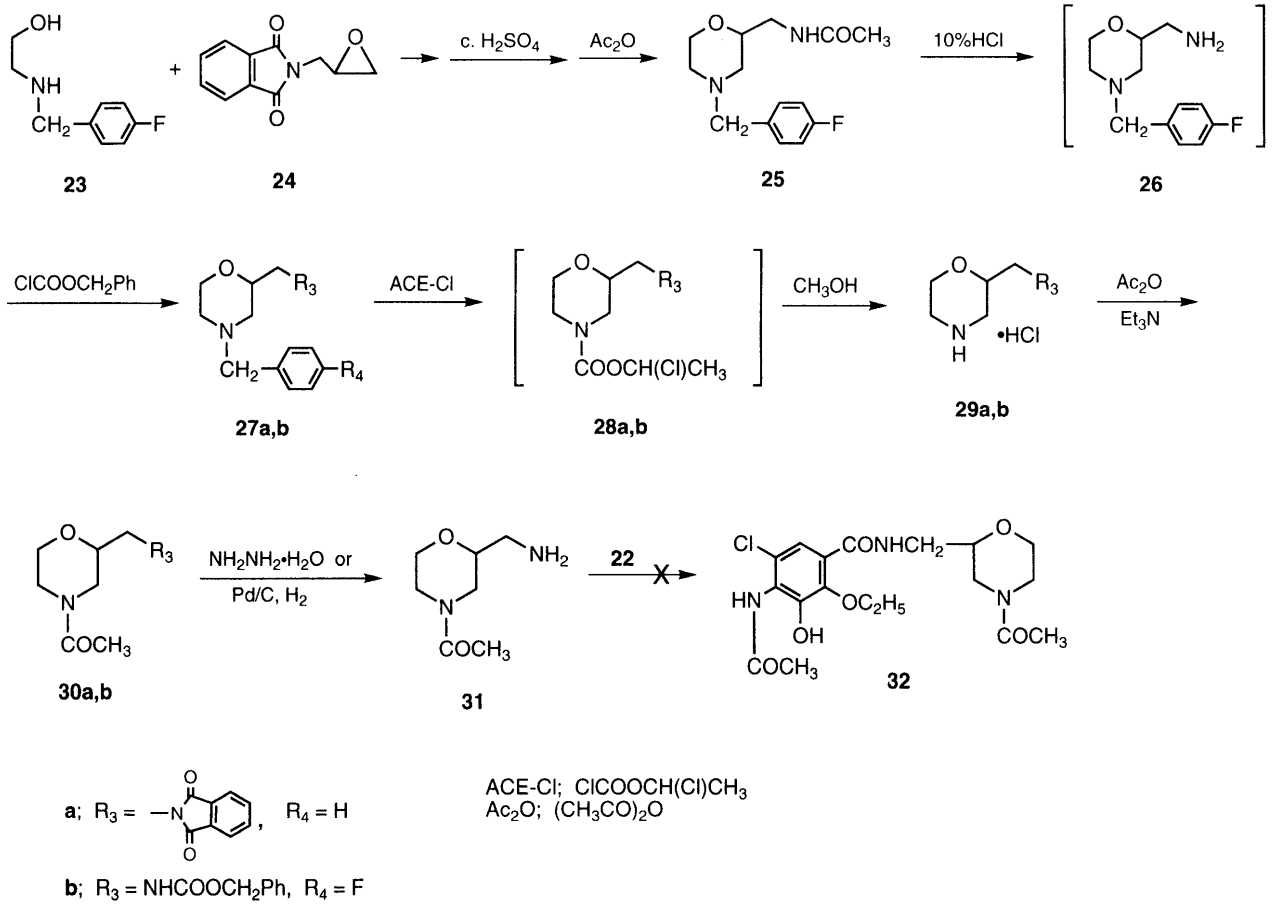


Chart 4

(aminomethyl)morpholine (**31**) as an intermediate amine for the preparation of compound **3**. Since hydrogenation of *N*-[(4-benzyl-2-morpholinyl)methyl]phthalimide[1] (**27a**) did not proceed, debenzylation employing α-chloroethyl chloroformate[13] (ACE-Cl) was examined. Reaction of **27a** with ACE-Cl in CH_2Cl_2 proceeded to generate the carbamate **28a**, which, without isolation, was treated with MeOH to afford the debenzylated phthalimido **29a** as its hydrochloride. This compound was acetylated with Ac_2O in the presence of Et_3N to give *N*-[(4-acetyl-2-morpholinyl)methyl]phthalimide (**30a**) in a good yield. Treatment of **30a** with hydrazine produced the intermediate amine **31**. Compound **31** was alternatively prepared as follows. 2-(Aminomethyl)-4-(4-fluorobenzyl)-morpholine (**26**), the amine moiety of mosapride, was prepared from 2-(4-fluorobenzylamino)ethanol (**23**) and *N*-(2,3-epoxypropyl)phthalimide (**24**) in a similar manner to that described in our previous paper.[14] Thus, reaction of **23** and **24** at 80 °C, followed by treatment of concentrated sulfuric acid, gave the crude **26**. This was acetylated with Ac_2O to afford the acetamide **25** in a moderate yield. After acid hydrolysis of **25**, the amino group of the resulting **26** was protected using benzyl chloroformate to provide 2-[(benzyloxycarbonylamino)-methyl]-4-(4-fluorobenzyl)morpholine (**27b**). Debenzylation of **27b** using ACE-Cl and MeOH, and subsequent acetylation with Ac_2O produced the 4-acetyl-2-[(benzyloxycarbonyl)amino]morpholine (**30b**) (84% yield from **27b** through **28b** and **29b**). Catalytic hydrogenation of **30b** afforded compound **31**. To obtain the benzamide **32**, various condensations of the benzoic acid **22** with the amine **31** *via* the corresponding acid chloride or anhydride and using coupling reagents were attempted, but without success for some unknown reason (Chart 4). On the other hand, the intermediate 2-ethoxy-3-hydroxy-4-nitrobenzoic acid (**15**) was treated with thionyl chloride, followed by reaction of the resultant corresponding benzoyl chloride **33** with the amine **31** in the presence of Et_3N, affording the benzamide **34** in a good yield. Hydrogenation of **34** in the presence of Ac_2O, and then chlorination of the resultant 4-(acetylamino)-2-ethoxy-3-hydroxybenzamide **35** using NCS gave only a messy mixture. After this unsuccessful trial to isolate **36**, we chose the *tert*-butoxycarbonyl group as an *N*-protecting group. The key compound **39** was obtained as follows. Reaction of **33** with **26** in the presence of Et_3N gave the benzamide **37**

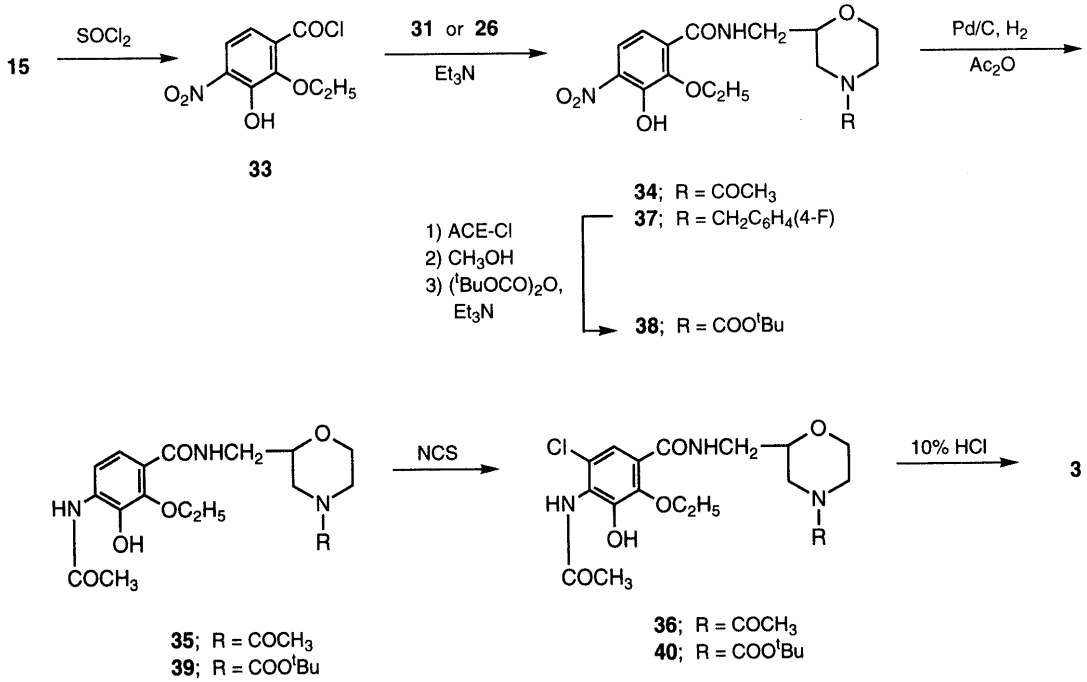


Chart 5

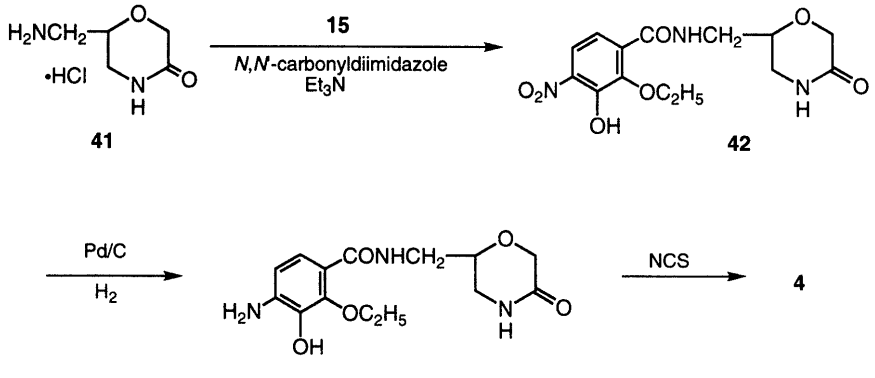


Chart 6

Table 1. Serotonin-4 Receptor Binding Assay

Compound	[^{3}H]GR113808 binding affinity[a] IC_{50} (nM)
3	641
4	>1000
Mosapride[b]	113

a) The experiment was performed as described under Experimental. *b*) Mosapride was used as the citric acid salt.

in an excellent yield. Debenzylation of **37** using ACE-Cl and MeOH gave 2-ethoxy-3-hydroxy-*N*-(2-morpholinylmethyl)-4-nitrobenzamide, which was protected with di-*tert*-butoxy carbonate to produce the *N*-*tert*-butoxycarbonylbenzamide **38**. Hydrogenation of **38** in the presence of Ac_2O gave the 4-(acetylamino)benzamide **39** in an excellent yield. Treatment of **39** with NCS afforded the 5-chlorobenzamide **40** in only 16% yield. Finally, compound **40** was transformed by acid hydrolysis into the desired benzamide **3** (Chart 5).

Compound **4** was prepared by a similar method to that described above (Chart 6). Condensation of **15** with 2-(aminomethyl)-5-oxomorpholine hydrochloride[5] (**41**) in the presence of Et_3N using *N*,*N*′-carbonyldiimidazole afforded the 5-oxomorpholinylbenzamide **42** in 67% yield. Catalytic hydrogenation of **42** and chlorination of the resultant 4-aminobenzamide **43** with NCS provided the target benzamide **4** in a poor yield. The ^{1}H-NMR spectra of these products **3** and **4** were very similar to those of the isolated metabolites, respectively. The HPLC analysis, however, the isolated metabolites showed a more polar character than the prepared compounds. Final characterization of the isolated metabolites as conjugates of **3** and **4** was performed by using negative ion MS and/or HPLC comparison with the synthetic compound after enzymatic treatment of the isolated metabolite. The details of characterization of the isolated metabolites will be reported elsewhere.

Biological Activity The gastroprokinetic activity of mosapride is believed to be correlated with agonistic activity at a serotonin-4 receptor subtype. The affinity for serotonin-4 receptor ([^{3}H]GR113808) of mosapride and the metabolites **3** and **4** was measured, and the results are shown in Table 1. The metabolites **3** and **4**, like the metabolites **1** and **2**,[5] were much less active than mosapride.

Experimental

Chemistry All melting points were determined on a Yanagimoto micromelting point apparatus without correction. IR spectra were recorded on a Hitachi 260-10 spectrometer and a Shimadzu FTIR-8200 PC spectrometer with KBr disks unless otherwise specified. Electron ionization, chemical ionization, and secondary ion mass spectra were obtained on a JEOL JMS D-300 or a Hitachi M-80B spectrometer. ^{1}H-NMR spectra were taken at 200 MHz with a Varian Gemini-200 spectrometer unless otherwise specified. ^{1}H-NMR spectra (300 MHz) and ^{13}C-NMR spectra were recorded on a Varian XL-300 spectrometer. Chemical shifts are expressed as δ (ppm) values from tetramethylsilane as an internal standard, and coupling constants (J) are given in hertz (Hz). Organic extracts were dried over anhydrous $MgSO_4$ or anhydrous Na_2SO_4, and the solvent was evaporated under reduced pressure. Merck Silica gel 60 (70—230 mesh) and Chromatorex NH-DM 1020 (100—200 mesh, Fuji Silysia Chemical Ltd.) were used for column chromatography.

3-(Benzoyloxy)-5-chloro-2-hydroxybenzoic Acid (6) A solution of 3-(benzoyloxy)-2-hydroxybenzoic acid[7] (**5**, 5.2 g, 20 mmol) and NCS (3.2 g, 24 mmol) in AcOH (100 ml) was heated at 80 °C for 3 h. The reaction mixture was concentrated to dryness. The residue was dissolved in $CHCl_3$ and washed successively with water (50 ml × 4) and brine. The solvent was evaporated to afford a solid, which was recrystallized from toluene to give 4.9 g (83%) of **6**, mp 154—157 °C. ^{1}H-NMR ($CDCl_3$) δ: 7.20 (1H, m, arom. H), 7.45 (1H, d, J=2.5, arom. 4-H), 7.53 (1H, m, arom. H), 7.67 (1H, m, arom. H), 7.80 (1H, d, J=2.5, arom. 6-H), 8.18—8.27 (2H, m, arom. H), 8.60 (1H, br s), 10.50 (1H, s, OH). MS m/z: 293 (MH^+), 275. IR ν cm^{-1}: 1735, 1662, 1220. *Anal.* Calcd for $C_{14}H_9ClO_5$: C, 57.45; H, 3.10; Cl, 12.11. Found: C, 57.74; H, 3.02; Cl, 11.83.

Ethyl 3-(Benzoyloxy)-5-chloro-2-ethoxybenzoate (7) A mixture of **6** (18.2 g, 62 mmol), anhydrous K_2CO_3 (27.6 g, 0.20 mol), diethyl sulfate (19.2 g, 0.12 mol), and methyl ethyl ketone (300 ml) was heated to reflux for 7 h and then cooled to room temperature. The reaction mixture was concentrated to dryness, and the residue was dissolved in water and $CHCl_3$. The organic layer was separated and washed with brine. The solvent was evaporated to leave an oil, which was chromatographed on silica gel with $CHCl_3$ to give 19.5 g (90%) of **7** as an oil. ^{1}H-NMR ($CDCl_3$) δ: 1.22 (3H, t, J=7.0, $CH_2C\underline{H}_3$), 1.38 (3H, t, J=7.0, $CH_2C\underline{H}_3$), 4.04 (2H, q, J=7.0, $C\underline{H}_2CH_3$), 4.37 (2H, q, J=7.0, $C\underline{H}_2CH_3$), 7.39 (1H, d, J=2.5, arom. 4-H), 7.45—7.65 (2H, m, arom. H), 7.71 (1H, d, J=2.5, arom. 6-H), 8.15—8.25 (3H, m, arom. H). MS m/z: 349 (MH^+), 303, 272, 227. IR (neat) ν cm^{-1}: 2975, 1735, 1720, 1240, 1050.

5-Chloro-2-ethoxy-3-hydroxybenzoic Acid (8) A mixture of **7** (16.9 g, 48 mmol), 2 N aqueous NaOH solution (61 ml), and EtOH (150 ml) was heated to reflux for 4 h and then cooled to 5 °C. The resulting precipitates were collected by filtration and dissolved in hot water. The aqueous solution was acidified with 35% aqueous HCl. The resulting precipitates were collected by filtration, washed with water, and dried to give 2.7 g (26%) of **8**, mp 202—203 °C. ^{1}H-NMR (DMSO-d_6) δ: 1.34 (3H, t, J=7.0, $CH_2C\underline{H}_3$), 4.07 (2H, q, J=7.0, $C\underline{H}_2CH_3$), 7.22 (1H, d, J=2.5, arom. 4-H), 7.30 (1H, d, J=2.5, arom. 6-H), 12.5 (2H, br s, OH, COOH). MS m/z: 217 (MH^+), 199. IR ν cm^{-1}: 3180, 1680, 1250. *Anal.* Calcd for $C_9H_9ClO_4$: C, 49.90; H, 4.19; Cl, 16.37. Found: C, 49.83; H, 4.11; Cl, 16.26.

2-Ethoxy-3-hydroxybenzaldehyde (11) Compound **11** was prepared by a modification of the method of Kessar *et al.*[8] A solution of commercial 97% 2,3-dihydroxybenzaldehyde (**10**, 325 g, 2.3 mol) in anhydrous DMSO (4.6 l) was treated portionwise with NaH (105 g of *ca.* 60% oil dispersion, 2.6 mol) at room temperature. The reaction mixture was heated at *ca.* 110 °C for 2 h. It was cooled to room temperature, and then ethyl iodide (392 g, 2.5 mol) was added. The mixture was stirred at room temperature for 18 h and poured into a mixture of ice-water (10 l) and 20% aqueous NaOH solution (200 ml). The solution was washed with $CHCl_3$ (1 l × 3), and the aqueous layer was acidified with 35% aqueous HCl and extracted with ethyl acetate (AcOEt, 2.5 l × 3). The extract was washed successively with water and brine, and evaporated to give a brown oil, which was crystallized from toluene to furnish 90 g (24%) of **11** as a pale brown powder. The mother liquid was chromatographed on silica gel with a gradient of toluene to toluene : AcOEt = 1 : 1 to give 119 g (31%) of **11** as pale yellow crystals. An analytical sample was obtained by recrystallization from toluene, mp 89—90 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.46 (3H, t, J=7.0, $CH_2C\underline{H}_3$), 4.17 (2H, q, J=7.0, $C\underline{H}_2CH_3$), 5.85 (1H, d, J=0.8, OH, disappeared with D_2O), 7.13 (1H, ddd, J=0.8, 7.5, 8.0, arom. 5-H), 7.22 (1H, dd, J=8.0, 2.0, arom. 4-H), 7.37 (1H, dd, J=7.5, 2.0, arom. 6-H), 10.27 (1H, s, CHO). ^{1}H-NMR (DMSO-d_6) δ: 9.85 (1H, s, OH). MS m/z: 166 (M^+), 137 ($M^+ - C_2H_5$). IR ν cm^{-1}: 3160, 1660, 1220. *Anal.* Calcd for $C_9H_{10}O_3$: C, 65.05; H, 6.07. Found: C, 65.01; H, 6.01.

2-Ethoxy-3-hydroxy-4-nitrobenzaldehyde (12) A 70% HNO_3 solution (d=1.42, 12.8 ml, 0.20 mol) was added dropwise to a solution of **11** (25.7 g, 0.15 mol) in a mixture of toluene (250 ml) and AcOH (50 ml) at 5—10 °C. The reaction mixture was stirred for *ca.* 5 min at the same temperature, and then poured into a stirred mixture of ice-water and AcOEt (250 ml). The organic layer was separated and washed successively with water (100 ml × 5) and brine. The solvent was evaporated to give a brown solid, which was chromatographed on silica gel with $CHCl_3$ to AcOEt to afford the desired 4-nitrobenzaldehyde **12** (7.3 g, 22%) as a pale brown solid, the 6-nitro regioisomer (**13**, 8.3 g, 25%) as a yellow solid, and 2,3-dihydroxy-6-nitrobenzaldehyde (**14**, 8.6 g, 30%) as a pale

brown solid in that order.

Compound **12**: mp 94—95 °C (toluene–*n*-hexane). ^{1}H-NMR (300 MHz, $CDCl_3$) δ: 1.48 (3H, t, J=7.1, $CH_2C\underline{H}_3$), 4.39 (2H, q, J=7.1, $C\underline{H}_2CH_3$), 7.40 (1H, d, J=9.0, arom. 6-H), 7.91 (1H, dd, J=9.0, 0.9, arom. 5-H), 10.48 (1H, d, J=0.9, CHO), 10.82 (1H, s, OH, disappeared with D_2O). ^{1}H-NMR (DMSO-d_6) δ: 10.94 (1H, s, OH). MS *m/z*: 211 (M^+), 183, 167, 136, 107. IR ν cm^{-1}: 3255, 1685, 1520, 1215. *Anal.* Calcd for $C_9H_9NO_5$: C, 51.19; H, 4.30; N, 6.63. Found: C, 51.28; H, 4.17; N, 6.63.

Compound **13**: mp 139—140 °C (EtOH–*n*-hexane). ^{1}H-NMR ($CDCl_3$) δ: 1.43 (3H, t, J=7.1, $CH_2C\underline{H}_3$), 4.14 (2H, q, J=7.1, $C\underline{H}_2CH_3$), 6.40 (1H, s, OH, disappeared with D_2O), 7.16 (1H, d, J=9.0, arom. 4-H), 7.98 (1H, d, J=9.0, arom. 5-H), 10.34 (1H, s, CHO). ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 1.32 (3H, t, J=7.1, $CH_2C\underline{H}_3$), 4.06 (2H, q, J=7.1, $C\underline{H}_2CH_3$), 7.13 (1H, d, J=9.0, arom. 4-H), 7.87 (1H, dd, J=9.0, 0.4, arom. 5-H), 10.30 (1H, d, J=0.3, CHO), 11.40 (1H, s, OH). MS *m/z*: 211 (M^+), 137, 79. IR ν cm^{-1}: 3120, 1675, 1515, 1300. *Anal.* Calcd for $C_9H_9NO_5$: C, 51.19; H, 4.30; N, 6.63. Found: C, 51.23; H, 4.19; N, 6.64.

Compound **14**: mp 136—138 °C (toluene–*n*-hexane). ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 7.05 (1H, d, J=8.8, arom. 4-H), 7.55 (1H, dd, J=8.8, 0.3, arom. 5-H), 10.28 (1H, d, J=0.3, CHO), 10.58 (1H, br s, 2-OH, disappeared with D_2O), 11.26 (1H, br s, 3-OH, disappeared with D_2O). [lit.[11] mp 120—124 °C (H_2O). ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 7.03 (1H, d, J=8.8), 7.54 (1H, d, J=8.8), 10.26 (1H, s)]. MS *m/z*: 183 (M^+), 137 ($M^+ - NO_2$), 79. IR ν cm^{-1}: 3180, 1635, 1515, 1440, 1290. *Anal.* Calcd for $C_7H_5NO_5$: C, 45.91; H, 2.75; N, 7.65. Found: C, 45.76; H, 2.84; N, 7.64.

2-Ethoxy-3-hydroxy-4-nitrobenzoic Acid (15) A mixture of $AgNO_3$ (9.2 g, 54 mmol), **12** (10.2 g, 48 mmol), 50% aqueous MeOH (160 ml), and 1 N aqueous NaOH solution (800 ml) was heated at 85 °C for 6 h, cooled to room temperature, and then filtered through Celite. The filtrate was concentrated to leave an aqueous solution, acidified with 10% aqueous HCl, and then extracted with AcOEt (50 ml × 3). The extract was washed with brine and evaporated to give a solid, which was recrystallized from toluene to afford 8.5 g (77%) of **15**, mp 125—127 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.31 (3H, t, J=7.0, $CH_2C\underline{H}_3$), 4.07 (2H, q, J=7.0, $C\underline{H}_2CH_3$), 7.19 (1H, d, J=9.0, arom. 5-H), 7.68 (1H, d, J=9.0, arom. 6-H), 10.70 (1H, br s, OH, disappeared with D_2O), 13.35 (1H, br s, COOH). MS *m/z*: 228 (MH^+), 210 ($M^+ - OH$). IR ν cm^{-1}: 3190, 2960, 1698, 1677, 1310, 1210. *Anal.* Calcd for $C_9H_9NO_6$: C, 47.58; H, 3.99; N, 6.17. Found: C, 47.76; H, 3.99; N, 6.00.

Methyl 2-Ethoxy-3-hydroxy-4-nitrobenzoate (17) Thionyl chloride (12.3 g, 0.10 mol) was added dropwise to a solution of **15** (7.8 g, 34 mmol) in MeOH (250 ml) at *ca.* 10 °C. The mixture was heated to reflux for 3 h, then cooled to room temperature, and concentrated to leave a solid, which was dissolved in $CHCl_3$. The solution was washed successively with 5% aqueous NH_4OH solution, water, and brine. The solvent was evaporated to give an oil, which was chromatographed on silica gel with AcOEt to afford a solid. The solid was recrystallized from toluene–*n*-hexane to furnish 8.2 g (99%) of **17**, mp 77—78 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.44 (3H, t, J=7.0, $CH_2C\underline{H}_3$), 3.95 (3H, s, $COOCH_3$), 4.23 (2H, q, J=7.0, $C\underline{H}_2CH_3$), 7.25 (1H, d, J=9.0, arom. 5-H), 7.87 (1H, d, J=9.0, arom. 6-H), 10.72 (1H, s, OH, disappeared with D_2O). MS *m/z*: 242 (MH^+), 210 ($M^+ - OCH_3$). IR ν cm^{-1}: 2980, 1720, 1540, 1330, 1260, 1230, 1140. *Anal.* Calcd for $C_{10}H_{11}NO_6$: C, 49.80; H, 4.60; N, 5.81. Found: C, 49.91; H, 4.53; N, 5.78.

Methyl 4-Amino-2-ethoxy-3-hydroxybenzoate (18) A solution of **17** (8.3 g, 34 mmol) in 10% aqueous EtOH (200 ml) was hydrogenated at 4.0 kg/cm^2 over 10% palladium on carbon (0.8 g) at room temperature. When no further change was observed in the pressure of hydrogen (*ca.* 1 h), the catalyst was filtered off. The filtrate was concentrated to dryness, and the solid was recrystallized from EtOH to give 4.7 g (65%) of **18**, mp 114—115 °C. ^{1}H-NMR (DMSO-d_6) δ: 1.30 (3H, t, J=7.0, $CH_2C\underline{H}_3$), 3.71 (3H, s, $COOCH_3$), 3.88 (2H, q, J=7.0, $C\underline{H}_2CH_3$), 5.40 (2H, s, NH), 6.38 (1H, d, J=8.7, arom. 5-H), 7.15 (1H, d, J=8.7, arom. 6-H), 8.47 (1H, s, OH). MS *m/z*: 212 (MH^+), 180 ($M^+ - OCH_3$). IR ν cm^{-1}: 3420, 3320, 1710, 1610, 1450. *Anal.* Calcd for $C_{10}H_{13}NO_4$: C, 56.87; H, 6.20; N, 6.63. Found: C, 56.87; H, 6.23; N, 6.59.

Methyl 4-(Acetylamino)-2-ethoxy-3-hydroxybenzoate (19) A) A solution of **18** (4.0 g, 19 mmol) and Ac_2O (2.2 g, 22 mmol) in MeOH (150 ml) was stirred at room temperature for 15 h. The reaction mixture was concentrated to dryness, and the residue was dissolved in $CHCl_3$. The solution was washed successively with 10% aqueous NaOH solution, water, and brine. The solvent was evaporated to leave an amorphous solid, which was crystallized from acetone–toluene to give 4.6 g (96%) of **19**, mp 125—126 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.42 (3H, t, J=7.0, $CH_2C\underline{H}_3$), 2.24 (3H, s, $COCH_3$), 3.90 (3H, s, $COOCH_3$), 4.12 (2H, q, J=7.0, $C\underline{H}_2CH_3$), 6.51 (1H, br s, OH), 7.48 (1H, d, J=8.7, arom. 5-H), 7.73 (1H, br s, NHCO), 8.01 (1H, d, J=8.7, arom. 6-H). MS *m/z*: 254 (MH^+), 222 ($M^+ - OCH_3$). IR ν cm^{-1}: 3330, 1720, 1670, 1600, 1530, 1440. *Anal.* Calcd for $C_{12}H_{15}NO_5$: C, 56.91; H, 5.97; N, 5.53. Found: C, 57.08; H, 5.92; N, 5.51.

B) A solution of **17** (11.4 g, 47 mmol) and Ac_2O (7.2 g, 71 mmol) in EtOH (500 ml) was hydrogenated at 3.5 kg/cm^2 over 10% palladium on carbon (1.0 g) at room temperature. After *ca.* 24 h, the catalyst was filtered off, and the filtrate was concentrated to dryness. The residue was dissolved in AcOEt, and the solution was washed successively with 10% aqueous NaOH solution and water. The solvent was evaporated, and the residue was chromatographed on silica gel with AcOEt to give 10.4 g (87%) of **19** as an amorphous solid, which was identical with the sample obtained above.

Methyl 3-Acetoxy-4-(acetylamino)-2-ethoxybenzoate (20) A) A suspension of **19** (4.8 g, 19 mmol), Ac_2O (2.3 g, 23 mmol), DMAP (2 mg), and toluene (150 ml) was heated to reflux for 2 h and cooled to 5 °C. The resulting precipitates were collected by filtration, washed with water, and recrystallized from acetone–*n*-hexane to give 4.6 g (82%) of **20**, mp 163—164 °C. ^{1}H-NMR (DMSO-d_6) δ: 1.26 (3H, t, J=7.0, $CH_2C\underline{H}_3$), 2.12 (3H, s, $COCH_3$), 2.35 (3H, s, $COCH_3$), 3.82 (3H, s, $COOCH_3$), 3.90 (2H, q, J=7.0, $C\underline{H}_2CH_3$), 7.61 (1H, d, J=8.7, arom. 5-H), 7.97 (1H, d, J=8.7, arom. 6-H), 9.60 (1H, br s, NHCO). MS *m/z*: 296 (MH^+), 254, 222. IR ν cm^{-1}: 3340, 2980, 1730, 1700, 1610, 1520, 1430. *Anal.* Calcd for $C_{14}H_{17}NO_6$: C, 56.95; H, 5.80; N, 4.74. Found: C, 57.25; H, 5.66; N, 4.77.

B) A mixture of **18** (4.0 g, 19 mmol), Ac_2O (9.6 g, 94 mmol), DMAP (0.1 g), and acetone (200 ml) was heated to reflux for 18 h. The solvent was evaporated to leave a solid, which was recrystallized from acetone–*n*-hexane to give 4.8 g (86%) of **20**, which was identical with the sample obtained above.

Methyl 3-Acetoxy-4-(acetylamino)-5-chloro-2-ethoxybenzoate (21) A mixture of **20** (4.8 g, 16 mmol), NCS (3.3 g, 25 mmol), and *N*,*N*-dimethylformamide (DMF, 80 ml) was heated at 80 °C for 3 h. The reaction mixture was poured into ice-water and extracted with AcOEt. The extract was washed with brine and concentrated to dryness. The residue was crystallized from Et_2O to afford 4.1 g (76%) of **21**. An analytical sample was obtained by recrystallization from toluene–*n*-hexane, mp 123—125 °C. ^{1}H-NMR (DMSO-d_6) δ: 1.26 (3H, t, J=7.0, $CH_2C\underline{H}_3$), 2.01 (3H, s, $COCH_3$), 2.32 (3H, s, $COCH_3$), 3.87 (3H, s, $COOCH_3$), 3.94 (2H, q, J=7.0, $C\underline{H}_2CH_3$), 7.72 (1H, s, arom. 6-H), 9.78 (1H, br s, NHCO). ^{13}C-NMR (DMSO-d_6) δ: 15.26 ($CH_2\underline{C}H_3$), 20.16 ($OCO\underline{C}H_3$), 22.29 ($NHCO\underline{C}H_3$), 52.51 ($COO\underline{C}H_3$), 70.71 ($\underline{C}H_2CH_3$), 124.16 (arom. 1-C), 126.19 (arom. 5-C), 127.42 (arom. 6-C), 133.47 (arom. 4-C), 142.06 (arom. 3-C), 150.31 (arom. 2-C), 164.02 ($\underline{C}OOCH_3$), 167.44 ($O\underline{C}OCH_3$), 168.00 ($NH\underline{C}OCH_3$). MS *m/z*: 330 (MH^+), 288, 270 ($M^+ - OCOCH_3$), 256. IR ν cm^{-1}: 3340, 2980, 1770, 1735, 1680, 1510, 1420. *Anal.* Calcd for $C_{14}H_{16}ClNO_6$: C, 51.00; H, 4.89; Cl, 10.75; N, 4.25. Found: C, 50.93; H, 4.82; Cl, 10.83; N, 4.23.

4-(Acetylamino)-5-chloro-2-ethoxy-3-hydroxybenzoic Acid (22) A mixture of **21** (4.9 g, 15 mmol), iso-PrOH (100 ml), H_2O (50 ml), and 48% aqueous NaOH solution (3.7 g, 44 mmol) was heated to reflux for 5 h and cooled to room temperature. The solvent was evaporated off, and the resulting aqueous solution was acidified with 35% aqueous HCl and then extracted with AcOEt. The extract was washed with brine and concentrated to dryness. The residue was triturated with Et_2O to give 3.7 g (91%) of **22**. An analytical sample was obtained by recrystallization from acetone–*n*-hexane, mp 123—125 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.48 (3H, t, J=7.0, $CH_2C\underline{H}_3$), 2.41 (3H, s, $COCH_3$), 4.51 (2H, q, J=7.0, $C\underline{H}_2CH_3$), 7.79 (1H, s, arom. 6-H), 7.85 (1H, br s, NHCO), 9.89 (1H, s, OH, disappeared with D_2O), 11.48 (1H, br s, COOH). MS *m/z*: 274 (MH^+), 256 ($M^+ - OH$). IR ν cm^{-1}: 3365, 2980, 1700, 1675, 1655, 1510, 1445. *Anal.* Calcd for $C_{11}H_{12}ClNO_5$: C, 48.28; H, 4.42; Cl, 12.95; N, 5.12. Found: C, 48.20; H, 4.30; Cl, 12.70; N, 5.10.

***N*-(2-Morpholinylmethyl)phthalimide Hydrochloride (29a)** A solution of *N*-[(4-benzyl-2-morpholinyl)methyl]phthalimide[1] (**27a**, 70.0 g, 0.21 mol) and ACE-Cl (32.8 g, 0.23 mol) in CH_2Cl_2 (400 ml) was heated to reflux for 1 h and then cooled to room temperature. The solvent was evaporated to leave a residue containing *N*-[4-(α-chloroethoxycarbonyl-2-morpholinyl)methyl]phthalimide (**28a**). The residue was dissolved in a mixture of toluene (300 ml) and MeOH (100 ml). The solution was

heated to reflux for 1 h, cooled to room temperature, and evaporated to give a solid, which was triturated with $CHCl_3$. The powder was recrystallized from MeOH to afford 54.7 g (91%) of **29a**, mp 254—257 °C. ^{1}H-NMR (DMSO-d_6) δ: 2.82 (1H, dd, J=11.0, 12.5, 3-H$_{ax}$), 2.95 (1H, dt, J=3.8, 12.0, 5-H$_{ax}$), 3.12 (1H, d-like, J=12, C$\underline{H}_2$N), 3.36 (1H, d-like, J=12, C$\underline{H}_2$N), 3.60—4.10 (5H, m), 7.80—7.95 (4H, m, arom. H), 9.60 (2H, br s, $NH_2^+Cl^-$). MS m/z: 247 (MH^+), 198. IR ν cm^{-1}: 3400, 2900, 2770, 1760, 1705. *Anal.* Calcd for $C_{13}H_{14}N_2O_3 \cdot HCl \cdot 1/4H_2O$: C, 55.23; H, 5.35; Cl, 12.54; N, 9.91. Found: C, 55.36; H, 5.44; Cl, 12.34; N, 9.75.

***N*-[(4-Acetyl-2-morpholinyl)methyl]phthalimide (30a)** A solution of **29a** (8.0 g, 28 mmol), Ac_2O (6.1 g, 60 mmol), and Et_3N (9.0 g, 89 mmol) in MeOH (100 ml) was stirred at room temperature for 2 h and concentrated to dryness. The residue was dissolved in $CHCl_3$, and the solution was washed successively with water, 10% aqueous NaOH solution, water, and brine. The solvent was evaporated to leave a solid, which was recrystallized from toluene to give 7.4 g (91%) of **30a**, mp 129—130 °C. ^{1}H-NMR ($CDCl_3$) δ: 2.10 (3H, s, $COCH_3$), 2.5—4.6 (9H, m), 7.65—7.95 (4H, m, arom. H). MS m/z: 289 (MH^+). IR ν cm^{-1}: 1770, 1710, 1635. *Anal.* Calcd for $C_{15}H_{16}N_2O_4$: C, 62.49; H, 5.59; N, 9.72. Found: C, 62.48; H, 5.55; N, 9.73.

2-[(Acetylamino)methyl]-4-(4-fluorobenzyl)morpholine (25) A mixture of 2-(4-fluorobenzylamino)ethanol[15] (**23**, 33.8 g, 0.20 mol) and *N*-(2,3-epoxypropyl)phthalimide (**24**, 41.4 g, 0.20 mol) was stirred at 80 °C for 3 h. Concentrated H_2SO_4 (107.8 g, 1.1 mol) was gradually added to the resultant brown oil, and the mixture was rapidly heated to 150 °C and kept at the same temperature for 2 h. The dark brown solution was cooled to room temperature, poured into ice-water, basified with 48% aqueous NaOH solution, and extracted with $CHCl_3$ (200 ml × 3). The extract was washed successively with water and brine. Acetic anhydride (20.4 g, 0.2 mol) was added to the dry $CHCl_3$ solution, and the mixture was stirred at room temperature for 2 h. The solution was washed successively with water, 20% aqueous NaOH solution, water, and brine. The solvent was evaporated to leave a residual solid, which was recrystallized from toluene to give 30.8 g (58%) of **25**, mp 120—122 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.88 (1H, dd, J=10, 11, 3-H$_{ax}$), 1.98 (3H, s, $COCH_3$), 2.14 (1H, dt, J=3.5, 11, 5-H$_{ax}$), 2.57—2.75 (2H, m), 3.10 (1H, ddd, J=4.5, 7.5, 13.5, C$\underline{H}_2$N), 3.43 (2H, s, C$\underline{H}_2$C$_6$H$_4$F), 3.55—3.72 (3H, m), 3.84 (1H, ddd, J=2.0, 3.5, 9.5, 6-H$_{eq}$), 5.80 (1H, br s, CONH), 6.94—7.06 (2H, m, arom. H), 7.20—7.32 (2H, m, arom. H). MS m/z: 267 (MH^+), 109. IR ν cm^{-1}: 3290, 1645, 1602, 1556. *Anal.* Calcd for $C_{14}H_{19}FN_2O_2$: C, 63.14; H, 7.19; F, 7.13; N, 10.52. Found: C, 63.06; H, 7.31; F, 7.24; N, 10.51.

2-[(Benzyloxycarbonylamino)methyl]-4-(4-fluorobenzyl)morpholine (27b) A mixture of **25** (13.3 g, 50 mmol) and 10% aqueous HCl (80 ml) was heated to reflux for 2 h and then cooled to 5 °C. The solution was basified with 20% aqueous NaOH solution and extracted with $CHCl_3$. A solution of benzyl chloroformate (9.4 g, 55 mmol) in $CHCl_3$ (10 ml) was added dropwise to a vigorously stirred mixture of the resulting extract including 2-(aminomethyl)-4-(4-fluorobenzyl)morpholine (**26**) and 2 N aqueous NaOH solution (50 ml) at *ca.* 5 °C. The resulting mixture was vigorously stirred at the same temperature for 1 h and at room temperature for 2 h. The organic layer was separated and washed successively with water and brine. The solvent was evaporated to give quantitatively 17.9 g of **27b** as a pale yellow oil. ^{1}H-NMR ($CDCl_3$) δ: 1.88 (1H, t-like, J=10.5, 3-H$_{ax}$), 2.11 (1H, dt, J=3.5, 10.5, 5-H$_{ax}$), 2.52—2.75 (2H, m), 3.12 (1H, ddd, J=5.1, 7.1, 13.9, C$\underline{H}_2$N), 3.33 (1H, m), 3.42 (2H, s, C$\underline{H}_2$C$_6$H$_4$F), 3.50—3.70 (2H, m), 3.82 (1H, ddd, J=1.8, 3.3, 11.0, 6-H$_{eq}$), 5.09 (2H, s, C$\underline{H}_2$Ph), 5.17 (1H, br t, J=7, NHCO), 6.90—7.05 (2H, m, arom. H), 7.18—7.43 (7H, m, arom. H). MS m/z: 359 (MH^+), 267 ($M^+ - CH_2Ph$), 109, 91. IR (neat) ν cm^{-1}: 3338, 2937, 2812, 1722, 1511. *Anal.* Calcd for $C_{20}H_{23}FN_2O_3$: C, 67.02; H, 6.47; F, 7.82; N, 5.30. Found: C, 67.03; H, 6.47; F, 7.83; N, 5.32.

4-Acetyl-2-[(benzyloxycarbonylamino)methyl]morpholine (30b) A mixture of **27b** (51.5 g, 0.14 mol), ACE-Cl (22.6 g, 0.16 mol), and CH_2Cl_2 (900 ml) was heated to reflux for 4 h and then cooled to room temperature. The solvent was evaporated, and the residue containing the intermediate carbamate **28b** was dissolved in a mixture of toluene (500 ml) and MeOH (200 ml). The solution was heated to reflux for 0.5 h and cooled to room temperature. The solvent was evaporated to leave a residue containing **29b**, which was dissolved in $CHCl_3$ (900 ml). This solution was cooled (*ca.* 10 °C), then Et_3N (43.6 g, 0.43 mol) and Ac_2O (17.6 g, 0.17 mol) were added successively. The whole was stirred at room temperature for 18 h and washed successively with water, 1 N aqueous NaOH solution, 5% aqueous citric acid solution, and brine. The solvent was evaporated to afford an oily residue, which was chromatographed on silica gel with $CHCl_3$: MeOH = 19 : 1 to give 35.4 g (84%) of **30b** as a pale brown oil. ^{1}H-NMR ($CDCl_3$) δ: 2.08 (3H, s, $COCH_3$), 2.50 (0.5H, dd, J=9, 13), 2.74 (0.5H, dt, J=7, 9), 2.98 (0.5H, dd, J=9, 13), 3.10—3.32, 3.32—3.68 (5.5H, m), 3.91 (1H, m), 4.41 (1H, dd, J=13, 13, 6-H$_{eq}$), 5.12 (3H, s, C$\underline{H}_2$Ph, NHCO), 7.30—7.40 (5H, m, arom. H). MS m/z: 293 (MH^+). IR (neat) ν cm^{-1}: 3315, 1717, 1636, 1537, 1439. *Anal.* Calcd for $C_{15}H_{20}N_2O_4 \cdot 1/4H_2O$: C, 60.69; H, 6.96; N, 9.44. Found: C, 60.62; H, 7.12; N, 9.45.

4-Acetyl-2-(aminomethyl)morpholine (31) A) A mixture of **30a** (22.6 g, 78 mmol), 100% $NH_2NH_2 \cdot H_2O$ (4.1 g, 82 mmol), and EtOH (80 ml) was heated to reflux for 1 h and then cooled to room temperature. The reaction mixture was concentrated and diluted with $CHCl_3$ (500 ml). The insoluble materials were filtered off, and the filtrate was washed successively with small amounts of water and brine. The solvent was evaporated to give 8.8 g (71%) of **31** as an unstable pale yellow oil. ^{1}H-NMR ($CDCl_3$) δ: 1.48 (2H, s, NH_2), 2.10 (3H, s, $COCH_3$), 2.50 (0.5H, dd, J=10.3, 12.6), 2.68—2.88 (2H, m), 3.02 (0.5H, dd, J=10.3, 12.6), 3.25—3.75 (4H, m), 3.96 (1H, m), 4.42 (1H, m). MS m/z: 159 (MH^+). IR (neat) ν cm^{-1}: 1642, 1441.

B) A solution of **30b** (13.4 g, 46 mmol) in 10% aqueous EtOH (300 ml) was hydrogenated over 10% palladium on carbon (1.0 g) at room temperature, until TLC indicated that almost no starting material remained. The catalyst was filtered off, and the filtrate was evaporated to give quantitatively 7.2 g of **31**, which was identical with the sample obtained above.

***N*-[(4-Acetyl-2-morpholinyl)methyl]-2-ethoxy-3-hydroxy-4-nitrobenzamide (34)** A mixture of **15** (6.0 g, 26 mmol), $SOCl_2$ (4.7 g, 39 mmol), DMF (2 drops), and CH_2Cl_2 (200 ml) was heated to reflux for 1.5 h and then cooled to room temperature. The solvent was evaporated to leave an oily residue containing 2-ethoxy-3-hydroxy-4-nitrobenzoyl chloride (**33**), which was dissolved in CH_2Cl_2 (200 ml). To this solution was added dropwise a solution of **31** (4.6 g, 29 mmol) and Et_3N (8.0 g, 79 mmol) in CH_2Cl_2 (50 ml) at *ca.* 5 °C. The mixture was stirred at room temperature for 3 h and then washed successively with water, 5% aqueous AcOH solution, water, and brine. The solvent was evaporated to leave a residue, which was chromatographed on silica gel with AcOEt : MeOH = 9 : 1 to give 7.4 g (76%) of **34** as an amorphous solid. An analytical sample was obtained by crystallization from acetone–*n*-hexane, mp 106—108 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.49 (3H, t, J=7.3, $CH_2C\underline{H}_3$), 2.11 (3H, s, $COCH_3$), 2.57 (0.5H, dd, J=10.5, 13.2), 2.77 (0.5H, td, J=12.8, 3.7), 3.05 (0.5H, dd, J=10.5, 13.2), 3.20—3.90, 3.90—4.05 (6.5H, m), 4.29, 4.31 (each 1H, q, J=7.3, C$\underline{H}_2$CH$_3$), 4.49 (1H, tdd, J=1.0, 13.5, 21.3), 7.71 (1H, d, J=9.1, arom. 5-H), 7.93 (1H, dd, J=9.1, 1.5, arom. 6-H), 8.43 (1H, br td, J=5, 25, CONH), 10.75 (1H, br s, OH). MS m/z: 368 (MH^+). IR ν cm^{-1}: 3381, 1678, 1585, 1520, 1510, 1320. *Anal.* Calcd for $C_{16}H_{21}N_3O_7$: C, 52.31; H, 5.76; N, 11.44. Found: C, 52.45; H, 5.70; N, 11.42.

***N*-[(4-Acetyl-2-morpholinyl)methyl]-4-(acetylamino)-2-ethoxy-3-hydroxybenzamide (35)** A solution of **34** (7.4 g, 20 mmol) and Ac_2O (3.1 g, 30 mmol) in EtOH (300 ml) was hydrogenated at 3.8 kg/cm^2 over 10% palladium on carbon (0.5 g) at room temperature for 5 h. The catalyst was filtered off, and the filtrate was evaporated to give a residue, which was dissolved in $CHCl_3$. The solution was washed successively with water, 1 N aqueous NaOH solution, water, and brine. The solvent was evaporated to leave a residue, which was chromatographed on silica gel with AcOEt : MeOH = 9 : 1 to afford 3.6 g (47%) of **35** as an amorphous solid. ^{1}H-NMR ($CDCl_3$) δ: 1.42 and 1.44 (each 1.5H, each t, J=7.3, $CH_2C\underline{H}_3$), 2.10 (3H, s, $NCOCH_3$), 2.26 (3H, s, $NHCOC\underline{H}_3$), 2.56 (0.5H, dd, J=10.5, 13.0), 2.75 (0.5H, dt, J=3.7, 13.0), 3.03 (0.5H, dd, J=10.5, 13.0), 3.15—3.85, 3.85—4.05 (6.5H, m), 4.18 (2H, q, J=7.3, C$\underline{H}_2$CH$_3$), 4.47 (1H, dd, J=14, 22), 7.22 (1H, d, J=9.0, 3.5, arom. 6-H), 7.57 (1H, dd, J=9.0, 5.0, arom. 5-H), 8.00—8.65 (2H, m, NHCO), 10.55 (1H, d, J=5.0, OH). MS m/z: 380 (MH^+), 362 ($M^+ - OH$). IR ν cm^{-1}: 3373, 1641, 1522, 1435.

2-Ethoxy-*N*-{[4-(4-fluorobenzyl)-2-morpholinyl]methyl}-3-hydroxy-4-nitrobenzamide (37) In a similar manner to that described for **34**, compound **37** was prepared from **15** (10.0 g, 44 mmol) and **26** (10.4 g, 46 mmol) in 95% yield. mp 119—121 °C (toluene–*n*-hexane). ^{1}H-NMR ($CDCl_3$) δ: 1.48 (3H, t, J=7.0, $CH_2C\underline{H}_3$), 1.97 (1H, dd, J=10.0, 11.0, 3-H$_{ax}$), 2.18 (1H, td, J=11.0, 3.5, 5-H$_{ax}$), 2.67 (1H, qd, J=1.5, 11.0, 3-H$_{eq}$), 2.77 (1H, td, J=1.8, 11.0, 5-H$_{eq}$), 3.48 (1H, m), 3.48 (2H, s, C$\underline{H}_2$C$_6$H$_4$F), 3.61—3.81 (3H, m), 3.90 (1H, ddd, J=1.8, 3.5, 11.2, 6-H$_{eq}$), 4.25 and 4.27 (each 1H, each q, J=7.0, C$\underline{H}_2$CH$_3$), 6.94—7.07 (2H, m,

$CH_2C_6\underline{H}_4F$), 7.22—7.33 (2H, m, $CH_2C_6\underline{H}_4F$), 7.70 (1H, d, J=9.2, arom. 5-H), 7.92 (1H, dd, J=9.2, 0.8, arom. 6-H), 8.41 (1H, br t, J=5, CONH), 10.85 (1H, br s, OH). MS m/z: 434 (MH^+). IR νcm^{-1}: 3381, 1678, 1585, 1520, 1510, 1319, 1222. *Anal.* Calcd for $C_{21}H_{24}FN_3O_6$: C, 58.19; H, 5.58; F, 4.38, N, 9.69. Found: C, 58.33; H, 5.55; F, 4.52; N, 9.56.

***N*-{[4-(*tert*-Butoxycarbonyl)-2-morpholinyl]methyl}-2-ethoxy-3-hydroxy-4-nitrobenzamide (38)** A mixture of **37** (4.6 g, 11 mmol), ACE-Cl (2.3 g, 16 mmol), and toluene (250 ml) was heated to reflux for 10 h and then cooled to room temperature. Then MeOH (50 ml) was added, and the whole was heated to reflux for an additional 2 h. The solvent was evaporated to leave a residue, which was dissolved in MeOH (250 ml). Di-*tert*-butyl dicarbonate (2.9 g, 13 mmol) and Et_3N (3.2 g, 32 mmol) were added to the solution at *ca.* 10 °C. The mixture was stirred at room temperature for 18 h and concentrated to dryness. The residue was dissolved in $CHCl_3$ and washed successively with water, 5% aqueous citric acid solution, and brine. The solvent was evaporated to afford an oily residue, which was chromatographed on silica gel with AcOEt to give 4.1 g (91%) of **38** as a pale brown amorphous solid. An analytical sample was obtained by crystallization from toluene, mp 123—124 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.47 (9H, s, $C(CH_3)_3$), 1.49 (3H, t, J=7.0, $CH_2C\underline{H}_3$), 2.72 (1H, dd, J=11.0, 13.0, 3-H_{ax}), 2.94 (1H, br t, J=13, 5-H_{ax}), 3.35 (1H, ddd, J=4.7, 8.0, 9.3), 3.47—3.68 (2H, m), 3.73—4.00 (4H, m), 4.28, 4.30 (each 1H, q, J=7.0, $C\underline{H}_2CH_3$), 7.71 (1H, d, J=9.2, arom. 5-H), 7.93 (1H, d, J=9.2, 0.8, arom. 6-H), 8.43 (1H, br t, J=5, CONH), 10.84 (1H, br s, OH). MS m/z: 426 (MH^+), 370, 326. IR νcm^{-1}: 3379, 2984, 1697, 1668, 1589, 1529, 1406, 1242, 1222, 1169. *Anal.* Calcd for $C_{19}H_{27}N_3O_8$: C, 53.64; H, 6.40; N, 9.88. Found: C, 53.64; H, 6.40; N, 9.85.

4-(Acetylamino)-*N*-{[4-(*tert*-butoxycarbonyl)-2-morpholinyl]methyl}-2-ethoxy-3-hydroxybenzamide (39) A solution of **38** (1.5 g, 3.5 mmol) and Ac_2O (0.7 g, 6.9 mmol) in EtOH (80 ml) was hydrogenated at 2.5 kg/cm^2 over 10% palladium on carbon (0.1 g) at room temperature for 6 h. The catalyst was filtered off, and the filtrate was evaporated to give a residue, which was dissolved in $CHCl_3$. The solution was washed successively with water, 1 N aqueous NaOH solution, water, and brine. The solvent was evaporated to leave a residue, which was chromatographed on silica gel with AcOEt to afford 1.5 g (97%) of **39** as an amorphous solid. ^{1}H-NMR ($CDCl_3$) δ: 1.26 (3H, t, J=7.0, $CH_2C\underline{H}_3$), 1.47 (9H, s, $C(CH_3)_3$), 2.27 (3H, s, $COCH_3$), 2.71 (1H, br t, J=12, 3-H_{ax}), 2.92 (1H, br t, J=12, 5-H_{ax}), 3.35 (1H, m), 3.42—3.68 (2H, m), 3.68—4.05 (4H, m), 4.17 (2H, q, J=7.0, $C\underline{H}_2CH_3$), 7.23 (1H, d, J=9, arom. 5-H), 7.58 (1H, dd, J=9, 0.8, arom. 6-H), 7.98 (1H, br s like), 8.29 (2H, br s like). MS m/z: 438 (MH^+), 382, 339, 320. IR νcm^{-1}: 3366, 2980, 1697, 1655, 1528, 1431.

4-(Acetylamino)-*N*-{[4-(*tert*-butoxycarbonyl)-2-morpholinyl]methyl}-5-chloro-2-ethoxy-3-hydroxybenzamide (40) A mixture of **39** (3.5 g, 8.0 mmol), NCS (1.1 g, 8.2 mmol), $CHCl_3$ (100 ml), and DMF (30 ml) was stirred at room temperature for 5 h. The reaction mixture was washed successively with water and brine. The solvent was evaporated to leave a residue, which was chromatographed on silica gel with AcOEt : *n*-hexane = 1 : 1 to give 0.6 g (16%) of **40** as a solid. An analytical sample was obtained by recrystallization from $CHCl_3$–*n*-hexane, mp 158—159 °C. ^{1}H-NMR (DMSO-d_6) δ: 1.29 (3H, t, J=7.0, $CH_2C\underline{H}_3$), 1.40 (9H, s, $C(CH_3)_3$), 2.56 (3H, s, $COCH_3$), 2.87 (1H, m), 3.20—3.55 (4H, m), 3.65—3.92 (4H, m), 4.02 (2H, q, J=7.0, $C\underline{H}_2CH_3$), 7.13 (1H, s, arom. 6-H), 8.43 (1H, t, J=6, NHCO), 9.49 (1H, br s, 4-NHCO), 11.06 (1H, br s, OH). MS m/z: 472 (MH^+), 256, 238, 204. IR νcm^{-1}: 1661, 1522. *Anal.* Calcd for $C_{21}H_{30}ClN_3O_7$: C, 53.45; H, 6.41; Cl, 7.51; N, 8.90. Found: C, 53.66; H, 6.61; Cl, 7.15; N, 8.72.

4-Amino-5-chloro-2-ethoxy-3-hydroxy-*N*-(2-morpholinylmethyl)benzamide (3) A mixture of **40** (1.8 g, 3.8 mmol) and 10% aqueous HCl (60 ml) was heated to reflux for 3 h and cooled to room temperature. The solution was basified with 28% aqueous NH_4OH solution, and the mixture was concentrated to dryness. Then EtOH was added to the residue, and the insoluble materials were filtered off. The filtrate was concentrated to leave an oily residue, which was chromatographed on silica gel (Chromatorex NH-DM 1020) with $CHCl_3$: MeOH : 28% aqueous NH_4OH solution = 90 : 10 : 1 to afford 0.9 g (72%) of **3** as an amorphous solid. An analytical sample was obtained by crystallization from EtOH–*n*-hexane, mp 133—135 °C. ^{1}H-NMR (300 MHz, DMSO-d_6) δ: 1.34 (3H, t, J=7.0, $CH_2C\underline{H}_3$), 2.39 (1H, dd, J=10.0, 12.3), 2.55—2.71 (2H, m), 2.77 (1H, dd, J=2.3, 12.3), 3.16 (1H, m), 3.30—3.55 (3H, m), 3.74 (1H, td, J=1.9, 10.9), 3.95 (2H, q, J=7.0, $C\underline{H}_2CH_3$), 4.3 (1H, br s, 4-NH), 5.28 (2H, br s, NH_2), 7.28 (1H, s, arom. 6-H), 8.12 (1H, t, J=5.5, NHCO).[16] MS m/z: 330 (MH^+). IR νcm^{-1}: 3423, 3333, 1624, 1537, 1448, 1425, 1304. *Anal.* Calcd for $C_{14}H_{20}ClN_3O_4 \cdot 3/10H_2O$: C, 50.17; H, 6.19; Cl, 10.58; N, 12.54. Found: C, 50.11; H, 6.20; Cl, 10.30; N, 12.34.

2-Ethoxy-3-hydroxy-4-nitro-*N*-[(5-oxo-2-morpholinyl)methyl]benzamide (42) A solution of **15** (12.3 g, 54 mmol) and *N*,*N*′-carbonyldiimidazole (10.5 g, 65 mmol) in tetrahydrofuran (600 ml) was stirred at room temperature for 0.5 h. The mixture was added to a mixture of 2-(aminomethyl)-5-oxomorpholine hydrochloride[5] (**41**, 9.9 g, 59 mmol), Et_3N (7.1 g, 70 mmol), tetrahydrofuran (250 ml), and DMF (100 ml) and the whole was stirred at room temperature for 18 h. The solvent was evaporated to leave an oily residue, which was dissolved in AcOEt and a small amount of water. The resulting precipitates were collected by filtration and washed with AcOEt to afford 8.8 g (48%) of **42** as pale yellow crystals. The organic layer of the filtrate was separated. The solvent was evaporated to give a residue, which was chromatographed on silica gel with $CHCl_3$: MeOH = 9 : 1 to provide a solid. The solid was triturated with acetone–EtOH to afford 3.5 g (19%) of **42**. An analytical sample was obtained by recrystallization from MeOH, mp 209—210 °C. ^{1}H-NMR (DMSO-d_6) δ: 1.29 (3H, t, J=7.0, $CH_2C\underline{H}_3$), 3.05—3.55 (4H, m), 3.75—4.15 (5H, m), 7.07 (1H, d, J=9.0, arom. 5-H), 7.71 (1H, d, J=9.0, arom. 6-H), 8.03 (1H, d, J=4.0, 4-NH), 8.56 (1H, t, J=5.0, NHCO), 10.68 (1H, br s, OH). MS m/z: 340 (MH^+). IR νcm^{-1}: 3350, 3170, 1675, 1660, 1517. *Anal.* Calcd for $C_{14}H_{17}N_3O_7$: C, 49.56; H, 5.05; N, 12.38. Found: C, 49.77; H, 5.29; N, 12.23.

4-Amino-2-ethoxy-3-hydroxy-*N*-[(5-oxo-2-morpholinyl)methyl]benzamide (43) A solution of **42** (0.8 g, 2.4 mmol) in 10% aqueous EtOH (150 ml) was hydrogenated at 4.0 kg/cm^2 over 10% palladium on carbon (0.08 g) at room temperature. When no further change was observed in the pressure of hydrogen, the catalyst was filtered off. The filtrate was concentrated to dryness, and the solid was recrystallized from EtOH to give 0.6 g (82%) of **43**, mp 215—216 °C. ^{1}H-NMR (DMSO-d_6) δ: 1.33 (3H, t, J=7.0, $CH_2C\underline{H}_3$), 3.0—3.45 (3H, m), 3.6 (1H, m), 3.75—4.1 (3H, m), 3.94 (2H, q, J=7.0, $C\underline{H}_2CH_3$), 5.21 (2H, br s, NH_2), 6.42 (1H, d, J=8.0, arom. 5-H), 7.29 (1H, d, J=8.0, arom. 6-H), 7.99 (1H, d, J=4.0, 4-NH), 8.15 (1H, t, J=5.0, NHCO), 8.48 (1H, br s, OH). MS m/z: 310 (MH^+). IR νcm^{-1}: 3400, 3265, 1680, 1670, 1590, 1545, 1280. *Anal.* Calcd for $C_{14}H_{19}N_3O_5$: C, 54.36; H, 6.19; N, 13.58. Found: C, 54.33; H, 6.25; N, 13.33.

4-Amino-5-chloro-2-ethoxy-3-hydroxy-*N*-[(5-oxo-2-morpholinyl)methyl]benzamide (4) A mixture of **43** (12.8 g, 41 mmol), NCS (6.1 g, 46 mmol), and DMF (300 ml) was heated at 80 °C for 2.5 h and then cooled to room temperature. The reaction mixture was concentrated to dryness. The residue was chromatographed on silica gel with $CHCl_3$: MeOH = 9 : 1 to give a solid, which was recrystallized from EtOH–$CHCl_3$ to afford 2.5 g (17%) of **4**, mp 165.5—166.5 °C. ^{1}H-NMR (DMSO-d_6) δ: 1.34 (3H, t, J=7.0, $CH_2C\underline{H}_3$), 3.04—3.45 (3H, m), 3.58 (1H, m), 3.85 (1H, m), 3.96 (2H, q, J=7.0, $C\underline{H}_2CH_3$), 4.04 (1H, d, J=16.0, 6-$\underline{H}_2$), 4.11 (1H, d, J=16.0, 6-$\underline{H}_2$), 5.33 (2H, br s, NH_2), 7.28 (1H, d, J=8.7, arom. 6-H), 8.00 (1H, d, J=3.0, 4-NH), 8.20 (1H, t, J=5.5, CONH), 9.14 (1H, br s, OH). MS m/z: 344 (MH^+), 213. IR νcm^{-1}: 3485, 3380, 3330, 1677, 1665, 1600, 1550. *Anal.* Calcd for $C_{14}H_{18}ClN_3O_5 \cdot 1/2H_2O$: C, 47.67; H, 5.43; Cl, 10.05; N, 11.91. Found: C, 47.49; H, 5.47; Cl, 9.82; N, 11.78.

Serotonin-4 Receptor Binding Assay The binding assay was carried out according to the method of Grossman *et al.*[17] All determinations were performed in triplicate. Assay tubes contained 300 μl of HEPES buffer (pH 7.4), 200 μl of a solution of either a competing agent (for drug competition studies), or serotonin to give a final concentration of 30 μM (to determine non-specific binding) or buffer (for determination of total binding), 400 μl of [^{3}H]GR113808 in HEPES buffer to give a final concentration of 0.1 nM and 100 μl of tissue preparation. Assay tubes were incubated at 37 °C. The reaction was terminated by rapid vacuum filtration and washing (4 ml × 1) with ice-cold buffer through Whatman GF/B filter paper using a Brandel Cell Harvester. Filters were presoaked in a solution of polyethylenimine (*ca.* 0.1%) to reduce filter binding. For drug competition studies, assay tubes were incubated at 37 °C for 30 min and the reaction was terminated as above. Filters were placed in 10 ml of ACS II scintillator (Amersham) before scintillation counting.

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References and Notes

1) Kato S., Morie T., Hino K., Kon T., Naruto S., Yoshida N., Karasawa T., Matsumoto J., *J. Med. Chem.*, **33**, 1406—1413 (1990).
2) *a*) Kato S., Morie T., Kon T., Yoshida N., Karasawa T., Matsumoto J., *J. Med. Chem.*, **34**, 616—624 (1991); *b*) Yoshida N., Kato S., Ito T., *Drugs Future*, **18**, 513—515 (1993).
3) *a*) Dumuis A., Sebben M., Bockaert J., *Naunyn-Schmiedeberg's Arch. Pharmacol.*, **340**, 403—410 (1989); *b*) Yoshida N., Ito T., *Neurogastroenterol. Mot.*, **6**, 197—204 (1994).
4) Matsumoto S., Yoshida K., Itokawa A., Tagawa M., Fujii T., Miyazaki H., Sekine Y., *Arzeim.-Forsch./Drug Res.*, **43**, 1095—1102 (1993).
5) Kato S., Morie T., Yoshida N., *Chem. Pharm. Bull.*, **43**, 699—702 (1995).
6) *a*) Meuldermans W., Peer A. van., Hendrickx J., Lauwers W., Swysen E., Bockx M., Woestenborghs R., Heykants J., *Drug Metab. Dispos.*, **16**, 403—409 (1988); *b*) Meuldermans W., Hendrickx J., Lauwers W., Hurkmans R., Mostmans E., Swysen E., Bracke J., Knaeps A., Heykants J., *ibid.*, **16**, 410—419 (1988).
7) Högbeg T., de Pauli T., Johansson L., Kumar Y., Hall H., Ögren S. V., *J. Med. Chem.*, **33**, 2305—2309 (1990).
8) Kessar S. V., Gupta Y. P., Mohammad T., Goyal M., Sawal K. K., *J. Chem. Soc., Chem. Commun.*, **1983**, 400—401.
9) 3-Ethoxysalicylaldehyde is commercially available. ^{1}H-NMR ($CDCl_3$) δ: 1.48 (3H, t, J=7.0, $CH_2C\underline{H}_3$), 4.12 (2H, q, J=7.0, $C\underline{H}_2CH_3$), 6.94 (1H, dd, J=7.7, 7.7, arom. 5-H), 7.11 (1H, dd, J=7.7, 1.6, arom. 4-H), 7.17 (1H, dd, J=7.7, 1.6, arom. 6-H), 9.91 (1H, s, CHO), 11.04 (1H, s, OH, disappeared with D_2O).
10) Aimi N., Ueno M., Hoshino H., Sakai S., *Tetrahedron Lett.*, **33**, 5403—5404 (1992).
11) *a*) Bhattacharyya S. C., Seymour D. E., *J. Chem. Soc.*, **1950**, 1139—1140; *b*) Berti G., Carboni S., Da Settimo A., *Gazz. Chim. Ital.*, **85**, 1632—1645 (1955) [*Chem. Abstr.*, **50**, 10041*i* (1956)].
12) Pérez R. A., Fernández-Alvarez E., Nieto O., Piedrafita F. J., *J. Med. Chem.*, **35**, 4584—4588 (1992).
13) *a*) Olofson R. A., Martz J. T., Senet J.-P., Piteau M., Malfroot T., *J. Org. Chem.*, **49**, 2081—2082 (1984); *b*) DeShong P., Kell D. A., *Tetrahedron Lett.*, **27**, 3979—3982 (1986); *c*) Olofson R. A., *Pure Appl. Chem.*, **60**, 1715—1724 (1988); *d*) Gubert S., Braojos C., Sacristán A., Ortiz J. A., *Synthesis*, **1991**, 318—320; *e*) Yang B. V., O'Rourke D., Li J., *Synlett*, **1993**, 195—196.
14) Kato S., Morie T., Harada H., Yoshida N., Matsumoto J., *Chem. Pharm. Bull.*, **40**, 652—660 (1992).
15) Morie T., Kato S., Harada H., Yoshida N., Matsumoto J., *Chem. Pharm. Bull.*, **42**, 877—882 (1994).
16) The proton signal for the 3-OH group was not observed owing to the intra- and/or intermolecular hydrogen bonding.
17) Grossman C. J., Kilpatrick G. J., Bunce K. T., *Br. J. Pharmacol.*, **109**, 618—624 (1993).