Synthesis and Biological Activities of Metabolites of Mosapride, a New Gastroprokinetic Agent

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In order to confirm the proposed structures of two metabolites 3 and 4 of the gastroprokinetic agent mosapride [4-amino-5-chloro-2-ethoxy-*N*-{[4-(4-fluorobenzyl)-2-morpholinyl]methyl}benzamide, 2], the compounds were synthesized and their biological activity was examined. The structures of the metabolites were confirmed by means of comparison with the synthetic compounds. The serotonin 5-HT_4 receptor agonistic activities of the metabolites were found to be less than that of mosapride.

Key words mosapride; gastroprokinetic activity; metabolite; 4-amino-5-chloro-2-ethoxy-*N*-{[4-(4-fluorobenzyl)-2-morpholinyl]methyl}benzamide; serotonin 5-HT_4 receptor agonistic activity

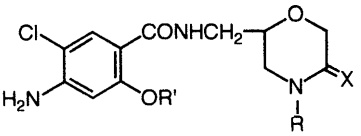
We have previously reported the synthesis of 4-amino-*N*-[(4-benzyl-2-morpholinyl)methyl]-5-chloro-2-methoxybenzamide (**1**) and its derivatives as potential gastroprokinetic agents without dopamine D_2 receptor antagonistic activity.[1] Among them, 4-amino-5-chloro-2-ethoxy-*N*-{[4-(4-fluorobenzyl)-2-morpholinyl]methyl}benzamide (**2**, mosapride) was found to show the most potent gastroprokinetic activity and is presently under clinical study.[2] During the course of the metabolic studies of mosapride, two major metabolites were isolated from rat urine, and their structures were proposed to be the des-4-fluorobenzylated mosapride **3** and its oxo analogue **4** (Chart 1) on the basis of their MS and ^{1}H-NMR spectra.[3] The metabolites **3** and **4** were detected in the plasma and/or urine of mice, rats, dogs and/or monkeys, and the metabolite **3** has also been found in human plasma.[4]

The present study was undertaken to confirm the structures of the two metabolites by chemical synthesis and to compare their biological activity with that of mosapride (**2**).

Synthesis of the Des-4-fluorobenzylated Mosapride 3 For the mild and selective *N*-debenzylation of tertiary amines, α-chloroethyl chloroformate is useful.[5] Thus, *N*-debenzylation of **2** using this reagent was first attempted. The treatment of **2** with α-chloroethyl chloroformate in 1,2-dichloroethane at 80 °C, followed by the decomposition of the resulting *N*-carbamate by MeOH gave a complex mixture, presumably because of the existence of the 4-amino group. We then tried the hydrogenolysis of **2**. Hydrogenation of **2** with 10% palladium-on-carbon in a mixture of 10% aqueous EtOH and acetic acid (AcOH) at *ca.* 50 °C caused not only debenzylation but also removal of the chlorine atom, thereby affording 4-amino-2-ethoxy-*N*-(2-morpholinylmethyl)benzamide (**5**) in an excellent yield. In this reaction, the dechlorination was faster than the debenzylation, and without acetic acid as a cosolvent the debenzylation was very slow. The reaction of **5** with acetic anhydride, followed by the chlorination of the resulting diacetyl derivative **6** with *N*-chlorosuccinimide (NCS) produced *N*-[(4-acetyl-2-morpholinyl)methyl]-4-(acetylamino)-5-chloro-2-ethoxybenzamide (**7**) as the precursor **3** of in a good yield. Compound **7** was finally hydrolyzed with 10% HCl to give compound **3** as its hydrochloride (Chart 2).

This synthetic compound was identical with the proposed metabolite **3**, on the basis of the MS, ^{1}H-NMR, and HPLC comparisons.

Synthesis of the Oxidized Metabolite 4 The synthetic route to the metabolite **4** from *N*-(2,3-epoxypropyl)phthalimide (**8**) *via* the amine **14** is shown in Chart 3. The reaction of **8** with dibenzylamine without solvent afforded the 2-aminoethanol derivative **9** in an excellent yield. The removal of the phthaloyl group of **9** by employing hydrazine afforded 1-amino-3-(dibenzylamino)-2-propanol (**10**) in a poor yield. On the other hand, the treatment of **9** with concentrated HCl gave the aminopropanol **10** in 84% yield. The ring closure reaction of **10** was achieved by using a method previously described[6]; **10** was allowed to react with chloroacetyl chloride in a mixture of $CHCl_3$



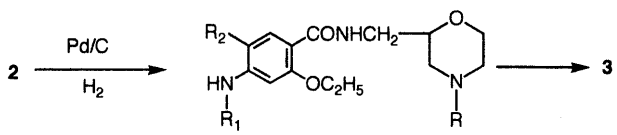
1: R = $C_6H_5CH_2$, R' = CH_3, X = H_2

2: R = 4-$FC_6H_4CH_2$, R' = C_2H_5, X = H_2 (mosapride)

3: R = H, R' = C_2H_5, X = H_2

4: R = H, R' = C_2H_5, X = O

Chart 1



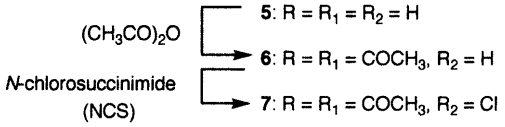


Chart 2

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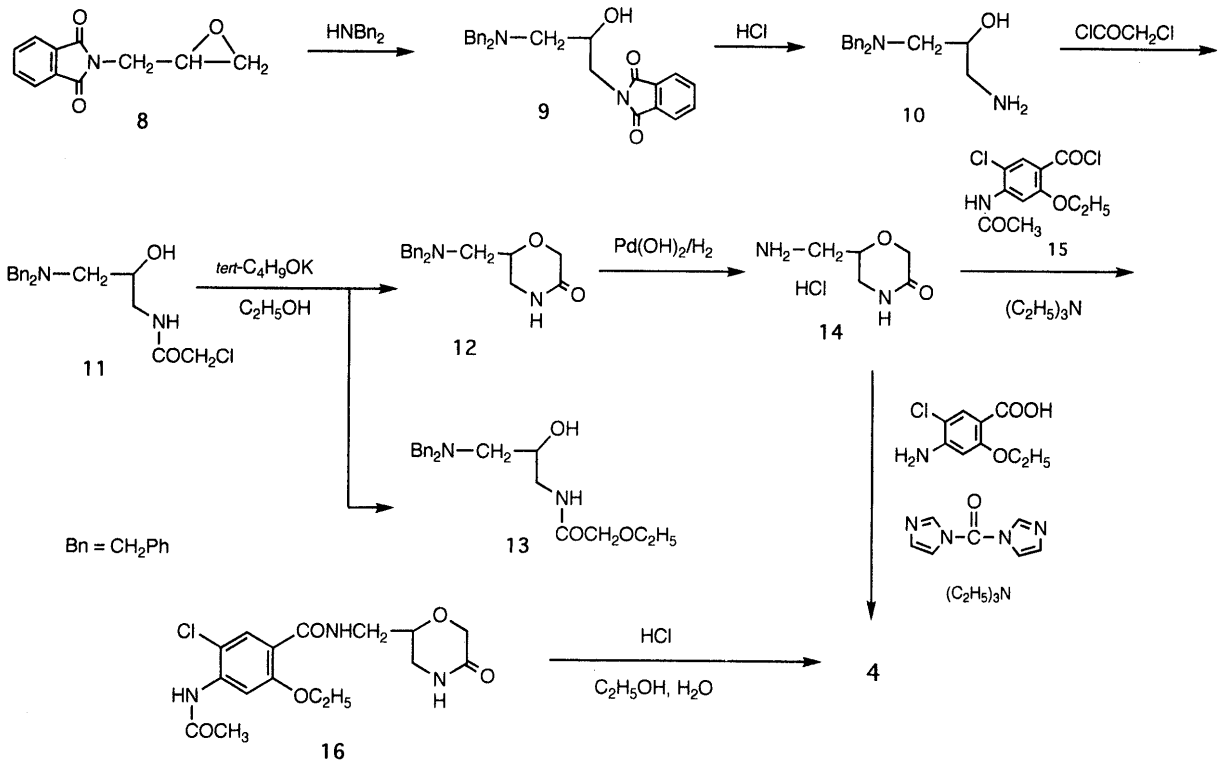

Chart 3

and 3% aqueous NaOH to afford the *N*-chloroacetyl derivative **11**, which was subsequently treated with potassium *tert*-butoxide in EtOH to furnish the desired 5-oxomorpholine **12** in 53% yield together with the *N*-ethoxyacetyl derivative **13** in 22% yield. The dibenzyl group of **12** was hydrogenated with Pearlman's catalyst[7] (palladium hydroxide-on-carbon), and treatment of the resultant amine with ethanolic HCl afforded the hydrochloride of **14** as crystals in a good yield. The reaction of 4-(acetylamino)-5-chloro-2-ethoxybenzoic acid[2] with thionyl chloride gave the corresponding benzoyl chloride **15**, which was transformed into 4-(acetylamino)-5-chloro-2-ethoxy-*N*-[(5-oxo-2-morpholinyl)methyl]benzamide (**16**) on treatment with the hydrochloride of **14** in the presence of triethylamine in a good yield. Compound **16** was hydrolyzed with 10% HCl to give the desired product **4**. The reaction of the hydrochloride of **14** with 4-amino-5-chloro-2-ethoxybenzoic acid[2] in the presence of triethylamine using *N*,*N*'-carbonyldiimidazole straightforwardly gave **4** in a good yield, but the use of 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide hydrochloride as a coupling agent resulted in a poor yield.

This synthetic compound was identical with the proposed metabolite **4**, on the basis of the MS, ^{1}H-NMR, and HPLC comparisons.

Biological Activity The gastroprokinetic activity of mosapride (**2**) is believed to be correlated with agonistic activity at a new serotonin receptor subtype (5-HT$_4$). The serotonin 5-HT$_4$ receptor agonistic activities of mosapride (**2**) and the metabolites **3** and **4** were measured in isolated guinea pig ileum.[8] The EC_{50} values for these compounds are shown in Table 1. As is clear from the results, the *in vitro* activity of the metabolite **3** was approximately half that of **2**, and the metabolite **4** was much less active.

Table 1. Serotonin 5-HT$_4$ Receptor Agonistic Activity of Mosapride (**2**) and Its Metabolites **3** and **4**

Compound	5-HT$_4$ agonistic activity[a] (EC_{50}, μM)
Mosapride (**2**)[b]	0.074 (0.053—0.104)
3	0.119 (0.084—0.172)
4	1.007 (0.597—2.204)

a) Potency of compounds was estimated on the basis of enhancement of the electrically evoked contractions in isolated guinea pig ileum. The EC_{50} value for each compound represents the concentration causing 50% enhancement. The 95% confidence limit is indicated in parentheses. *b*) Mosapride was used as the citric acid salt.

Yoshida *et al.*[9] of our laboratories reported that **3** and **4** showed weaker gastric emptying activity than **2** with semi-solid meal and solid meal. Therefore, it is suggested that the metabolites **3** and **4** do not play a crucial role in the gastroprokinetic effect of mosapride (**2**).

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 with KBr disks unless otherwise specified. Electron ionization (EI), chemical ionization (CI), and secondary ion (SI) 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. Chemical shifts are expressed as δ (ppm) values with tetramethylsilane as an internal standard, and coupling constants (J) are given in hertz (Hz). Organic extracts were dried over anhydrous $MgSO_4$. The solvent was evaporated under reduced pressure. Merck Silica gel 60 (70—230 mesh) was used for column chromatography.

4-Amino-2-ethoxy-*N*-(2-morpholinylmethyl)benzamide (5) A mixture of **2** (10.0 g, 24 mmol), 10% palladium-on-carbon (1.0 g), and a mixture of 10% aqueous EtOH (200 ml) and AcOH (20 ml) was hydrogenated at *ca.* 50 °C and atmospheric pressure, until no more hydrogen was

consumed (*ca.* 4 h). The catalyst was filtered off, and the filtrate was concentrated to dryness. The residual oil was dissolved in water and then basified with 10% NaOH. The resulting precipitates were collected by filtration, washed with water, and dried to give 6.2 g (94%) of **5**, mp 209—210 °C (MeOH). ^{1}H-NMR [dimethyl sulfoxide (DMSO)-d_6] δ: 1.41 (3H, t, J=7.0, $OCH_2C\underline{H}_3$), 2.3—3.8 (10H, m), 4.05 (2H, q, J=7.0, $OC\underline{H}_2CH_3$), 5.70 (2H, s, NH_2), 6.17 (1H, dd, J=2.0, 8.5, arom. 5-H), 6.21 (1H, d, J=2.0, arom. 3-H), 8.05 (1H, d, J=8.5, arom. 6-H), 8.08 (1H, br t, J=5.5, CONH). SIMS m/z: 280 (MH^+), 206. IR ν cm^{-1}, 3360, 3150, 1650. *Anal.* Calcd for $C_{14}H_{21}N_3O_3$: C, 60.20; H, 7.58; N, 15.04. Found: C, 60.08; H, 7.67; N, 14.75.

***N*-[(4-Acetyl-2-morpholinyl)methyl]-4-(acetylamino)-2-ethoxybenzamide (6)** A mixture of **5** (4.0 g, 14 mmol), acetic anhydride (3.2 g, 31 mmol), MeOH (200 ml), and $CHCl_3$ (50 ml) was stirred at room temperature for 4 h. The solvent was evaporated, then the residue was taken up in $CHCl_3$ and the solution was washed successively with 10% NaOH, water, and brine. The solvent was evaporated to give a solid, which was recrystallized from iso-PrOH–*n*-hexane to afford 4.7 g (90%) of **6**, mp 154—156 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.50 (3H, t, J=7.0, $OCH_2C\underline{H}_3$), 2.09 (3H, s, $NCOCH_3$), 2.19 (3H, s, $NHCOC\underline{H}_3$), 3.35—4.05 (9H, m), 4.19 (2H, q, J=7.0, $OC\underline{H}_2CH_3$), 6.77 (1H, dd, J=8.5, 2.0, arom. 5-H), 7.84 (1H, d, J=2.0, arom. 3-H), 8.02 (1H, br s, NHCO), 8.09 (1H, d, J=8.5, arom. 6-H), 8.43 (1H, br s, CONH). EIMS m/z: 363 (M^+), 320 ($M^+ - COCH_3$). IR ν cm^{-1}: 3370, 3290, 1680, 1620. *Anal.* Calcd for $C_{18}H_{25}N_3O_5$: C, 59.49; H, 6.93; N, 11.56. Found: C, 59.24; H, 6.99; N, 11.47.

***N*-[(4-Acetyl-2-morpholinyl)methyl]-4-(acetylamino)-5-chloro-2-ethoxybenzamide (7)** A solution of **6** (60.0 g, 0.17 mol) and NCS (24.3 g, 0.18 mol) in *N*,*N*-dimethylformamide (DMF, 300 ml) was heated at 70 °C for 1 h. The reaction mixture was concentrated to dryness. The residue was triturated with water, and the resulting precipitates were collected by filtration and then recrystallized from MeOH to give 59.9 g (91%) of **7**, mp 204—205 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.52 (3H, t, J=7.0, $OCH_2C\underline{H}_3$), 2.10 (3H, s, $NCOCH_3$), 2.26 (3H, s, $NHCOC\underline{H}_3$), 2.5—4.05 (9H, m), 4.22 (2H, q, J=7.0, $OC\underline{H}_2CH_3$), 7.78 (1H, br s, NHCO), 8.22 (1H, s, arom. 3-H), 8.30 (1H, s, arom. 6-H), 8.37 (1H, br s, CONH). CIMS m/z: 398 (MH^+), 354 ($M^+ - COCH_3$), 240. IR ν cm^{-1}: 3380, 3260, 1690, 1665, 1635. *Anal.* Calcd for $C_{18}H_{24}ClN_3O_5$: C, 54.34; H, 6.08; Cl, 8.91; N, 10.56. Found: C, 54.15; H, 6.05; Cl, 9.07; N, 10.49.

4-Amino-5-chloro-2-ethoxy-*N*-(2-morpholinylmethyl)benzamide Hydrochloride (3) A suspension of **7** (25.0 g, 63 mmol) in 10% HCl (170 ml) was heated to reflux for 3 h and then cooled to *ca.* 5 °C. The resulting precipitates were collected by filtration and recrystallized from EtOH to give 16.3 g (83%) of **3** as slightly hygroscopic crystals, mp 220—222 °C. ^{1}H-NMR (DMSO-d_6) δ: 1.40 (3H, t, J=7.0, $OCH_2C\underline{H}_3$), 2.76 (1H, t-like, J=12.0, 3-H_{ax}), 2.95 (1H, td, J=12.0, 4.0, 5-H_{ax}), 3.1—4.1 (7H, m), 3.3 (s, H_2O), 4.08 (2H, q, J=7.0, $OC\underline{H}_2CH_3$), 5.99 (2H, s, NH_2), 6.49 (1H, s, arom. 3-H), 7.70 (1H, s, arom. 6-H), 8.09 (1H, t, J=5.5, CONH), 9.45 (2H, br s, $NH_2^+Cl^-$). SIMS m/z: 314 (MH^+). IR ν cm^{-1}: 3360, 1625. *Anal.* Calcd for $C_{14}H_{20}ClN_3O_3 \cdot HCl \cdot 1/4H_2O$: C, 47.40; H, 6.11; Cl, 19.99; N, 11.84. Found: C, 47.35; H, 6.06; Cl, 19.80; N, 11.73.

1-(Dibenzylamino)-3-phthalimido-2-propanol (9) A mixture of 98% *N*-(2,3-epoxypropyl)phthalimide (**8**, 149.4 g, 0.72 mol) and dibenzylamine (145.2 g, 0.74 mol) was heated at 80 °C for 20 h and then cooled to room temperature. The resulting solid was recrystallized from EtOH to give 284.3 g (95%) of **9**, mp 115—117 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.58 (1H, s, OH), 2.56 (2H, d, J=6.5, $(PhCH_2)_2NC\underline{H}_2$), 3.45 (2H, d, J=13.5, $PhC\underline{H}_2 \times 2$), 3.59 (1H, d, J=7.0, $C\underline{H}_2N(CO)_2$), 3.66 (1H, d, J=7.0, $C\underline{H}_2N(CO)_2$), 3.74 (2H, d, J=13.5, $PhC\underline{H}_2 \times 2$), 4.05 (1H, m), 7.15—7.45 (10H, m), 7.65—7.8, 7.8—7.9 (4H, m). SIMS m/z: 401 (MH^+), 210 $[CH_2N(CH_2Ph)_2]^+$. IR ν cm^{-1}: 3450, 1695. *Anal.* Calcd for $C_{25}H_{24}N_2O_3$: C, 74.98; H, 6.04; N, 7.00. Found: C, 74.76; H, 5.97; N, 6.93.

1-Amino-3-(dibenzylamino)-2-propanol (10) A mixture of **9** (55.9 g, 0.14 mol) and concentrated HCl (300 ml) was heated to reflux for 24 h and then cooled to 0 °C. The resulting precipitates were filtered off, and the filtrate was washed with $CHCl_3$ and then basified with 30% aqueous NaOH. The resulting precipitates were collected by filtration, washed with water, and recrystallized from toluene–*n*-hexane to give 31.7 g (84%) of **10**, mp 90—92 °C. ^{1}H-NMR ($CDCl_3$) δ: 1.95 (3H, br s, NH_2, OH), 2.35—2.8 (4H, m), 3.47 (2H, d, J=13.5, $PhC\underline{H}_2 \times 2$), 3.77 (2H, d, J=13.5, $PhC\underline{H}_2 \times 2$), 3.7 (1H, m), 7.15—7.45 (10H, m). CIMS m/z: 271 (MH^+), 210. *Anal.* Calcd for $C_{17}H_{22}N_2O$: C, 75.52; H, 8.20; N, 10.36. Found: C, 75.53; H, 8.29; N, 10.29.

1-[(Chloroacetyl)amino]-3-(dibenzylamino)-2-propanol (11) A solution of chloroacetyl chloride (5.0 g, 44 mmol) in $CHCl_2$ (20 ml) was added dropwise to a mixture of **10** (10.0 g, 37 mmol), 3% aqueous NaOH (100 ml), and $CHCl_3$ (80 ml) at such a rate that the temperature did not rise above 5 °C (about 15 min). The mixture was vigorously stirred at the same temperature for 1 h and then at room temperature for an additional 3 h. The organic layer was separated and washed successively with water and brine. The solvent was evaporated to leave a crude oil, which was chromatographed on silica gel with AcOEt : $CHCl_3$ = 1 : 1 to give 11.1 g (86%) of **11** as an oil. ^{1}H-NMR ($CDCl_3$) δ: 1.6 (1H, br s, OH), 2.48 (2H, d, J=6.5, $(PhCH_2)_2NC\underline{H}_2$), 3.14 (1H, dt, J=14.0, 6.0, $C\underline{H}_2NH$), 3.3—3.7 (2H, m), 3.82 (2H, s, CH_2COCl), 3.90 (2H, d, J=13.5, $PhC\underline{H}_2 \times 2$), 3.93 (2H, d, J=13.5, $PhC\underline{H}_2 \times 2$), 6.75 (1H, br s, CONH), 7.15—7.6 (10H, m). SIMS m/z: 347 (MH^+), 210. IR (neat) ν cm^{-1}: 3400, 3300, 1665.

2-[(Dibenzylamino)methyl]-5-oxomorpholine (12) A solution of **11** (10.5 g, 30 mmol) and potassium *tert*-butoxide (4.1 g, 37 mmol) in EtOH (200 ml) was heated to reflux for 10 h. The reaction mixture was concentrated to give a residue, which was chromatographed on silica gel with AcOEt : *n*-hexane = 1 : 1 to 2 : 1 to afford 1-[(ethoxyacetyl)amino]-3-(dibenzylamino)-2-propanol (**13**, 2.4 g, 22%) as an oil and **12** (5.0 g, 53%) as a solid in that order. Compound **12** was recrystallized from toluene.

Compound **12**: mp 111—112 °C. ^{1}H-NMR ($CDCl_3$) δ: 2.58 (1H, dd, J=6.5, 13.5, 3-H_{ax}), 2.68 (1H, dd, J=5.5, 13.5, 3-H_{eq}), 3.08 (1H, dd, J=10.5, 12.0, $C\underline{H}_2N$), 3.30 (1H, dt, J=12.0, 3.5, $C\underline{H}_2N(CH_2Ph)_2$), 3.52 (2H, d, J=13.5, $C\underline{H}_2Ph \times 2$), 3.75 (2H, d, J=13.5, $C\underline{H}_2Ph \times 2$), 3.7 (1H, m, 2-H), 4.04 (1H, d, J=17.0, 6-H_{ax}), 4.21 (1H, d, J=17.0, 6-H_{eq}), 6.15 (1H, br s, NH), 7.1—7.4 (10H, m). SIMS m/z: 311 (MH^+), 210. IR ν cm^{-1}: 3175, 3060, 2850, 2820, 1680. *Anal.* Calcd for $C_{19}H_{22}N_2O_2$: C, 73.52; H, 7.14; N, 9.03. Found: C, 73.33; H, 7.19; N, 8.98.

Compound **13**: ^{1}H-NMR ($CDCl_3$) δ: 1.17 (3H, t, J=7.0, $OCH_2C\underline{H}_3$), 1.6 (1H, br s, OH), 2.48 (2H, d, J=6.5, $(PhCH_2)_2NC\underline{H}_2$), 3.12 (1H, dt, J=14.0, 6.0, $C\underline{H}_2NH$), 3.2—3.7 (4H, m), 3.80 (2H, s, OCH_2CO), 3.83 (2H, d, J=13.5, $PhC\underline{H}_2 \times 2$), 3.85 (2H, d, J=13.5, $PhC\underline{H}_2 \times 2$), 6.82 (1H, br s, CONH), 7.1—7.5 (10H, m). SIMS m/z: 357 (MH^+), 210. IR (neat) ν cm^{-1}: 3400, 3300, 1665.

2-(Aminomethyl)-5-oxomorpholine (14) A solution of **12** (3.3 g, 11 mmol) in EtOH (150 ml) was hydrogenated over 10% palladium hydroxide-on-carbon (3.0 g) at room temperature and atmospheric pressure. After the theoretical amount of hydrogen was absorbed, the catalyst was filtered off. Then *ca.* 30% HCl in EtOH (1.9 g) was added to the filtrate, and the solvent was evaporated. The residual solid was recrystallized from iso-PrOH–Et_2O to give 1.4 g (79%) of **14**·HCl, mp 275—276 °C. ^{1}H-NMR (DMSO-d_6) δ: 2.75—3.45 (4H, m), 4.0 (1H, m), 3.99 (1H, d, J=16.0, 6-H_{ax}), 4.15 (1H, d, J=16.0, 6-H_{eq}), 8.10 (1H, d, J=4.0, CONH). EIMS m/z: 130 (M^+), 101. IR ν cm^{-1}: 3170, 1650. *Anal.* Calcd for $C_5H_{10}N_2O_2 \cdot HCl$: C, 36.05; H, 6.66; Cl, 21.28; N, 16.81. Found: C, 36.10; H, 6.67; Cl, 21.19; N, 16.66.

4-(Acetylamino)-5-chloro-2-ethoxy-*N*-[(5-oxo-2-morpholinyl)methyl]-benzamide (16) A mixture of 4-(acetylamino)-5-chloro-2-ethoxybenzoic acid[2a] (1.7 g, 6.6 mmol), thionyl chloride (2.4 g, 20 mmol), DMF (2 drops), and toluene (70 ml) was heated at 80 °C for 2 h. The solvent was evaporated, and the residue including 4-(acetylamino)-5-chloro-2-ethoxybenzoyl chloride (**15**) was dissolved in $CHCl_3$ (50 ml). To this solution was added dropwise a mixture of **14**·HCl (1.1 g, 6.6 mmol), Et_3N (2.8 g, 28 mmol), and $CHCl_3$ (100 ml) at 5 °C. The reaction mixture was stirred at room temperature for 5 h, then the solvent was evaporated, and the residue was triturated with water. The resulting precipitates were collected by filtration and recrystallized from MeOH to afford 1.7 g (70%) of **16**, mp 249—251 °C. ^{1}H-NMR (DMSO-d_6) δ: 1.40 (3H, t, J=7.0, $OCH_2C\underline{H}_3$), 2.15 (3H, s, $COCH_3$), 3.05—3.45 (3H, m), 3.5 (1H, m), 3.8 (1H, m), 4.07 (2H, s, 6-H), 4.12 (2H, q, J=7.0, $OC\underline{H}_2CH_3$), 7.79 (1H, s, arom. 3-H), 7.84 (1H, s, arom. 6-H), 8.01 (1H, d, J=4.0, 4-NH), 8.30 (1H, t, J=5.0, CONH), 9.56 (1H, s, $N\underline{H}COCH_3$). SIMS m/z: 370 (MH^+), 240. IR ν cm^{-1}: 3360, 3260, 1668, 1628. *Anal.* Calcd for $C_{16}H_{20}ClN_3O_5$: C, 51.97; H, 5.45; Cl, 9.59; N, 11.36. Found: C, 51.73; H, 5.39; Cl, 9.30; N, 11.24.

4-Amino-5-chloro-2-ethoxy-*N*-[(5-oxo-2-morpholinyl)methyl]benzamide (4) A) A mixture of **16** (1.1 g, 3.0 mmol), *ca.* 30% HCl in EtOH (20 ml), and 10% aqueous EtOH (50 ml) was heated to reflux for 1.5 h. The solvent was evaporated, and *ca.* 28% NH_4OH (10 ml) was added to the residue. The resulting precipitates were collected by filtration, washed with water, and recrystallized from MeOH to give 0.5 g (51%) of **4**, mp 212—214 °C. ^{1}H-NMR (DMSO-d_6) δ: 1.39 (3H, t, J=7.0,

$OCH_2C\underline{H}_3$), 3.0—3.4 (3H, m), 3.6 (1H, m), 3.8 (1H, m), 4.05 (2H, s, 6-H), 4.07 (2H, q, J=7.0, $OC\underline{H}_2CH_3$), 5.96 (2H, s, NH_2), 6.48 (1H, s, arom. 3-H), 7.71 (1H, s, arom. 6-H), 7.99 (1H, d, J=4.0, 4-NH), 8.10 (1H, t, J=5.0, CONH). SIMS m/z: 328 (MH^+), 198. IR ν cm^{-1}: 3345, 3340, 3200, 1670, 1620. *Anal.* Calcd for $C_{14}H_{18}ClN_3O_4$: C, 51.30; H, 5.54; Cl, 10.82; N, 12.82. Found: C, 51.15; H, 5.50; Cl, 10.58; N, 12.63.

B) A solution of 4-amino-5-chloro-2-ethoxybenzoic acid[2a] (1.0 g, 4.6 mmol) and *N,N'*-carbonyldiimidazole (0.83 g, 5.6 mmol) in tetrahydrofuran (THF, 50 ml) was stirred at room temperature for 2 h. This reaction mixture was added to a suspension of **14**·HCl (0.77 g, 4.8 mmol), Et_3N (0.7 g, 5.9 mmol), THF (20 ml), and DMF (5 ml) at room temperature. The whole was stirred at room temperature for 18 h. The solvent was evaporated, and the residue was triturated with 10% HCl. The resulting precipitates were collected by filtration, washed with water and dried to afford a solid, which was recrystallized from MeOH to give 1.0 g (77%) of **4**. This compound was confirmed to be identical with the sample obtained from A), on the basis of TLC, IR, ^{1}H-NMR comparisons.

Biological Method Male guinea pigs of the Hartley strain (Nihon Animals Co.) weighing 300—500 g were used. The guinea pigs were killed by a blow on the head. The distal ileum was removed at least 10 cm proximal to the cecum, and the longitudinal muscles with the mysenteric plexus (2—3 cm) were prepared according to the method of Craig and Clarke.[8] Each preparation was mounted between two parallel platinum wire electrodes in a 10 cm organ bath containing Krebs–Henseleit solution (NaCl 118 mM, KCl 4.75 mM, $CaCl_2$ 2.5 mM, KH_2PO_4 1.2 mM, $MgSO_4$ 1.2 mM, $NaHCO_3$ 2.5 mM, and glucose 10 mM). The solution was maintained at 37 °C and saturated with 95% O_2 and 5% CO_2. A resting tension of 1 g was applied, and the response was recorded isometrically through a force displacement transducer. The strips were equilibrated for 60 min. The strips were then stimulated through two parallel platinum electrodes with square-wave pulses (1 ms duration, supramaximal voltage, 0.2 Hz frequency) from an electrical stimulator. Therefore, the strips were treated with phenoxybenzamine (3×10^{-7} M) for 30 min. After washing out of the phenoxybenzamine several times, the stimulus voltage was adjusted to a submaximal voltage inducing approximately 50% of the maximal contractile response. Mosapride (**2**, as its citrate) and the metabolites **3** and **4**, dissolved in deionized water containing 1% lactic acid, were cumulatively applied.

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