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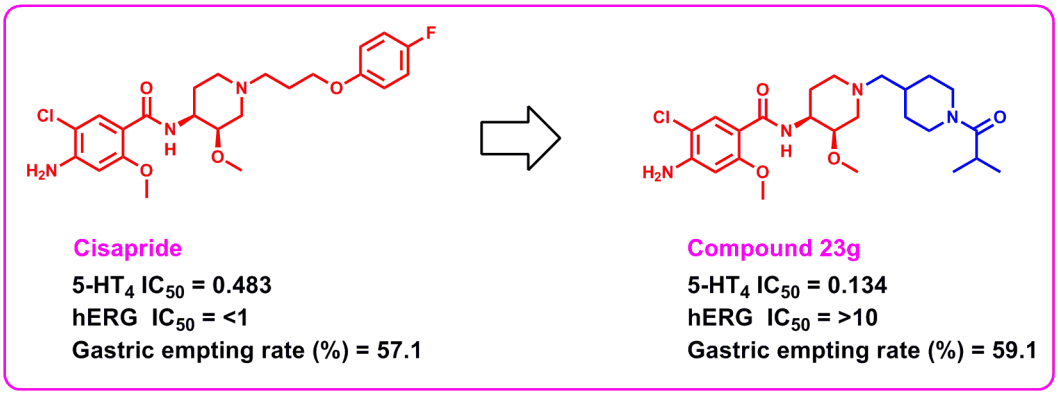
Graphical abstract



Discovery and SAR of N-(1-((Substituted piperidin-4-yl)methyl)-3-methoxypiperidin-4-yl)-2-methoxybenzamide Derivatives; 5-Hydroxytryptamine receptor 4 Agonist as a Potent Prokinetic Agent

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Abstract

A series of novel benzamide derivatives, altering the 4-fluorophenylalkyl moiety in cisapride, were synthesized as 5-HT_4 receptor agonists, and SAR of these analogs was examined on *in vitro* and *in vivo* prokinetic activities. These compounds were synthesized for high 5-HT_4 receptor binding affinities and low hERG affinities. Several types of analogs were obtained and screened for 5-HT_4 binding, hERG blocking, agonism, and gastric emptying assessment. Among the analogues, compound **23g** showed promising results compared with the other analogs with respect to gastric emptying rates in rats. Therefore, we suggest that it may be a clinical candidate for the development of a potent prokinetic agent to treat GI disorders.

Keywords: 5-HT_4, hERG inhibition, gastric emptying rate, prokinetic, GI disorder

1. Introduction

Serotonin (5-hyroxytryptamine, 5-HT) functions as both a hormone and a neurotransmitter, interacting with different receptors [1-4]. There exist several members of the serotonin receptor family, which all play important roles in various disorders. In particular, the role and distribution of 5-HT_4 receptors within the gastrointestinal (GI) tract have been well-established in different species including humans, guinea pigs [5], mice [6], and rats [7]. Due to its potential roles in central and peripheral disorders, 5-HT_4 has been an attractive target for the treatment of GI disorders such as irritable bowel syndrome, chronic constipation, gastroparesis, dyspepsia, and gastroesophageal reflux disease [8].

Dyspepsia is defined as pain or discomfort in the upper abdomen [9]. In particular, functional dyspepsia is associated with abnormalities in gastric or duodenal physiology, including abnormal sensitivity to gastric acid, delayed gastric emptying, impaired gastric accommodation, and visceral hypersensitivity [9-11]. Prokinetic agents such as 5-HT_4 agonists are potential therapeutic agents to treat GI tract disorders including

functional dyspepsia (Figure 1)[12, 13]. Of the representative prokinetic agents, cisapride (Propulsid^{TM}) was launched in 1988 and has shown potent effects in patients with GI disorders [14-16]. However, cisapride was withdrawn from the US market in 2000 as a result of adverse cardiovascular effects associated with QT prolongation due to the fact that it is a potent blocker of the hERG (human ether-a-go-go related gene) channel [17, 18].

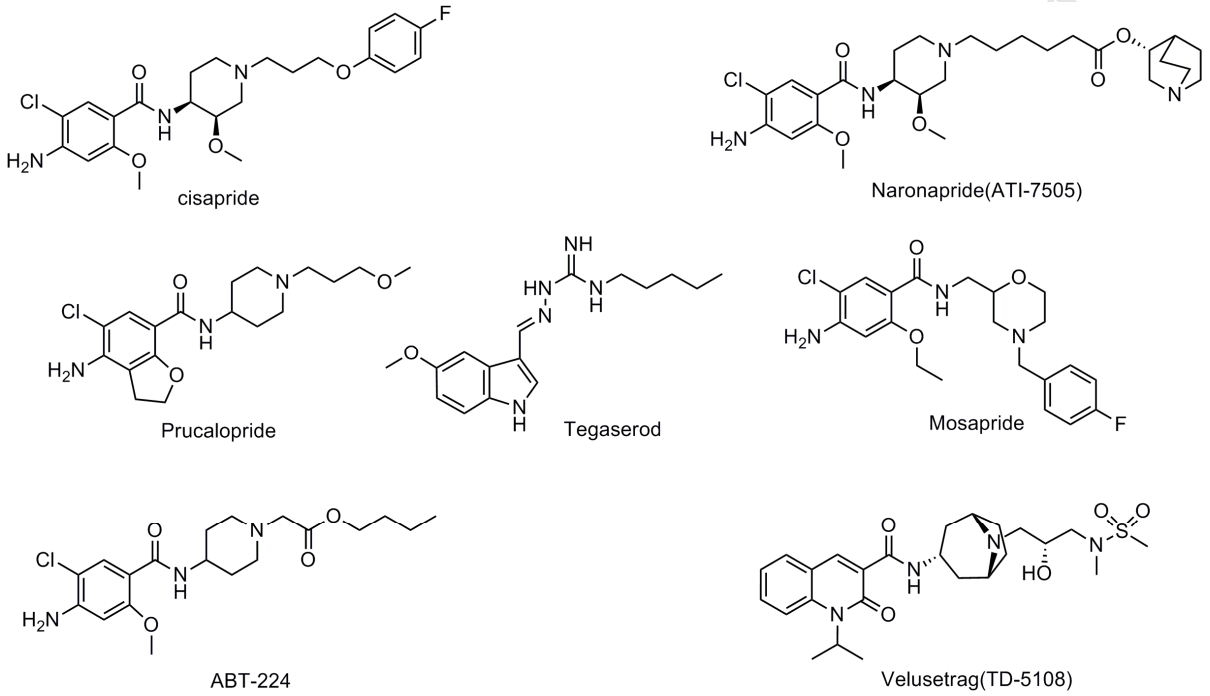


Figure 1. Examples of known prokinetic agents (5-HT$_4$ agonists).

cis-4-Amino-5-chloro-2methoxy-N-(3-methoxypiperidin-4-yl)benzamide (*cis*-norcisapride) is a metabolite of cisapride [19], and a pharmacophore that is a potential 5-HT$_4$ receptor agonist and a potential 5-HT$_3$ receptor antagonist [20]. Moreover, *cis*-norcisapride does not bind the hERG channel (IC_{50} > 10 μM). The portion of cisapride that binds to the 5-HT$_4$ receptor can be divided into parts A and B (Figure 2) [21]. Part A contains *cis*-norcisapride and part B contains linkers with functional groups. We expected, as shown in figure 2 (c), that modifying part B into adequate structures may result in an enhanced binding affinity to the 5-HT$_4$ receptor and a reduced inhibition of the hERG potassium ion channel.

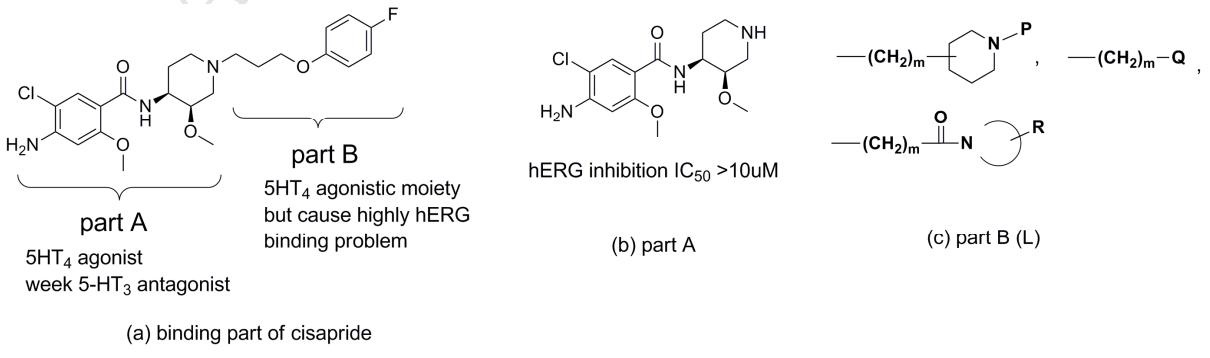
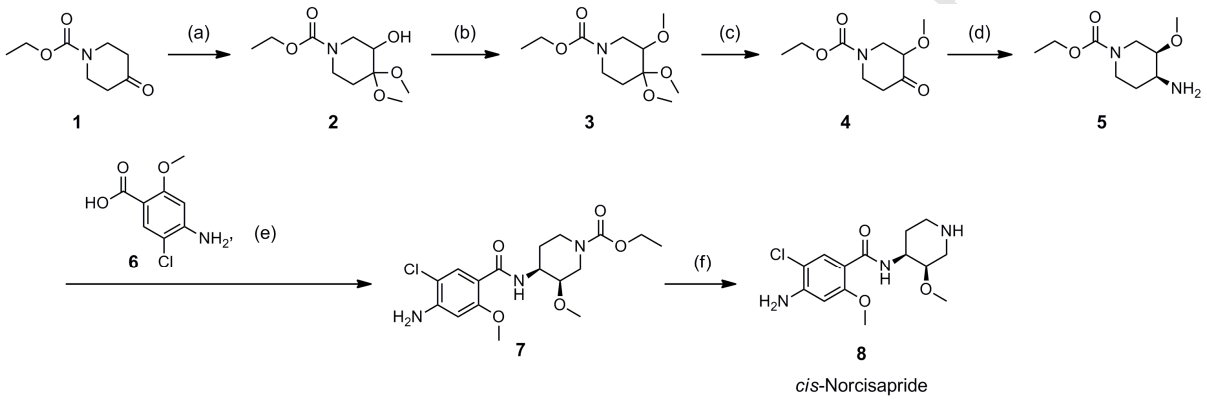


Figure 2. (a) The pharmacophore parts of cisapride, known as prokinetics, bind to the 5-HT_4 receptor; (b) part A, *cis*-norcisapride is a known 5-HT_4 receptor agonist and a partial 5-HT_3 receptor antagonist; (c) part B, three main structures of novel benzamide derivatives.

2. Results and discussion

2.1. Chemistry

Our approach was to examine various linkers and functional groups in cisapride part B that show effective binding affinity and agonism to the 5-HT_4 receptor, but are not hERG blockers. Herein we describe the synthetic strategy and structure-activity relationships (SAR) of diverse benzamide derivatives as 5-HT_4 receptor agonists.

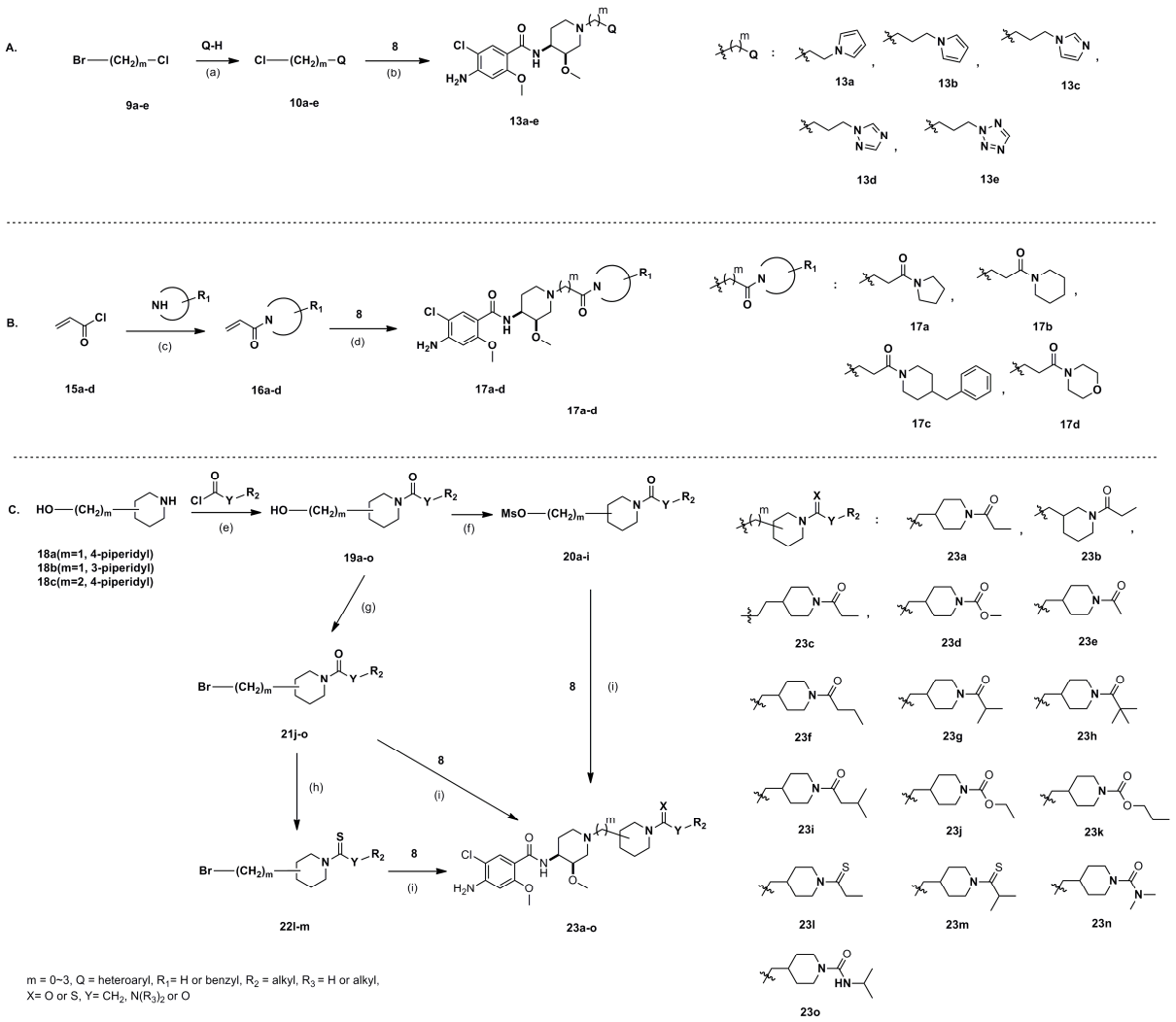


Scheme 1. Reagents and conditions: (a) $PhI(OAc)_2$, KOH, MeOH, 0°C for 30 min, then r.t. 3 h, 71%; (b) NaH, MeI, DMF, 0°C for 30 min, then r.t. 4 h, 75%; (c) 5% H_2SO_4, r.t., 78%; (d) $NaBH_3CN$, NH_4OAc, MeOH, 80°C, 3 h, 35%; (e) TEA, EDC, HOBt, DMF, r.t., 5 h, 83%; (f) KOH, 2-propanol, 0°C for 15 min, then reflux for 6 h, 72%. Compound 1 and 6 are commercially available.

Initially, we synthesized compound **8**, well-known as cis-norcisapride by following the route as depicted in Scheme 1 [22, 23]. Further, we modified part B of the pharmacophore by incorporating three different types of moieties such as alkyl heteroaryl, alkyl amide, and alkyl piperidinyl groups into compound **8** as shown in Scheme 2. Compounds **13a-e** containing alkyl heteroaryl moieties were synthesized as shown in Scheme 2A. 1-Bromo-3-chloropropane underwent S_N2 reaction with heteroaryl compounds like pyrrole, imidazole, triazole, and tetrazole to provide compounds **10a-e,** which were coupled with compound **8** to give the desired products **13a**–**e**. Subsequently, we synthesized the alkyl amide derivatives **17a**–**d** using the strategy shown in Scheme 2B. Acryloyl chloride in the presence of triethylamine reacted with cyclic amines to provide the corresponding amides **16a**–**d**, which upon coupling with compound **8** in ethanol at room temperature furnished the desired compounds **17a**–**d**.

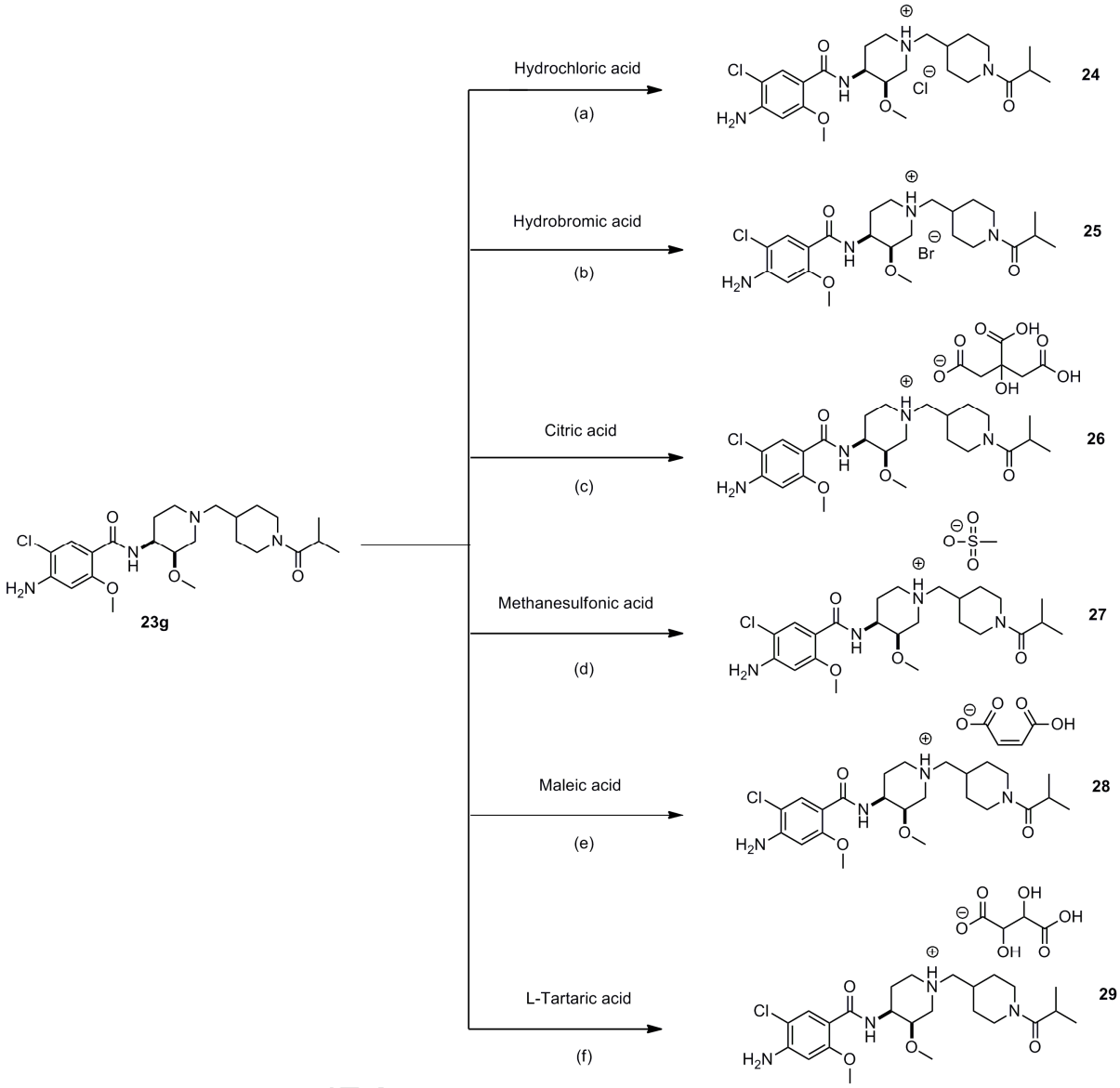
In addition, compounds **23a**–**o** containing alkyl piperidinyl groups were synthesized using the synthetic routes depicted in Scheme 2C. Substituted piperidinyl alcohols **18a**–**c** were reacted with different acyl chlorides to afford the corresponding amides **19a**–**o**. Subsequently, the free hydroxyl group of the amides was substituted with either mesylates **20a**–**i** or halides **21j**–**o**. Compounds **20a**–**i**, **21j**–**k,** and **21n**–**o** were coupled with compound **8** to afford the products **23a**–**i**, **23j**–**k,** and **23n**–**o,** respectively. Additionally, compounds **21l**–**m** were converted to the sulfur analogues by Lawesson's reagent to furnish the thioamides **22l**–**m,** which on reaction with compound **8** provide the thio-substituted compounds **23l**–**m**. All compounds were purified by

column chromatography and characterized by ^{1}H and ^{13}C NMR. Detailed experimental procedures and characterization are provided in the experimental section.

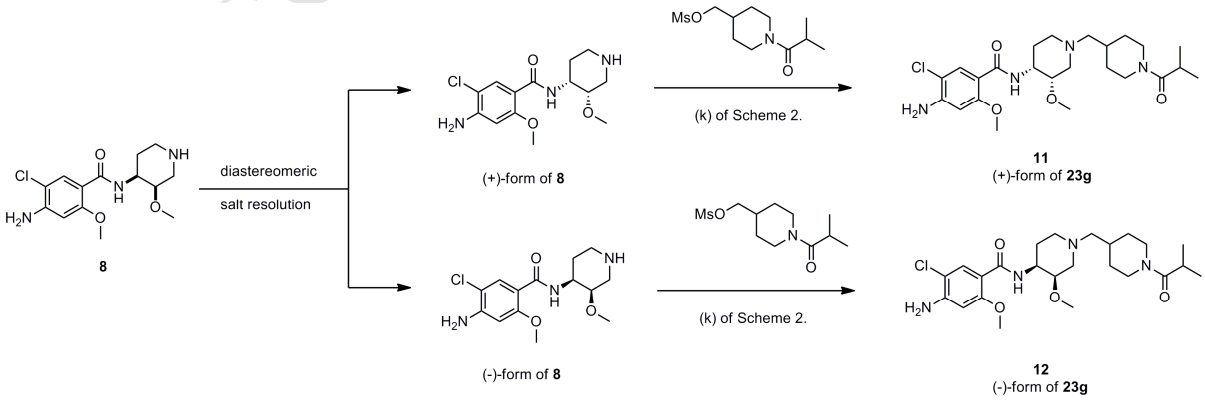


Scheme 2. Reagents and conditions: (a) NaH, DMF, 0°C for 20 min, then r.t. 8 h; (b) K_2CO_3, KI, DMF, 90°C, 12 h; (c) Et_3N, CH_2Cl_2, from 0°C to r.t., 2 h; (d) EtOH, r.t.; (e) DIPEA, MeOH, CH_2Cl_2, from 0°C to r.t., 3 h; (f) MsCl, Et_3N, CH_2Cl_2, from 0°C to r.t.; (g) PPh_3, NBS, DCM, from 0°C to r.t., 12 h; (h) Lawesson's reagent, THF, from 0°C to reflux, 20 h; (i) K_2CO_3, KI, DMF, 80─100°C.

Further, we also synthesized various salts of benzamide derivative **23g**. As depicted in Scheme 3, compound **23g** was reacted with several acids like hydrochloric acid, hydrobromic acid, citric acid, methanesulfonic acid, maleic acid, and L-tartaric acid in appropriate solvent to furnish the corresponding salts **24**–**29**. These acid salts showed good solubility in distilled water. In addition, single enantiomers of **23g** were prepared using the diastereomeric salt resolution method as described in Scheme 4. *cis*-Norcisapride (**8**) was separated into the (+)-form and (–)-form using suitable optically active acids ((+)-DBTA and (–)-DBTA) [24], and then S_N2 reactions between **20g** and a single enantiomer of cis-norcisapride were performed to obtain compound **11** and **12**, i.e. the (+)-form and (–)-form of **23g,** respectively**.**



Scheme 3. Diverse salt forms of compound 23g: (a) 2-Propanol, from r.t. to 0°C for 2 h; (b) 2-Propanol, from r.t. to 0°C for 2 h; (c) Acetone, reflux for 1 h, cooled to 0°C for 12 h; (d) 2-Propanol, from r.t. to 0°C for 2 h; (e) 2-Propanol, from r.t. to 0°C for 3 h; (f) Acetone, r.t. for 2 h.



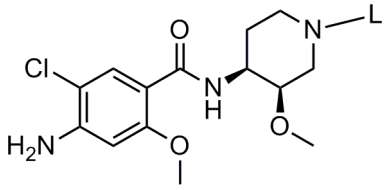
Scheme 4. Preparation of each single enantiomer of compound **23g** by diastereomeric salt resolution.

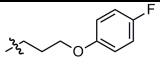
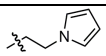
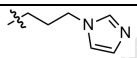
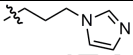
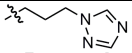
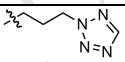
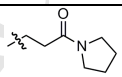
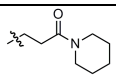
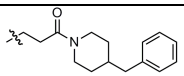
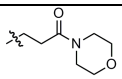
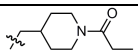
2.2. Pharmacological evaluation

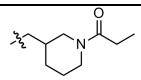
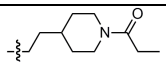
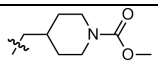
2.2.1. Structure-activity relationship (in vitro and in vivo efficacy, and hERG binding assay)

Structure-activity relationship (SAR) data based on 5-HT_4 binding affinity (IC_{50}), hERG binding affinity (IC_{50}), and functional potency (pEC_{50}) for the compounds (**13a**–**23d**) are shown in Table 1. Compounds containing alkyl heteroaryl functional groups (**13a**–**e**) showed relatively low binding affinities for the 5-HT_4 receptor. Moreover, compounds **13b**, **13c**, and **13d** weakly inhibited hERG. Compounds containing alkyl amide functional groups (**17a**, **17b**, and **17d**) showed lower binding affinities for the 5-HT_4 receptor than that of cisapride. However, compound **17c**, containing a benzyl group along with an alkylamide functional group, showed dramatically higher binding affinity to the 5-HT_4 receptor than that of cisapride, however, it also strongly inhibited hERG.

Table 1. SAR for 13a–23d, based on 5-HT_4 binding affinity (IC_{50}), hERG binding (IC_{50}), and functional potency (pEC_{50}).

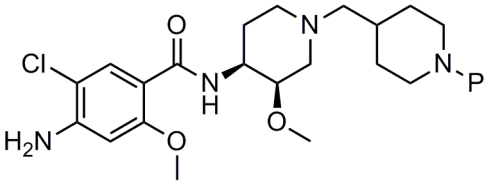


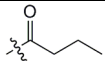
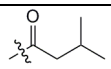
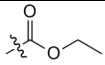
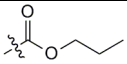
Compound	L	5-HT_4 (IC_{50}, μM)	hERG (IC_{50}, μM)	Functional potency (pEC_{50}, μM)
cisapride		0.483	<1	6.99
13a		5.759	>10	-
13b		0.648	6.835	6.17
13c		1.165	7.261	6.60
13d		1.271	7.711	6.15
13e		1.121	>10	-
17a		0.920	>10	-
17b		0.560	>10	5.98
17c		0.066	3.701	5.22
17d		0.954	>10	-
23a		0.329	>10	6.42

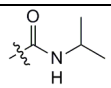
23b		0.493	>10	5.81
23c		0.805	>10	-
23d		0.190	10	-

In accordance with the SAR data for compounds **23a**–**d**, consisting of amide derivatives of the alkyl piperidinyl group in part B, they have higher binding affinities for the 5-HT_4 receptor than that of cisapride and lower affinities for hERG. The 4-piperidyl analog of compound **23a** displayed a higher binding affinity and functional potency value (pEC_{50}) for the 5-HT_4 receptor than that of 3-piperidyl derivative **23b**. Furthermore, a comparison between **23a** and **23c** illustrated that the –CH_2– linker was better for 5-HT_4 binding than –CH_2CH_2– in these series. Interestingly, introducing carbamate protection to the piperidinyl nitrogen (**23d**) improved 5-HT_4 receptor binding affinity and did not affect hERG binding. Thus, the initial SAR results for compounds **13a**–**23d** revealed that benzamide derivatives, which are composed of *cis*-norcisapride (part A) and piperidine analogs, have potential as long as the nitrogen does not act as a tertiary amine (part B).

Table 2. Pharmacological evaluation of N-substituted piperidinyl methoxypiperidine benzamide derivatives.



Compound	**P**	**5-HT_4 IC_{50} (μM)**	**hERG (IC_{50}, μM)**	**Gastric empting rate % (rat iv 5mg/kg, normal model)**
cisapride	**-**	0.483	<1	57.1
23e		0.592	>10	39.5
23a		0.329	>10	57.8
23f		0.178	>10	48.6
23g		0.134	>10	59.1
23h		0.118	>10	56.4
23i		0.078	10	54.3
23d		0.190	>10	56.2
23j		0.377	4.580	55.9
23k		0.134	<1	57.7

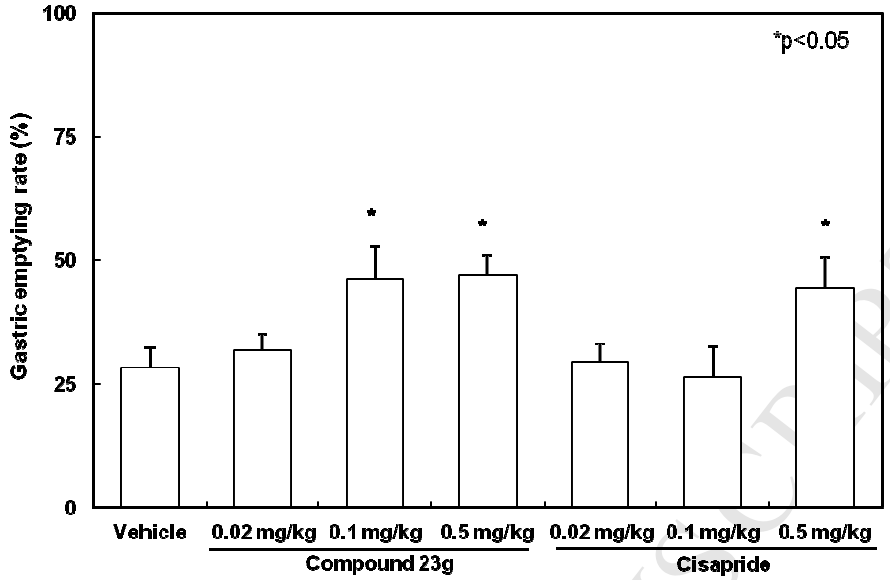
23l		0.593	2.862	61.0
23m		0.155	1.132	67.3
23n		0.052	>10	46.0
23o		0.073	7.551	59.9

Next, various N-substituted analogs of compound **23a** were tested for hERG and 5-HT_4 receptor binding affinities as well as for gastric emptying rate (Table 2). Biological assays of compounds **23a** and **23e–i** indicated that the binding affinities for 5-HT_4 receptor increased gradually by lengthening or branching of the alkyl chain of the amide group. However, the gastric emptying results were not always relevant to homologation. The gastric emptying rates of compounds **23a** and **23g** were comparatively equal or superior to that of cisapride, whereas that of compound **23h** was slightly less than that of cisapride. However, compounds **23g** and **23h** were found to have good binding affinities for the 5-HT_4 receptor and negligible inhibition of hERG (IC_{50} > 10 µM) relative to cisapride.

Compounds containing piperidyl carboxylates (**23d**, **23j**, and **23k)** had similar binding affinities to those of the compounds **23a** and **23e–i**, however, their hERG binding affinities were relatively higher and gastric emptying rates were not improved from that of compound **23g**. Pharmacological evaluation results of thio-derivatives **23l** and **23m** were inferior to compound **23g** in terms of hERG binding. Although **23n** of the piperidyl carboxamide derivatives showed the highest binding affinity (0.052 µM of 50% inhibition concentration) of all the compounds, it showed a very low gastric emptying rate (46%). On the other hand, **23o** showed a high binding affinity (0.073 µM of 50% inhibition concentration) for the 5-HT_4 receptor, however, also slightly inhibited hERG (7.551 µM of 50% inhibition concentration).

Thus, after screening all compounds for 5-HT_4 and hERG binding affinities, agonism, and gastric emptying rates in rats, compound **23g** was found to be the best pre-candidate for a novel prokinetic agent.

Figure 3 shows that a 0.1 mg/kg oral dose of compound **23g** minimally enhanced gastric emptying, whereas that of cisapride was 0.5 mg/kg. The mean gastric emptying rates were 46.2% and 46.9% in the rat model at 0.1 and 0.5 mg/kg doses of compound **23g**, respectively. However, the gastric emptying rate was 44.5% in the same model at a 0.5 mg/kg dose of cisapride [21]. Consequently, compound **23g** produced more potent gastric emptying stimulation in rats than that of cisapride.



* P < 0.05

Figure 3. Gastric emptying rates of a semi-solid meal in rats.

In addition to the study as described above, the chiral (+)-form of **23g, 11** was more potent than the racemic mixture, in binding affinity to the 5-HT_4 receptor and *in vivo* gastric emptying rate as shown in Table 3. The melting point and solubility in aqueous conditions of the different salts **24**–**29** are shown in Table 4. Some salts of **23g,** hydrochlorate **24**, methansulfonate **27**, and maleate **28,** synthesized as shown in scheme 3, were found to be stable and soluble in aqueous conditions.

Table 3. 5-HT_4 binding affinities and GE (%) rates of **23g** and each single enantiomer of compound **23g.**

Compound	5-HT_4 IC_{50} (μM)	Gastric empting rate % (rat iv 5 mg/kg, normal model)
23g	0.134	59.1
11 ((+)-isomer of **23g**)	0.068	.61.7
12 ((−)-isomer of **23g**)	>10	56.5

Table 4. Melting point and solubility of several salts of **23g**.

	24	**25**	**26**	**27**	**28**	**29**
Melting point (℃)	234.9	208.4	120.1	273	210.2	124.7
Solubility in D.W. (mg/ml)	> 15	< 5	> 30	> 10	> 10	> 30

*2.2.2. Pharmacokinetic properties of **23g** and **23h***

Compounds **23g** and **23h** are illustrated in Table 5 for *in vivo* efficacy, functional potency, and rat pharmacokinetic (PK) tests. As shown in Table 5, compound **23g** displayed gastric emptying results superior to

those of compound **23h** and cisapride in the rat model (normal model: 59.1% compared with 56.4% and 57.1%; cisplatin model: 58.6% compared with 56.6% and 57.2%). Furthermore, with respect to pharmacokinetic properties, **23g** showed longer $T_{1/2}$ and higher C_{max} and AUC_{inf} values than **23h** in a rat PK study.

Table 5. Functional potency, hERG binding, *in vivo* efficacy and oral PK data of **23g** and **23h** in rats.

Item / parameters	Functional potency pEC_{50}	hERG (IC_{50}, μM)	*In vivo* efficacy (rat gastric emptying rate (%))		Rat PK (oral, 5 mg/kg)			
			normal	cisplatin	$T_{1/2}$ (h)	T_{max} (h)	C_{max} (ng/ml)	AUCinf (ng/h/ml)
cisapride	6.99	<1	57.1	57.2	1.48	0.25	104	142
23g	7.34	>10	59.1	58.6	0.98	0.25	572	483
23h	7.00	>10	56.4	56.6	0.77	0.25	345	263

*2.2.3. Off-target screening study of **23g***

We examined compound **23g** (1–2 μM) for binding affinities to other pharmacologically relevant receptors. Figure 4 shows that compound **23g** selectively bound to the 5-HT_4 receptor, whereas it did not show even 50% inhibition of any other receptors. Therefore, it is expected that compound **23g** is a potent prokinetic agent without concern for side effects arising from non-specificity.

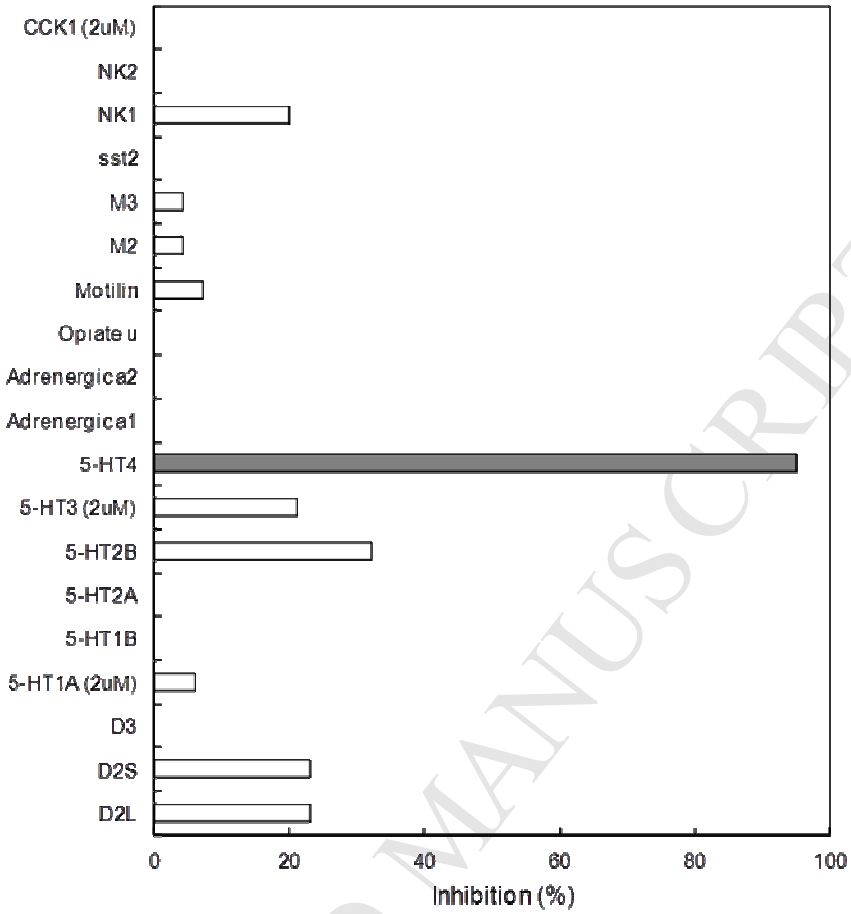


Figure 4. Binding affinities of compound **23g** (1–2μM) for other receptors.

3. Conclusion

In this study, we intended to find compounds that retained the prokinetic efficacies of cisapride but did not block the hERG channel. We designed novel benzamide derivatives of 5-HT_4 receptor agonists and tested diverse compounds. We successfully found part B of a compound with negligible hERG binding and an enhanced gastric emptying rate. We determined that compound **23g** was better than cisapride in terms of high 5-HT_4 receptor binding, lower cardiac adverse effects due to lower hERG binding, and enhanced gastric emptying in various models. In particular, the chiral (+)-form of **23g** was more potent than the racemic mixture with respect to binding affinity to the 5-HT_4 receptor and *in vivo* gastric emptying rate. Thus, compound **23g** or (+)-form of **23g** could be a good candidate to treat GI disorders, particularly functional dyspepsia. We plan to investigate additional *in vivo* efficacy and toxicity for the single enantiomer of **23g**.

4. Experimental Section

4.1. Chemistry

Reagents and solvents were purchased from commercial suppliers (Sigma-Aldrich and TCI) used as provided, unless indicated otherwise. All other solvents used for reactions were analytical grade and used as provided. Column chromatography was carried out using Merck silica gel 60 (230-400 mesh). The melting points were determined on Mettler Toledo FP900 thermosystem. ^{1}H NMR and ^{13}C NMR spectra were recorded on Varian 400MHz spectrophotometer by $CDCl_3$ or DMSO-d_6 and the chemical shifts are reported in δ (ppm) and are relative to the central peak of these solvents. Mass spectra were obtained with JMS-700 Mstation mass spectrometer. (JEOL Ltd.)

*4.1.1. 1-(3-Chloropropyl)-1H-1,2,4-triazole (**10d**)*

1,2,4-Triazole sodium derivative (1 g, 10.983 mmol) was dissolved in N,N-dimethylformamide (10mL) and cooled to 0 ºC. Sodium hydride (60% dispersion in mineral oil, 570 mg, 14.25 mmol) was added to the reaction mixture and stirred for 20 minutes at 0 ºC. 1-Bromo-3-chloropropane (2.08 g, 13.2mmol) was added dropwise to the reaction mixture. The reaction mixture was stirred for 12h at room temperature and extracted with ethyl acetate and water. The organic layer was dried over anhydrous $MgSO_4$ and evaporated *in vacuo*. The residue was purified by column chromatography (hexane:ethyl acetate = 4:1) to obtain the target compound (600 mg, 38% yield).

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.09 (s, 1H), 7.91 (s, 1H), 4.33 (t, *J* = 6 Hz, 2H), 3.43(t, *J* = 6 Hz, 2H), 2.33–2.26 (m, 2H). ^{13}C NMR (400 MHz, $CDCl_3$): δ 152.35, 143.59, 46.03, 41.09, 31.80.

*4.1.2. cis-4-Amino-5-chloro-N-[1-(3-(1H-pyrrol-1-yl)ethyl)-3-methoxypiperidin-4-yl]-2-methoxybenzamide (**13a**)*

1-(2-Bromoethyl)-1*H*-pyrrole (717 mg, 4.12 mmol), potassium carbonate (660 mg, 4.775 mmol) and potassium iodide (106 mg, 0.64 mmol) were added to a stirred solution of *cis*-4-amino-5-chloro-2-methoxy-N-(3-methoxypiperidin-4-yl)benzamide (1 g, 3.188 mmol) in N,N-dimethylformamide (20mL) in order. The reaction mixture was heated to 110 ºC for 15 hours and then cooled to room temperature and extracted with ethyl acetate and water. The organic layer was dried over anhydrous $MgSO_4$ and evaporated *in vacuo*. The residue was purified by column chromatography (chloroform:acetone = 4:1) to obtain the target compound (430 mg, 33% yield).

^{1}H NMR($CDCl_3$): δ 8.21 (d, *J* = 8Hz, 1H), 8.08 (s, 1H), 6.69 (s, 2H), 6.29 (s, 1H), 6.13 (s, 2H), 4.40 (s, 2H), 4.20–4.12 (m, 1H), 4.04 (t, *J* = 7.2Hz, 2H), 3.87 (s, 3H), 3.45–3.37 (m, 4H), 3.02–2.93 (m, 1H), 2.85–2.73 (m, 3H), 2.34–2.18 (m, 2H), 1.93–1.78 (m, 2H). ^{13}C NMR (400 MHz, $CDCl_3$): δ 163.75, 157.57, 146.69, 132.96, 120.76, 112.56, 111.50, 108.20, 97.89, 76.66, 59.20, 57.17, 55.97, 53.82, 52.02, 47.98, 47.55, 27.78. HRMS (FAB): calcd for $C_{20}H_{27}ClN_4O_3$ $[M + H]^+$ 407.1845, found 407.1845.

*4.1.3. cis-4-Amino-5-chloro-N-[1-(3-(1H-pyrrol-1-yl)propyl)-3-methoxypiperidin-4-yl]-2-methoxybenzamide (**13b**)*

1-(3-Bromopropyl)-1*H*- pyrrole (775 mg, 4.12 mmol), potassium carbonate (660 mg, 4.775 mmol) and potassium iodide (106 mg, 0.64 mmol) were added to a stirred solution of *cis*-4-amino-5-chloro-2-methoxy-N-(3-methoxypiperidin-4-yl)benzamide (1g, 3.188 mmol) in N,N-dimethylformamide (20mL) in order. The

reaction mixture was heated to 90 ºC for 8 hours and then cooled to room temperature and extracted with ethyl acetate and water. The organic layer was dried over anhydrous $MgSO_4$ and evaporated *in vacuo*. The residue was purified by column chromatography (chloroform:acetone = 4:1) to obtain the target compound (800 mg, 60% yield).

^{1}H NMR($CDCl_3$): δ 8.21 (d, *J* = 8.0Hz, 1H), 8.07 (s, 1H), 6.65 (s, 2H), 6.28 (s, 1H), 6.13 (s, 2H), 4.56 (s, 2H), 4.21–4.10 (m, 1H), 3.97–3.87 (m, 2H), 3.85(s, 3H), 3.45–3.37 (m, 4H), 3.05–2.92 (m, 1H), 2.80–2.68 (m, 1H), 2.38–2.22 (m, 2H), 2.20–2.05 (m, 2H), 2.01–1.73 (m, 4H). ^{13}C NMR (400 MHz, $CDCl_3$): δ 163.75, 157.57, 146.71, 132.93, 120.61, 112.56, 111.46, 107.89, 97.89, 76.72, 57.02, 55.97, 55.03, 53.55, 51.77, 48.15, 47.38, 28.83, 27.83. HRMS (FAB): calcd for $C_{21}H_{29}ClN_4O_3$ $[M + H]^+$ 421.2001, found 421.2010.

*4.1.4. cis-4-Amino-5-chloro-N-[1-(3-(1H-imidazol-1-yl)propyl)-3-methoxypiperidin-4-yl]-2-methoxybenzamide (**13c**)*

1-(3-Chloropropyl)-1*H*-imidazole (596 mg, 4.12 mmol), potassium carbonate (660 mg, 4.775 mmol) and potassium iodide (106 mg, 0.64 mmol) were added to a stirred solution of *cis*-4-amino-5-chloro-2-methoxy-N-(3-methoxypiperidin-4-yl)benzamide (1g, 3.188 mmol) in N,N-dimethylformamide (20mL) in order. The reaction mixture was heated to 90 ºC for 12 hours and then cooled to room temperature and extracted with ethyl acetate and water. The organic layer was dried over anhydrous $MgSO_4$ and evaporated *in vacuo*. The residue was purified by column chromatography (chloroform:acetone = 4:1) to obtain the target compound (700 mg, 52% yield).

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.19 (d, *J* = 8.4 Hz, 1H), 8.06 (s, 1H), 7.49 (s, 1H), 7.03 (s, 1H), 6.92(s, 1H), 6.28 (s, 1H), 4.49 (bs, 2H), 4.19–4.08 (m, 1H), 4.08–3.92 (m, 2H), 3.85(s, 3H), 3.44–3.38 (m, 4H), 2.99–2.83(m, 1H), 2.78–2.65 (m, 1H), 2.35–2.18(m, 2H), 2.18–2.03 (m, 2H), 1.99–1.67(m, 4H). ^{13}C NMR (400 MHz, $CDCl_3$): δ 163.75, 157.56, 146.76, 137.41, 132.92, 129.28, 112.45, 111.44, 97.85, 57.14, 55.96, 54.19, 53.74, 51.44, 48.15, 44.53, 28.14, 27.73. HRMS (FAB): calcd for $C_{20}H_{28}ClN_5O_3$ $[M + H]^+$ 422.1954, found 422.1955.

*4.1.5. cis-4-Amino-5-chloro-N-[1-(3-(1H-1,2,4-triazol-1-yl)propyl)-3-methoxypiperidin-4-yl]-2-methoxybenzamide (**13d**)*

1-(3-Chloropropyl)-1*H*-1,2,4-triazole (600 mg, 4.12 mmol), potassium carbonate (660 mg, 4.775 mmol) and potassium iodide (106 mg, 0.64 mmol) were added to a stirred solution of *cis*-4-amino-5-chloro-2-methoxy-N-(3-methoxypiperidin-4-yl)benzamide (1g, 3.188 mmol) in N,N-dimethylformamide (20mL) in order. The reaction mixture was heated to 90 ºC for 12 hours and then cooled to room temperature and extracted with ethyl acetate and water. The organic layer was dried over anhydrous $MgSO_4$ and evaporated *in vacuo*. The residue was purified by column chromatography (chloroform:acetone = 4:1) to obtain the target compound (610 mg, 45% yield).

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.17 (d, *J* = 8 Hz, 1H), 8.11 (s, 1H), 8.0 (s, 1H), 7.91 (s, 1H), 6.27 (s, 1H), 4.48 (s, 2H), 4.28–4.06 (m, 3H), 3.84(s, 3H), 3.43–3.37 (m, 4H), 2.99–2.83(m, 1H), 2.77–2.64 (m, 1H), 2.34–1.97 (m, 6H), 1.90–1.65(m, 2H). ^{13}C NMR (400 MHz, $CDCl_3$): δ 163.75, 157.55, 151.92, 146.76, 143.65, 132.91, 112.44,

111.43, 97.83, 57.16, 55.97, 53.99, 53.71, 51.26, 48.17, 47.11, 27.69, 26.56. HRMS (FAB): calcd for $C_{19}H_{27}ClN_6O_3$ $[M + H]^+$ 423.1906, found 423.1911.

*4.1.6. cis-4-Amino-5-chloro-N-[1-(3-(1H-tetrazol-1-yl)propyl)-3-methoxypiperidin-4-yl]-2-methoxybenzamide (**13e**)*

1-(3-Chloropropyl)-1*H*-tetrazole (604 mg, 4.12 mmol), potassium carbonate (660 mg, 4.775 mmol) and potassium iodide (106 mg, 0.64 mmol) were added to a stirred solution of *cis*-4-amino-5-chloro-2-methoxy-N-(3-methoxypiperidin-4-yl)benzamide (1g, 3.188 mmol) was dissolved in N,N-dimethylformamide (20mL) in order. The reaction mixture was heated to 100 ºC for 12 hours and then cooled to room temperature and extracted with ethyl acetate and water. The organic layer was dried over anhydrous $MgSO_4$ and evaporated *in vacuo*. The residue was purified by column chromatography (chloroform:acetone = 4:1) to obtain the target compound (795 mg, 59% yield).

^{1}H NMR(DMSO-d_6): δ 9.38 (s, 1H), 8.08 (d, *J*=7.6Hz, 1H), 7.72 (s, 1H), 6.49 (s, 1H), 5.99 (s, 2H), 4.47 (t, *J* = 6.8Hz, 2H), 4.00 (bs, 1H), 3.85 (s, 3H), 3.39–3.28 (m, 5H), 2.75 (bs, 1H), 2.25 (t, *J* = 6.6 Hz, 2H), 2.22–2.03 (m, 2H), 2.00 (t, *J* = 6.6 Hz, 2H), 1.75–1.58 (m, 2H). ^{13}C NMR (400 MHz, $CDCl_3$): δ 163.11, 157.85, 149.11, 144.57, 132.06, 110.38, 109.62, 98.09, 76.30, 56.54, 56.35, 54.25, 53.40, 50.73, 47.77, 46.17, 28.08, 26.81. HRMS (FAB): calcd for $C_{18}H_{26}ClN_7O_3$ $[M + H]^+$ 424.1859, found 424.1872.

*4.1.7. 1-(Piperidin-1-yl)prop-2-en-1-one (**16b**)*

Piperidine (2 g, 23.49 mmol) was dissolved in dichloromethane (20mL) and cooled to 0 ºC. Triethylamine (6.6mL, 46.98 mmol) was added to the reaction mixture and stirred for 20 minutes at 0 ºC. Acryloyl chloride (2.34 g, 25.84mmol) was added dropwise to the reaction mixture and stirred for 1h at 0 ºC. The reaction mixture was extracted with ethyl acetate and water. The organic layer was dried over anhydrous $MgSO_4$ and evaporated *in vacuo*. The residue was purified by column chromatography (ethyl acetate:hexane = 4:1) to obtain the target compound (1.32 g, 40% yield).

^{1}H NMR (400 MHz, $CDCl_3$): δ 6.58 – 6.47 (m, 1H), 6.18 (d, *J* = 16.8Hz, 1H), 5.59 (d, *J* = 10.4Hz, 1H), 3.64–3.36 (m, 4H), 1.69–1.38 (m, 6H).

*4.1.8. cis-4-Amino-5-chloro-N-[1-(3-oxo-3-(pyrrolidin-1-yl)propyl)-3-methoxypiperidin-4-yl]-2-methoxybenzamide (**17a**)*

1-(Pyrrolidin-1-yl)prop-2-en-1-one (400 mg, 3.19 mmol) was slowly added to a stirred solution of *cis*-4-amino-5-chloro-2-methoxy-N-(3-methoxypiperidin-4-yl)benzamide (500 mg, 1.59 mmol) in ethanol (5 mL). The reaction mixture was stirred for 12 hours at room temperature and extracted with ethyl acetate and water. The organic layer was dried over anhydrous $MgSO_4$ and evaporated *in vacuo*. The residue was purified by column chromatography (chloroform:methanol = 20:1) to obtain the target compound (370 mg, 53% yield).

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.20 (d, *J* = 8.4Hz, 1H), 8.07 (s, 1H), 6.29 (s, 1H), 4.42 (s, 2H), 4.23–4.13 (m, 1H), 3.86(s, 3H), 3.51–3.38 (m, 8H), 3.10–2.97 (m, 1H), 2.87–2.71 (m, 3H), 2.58–2.46 (m, 2H), 2.34–2.18 (m, 2H), 1.99–1.78 (m, 6H). ^{13}C NMR (400 MHz, $CDCl_3$): δ 170.15, 163.70, 157.54, 146.61, 132.98, 112.66,

111.52, 97.88, 57.11, 55.95, 53.83, 53.72, 51.55, 47.86, 46.64, 45.64, 32.31, 27.79, 26.07, 24.40. HRMS (FAB): calcd for $C_{21}H_{31}ClN_4O_4$ $[M + H]^+$ 439.2107, found 439.2108.

*4.1.9. cis-4-Amino-5-chloro-N-[1-(3-oxo-3-(piperidin-1-yl)propyl)-3-methoxypiperidin-4-yl]-2-methoxybenzamide (**17b**)*

1-(Piperidin-1-yl)prop-2-en-1-one (405 mg, 2.91 mmol) was slowly added to a stirred solution of *cis*-4-amino-5-chloro-2-methoxy-N-(3-methoxypiperidin-4-yl)benzamide (500 mg, 1.59 mmol) in ethanol (5 mL). The reaction mixture was stirred for 12 hours at room temperature and extracted with ethyl acetate and water. The organic layer was dried over anhydrous $MgSO_4$ and evaporated *in vacuo*. The residue was purified by column chromatography (chloroform:methanol = 20:1) to obtain the target compound (360mg, 50% yield).

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.20 (d, *J* = 8.4Hz, 1H), 8.07 (s, 1H), 6.28 (s, 1H), 4.43 (s, 2H), 4.23–4.11 (m, 1H), 3.86(s, 3H), 3.52(t, *J* = 5.2Hz, 2H), 3.46–3.37 (m, 4H), 3.09–2.98 (m, 1H), 2.85–2.69 (m, 3H), 2.61–2.50 (m, 2H), 2.34–2.18 (m, 2H), 1.91–1.77(m, 2H), 1.67–1.46 (m, 6H). ^{13}C NMR (400 MHz, $CDCl_3$): δ 169.75, 163.70, 157.53, 146.62, 132.97, 112.63, 111.51, 97.87, 57.13, 55.94, 54.15, 53.85, 51.60, 47.86, 46.63, 42.62, 30.94, 27.80, 26.50, 25.53, 24.53. HRMS (FAB): calcd for $C_{22}H_{33}ClN_4O_4$ $[M + H]^+$ 453.2263, found 453.2265.

*4.1.10. cis-4-Amino-5-chloro-N-[1-(3-(4-benzylpiperidin-1-yl)-3-oxopropyl)-3-methoxypiperidin-4-yl]-2-methoxybenzamide (**17c**)*

1-(4-Benzylpiperidin-1-yl)prop-2-en-1-one (438 mg, 1.908 mmol) was slowly added to a stirred solution of *cis*-4-amino-5-chloro-2-methoxy-N-(3-methoxypiperidin-4-yl)benzamide (500 mg, 1.59 mmol) in ethanol (5 mL). The reaction mixture was stirred for 12 hours at room temperature and extracted with ethyl acetate and water. The organic layer was dried over anhydrous $MgSO_4$ and evaporated *in vacuo*. The residue was purified by column chromatography (chloroform:methanol = 20:1) to obtain the target compound (318 mg, 37% yield).

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.19 (d, *J* = 8.4Hz, 1H), 8.06 (s, 1H), 7.30–7.22 (m, 2H), 7.22–7.13 (m, 1H), 7.13–7.07 (m, 2H), 6.27 (s, 1H), 4.56 (d, *J* = 13.2Hz, 1H), 4.43 (s, 2H), 4.21–4.12 (m, 1H), 3.87–3.76 (m, 4H), 3.38 (m, 4H), 3.09–2.99 (m, 1H), 2.99–2.83 (m, 1H), 2.59–2.40 (m, 5H), 2.31–2.17 (m, 2H), 1.91–1.62 (m, 5H), 1.20–1.02 (m, 2H) . ^{13}C NMR (400 MHz, $CDCl_3$): δ 170.00, 163.72, 157.55, 146.67, 139.91, 132.97, 129.08, 128.30, 126.06, 124.96, 112.59, 111.50, 97.88, 57.14, 55.94, 54.15, 53.91, 53.86, 53.47, 51.60, 47.87, 45.86, 42.93, 41.94, 38.25, 32.55, 31.76, 27.80. HRMS (FAB): calcd for $C_{29}H_{39}ClN_4O_4$ $[M + H]^+$ 543.2733, found 543.2739.

*4.1.11. cis-4-Amino-5-chloro-N-[1-(3-oxo-3-(morpholino-1-yl)propyl)-3-methoxypiperidin-4-yl]-2-methoxybenzamide (**17d**)*

1-Morpholinoprop-2-en-1-one (269 mg, 1.908 mmol) was slowly added to a stirred solution of *cis*-4-amino-5-chloro-2-methoxy-N-(3-methoxypiperidin-4-yl)benzamide (500 mg, 1.59 mmol) in ethanol (5 mL). The reaction mixture was stirred for 12 hours at room temperature and extracted with ethyl acetate and water. The organic layer was dried over anhydrous $MgSO_4$ and evaporated *in vacuo*. The residue was purified by column chromatography (chloroform:methanol = 20:1) to obtain the target compound (292 mg, 40% yield).

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.19 (d, *J* = 8.4Hz, 1H), 8.06 (s, 1H), 6.28 (s, 1H), 4.44 (s, 2H), 4.20–4.12 (m, 1H), 3.85 (s, 3H), 3.68–3.56 (m, 6H), 3.47–3.39 (m, 6H), 3.09–2.97 (m, 1H), 2.84–2.67 (m, 3H), 2.54 (t, *J* = 7.6Hz, 2H), 2.28–2.17 (m, 2H), 1.86–1.77 (m, 2H). ^{13}C NMR (400 MHz, $CDCl_3$): δ 170.31, 163.71, 157.53, 146.67, 132.95, 112.54, 111.49, 97.85, 66.87, 66.61, 60.40, 57.17, 55.94, 53.99, 53.93, 51.67, 47.92, 45.94, 41.87, 30.78, 27.75. HRMS (FAB): calcd for $C_{21}H_{31}ClN_4O_5$ $[M + H]^+$ 455.2056, found 455.2065.

*4.1.12. 1-(4-(Hydroxymethyl)piperidin-1-yl)-2-methylpropan-1-one (**19g**)*

Diisopropylethylamine(DIPEA) was slowly added to a stirred solution of piperidin-4-ylmethanol (1.3 g, 11.29 mmol) in methanol (20mL) and the reaction mixture was stirred for 30 minutes at 0ºC. Isobutyryl chloride (1.42 mL, 13.55 mmol) was added dropwise to the reaction mixture at the same temperature. The reaction mixture stirred for more 2 h at 0ºC and 1 h at room temperature and extracted with ethyl acetate and water. The organic layer was dried over anhydrous $MgSO_4$ and evaporated *in vacuo*. The residue was purified by column chromatography (chloroform:acetone = 2:1) to obtain the target compound (1.42 g, 7.68 mmol).

^{1}H NMR (400 MHz, $CDCl_3$): δ 4.68–4.47 (m, 1H), 4.03–3.79 (m, 1H), 3.44 (d, *J* = 5.2Hz, 2H), 2.83–2.64 (m, 2H), 2.62–2.37 (m, 1H), 1.90–1.62 (m, 3H), 1.19–0.97 (m, 8H).

*4.1.13. (1-Isobutyrylpiperidin-4-yl)methyl methanesulfonate (**20g**)*

1-(4-(Hydroxymethyl)piperidin-1-yl)-2-methylpropan-1-one (**19g**) (1 g, 5.398 mmol) was dissolved in dichloromethane 20mL and cooled to 0 ºC. Triethylamine (1.51 mL , 10.796 mmol) was added to the reaction mixture and stirred for 30 minutes at 0 ºC. Methanesulfonyl chloride (0.5 mL, 6.478 mmol) was added dropwise for 30 minutes and stirred for 3 hours at the same temperature. The reaction mixture was stirred for 1 hour at room temperature and extracted with dichloromethane and 1M citric acid aqueous solution. The organic layer was dried with $MgSO_4$ and evaporated in vacuo. The residue was purified by column chromatography (n-hexane:EtOAc = 1:1) to obtain the target compound (1.21 g, 85% yield)

^{1}H NMR (400 MHz, $CDCl_3$): δ 4.79–4.49 (m, 1H), 4.08–3.81 (m, 1H), 3.67–3.40 (m, 2H), 3.07–2.73 (m, 1H), 2.65–2.71 (m, 2H), 2.64–2.38 (m, 1H), 1.99–1.74 (m, 4H), 1.27–1.03 (m, 9H).

*4.1.14. cis-4-Amino-5-chloro-N-(1-((1-propionylpiperidin-4-yl)methyl)-3-methoxypiperidin-4-yl)-2-methoxybenzamide (**23a**)*

(1-Propionylpiperidin-4-yl)methyl methanesulfonate (**20a**) (381 mg, 1.53 mmol), potassium carbonate (246 mg, 1.78 mmol) and potassium iodide (42 mg, 0.25 mmol) were added to a solution of *cis*-4-amino-5-chloro-2-methoxy-N-(3-methoxypiperidin-4-yl)benzamide (400 mg, 1.275 mmol) in N,N-dimethylformamide (10mL) in order. The reaction mixture was heated to 100 ºC for 12 hours and then cooled to room temperature and extracted with ethyl acetate and water. The organic layer was dried over anhydrous $MgSO_4$ and evaporated *in vacuo*. The residue was purified by column chromatography (chloroform:acetone = 4:1) to obtain the target compound (115 mg, 19% yield).

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.18 (d, *J* = 8Hz, 1H), 8.08 (s, 1H), 6.29 (s, 1H), 4.60 (d, *J* = 13.2Hz, 1H), 4.41 (s, 2H), 4.25–4.16 (m, 1H), 3.96–3.78 (m, 4H), 3.42 (s, 4H), 3.04–2.83 (m, 2H), 2.78–2.61 (m, 1H), 2.54 (t, *J* = 13.2Hz, 1H), 2.39–2.07 (m, 6H), 1.96–1.64 (m, 6H), 1.20–0.99 (m, 5H). ^{13}C NMR (400 MHz, $CDCl_3$): δ

172.17, 163.76, 157.55, 146.60, 133.01, 112.68, 111.53, 97.86, 64.29, 56.82, 56.00, 54.02, 52.22, 47.91, 45.57, 41.76, 34.01, 31.54, 31.41, 30.64, 30.52, 27.80, 9.63. HRMS (FAB): calcd for $C_{23}H_{35}ClN_4O_4$ $[M + H]^+$ 467.2420, found 467.2425.

*4.1.15. cis-4-Amino-5-chloro-N-(1-((1-propionylpiperidin-3-yl)methyl)-3-methoxypiperidin-4-yl)-2-methoxybenzamide (**23b**)*

(1-Propionylpiperidin-3-yl)methyl methanesulfonate (**20b**) (190 mg, 0.765 mmol), potassium carbonate (123 mg, 0.89 mmol) and potassium iodide (21 mg, 0.125 mmol) were added to a solution of *cis*-4-amino-5-chloro-2-methoxy-N-(3-methoxypiperidin-4-yl)benzamide (200 mg, 0.638 mmol) in N,N-dimethylformamide (5mL) in order. The reaction mixture was heated to 100 ºC for 12 hours and then cooled to room temperature and extracted with ethyl acetate and water. The organic layer was dried over anhydrous $MgSO_4$ and evaporated *in vacuo*. The residue was purified by column chromatography (chloroform:acetone = 4:1) to obtain the target compound (30 mg, 10% yield). Four stereoisomers were blended at the ratio of 0.24 : 0.26 : 0.26 : 0.24 (1R3S4R : 1S3S4R : 1R3R4S : 1S3R4S); 4-amino-5-chloro-2-methoxy-N-((3S,4R)-3-methoxy-1-(((R)-1-propionylpiperidin-3-yl)methyl)piperidin-4-yl)benzamide : 4-amino-5-chloro-2-methoxy-N-((3R,4S)-3-methoxy-1-(((R)-1-propionylpiperidin-3-yl)methyl)piperidin-4-yl)benzamide : 4-amino-5-chloro-2-methoxy-N-((3S,4R)-3-methoxy-1-(((S)-1-propionylpiperidin-3-yl)methyl)piperidin-4-yl)benzamide : 4-amino-5-chloro-2-methoxy-N-((3R,4S)-3-methoxy-1-(((S)-1-propionylpiperidin-3-yl)methyl)piperidin-4-yl)benzamide

*4.1.16. cis-4-Amino-5-chloro-N-(1-(2-(1-propionylpiperidin-4-yl)ethyl)-3-methoxypiperidin-4-yl)-2-methoxybenzamide (**23c**)*

2-(1-Propionylpiperidin-4-yl)ethyl methanesulfonate (**20c**) (202 mg, 0.765 mmol), potassium carbonate (123 mg, 0.89 mmol) and potassium iodide (21 mg, 0.125 mmol) were added to a solution of *cis*-4-amino-5-chloro-2-methoxy-N-(3-methoxypiperidin-4-yl)benzamide (200 mg, 0.638 mmol) in N,N-dimethylformamide (50mL) in order. The reaction mixture was heated to 90 ºC for 16 hours and then cooled room temperature and extracted with ethyl acetate and water. The organic layer was dried over anhydrous $MgSO_4$ and evaporated *in vacuo*. The residue was purified by column chromatography (chloroform:acetone = 3:1) to obtain the target compound (83 mg, 27% yield).

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.20 (d, *J* = 8Hz, 1H), 8.06 (s, 1H), 6.28 (s, 1H), 4.57 (d, *J* = 12.8Hz, 1H), 4.46 (s, 2H), 4.21–4.09 (m, 1H), 3.88–3.76 (m, 4H), 3.41 (s, 4H), 3.12–2.91 (m, 2H), 2.85–2.70 (m, 1H), 2.58–2.26 (m, 5H), 2.23–2.03 (m, 2H), 1.93–1.62(m, 4H), 1.58–1.37 (m, 3H), 1.21–1.00 (m, 5H). ^{13}C NMR (400 MHz, $CDCl_3$): δ 172.09, 163.74, 157.57, 146.70, 132.94, 112.57, 111.47, 97.90, 76.64, 57.02, 56.03, 55.98, 55.95, 53.47, 53.41, 52.10, 48.09, 45.69, 41.90, 34.68, 33.42, 33.38, 32.91, 32.00, 27.78, 26.59, 9.64. HRMS (FAB): calcd for $C_{24}H_{37}ClN_4O_4$ $[M + H]^+$ 481.2576, found 481.2589.

*4.1.17. Methyl 4-((cis-4-(4-amino-5-chloro-2-methoxybenzamido)-3-methoxypiperidin-1-yl)methyl)piperidine-1-carboxylate (**23d**)*

Methyl 4-(((methylsulfonyl)oxy)methyl)piperidine-1-carboxylate (**20d**) (437 mg, 1.74 mmol), potassium

carbonate (281 mg, 2.03 mmol) and potassium iodide (48 mg, 0.29 mmol) were added to a solution of *cis*-4-amino-5-chloro-2-methoxy-N-(3-methoxypiperidin-4-yl)benzamide (455 mg, 1.45 mmol) in N,N-dimethylformamide (10mL) in order. The reaction mixture was heated to 100 ºC for 12 hours and then cooled to room temperature and extracted with ethyl acetate and water. The organic layer was dried over anhydrous $MgSO_4$ and evaporated *in vacuo*. The residue was purified by column chromatography (chloroform:acetone = 4:1) to obtain the target compound (189 mg, 28% yield).

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.18 (d, *J* = 7.6Hz, 1H), 8.04 (s, 1H), 6.27 (s, 1H), 4.50 (s, 2H), 4.23–3.08 (m, 3H), 3.83 (s, 3H), 3.65 (s, 3H), 3.38 (s, 4H), 2.98–2.83 (m, 1H), 2.79–2.58 (m, 3H), 2.29–2.07 (m, 5H), 1.90–1.56 (m, 5H), 1.14–0.97 (m, 2H). ^{13}C NMR (400 MHz, $CDCl_3$): δ 163.80, 157.55, 155.98, 146.77, 132.85, 112.40, 111.37, 97.83, 76.67, 64.36, 56.72, 55.94, 54.00, 52.45, 52.19, 47.93, 43.94, 33.75, 30.72, 30.60, 27.79, 14.16. HRMS (FAB): calcd for $C_{22}H_{33}ClN_4O_5$ $[M + H]^+$ 469.2212, found 469.2215.

*4.1.18. cis-4-Amino-5-chloro-N-(1-((1-acetylpiperidin-4-yl)methyl)-3-methoxypiperidin-4-yl)-2-methoxybenzamide (**23e**)*

(1-Acetylpiperidin-4-yl)methyl methanesulfonate (**20e**) (360 mg, 1.53 mmol), potassium carbonate (246 mg, 1.78 mmol) and potassium iodide (42 mg, 0.25 mmol) were added to a solution of *cis*-4-amino-5-chloro-2-methoxy-N-(3-methoxypiperidin-4-yl)benzamide (400 mg, 1.275 mmol) in N,N-dimethylformamide (10mL) in order. The reaction mixture was heated to 90 ºC for 12 hours and then cooled to room temperature and extracted with ethyl acetate and water. The organic layer was dried over anhydrous $MgSO_4$ and evaporated *in vacuo*. The residue was purified by column chromatography (chloroform:acetone = 4:1) to obtain the target compound (27 mg, 5 % yield).

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.18 (d, *J* = 8Hz, 1H), 8.07 (s, 1H), 6.29 (s, 1H), 4.57 (d, *J* = 13.2Hz, 1H), 4.44 (s, 2H), 4.23–4.14 (m, 1H), 3.86 (s, 3H), 3.78 (d, *J* = 13.2Hz, 1H), 3.41 (s, 4H), 3.07–2.82 (m, 2H), 2.75–2.59 (m, 1H), 2.53 (t, *J* = 13.2Hz, 1H), 2.26–2.09 (m, 4H), 2.07 (s, 3H), 1.94–1.65 (m, 5H), 1.18–0.98 (m, 2H). ^{13}C NMR (400 MHz, $CDCl_3$): δ 168.83, 163.75, 157.55, 146.65, 132.97, 112.61, 111.48, 97.86, 64.27, 56.79, 55.98, 54.15, 52.20, 47.91, 46.55, 41.65, 41.61, 33.96, 31.44, 31.31, 30.56, 30.42, 27.83, 21.58. HRMS (FAB): calcd for $C_{22}H_{33}ClN_4O_4$ $[M + H]^+$ 453.2263, found 453.2267.

*4.1.19. cis-4-Amino-5-chloro-N-(1-((1-butyrylpiperidin-4-yl)methyl)-3-methoxypiperidin-4-yl)-2-methoxybenzamide (**23f**)*

(1-Butyrylpiperidin-4-yl)methyl methanesulfonate (**20f**) (381 mg, 1.53 mmol), potassium carbonate (246 mg, 1.78 mmol) and potassium iodide (42 mg, 0.25 mmol) were added to a solution of *cis*-4-amino-5-chloro-2-methoxy-N-(3-methoxypiperidin-4-yl)benzamide (400 mg, 1.275 mmol) in N,N-dimethylformamide (10mL) in order. The reaction mixture was heated to 100 ºC for 12 hours and then cooled to room temperature and extracted with ethyl acetate and water. The organic layer was dried over anhydrous $MgSO_4$ and evaporated *in vacuo*. The residue was purified by column chromatography (chloroform:acetone = 4:1) to obtain the target compound (115 mg, 19% yield).

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.16 (d, *J* = 7.6Hz, 1H), 8.02 (s, 1H), 6.28 (s, 1H), 4.63–4.47 (m, 3H), 4.28–4.07 (m, 1H), 3.85–3.63 (m, 4H), 3.37 (s, 4H), 2.99–2.83 (m, 2H), 2.68–2.55 (m, 1H), 2.54–2.44 (m, 1H), 2.29–2.04

(m, 6H), 1.88–1.53 (m, 7H), 1.12–0.96 (m, 3H), 0.91 (t, *J* = 7.6Hz, 3H). ^{13}C NMR (400 MHz, $CDCl_3$): δ 171.40, 163.82, 157.56, 146.91, 132.78, 112.25, 111.30, 97.83, 76.66, 64.23, 64.18, 56.73, 55.92, 54.06, 53.99, 52.12, 47.92, 45.77, 41.71, 41.68, 35.39, 33.96, 31.53, 31.42, 30.62, 30.50, 27.77, 18.84, 14.00. HRMS (FAB): calcd for $C_{24}H_{37}ClN_4O_4$ $[M + H]^+$ 481.2576, found 481.2584.

*4.1.20. cis-4-Amino-5-chloro-N-(1-((1-isobutyrylpiperidin-4-yl)methyl)-3-methoxypiperidin-4-yl)-2-methoxybenzamide (**23g**)*

(1-Isobutyrylpiperidin-4-yl)methyl methanesulfonate (**20g**) (403 mg, 1.53 mmol), potassium carbonate (246 mg, 1.78 mmol) and potassium iodide (42 mg, 0.25 mmol) were added to a solution of *cis*-4-amino-5-chloro-2-methoxy-N-(3-methoxypiperidin-4-yl)benzamide (400 mg, 1.275 mmol) in N,N-dimethylformamide (10mL) in order. The reaction mixture was heated to 100 ºC for 12 hours and then cooled to room temperature and extracted with ethyl acetate and water. The organic layer was dried over anhydrous $MgSO_4$ and evaporated *in vacuo*. The residue was purified by column chromatography (chloroform:acetone = 4:1) to obtain the target compound (428 mg, 70% yield).

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.18 (d, *J* = 7.6Hz, 1H), 8.06 (s, 1H), 6.29 (s, 1H), 4.60 (d, *J* = 12.4Hz, 1H), 4.49(s, 2H), 4.24–4.13 (m, 1H), 3.99–3.80(m, 4H), 3.40(s, 4H), 3.05–2.44(m, 3H), 2.30–1.99(m, H), 1.97–1.62 (m, 5H), 1.24–0.96 (m, 8H). ^{13}C NMR (400 MHz, $CDCl_3$): δ 175.24, 163.77, 157.54, 146.73, 132.89, 112.47, 111.41, 97.84, 64.26, 56.77, 55.95, 54.11, 53.99, 52.25, 52.15, 47.92, 45.49, 41.90, 34.09, 31.78, 31.66, 30.69, 30.57, 30.06, 27.80, 19.57, 19.32. HRMS (FAB): calcd for $C_{24}H_{37}ClN_4O_4$ $[M + H]^+$ 481.2576, found 481.2579.

*4.1.21. cis-4-Amino-5-chloro-N-(1-((1-pivaloylpiperidin-4-yl)methyl)-3-methoxypiperidin-4-yl)-2-methoxybenzamide (**23h**)*

(1-Pivaloylpiperidin-4-yl)methyl methanesulfonate (**20h**) (530 mg, 1.912 mmol), potassium carbonate (308 mg, 2.23 mmol) and potassium iodide (53 mg, 0.32 mmol) were added to a solution of *cis*-4-amino-5-chloro-2-methoxy-N-(3-methoxypiperidin-4-yl)benzamide (500 mg, 1.593 mmol) in N,N-dimethylformamide (10mL) in order. The reaction mixture was heated to 90 ºC for 12 hours and then cooled to room temperature and extracted with ethyl acetate and water. The organic layer was dried over anhydrous $MgSO_4$ and evaporated *in vacuo*. The residue was purified by column chromatography (chloroform:acetone = 4:1) to obtain the target compound (228 mg, 29% yield).

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.17 (d, *J* = 8.0Hz, 1H), 8.04 (s, 1H), 6.28 (s, 1H), 4.54 (s, 2H), 4.43–4.30 (m, 2H), 4.22–4.11 (m, 1H), 3.82 (s, 3H), 3.38 (m, 4H), 2.97–2.58 (m, 4H), 2.24–2.08 (m, 4H), 1.92–1.67 (m, 5H), 1.34–1.19 (m, 10H), 1.15–0.97 (m, 2H). ^{13}C NMR (400 MHz, $CDCl_3$): δ 176.13, 163.78, 157.54, 146.82, 132.82, 112.35, 111.34, 97.83, 76.67, 64.26, 60.36, 56.77, 55.93, 53.95, 52.20, 47.90, 45.31, 38.65, 34.04, 31.16, 31.10, 28.39, 27.80, 21.03, 14.16. HRMS (FAB): calcd for $C_{25}H_{39}ClN_4O_4$ $[M + H]^+$ 495.2733, found 495.2739.

*4.1.22. cis-4-Amino-5-chloro-N-(1-((1-(3-methylbutanoyl)piperidin-4-yl)methyl)-3-methoxypiperidin-4-yl)-2-methoxybenzamide (**23i**)*

(1-(3-Methylbutanoyl)piperidin-4-yl)methyl methanesulfonate (**20i**) (424 mg, 1.53 mmol), potassium carbonate

(246 mg, 1.78 mmol) and potassium iodide (42 mg, 0.25 mmol) were added to a solution of *cis*-4-amino-5-chloro-2-methoxy-N-(3-methoxypiperidin-4-yl)benzamide (400 mg, 1.275 mmol) in N,N-dimethylformamide (10mL) in order. The reaction mixture was heated to 90 ºC for 12 hours and then cooled to room temperature and extracted with ethyl acetate and water. The organic layer was dried over anhydrous $MgSO_4$ and evaporated *in vacuo*. The residue was purified by column chromatography (chloroform:acetone = 4:1) to obtain the target compound (148 mg, 24% yield).

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.18 (d, *J* = 8.0Hz, 1H), 8.08 (s, 1H), 6.29 (s, 1H), 4.62 (d, *J* = 13.2Hz, 1H), 4.43 (s, 2H), 4.25–4.13 (m, 1H), 3.90–3.78 (m, 4H), 3.41 (s, 4H), 3.04–2.85 (m, 2H), 2.76–2.47 (m, 1H), 2.52 (t, *J* = 13.2Hz, 1H), 2.47–2.01 (m, 7H), 1.97–1.66 (m, 6H), 1.15–0.98 (m, 2H), 0.95 (d, *J* = 6.0Hz, 6H). ^{13}C NMR (400 MHz, $CDCl_3$): δ 170.89, 163.75, 157.54, 146.61, 132.97, 112.62, 111.49, 97.85, 64.25, 56.78, 55.97, 54.15, 54.00, 52.23, 47.89, 46.00, 42.23, 41.74, 41.71, 34.02, 31.65, 31.53, 30.62, 27.80, 25.84, 25.82, 22.80, 22.70. HRMS (FAB): calcd for $C_{25}H_{39}ClN_4O_4$ $[M + H]^+$ 495.2733, found 495.2737.

*4.1.23. Ethyl 4-((cis-4-(4-amino-5-chloro-2-methoxybenzamido)-3-methoxypiperidin-1-yl)methyl)piperidine-1-carboxylate (**23j**)*

Ethyl 4-(bromomethyl)piperidine-1-carboxylate (410 mg, 1.64 mmol), potassium carbonate (281 mg, 2.03 mmol) and potassium iodide (48 mg, 0.29 mmol) were added to a stirred solution of *cis*-4-amino-5-chloro-2-methoxy-N-(3-methoxypiperidin-4-yl)benzamide (455 mg, 1.45 mmol) in N,N-dimethylformamide (10mL) in order. The reaction mixture was heated to 90 ºC for 12 hours and then cooled to room temperature and extracted with ethyl acetate and water. The organic layer was dried over anhydrous $MgSO_4$ and evaporated *in vacuo*. The residue was purified by column chromatography (chloroform:acetone = 5:1) to obtain the target compound (222 mg, 32% yield).

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.18 (d, *J* = 7.2Hz, 1H), 8.09 (s, 1H), 6.29 (s, 1H), 4.39 (s, 2H), 4.30–4.02 (m, 5H), 3.88 (s, 3H), 3.42 (s, 4H), 3.02–2.84 (m, 1H), 2.81–2.60 (m, 3H), 2.34–2.06 (m, 4H), 1.98–1.58 (m, 6H), 1.25 (t, *J* = 7.2Hz, 3H), 1.09–0.99 (m, 2H). ^{13}C NMR (400 MHz, $CDCl_3$): δ 163.73, 157.56, 155.61, 146.55, 133.04, 112.75, 111.56, 97.88, 64.43, 61.16, 56.78, 56.02, 54.05, 52.29, 47.88, 43.87, 33.85, 30.81, 30.69, 27.84, 14.72. HRMS (FAB): calcd for $C_{23}H_{35}ClN_4O_5$ $[M + H]^+$ 483.2369, found 483.2379.

*4.1.24. Propyl 4-((cis-4-(4-amino-5-chloro-2-methoxybenzamido)-3-methoxypiperidin-1-yl)methyl)piperidine-1-carboxylate (**23k**)*

Propyl 4-(bromomethyl)piperidine-1-carboxylate (434 mg, 1.64 mmol), potassium carbonate (281 mg, 2.03 mmol) and potassium iodide (48 mg, 0.29 mmol) were added to a stirred solution of *cis*-4-amino-5-chloro-2-methoxy-N-(3-methoxypiperidin-4-yl)benzamide (455 mg, 1.45 mmol) in N,N-dimethylformamide (10mL) in order. The reaction mixture was heated to 90 ºC for 12 hours and then cooled room temperature and extracted with ethyl acetate and water. The organic layer was dried over anhydrous $MgSO_4$ and evaporated *in vacuo*. The residue was purified by column chromatography (chloroform:acetone = 4:1) to obtain the target compound (200 mg, 28% yield).

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.18 (d, *J* = 8.0Hz, 1H), 8.06 (s, 1H), 6.28 (s, 1H), 4.45 (s, 2H), 4.24–4.03 (m, 3H), 4.00 (t, *J* = 6.8Hz, 2H), 3.85 (s, 3H), 3.40 (s, 4H), 2.99–2.84 (m, 1H), 2.80–2.59 (m, 3H), 2.28–1.57 (m,

13H), 1.16–1.00 (m, 2H), 0.92 (t, *J* = 7.6Hz, 3H). ^{13}C NMR (400 MHz, $CDCl_3$): δ 163.79, 157.55, 155.68, 146.67, 132.91, 112.53, 111.44, 97.84, 76.66, 66.80, 64.38. 56.74, 55.96, 53.98, 52.20, 47.88, 43.88, 33.80, 30.75, 30.65, 27.74, 22.35, 10.43. HRMS (FAB): calcd for $C_{24}H_{37}ClN_4O_5$ $[M + H]^+$ 497.2525, found 497.2529.

*4.1.25. 4-((cis-4-(4-Amino-5-chloro-2-methoxybenzamido)-3-methoxypiperidin-1-yl)methyl)-N,N-dimethylpiperidine-1-carboxamide (**23n**)*

4-(Bromomethyl)-N,N-dimethylpiperidine-1-carboxamide (719 mg, 2.886 mmol), potassium carbonate (465 mg, 3.364 mmol) and potassium iodide (80 mg, 0.482 mmol) were added to a stirred solution of *cis*-4-amino-5-chloro-2-methoxy-N-(3-methoxypiperidin-4-yl)benzamide (755 mg, 2.406 mmol) in N,N-dimethylformamide (15mL) in order. The reaction mixture was heated to 90 ºC for 12 hours and then cooled to room temperature and extracted with ethyl acetate and water. The organic layer was dried over anhydrous $MgSO_4$ and evaporated *in vacuo*. The residue was purified by column chromatography (chloroform:acetone = 3:1) to obtain the target compound (310 mg, 27% yield).

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.17 (d, *J* = 8.0Hz, 1H), 8.02 (s, 1H), 6.27 (s, 1H), 4.56 (s, 2H), 4.21–4.08 (m, 1H), 3.81 (s, 3H), 3.60 (d, *J* = 13.2Hz, 2H), 3.37 (s, 4H), 2.98–2.83 (m, 1H), 2.76 (s, 6H), 2.73–2.56 (m, 4H), 2.50–2.31 (m, 1H), 2.22–2.01 (m, 4H), 1.90–1.55 (m, 5H), 1.09–1.02 (m, 2H). ^{13}C NMR (400 MHz, $CDCl_3$): δ 165.17, 163.82, 157.55, 146.89, 132.79, 112.26, 111.30, 97.82, 76.66, 64.44, 56.73, 55.92, 53.89, 52.20, 47.93, 46.97, 38.51, 34.01, 30.81, 30.71, 27.78. HRMS (FAB): calcd for $C_{23}H_{36}ClN_5O_4$ $[M + H]^+$ 482.2529, found 482.2531.

*4.1.26. 4-((cis-4-(4-Amino-5-chloro-2-methoxybenzamido)-3-methoxypiperidin-1-yl)methyl)-N,N-dimethylpiperidine-1-carboxamide (**23o**)*

4-(Bromomethyl)-N-isopropylpiperidine-1-carboxamide (3.76 g, 14.287 mmol), potassium carbonate (2.18 g, 15.773 mmol) and potassium iodide (373 mg, 2.247 mmol) were added to a stirred solution of *cis*-4-amino-5-chloro-2-methoxy-N-(3-methoxypiperidin-4-yl)benzamide (3.53 g, 11.25 mmol) in N,N-dimethylformamide (70mL) in order. The reaction mixture was heated to 90 ºC for 12 hours and then cooled to room temperature and extracted with ethyl acetate and water. The organic layer was dried over anhydrous $MgSO_4$ and evaporated *in vacuo*. The residue was purified by column chromatography (chloroform:acetone = 3:1) to obtain the target compound (2.02 g, 36% yield).

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.17 (d, *J* = 7.6Hz, 1H), 8.04 (s, 1H), 6.29 (s, 1H), 4.54 (s, 2H), 4.38–4.10 (m, 2H), 4.04–3.77 (m, 6H), 3.38 (s, 4H), 3.00–2.81 (m, 1H), 2.79–2.57 (s, 3H), 2.30–2.03 (m, 4H), 1.93–1.55 (m, 5H), 1.27–0.96 (m, 8H). ^{13}C NMR (400 MHz, $CDCl_3$): δ 163.78, 157.55, 157.15, 132.84, 146.89, 132.79, 112.37, 111.35, 97.84, 76.67, 64.35, 56.72, 55.94, 54.00, 43.99, 42.46, 33.80, 30.66, 30.53, 27.80, 23.48. HRMS (FAB): calculated for $C_{24}H_{38}ClN_5O_4$ $[M + H]^+$ 496.2685, found 496.2696.

*4.1.27. 1-(4-(Bromomethyl)piperidin-1-yl)-2-methylpropane-1-thione (**22m**)*

Lawesson's reagent (724 mg, 1.79 mmol) was added to a stirred solution of 1-(4-(bromomethyl)piperidin-1-yl)-2-methylpropane-1-one (740 mg, 2.982 mmol) in tetrahydrofuran (10mL) at 0 ºC. The reaction mixture was

refluxed for 20 hours and then cooled to room temperature and extracted with ethyl acetate and water. The organic layer was dried over anhydrous $MgSO_4$ and evaporated *in vacuo*. The residue was purified by column chromatography (chloroform:acetone = 8:1) to obtain the target compound (730 mg, 93 % yield).

^{1}H NMR (400 MHz, $CDCl_3$): δ 5.77 (d, *J* = 12.0Hz, 1H), 4.44 (d, *J* = 12.8Hz, 1H), 3.49–3.05 (m, 4H), 2.93 (t, *J* = 12.0Hz, 1H), 2.13–1.92 (m, 3H), 1.47–1.15 (m, 8H).

*4.1.28. cis-4-Amino-5-chloro-N-(1-((1-(2-methylpropanethioyl)piperidin-4-yl)methyl)-3-methoxypiperidin-4-yl)-2-methoxybenzamide (**23m**)*

1-(4-(Bromomethyl)piperidin-1-yl)-2-methylpropane-1-thione (730 mg, 2.763 mmol), potassium carbonate (480 mg, 3.473 mmol) and potassium iodide (74 mg, 0.446 mmol) were added to a stirred solution of *cis*-4-amino-5-chloro-2-methoxy-N-(3-methoxypiperidin-4-yl)benzamide (722 mg, 2.3 mmol) in N,N-dimethylformamide (15mL) in order. The reaction mixture was heated to 90 ºC for 12 hours and then cooled to room temperature and extracted with ethyl acetate and water. The organic layer was dried over anhydrous $MgSO_4$ and evaporated *in vacuo*. The residue was purified by column chromatography (chloroform:acetone = 4:1) to obtain the target compound (380 mg, 33 % yield).

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.17 (d, *J* = 7.6Hz, 1H), 8.03 (s, 1H), 6.28 (s, 1H), 5.64 (d, *J* = 12.4Hz, 1H), 4.49 (s, 2H), 4.36 (d, *J* = 13.2Hz, 1H), 4.21–4.08 (m, 1H), 3.83 (s, 3H), 3.38 (s, 4H), 3.23–3.03 (m, 2H), 3.01–2.78 (m, 2H), 2.72–2.57 (m, 1H), 2.26–1.68 (m, 9H), 1.34–1.03 (m, 8H). ^{13}C NMR (400 MHz, $CDCl_3$): δ 208.95, 208.90, 163.79, 157.56, 146.77, 132.83, 112.38, 111.36, 97.87, 76.64, 63.76, 63.73. 56.80, 56.76, 55.98, 54.28, 54.05, 52.26, 51.95, 50.71, 50.67, 49.06, 49.04, 47.96, 36.42, 33.89, 31.96, 31.86, 30.21, 30.10, 27.77, 23.12, 23.08, 23.03, 23.01. HRMS (FAB): calcd for $C_{24}H_{37}ClN_4O_3S$ $[M + H]^+$ 497.2348, found 497.2355.

*4.1.29. cis-4-Amino-5-chloro-N-(1-((1-propanethioylpiperidin-4-yl)methyl)-3-methoxypiperidin-4-yl)-2-methoxybenzamide (**23l**)*

1-(4-(Bromomethyl)piperidin-1-yl)propane-1-thione (658 mg, 2.763 mmol), potassium carbonate (480 mg, 3.473 mmol) and potassium iodide (74 mg, 0.446 mmol) were added to a stirred solution of *cis*-4-amino-5-chloro-2-methoxy-N-(3-methoxypiperidin-4-yl)benzamide (722 mg, 2.3 mmol) in N,N-dimethylformamide (15mL) in order. The reaction mixture was heated to 90 ºC for 12 hours and then cooled to room temperature and extracted with ethyl acetate and water. The organic layer was dried over anhydrous $MgSO_4$ and evaporated *in vacuo*. The residue was purified by column chromatography (chloroform:acetone = 4:1) to obtain the target compound (400 mg, 36 % yield).

^{1}H NMR (400 MHz, $CDCl_3$): δ 8.18 (d, *J* = 8.0Hz, 1H), 8.06 (s, 1H), 6.29 (s, 1H), 5.52 (d, *J* = 13.2Hz, 1H), 4.44 (s, 2H), 4.25–4.13 (m, 2H), 3.86 (s, 3H), 3.40 (s, 4H), 3.17 (t, *J* = 13.2Hz, 1H), 3.04–2.82 (m, 4H), 2.75–2.63 (m, 1H), 2.31–2.10 (m, 4H), 2.04–1.73 (m, 6H), 1.35–1.11 (m, 5H). ^{13}C NMR (400 MHz, $CDCl_3$): δ 203.64, 203.60, 163.78, 157.54, 146.67, 132.91, 112.53, 111.45, 97.87, 76.73, 63.72. 56.83, 56.78, 55.99, 54.28, 54.06, 52.25, 51.98, 50.47, 50.44, 49.60, 49.58, 47.94, 37.27, 37.25, 33.57, 31.69, 31.57, 30.19, 30.06, 27.72, 13.47. HRMS (FAB): calcd for $C_{23}H_{35}ClN_4O_3S$ $[M + H]^+$ 483.2191, found 483.2202.

4.2. Biological evaluation

4.2.1. 5-HT4 receptor binding assay

5-HT_4 receptor binding assay were performed using membrane preparations form Cos-7 cells expressing human 5-HT_4. The membrane protein preparation was conducted as described previously. [25] Briefly, cells were washed with phosphate buffered saline (PBS) and centrifuged at 300 g for 5min. The resulting pellet was suspended in ice-cold HEPES buffer (50mM, pH7.4), centrifuged at 40,000 g for 30 min at 4 ºC. The final pellet was resuspended in HEPES buffer, and protein quantification assay. Competitive (30-4000 nM) of test compounds and 30nM of [^{3}H]-GR113808. Nonspecific binding was defined using 10 µM of 5-HT. The IC_{50} value (the concentration of test compound that inhibits the binding of the radioactive ligand by 50%) was determined by linear regression of the displacement curve.

4.2.2. hERG channel assay

hERG binding affinities of test compounds were obtained using Predictor^{TM} hERG fluorescence polarization assay (Invitrogen, Carlsbad, CA) according to the manufacturer's protocol. The IC_{50} values of compounds (10 – 10,000 nM) were calculated by eliminating maximum polarization value of 30 µM E-4031, a specific hERG blocker.

4.2.3. Functional potency evaluation; 5-HT4receptor agonistic activity on carbachol-induced contraction of rat esophageal thoracic muscularis mucosae (TMM) preparations

Rats were sacrificed by a blow on the head, and the most distal 1.2 cm of the esophagus was isolated. The esophageal segments were prepared as described previously. [26] Briefly, the external muscularis propria, containing the outer longitudinal and circular muscle layers of the esophagus, was carefully removed in order to isolate the smooth muscle of the tunica muscularis mucosae. The preparations were suspended longitudinally under an initial tension of approximately 0.5 g in modified Krebs–Henseleit solution at 37 ºC and saturated with 95% O_2 and 5% CO_2. The ionic composition of the Krebs–Henseleit solution (mM) was NaCl 118, KCl 4.75, $CaCl_2$ 2.5, KH_2PO_4 1.2, $MgSO_4$ 1.2, $NaHCO_3$ 25 and glucose 10. This solution routinely contained indomethacin (3 µM) to prevent the relaxation effects of prostanoids, methysergide(1 µM) to block 5-HT_1 and 5-HT_2 receptor.

Tissues were left to equilibrate with Krebs–Henseleit solution for 60 min (with washing every 15 min) before starting the experiment. Responses were recorded isometrically through a force displacement transducer (FT03, GRASS technology, U.S.A.) coupled to a chart recorder (Labchart 5, AD Instruments, Australia).

The preparations were contracted by addition of a submaximal concentration of carbachol (3 µM) into the bathing solution. Upon establishing a stable contraction, accumulative concentration–effect curve for relaxation to 5-HT was constructed. After construction of the control curve, the tissue was washed with fresh modified Krebs–Henseleit solution and allowed to recover for 60 min before recontracting with carbachol. Potency relative to 5-HT was calculated from experiments in which two concentration–effect curves were constructed in the same preparation: the first to5-HT itself and the second to a test compound.

4.2.4. Gastric emptying evaluation

Gastric emptying was measured according to the method [27] of with some modifications. Male Sprague-Dawley rats (220—250 g) were fasted for 18 h with ad libitum access to water. (i) Normal rats were given 2 mL of semisolid meals by gavages at 50 min after drug administration. Following 30 min animals were sacrificed, and the weights of stomachs and contents in the stomachs were measured to determine gastric emptying. Gastric emptying (%) = [1−weight of test stomach/weight of 0 time control stomach]×100. (ii) Animals were given 2 mL of semisolid meal at 30 min after drug administration, and simultaneously injected with cisplatin (i.v., 5 mg/kg). Following 40 min, gastric emptying was determined by the same method described above.

4.2.5. Other receptor binding assay

Other receptor binding assays were performed by MDS Pharma Services, Taiwan Ltd., using compound **23g** at a screening concentration of 1 or 2 uM. The radioligand binding affinities of 23g were determined at the following receptors: 5-HT receptors (5-HT_{1A}, 5-HT_{1B}, 5-HT_{2A}, 5-HT_{2B}, 5-HT_3, 5-HT_4 receptors), non-5-HT receptors (Adrenergic α1, Adrenergic α2, Adrenergic β1, Cholecystokininin 1, Dopamine 2L, Dopamine 2S, Dopamine 3, Motilin, Muscarinic M2, Muscarinic M3, Opiate κ (OP2), Opiate μ (OP3), Somatostatin sst2, Tachykinin NK1, Tachykinin NK2)

4.3. Chiral HPLC analyses

4.3.1. Instruments

Analytical HPLC apparatus consisted on a Agilent G1311A quaternary pump, a G1319A autosampler, a G1316A column oven, a DAD G1315B UV detector, data were acquired and processed by a ChemStation Datasystem. (Agilent)

4.3.2. HPLC operating condition

Analytical chromatographic separations were carried out on a Chiralpak IA column (250 mm × 4.6 mm I.D.) with a mobile phase consisting of heptane : isopropanol : ethanol : diethylamine in the ratio 80 : 10 : 10 : 0.1 (v/v/v/v) at a flow rate of 1.0 mL/min and maintaining the column temperature at 30 ºC. The injection volume was 10 μL and the detection wavelength was set at 220 nm.

4.3.3. Preparation of sample

Sample was prepared by dissolving of accurate weight of 25 mg in 50 mL of methanol (0.5 mg/mL)

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Highlights

- 26 novel benzamide derivatives of cisapride were synthesized as potential prokinetic agents
- Amide derivatives of the alkyl piperidinyl group showed good binding affinities for the 5-HT_4 receptor and low affinities for hERG.
- Compound **23g** could be a good candidate to treat GI disorders, particularly functional dyspepsia.