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Short communication

Synthesis and gastroprokinetic activity of *N*-(4-amino-5-chloro-2-methoxyphenyl)-4-benzyl-2-morpholineacetamide and related compounds

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Summary — 4-Amino-*N*-[(4-benzyl-2-morpholinyl)methyl]-5-chloro-2-methoxybenzamide **1a** and its 2-ethoxy analogue **1b** show a potent gastroprokinetic activity. To examine the effect of reversal of the amide linkage of **1a** and **1b**, *N*-(4-amino-5-chloro-2-methoxyphenyl)-4-benzyl-2-morpholineacetamide and related compounds (**2** and **8–13**) were prepared and evaluated for gastroprokinetic activity by determining their effects on gastric emptying of a phenol red semisolid meal and a serotonin-4 receptor binding assay. Reversal of the amide bond resulted in a fall in activity; the amide bond of the 2-morpholinyl benzamides is essential for a potent gastroprokinetic activity. Molecular superimposition of **2c** upon **1b** using computer graphics suggested that the location of the morpholine ring and *N*-benzyl group is crucial for the activity.

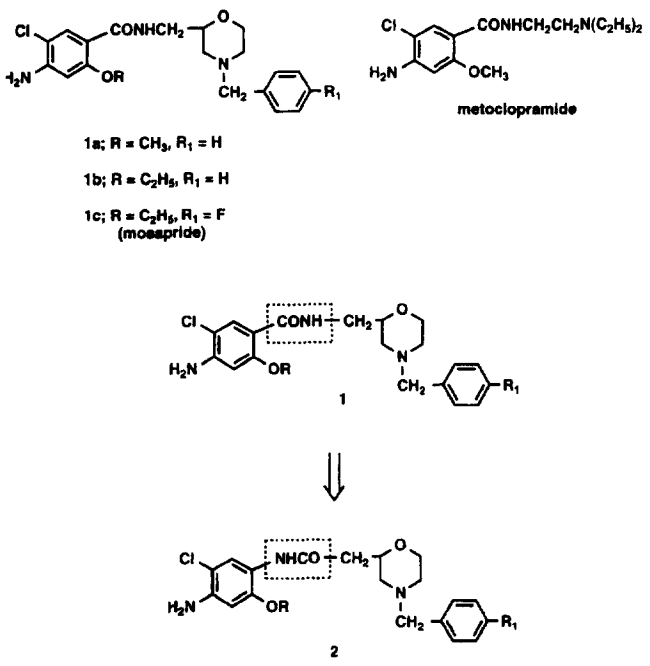
gastroprokinetic activity / 2-morpholineacetamide / molecular superimposition / mosapride / 2-morpholinyl benzamide

Introduction

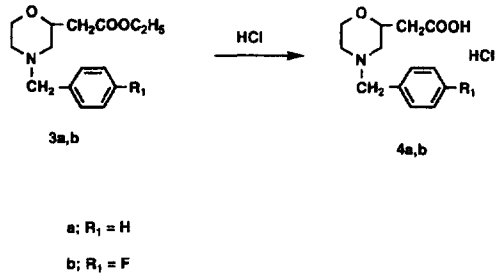
Our previous papers [1] reported that 4-amino-*N*-[(4-benzyl-2-morpholinyl)methyl]-5-chloro-2-methoxybenzamide **1a** and its 2-ethoxy analogue **1b** exhibited a potent gastroprokinetic activity without dopamine D_2 receptor antagonistic activity. These compounds showed more potent gastroprokinetic activity than the standard compound, metoclopramide. Subsequent modifications of the benzoyl moiety and the benzyl group of **1a** and **1b** led to the finding of 4-amino-5-chloro-2-ethoxy-*N*-[[4-(4-fluorobenzyl)-2-morpholinyl]methyl]benzamide (**1c**, mosapride) with more potent gastroprokinetic activity than **1a** and **1b** [2, 3]. The gastroprokinetic action of mosapride is accepted to be due to agonistic activity at a new serotonin receptor subtype (5-HT_4) [4, 5]. As an extension of that work, we have focused on replacing the benzamide moiety of 2-morpholinyl benzamides **1** with acetamide moiety (**1** → **2**) to define the structural requirements for gastroprokinetic activity of this series; the effect of reversal of amide group was investigated (scheme 1). This modification may cause a change not only in the electronic distribution in the region of the amide group and within the aromatic ring but also in the preferred stable conformations of the molecule. The present paper deals with synthesis and biological activity of *N*-(4-amino-5-chloro-2-methoxyphenyl)-4-benzyl-2-morpholineacetamide and its related compounds (**2** and **8–13**).

Chemistry

The known 2-morpholineacetates **3a** [6] and **3b** (Kato *et al*, submitted for publication) were hydrolyzed to give the requisite acetic acids **4a** and **4b**, respectively (scheme 2). A coupling reaction between the acetic acids **4a** and **4b** and 5-chloro-2-methoxyaniline was performed by using 1-ethyl-3-[3-(dimethylamino)-propyl]carbodiimide hydrochloride (WSC) to give the acetamides **5a** and **5b**, respectively. On the other hand, the 2-ethoxy analogue **5c** was prepared by the reaction of 5-chloro-2-hydroxyaniline with **4a**, followed by successive treatment of the resulting **6** with ethyl iodide. An amino group at the 4 position of compounds **5a–c** was then introduced; compound **5a–c** were nitrated with a mixed acid (fuming nitric acid and concentrated sulfuric acid) to give the 4-nitro derivatives **7a–c** in excellent yields, and subsequent reduction of **7a–c** with stannous chloride in concentrated hydrochloric acid gave the desired 4-amino derivatives **2a–c** (scheme 3, *Method A*).



Scheme 1.

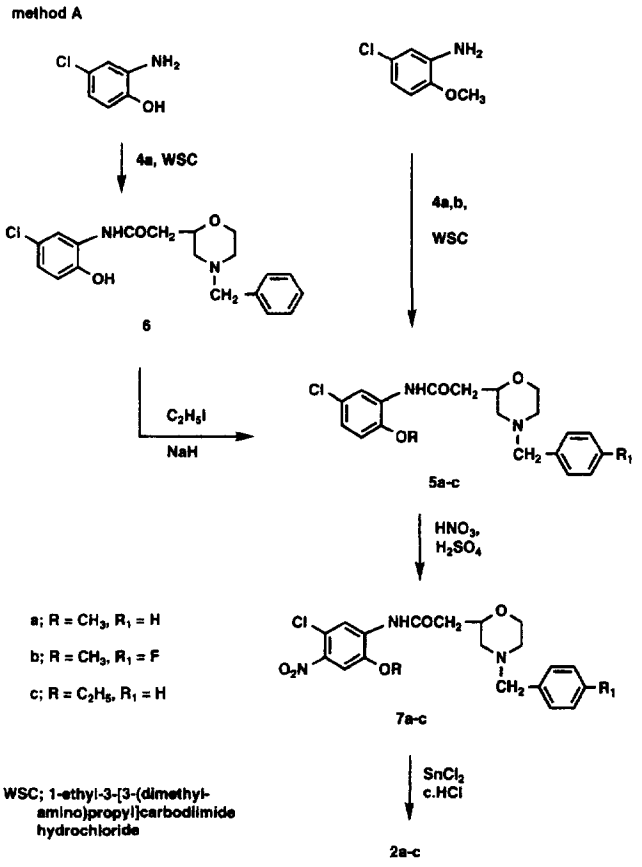


Scheme 2.

The 4-acetylamino analogues **9a–c** were derived from **2a–c** by acetylation with acetic anhydride (scheme 4, *Method B*). The reaction of **2a** with an appropriate acid chloride in the presence of triethylamine afforded the corresponding 4-substituted amino derivatives **10–13** in good yields (scheme 4, *Method C*). The treatment of **2a** with formic acid gave the 4-formylamino derivative **8**.

Biological results and discussion

Compounds **2** and **8–13** were evaluated for gastroprokinetic activity by determining their effects on the gastric emptying rates of a phenol red semisolid meal

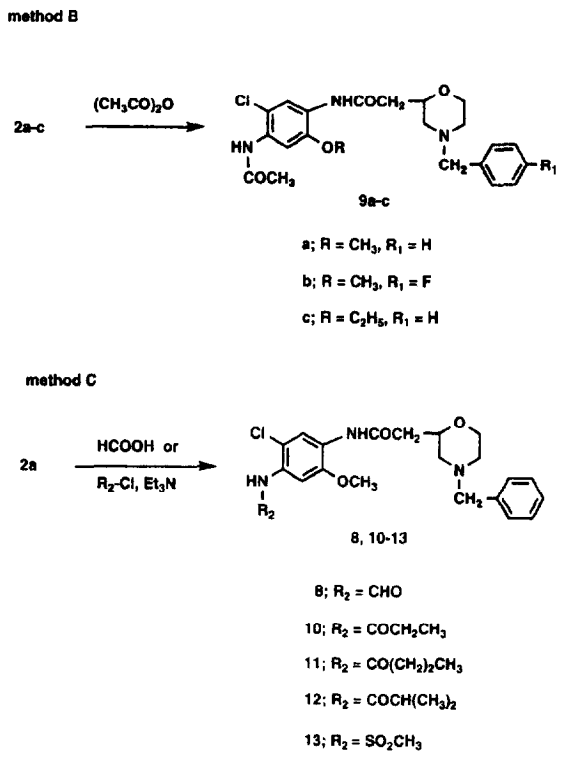


Scheme 3.

through the stomach. The biological data at an oral dose of 2.0 mg/kg in rats are given in table I, which includes, for comparison, data for 4-amino-*N*-[(4-benzyl-2-morpholinyl)methyl]-5-chloro-2-methoxybenzamide **1a**, its 2-ethoxy analogue **1b** [1] and metoclopramide.

Reversal of the amide bond of **1a** and **1b** considerably reduced the activity (**1a** (39%) *vs* **2a** (19%), **1b** (54%) *vs* **2b** (16%), and **1b** (54%) *vs* **2c** (22%)). This may be due to the electronic and/or conformational changes caused by amide reversal. Blaney *et al* [7] studied clebopride, which is stimulant of gastric motility and a potent central dopamine D_2 antagonist, and the corresponding amide reversal and reported similar results.

In a previous paper [2], we reported that introduction of a fluorine atom at the 4 position of the benzyl group of **1a** and **1b** and an acetyl group on the 4-amino group of **1c** caused an increase in activity. This finding led us to examine the effect of a fluorine atom or the substituent on the amino group of **2**. Compounds **9a** and **9c** with an acetyl group were



Scheme 4.

somewhat superior to metoclopramide. On the other hand, the formyl **8**, propionyl **10**, butyryl **11**, isobutyryl **12**, and methanesulfonyl **13** derivatives decreased the activity compared with **2a**. Introduction of a fluorine atom at benzyl group of **2a** and **9a** (giving **2b** and **9b**, respectively) also caused a decrease in activity. Compounds **8** and **9b** were inactive at this screening dose. Overall, we could not find a compound with a potent gastroprokinetic activity.

Compounds **1b** and **2c** were evaluated for 5-HT_4 receptor binding affinity *versus* [^{3}H]GR 113808. Compound **1b** showed high binding affinity (IC_{50} = 57 nM) compared to **2c** (IC_{50} > 1000 nM). These results suggest that the amide moiety of the morpholinyl benzamides is important for a potent gastroprokinetic activity (5-HT_4 receptor agonistic activity).

In order to get insight into the cause of the difference in activities between compounds **1b** and **2c**, first the electrostatic potentials of **1b** and **2c** were calculated. As is clear from figure 1, their electrostatic potentials are resemble each other. The three-dimensional structures of **1b** and **2c** were examined by computer-assisted superimposition (fig 2). The structure of **1b** derived from X-ray crystal structure of **1c** [8] was employed as a template for molecular superimpositions. Constructing the molecular model of compound **2c**, a systematic conformational search was carried out to find the most stable conformation of **2c**.

Table I. Effect of 2-morpholineacetamide derivatives (**2** and **8–13**) on gastric emptying of a phenol red semisolid meal in rats.

Compd	Gastric emptying rate[a] (%)		
	Control (mean ± S.E.M.) (N[b])	2.0 mg/kg, *p.o.* (mean ± S.E.M.) (N)	% change
2a	54.3 ± 1.4 (6)	64.6 ± 2.0 (4)	19[c]
2b	58.6 ± 2.9 (4)	67.8 ± 5.4 (4)	16
2c	54.3 ± 1.4 (6)	66.0 ± 4.9 (4)	22[c]
8	58.6 ± 2.9 (4)	58.7 ± 4.2 (4)	0
9a	45.0 ± 5.2 (6)	58.1 ± 3.3 (4)	29[d]
9b	58.6 ± 2.9 (4)	58.5 ± 3.5 (4)	0
9c	45.0 ± 5.2 (6)	57.2 ± 4.4 (4)	27[d]
10	58.6 ± 2.9 (4)	65.4 ± 11.2 (4)	12
11	58.6 ± 2.9 (4)	64.2 ± 5.2 (4)	10
12	58.6 ± 2.9 (4)	66.7 ± 5.4 (4)	14
13	58.6 ± 2.9 (4)	60.7 ± 5.4 (4)	4
1a	54.5 ± 3.8 (5)	75.9 ± 3.6 (4)	39[d]
1b	51.5 ± 3.1 (5)	79.5 ± 3.7 (4)	54[d]
metoclopramide	58.9 ± 2.1 (5)	72.3 ± 3.8 (5)	21[c]

[a]Each value represents the mean ± SEM. [b]Number of rats used. A statistically significant difference from the control group: [c]$p < 0.05$; [d]$p < 0.01$ (Duncan's multiple range test).

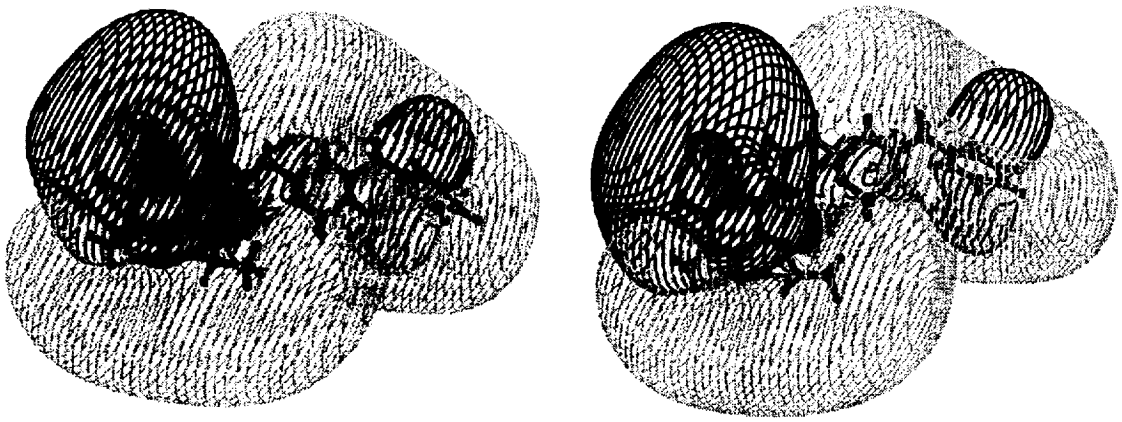


Fig 1. Electrostatic potentials of **1b** and **2c** based on the AM1 charge. Bold and dashed lines represent the isopotential contours at −1 and +1 kcal/mol, respectively.

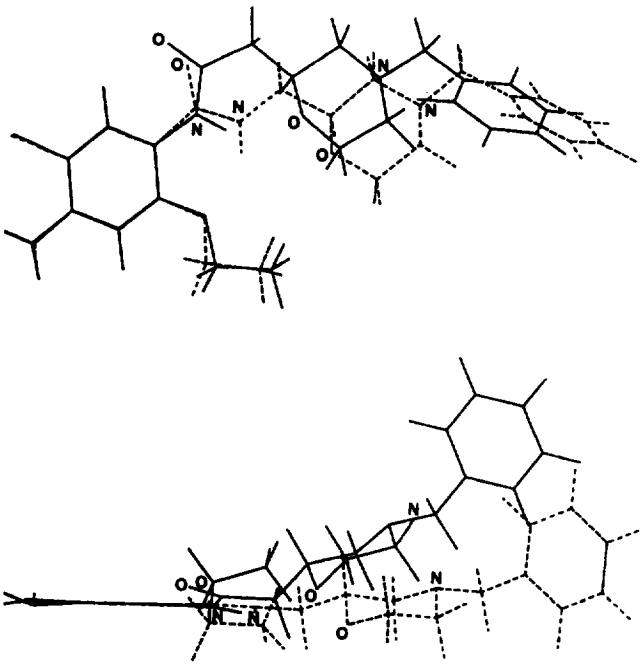
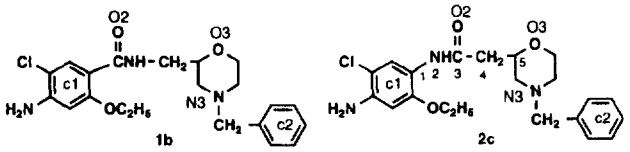


Fig 2. Orthogonal stereoscopic view of the superimposed structures **1b** (dashed lines) and **2c** (bold lines). The left-hand benzene ring was subjected to a least-squares fit.

Semiempirical molecular orbital calculations using AM1 method in MOPAC were performed for the structure **1b** and the selected conformer of **2c** with full geometry optimization to refine our molecular models (see *Experimental protocols* for details). Figure 2 shows the result of molecular superimposition, where the six carbon atoms in the left-hand benzene ring were superimposed. The distances between the points c1–O2, c1–O3, O2–N3, c1–N3, and c1–c2 as values specifying geometric features of **1b** and **2c** were measured and are listed in table II. The five points, c1, O2, O3, N3, and c2, were selected on the assumption that they are involved in the molecular interaction with the 5-HT_4 receptor. Comparing **1b** and **2c**, the *N*-benzyl group of **2c** extends to the same direction as that of **1b**. However, the overall spatial structures of the two molecules are somewhat different; every distance between c1–N3, c1–O3, O2–N3, and c1–c2 of **2c** is shorter than that of **1b** except for c1–O2. Thus, compound **2c** has a relatively compact and folded conformation compared to **1b**. Judging from these observations, the reason that **2c** is far less active may be attributable to an unfavorable location of its morpholine ring and *N*-benzyl group.

In summary, the reversal of the amide bond of **1a** and **1b** resulted in a fall in activity, and the modification of 2-morpholineacetamides provided no favorable influence on activity; it was found that the 2-alkoxy-4-amino-5-chlorobenzamide moiety was essential for a potent gastroprokinetic activity (5-HT_4 receptor agonistic activity). From the results of superimposition of the energy-minimized structure of **2c** upon that of **1b** and the electrostatic potentials of **1b** and **2c**, the reduction in activity caused by amide reversal of **1b** is probably due to conformational changes. In particular, the location of the morpholine ring and *N*-benzyl group have significant influences on activity.

Table II. Distances between c1–O2, c1–N3, c1–O3, O2–N3 and c1–c2 for energy-minimized conformations of **1b** and **2c**.



Compound	distances (Å)				
	c1—O2	c1—N3	c1—O3	O2—N3	c1—c2
1b	3.67	8.63	6.17	6.51	12.20
2c	4.03	7.90	5.43	5.86	11.18

The atom numbering adopted to use in the conformational search for **2c** is arbitrary, c1 and c2 denote the centers of the benzene ring.

Experimental protocols

Chemistry

All melting points were determined on a Yanagimoto micro-melting point apparatus and are uncorrected. IR spectra were recorded on a Hitachi 260-10 spectrometer with potassium bromide disks unless otherwise specified, and electron ionization mass spectra were recorded on a Jeol LMS D-300 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). Elemental analyses were within ±0.4% of the theoretical values. Organic extracts were dried over anhydrous magnesium sulfate or anhydrous sodium sulfate. The solvent was evaporated under reduced pressure. The physical data of compounds **2a–c** and **8–13** are shown in table III.

*4-Benzyl-2-morpholineacetic acid hydrochloride **4a***
A solution of ethyl 4-benzyl-2-morpholineacetate [6] (**3a**, 65.0 g, 0.25 mol) in 20% hydrochloric acid solution (200 ml) was heated to reflux for 6 h and then cooled to room temperature. The reaction mixture was concentrated to leave an oil, which was dissolved in acetone. The resulting precipitates were collected and recrystallized from 5% aqueous ethanol/ether to afford 65.5 g (98%) of **4a**, mp 200–206°C. ^{1}H-NMR (dimethylsulfoxide (DMSO)-d_6) δ: 2.29 (1H, dd, J = 8.0, 15.0, 3-H_{ax}), 2.60 (1H, d, J = 5.0, 5-H_{ax}), 2.7–3.2 (2H, m, $COCH_2$), 3.15–3.35 (2H, m, 5-H_{eq}, 3-H_{eq}), 3.7–4.1 (2H, m, $CH_2C_6H_5$), 4.1–4.5 (3H, m, 6-H, 2-H), 7.3–7.55, 7.55–7.8 (5H, m, arom H), 12.43 (1H, br s, COOH). IR ν cm^{-1}: 1710 (COOH). Anal $C_{13}H_{18}ClNO_3$ (C, H, Cl, N).

*4-(4-Fluorobenzyl)-2-morpholineacetic acid hydrochloride **4b***
In similar manner to that described above, **4b** was prepared from **3b** in 83% yield, mp 173–177°C (ethanol/ether). ^{1}H-NMR (DMSO-d_6) δ: 2.30 (1H, dd, J = 8.0, 15.0, 3-H_{ax}), 2.60 (1H, d, J = 5.0, 5-H_{ax}), 2.65–3.2 (2H, m, CH_2CO), 3.12–3.32 (2H, m, 5-H_{eq}, 3-H_{eq}), 3.7–4.05 (2H, m, $CH_2C_6H_4F$), 4.05–4.5 (3H, m, 6-H, 2-H), 7.15–7.4 (2H, m, arom H), 7.55–7.8 (2H, m, arom H), 12.44 (1H, br s, COOH). IR ν cm^{-1}: 1710 (COOH). Anal $C_{13}H_{17}ClFNO_3$ (C, H, Cl, F, N).

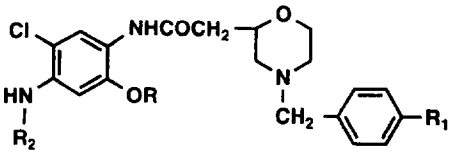
*2-Morpholineacetamide derivatives **2** and **8–13**. Method A. 4-Benzyl-N-(5-chloro-2-methoxyphenyl)-2-morpholineacetamide **5a***
A mixture of **4a** (3.0 g, 11 mmol), 5-chloro-2-methoxyaniline (2.0 g, 13 mmol), 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide hydrochloride (2.4 g, 13 mmol), and dichloromethane (60 ml) was stirred at room temperature for 6 h. The reaction mixture was washed successively with water, 10% potassium carbonate, water and brine, and concentrated to dryness. The residue was recrystallized from ethanol to afford 2.5 g (60%) of **5a**, mp 111–114°C. ^{1}H-NMR ($CDCl_3$) δ: 2.08 (1H, m, 3-H_{ax}), 2.3 (1H, m, 5-H_{ax}), 2.4–2.6 (2H, m, $COCH_2$), 2.70–2.90 (2H, m, 5-H_{eq}, 3-H_{eq}), 3.56 (2H, s, $CH_2C_6H_5$), 3.85 (3H, s, OCH_3), 3.7–4.15 (3H, m, 6-H, 2-H), 6.76 (1H, d, J = 8.5, arom 3-H), 6.98 (1H, dd, J = 2.5, 8.5, arom 4-H), 7.2–7.45 (5H, m, $CH_2C_6H_5$), 8.43 (1H, d, J = 2.5, arom 6-H), 8.97 (1H, br s, CONH). IR ν cm^{-1}: 3270, 1665, 1525. Anal $C_{20}H_{23}ClN_2O_3$ (C, H, Cl, N).

*N-(5-Chloro-2-methoxyphenyl)-4-(4-fluorobenzyl)-2-morpholineacetamide **5b***
In a similar manner to that described above, **5b** was prepared from **4b** and 5-chloro-2-methoxyaniline: mp 100–102°C (ethanol/hexane). ^{1}H-NMR ($CDCl_3$) δ: 2.00 (1H, t-like, J = 10.5, 3-H_{ax}), 2.22 (1H, td, J = 3.5, 11.5, 5-H_{ax}), 2.47–2.57 (2H, m, $COCH_2$), 2.62–2.82 (2H, m, 5-H_{eq}, 3-H_{eq}), 3.48 (2H, s, $CH_2C_6H_4F$), 3.85 (3H, s, OCH_3), 3.77 (1H, td, J = 2.5, 11.5, 6-H_{ax}), 3.88–4.05 (2H, m, 2-H, 6-H_{eq}), 6.76 (1H, d, J = 8.5, arom 3-H), 6.98 (1H, dd, J = 2.5, 8.5, arom 4-H), 6.95–7.07 (2H, m, C_6H_4F), 7.22–7.34 (2H, m, C_6H_4F), 8.43 (1H, d, J = 2.5, arom 6-H), 8.97 (1H, br s, CONH). IR ν cm^{-1}: 3250, 1665, 1530. Anal $C_{20}H_{22}ClFN_2O_3$ (C, H, Cl, F, N).

*4-Benzyl-N-(5-chloro-2-hydroxyphenyl)-2-morpholineacetamide **6***
In a manner similar to that described for **5a**, a solution of 5-chloro-2-hydroxyaniline (16.4 g, 11 mmol) and **4a** (31.0 g, 11 mmol) in dichloromethane was treated with 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide hydrochloride (21.8 g, 11 mmol) and then workup, giving 22.7 g (55%) of **6**, mp 188–191°C (acetone/hexane). ^{1}H-NMR ($CDCl_3$) δ: 2.04 (1H, t-like, J = 10.5, 3-H_{ax}), 2.28 (1H, td, J = 3.5, 11.0, 5-H_{ax}), 2.45–2.65 (2H, m), 2.65–2.85 (2H, m), 3.55 (2H, s, $CH_2C_6H_5$), 3.70 (1H, td, J = 3.0, 11.0, 6-H_{ax}), 3.90–4.10 (2H, m), 6.90 (1H, m), 7.00–7.10 (2H, m), 7.20–7.40 (5H, s, $CH_2C_6H_5$), 8.88 (1H, br s, CONH). IR ν cm^{-1}: 3255, 1640, 1535. Anal $C_{19}H_{21}ClN_2O_3$ (C, H, Cl, N).

*4-Benzyl-N-(5-chloro-2-ethoxyphenyl)-2-morpholineacetamide **5c***
To a solution of **6** (18.0 g, 50 mmol) in *N,N*-dimethylformamide (200 ml) was added sodium hydride (60% dispersion in mineral oil, 2.2 g, 55 mmol) under ice cooling, and the mixture was stirred at the same temperature for 1 h. Ethyl iodide (10.1 g, 65 mmol) was then added. The reaction mixture was stirred at room temperature for 6 h, poured into ice-water, and extracted with chloroform. The extract was washed successively with water and brine and concentrated to leave a solid, which was recrystallized from ethanol to afford 13.2 g (68%) of **5c**, mp 108–110°C. ^{1}H-NMR ($CDCl_3$) δ: 1.43 (3H, t, J = 7.0, OCH_2CH_3), 2.02 (1H, t-like, J = 10.5, 3-H_{ax}), 2.24 (1H, td, J = 11.5, 3.5, 5-H_{ax}), 2.46 (1H, dd, J = 3.5, 16.0, $COCH_2$), 2.59

Table III. Physical data for 2-morpholineacetamide derivatives (**2** and **9–14**).



Compd	R	R_1	R_2	Yield (%) Method[a]	mp (°C) (Recryst. solvent)	Formula
2a	CH_3	H	H	70 A	124—127 (ethanol)	$C_{20}H_{24}ClN_3O_3$
2b	CH_3	F	H	72 A	98—101 (toluene/hexane)	$C_{20}H_{23}ClFN_3O_3$[b]
2c	C_2H_5	H	H	76 A	114—116 (isopropanol)	$C_{21}H_{26}ClN_3O_3$
9	CH_3	H	CHO	75 e	145—147 (ethanol/hexane)	$C_{21}H_{24}ClN_3O_4$
10a	CH_3	H	MeCO	77 B	177—179 (acetone/hexane)	$C_{22}H_{26}ClN_3O_4$
10b	CH_3	F	MeCO	73 B	171—173 (acetone/ethanol)	$C_{22}H_{25}ClFN_3O_4$[c]
10c	C_2H_5	H	MeCO	81 B	146—148 (ethanol)	$C_{23}H_{28}ClN_3O_4$
11	CH_3	H	EtCO	70 C	144—148 (ethanol)	$C_{23}H_{28}ClN_3O_4$
12	CH_3	H	*n*-PrCO	87 C	151—154 (ethanol)	$C_{24}H_{30}ClN_3O_4$
13	CH_3	H	*iso*-PrCO	76 C	167—170 (acetone/ethanol)	$C_{24}H_{30}ClN_3O_4$
14	CH_3	H	CH_3SO_2	69 C	162—164 (ethanol)	$C_{21}H_{26}ClN_3O_5S$[d]

[a]Capital letters refer to the methods described in *Experimental protocols*. [b]Calcd for F: 4.66, Found: 4.58. [c]Calcd for F: 4.22, Found: 4.19. [d]Calcd for S: 6.85, found: 6.58. [e]See *Experimental protocols*.

(1H, dd, J = 8.0, 16.0, $COCH_2$), 2.65–2.85 (2H, m, 5-H_{eq}, 3-H_{eq}), 3.52 (2H, s, $CH_2C_6H_5$), 3.78 (1H, m), 3.90–4.1 (2H, m), 4.05 (2H, q, J = 7.0, OCH_2CH_3), 6.75 (1H, d, J = 8.5, arom 3-H), 6.95 (1H, dd, J = 2.5, 8.5, arom 4-H), 7.20–7.35 (5H, s, $CH_2C_6H_5$), 8.48 (1H, d, J = 2.5, arom 6-H), 9.04 (1H, br s, CONH). IR ν cm^{-1}: 3280, 1670, 1520. Anal $C_{21}H_{25}ClN_2O_3$ (C, H, Cl, N).

4-Benzyl-N-(5-chloro-2-methoxy-4-nitrophenyl)-2-morpholineacetamide 7a

To a stirred solution of acetic acid (100 ml) and concentrated sulfuric acid (5 ml) was added portionwise **5a** (14.6 g, 39 mmol) at room temperature. After complete addition, fuming nitric acid (d = 1.50, 2.1 ml) was added to the mixture, while the temperature was maintained at 20–30°C. The reaction mixture was stirred at room temperature for 3 h, poured into ice-water, and extracted with chloroform. The extract was washed successively with 10% sodium hydroxide solution, water, and brine and concentrated to leave a solid, which was recrystallized from ethanol to afford 15.4 g (94%) of **7a**, mp 97–98°C. ^{1}H-NMR ($CDCl_3$) δ: 2.05 (1H, m, 3-H_{ax}), 2.27 (1H, m, 5-H_{ax}), 2.4–2.65 (2H, m, $COCH_2$), 2.65–2.9 (2H, m, 5-H_{eq}, 3-H_{eq}), 3.53 (2H, s, $CH_2C_6H_5$), 3.96 (3H, s, OCH_3), 3.7–4.15 (3H, m, 6-H, 2-H), 7.2–7.4 (5H, m, $CH_2C_6H_5$), 7.52 (1H, s, arom 3-H), 8.67 (1H, s, arom 6-H), 9.40 (1H, br s, CONH). IR ν cm^{-1}: 3250, 1680, 1530, 1320. Anal $C_{20}H_{22}ClN_3O_5$ (C, H, Cl, N).

N-(5-Chloro-2-methoxy-4-nitrophenyl)-4-(4-fluorobenzyl)-2-morpholineacetamide 7b

In a similar manner to that described above, **7b** was prepared from **5b**, mp 149–150°C (acetone/hexane). ^{1}H-NMR ($CDCl_3$)

δ: 2.0 (1H, t-like, J = 10.0, 3-H_{ax}), 2.24 (1H, td, J = 3.5, 11.0, 5-H_{ax}), 2.4–2.6 (2H, m, CH_2CO), 2.65–2.85 (2H, m, 5-H_{eq}, 3-H_{eq}), 3.49 (2H, s, $CH_2C_6H_4F$), 3.95 (3H, s, OCH_3), 3.7–4.05 (3H, m, 6-H, 2-H), 6.93–7.08 (2H, m, C_6H_4F), 7.20–7.38 (2H, m, C_6H_4F), 7.52 (1H, s, arom 3-H), 8.68 (1H, s, arom 6-H), 9.39 (1H, br s, CONH). IR ν cm^{-1}: 3230, 1675, 1520, 1310. Anal $C_{20}H_{21}ClFN_3O_5$ (C, H, Cl, F, N).

4-Benzyl-N-(5-chloro-2-ethoxy-4-nitrophenyl)-2-morpholineacetamide 7c

In a similar manner to that described for **7a**, **7c** was prepared from **5c**, mp 128–130°C (ethanol). ^{1}H-NMR ($CDCl_3$) δ: 1.50 (3H, t, J = 7.0, OCH_2CH_3), 2.02 (1H, m), 2.27 (1H, m), 2.4–2.65 (2H, m), 2.65–2.9 (2H, m), 3.54 (2H, s $CH_2C_6H_5$), 3.7–4.15 (3H, m), 4.17 (2H, q, J = 7.0, OCH_2CH_3), 7.2–7.4 (5H, m, $CH_2C_6H_5$), 7.50 (1H, s arom 3-H), 8.72 (1H, s, arom 6-H), 9.39 (1H, br s, CONH). IR ν cm^{-1}: 3250, 1685, 1575, 1525. Anal $C_{21}H_{24}ClN_3O_5$ (C, H, Cl, N).

N-(4-Amino-5-chloro-2-methoxyphenyl)-4-benzyl-2-morpholineacetamide 2a

To a stirred solution of **7a** (12.4 g, 36 mmol) in acetic acid (100 ml) was added dropwise a solution of stannous chloride (24.8 g 0.11 mol) in concentrated hydrochloric acid solution (50 ml), while the temperature was maintained at 20–30°C. The mixture was stirred at room temperature for 15 h, poured into ice-water, basified with 20% sodium hydroxide solution, and extracted with chloroform. The extract was washed successively with water and brine and concentrated to leave a solid, which was recrystallized to give 9.8 g (70%) of **2a**. ^{1}H-NMR ($CDCl_3$) δ: 1.95–2.9 (6H, m), 3.56 (2H, s $CH_2C_6H_5$), 3.80 (3H, s, OCH_3), 3.91 (2H, s NH_2), 3.7–4.2 (3H, m), 6.31 (1H, arom 3-H), 7.15–7.45 (5H, s, $CH_2C_6H_5$), 8.26 (1H, s, arom 6-H), 8.57 (1H, br s, CONH). MS *m/z*: 389 (M^+), 358 ($M^+ - OCH_3$), 298 ($M^+ - CH_2C_6H_5$). IR ν cm^{-1}: 3455, 3355, 3275, 1655, 1520.

N-(4-Amino-5-chloro-2-methoxyphenyl)-4-(4-fluorobenzyl)-2-morpholineacetamide 2b

In a similar manner to that described above, **2b** was prepared from **7b**. ^{1}H-NMR ($CDCl_3$) δ: 1.9–2.6 (4H, m), 2.65–2.85 (2H, m, 5-H_{eq}, 3-H_{eq}), 3.52 (2H, s, $CH_2C_6H_4F$), 3.80 (3H, s, OCH_3), 3.65–4.1 (5H, m), 6.31 (1H, s, arom 3-H), 6.95–7.10 (2H, m, C_6H_4F), 7.23–7.37 (2H, m, C_6H_4F), 8.27 (1H, s, arom 6-H), 8.57 (1H, br s, CONH). MS *m/z*: 408 (M^+). IR ν cm^{-1}: 3460, 3330, 3250, 1645, 1530.

N-(4-Amino-5-chloro-2-ethoxyphenyl)-4-benzyl-2-morpholineacetamide 2c

In a similar manner to that described for **2a**, **2c** was prepared from **7c**. ^{1}H-NMR ($CDCl_3$) δ: 1.42 (3H, t, J = 7.0, OCH_2CH_3), 1.95–2.65 (4H, m), 2.72 (1H, br d, J = 13.0, 3-H_{eq}), 2.77 (1H, t-like, J = 13.0, 5-H_{eq}), 3.55 (2H, s $CH_2C_6H_5$), 3.65–4.2 (5H, m), 3.98 (2H, q, J = 7.0, OCH_2CH_3), 6.29 (1H, s arom 3-H), 7.2–7.4 (5H, m, $CH_2C_6H_5$), 8.31 (1H, s, arom 6-H), 8.68 (1H, br s, CONH). IR cm^{-1}: 3450, 3355, 3295, 1655, 1525.

Method B. N-[4-(Acetylamino)-5-chloro-2-methoxyphenyl]-4-benzyl-2-morpholineacetamide 9a

A mixture of **2a** (4.0 g 10 mmol) and acetic anhydride (30 ml) was stirred at room temperature for 20 h. The reaction mixture was poured into ice-water, basified with 20% sodium hydroxide solution, and extracted with chloroform. The extract was washed successively with water and brine and concentrated to leave a solid, which was recrystallized from acetone/hexane to afford 3.4 g of **9a**. ^{1}H-NMR ($CDCl_3$) δ: 2.05 (1H, m), 2.23 (3H, s, $COCH_3$), 2.25 (1H, m), 2.45–2.6 (2H, m), 2.65–2.85 (2H, m, 5-H_{eq}, 3-H_{eq}), 3.55 (2H, s, $CH_2C_6H_5$), 3.88 (3H, s, OCH_3), 3.7–4.1 (3H, m), 7.2–7.4 (5H, m, $CH_2C_6H_5$), 7.59 (1H, br s, $NHCOCH_3$), 8.10 (1H, s, arom 3-H), 8.47 (1H, s, arom 6-H), 8.94 (1H, br s, CONH). IR ν cm^{-1}: 3280, 1645, 1535.

Method C. 4-Benzyl-N-[5-chloro-2-methoxy-4-(propionylamino)-phenyl]-2-morpholineacetamide 10

To a solution of **2a** (3.0 g, 7.7 mmol) and triethylamine (0.9 g, 8.9 mmol) in chloroform (50 ml) was added dropwise a solution of propionyl chloride (0.8 g, 8.6 mmol) in chloroform (10 ml) at 5°C. The mixture was stirred at same temperature for 1 h and then room temperature for 5 h. The reaction mixture was washed successively with water, 10% sodium hydroxide solution, water and brine, and concentrated to leave a solid, which was recrystallized from ethanol to afford 2.4 g of **10**. ^{1}H-NMR ($CDCl_3$) δ: 1.27 (3H, t, J = 7.0, $COCH_2CH_3$), 2.05 (1H, m), 2.20 (1H, m), 2.35–2.6 (4H, m), 2.65–2.85 (2H, m, 5-H_{eq}, 3-H_{eq}), 3.55 (2H, s, $CH_2C_6H_5$), 3.7–4.1 (3H, m), 3.88 (3H, s, OCH_3), 7.2–7.4 (5H, m, $CH_2C_6H_5$), 7.61 (1H, br s, $NHCOCH_2CH_3$), 8.17 (1H, s, arom 3-H), 8.47 (1H, s, arom 6-H), 8.94 (1H, br s, CONH). IR ν cm^{-1}: 3275, 1650, 1530.

4-Benzyl-N-[5-chloro-4-(formylamino)-2-methoxyphenyl]-2-morpholineacetamide 8

A mixture of **2a** (3.0 g, 7.7 mmol) and formic acid (30 ml) was heated at 95–100°C for 1 h. The reaction mixture was poured into ice-water, basified with 20% sodium hydroxide solution, and extracted with chloroform. The extract was washed successively with water and brine and concentrated to leave a solid, which was recrystallized from ethanol/hexane to afford 2.4 g of **8**. ^{1}H-NMR ($CDCl_3$) δ: 2.04 (1H, t-like, J = 10.5, 3-H_{ax}), 2.25 (1H, td, J = 3.0, 11.0, 5-H_{ax}), 2.51 (1H, dd, J = 4.0, 15.5, $COCH_2$), 2.53 (1H, dd, J = 7.5, 15.5, $COCH_2$), 2.67–2.87 (2H, m, 5-H_{eq}, 3-H_{eq}), 3.53 (2H, s, $CH_2C_6H_5$), 3.90 (3H, s, OCH_3), 3.7–4.1 (3H, m), 7.2–7.4 (5H, m, $CH_2C_6H_5$), 7.66 (1H, br s, NHCHO), 8.12 (1H, s, arom 3-H), 8.45 (1H, d, J = 2.0, CHO), 8.49 (1H, s, arom 6-H), 8.98 (1H, br s, CONH). IR ν cm^{-1}: 3250, 1655, 1530.

Pharmacology

Male rats of Wistar strain (Japan SLC Inc) weighing 130–150 g were used. The rats were fasted for 18 h before the experiment. A test meal (0.05% phenol red in 1.5% aqueous methylcellulose solution) of 1.5 ml per rat was given through a gastric tube. Fifteen minutes later, the animals were sacrificed. The stomach was removed, and the amount of phenol red remaining in it was measured according to the method of Scarpignato *et al* [9]. The test compounds, suspended in a 0.5% tragacanth solution, were orally administered 60 min before administration of the test meal.

5-HT_4 receptor binding assay

The binding assay was carried out according to the method of Grossman *et al* [10]. All determinations were performed in triplicate. Assay tubes contained 300 μl HEPES buffer at pH 7.4, 200 μl of a solution of either a competing agent (for drug competition studies), 5-HT 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 (1 x 4 ml) with ice-cold buffer through Whatman GF/B filter paper using a Brandel Cell Harvester. Filters were presoaked in a solution of polyethylenimine (~ 0.1%) to reduce filter binding. For drug competition studies, assay tubes were incubated at 37°C for 30 min and the reaction terminated as above. Filters were placed in 10 ml ACS II scintillator (Amersham) before scintillation counting.

Electrostatic potential

The value of electrostatic potential V at a grid p surrounding molecule in a medium dielectric constant ε is given by the classical formula

$$V = 332 \sum_i \left(\frac{Q_i}{\varepsilon \bullet | r_i - p |} \right)$$

where $| r_i - p |$ is a distance between point p and ith atom with charge Q_i; a distance-dependent dielectric constant, $\varepsilon = | r_i - p |$, was used. Net atomic charges obtained from AM1 calculation were used to represent Q_i. The isopotential surfaces of electrostatic potential are shown for two contour levels of potential in figure 1.

Molecular modeling

The molecular modeling of compounds **1b** and **2c** was performed using Sybyl version 5.5 [11] on a Silicon Graphics IRIS 4D/35TG workstation. Computational procedures are as follows. The initial atomic coordinate of structure **1b** for molecular orbital calculation was obtained by replacement of fluorine atom in X-ray crystal structure of **1c** [8] by hydrogen. The AM1 [12] calculation with geometry optimization by Mopac version 5.0 [13] for all geometric variables was carried out with the keyword MMOK. This optimized structure was used as a template for the molecular superimposition with structure **2c**. The molecular model of **2c** was built using three-dimensional fragments, 4-amino-5-chloro-2-ethoxybenzamide and *N*-benzyl morpholine moieties in the X-ray crystal structure of **1c**; the other remaining substructure was constructed with standard bond lengths and bond angles. After energy minimization using Maximin2 routine implemented in Sybyl neglecting the electrostatic term of Tripos force field [14], a systematic conformational search with energy calculation was carried out for the three bonds, 1(C)-2(N), 3(C)-4(C), and 4(C)-5(C) of **2c**, for all torsional angles in the range of 0–360° with increments of 10, 30 and 30°, respectively (see table II for atom labeling). We selected the most stable conformer from 724 rotamers (E_{min} = 7.64 kcal/mol, E_{max} = 18.14 kcal/mol) generated from this conformational analysis. Finally, semiempirical molecular orbital calculation of the selected conformer using Mopac/AM1 with full geometry optimization was done to refine our molecular model of compound **2c**. Molecular superimposition was performed with the Fit command in Sybyl.

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