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Design and Synthesis of Orally Active Benzamide Derivatives as Potent Serotonin 4 Receptor Agonist

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Abstract—A series of 4-amino-5-chloro-2-methoxy-*N*-(piperidin-4-ylmethyl)benzamide derivatives bearing an aralkylamino, alkylamino, benzoyl or phenylsulfonyl group at its side chain part at the 1-position on the piperidine ring was synthesized. They were evaluated for serotonin **4** (5-HT_4) receptor agonist activity by testing their ability to contract the isolated guinea-pig ascending colon. 4-Amino-5-chloro-2-methoxy-*N*-[1-[5-(1-methylindol-3-ylcarbonylamino)pentyl]piperidin-4-ylmethyl]benzamide (**1a**, Y-34959) and its related compounds possessed favorable pharmacological profiles for gastrointestinal motility. Unfortunately, the compound **1a** showed low bioavailability when given orally presumably due to its poor intestinal absorption rate. Replacement of the 1-methylindol-3-yl carbonylamino moiety of **1a** with an aralkylamino (or alkylamino) group did not improve the intestinal absorption rate. Replacement of the 1-methylindol-3-ylcarbonylamino moiety with a benzoyl or phenylsulfonyl group increased the intestinal absorption rate compared with **1a**. These compounds revealed good pharmacological profiles for gastrointestinal motility and were superior to **1a** in oral bioavailability.

Introduction

Serotonin **4** (5-HT_4) receptor, which was discovered by Dumuis et al. in 1989,[1] regulates cAMP concentration in neuron synapse, and contracts intestinal smooth muscle.[2,3] It is known that the stimulation of this receptor exerts activation of gastric motility, ileal motility and colonic transit.

Benzamides (metoclopramide,[4] cisapride,[5] etc.) have been used clinically for the treatment of gastric disorder, gastroesophageal reflux disease (GERD) and intestinal pseudoobstruction, and characterized as the 5-HT_4 receptor agonist. However, these benzamides have the binding affinities for not only the 5-HT_4 receptor but also the dopamine D_2, serotonin 5-HT_2 and 5-HT_3 receptors.[6] Antagonisms against the dopamine D_2, serotonin 5-HT_2 and 5-HT_3 receptors would reduce the prokinetic effect and cause unfavorable side effects.[7] Therefore, we have made an effort to find out selective and potent 5-HT_4 receptor agonists without other receptor binding affinities.[7,8]

In our previous papers, we reported a new type of benzamides as the 5-HT_4 receptor agonists.[7,8] The benzamide derivative, 4-amino-5-chloro-2-methoxy-*N*-[1-[5-(1-methylindol-3-yl carbonylamino)pentyl]piperidin-4-ylmethyl]benzamide (**1a**, Y-34959) and its related compounds

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demonstrated good pharmacological profiles. Those compounds also showed very high and selective binding affinity for the 5-HT_4 receptor, and had potent agonistic activities. Accordingly, we proposed that **1a** was a novel gastrointestinal motility stimulant which can enhance both upper and lower gastrointestinal motility with few side effects.[8]

Unfortunately, compound **1a** showed low oral bioavailability (5.1%) in dogs.[9] Cisapride reveals higher oral bioavailability in dogs (53%).[6] We assumed that compound **1a** revealed poor oral bioavailability because it has a 1-methylindol-3-ylcarbonylamino frame (A part) on the terminal of the straight alkyl chain at the 1-position on the piperidine ring as shown in Figure 1. On the other hand, cisapride has a phenoxy frame at the corresponding part. Therefore, modification of the 1-methylindol-3-ylcarbonylamino moiety (A part) would improve the oral absorption although we described the importance of the 1-methylindol-3-ylcarbonylamino for 5-HT_4 receptor agonistic activity in previous paper.[8]

Here, we replaced the 1-methylindol-3-ylcarbonylamino moiety with the aralkylamino (or alkylamino) group, benzoyl or phenylsulfonyl group for improvement of oral bioavailability. (It is known that alkylamine and carbony groups are useful as a bioisostere of an amide bond.[10,11]) As a result we discovered derivatives with

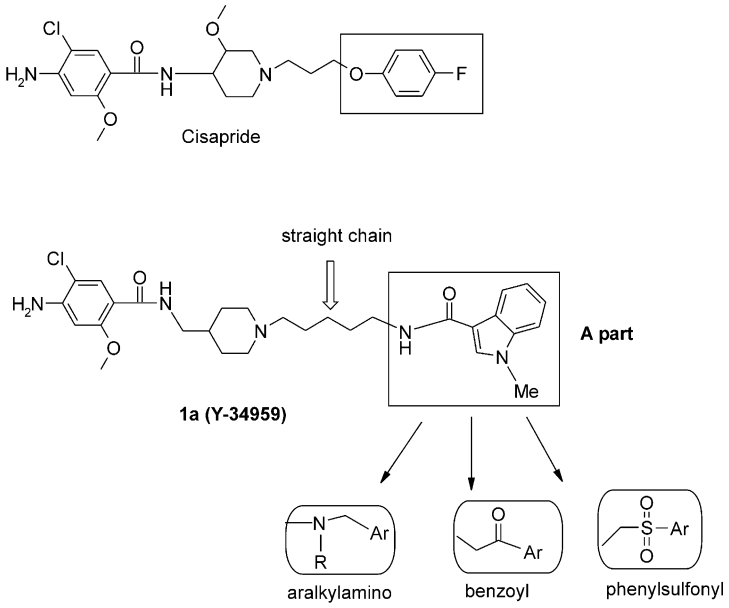
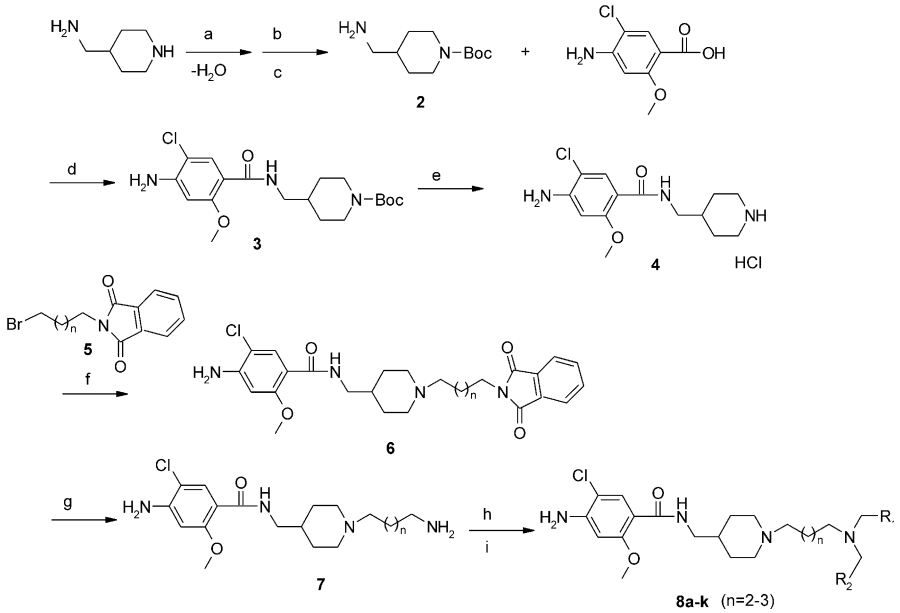
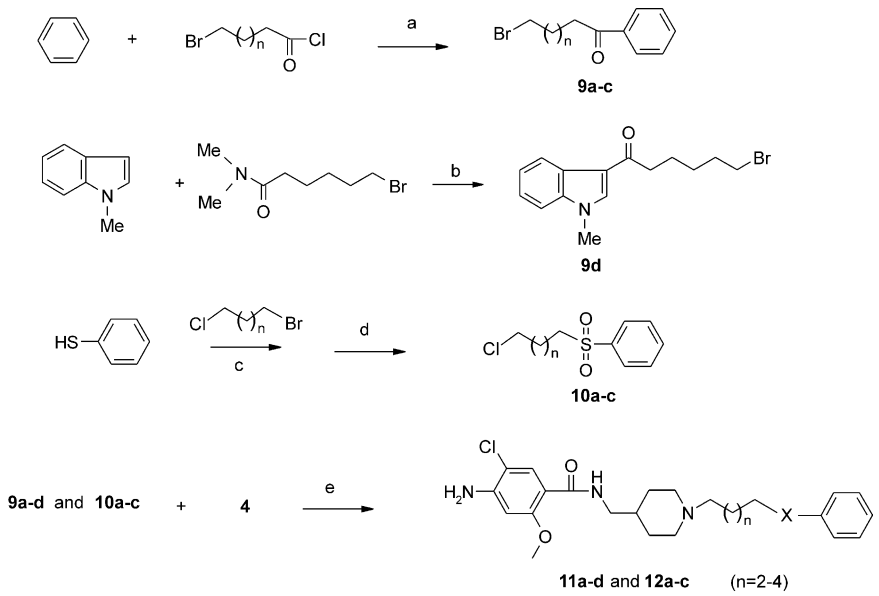


Figure 1. Design of orally active benzamine derivatives.

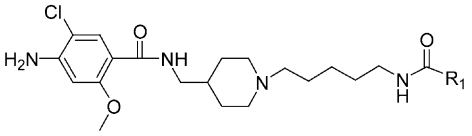


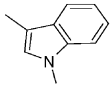
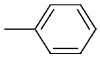
Scheme 1. Reagents: (a) benzaldehyde, toluene, reflux; (b) $(Boc)_2O$; (c) $KHSO_4$, H_2O, rt, 12 h (92% in three steps); (d) EDC, HOBT, Et_3N, 25 °C, 24 h (92%); (e) HCl/dioxane, 25 °C, 4 h (82%); (f) K_2CO_3, DMF, 70–75 °C; (g) NH_2NH_2–H_2O, EtOH, reflux, 6 h; (h) R_1CHO,$NaBH_3CN$; (i) R_2CHO, $NaBH_3CN$, EtOH or $NaBH_4$, EtOH.



Scheme 2. Reagents: (a) $AlCl_3$, CH_2Cl_2, 25 °C; (b) $POCl_3$, $CHCl_3$, reflux (38%); (c) K_2CO_3, DMF, 50–60 °C; (d) *m*-CPBA or H_2O_2, HCOOH; (e) K_2CO_3, DMF, 70–75 °C, 5–12 h.

Table 1. Pharmacological data of **1a** and **1b**



Compd	R1	Binding affinities[a]		Potency[b]	
		5-HT4, K_i (nM)	5-HT3, IC_{50} (nM)	EC_{50} (nM)[c]	% Absorption[d]
1a (Y-34959)		0.3	> 1000	1.2	6.9
1b		1.7	> 1000	3.7	18.9
Cisapride		70	200	29	97.0

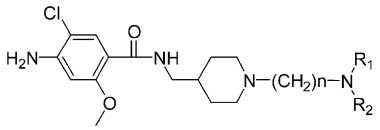
[a]Each value is the mean from triplicate assay in a single experiment.
[b]5-HT4 receptor agonistic activities; contractile effects in guinea pigs ascending colon.
[c]EC_{50} values were determined by linear regression.
[d]Intestinal absorption rate.

good oral bioavailability, very high and selective binding affinity for the 5-HT_4 receptor, and potent 5-HT_4 receptor agonistic activity. This paper outlines the preparation of aralkylamino (or alkylamino), benzoyl, and phenylsulfonyl derivatives, as well as pharmacological profiles for gastrointestinal motility and pharmacokinetic properties of compounds.

Chemistry

The preparation of all compounds described in this paper is illustrated in Schemes 1 and 2. 4-(Aminomethyl)piperidine was treated with benzaldehyde for protection of the primary amino group at the 4-position to give a benzylidene derivative, which was reacted with di-*tert*-butyldicarbonate followed by aqueous $KHSO_4$ to deprotect the benzylidene group to give 4-aminomethyl-1-(*tert*-butoxycarbonyl) piperidine (**2**).[11] The compound **2** was condensed with 4-amino-5-chloro-2-methoxybenzoic acid using 1-ethyl-3-[3-(dimethylamino)-propyl]carbodiimide hydrochloride (EDC) and 1-hydroxybenzotriazole (HOBT). The resulting **3** was treated with HCl-dioxane to afford 4-amino-5-chloro-2-methoxy-*N*-(piperidin-4-ylmethyl)benzamide hydrochloride (**4**). The compound **4** was converted into **6** by treating with **5**. Compound **7** was obtained from compound **6** by treating with hydrazine hydrate for the removal of the phthaloyl group. The secondary (or tertiary) amine derivatives **8a–k** were prepared by reductive amination of the primary amine derivative **7** with the corresponding aldehydes using $NaBH_4$ or $NaBH_3CN$.

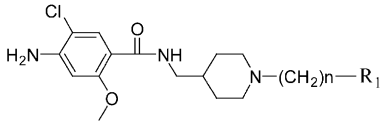
Table 2. Pharmacological data of aralkylamino and alkylamino derivatives **8a–k**

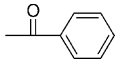
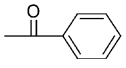
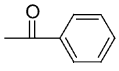
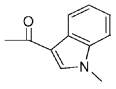
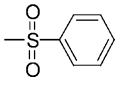
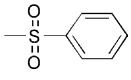
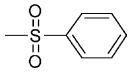


Compd	*n*	R1	R2	Binding affinities[a]		Potency[b]	
				5-HT4, K_i (nM)	5-HT3, IC_{50} (nM)	EC_{50} (nM)[c]	% Absorption[d]
8a	4	3,4-Dichlorobenzyl	Et	0.62	>1000	No effect	47.5
8b	5	Benzyl	H	1.7	>1000	3.7	26.5
8c	5	Benzyl	Me	3.5	>1000	29.9	24.8
8d	5	Benzyl	Propyl	0.84	>1000	12.0	33.6
8e	5	4-Methoxybenzyl	Et	1.2	>1000	9.2	8.3
8f	5	2-Thienylmethyl	H	2.8	>1000	22.3	9.3
8g	5	2-Thienylmethyl	Ethyl	2.2	>1000	62.5	57.9
8h	5	1-Naphthylmethyl	Et	2.7	>1000	54.0	28.3
8i	5	2-Naphthylmethyl	Et	1.6	>1000	6.7	21.6
8j	5	Cylohexylmethyl	H	0.51	>1000	9.0	NT[e]
8k	5	Cylohexylmethyl	Benzyl	1.1	>1000	No effect	NT[e]

[a]Each value is the mean from triplicate assay in a single experiment.
[b]5-HT4 receptor agonistic activities; contractile effects in guinea pigs ascending colon.
[c]EC_{50} values were determined by linear regression.
[d]Intestinal absorption rate.
[e]NT, not tested.

Table 3. Pharmacological data of benzoyl and phenylsulfonyl derivatives



Compd	*n*	R1	Binding affinities[a]		Potency[b]	
			5-HT4, K_i (nM)	5-HT3, IC_{50} (nM)	EC_{50}(nM)[c]	% Absorption[d]
11a	4		4.0	>1000	9.1	87.0
11b	5		2.4	>1000	10.0	84.9
11c	6		1.5	>1000	6.5	61.6
11d	5		0.34	>1000	4.3	26.0
12a	4		3.0	>1000	12.0	71.6
12b	5		1.4	>1000	6.3	60.4
12c	6		3.4	>1000	6.5	45.1

[a]Each value is the mean from triplicate assay in a single experiment.
[b]5-HT4 receptor agonistic activities; contractile effects in guinea pigs ascending colon.
[c]EC_{50} values were determined by linear regression.
[d]Intestinal absorption rate.

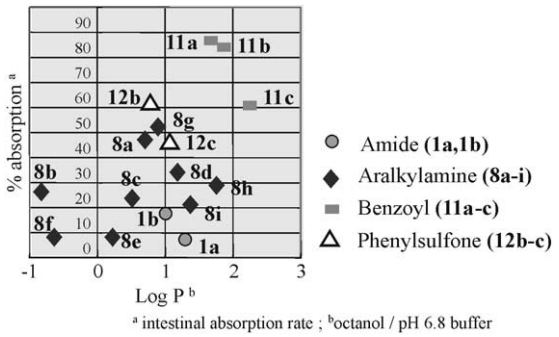


Figure 2. Relationship between logP and % absorption of compounds. Physical properties and spectral data of the compounds are given in Tables 4–6.

The benzoyl type compounds **11a–d** and phenylsulfonyl type compounds **12a–c** were synthesized by coupling reaction of compound **4** with compounds **9a–d** and **10a–c** in K_2CO_3–DMF, respectively. The compounds **9a–c** were prepared by Friedel–Craft reaction. The indole derivative **9d** were prepared using phosphorus oxychloride by the Vilsmeier–Haack reaction of 1-methylindole with 6-bromoheptyl-*N*,*N*-dimethylamide. The compounds **10a–c** were synthesized by coupling of benzenethiol and bromochloroalkanes followed by oxidation with *m*-CPBA or H_2O_2/HCOOH.

Result and discussion

Synthesized compounds were evaluated for 5-HT_4 receptor binding affinity by [^{3}H]GR-113808 binding assay in guinea-pig striatum membranes[12] and for in vitro 5-HT_4 receptor agonistic activities (EC_{50} values) by their ability to contract isolated guinea-pig ascending colon.[13] The compounds were evaluated for 5-HT_3 receptor binding affinity in rat cerebrocortical membranes by [^{3}H] Granisetron binding.[13] The bioavailabilities were determined using male dogs.[9,14] The intestinal absorption rate (% absorption) was evaluated using the in situ rat loop method in which the absorption rate of compounds was measured 2 h after the injection. 1-Methylindol-3-yl carbonylamino derivative 1a showed low oral bioavailability (5.1%) in dogs.[9] In our previous report, when compound 1a was given intravenously to dogs at 0.01 mg/kg, the motility of gastric antrum and ascending colon was clearly enhanced,[8] suggesting that compound **1a** was relatively stable in plasma. The intestinal absorption rate of **1a** (6.9%) was much lower than that of cisapride (97.0%) as shown in Table 1. Therefore, low oral bioavailability of **1a** would be due to its poor intestinal absorption rate rather than susceptibility to metabolism. Accordingly, the pharmocokinetic evaluation of compounds started with measuring intestinal

Table 4. Physical data of compounds

Compd	Formula	Elemental analysis (%) found (calculated)			
		C	H	N	MP (°C)
8a	$C_{27}H_{37}N_4O_2Cl_3 \cdot 3/2C_2H_2O_4 \cdot 1/2H_2O$	51.56 (51.47	5.94 5.9	8.00 8.00)	201–202
8b	$C_{26}H_{37}N_4O_2Cl \cdot 1/2H_2O$	64.98 (64.78	7.77 7.95	11.60 11.62)	Amorphous
8c	$C_{27}H_{39}N_4O_2Cl \cdot 6/5H_2O$	63.47 (63.75	7.92 8.20	11.01 11.01)	Amorphous
8d	$C_{29}H_{43}N_4O_2Cl \cdot 1/2H_2O$	66.15 (66.45	8.48 8.46	10.67 10.69)	Amorphous
8e	$C_{29}H_{43}N_4O_3Cl \cdot 2C_2H_2O_4 \cdot H_2O$	54.06 (54.35	6.88 6.77	7.39 7.68)	Amorphous
8f	$C_{24}H_{35}N_4O_2SCl \cdot 7/4H_2O$	56.14 (56.45	7.23 7.6	10.76 10.97)	67–69
8g	$C_{26}H_{39}N_4O_2SCl \cdot 2C_2H_2O_4 \cdot 3/2H_2O$	50.66 (50.45	6.28 6.49	7.93 7.84)	104–108
8h	$C_{32}H_{43}N_4O_2Cl \cdot 2H_2O$	65.23 (65.45	7.87 8.07	9.65 9.54)	Amorphous
8i	$C_{32}H_{43}N4O_2Cl_2/5H_2O$	68.97 (68.83	7.81 7.91	10.04 10.03)	Amorphous
8j	$C_{26}H_{43}N_4O_2Cl \cdot 2C_2H_2O_4 \cdot H_2O$	53.18 (53.21	7.16 7.29	8.26 8.27)	125–129
8k	$C_{33}H_{49}N_4O_2Cl \cdot H_2O$	67.61 (67.49	8.69 8.75	9.44 9.54)	Amorphous
11a	$C_{25}H_{32}N_3O_3Cl \cdot 4/5H_2O$	63.58 (63.56	7.10 7.17	8.84 8.89)	100–102
11b	$C_{26}H_{34}N_3O_3Cl \cdot 2/5H_2O$	65.06 (65.16	7.19 7.32	9.05 8.77)	138–141
11c	$C_{27}H_{36}N_3O_3Cl \cdot 5/4H_2O$	63.88 (63.77	7.49 7.63	8.29 8.26)	128–130
11d	$C_{29}H_{37}N_4O_3Cl \cdot 1/5H_2O$	65.98 (65.88	7.20 7.13	10.56 10.60)	125–127
12a	$C_{24}H_{32}N_3O_4SCl \cdot HCl \cdot 1/2H_2O$	53.57 (53.43	6.39 6.35	7.71 7.79)	201–202
12b	$C_{25}H_{34}N_3O_4SCl \cdot HCl \cdot H_2O$	55.56 (53.38	6.61 6.63	7.51 7.47)	114–117
12c	$C_{26}H_{36}N_3O_4SCl \cdot HCl \cdot 5/2H_2O$	51.96 (51.74	6.83 7.01	6.88 6.96)	90–92

Table 5. ^{1}H NMR spectra.l data for alkylamino and aralkylamino type compounds **8a–k**

Compd No.	d (ppm)
8a (DMSO-d_6)	1.07 (3H, t, J = 7.3 Hz), 1.35–1.90 (9H, m), 2.55–3.50 (10H, m), 3.83 (3H, s), 5.75–6.05 (2H, broad), 6.50 (1H, s), 7.42 (1H, d, J = 7.9 Hz), 7.62–7.68 (2H, m), 7.67 (1H, s), 8.00–8.02 (1H, m).
8b ($CDCl_3$)	1.25–2.00 (14H, m), 2.30 (2H, t, J = 7.7 Hz,), 2.62 (2H, t, J = 7.3 Hz), 2.85–2.98 (2H, m), 3.32 (2H, t, J = 5.7 Hz), 3.78 (2H, s), 3.88 (3H, s), 4.44 (2H, brS), 6.29 (1H, s), 7.20–7.36 (5H, m), 7.73–7.77 (1H, m), 8.09 (1H, s).
8c ($CDCl_3$)	1.24–2.05 (12H, m), 2.17 (2H, s), 2.25–2.40 (4H, m), 2.90–3.01 (2H, t, J = 5.7 Hz), 3.32 (2H, t, J = 6.1 Hz), 3.47 (4H, d, J = 3.3 Hz), 3.89 (3H, s), 4.38 (2H, brs), 6.29 (1H, s), 7.19–7.74 (5H, m), 7.72–7.74 (1H, m), 8.10 (1H, s).
8d ($CDCl_3$)	0.85 (3H, t, J = 3.5 Hz), 1.20–1.75 (13H, m), 1.85–1.96 (2H, m), 2.24–2.43 (6H, m), 2.88–2.96 (2H, m), 3.31 (2H, t, J = 6.6 Hz), 3.53 (2H, s), 3.87 (3H, s), 4.51 (2H, s), 6.30 (1H, s), 7.16–7.35 (5H, m), 7.71–7.79 (1H, m), 8.09 (1H, s).
8e (DMSO-d_6)	1.06 (3H, t, J = 6.9 Hz), 1.15–1.90 (11H, m), 2.70–3.50 (12H, m), 3.78 (3H, s), 3.83 (3H, s), 4.17 (2H, s), 5.70–6.15 (2H, broad), 6.50 (1H, s), 6.99 (2H, d, J = 8.6 Hz), 7.45 (2H, d, J = 8.6 Hz), 7.66 (1H, s), 8.00–8.03 (1H, m).
8f ($CDCl_3$)	1.25 (11H, m), 2.01–2.16 (2H, m), 2.35–2.45 (2H, m), 2.65 (2H, t, J = 6.9 Hz), 2.85–3.10 (2H, m), 3.32 (2H, t, J = 6.2 Hz), 3.89 (3H, s), 3.97 (3H, s), 4.45 (2H, brs), 6.31 (1H, s), 6.90–7.30 (3H, m), 7.77–7.87 (1H, m), 8.09 (1H, s).
8g ($CDCl_3$)	1.16 (3H, t, J = 7.3 Hz), 1.20–1.90 (11H, m), 2.70–3.50 (12H, m), 3.82 (3H, s), 4.29 (2H, s), 5.80–6.10 (2H, broad), 6.49 (1H, s), 7.07–7.09 (1H, m), 7.24–7.25 (1H, m), 7.60–7.62 (1H, m), 7.66 (1H, s), 8.00–8.02 (1H, m).
8h ($CDCl_3$)	1.05 (3H, t, J = 7.3 Hz,), 1.18–2.02 (13H, m), 2.22 (2H, t, J = 7.9 Hz), 2.45 (2H, t, J = 7.9 Hz), 2.55 (2H, q, J = 7.2 Hz), 2.81–2.94 (2H, m), 3.31 (2H, t, J = 6.3 Hz), 3.87 (3H, s), 3.95 (2H, s), 4.39 (2H, brs), 6.27 (1H, s), 7.38–7.55 (4H, m), 7.70–7.87 (3H, m), 8.10 (1H, s), 8.30–8.34 (1H, m).
8i ($CDCl_3$)	1.05 (3H, t, J = 7.0 Hz,), 1.19–1.95 (15H, m), 2.26 (2H, t, J = 7.9 Hz), 2.45 (2H, t, J = 7.9 Hz), 2.55 (2H, q, J = 7.2 Hz), 2.81–2.94 (2H, m), 3.31 (2H, t, J = 6.3 Hz), 3.87 (3H, s), 4.39 (2H, brs), 6.27 (1H, s), 7.42–7.52 (3H, m), 7.70–7.84 (5H, m), 8.11 (1H, s).
8j (DMSO-d_6)	0.86–1.90 (21H, m), 2.60–3.50 (14H, m), 3.82 (3H, s), 5.92 (2H, brs), 6.48 (1H, s), 7.66 (1H, s), 7.96–8.01 (1H, m).
8k ($CDCl_3$)	0.72–0.84 (2H, m), 1.09–1.80 (20H, m), 2.01–2.13 (2H, m), 2.16 (2H, d, J = 7.3 Hz), 2.32 (2H, t, J = 7.3 Hz), 2.36–2.44 (2H, m), 2.99–3.07 (2H, m), 3.32 (2H, t, J = 5.9 Hz), 3.48 (2H, s), 3.90 (3H, s), 4.40 (2H, s), 6.30 (1H, s), 7.17–7.33 (5H, m), 7.73–7.80 (1H, m), 8.10 (1H, s).

absorption rate of analogues. [The compound having a benzene group instead of the 1-methylindole, **1b**[8] showed low intestinal absorption rate (18.9%) as shown in Table 1].

The aralkylamine and alkylamine type compounds **8a–k** had high and selective 5-HT_4 receptor binding affinity as shown in Table 2. The secondary amine type, benzylamino derivative 8b revealed potent 5-HT_4 receptor agonistic activity (EC_{50} = 3.7 nM), although it showed poor intestinal absorption rate (26.5%). The tertiary amine type, benzyl(methyl)amino derivative **8c** and benzyl(propyl)amino derivative **8d** also showed poor intestinal absorption rate (24.8 and 33.6%, respectively). A decrease in intestinal absorption rate was observed when a methoxy group was at the 4-position of the benzene ring (compound **8e**, 8.3%). Introduction of chlorine atoms into the 3,4-positions of the benzene ring resulted in a moderately increased intestinal absorption rate (**8a**, 47.5%). However, compound **8a** did not show 5-HT_4 receptor agonistic activity. The compound **8k** with benzyl(cyclohexylmethyl)amino group showed no 5-HT_4 receptor agonistic activity. On the other hand, the benzylamino derivative **8b** and cyclohexylmethylamino derivative **8j** possessed 5-HT_4 receptor agonistic activities. These results indicated that the compounds with a bulky substituent group (such as (3,4-dichlorobenzyl)ethylamino or benzyl(cyclohexylmethyl)amino) on the terminal of the straight alkyl chain at the 1-position on the piperidine ring did not show 5-HT_4 receptor agonistic activity. The tertiary amine type, ethyl(2-thienylmethyl)amino derivative **8g** possessed better intestinal absorption rate (57.9%) than the secondary amine type, (2-thienylmethyl)amino derivative **8f** (9.3%).

A correlation between the logP value and the % absorption of aralkylamine derivatives was low as shown in Figure 2. Although naphthylmethyl derivatives **8h** (logP = 1.8) and **8i** (logP = 1.4) were more lipophilic than **8a–g** (logP = −0.9–1.1, octanol/pH 6.8 buffer), a modest increase in lipophilicity was not sufficient to confer improved intestinal absorption rate (Fig. 2). In the aralkylamine and alkylamine derivatives series **8a–k**, the potency (EC_{50} = 3.7–62.5 nM) of tested compounds was weaker than that of compounds 1a (EC_{50} = 1.2 nM) and **1b** (EC_{50} = 3.7 nM). Furthermore, the intestinal absorption rates of tested compounds were also as poor as those of compound **1a** and **1b** (Table 1).

The benzoyl type compounds **11a–d** and phenylsulfonyl type compounds **12a–c** also showed selective and high 5-HT_4 receptor binding affinity as depicted in Table 3. Compounds **11a–d** and **12a–c** revealed potent 5-HT_4 receptor agonistic activities. These results suggested that the benzoyl and phenylsulfonyl moiety at its side-chain part at the 1-position on the piperidine ring played an important role in enhancing 5-HT_4 receptor agonistic activity as well as the 1-methylindol-3-yl carbonylamino

Table 6. ^{1}H NMR for benzoyl and phenylsulfonyl type compounds **11a–d** and **12a–c**

Compd No.	δ (ppm)
11a (DMSO-d_6)	1.05–1.22 (2H, m), 1.37–1.70 (7H, m), 1.72–1.83 (2H, m), 2..25–2.38 (2H, m), 2.82–2.90 (2H, m), 3.02 (2H, t, J = 7.3 Hz), 3.14 (2H, t, J = 6.2 Hz), 3.82 (3H, s), 5.90 (2H, brs), 6.47 (1H, s), 7.52-7.54 (2H, m), 7.60–7.64 (2H, m), 7.84–7.88 (1H, m), 7.95–7.98 (2H, m).
11b ($CDCl_3$)	1.30–1.94 (13H, m), 2.27–2.34 (2H, m), 2.90–2.99 (4H, m), 3.32 (2H, t, J = 6.3 Hz), 3.89 (3H, s), 4.38 (2H, brs), 6.29 (1H, s), 7.46–7.58 (3H, m), 7.65–7.80 (1H, m), 7.93–7.95 (2H, m), 8.11 (1H, s).
11c ($CDCl_3$)	1.28–1.81 (13H, m), 1.90–2.10 (2H, m), 2.25–2.41 (2H, m), 2.88–3.02 (4H, m), 3.32 (2H, t, J = 6.3 Hz), 3.89 (3H, s), 4.38 (2H, brs), 6.29 (1H, s), 7.42–7.54 (3H, m), 7.70–7.81 (1H, m), 7.95 (2H, dd, J = 6.6 Hz, 2.0 Hz), 8.10 (1H, s).
11d (DMSO-d_6)	1.25–1.91 (15H, m), 2.25–2.40 (2H, m), 2.89–2.97 (4H, m), 3.32 (2H, t, J = 6.6 Hz), 3.85 (3H, s), 3.89 (3H, s), 4.35 (2H, s), 6.28 (1H, s), 7.24–7.35 (3H, m), 7.71 (1H, s), 7.77–7.79 (1H, m), 8.11 (1H, s), 8.37–8.39 (1H, m).
12a (DMSO-d_6)	1.39–2.00 (13H, m), 2.67–2.89 (2H, m), 2.94-3.00 (2H, m), 3.01–3.11 (2H, m), 3.82 (3H, s), 5.93 (2H, brs), 6.48 (1H, s), 7.60–7.67 (3H, m), 7.76–7.78 (1H, m), 7.87–7.89 (2H, m), 7.90–7.99 (1H, m), 9.98–10.03 (1H, m).
12b ($CDCl_3$)	1.30–1.85 (11H, m), 2.75–350 (10H, m), 3.82 (3H, s), 5.93 (2H, bs), 6.48 (1H, s), 7.64 (1H, s), 7.65–7.94 (5H,m), 7.95–8.03 (1H, m), 9.65–9.90 (1H, broad).
12c (DMSO-d_6)	1.14–1.99 (13H, m), 2.77–2.99 (4H, m), 3.16–3.24 (2H, m), 3.25–350 (4H, m), 3.82 (3H, s), 3.90–4.01 (2H, brs), 6.50 (1H, s), 7.64 (1H, s) 7.68 (2H, d, J = 7.7 Hz), 7.73–7.75 (1H, m), 7.90 (2H, d, J = 7.7 Hz), 8.00 (1Hm).

moiety of compound **1a**.[8] These compounds except for 11d showed better intestinal absorption rate than **1a**, **1b** and aralkyl (or alkyl) amine derivative **8a–k**. Conversion of the benzoyl moiety in **11b** to the 1-methylindole carbonyl (**11d**) resulted in considerably increased 5-HT$_4$ receptor binding affinity (K_i = 0.34 nM) and agonistic activity (EC_{50} = 4.3 nM) but decreased the intestinal absorption rate (26.0%). This result suggested that the compounds with 1-methylindole moiety showed lower intestinal absorption rate than the benzene derivatives (**1a** vs **1b**; **11d** vs **11b**, Tables 1 and 3). The benzoyl type compounds possessed better intestinal absorption rate than phenylsulfonyl derivatives (**11a** vs **12a**; **11b** vs **12b**; **11c** vs **12c**, Table 3). As exemplified by compounds **11a–c**, the length of alkyl linker (n = 4, 5 and 6) appeared to contribute to intestinal absorption rates (87.0, 84.9 and 61.6%, respectively).

In also the phenylsulfonyl type derivatives such as **12a–c**, a variety of the length of alkyl linker (n = 4–6) affected to intestinal absorption rates (71.6, 60.4 and 45.1%, respectively). In benzoyl derivatives and phenylsulfonyl derivatives, lipophilicity could be increased by manipulation of the straight alkyl side chain at the 1-position on the piperidine ring. The relationship with intestinal absorption rate and lipophilicity of compounds was illustrated in Figure 2. In benzoyl derivatives **11a–c**, intestinal absorption rate correlated with logP.

The carbonyl and sulfonyl groups were good bioisosteres of the amide bond in order to improve intestinal absorption rate and to show potent 5-HT$_4$ receptor agonistic effect. Especially, 4-amino-5-chloro-2-methoxy-*N*-[1-(6-oxo-6-phenylhexyl)piperidin-4-ylmethyl]benzamide (11b) possessed good intestinal absorption rate (84.9%). As might be expected, compound **11b** showed sufficient oral bioavailability in dogs (54%) because of its good intestinal absorption rate. Compound **11b** also showed high and selective 5-HT$_4$ receptor binding affinity (K_i = 2.4 nM), and potent agonistic activity (EC_{50} = 10 nM).

Conclusion

We described the preparation, biological evaluation and pharmacokinetic profile of a series of 4-amino-5-chloro-2-methoxy-*N*-(piperidin-4-ylmethyl)benzamides as selective 5-HT$_4$ receptor agonists. Among them, 4-amino-5-chloro-2-methoxy-*N*-[1-(6-oxo-6-phenylhexyl)piperidin-4-yl methyl]benzamide (**11b**) and its related compounds with a benzoyl or phenylsulfonyl moiety at its side chain part at the 1-position on the piperidine ring showed improved intestinal absorption rate compared to compound **1a**. In our studies, replacement of the 1-methylindol-3-yl carbonylamino moiety of compound **1a** with a benzoyl or phenylsulfonyl moiety significantly improved oral bioavailability.

Experimental

Melting points were determined in open capillaries and are uncorrected. Proton nuclear magnetic resonance (^{1}H NMR) spectra were recorded on JEOL JNM-EX270 spectrometer. Coupling constants are reported in hertz (Hz) and chemical shifts were expressed in ppm downfield from tetramethylsilane as an internal standard. Mass spectra (MS) were obtained by a JMS-O1SG spectrometer. Elementary analysis was performed for C, H and N by our laboratory.

Chemistry

4-Aminomethyl-1-(*tert*-butoxycarbony) piperidine (2). 4-(Aminomethyl)piperidine (137 g, 1.20 mol) was refluxed with benzaldehyde (127 g, 1.20 mol) in toluene (1.0 L) with azeotropic removal of water for 12 h. To the reaction mixture was added di-*tert*-butyldicarbonate (288 g, 1.32 mol) below 10 °C, then stirring continued at room temperature for 6 h. The reaction mixture was reacted with aqueous $KHSO_4$ at room temperature for 12 h. It was washed with diisopropylether and extracted with

chloroform. The extracts were washed with brine and dried over $MgSO_4$. The solvent was removed in vacuo to give **2** (273 g, 92%).

^{1}H NMR ($CDCl_3$) δ: 1.01–1.30 (5H, m), 1.45 (9H, s), 1.65–1.75 (2H, m), 2.58 (2H, d, J = 6.6 Hz), 2.60–2.85 (2H, m), 4.07–4.16 (2H, m); MS EI-MS m/z: 214 (M^+).

4-Amino-*N*-[1-(*tert*-butoxycarbonyl)piperidin-4-ylmethyl]-5-chloro-2-methoxybenzamide (3). To the mixture of **2** (100 g, 467 mmol), 4-amino-5-chloro-2-methoxybenzoic acid (94.1 g, 467 mmol) and Et_3N (67.9 mL, 467 mmol) in DMF (1000 mL) were added 1-ethyl-3-[3-(dimethylamino)propyl]carbodiimide hydrochloride (EDC) (93.9 g, 467 mmol) and 1-hydroxybenzotriazole (HOBT) (66.2 g, 467 mmol) at 5 °C. The reaction mixture was stirred at 25 °C for 24 h and concentrated in vacuo. The resulting residue was extracted with AcOEt. The combined organic layers were washed with aqueous K_2CO_3 and dried over $MgSO_4$. The solvent was removed in vacuo to give **3** (170 g, 92%).

^{1}H NMR ($CDCl_3$) δ 1.01–1.27 (5H, m), 1.45 (9H, s), 1.65–1.85 (2H, m), 2.62–2.75 (2H, m), 3.27–3.37 (2H, m), 3.88 (3H, s), 4.61 (2H, s), 6.34 (1H, s), 7.72–7.82 (1H, m), 8.07 (1H, s); EI-MS m/z: 397 (M^+).

4-Amino-5-chloro-2-methoxy-*N*-(piperidin-4-ylmethyl)-benzamide hydrochloride (4). To the mixture of **3** (170 g, 430 mmol) in dioxane was added 10%HCl/dioxane portionwise at 5 °C. The reaction mixture was stirred at 25 °C for 4 h and evaporated in vacuo to afford pale yellow solid. The resulting solid was recrystallized from IPE-EtOH to give **4** (118.5 g, 82%). ^{1}H NMR (DMSO-d_6) δ 1.30–1.50 (2H, m), 1.65–1.90 (3H, m), 2.60–2.90 (2H, m), 3.12–3.29 (4H, m), 3.83 (3H, s), 4.94 (3H, broad), 6.53 (1H, s), 7.66 (1H, s), 7.96–8.03 (1H, m), 8.80–9.00 (1H, broad), 9.10–9.25 (1H, broad); EI–MS m/z: 297 (M^+). Anal. calcd for $C_{14}H_{20}N_3O_2Cl{\cdot}2HCl{\cdot}3/4H_2O$: C, 43.77; H, 6.16 ; N, 10.94. Found C, 43.73; H, 6.23; N, 10.94.

4-Amino-5-chloro-*N*-[1-[5-(2,3-dihydro-1,3-dioxo-*1H*-isoindole-2-yl)pentyl]piperidin-4-yl methyl]-2-methoxybenzamide (6). The compound **4** (67.7 g, 203 mmol) was stirred with *N*-(5-bromopentyl)phthalimide (60.0 g, 203 mmol) at 70–75 °C in K_2CO_3 (84.0 g, 609 mmol)/DMF (700 mL) for 12 h. The reaction mixture was evaporated and extracted with AcOEt. The extracts were washed with aqueous K_2CO_3 and dried over $MgSO_4$ and concentrated in vacuo. The mixture was purified by silica gel column chromatography ($CHCl_3$/MeOH, 10/1) to give **6** (39.0 g, 37%). ^{1}H NMR ($CDCl_3$) δ 1.23–1.76 (11H, m), 1.83–1.94 (2H, m), 2.30 (2H, t, J = 7.3 Hz), 2.87–2.95 (2H, m), 3.31 (2H, t, J = 6.6 Hz), 3.68 (2H, t, J = 7.3 Hz), 3.89 (3H, s), 4.42 (2H, s), 6.30 (1H, s), 7.67–7.86 (5H, m), 8.10 (1H, s); EI-MS m/z: 512 (M^+). Anal. calcd for $C_{27}H_{33}N_4O_4Cl{\cdot}1/2H_2O$: C, 62.12; H, 6.56; N, 10.73. Found C, 62.19; H, 6.63; N, 10.63.

4-Amino-*N*-[1-(5-aminopentyl)piperidin-4-ylmethyl]-5-chloro-2-methoxybenzamide (7). The compound **5** (26.0 g, 50.7 mmol) was refluxed with hydrazine hydrate (3.5 mL) in EtOH (250 mL) for 6 h. After cooling, insoluble compound was removed by filtration and solution was evaporated in vacuo. The residue was purified by silica gel column chromatography ($CHCl_3$/MeOH/NH_4OH) to give compound **6** (17.0 g, 88%). ^{1}H NMR($CDCl_3$) δ 1.23–1.74 (9H, m), 1.76–1.90 (2H, m), 1.95–2.10 (2H, m), 2.30–2.41 (2H, m), 2.72 (2H, d, J = 7.3 Hz), 2.90–3.05 (2H, m), 3.31 (2H, t, J = 6.6 Hz), 3.91 (3H, s), 4.42 (2H, s), 4.69–4.85 (2H, m), 6.37 (1H, s), 7.84–7.97 (1H, m), 8.00 (1H, s); EI–MS m/z: 382 (M^+). Anal. calcd for $C_{19}H_{31}N_4O_2Cl{\cdot}H_2O$/C, 56.92; H, 8.30; N, 13.97. Found C, 56.84; H, 8.42; N, 13.78.

General procedure for preparation of amine type benzamide derivatives (8a–k). The compound was stirred with aldehyde at 60–65 °C in EtOH for 2 h. To the reaction mixture which was cooled to 5 °C was added $NaBH_4$ and stirred at 25 °C for 2–4 h. The reaction mixture was poured into ice water, extracted with $CHCl_3$ and washed with saturated brine. The organic layer was dried over $MgSO_4$ and concentrated in vaccuo. The residue was purified by silica gel column chromatography ($CHCl_3$/MeOH). The resulting oil was transformed into oxalate and recrystallized from AcOEt to give colorless crystals.

General procedure for preparation of derivatives (9a–c)

A cooled (5 °C) solution of benzene and 6-chlorohexanoyl chloride in CH_2Cl_2 was treated with $AlCl_3$ and stirred at 25 °C for 2–4 h. The reaction mixture was poured into ice water and extracted with CH_2Cl_2. The organic layer was concentrated in vaccuo and purified by silica gel column chromatography ($CHCl_3$/MeOH) to give a colorless oil.

6-Bromo-1-(1-methyl-1H-indol-3-yl)-1-hexanone (9d). A mixture of 1-methyl-*1H*-indole (1.5 g, 11 mmol), *N*,*N*-dimethyl-5-bromopentylamide (2.5 g, 11 mmol), $POCl_3$ (1.2 mL, 13 mmol) in $CHCl_3$ (20 mL) was refluxed for 7 h. The reaction mixture was poured into ice and was extracted with $CHCl_3$. The extract was washed with brine and was dried over $MgSO_4$. After evaporation, the residue was chromatographed on silica gel ($CHCl_3$) to give **9d** (1.3 g, 38%); ^{1}H NMR ($CDCl_3$) δ 1.48–1.60 (2H, m), 1.75–2.00 (4H, m), 2.83 (2H, t, J = 7.7 Hz), 3.50 (2H, t, J = 7.7 Hz), 3.82 (3H, s), 7.27–7.32 (3H, m), 7.69 (1H, s), 8.35–8.39 (1H, m), EI-MS m/z: 309 ($M^+ + 2$), 307 (M^+).

General procedure for preparation of derivatives (10a–c)

A solution of benzenethiol and bromochloroalkane in DMF was stirred at 50–60 °C for 3–4 h in the presence of K_2CO_3. The reaction mixture was poured into ice water and extracted with AcOEt. The combined organic extracts were concentrated in vacuo and purified by silica gel column chromatography ($CHCl_3$/MeOH) to give thioether compound. H_2O_2 was added to the thioether compound in HCOOH, and the reaction mixture was stirred at 25 °C for 12 h. The reaction mixture was poured into ice water and filtrate to afford white solid.

General procedure for preparation of benzamide derivatives (11a–d, 12a–c)

The compound was stirred with halides (9a–d, 10a–c) at 70–75 °C in K_2CO_3/DMF for 5–12 h. The reaction mixture was evaporated and extracted with AcOEt. The extracts were washed with aqueous K_2CO_3. The organic layer was dried over $MgSO_4$ and concentrated in vaccuo. The residue was purified by silica gel column chromatography ($CHCl_3$/MeOH) and recrystallized to give colorless crystals.

Pharmacology

5-HT$_4$ receptor binding assay. Male Hartley guinea pigs (Japan SLC, Ltd., Shizuoka, Japan) were sacrificed by cervical dislocation and the striatum was separated from each brain. The striatum was homogenized in 15 volume of 50 mmol/L ice-cold HEPES buffer (pH 7.4) with Polytron PT-10 and then centrifuged at 35,000*g* for 20 min. The resulting pellet was resuspended in the HEPES buffer and finally diluted to the appropriate concentration for assay (6 mg wet weight per assay tube). This suspension was used as the tissue preparation. Assay tube contained 50 μL of HEPES buffer or a solution of the test agents, 50 μL solution of [^{3}H]GR113808 (Amersham International, UK) to give a final concentration of 0.1 nmol/L and 900 μL of tissue preparation. Each tube was incubated for 30 min at 37 °C and the reaction was terminated by rapid filtration through a Whatmann GF/B filter (presoaked in 0.01% v/v polyethyleneimine) followed by washing with 1×4 mL of ice-cold HEPES buffer. Then the filter was placed in 3 mL of scintillator and the radioactivity was determined by scintillation counting in a Beckman model LS3801 scintillation counter. Non specific binding was defined in the presence of unlabelled GR113808 to give a final concentration of 1 μmol/L. The IC_{50} value was determined by non-linear regression of the displacement curve, and the K_i value was calculated according to the formula $[K_i = IC_{50}/(1 + L/K_d)]$, where L is the concentration of radioligand and K_d is the dissociation constant of the radioligand.

5-HT$_3$ receptor binding assay

[^{3}H]Granisetron binding assay was performed according to the method of Nelson and Thomas.[15] Male Wistar rat (Japan SLC, Ltd., Shizuoka, Japan) cerebral cortex was homogenized in 20 volumes of 0.32 mol/L sucrose and the centrifuged at 1000*g* for 10 min. The supernatant was centrifuged at 40,000*g* for 15 min. The pellet was suspended in 20 volumes of HEPES buffer (50 mmol/L, pH 7.4) and suspension was incubated at 37 °C for 10 min, was centrifuged at 40,000*g* for 15 min. The pellet was washed and centrifuged (40,000*g* for 15 min). The final pellet was resuspended in 30 volumes of HEPES buffer and used as tissue homogenate. The binding assay consisted of 50 μmmol/L of [^{3}H]Granisetron, 50 μL of displacing drugs and 900 μL of tissue homogenate. Following a 30-min incubation at 25 °C, the assay mixture was rapidly filtered under reduced pressure through Whatman GF/B glass filters which had been presoaked in 0.1% polyethyleneimie. Filters were washed immediately with 3×3 mL of ice-cold Tris–HCl buffer (50 mM, pH 7.4). ICS 205930 (100 μmmol/L) was used for the determination of nonspecific binding.

5-HT$_4$ receptor agonistic activities in vitro contraction of isolated guinea-pig ascending colon

Male Hartley guinea pigs (Japan SLC, Ltd., Shizuoka, Japan) were killed by cervical dislocation and the ascending colon (a 10-cm segment starting 1 cm from the caecum) was removed. The longitudinal muscle layer was separated from the underlying circular muscle and divided into four segments of about 2.5 cm. Four muscle strip preparations were individually mounted vertically for isotonic measurement into a tissue bath containing 10 mL Tyrode solution. Only 5-HT was tested in the Tyrode solution with containing methysergide (1 μmol/L) and granisetron (1 μmol/L) to inhibit responses mediated by 5-HT$_2$ and 5-HT$_1$-like and 5-HT$_3$ receptors, respectively. This solution was kept at 37 °C and gassed with 95% O_2, 5% CO_2. The strips were subjected to a preload of 1 g and allowed to stabilize for 20 min. After stabilization, the response of the longitudinal muscle to 30 μmol/L methacholine was measured. Agonist concentration–effect curves were constructed using sequential dosing, leaving 15 min between doses. A 15-min dosing cycle was required to prevent desensitization. The agonist was left in contact with a preparation until the response had reached a maximum, the preparation was washed. 40 min was left between the determination of concentration–effect curves. GR113808 (10 nmol/L) were incubated for 10 min before repeating agonist concentration–effect curves. After each determination of concentration–effect curve, 30 μmol/L of methacholine was added to the tissue bath again. All responses were expressed as a percentage of the mean of the two contractions induced by 30 μmol/L methacholine. The EC_{50} value, the concentration causing 50% of the maximal response, was determined by linear regression analysis.

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