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Synthesis and pharmacological evaluation of benzamide derivatives as selective 5-HT$_4$ receptor agonists

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Abstract—It is thought that selective 5-HT$_4$ receptor agonists—such as 4-amino-5-chloro-2-methoxy-*N*-[1-(6-oxo-6-phenylhexyl)piperidin-4-ylmethyl]benzamide (**2**)—have the ability to enhance both upper and lower gastrointestinal motility without any significant adverse effects.

Modification of **2** was performed. Variation of the piperidin-4-ylmethyl moiety of **2** led to a decrease in the binding affinity for the 5-HT$_4$ receptor. Following conversion of the carbonyl group on the benzoyl part to a hydroxyl or sulfoxide group, the binding affinity for the 5-HT$_4$ receptor was retained although the effect on defecation was reduced. Many of the 4-amino-5-chloro-2-methoxy-*N*-(piperidin-4-ylmethyl)benzamides that had a ether or sulfide moiety in the side-chain part at the 1-position of the piperidine exhibited high affinity for the 5-HT$_4$ receptor.

Among these, phenylthio **41c** and benzylthio derivative **44** were selective 5-HT$_4$ receptor agonists, and had a similar effect on defecation to compound **2**.

1. Introduction

The 5-HT$_4$ receptor is localized in the central nervous system and peripheral tissues.[1,2] At the periphery, stimulation of the 5-HT$_4$ receptor mediates gastric motility, ileal motility and colonic transit. Recently, Grider et al. indicated that 5-HT$_4$ receptor agonists initiate the peristaltic reflex in human jejunum, rat and guinea-pig intestine.[3] Various compounds have been reported to act as agonists or antagonists to 5-HT$_4$ receptors.[4–12] A number of benzamides known as 5-HT$_4$ receptor agonists (cisapride, BIMU-8, metoclopramide, etc.) have binding affinity for other receptors, notably the 5-HT$_3$ and dopamine D$_2$ receptors.[13–16]

It is known that 5-HT$_3$ receptor antagonism reduces colonic transit[18] and that binding affinity for the dopamine D$_2$ receptor produces unfavourable side effects, such as extrapyramidal syndrome including parkinsonism, dystonia, dyskinesia and akathisia in the central nervous system. It is therefore thought that a selective 5-HT$_4$ receptor agonist with no binding affinity for the 5-HT$_3$ and dopamine D$_2$ receptors should enhance both upper and lower gastrointestinal motility without serious side effects.

In our previous reports, we have described the design and characterization of a number of selective 5-HT$_4$ receptor agonists.[17–21] The first step of our strategy was the optimization of the aromatic ring and cyclic amine moiety of the benzamide.[18] Selectivity for the 5-HT$_4$ receptor was induced by using the piperidin-4-ylmethyl group as the cyclic amine part [e.g., 4-amino-5-chloro-2-methoxy-*N*-[1-[5-(1-methylindol-3-ylcarbonylamino)pentyl]piperidin-4-ylmethyl]benzamides (**1**) showed binding affinity for 5-HT$_4$ (K_i = 0.3 nM), 5-HT$_3$ (IC_{50} > 1000 nM) and dopamine D$_2$ (IC_{50} > 1000 nM) Fig. 1]. It had been supposed that compound **1** was a gastrointestinal motility stimulant, which could enhance both upper and lower

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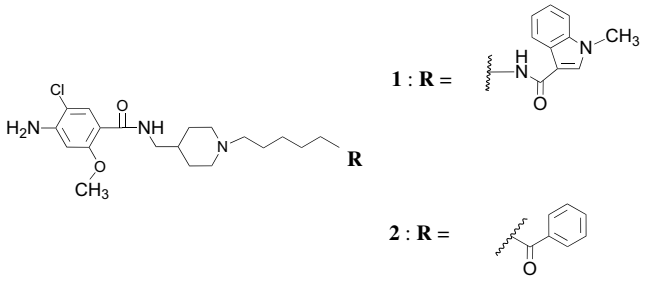


Figure 1.

gastrointestinal motility with few side effects, but it displayed poor bioavailability in dogs (5.1%) because of the low intestinal absorption rate.

In the next step, we replaced the 1-methylindol-3-ylcarbonylamino moiety of **1** with aralkylamino (or alkylamino), benzoyl and phenylsulfonyl group to raise oral bioavailability.[20] Compounds with a benzoyl moiety on the side-chain part at the 1-position of the piperidine ring showed satisfactory intestinal absorption. Among these, compound **2** showed 54% oral bioavailability in dogs and displayed selective binding affinity for the 5-HT_4 receptor (K_i = 2.4 nM).

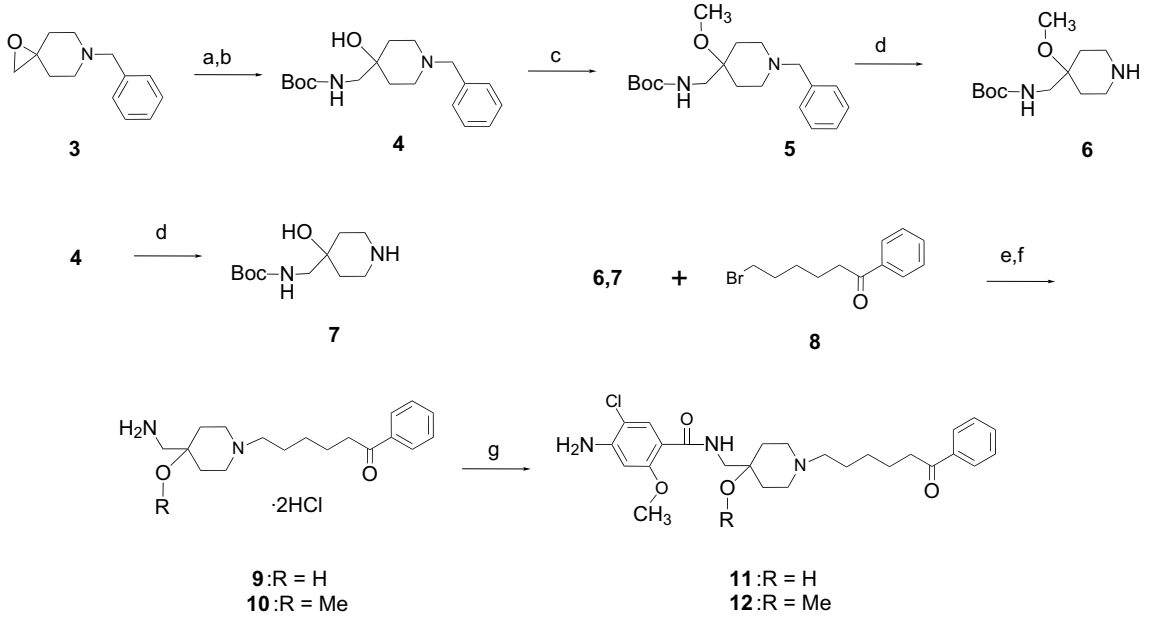
We therefore carried out further optimization of compound **2** to explore the structure–activity relationship. In the present paper, we report the modification of the piperidine and benzoyl moieties.

2. Chemistry

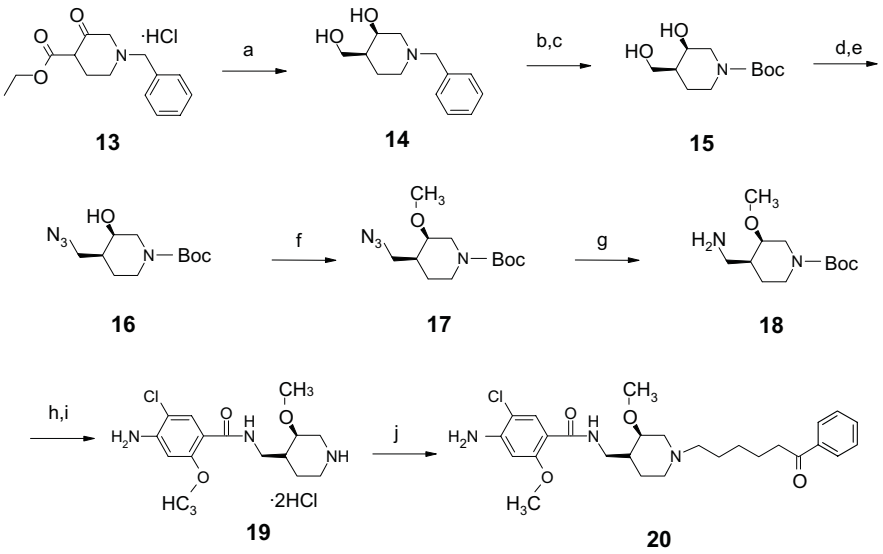
The synthesis of derivatives **11** and **12** is outlined in Scheme 1. The oxirane **3** was prepared from commercially available 1-benzylpiperidin-4-one using $Me_3S(O)I$ in a good yield. The alcohol **4** was obtained by ring-opening reaction of **3** with NH_4OH followed by Boc-protection of the amino group. Methylation of the 4-hydroxyl group on the piperidine ring of **4** provided compound **5**, from which the intermediate **6** was derived by removing the benzyl group at the 1-position of the piperidine under reductive conditions. Similarly, compound **7** was synthesized by benzyl-deprotection of the intermediate **4**. The piperidines **6** and **7** were treated with 6-bromo-1-phenylhexan-1-one (**8**) in K_2CO_3/DMF followed by Boc-deprotection with hydrochloric acid to afford the corresponding amine derivatives **9** and **10**, respectively. Condensation of these with 4-amino-5-chloro-2-methoxybenzoic acid using EDC·HCl and HOBt afforded the benzamides **11** and **12**, respectively.

The derivative **20**, which had a methoxy group at the 3-position of the piperidine part, was prepared from ethyl 1-benzyl-3-oxo-4-piperidinecarboxylate hydrochloride (**13**) as the starting material according to the synthetic pathway described in Scheme 2. The compound **13** was converted into the *cis*-diol **14** by reduction with $NaBH_4$. After removal of the benzyl group under reductive conditions, compound **14** was protected with the *tert*-butoxycarbonyl group to give compound **15**, from which **16** was obtained by selective conversion through mesylation of the hydroxylmethyl group at the 4-position of the piperidine followed by azidation. Methylation of the hydroxyl group at the 3-position of **16** with methyliodide provided derivative **17**, hydrogenation of which under atmospheric pressure in the presence of $Pd(OH)_2$ gave the amine **18** in a good yield.

Compound **18** was condensed with 4-amino-5-chloro-2-methoxybenzoic acid and treated with hydrochloric acid to afford compound **19**. A 6-oxo-6-phenylhexyl moiety was introduced by alkylation of **19** with **8** to give the desired benzamide **20**.



Scheme 1. Reagents and conditions: (a) NH_4OH; (b) $(BOC)_2O$; (c) MeI, 60% NaH; (d) 10% Pd–C, NH_2NH_2–H_2O/EtOH; (e) K_2CO_3/DMF; (f) HCl/2-propanol; (g) 4-amino-5-chloro-2-methoxybenzoic acid, EDC–HCl, HOBt, Et_3N.



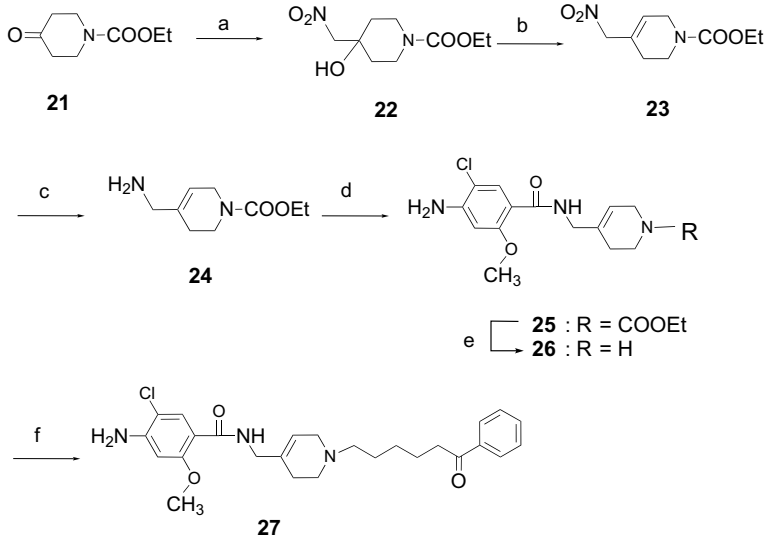
Scheme 2. Reagents and conditions: (a) $NaBH_4$; (b)10% Pd–C, NH_2NH_2–H_2O; (c) $(BOC)_2O$; (d) MsCl, Et_3N; (e) NaN_3, NH_4Cl/DMF; (f) MeI, 60% NaH/DMF; (g) H_2, $Pd(OH)_2$; (h) 4-amino-5-chloro-2-methoxybenzoic acid, EDC–HCl, HOBt, Et_3N; (i) HCl/2-propanol; (j) **8**, K_2CO_3/DMF.

The derivative **27**, which possessed an unsaturated piperidine, was synthesized from ethyl 4-oxopiperidine-1-carboxylate (**21**) as shown in Scheme 3. The derivative **22** was prepared by nitro-aldol reaction with nitromethane from **21**. The dehydroxylation of **22** in the presence of P_2O_5 provided the unsaturated piperidine **23**, the nitro group of which was reduced with Fe and NH_4Cl to give the amine derivative **24**. Condensation reaction of 4-amino-5-chloro-2-methoxybenzoic acid with **24** afforded compound **25**, which was treated with KOH/2-propanol to provide **26**. Coupling reaction of the benzamide **26** with compound **8** in the presence of K_2CO_3 gave the desired compound **27**.

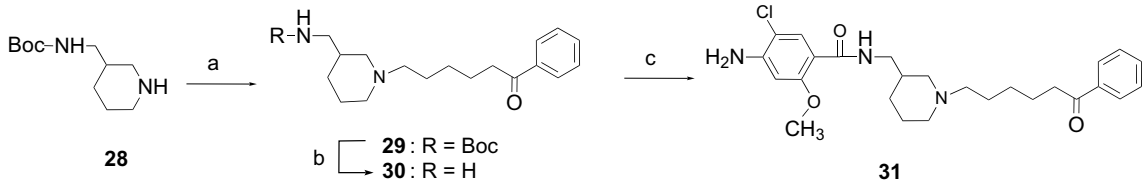
The piperidin-3-ylmethyl compound **31** was synthesized as shown in Scheme 4. Alkylation of the piperidine **28** with the bromide **8** under a typical condition (K_2CO_3/DMF) gave compound **29**. Compound **30**, produced from the intermediate **29** by deprotection of the Boc group with hydrochloric acid, was attached to 4-amino-5-chloro-2-methoxybenzoic acid under standard condensation conditions using EDC·HCl–HOBt to provide **31**.

Scheme 5 shows the preparation of the alcohol **32** and the oxime **33**. The reduction of the carbonyl group of compound **2** with $NaBH_4$ afforded the racemic secondary alcohol derivative **32**. Condensation of the carbonyl group of **2** with hydroxylamine hydrochloride using pyridine afforded the oxime derivative **33**.

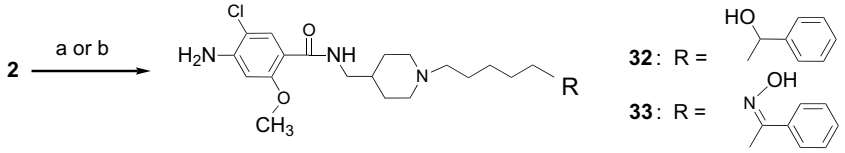
The preparation of the compounds in Table 3 proceeded as shown in Scheme 6. The ethers **34a**–**e** and sulfides **35a**–**d** were synthesized by alkylation of phenol or benzenethiol with the corresponding bromochloroalkanes (n = 2–6). The sulfide derivative **35c** was oxidized with 1 equiv of *m*-CPBA to give the sulfoxide **36**. The



Scheme 3. Reagents and conditions: (a) CH_3NO_2, Na/EtOH; (b) P_2O_5, benzene reflux; (c) Fe, NH_4Cl, H_2O/toluene reflux; (d) 4-amino-5-chloro-2-methoxybenzoic acid, EDC–HCl, Et_3N, HOBt; (e) KOH, 2-propanol reflux; (f) **8**, K_2CO_3/DMF.



Scheme 4. Reagents: (a) **8**, K_2CO_3/DMF; (b) HCl (aq); (c) 4-amino-5-chloro-2-methoxybenzoic acid, EDC–HCl, HOBt, Et_3N.

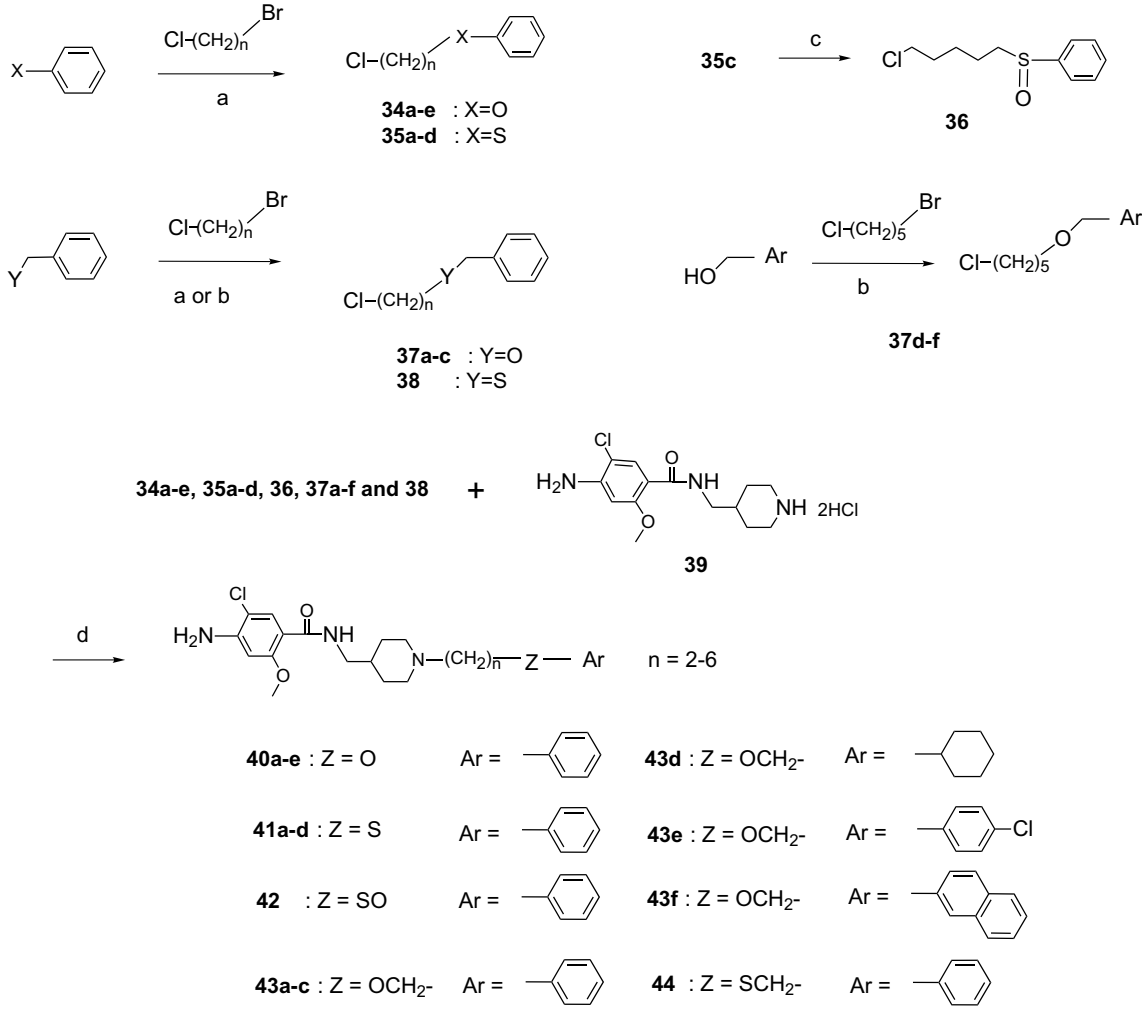


Scheme 5. Reagents and conditions: **32**: (a) $NaBH_4$; **33**: (b) NH_2OH–HCl, pyridine/MeOH reflux.

compounds **37a–c** and **37e–f** were synthesized from the corresponding benzyl alcohols (**37d**: from cyclohexylmethanol). Alkylation of benzylmercaptane with the corresponding bromochloroalkane in 60% NaH/THF afforded **38**. The key intermediate **39** was prepared using a method reported previously.[20] Coupling reaction of compound **39** with **34a–e**, **35a–d**, **36**, **37a–f** and **38** in K_2CO_3–DMF gave phenoxy (**40a–e**), phenylthio (**41a–d**), phenylsulfinyl (**42**), benzyloxy (**43a–c**,**e**,**f**), cyclohexylmethyl (**43d**) and benzylthio (**44**) derivatives, respectively.

3. Results and discussion

The synthesized compounds were evaluated for their 5-HT_4 receptors-binding affinity by use of [^{3}H]GR113808. Their affinity for the 5-HT_3 and dopamine D_2 receptors



Scheme 6. Reagents and conditions: (a) K_2CO_3/DMF; (b) 60% NaH; (c) *m*-CPBA; (d) K_2CO_3/DMF.

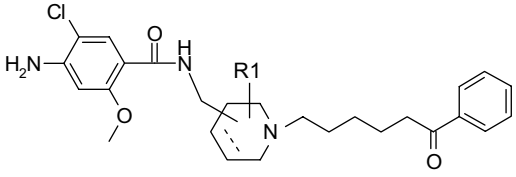
was similarly evaluated using [^{3}H]Granisetron and [^{3}H]Spiperone, respectively, as radioligands. Membrane preparations of guinea-pig striatum, rat cerebral cortex and rat striatum were used for the 5-HT_4, 5-HT_3 and dopamine D_2 receptor-binding assays, respectively.

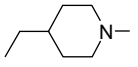
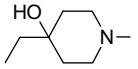
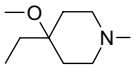
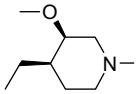
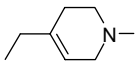
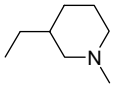
Initially, we proceeded with the modification of the piperidine moiety (or R1) of the parent compound **2** to investigate the influence of a steric hindrance (or electric atmosphere) by substituents such as OH, OCH_3, introduction of double bond or transformation to the piperidin-3-ylmethyl group.

The results of modification are shown in Table 1. Compound **2** showed high binding affinity and high selectivity for the 5-HT_4 receptor compared to the 5-HT_3 and dopamine D_2 receptors. Introduction of a hydroxyl group at the 4-position (**11**) of the piperidine ring resulted in a 3.7-fold decrease in the binding affinity for the 5-HT_4 receptor. The decrease in binding affinity was particularly drastic when the methoxy group occupied the 4-position of the piperidine ring (**12**).

A methoxy substituent (*cis* form) at the 3-position (**20**) caused an 18-fold decrease in binding affinity and a decline in the selectivity of the binding affinity for the 5-HT_4 receptor. Incorporation of a double bond at the 3,4-position of the piperidine ring (**27**) resulted in a loss of binding affinity for the 5-HT_4 receptor (K_i = 15 nM). These results suggest that the introduction of a steric hindrance (or electric atmosphere) at the 3-position of the piperidine ring could decrease selectivity for the 5-HT_4 receptor.

Table 1. Pharmacological data of benzoyl derivatives



Compd	R1	Binding affinity[a]		
		5-HT_4 K_i (nM)	5-HT_3 IC_{50} (nM)	D_2 IC_{50} (nM)
2		2.4	>1000	>1000
11		8.8	>1000	>1000
12		>100	>1000	>1000
20		44	180[b]	>1000
27		15	240[b]	NT[c]
31		28	>1000	>1000

[a] Each value is the mean from triplicate assay in a single experiment.
[b] K_i value.
[c] Not tested.

The piperidin-3-ylmethyl derivative **31** exhibited an 11-fold decrease in binding affinity compared to the piperidin-4-ylmethyl derivative **2**. These finding suggests that use of an unsubstituted piperidin-4-ylmethyl part as the cyclic amine on the benzamide is optimal for 5-HT_4 receptor-binding affinity.

The structure–activity relationships revealed by transformation to bioisosters of the carbonyl group (R2 part) on the benzamides are summarized in Table 2. The derivatives were measured for the rate of increase in defecation induced in mice at oral doses of 1 mg/kg (percentage increase in number, dry weight and wet weight of faecal deposits). Binding affinity for the 5-HT_4 receptor was retained after reduction of the carbonyl group of **2** (racemic **32**, K_i = 3.6 nM). Similarly, the oxime **33** and the racemic sulfoxide derivative **42** were nearly equipotent (K_i = 2.5 and 1.9, respectively) with the derivative **2**. However, the effect on defecation of **32** and **42** was considerably less than that of compound **2**. Compound **43b**, which possessed benzyloxy in the side-chain part of the 1-position of the piperidine ring, had moderate effect on defecation.

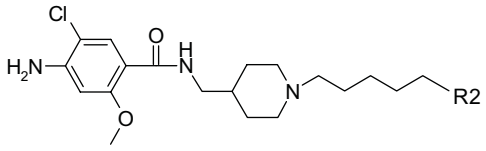
On the basis of the above results, the development of the structure–activity relationship was focused on the ether derivatives. As shown in Table 3, the synthesized phenoxy (or phenylthio) derivatives and benzyloxy (or benzylthio) derivatives possessed strong affinity for the 5-HT_4 receptor but showed little binding affinity for the 5-HT_3 receptor.

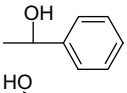
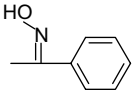
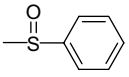
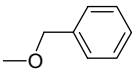
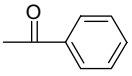
A series of phenoxy derivatives, **40a**–**e**, with 2–6 methylenes as a spacer between the piperidine and phenoxy moieties, possessed moderate binding affinity (K_i = 15, 4.5, 9.4, 6.2 and 4.1 nM) for the 5-HT_4 receptor. Compounds containing ethylene (**40a**) and propylene spacers (**40b**) displayed binding affinity for the dopamine D_2 receptor (43 and 89 nM, respectively), insertion of 4–6 methylenes as a spacer (**40c**–**e**) was effective in decreasing this affinity (IC_{50} > 1000 nM). These results suggest that in the hydrophobic region of the dopamine D_2 receptor there is not enough space available for a phenoxy group linked with 4–6 methylenes. The replacement of the phenyl group with a benzyl group increased the binding affinity for the 5-HT_4 receptor (**40c** vs **43a**, **40d** vs **43b** and **40e** vs **43c**).

Similarly, the effect on defecation (percentage increase in dry weight and wet weight of faecal deposits) of benzyloxy derivatives was greater than that of phenoxy compounds. The effect of **43c** linked with six methylenes was particularly strong (respective increases of 93%, 110% and 87% in the three items measured).

The saturation of the benzyl group of **43b** to the cyclohexylmethyl group resulted in a small decrease in binding affinity for the 5-HT_4 receptor (**43d**, K_i = 10 nM). This observation suggests that there may be a favourable interaction between the π-electron density of the

Table 2. Pharmacological data of benzamide derivatives



Compd	R2	Binding affinity[a]			Rate of increase in defecation[b] (%)		
		5-HT_4 K_i (nM)	5-HT_3 IC_{50} (nM)	D_2 IC_{50} (nM)	Number	Dry weight	Wet weight
32		3.6	>1000	NT[c]	−3	15	13
33		2.5	>1000	>1000		NT[c]	
42		1.9	>1000	>1000	40	33	25
43b		3.6	270[d]	>1000	59	81	68
2		2.4	>1000	>1000	49	90	76

[a] Each value is the mean from triplicate assay in a single experiment.
[b] Rate of increase in defecation induced in mice at oral dose of 1 mg/kg.
[c] Not tested.
[d] K_i value.

benzyl group of **43b** and the corresponding hydrophobic binding region of the 5-HT_4 receptor.

With introduction of a chlorine atom (**43e**), the binding affinity for the 5-HT_4 receptor was nearly retained than in the counterpart **43b**. Meanwhile, **43e** provided a potent effect on defecation (increases of 93% in number, 93% in dry weight and 77% in wet weight of faecal deposits). The naphthalen-2-ylmethoxy derivative **43f** also maintained binding affinity for the 5-HT_4 receptor (K_i = 4.7 nM) and displayed a moderate effect on defecation. This result demonstrates that the corresponding hydrophobic binding region of the 5-HT_4 receptor has enough space to fit a naphthalen-2-ylmethoxy moiety and this compound can produce agonistic activity.

The phenylthio derivatives **41a**–**d** possessed higher binding affinity (K_i = 2.6, 3.4, 4.1 and 2.3 nM) for the 5-HT_4 receptor than the corresponding phenoxy derivatives **40b**–**e**. The compound containing propylene spacer (**41a**) displayed a similar binding affinity for the dopamine D_2 receptor (K_i = 23 nM) to compounds **40a**,**b**. The replacement of the phenylthio group (**41c**) with a benzylthio group (**44**) increased the binding affinity (K_i = 1.7 nM). Compound **44** displayed moderate effect on defecation (increases of 80% in number, 89% in dry weight and 71% in wet weight of faecal deposits).

4. Conclusion

We performed modification of the parent compound **2**. The introduction of a hydroxyl or methoxy group at the 4-position of the piperidin-4-ylmethyl moiety led to decrease in the binding affinity for the 5-HT_4 receptor. Incorporation of a 3-methoxy group or double bond in the piperidine moiety also decreased the binding affinity. The conversion of the carbonyl group of the benzoyl part to a hydroxyl or sulfoxide (**32** and **42**) group maintained most of the binding affinity for the 5-HT_4 receptor, but the effect on defecation was reduced compared to compound **2**. Many of the ether and sulfide derivatives exhibited high affinity for the 5-HT_4 receptor. Among these, phenylthio **41c** and benzylthio derivative **44** were selective 5-HT_4 receptor agonists, and had a similar effect on defecation to compound **2**. Accordingly, these derivatives would be a useful tool for search of the 5-HT_4 receptor functions.

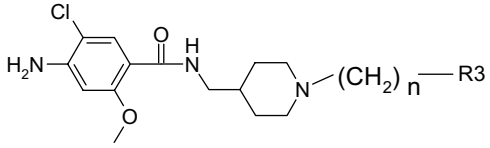
5. Experimental

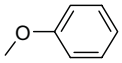
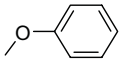
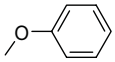
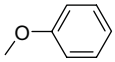
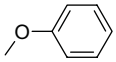
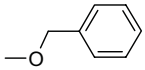
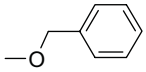
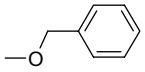
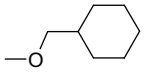
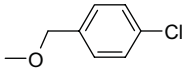
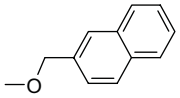
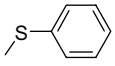
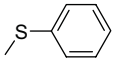
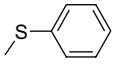
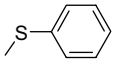
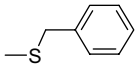
5.1. Chemistry

Melting points were determined in open capillaries and are uncorrected. Proton nuclear magnetic resonance (^{1}H NMR) spectra were recorded at 270 MHz on JEOL JNM-EX270 spectrometer. Coupling constants are reported in hertz (Hz) and chemical shifts are 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.

5.1.1. *tert*-Butyl (1-benzyl-4-hydroxypiperidin-4-yl)methylcarbamate (4). To a solution of 6-benzyl-1-oxa-6-azaspiro[2.5]octane (**3**) (39.1 g, 0.192 mmol) in ethanol (170 mL) was added 28% NH_4OH (117 mL). The reac-

Table 3. Pharmacological data of benzamide derivatives



Compd	R3	*n*	Binding affinity[a]			Rate of increase in defecation[b] (%)		
			5-HT_4 K_i (nM)	5-HT_3 IC_{50} (nM)	D_2 IC_{50} (nM)	Number	Dry weight	Wet weight
40a		2	15	>1000	43		NT[d]	
40b		3	4.5	140	89		NT[d]	
40c		4	9.4	>1000	>1000	76	31	24
40d		5	6.2	310[c]	>1000	73	73	66
40e		6	4.1	>1000	>1000	18	24	14
43a		4	2.2	>1000	>1000	46	64	56
43b		5	3.6	270[c]	>1000	59	81	68
43c		6	2.3	>1000	NT[d]	93	110	87
43d		5	10	>1000	>1000		NT[d]	
43e		5	5.2	>1000	NT[d]	93	93	77
43f		5	4.7	>1000	>1000	37	65	43
41a		3	2.6	200[c]	23	32	49	34
41b		4	3.4	>1000	>1000	64	86	91
41c		5	4.1	>1000	>1000	61	90	69
41d		6	2.3	>1000	>1000	32	49	34
44		5	1.7	340[c]	>1000	80	89	71
2			2.4	>1000	>1000	49	90	76

[a] Each value is the mean from triplicate assay in a single experiment.
[b] Rate of increase in defecation induced in mice at oral dose of 1 mg/kg.
[c] K_i value.
[d] Not tested.

tion mixture was stirred at room temperature for 9 h and evaporated to give crude aminoalcohol derivative. This material was dissolved in CH_2Cl_2 (250 mL) and di-*tert*-butyl dicarbonate (43.5 g, 0.199 mmol) was added at 0 °C. The mixture was stirred at room temperature for 3 h, washed with aqueous K_2CO_3 and dried over $MgSO_4$. The solvent was removed, and the residue was purified by column chromatography ($CHCl_3$–MeOH) to afford the title compound **4** as a pale yellow solid (36.5 g, 59% from **3**). ^1H NMR ($CDCl_3$): δ 1.43

(9H, s), 1.54–1.68 (4H, m), 2.28–2.45 (3H, m), 2.55–2.70 (2H, m), 3.05–3.11 (2H, m), 3.52 (2H, s), 4.83–5.00 (1H, m), 7.18–7.36 (5H, m); MS (EI) *m*/*z* 320 (M^+).

5.1.2. *tert*-Butyl (1-benzyl-4-methoxypiperidin-4-yl)methylcarbamate (5). To a suspension of 60% NaH (1.87 g, 77.9 mmol) in DMF (50 mL) was added a solution of **4** (15.0 g, 46.8 mmol) in DMF (100 mL) at 0 °C, and resulting solution was stirred at room temperature for 1 h. The solution was cooled to 0 °C, and MeI (6.64 g, 46.8 mmol) was added slowly. The reaction mixture was stirred at room temperature for 5 h and diluted with water. The product was extracted with ethyl acetate. The combined extracts were washed with brine and dried over $MgSO_4$. The solvent was evaporated, and the residue was purified by column chromatography (ethyl acetate–methanol) to afford the title compound **5** as a pale yellow solid (6.0 g, 38%). ^1H NMR ($CDCl_3$): δ 1.43 (9H, s), 1.49–1.85 (4H, m), 2.20–2.40 (2H, m), 2.45–2.68 (2H, m), 3.16 (3H, s), 3.17–3.22 (2H, m), 3.52 (2H, s), 4.59–4.74 (1H, m), 7.19–7.39 (5H, m); MS (EI) *m*/*z* 334 (M^+).

5.1.3. *tert*-Butyl (4-methoxypiperidin-4-yl)methylcarbamate (6). A mixture of **5** (6.0 g, 17.9 mmol), 10% Pd–C (2.0 g) and hydrazine hydrate (0.87 mL) in ethanol (70 mL) was refluxed for 3 h. The mixture was cooled to room temperature and the resulting precipitate was filtered. The filtrate was evaporated to give **6** (3.8 g, 87%) as a colourless oil. ^1H NMR ($CDCl_3$): δ 1.44 (9H, s), 1.46–1.84 (4H, m), 2.85–3.01 (4H, m), 3.18 (3H, s), 3.19–3.28 (1H, m), 3.29–3.56 (2H, m), 4.61–4.80 (1H, m); MS (EI) *m*/*z* 244 (M^+).

5.1.4. *tert*-Butyl (4-hydroxypiperidin-4-yl)methylcarbamate (7). The same procedure, as described for synthesis of **6**, was followed using **4** (4.0 g, 12.5 mmol), 10% Pd–C (1.0 g) and hydrazine hydrate (0.60 mL) in ethanol (40 mL). The filtrate was concentrated to give **7** (2.8 g, 97%) as a colourless oil. ^1H NMR ($CDCl_3$): δ 1.44 (9H, s), 1.50–1.62 (4H, m), 2.55–2.75 (2H, m), 2.80–3.02 (4H, m), 3.09–3.19 (2H, m), 5.05–5.19 (1H, m); MS (EI) *m*/*z* 242 (M^+).

5.1.5. 6-(4-Aminomethyl-4-hydroxypiperidin-1-yl)-1-phenylhexan-1-one dihydrochloride (9). A mixture of **7** (2.50 g, 10.2 mmol), 6-bromo-1-phenylhexan-1-one (**8**) (2.90 g, 11.4 mmol) and K_2CO_3 (3.00 g, 21.7 mmol) in DMF (40 mL) was stirred at 60 °C for 2 h. The mixture was cooled to room temperature and diluted with water. The product was extracted with $CHCl_3$. The combined extracts were washed with brine and dried over $MgSO_4$. The solvent was removed, the residue was purified by column chromatography ($CHCl_3$–MeOH) to afford *tert*-butyl [4-hydroxy-1-(6-oxo-6-phenyl)piperidine-4-ylmethyl]carbamate as an oil. To the solution of this material in 2-propanol (30 mL) was added 15% HCl/2-propanol (10 mL) and stirred at 60 °C for 3 h. After cooling to room temperature, the resulting crystals were filtrated to give the title compound **9** (2.92 g, 76%) as a colourless solid. ^1H NMR (DMSO-d_6): δ 1.20–1.47 (2H, m), 1.55–2.05 (8H, m), 2.68–2.87 (2H, m), 2.94–3.18 (8H, m), 5.40–5.74 (1H, m), 7.45–7.57 (2H, m), 7.59–7.70 (1H, m), 7.90–7.99 (2H, m), 8.12 (3H, br s), 10.60 (1H, br).

5.1.6. 6-(4-Aminomethyl-4-methoxypiperidin-1-yl)-1-phenylhexan-1-one dihydrochloride (10). A mixture of **6** (2.30 g, 9.41 mmol), **8** (2.50 g, 9.80 mmol) and K_2CO_3 (2.60 g, 18.8 mmol) in DMF (40 mL) was stirred at 60 °C for 2 h. The mixture was cooled to room temperature and diluted with water. The product was extracted with $CHCl_3$. The combined extracts were washed with brine, dried over $MgSO_4$ and the solvent was evaporated. The residue was purified by column chromatography ($CHCl_3$–MeOH) to afford *tert*-butyl [4-methoxy-1-(6-oxo-6-phenylhexyl)piperidin-4-ylmethyl]carbamate (3.4 g, 85%) as an oil.

To the solution of this compound (3.35 g, 8.0 mmol) in 2-propanol (15 mL) was added 15% HCl/2-propanol (15 mL). This mixture was stirred at 60 °C for 1 h and evaporated to give the title compound **10** as a colourless solid (2.25 g, 72%); mp 185–188 °C. ^1H NMR (DMSO-d_6): δ 1.22–2.16 (10H, m), 2.78–3.13 (10H, m), 3.14 (3H, s), 7.48–7.58 (2H, m), 7.59–7.70 (1H, m), 7.95–8.02 (2H, m), 8.05–8.19 (2H, m), 8.75–9.04 (m, 1H), 10.71–11.05 (1H, m).

5.1.7. 4-Amino-5-chloro-*N*-[4-hydroxy-1-(6-oxo-6-phenylhexyl)piperidin-4-ylmethyl]-2-methoxybenzamide (11). To a solution of **9** (2.90 g, 7.69 mmol), 4-amino-5-chloro-2-methoxybenzoic acid (1.55 g, 7.67 mmol), Et_3N (3.2 mL, 23.0 mmol) and HOBt (1.09 g, 7.12 mmol) in DMF (50 mL) was added EDC hydrochloride (1.55 g, 8.09 mmol) at 0 °C and stirred at room temperature for 6 h. The mixture was diluted with water and extracted with $CHCl_3$. The combined extracts were washed with brine and dried over $MgSO_4$. The solvent was evaporated, and purified by column chromatography ($CHCl_3$–MeOH). Crystallization from ethyl acetate gave the title compound **11** (2.38 g, 63%) as a colourless solid; mp 102–103 °C. ^1H NMR ($CDCl_3$): δ 1.29–1.84 (10H, m), 2.26–2.49 (4H, m), 2.54–2.69 (2H, t, J = 7.6 Hz), 2.97 (2H, t, J = 7.6 Hz), 3.36 (1H, br s), 3.46 (2H, d, J = 6.0 Hz), 3.89 (3H, s), 4.47 (2H, br s), 6.30 (1H, s), 7.40–7.50 (2H, m), 7.51–7.60 (1H, m), 7.90–7.98 (2H, m), 7.99–8.06 (1H, m), 8.08 (1H, s); MS (EI) *m*/*z* 487 (M^+). Anal. Calcd for $C_{26}H_{34}ClN_3O_4 \cdot 1/4H_2O$: C, 63.40; H, 7.06; N, 8.53. Found: C, 63.32; H, 7.10; N, 8.20.

5.1.8. 4-Amino-5-chloro-2-methoxy-*N*-[4-methoxy-1-(6-oxo-6-phenylhexyl)piperidin-4-ylmethyl]benzamide (12). To a solution of **10** (2.25 g, 5.75 mmol), 4-amino-5-chloro-2-methoxybenzoic acid (1.16 g, 5.75 mmol), Et_3N (2.40 mL, 17.2 mmol) and HOBt (0.815 g, 5.32 mmol) in DMF (50 mL) was added EDC hydrochloride (1.16 g, 6.05 mmol) at 0 °C. The reaction mixture was stirred at room temperature for 4 h and poured into ice/water. The product was extracted with $CHCl_3$. The combined extracts were washed with brine, dried over $MgSO_4$ and the solvent was evaporated to obtain a crude oil. This oil was purified by column chromatography ($CHCl_3$–MeOH). Crystallization from ethyl acetate/*n*-hexane gave the title compound **12**

(1.43 g, 50%) as a colourless solid; mp 91–93 °C. ^{1}H NMR ($CDCl_3$): δ 1.30–1.91 (11H, m), 2.23–2.43 (4H, m), 2.47–2.65 (2H, m), 2.97 (2H, t, J = 7.3 Hz), 3.22 (3H, s), 3.51 (2H, d, J = 5.3 Hz), 3.89 (3H, s), 4.40 (1H, br s), 6.29 (1H, br s), 7.40–7.61 (3H, m), 7.85–8.01 (3H, m), 8.10 (1H, s); MS (EI) m/z 501 (M^+). Anal. Calcd for $C_{27}H_{36}ClN_3O_4 \cdot 0.15H_2O$: C, 64.25; H, 7.25; N, 8.32. Found: C, 64.26; H, 7.23; N, 8.36.

5.2. General procedure for the preparation of compounds 20, 27

A suspension of benzamide (2.70 mmol), **8** (758 mg, 2.97 mmol) and K_2CO_3 (0.41 g, 2.97 mmol) in DMF (20 mL) stirred at 60–70 °C for 2–8 h. The resulting solution was cooled, then treated with aqueous K_2CO_3 and extracted with $CHCl_3$. The combined extracts were evaporated and residue was purified by column chromatography. Crystallization from ethyl acetate or ethanol gave the title compound as a colourless solid.

5.2.1. (3*R,4*S**)-1-Benzyl-4-(hydroxymethyl)piperidin-3-ol (14).** To a solution of **13** (21.0 g, 80.4 mmol) in EtOH (200 mL) was added $NaBH_4$ (9.1 g, 0.24 mol) at 0 °C and stirred at room temperature for 9 h. The mixture was diluted with water and extracted with $CHCl_3$. The combined extracts were washed with brine, dried over $MgSO_4$ and the solvent was evaporated. The resulting residue was purified by silica gel column chromatography ($CHCl_3$–MeOH) to afford the title compound **14** (10.6 g, 59%) as a colourless oil. (The configuration of compound **14** was assessed by NOESY–NMR experiment.)

5.2.2. (3*R,4*S**)-1-(*tert*-Butoxycarbonyl)-4-(hydroxymethyl)piperidin-3-ol (15).** A mixture of **14** (7.1 g, 32.1 mmol), hydrazine monohydrate (3.12 mL, 64.3 mmol) and 10% Pd–C (6.0 g) in ethanol (120 mL) was refluxed for 5 h. The reaction mixture was cooled to room temperature and resulting solid was filtered. The filtrate was concentrated to give 4-(hydroxymethyl)piperidin-3-ol as a colourless oil. To a solution of this material in DMF (100 mL) was added di-*tert*-butyl dicarbonate (8.4 g, 38.5 mmol), and the solution was stirred at room temperature for 22 h. The solvent was evaporated and the residue was purified by column chromatography ($CHCl_3$–MeOH) to afford the title compound **15** (6.50 g, 88%) as a colourless oil. ^{1}H NMR (DMSO-d_6): δ 1.55 (9H, s), 1.57–1.85 (2H, m), 2.48–2.95 (4H, m), 3.62–3.79 (3H, m), 4.01–4.27 (2H, m).

5.2.3. (3*R,4*S**)-4-(Azidomethyl)-1-(*tert*-butoxycarbonyl)piperidin-3-ol (16).** To a solution of **15** (6.5 g, 28 mmol), Et_3N (5.57 mL) in CH_2Cl_2 (100 mL) was added methanesulfonyl chloride (3.54 g, 30.9 mmol) and the mixture was stirred at room temperature for 6 h. The mixture was diluted with water and extracted with $CHCl_3$. The combined extracts were washed with brine, dried over $MgSO_4$ and evaporated to obtain mesyl compound as a crude oil. A mixture of mesyl compound (4.44 g, 14.4 mmol), NaN_3 (1.40 g, 21.5 mmol) and NH_4Cl (1.15 g, 21.5 mmol) in DMF (60 mL) was stirred at 60 °C for 6 h. The reaction mixture was cooled to room temperature and diluted with water. The product was extracted with $CHCl_3$. The combined extracts were washed with brine, dried over $MgSO_4$ and the solvent was removed in vacuo. The resulting residue was purified by column chromatography ($CHCl_3$–MeOH) to give **16** (3.4 g, 92%) as a colourless oil. ^{1}H NMR ($CDCl_3$): δ 1.46 (9H, s), 1.50–1.85 (3H, m), 2.65–2.88 (2H, m), 2.99–3.16 (1H, m), 3.19–3.30 (1H, m), 3.40–3.50 (1H, m), 4.05–4.64 (3H, m).

5.2.4. (3*R,4*S**)-4-(Azidomethyl)-1-(*tert*-butoxycarbonyl)-3-methoxypiperidine (17).** To a suspension of 60% NaH (580 mg, 24.1 mol) in DMF (10 mL) was added **16** (3.4 g, 13.3 mmol) in DMF (10 mL) and the mixture was stirred at room temperature for 1 h. To this solution was added MeI (3.72 mL, 59.8 mmol) and mixture was stirred at room temperature for 17 h. The mixture was diluted with water and extracted with $CHCl_3$. The combined organic extracts were washed with brine, dried over $MgSO_4$ and the solvent was evaporated. The residue was purified by column chromatography eluted with $CHCl_3$ to afford the title compound **17** (1.50 g, 42%) as a colourless oil. ^{1}H NMR ($CDCl_3$): δ 1.47 (9H, s), 1.51–1.62 (2H, m), 1.68–1.85 (1H, m), 2.51–2.83 (2H, m), 3.08–3.23 (1H, m), 3.28–3.39 (1H, m), 3.40–3.55 (1H, m), 3.36 (3H, s), 3.89–4.59 (2H, m).

5.2.5. (3*R,4*S**)-4-(Aminomethyl)-1-(*tert*-butoxycarbonyl)-3-methoxypiperidine (18).** A mixture of **17** (1.50 g, 5.55 mmol) and 10% $Pd(OH)_2$ (0.6 g) in MeOH (50 mL) was stirred at room temperature for 2 h. The mixture was filtered and filter pad was washed with MeOH. The filtrate and washings were concentrated to give **18** (1.20 g, 88%) as a colourless oil. ^{1}H NMR (DMSO-d_6): δ 1.39 (9H, s), 1.50–2.15 (3H, m), 2.55–2.97 (4H, m), 2.98–3.10 (1H, m), 3.30 (3H, s), 3.45–4.65 (4H, m).

5.2.6. 4-Amino-5-chloro-2-methoxy-*N*-[(3*R,4*S**)-3-methoxypiperidin-4-ylmethyl]benzamide dihydrochloride (19).** To a mixture of 4-amino-5-chloro-2-methoxybenzoic acid (0.99 g, 4.9 mmol), **18** (1.2 g, 4.9 mmol) and HOBt (0.70 g, 5.2 mmol) in DMF (30 mL) was added EDC hydrochloride (0.99 g, 5.2 mmol) at 0 °C and the mixture was stirred at room temperature for 4 h. The solution was diluted with water and extracted with $CHCl_3$. The combined extracts were washed with brine, dried over $MgSO_4$ and the solvent was evaporated in vacuo. The residue was purified by column chromatography ($CHCl_3$–MeOH) to afford pure oil. To the solution of this compound in 2-propanol was added 15% HCl/2-propanol. This mixture was stirred at 60 °C for 1 h and evaporated to give the title compound **19** (0.88 g, 45% from **18**) as a colourless solid. ^{1}H NMR (DMSO-d_6): δ 1.45–1.71 (2H, m), 1.86–2.05 (1H, m), 2.70–2.95 (2H, m), 3.00–3.58 (6H, m), 3.35 (3H, m), 3.84 (3H, s), 5.92 (2H, br s), 6.54 (1H, s), 7.67 (1H, s), 7.90–8.05 (1H, m), 8.14–8.50 (1H, m), 9.48–9.75 (1H, m).

5.2.7. 4-Amino-5-chloro-2-methoxy-*N*-[3-methoxy-1-(6-oxo-6-phenylhexyl)piperidin-4-yl-methyl]benzamide fumalate (20). Prepared from **19** and **8** according to the general procedure. The resulting oil was transformed into

fumalate and recrystallized from ethanol/EtOAc to give the title compound **20** (84%) as a colourless solid; mp 99–101 °C. ^{1}H NMR (DMSO-d_6): δ 1.21–1.72 (9H, m), 2.28–2.45 (2H, m), 2.51–2.65 (2H, m), 2.92–3.10 (3H, m), 3.17–3.35 (6H, m), 3.39–3.48 (1H, m), 3.83 (3H, s), 5.92 (2H, br s), 6.48 (1H, s), 7.46–7.58 (2H, m), 7.59–7.71 (2H, m), 7.85–8.04 (3H, m). Anal. Calcd for $C_{27}H_{36}ClN_3O_4{\cdot}C_4H_4O_4{\cdot}3/2H_2O$: C, 57.71; H, 6.72; N, 6.51. Found: C, 57.78; H, 6.54; N, 6.41.

5.2.8. 1-Ethoxycarbonyl-4-hydroxy-(4-nitromethyl)piperidine (22). To a solution of Na (4.6 g, 0.20 mol) in ethanol (80 mL), was added 1-ethoxycarbonylpiperidine-4-one (**21**) (34.2 g, 0.20 mol), nitromethane (16 g, 0.26 mol) and stirred at 50 °C for 1 h. The mixture was diluted with water and extracted with ethyl acetate The combined extracts were washed with brine, dried over $MgSO_4$ and evaporated to obtain compound **22** as a crude oil. ^{1}H NMR ($CDCl_3$): δ 1.26 (3H, t, J = 7.3 Hz), 1.51–1.78 (4H, m), 2.95–3.32 (3H, m), 3.87–4.05 (2H, m), 4.13 (2H, q, J = 7.3 Hz), 4.43 (2H, s); MS (EI) m/z 232 (M^+).

5.2.9. 1-Ethoxycarbonyl-4-nitromethyl-1,2,3,6-tetrahydropyridine (23). A mixture of **22** (10.0 g, 43.1 mmol) and P_2O_5 (30.6 g, 108 mmol) in benzene (130 mL) was refluxed for 2.5 h. The mixture was cooled to room temperature and was filtered. The filtrate was washed with brine, dried over $MgSO_4$ and the solvent was evaporated to obtain **23** (4.27 g, 46%) as a colourless oil. ^{1}H NMR ($CDCl_3$): δ 1.27 (3H, t, J = 7.3 Hz), 2.15–2.30 (2H, m), 3.51–3.68 (2H, m), 3.99–4.10 (2H, m), 4.16 (2H, q, J = 7.3 Hz), 4.89 (2H, br s), 5.85–6.00 (1H, m); MS (EI) m/z 214 (M^+), 168 ([M-NO_2]$^+$).

5.2.10. 4-Aminomethyl-1-ethoxycarbonyl-1,2,3,6-tetrahydropyridine (24). A mixture of **23** (2.0 g, 9.3 mmol), Fe (2.5 g), NH_4Cl (2.5 g, 47 mmol) and water (20 mL) in toluene (100 mL) was refluxed for 6 h. The mixture was cooled to room temperature and filtered. The filtrate was washed with aqueous NaOH, dried over $MgSO_4$ and the solvent was evaporated to obtain **24** (1.53 g, 89%) as a colourless oil. ^{1}H NMR ($CDCl_3$): δ 1.26 (3H, t, J = 7.0 Hz), 1.91–2.21 (2H, m), 3.14–3.26 (2H, m), 3.40–3.65 (2H, m), 3.81–4.00 (2H, m), 4.13 (2H, q, J = 7.0 Hz), 5.35–5.69 (1H, m), 7.00–7.32 (2H, m); MS (EI) m/z 184 (M^+), 167 ([M$-NH_3$]$^+$).

5.2.11. 4-Amino-5-chloro-2-methoxy-*N*-[1-ethoxycarbonyl-(1,2,3,6-tetrahydropyridin)-4-ylmethyl]benzamide (25). To a solution of **24** (1.53 g, 8.3 mmol), 4-amino-5-chloro-2-methoxybenzoic acid (1.67 g, 8.3 mmol) and HOBt (1.18 g, 8.7 mmol) in DMF (50 mL) was added EDC hydrochloride (1.67 g, 8.71 mmol) and the mixture was stirred at room temperature for 14 h. The mixture was diluted with water and extracted with $CHCl_3$. The combined extracts were washed with aqueous K_2CO_3, dried over $MgSO_4$ and the solvent was evaporated to obtain. The residue was purified by column chromatography ($CHCl_3$–MeOH) to afford **25** (2.18 g, 71%) as a colourless solid. ^{1}H NMR ($CDCl_3$): δ 1.26 (3H, t, J = 7.2 Hz), 1.55–1.68 (2H, m), 2.00–2.19 (2H, m), 3.47–3.61 (2H, m), 3.91 (3H, s), 3.85–4.05 (2H, m), 4.15 (2H, q, J = 7.2 Hz), 4.31–4.49 (2H, br s), 5.49–5.62 (1H, m), 6.31 (1H, s), 7.66–7.81 (1H, m), 8.12 (1H, s); MS (EI) m/z 367 (M^+).

5.2.12. 4-Amino-5-chloro-2-methoxy-*N*-[(1,2,3,6-tetrahydropyridin)-4-ylmethyl]benzamide (26). A mixture of **25** (1.1 g, 3.0 mmol), 85% KOH (0.84 g, 13 mmol) in 2-propanol (11 mL) was refluxed for 2.5 h. The mixture was cooled to room temperature and diluted with water. The product was extracted with $CHCl_3$. The combined extracts were washed with brine, dried over $MgSO_4$ and the solvent was evaporated. The residue was purified by column chromatography ($CHCl_3$–MeOH) to afford **26** (0.40 g, 31%) as a yellow oil. ^{1}H NMR ($CDCl_3$): δ 1.60–1.85 (1H, m), 2.00–2.10 (2H, m), 2.97 (2H, t, J = 6.0 Hz), 3.20–3.39 (2H, m), 3.90 (3H, s), 3.94–4.02 (2H, m), 4.39 (2H, br s), 5.57–5.68 (1H, m), 6.30 (1H, s), 7.64–7.80 (1H, m), 8.12 (1H, s); MS (EI) m/z 295 (M^+).

5.2.13. 4-Amino-5-chloro-2-methoxy-*N*-[1-(6-oxo-6-phenylhexyl)-(1,2,3,6-tetrahydropyridin)-4-yl-methyl]benzamide (27). Compound **27** was prepared in 60% yield according to the general procedure from **26** and **8**: colourless solid; mp 112–114 °C (ethyl acetate). ^{1}H NMR ($CDCl_3$): δ 1.34–1.49 (2H, m), 1.52–1.68 (2H, m), 1.70–1.85 (2H, m), 2.12–2.25 (2H, m), 2.38–2.50 (2H, m), 2.55–2.69 (2H, m), 2.92–3.04 (4H, m), 3.88 (3H, s), 3.95–4.05 (2H, m), 4.42 (2H, br s), 5.53–5.63 (1H, m), 6.30 (1H, s), 7.39–7.41 (2H, m), 7.51–7.61 (1H, m), 7.65–7.79 (1H, m), 7.89–8.01 (2H, m), 8.11 (1H, s); MS (EI) m/z 469 (M^+). Anal. Calcd for $C_{26}H_{32}ClN_3O_3{\cdot}1/2H_2O$: C, 65.19; H, 6.94; N, 8.77. Found C, 65.33; H, 6.85; N, 8.87.

5.2.14. *tert*-Butyl [1-(6-oxo-6-phenylhexyl)piperidin-3-ylmethyl]carbamate (29). A mixture of **28** (5.66 g, 26.4 mmol), 6-bromo-1-phenylhexan-1-one (**8**) (6.10 g, 29.1 mmol) and K_2CO_3 (8.03 g, 58.1 mmol) in DMF (40 mL) was stirred at 70 °C for 10 h. The mixture was cooled to room temperature and diluted with water. The product was extracted with ethyl acetate. The combined extracts were washed with brine and dried over $MgSO_4$. The solvent was removed and the residue was purified by column chromatography ($CHCl_3$–MeOH) to afford title compound **29** (8.30 g, 81%) as a colourless oil. ^{1}H NMR ($CDCl_3$): δ 1.25–1.96 (11H, m), 1.44 (9H, s), 2.21–2.41 (2H, m), 2.75–2.90 (2H, m), 2.94–3.10 (4H, m), 4.54–4.74 (2H, m), 7.41–7.51 (2H, m), 7.52–7.62 (2H, m), 7.92–8.04 (2H, m).

5.2.15. 6-(3-Aminomethylpiperidin-1-yl)-1-phenylhexan-1-one 2hydrochloride (30). To a solution of **29** (8.29 g, 21.4 mmol) in water (50 mL) was added hydrochloric acid (50 mL) and mixture was stirred at room temperature for 4 h. The mixture was evaporated to obtain crude oil. Crystallization from ethanol gave the title compound **30** (4.29 g, 62%) as a colourless solid. ^{1}H NMR ($CDCl_3$/CD_3OD): δ 1.25–1.55 (3H, m), 1.70–2.19 (8H, m), 2.41–2.68 (1H, m), 2.78–3.18 (9H, m), 2.41–2.68 (1H, m), 2.78–3.18 (1H, m), 3.45–3.60 (1H, m), 3.65–3.79 (1H, m), 7.39–7.52 (2H, m), 7.57–7.65 (1H, m), 7.91–8.04 (2H, m).

5.2.16. 4-Amino-5-chloro-2-methoxy-*N*-[1-(6-oxo-6-phenylhexyl)piperidin-3-ylmethyl]benzamide (31). To a solution of **30** (4.25 g, 11.8 mmol), 4-amino-5-chloro-2-methoxybenzoic acid (2.62 g, 13.0 mmol), Et_3N (5.26 g, 52.0 mmol) and HOBt (1.76 g, 13.0 mmol) in DMF (100 mL) was added EDC hydrochloride (2.49 g, 13.0 mmol) and stirred at room temperature for 22 h. The mixture was diluted with water and extracted with $CHCl_3$. The combined extracts were washed with aqueous K_2CO_3, dried over $MgSO_4$ and the solvent was evaporated. Crystallization from ethyl acetate/*n*-hexane gave the title compound **31** (3.46 g, 62%) as a colourless solid; mp 73–76 °C. ^{1}H NMR ($CDCl_3$): δ 0.91–1.97 (13H, m), 2.25–2.41 (2H, m), 2.75–3.02 (4H, m), 3.22–3.44 (2H, m), 3.88 (3H, s), 4.42 (2H, br s), 6.29 (1H, s), 7.39–7.50 (2H, m), 7.51–7.59 (1H, m), 7.65–7.75 (1H, m), 7.90–7.99 (2H, m), 8.10 (1H, s). Anal. Calcd for $C_{26}H_{34}ClN_3O_3\cdot 1/2H_2O$: C, 64.92; H, 7.12; N, 8.74. Found: C, 64.96; H, 7.38; N, 8.74.

5.2.17. 4-Amino-5-chloro-*N*-[1-(6-hydroxy-6-phenylhexyl)piperidin-4-ylmethyl]-2-methoxybenzamide (32). To a solution of **2** (1.0 g, 2.1 mmol) in ethanol (20 mL) was added $NaBH_4$ (0.16 g, 4.2 mmol) and mixture was stirred at room temperature for 1 h. The mixture was diluted with water and extracted with $CHCl_3$. The combined extracts were washed with brine, dried over $MgSO_4$ and the solvent was evaporated to obtain crude oil. Crystallization from ethyl acetate gave the title compound **32** (0.95 g, 95%) as a colourless solid; mp 157–158 °C. ^{1}H NMR (DMSO-d_6): δ 1.20–1.95 (15H, m), 2.12–2.40 (3H, m), 2.81–2.99 (2H, m), 3.30 (2H, t, J = 6.3 Hz), 3.88 (3H, s), 4.40 (2H, br s), 4.60–4.72 (1H, m), 6.28 (1H, s), 7.19–7.40 (5H, m), 7.66–7.82 (1H, m), 8.10 (1H, m). Anal. Calcd for $C_{26}H_{36}ClN_3O_3\cdot 1/3H_2O$: C, 65.13; H, 7.69; N, 8.76. Found: C, 65.01; H, 7.60; N, 8.73.

5.2.18. 4-Amino-5-chloro-*N*-[1-[6-(hydroxyimino)-6-phenylhexyl]piperidin-4-ylmethyl]-2-methoxybenzamide (33). To a solution of **2** (1.0 g, 2.1 mmol) and pyridine (2 mL) in MeOH (10 mL) was added hydroxylamine hydrochloride (0.15 g, 2.1 mmol), and refluxed for 6 h. The mixture was cooled to room temperature, diluted with aqueous K_2CO_3 and extracted with MeOH–$CHCl_3$. The combined extracts were washed with brine, dried over $MgSO_4$ and evaporated to obtain crude oil. Crystallization from ethanol gave the title compound **33** (0.80 g, 76%) as a colourless solid; mp 150–151 °C. ^{1}H NMR ($CDCl_3$): δ 1.28–1.78 (10H, m), 1.80–2.03 (3H, m), 2.25–2.39 (2H, m), 2.70–2.83 (2H, m), 2.89–3.05 (2H, m), 3.32 (2H, t, J = 5.9 Hz), 3.85 (3H, s), 4.40 (2H, br s), 6.26 (1H, s), 7.30–7.41 (3H, m), 7.55–7.65 (2H, m), 7.71–7.84 (1H, m), 8.10 (1H, s), 10.08 (1H, br s). Anal. Calcd for $C_{26}H_{35}ClN_4O_3$: C, 64.12; H, 7.24; N, 11.50. Found: C, 63.90; H, 7.24; N, 11.44.

5.3. General procedure for the preparation of compounds 40a–e, 41a–d, 42, 43a–f, 44

A suspension of **39** (1.00 g, 2.70 mmol), aralkyl chloride (2.97 mmol) and K_2CO_3 (0.41 g, 2.97 mmol) in DMF (20 mL) was stirred at 60–70 °C for 2–8 h. The resulting solution was cooled, then treated with aqueous K_2CO_3 and extracted with $CHCl_3$. The combined extracts were evaporated and residue was purified by column chromatography. Crystallization from ethyl acetate or ethanol gave the title compound as a colourless solid.

5.3.1. 4-Amino-5-chloro-2-methoxy-*N*-[1-[5-(phenylsulfinyl)pentyl]piperidin-4-ylmethyl]benzamide (42). Compound **42** was prepared according to the general procedure from **39** and **36**; amorphous. ^{1}H NMR ($CDCl_3$): δ 1.20–2.02 (13H, m), 2.24–2.39 (2H, m), 2.78 (2H, t, J = 7.9 Hz), 2.85–2.98 (2H, m), 3.32 (2H, t, J = 6.3 Hz), 3.90 (3H, s), 4.38 (2H, br s), 6.29 (1H, s), 7.45–7.58 (3H, m), 7.59–7.65 (2H, m), 7.69–8.22 (1H, m), 8.10 (1H, m); MS (FAB) m/z 492 ($M+H^+$). Anal. Calcd for $C_{25}H_{34}ClN_3O_3S\cdot 1.1H_2O$: C, 58.66; H, 7.13; N, 8.22. Found: C, 58.37; H, 6.88; N, 8.07.

5.3.2. 4-Amino-5-chloro-2-methoxy-*N*-[1-(4-phenoxyethyl)piperidin-4-ylmethyl]benzamide (40a). Compound **40a** was prepared according to the general procedure from **39** and **34a**: colourless solid; mp 71–73 °C (ethyl acetate). ^{1}H NMR ($CDCl_3$): δ 1.26–1.45 (2H, m), 1.51–1.97 (3H, m), 2.01–2.21 (2H, m), 2.74–2.85 (2H, m), 2.94–3.05 (2H, m), 3.33 (2H, t, J = 6.3 Hz), 3.89 (3H, s), 4.10 (2H, t, J = 6.3 Hz), 4.40 (2H, br s), 6.29 (1H, s), 6.87–6.97 (3H, m), 7.23–7.31 (2H, m), 7.66–7.84 (1H, m), 8.11 (1H, s). Anal. Calcd for $C_{22}H_{28}ClN_3O_3\cdot 1/5H_2O$: C, 62.68; H, 6.79; N, 9.97. Found: C, 62.59; H, 6.76; N, 9.98.

5.3.3. 4-Amino-5-chloro-2-methoxy-*N*-[1-(4-phenoxypropyl)piperidin-4-ylmethyl]benzamide (40b). Compound **40b** was prepared in 46% yield according to the general procedure from **39** and **34b**: colourless solid; mp 132–134 °C (ethyl acetate). ^{1}H NMR ($CDCl_3$): δ 1.26–1.46 (2H, m), 1.54–1.80 (3H, m), 1.90–2.08 (4H, m), 2.48–2.59 (2H, m), 2.90–3.04 (2H, m), 3.33 (2H, t, J = 6.3 Hz), 3.89 (3H, s), 4.00 (2H, t, J = 6.3 Hz), 4.40 (2H, br s), 6.29 (1H, s), 6.85–6.96 (3H, m), 7.21–7.30 (2H, m), 7.70–7.84 (1H, m), 8.10 (1H, s); MS (EI) m/z 431 (M^+). Anal. Calcd for $C_{23}H_{30}ClN_3O_3\cdot 2/5H_2O$: C, 62.90; H, 6.89; N, 9.57. Found: C, 62.98; H, 7.01; N, 9.53.

5.3.4. 4-Amino-5-chloro-2-methoxy-*N*-[1-(4-phenoxybutyl)piperidin-4-ylmethyl]benzamide (40c). Compound **40c** was prepared according to the general procedure from **39** and **34c**: colourless solid; mp 71–73 °C (ethyl acetate). ^{1}H NMR (DMSO-d_6): δ 1.22–2.02 (11H, m), 2.30–2.45 (2H, m), 2.85–3.03 (2H, m), 3.32 (2H, t, J = 6.3 Hz), 3.88 (3H, s), 3.97 (2H, t, J = 6.0 Hz), 4.41 (2H, br s), 6.28 (1H, s), 6.83–6.99 (3H, m), 7.20–7.36 (2H, m), 7.66–7.82 (1H, m), 8.10 (1H, s). Anal. Calcd for $C_{24}H_{32}ClN_3O_3\cdot 1/4H_2O$: C, 63.99; H, 7.27; N, 9.33. Found: C, 63.98; H, 7.31; N, 9.41.

5.3.5. 4-Amino-5-chloro-2-methoxy-*N*-[1-(5-phenoxypentyl)piperidin-4-ylmethyl]benzamide (40d). Compound **40d** was prepared according to the general procedure from **39** and **34d**: colourless solid; mp 129–132 °C (ethyl acetate). ^{1}H NMR ($CDCl_3$): δ 1.34–1.86 (11H, m), 1.94–2.06 (2H, m), 2.36–2.45 (2H, m), 2.96–3.04 (2H, m),

3.33 (2H, t, $J = 6.6$ Hz), 3.89 (3H, s), 3.94 (2H, t, $J = 6.6$ Hz), 4.40 (2H, s), 6.29 (1H, s), 6.85–6.96 (3H, m), 7.22–7.30 (2H, m), 7.71–7.79 (1H, m), 8.10 (1H, s); MS (EI) *m/z* 459 (M^+). Anal. Calcd for $C_{25}H_{34}ClN_3O_3 \cdot 1/2H_2O$: C, 64.02; H, 7.52; N, 8.96. Found: C, 63.83; H, 7.50; N, 8.99.

5.3.6. 4-Amino-5-chloro-2-methoxy-*N*-[1-(6-phenoxyhexyl)piperidin-4-ylmethyl]benzamide (40e). Compound **40e** was prepared according to the general procedure from **39** and **34e**: colourless solid; mp 113–115 °C (ethyl acetate). ^{1}H NMR ($CDCl_3$): δ 1.29–1.86 (13H, m), 1.95–2.16 (2H, m), 2.45–2.51 (2H, m), 2.97–3.13 (2H, m), 3.33 (2H, t, $J = 6.0$ Hz), 3.89 (3H, s), 3.94 (2H, t, $J = 6.6$ Hz), 4.39 (2H, br s), 6.30 (1H, s), 6.81–6.98 (3H, m), 7.19–7.33 (2H, m), 7.69–7.82 (1H, m), 8.10 (1H, s). Anal. Calcd for $C_{26}H_{36}ClN_3O_3 \cdot H_2O$: C, 63.47; H, 7.78; N, 8.54. Found: C, 63.67; H, 7.47; N, 8.54.

5.3.7. 4-Amino-*N*-[1-(4-benzyloxybutyl)piperidin-4-ylmethyl]-5-chloro-2-methoxybenzamide oxalate (43a). Prepared from **39** and **37a** according to the general procedure. The resulting oil was transformed into oxalate and recrystallized from ethanol to give **43a**: colourless solid; mp 175–176 °C. ^{1}H NMR (DMSO-d_6): δ 1.32–1.91 (9H, m), 2.69–2.89 (2H, m), 2.91–3.05 (2H, m), 3.12–3.27 (2H, m), 3.31–3.50 (4H, m), 3.82 (3H, s), 4.45 (2H, s), 5.81–6.06 (2H, br), 6.49 (1H, s), 7.22–7.42 (5H, m), 7.66 (1H, s), 7.94–8.05 (1H, s). Anal. Calcd for $C_{25}H_{34}ClN_3O_3 \cdot C_2H_2O_4 \cdot 1/5H_2O$: C, 58.58; H, 6.63; N, 7.59. Found: C, 58.58; H, 6.57; N, 7.61.

5.3.8. 4-Amino-*N*-[1-(5-benzyloxypentyl)piperidin-4-ylmethyl]-5-chloro-2-methoxybenzamide oxalate (43b). Prepared from **39** and **37b** according to the general procedure. The resulting oil was transformed into oxalate and recrystallized from ethanol to give **43b**: colourless solid; mp 185–188 °C. ^{1}H NMR (DMSO-d_6): δ 1.24–1.93 (10H, m), 2.70–3.03 (4H, m), 3.11–3.25 (2H, m), 3.30–3.53 (5H, m), 3.83 (3H, s), 4.44 (2H, s), 5.72–6.11 (2H, m), 6.49 (1H, s), 7.23–7.44 (5H, m), 7.67 (1H, s), 7.94–8.09 (1H, m). Anal. Calcd for $C_{26}H_{36}ClN_3O_3 \cdot C_2H_2O_4 \cdot 1/2H_2O$: C, 58.68; H, 6.86; N, 7.33. Found: C, 58.86; H, 6.78; N, 7.45.

5.3.9. 4-Amino-*N*-[1-(6-benzyloxyhexyl)piperidin-4-ylmethyl]-5-chloro-2-methoxybenzamide (43c). Compound **43c** was prepared according to the general procedure from **39** and **37c**: colourless solid; mp 91–95 °C (ethyl acetate). ^{1}H NMR ($CDCl_3$): δ 1.21–2.03 (15H, m), 2.21–2.36 (2H, m), 2.85–3.00 (2H, m), 3.32 (2H, t, $J = 6.3$ Hz), 3.46 (2H, t, $J = 6.3$ Hz), 3.88 (3H, s), 4.40 (2H, s), 4.49 (2H, s), 6.28 (1H, s), 7.21–7.42 (5H, m), 7.68–7.83 (1H, s), 8.10 (1H, s); MS (EI) *m/z* 487 (M^+). Anal. Calcd for $C_{27}H_{38}ClN_3O_3$: C, 66.44; H, 7.85; N, 8.61. Found: C, 66.29; H, 7.96; N, 8.58.

5.3.10. 4-Amino-5-chloro-*N*-[1-[5-(cyclohexylmethoxy)-pentyl]piperidin-4-ylmethyl]-2-methoxybenzamide hydrochloride (43d). Prepared from **39** and **37d** according to the general procedure. The resulting oil was treated with hydrochloric acid and recrystallized from ethanol to give **43d** as a colourless solid; mp 103–105 °C. ^{1}H NMR (DMSO-d_6): δ 0.78–2.00 (21H, m), 2.66–3.08 (4H, m), 3.11–3.22 (4H, m), 3.33 (2H, t, $J = 6.0$ Hz), 3.39–3.50 (2H, m), 3.83 (3H, s), 4.20–4.60 (2H, m), 6.52 (1H, s), 7.66 (1H, s), 7.91–8.09 (1H, m), 10.40–10.72 (1H, m). Anal. Calcd for $C_{26}H_{42}ClN_3O_3 \cdot HCl \cdot 3/2H_2O$: C, 57.45; H, 8.53; N, 7.73. Found: C, 57.46; H, 8.50; N, 7.80; MS (EI) *m/z* 479 (M^+).

5.3.11. 4-Amino-5-chloro-*N*-[1-[5-(4-chlorobenzyloxy)-pentyl]piperidin-4-ylmethyl]-2-methoxybenzamide (43e). Compound **43e** was prepared according to the general procedure from **39** and **37e**: colourless solid; mp 131–132 °C (ethyl acetate). ^{1}H NMR (DMSO-d_6): δ 1.01–1.64 (11H, m), 1.68–1.89 (2H, m), 2.11–2.29 (2H, m), 2.71–2.89 (2H, m), 3.06–3.19 (2H, m), 3.41 (2H, t, $J = 6.6$ Hz), 3.82 (3H, s), 4.43 (2H, br s), 5.90 (2H, br s), 6.50 (1H, s), 7.32 (2H, d, $J = 8.5$ Hz), 7.41 (2H, d, $J = 8.5$ Hz), 7.66 (1H, s), 7.82–7.95 (1H, m). Anal. Calcd for $C_{26}H_{35}Cl_2N_3O_3$: C, 61.41; H, 6.94; N, 8.26. Found: C, 61.20; H, 7.07; N, 8.27.

5.3.12. 4-Amino-5-chloro-2-methoxy-*N*-[1-[5-(naphthalen-2-ylmethoxy)pentyl]piperidin-4-ylmethyl]-benzamide hydrochloride (43f). Prepared from **39** and **37f** according to the general procedure. The resulting oil was treated with hydrochloric acid and recrystallized from ethanol to give **43f**: colourless solid; mp 161–165 °C. ^{1}H NMR (DMSO-d_6): δ 1.25–2.03 (11H, m), 2.65–3.62 (8H, m), 2.11–2.29 (2H, m), 3.83 (3H, s), 4.62 (2H, s), 5.65–6.25 (2H, br s), 6.50 (1H, s), 7.38–7.55 (3H, m), 7.67 (1H, s), 7.83 (1H, s), 7.90–7.95 (3H, m), 7.96–8.04 (1H, s), 10.12–10.46 (1H, m). Anal. Calcd for $C_{30}H_{38}ClN_3O_3 \cdot HCl \cdot H_2O$: C, 62.28; H, 7.14; N, 7.26. Found: C, 62.23; H, 7.27; N, 7.22.

5.3.13. 4-Amino-5-chloro-2-methoxy-*N*-[1-[3-(phenylthio)propyl]piperidin-4-ylmethyl]benzamide (41a). Compound **41a** was prepared in 47% yield according to the general procedure from **39** and **35a**: colourless solid; mp 146–148 °C (ethanol). ^{1}H NMR ($CDCl_3$): δ 1.19–1.40 (2H, m), 1.48–2.00 (7H, m), 2.34–2.48 (2H, m), 2.78–2.99 (4H, m), 3.31 (2H, t, $J = 6.3$ Hz), 3.88 (3H, s), 4.41 (2H, br s), 6.29 (1H, s), 7.09–7.39 (5H, m), 7.65–7.81 (1H, m), 8.10 (1H, s); MS (EI) *m/z* 447 (M^+). Anal. Calcd for $C_{23}H_{30}ClN_3O_2S \cdot 1/10H_2O$: C, 61.41; H, 6.67; N, 9.34. Found: C, 61.31; H, 6.78; N, 9.42.

5.3.14. 4-Amino-5-chloro-2-methoxy-*N*-[1-[4-(phenylthio)butyl]piperidin-4-ylmethyl]benzamide hydrochloride (41b). Prepared from **39** and **35b** according to the general procedure. The resulting oil was treated with hydrochloric acid and recrystallized from ethanol to give **41b** as a colourless solid; mp 102–105 °C. ^{1}H NMR (DMSO-d_6): δ 1.36–1.92 (11H, m), 2.63–3.57 (8H, m), 3.83 (3H, s), 5.60–6.17 (2H, m), 6.48 (1H, s), 7.11–7.27 (1H, m), 7.28–7.41 (4H, m), 7.66 (1H, s), 7.90–8.07 (1H, m), 9.65–10.00 (1H, m); MS (EI) *m/z* 461 (M^+). Anal. Calcd for $C_{24}H_{32}ClN_3O_2S \cdot HCl \cdot 3/2H_2O$: C, 54.85; H, 6.90; N, 8.00. Found: C, 54.71; H, 6.66; N, 8.06.

5.3.15. 4-Amino-5-chloro-2-methoxy-*N*-[1-[5-(phenylthio)pentyl]piperidin-4-ylmethyl]benzamide (41c). Compound **41c** was prepared according to the general

procedure from **39** and **35c**: colourless solid; mp 143–144 °C (ethyl acetate). ^{1}H NMR ($CDCl_3$): δ 1.21–2.00 (13H, m), 2.21–2.48 (2H, m), 2.82–3.00 (4H, m), 3.32 (2H, t, J = 6.3 Hz), 3.90 (3H, s), 4.36 (2H, br s), 6.29 (1H, s), 7.11–7.20 (1H, m), 7.22–7.38 (4H, m), 7.68–7.80 (1H, m), 8.11 (1H, s); MS (EI) *m/z* (M^+). Anal. Calcd for $C_{25}H_{34}ClN_3O_2S{\cdot}1/4H_2O$: C, 62.48; H, 7.18; N, 8.74. Found: C, 62.57; H, 7.16; N, 8.68.

5.3.16. 4-Amino-5-chloro-2-methoxy-*N*-[1-[6-(phenylthio)hexyl]piperidin-4-ylmethyl]benzamide (41d). Compound **41d** was prepared according to the general procedure from **39** and **35d**: colourless solid; mp 68–70 °C (ethyl acetate). ^{1}H NMR (DMSO-d_6): δ 1.21–2.05 (15H, m), 2.21–2.36 (2H, m), 2.82–2.98 (4H, m), 3.32 (2H, t, J = 6.4 Hz), 3.89 (3H, s), 4.41 (2H, br s), 6.29 (1H, s), 7.10–7.19 (2H, m), 7.21–7.38 (3H, m), 7.68–7.80 (1H, m), 8.10 (1H, s); MS (EI) *m/z* (M^+). Anal. Calcd for $C_{26}H_{36}ClN_3O_2S{\cdot}3/4H_2O$: C, 62.01; H, 7.51; N, 8.34. Found: C, 62.12; H, 7.49; N, 8.44.

5.3.17. 4-Amino-*N*-[1-[5-(benzylthio)pentyl]piperidin-4-ylmethyl]-5-chloro-2-methoxybenzamide oxalate (44). Prepared from **39** and **38** according to the general procedure. The resulting oil was transformed into oxalate and recrystallized from ethanol to give **44** as a colourless solid; mp 169–172 °C. ^{1}H NMR (DMSO-d_6): δ 1.21–1.92 (11H, m), 2.48–2.52 (2H, m), 2.71–3.01 (4H, m), 3.10–3.26 (2H, m), 3.32–3.49 (2H, m), 3.71 (2H, s), 3.83 (3H, s), 5.74–6.04 (2H, m), 6.49 (1H, s), 7.18–7.49 (5H, m), 7.66 (1H, s), 7.98–8.05 (1H, m); MS (EI) *m/z* (M^+). Anal. Calcd for $C_{26}H_{36}ClN_3O_2S{\cdot}C_2H_2O_4$: C, 57.61; H, 6.58; N, 7.19. Found: C, 57.60; H, 6.50; N, 7.29.

5.4. 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.

5.5. 5-HT$_3$ receptor-binding assay

[^{3}H]Granisetron binding assay was performed according to the method of Nelson and Thomas.[22] 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% polyethyleneimine. Filters were washed immediately with 3 × 3 mL of ice-cold Tris–HCl buffer (50 mM, pH 7.4). ICS 205930 (100 μm mol/L) was used for the determination of non-specific binding.

5.6. Dopamine D$_2$ receptor-binding assay

[^{3}H]Spiperone binding assay was performed according to the method of Creese et al. Male Wistar rat (Japan SLC, Ltd, Shizuoka, Japan) striatal membrane was homogenized in 100 volumes of ice-cold Tris–HCl buffer (50 mmol/L, pH 7.7) and centrifuged (500*g*, 10 min, 0 °C). The supernatant was centrifuged at 50,000*g* for 15 min. The pellet was suspended in 100 volumes of ice-cold Tris–HCl buffer (50 mmol/L, pH 7.7) and recentrifuged (500*g*, 10 min, 0 °C). The final pellet was resuspended in 150 volumes (50 mmol/L, pH 7.7) containing 120 mmol/L NaCl, 5 mmol/L KCl, 2 mmol/L $CaCl_2$, 1 mmol/L $MgCl_2$, 1.1 mmol/L ascorbic acid and 10 μmol/L pargyline, and incubated at 37 °C for 10 min. A portion of this membrane suspension (900 μmol/L) was placed in a tube, and 50 μmol/L of either test compound or vehicle solution was added, followed by 50 μL of [^{3}H]Spiperone (40 Ci/mmol) at a final concentration of 0.2 nmol/L. The tubes were incubated at 37 °C for 20 min and filtered through Whatman GF/B glass filters, which were then washed three times with 3 mL of Tris–HCl buffer (50 mmol/L, pH 7.7). Sulpiride (100 μmol/L) was used for the determination of non-specific binding. The radioactivity trapped on the filters was measured by liquid scintillation spectrometry.

5.7. Effect on defecation in mice

Male Crj:CD-1(ICR) mice were orally or subcutaneously administered test compounds after being adapted to experimental surroundings in partition box for 30 min. The number, wet weight and dry weight of feces excreted for 2 h from immediately after the administration were measured. Results are expressed as means ±SEM and were compared by Dunnett method.

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