Synthesis and Gastrointestinal Prokinetic Activity of Novel Benzamide Derivatives with Amphoteric Side Chains

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Novel benzamide derivatives (19—24, 32a—c, 43d—f), each possessing a cycloaminoalkanecarboxylic acid side chain, were synthesized and their gastrointestinal prokinetic and dopamine D_2 receptor antagonist activities were evaluated. 4-[(4-Amino-5-chloro-2-methoxybenzoyl)amino]-1-piperidineacetic acid (19) exhibited the most potent gastro- and colon-prokinetic activities, through intravenous administration to conscious dogs, and also showed the reduced dopamine D_2 receptor antagonistic activity. However, 19 showed only weak gastrointestinal prokinetic activity after oral administration. Several ester prodrugs (44—62) of 19 were tested for pharmacological activities as well as physicochemical and metabolic stability; the butyl ester (46) was consequently selected as a promising gastrointestinal prokinetic agent with reduced side effects.

Key words gastroprokinetic activity; colon-prokinetic activity; 5-HT_4 agonist; 4-[(4-amino-5-chloro-2-methoxybenzoyl)amino)]-1-piperidineacetic acid

Metoclopramide (**1a**), possessing a dopamine D_2 receptor antagonist and weak serotonin 5-HT_4 receptor agonist activities,[1a] is used clinically as a gastrointestinal prokinetic and an antiemetic agent.[1b] However, its clinical application is limited due to its side effects, such as extrapyramidal syndrome, parkinsonism and elevated serum prolactin levels, caused by blockage of the dopamine D_2 receptor.

We previously reported that the introduction of a methylenephenoxy group between the benzamide moiety and the terminal aminoalkyl groups of **1a** led to the discovery of itopride (**2**), with an appropriate dopamine D_2 receptor antagonist activity and a distinctive acetylcholine esterase inhibitory activity.[2] On the other hand, we suggested that amphoteric-ionization would be a useful approach to discovering a new drug, and actually succeeded in finding selective histamine H_1 antagonists by applying this method to classical antihistaminics.[3] We initially applied the amphoteric-ionization to **1a** in order to find a new type of gastrointestinal prokinetic agent. Although we preliminarily synthesized an amphoteric-ionized compound (**3**) by the introduction of an alkanecarboxylic acid moiety onto the terminal nitrogen atom of **1a**, it had no significant gastrointestinal prokinetic activity. Cisapride (**1b**),[4] which has a conformationally restricted amino moiety in comparison with **1a**, has been found to show more potent gastrointestinal prokinetic activity than **1a**. We therefore expected that some kind of conformational restriction of **3** would lead to an increased gastrointestinal prokinetic activity, and designed a series of [[(4-amino-5-chloro-2-methoxybenzoyl)amino]cycloamino]alkanecarboxylic acid derivatives (**4**).

In this paper, we describe the synthesis and gastrointestinal prokinetic activity of a series of [[(4-amino-5-chloro-2-methoxybenzoyl)amino]cycloamino]alkanecarboxylic acids (**4**). We also discuss conformational effects on gastrointestinal prokinetic activity and the evaluation of ester prodrugs of 4-[(4-amino-5-chloro-2-methoxybenzoyl)amino]-1-piperidineacetic acid (**19**), which exhibited the most excellent gastrointestinal prokinetic activity.

Chemistry New amphoteric compounds (**19**—**24**, **32a**—**c**, **38**, **43d**—**f**) and prodrugs (**44**—**60**) of compound **19** were synthesized as shown in Charts 2—6.

As shown in Chart 2, benzamides (**19**—**24**) with 1-

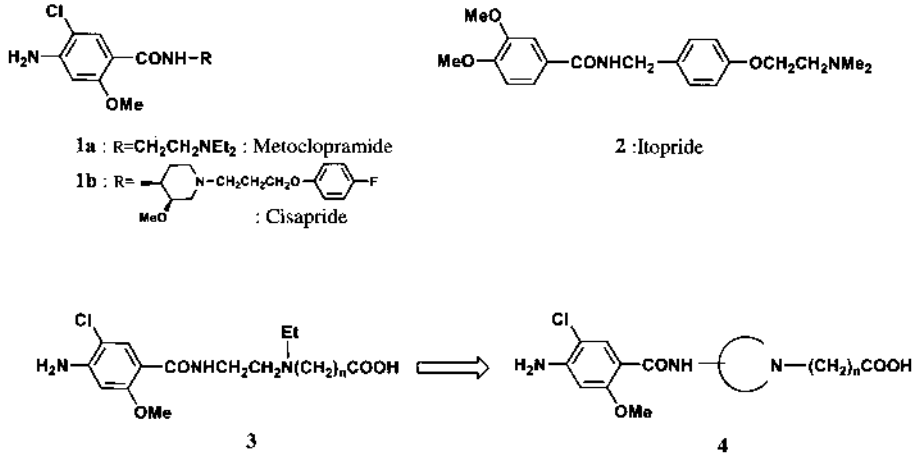

Chart 1

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Method A

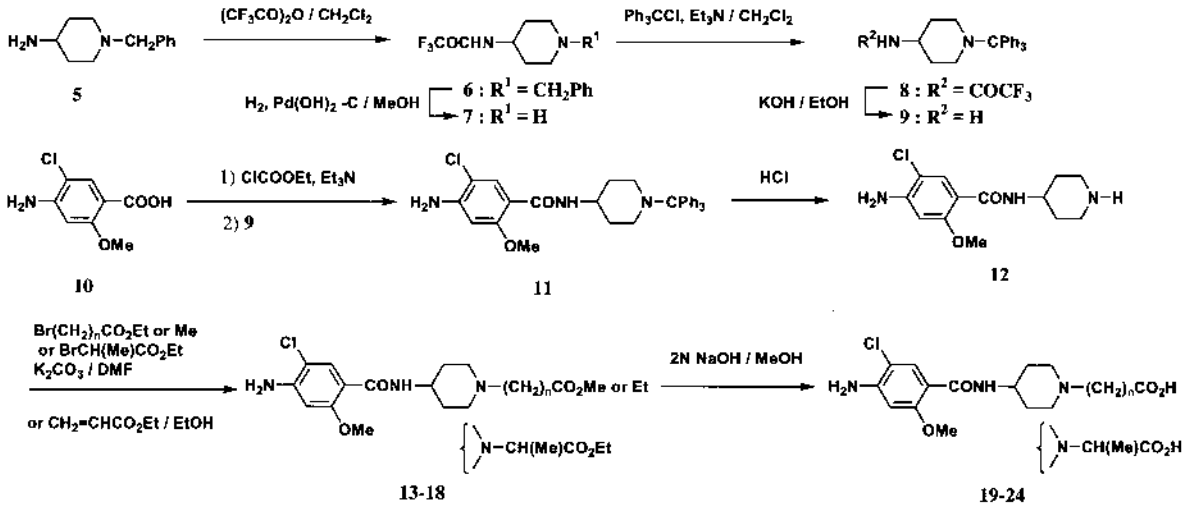


Chart 2

Method B

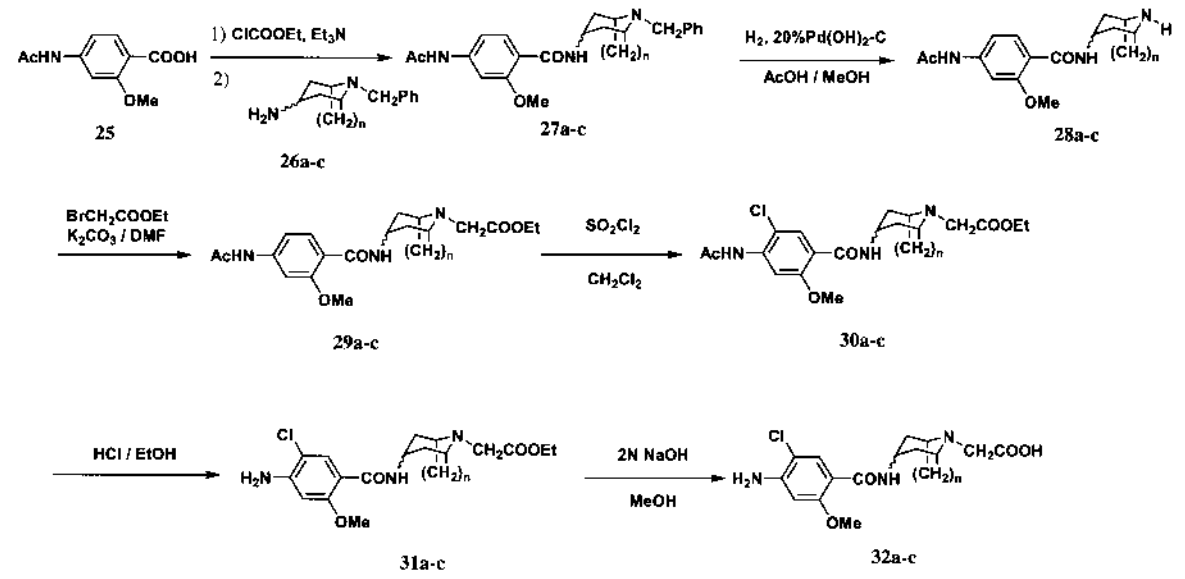


Chart 3

piperidinealkanecarboxylic acid side chains were prepared by method A. Firstly, 4-amino-1-benzylpiperidine (**5**) was treated with trifluoroacetic anhydride to give **6**. Catalytic hydrogenolysis of **6** with Pearlman's catalyst, followed by condensation with trytyl chloride, afforded **8** *via* **7**. Then deacylation of **8** with KOH gave 4-amino-1-tritylpiperidine (**9**). Commercially available 4-amino-5-chloro-2-methoxybenzoic acid (**10**) was condensed with **9** to afford **11**. Deprotection of **11** with hydrochloric acid gave the secondary amine (**12**),[5] which was treated with ethyl or methyl ω-bromoalkanecarboxylates, ethyl 2-bromopropionate or ethyl acrylate, to afford various esters (**13**—**18**). The esters (**13**—**18**) were hydrolyzed under basic conditions to provide the desired amphoteric benzamides (**19**—**24**).

Benzamides (**32a**—**c**), possessing *endo*- or *exo*-9-azabicyclo[3.3.1]nonane-9-acetic acid, or an *exo*-8-azabicyclo[3.2.1]octane-8-acetic acid side chain, were prepared by method B as shown in Chart 3. 4-Acetylamino-2-methoxybenzoic acid (**25**)[6] was converted with ethyl chloroformate to the activated ester, which was condensed with *endo*- or *exo*-3-amino-9-benzyl-9-azabicyclo[3.3.1]nonane (**26a**, **b**)[7] or *exo*-3-amino-8-benzyl-8-azabicyclo[3.2.1]octane (**26c**)[7] to afford benzamides **27a**—**c**. Catalytic hydrogenolysis of **27a**—**c** with Pearlman's catalyst gave secondary amines **28a**—**c**, and the subsequent treatment with ethyl bromoacetate afforded compounds **29a**—**c**. Chlorination of **29a**—**c**

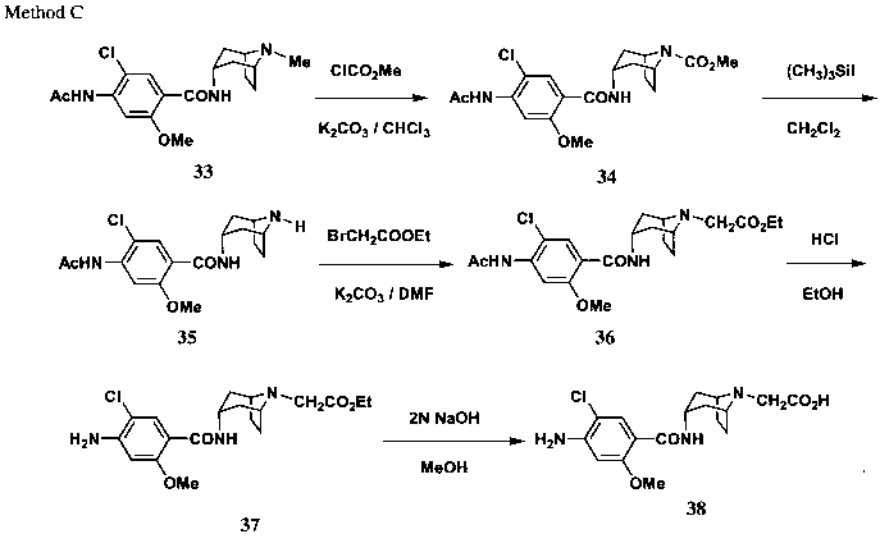


Chart 4

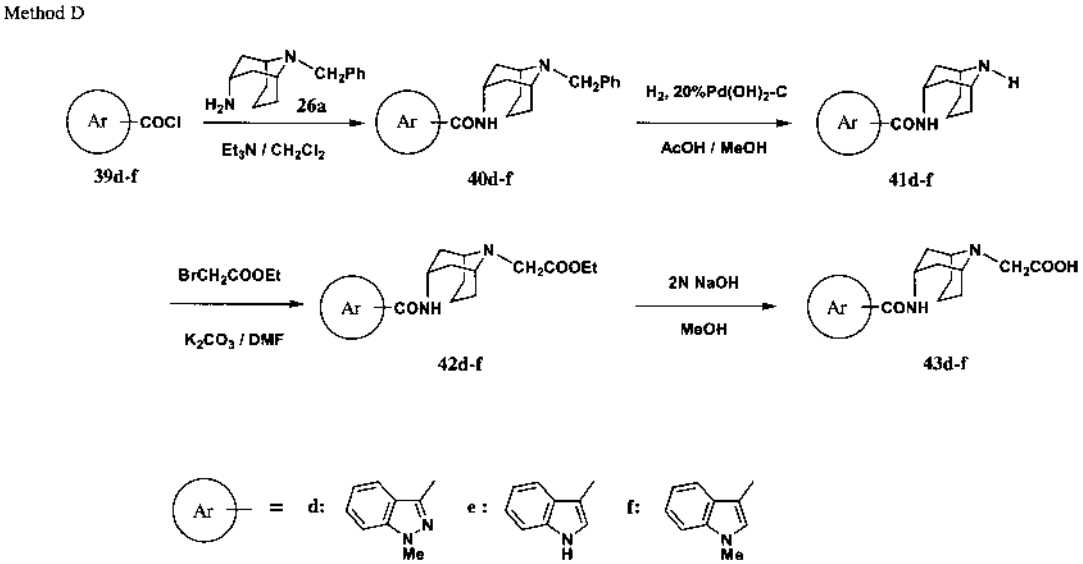


Chart 5

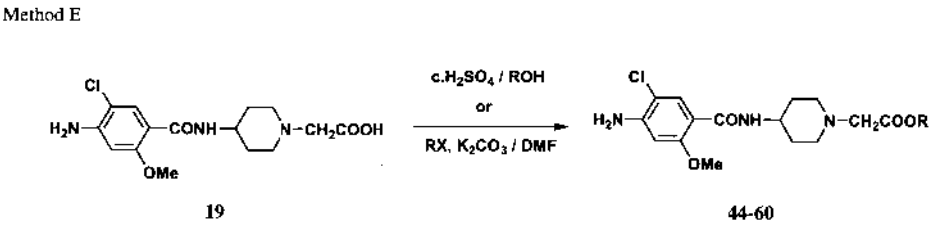


Chart 6

with sulfuryl chloride yielded **30a**—**c**, which was deacetylated with hydrochloric acid to give compounds **31a**—**c**. Basic hydrolysis of **31a**—**c** afforded the desired amphoteric benzamides (**32a**—**c**).

Benzamide (**38**), with an *endo*-8-azabicyclo[3.2.1]octane-8-acetic acid side chain, was synthesized by method C as shown in Chart 4. Treatment of *endo*-4-acetylamino-5-chloro-2-methoxy-*N*-(8-methyl-8-azabicyclo[3.2.1]oct-3-yl)benzamide (**33**)[7] with methyl chloroformate gave urethane (**34**), which was exposed to trimethylsilyl iodide to yield **35**. Compound **35** was treated with ethyl bromoacetate to afford **36**, which was subsequently deacetylated with hydrochloric acid to obtain ester (**37**). Compound **37** was hydrolyzed with base to provide the desired amphoteric benzamide (**38**).

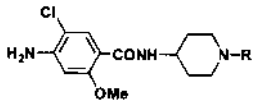
Heterocyclic carboxamides (**43d**—**f**), each possessing an amphoteric side chain, were prepared by method D as shown in Chart 5. Acid chloride (**39d**—**f**)[8,9] was condensed with amine (**26a**) to afford amides (**40d**—**f**). Catalytic hydrogenolysis of **40d**—**f** with Pearlman's catalyst, followed by conden-

sation with ethyl bromoacetate, afforded esters (**42d**—**f**) *via* secondary amines (**41d**—**f**). The esters (**42d**—**f**) were subsequently hydrolyzed with base to give the desired amphoteric carboxamides (**43d**—**f**).

The ester prodrugs (**44**—**60**) of **19** were prepared by method E as shown in Chart 6. Compound **19** was esterified with alcohol using an acid catalyst, or with various halides in *N*,*N*-dimethylformamide (DMF) in the presence of potassium carbonate, to give the desired prodrugs (**44**—**60**).

Physicochemical data for compounds **13**—**24**, **28a**—**c**, **29a**—**c**, **30a**—**c**, **31a**—**c**, **32a**—**c**, **36**—**38**, **42d**—**f**, **43d**—**f** and **44**—**60** are given in the Experimental section or in Tables 1—4.

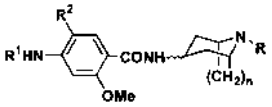
Table 1. Physicochemical Data for Monocyclic Esters and Carboxylic Acids **15**—**18**, **20**—**24**



Compd.	R	Method	mp, °C (recryst. solvent)[a]	Formula	Analysis (%) Calcd (Found)		
					C	H	N
15	$(CH_2)_3CO_2Et$	A	188.5—191.5 (E)	$C_{19}H_{28}ClN_3O_4 \cdot HCl$	52.54 (52.18	6.73 6.66	9.67 9.75)
16	$(CH_2)_4CO_2Et$	A	202—203.5 (E)	$C_{20}H_{30}ClN_3O_4 \cdot HCl \cdot 1 \cdot 4H_2O$	53.04 (52.99	7.01 6.95	9.28 9.21)
17	$(CH_2)_5CO_2Me$	A	208.5—210.5 (M)	$C_{20}H_{30}ClN_3O_4 \cdot HCl \cdot 5 \cdot 4H_2O$	51.01 (51.06	7.17 7.03	8.92 8.99)
18	$CH(Me)CO_2Et$	A	158—159 (E)	$C_{18}H_{26}ClN_3O_4 \cdot C_4H_4O_4$[b]	52.85 (52.67	6.05 5.94	8.40 8.39)
20	$(CH_2)_2CO_2H$	A	218—219.5 (W)	$C_{16}H_{22}ClN_3O_4 \cdot HCl$	48.99 (48.80	5.91 5.84	10.71 10.68)
21	$(CH_2)_3CO_2H$	A	228.5—231.5 (W)	$C_{17}H_{24}ClN_3O_4 \cdot HCl \cdot H_2O$	48.12 (47.97	6.41 6.34	9.90 10.12)
22	$(CH_2)_4CO_2H$	A	226—227.5 (W)	$C_{18}H_{26}ClN_3O_4 \cdot HCl \cdot H_2O$	49.32 (49.18	6.67 6.45	9.59 9.54)
23	$(CH_2)_5CO_2H$	A	223—225 (W)	$C_{19}H_{28}ClN_3O_4 \cdot HCl \cdot H_2O$	50.45 (50.33	6.91 6.84	9.29 9.25)
24	$CH(Me)CO_2H$	A	246—247 (W)	$C_{16}H_{22}ClN_3O_4 \cdot 5 \cdot 4H_2O$	50.79 (50.59	6.53 6.25	11.11 11.06)

a) E=EtOH, M=MeOH, W=Water. *b*) Fumaric acid.

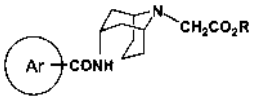
Table 2. Physicochemical Data for Bicyclic Compounds **28**—**32**



Compd.	*n*	R^1	R^2	R	*endo*/*exo*	Method	mp, °C (recryst. solvent)[a]	Formula	Analysis (%) Calcd (Found)		
									C	H	N
28b	3	Ac	H	H	*exo*	B	194—196 (dec.) (M)	$C_{18}H_{25}N_3O_3 \cdot C_4H_4O_4$[b] $\cdot 3/4H_2O$	57.32 (57.13	6.67 6.58	9.12 9.12)
28c	2	Ac	H	H	*exo*	B	223—225 (dec.) (M)	$C_{17}H_{23}N_3O_3 \cdot C_4H_4O_4$[b]	58.19 (58.05	6.28 6.45	9.69 9.71)
29b	3	Ac	H	CH_2CO_2Et	*exo*	B	oil	$C_{22}H_{31}N_3O_5$	417.2264[d] (417.2253)		
29c	2	Ac	H	CH_2CO_2Et	*exo*	B	oil	$C_{21}H_{29}N_3O_5$	403.2107[d] (403.2111)		
30b	3	Ac	Cl	CH_2CO_2Et	*exo*	B	125—128 (M)	$C_{22}H_{30}N_3O_5 \cdot 3/4\ C_4H_4O_4$[b] $\cdot 7/4H_2O$	52.63 (52.40	6.45 6.08	7.37 7.67)
30c	2	Ac	Cl	CH_2CO_2Et	*exo*	B	174—175 (E)	$C_{21}H_{28}ClN_3O_5 \cdot C_4H_4O_4$[c]	54.20 (54.03	5.82 5.85	7.58 7.56)
31b	3	H	Cl	CH_2CO_2Et	*exo*	B	161—164 (IP–DE)	$C_{20}H_{28}ClN_3O_4 \cdot 3/4H_2O$	56.73 (56.73	7.02 6.63	9.92 9.96)
31c	2	H	Cl	CH_2CO_2Et	*exo*	B	188.5—190 (E–DE)	$C_{19}H_{26}ClN_3O_4$	57.65 (57.60	6.62 6.57	10.61 10.49)
32b	3	H	Cl	CH_2CO_2H	*exo*	B	250—252 (dec.) (M)	$C_{18}H_{24}ClN_3O_4 \cdot 3/4H_2O$	54.68 (54.68	6.50 6.25	10.63 10.94)
32c	2	H	Cl	CH_2CO_2H	*exo*	B	273—276 (dec.) (M–DE)	$C_{17}H_{22}ClN_3O_4$	55.51 (55.20	6.03 6.05	11.42 11.20)

a) DE=Et_2O, DI=iso-Pr_2O, E=EtOH, IP=iso-PrOH, M=MeOH, W=Water. *b*) Fumaric acid. *c*) Maleic acid. *d*) High resolution mass spectra.

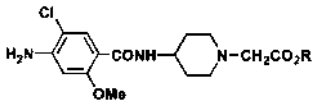
Table 3. Physicochemical Data for Heterocyclic Compounds **42**—**43**

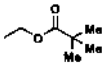
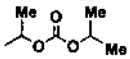
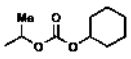


Compd.	Ar	R	Method	mp, °C (recryst. solvent)[a]	Formula	Analysis (%) Calcd (Found)		
						C	H	N
42e		Et	D	Oil	$C_{21}H_{27}N_3O_3$	369.2052[b] (369.2057)		
42f		Et	D	155—156 (E)	$C_{22}H_{29}N_3O_3$	68.90 (68.60	7.62 7.48	10.96 11.06)
43e		H	D	235—240 (dec.) (W)	$C_{19}H_{23}N_3O_3\cdot HCl$	60.39 (60.39	6.40 6.42	11.12 11.22)
43f		H	D	200—202 (W)	$C_{20}H_{25}N_3O_3\cdot HCl\cdot H_2O$	60.33 (60.27	7.36 7.39	9.59 9.71)

a) E=EtOH, W=Water. *b*) High resolution mass spectra.

Table 4. Physicochemical Data for Prodrugs **46**—**60**



Compd.	R	Method	mp, °C (recryst. solvent)[a]	Formula	Analysis (%) Calcd (Found)		
					C	H	N
46	*n*-Bu	F	209—211.5 (IP)	$C_{19}H_{28}ClN_3O_4\cdot CH_4O_3S$[b]	48.63 (48.37	6.53 6.81	8.51 8.44)
47	*n*-Pentyl	F	202.5—203 (IP)	$C_{20}H_{30}ClN_3O_4\cdot CH_4O_3S$[b]	49.65 (49.46	6.75 6.98	8.27 8.22)
48	*n*-Hex	F	133—137 (IP)	$C_{21}H_{32}ClN_3O_4\cdot HCl\cdot 1/2H_2O$	53.50 (53.26	7.27 7.27	8.91 8.82)
49	iso-Pr	F	125—130 (IP)	$C_{18}H_{26}ClN_3O_4\cdot HCl\cdot 2H_2O$	47.37 (47.46	6.85 6.67	9.21 9.14)
50	iso-Bu	F	207—208 (IP)	$C_{19}H_{28}ClN_3O_4\cdot CH_4O_3S$[b]$\cdot 1/4H_2O$	48.19 (48.14	6.57 6.80	8.43 8.36)
51	iso-Pentyl	F	199.5—201 (IP)	$C_{20}H_{30}ClN_3O_4\cdot CH_4O_3S$[b]$\cdot 3/4H_2O$	48.36 (48.24	6.86 6.64	8.06 8.02)
52	CH_2CH_2OMe	F	147—150 (E)	$C_{18}H_{26}ClN_3O_5\cdot C_4H_4O_4$[c]$\cdot 1/2H_2O$	50.34 (50.40	5.95 5.89	8.00 8.03)
53	Benzyl	F	174—175 (E)	$C_{22}H_{26}ClN_3O_4\cdot C_4H_4O_4$[c]	56.99 (56.98	5.52 5.72	7.67 7.67)
54	CH_2COMe	F	174—175 (E)	$C_{18}H_{24}ClN_3O_5\cdot CH_4O_3S$[b]$\cdot 3/4H_2O$	44.97 (44.88	5.86 5.75	8.28 8.19)
55	CH_2CO_2Et	F	196—201 (E)	$C_{19}H_{26}ClN_3O_6\cdot CH_4O_3S$[b]	45.84 (45.66	5.77 5.82	8.02 7.88)
56	CH_2CONMe_2	F	190—191 (A)	$C_{19}H_{27}ClN_4O_5$	53.46 (53.59	6.37 6.50	13.12 13.31)
57		F	124—129.5 (E)	$C_{19}H_{26}Cl_N3_O6\cdot C_4H_4O_4$[c]$\cdot 1/4H_2O$	50.37 (50.42	5.61 5.47	7.66 7.73)
58		F	160—164 (E)	$C_{21}H_{30}ClN_3O_6\cdot HCl\cdot 5/4H_2O$	48.98 (48.74	6.56 6.25	8.16 8.20)
59		F	152—156.5 (E)	$C_{21}H_{30}ClN_3O_7\cdot 2HCl\cdot 1/4H_2O$	45.91 (45.81	5.96 5.83	7.65 7.70)
60		F	118.5—126 (M–IP)	$C_{24}H_{34}ClN_3O_7\cdot HCl\cdot H_2O$	50.89 (50.88	6.58 6.29	7.42 7.42)

a) A=AcOEt, E=EtOH, IP=iso-PrOH, M=MeOH. *b*) Methanesulfonic acid. *c*) Fumaric acid.

Results and Discussion

The gastrointestinal prokinetic activities of all amphoteric compounds (**19**—**24**, **32a**—**c**, **38**, **43d**—**f**) in conscious dogs were determined using the method of Z. Itoh.[10] The gastric antrum motility of each compound was quantified by determining a motor index, which was equivalent to the integrated area between the contractile wave and the base line, and expressed as a symbol based on a percentage against the basal motor index. Colonic motility was expressed by the % ratio of the number of dogs, in which the giant contraction on the ascending colon was observed, *versus* the tested dogs. The dopamine D_2 receptor binding affinity, which was responsible for the side effect of benzamides, such as **1a** and **1b**, was estimated and expressed by pIC_{50} (-logarithm of inhibitory concentration 50%) values. The pharmacological results are given in Tables 5—7.

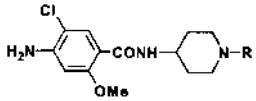
Firstly, we examined monocyclic amino compounds (Table 5). Amphoteric compounds (**19**—**23**) with a straight methylene chain, except for **22**, showed more potent gastro- and colon-prokinetic activities than *N*-methyl analog (**61**)[11] or **1a**, and elongation of the methylene chain led to a decrease in both prokinetic activities. Compound **19** especially exhibited excellent gastro- and colon-prokinetic activities in comparison with **1b**. In addition, the introduction of a methyl group (**24**) into the α-position of the carboxy group in **19** led to a slight reduction in gastro- and colon-prokinetic activities. These results suggested that the distance between the basic nitrogen atom and the carboxy group would be an important factor for gastro- and colon-prokinetic activities.

The dopamine D_2 receptor affinity of the *N*-methyl analog (**61**) was moderate ($pIC_{50}=5.7$), while none of the amphoteric compounds showed significant affinity. This result suggested that amphoteric-ionization caused a decrease in D_2 binding affinity.

We selected the acetic acid moiety ($n=1$) as a substituent on the basic nitrogen atom for further optimization, since **19** exhibited the most excellent gastro- and colon-prokinetic activities among monocyclic amphoteric compounds (**19**—**24**).

Secondly, we addressed our attention to the compounds with a bicyclic amine moiety. As shown in Table 6, bicyclic amphoteric compounds (**32a**—**c**, **38**) showed similar or less potent gastro- or colon-prokinetic activity in comparison with the corresponding *N*-methyl analogs (**62**—**65**)[7] in contrast to the case of monocyclic compounds. Especially, **32b**

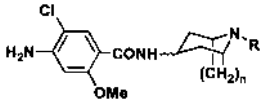
Table 5. Pharmacological Data for Monocyclic Benzamides



Compd.	R	Gastric antrum[a] (mg/kg, i.v.)		Ascending colon[b] (mg/kg, i.v.)		D_2 binding pIC_{50}
		0.1	1.0	0.1	1.0	
19	CH_2CO_2H	++	++	75	100	<4
20	$(CH_2)_2CO_2H$	++	++	0	25	<4
21	$(CH_2)_3CO_2H$	+	++	0	100	<4
22	$(CH_2)_4CO_2H$	NT	−	NT	0	<4
23	$(CH_2)_5CO_2H$	−	++	0	50	4.4
24	$CH(Me)CO_2H$	+	+	0	75	<4
61	Me	−	+	0	0	5.7
Metoclopramide (**1a**)		−	+	0	0	6.7
Cisapride (**1b**)		+	++	0	25	7.0

a) The symbols have the following meanings: ++, 150% or more; +, 125% to 150%; −, less than 125%; NT, not tested. *b*) % ratio of number of dogs in which giant contraction was observed versus the tested dogs.

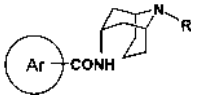
Table 6. Pharmacological Data for Bicyclic Benzamides

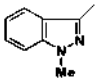
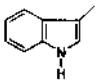
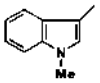


Compd.	n	R	*endo*/*exo*	Gastric antrum[a] (mg/kg, i.v.)		Ascending colon[b] (mg/kg, i.v.)		D_2 binding pIC_{50}
				0.1	1.0	0.1	1.0	
32a	3	CH_2CO_2H	*endo*	+	++	0	75	5.0
32b	3	CH_2CO_2H	*exo*	NT	−	NT	0	<4
32c	2	CH_2CO_2H	*exo*	+	++	33	67	<4
38	2	CH_2CO_2H	*endo*	−	++	0	100	<4
62	3	Me	*endo*	+	++	0	50	5.9
63	3	Me	*exo*	+	++	0	0	6.3
64	2	Me	*exo*	+	++	67	67	6.2
65	2	Me	*endo*	++	++	0	33	<4

a, *b*) See footnote *a* and *b* in Table 5.

Table 7. Pharmacological Data for Heterocyclic Carboxamides



Compd.	Ar	R	Gastric antrum[a] (mg/kg, i.v.)	Ascending colon[b] (mg/kg, i.v.)	D_2 binding pIC_{50}
			1.0	1.0	
66		Me	−	0	<4
43d		CH_2COOH	−	0	<4
67		Me	−	0	<4
43e		CH_2COOH	−	0	<4
68		Me	−	0	4.2
43f		CH_2COOH	−	0	<4

a, *b*) See footnote *a* and *b* in Table 5.

Table 8. Penetration of Compound **19** and Cisapride into Brain in Mice

Compd.	Plasma concentration (μg/ml)	Brain concentration (μg/ml)	Brain/Plasma
19	23.25±1.32	0.42±0.059	0.018
Cisapride (**1b**)	2.24±0.46	5.73±1.77	2.40

Table 9. Effect of SB-207266 on Gastrointestinal Contraction Induced by Intravenous Compound **19** Administration in Conscious Dogs

Compd.	Dose (mg/kg, i.v.)	Gastric antrum[a]	Ascending colon[b]
19	1.0	++	100
19+SB-207266	1.0+0.1	−	0

a, *b*) See footnote *a* and *b* in Table 5.

showed no significant gastro- and colon-prokinetic activities. Concerning D_2 binding affinity, bicyclic amphoteric compounds (**32a**—**c**, **38**) showed less potent affinity than the corresponding bicyclic *N*-methyl derivatives (**62**—**65**), and amphoteric-ionization of bicyclic compounds was found to cause the decrease in dopamine D_2 affinity. This observation was consistent with the previous result obtained from monocyclic compounds.

Also examined were the replacements of the benzene ring of **32a**, the most potent gastro- and colon-prokinetic active compound among the bicyclic amphoteric compounds, by some heterocyclic rings (Table 7). These replacements caused the disappearance in both gastrointestinal prokinetic activity and dopamine D_2 affinity in not only *N*-methyl analogs (**66**—**68**) but also the corresponding amphoteric compounds (**43d**—**f**). Consequently, the benzene ring of **32a** seemed to be essential for the gastrointestinal prokinetic activity.

On the basis of the above screening results, **19**, with an amphoteric monocyclic side chain, was selected as the candidate for a novel gastrointestinal prokinetic agent, exhibiting both the gastro- and colon-prokinetic activities without D_2 binding affinity. Furthermore, we examined its penetration into the central nervous systems (CNS). As shown in Table 8, **19** showed a 100 times lower brain/plasma ratio than **1b** and thus amphoteric-ionization was also found to be effective in lowering the penetration into the brain. Accordingly, **19** was expected to show no significant CNS side effect.

5-HT_4 Receptor Agonist Activity and Conformational Analysis In order to clarify the mode of action for **19**, the influence of the selective 5-HT_4 antagonist, SB-207266,[12] on the gastric and colonic contractions induced by **19** was evaluated. As shown in Table 9, the selective 5-HT_4 antagonist blocked both gastric and colonic contractions. This result suggested that the 5-HT_4 receptor agonist activity would participate in the mechanism for the gastrointestinal prokinetic effect of **19**.

The result shown in Table 9 allowed us to examine the relationship between gastrointestinal effects and 5-HT_4 agonist activity by conformational analysis.

Serotonin 5-HT_4 agonist activity of **19**, **24**, **32a**—**c** and **38** was evaluated by their effects on carbachol-induced tone in rat esophagus and expressed as the negative logarithm of the molar concentration that exhibited 50% relaxation to the carbachol-induced contraction (pEC_{50}), as shown in Table 10.

On the other hand, we implemented the conformational analysis of **19**, **24**, **32a**—**c** and **38** according to the method of López-Rodríguez *et al.*[13] The molecules were built *de novo* in their protonated forms, which are believed to be bioactive forms, using the CAChe system,[14] and their geometry was optimized by the MM2 force field of the CAChe system. For the first purpose, we applied a systematic conformational analysis around four rotatable bonds, as shown in Fig. 1, and calculated the energy by 15° stepwise increments of the dihedral angles. Then the lower energy conformers of each compound were energy-minimized by the MM2 force field. We tried to identify the lowest energy conformer of each compound accordingly. Generally, the oxygen atom substituted at

the *ortho* position of the benzamide is considered to form an intramolecular hydrogen bond with the NH of the amide group. Concerning the dihedral angle (τ), defined by [C1–C2–C3–O4] (see Fig. 1), we selected only the conformation of each compound whose τ was near 180°. López-Rodríguez *et al.* studied a 5-HT_4 pharmacophore model by their conformational analysis with various types of 5-HT_4 ligands. They proposed four essential criteria for active conformation as shown in Fig. 2: 1) the basic nitrogen atom situated at an average distance of 8.0±0.1 Å from the centroid of the benzene ring (Ar–N); 2) the oxygen atom of the amide's carbonyl group situated at an average distance of 3.6±0.1 Å from the centroid of the benzene ring (Ar–O); 3) the oxygen atom of the amide's carbonyl group situated at an average distance of 5.4±0.1 Å from the basic nitrogen atom (O–N); and (4) the basic nitrogen atom deviated at a distance of 3.6—4.0 Å from the plane of the benzene ring (h). To identify the active conformation, we actually performed a conformational analysis and energy minimization of each compound around the dihedral angle (θ), defined by [C3–N5–C6–C7] (see Fig. 1) of each compound, by 1° stepwise increments of the dihedral angle, and then examined to see if the selected conformers of each compound were adapted to four structural criteria in the pharmacophore model, as described above.

Except for the deviations (the criterion 4); h), the structural parameters of **19**, **24**, **32b** and **32c** corresponded well with the above three criteria (1 to 3) of the 5-HT_4 pharmacophore model, but the 5-HT_4 agonist activity of **24**, **32b** and **32c** was weaker than that of compound **19**. The α-methyl group of **24** and the alkylene-bridged chains of *exo*-bicyclic compounds (**32b**, **32c**) were presumed to situate at the same side of their amide carbonyl groups and which might disturb the tight interaction in the binding mode between the ligand and 5-HT_4 receptor for enhancing 5-HT_4 agonist activity (see Fig. 3). The distances Ar–N of *endo*-bicyclic compounds (**32a**, **38**) were also found to be shorter than that of monocyclic compound (**19**), and it seemed to be one of the reasons they showed weak 5-HT_4 agonist activity in comparison with **19**. On the other hand, 5-HT_4 agonist activities of all amphoteric compounds almost correlated with their *in vivo* gastrointestinal prokinetic activities. This result suggested that 5-HT_4 agonist activities of amphoteric compounds could contribute to their *in vivo* gastrointestinal prokinetic effects. Compound **19**, however, exhibited excellent *in vivo* gastrointestinal prokinetic activity after intravenous administration in dogs in spite of weaker 5-HT_4 agonist activity than **1b** (pEC_{50}=7.26).[15] Therefore, it was considered that other mechanisms contributed to the gastrointestinal activity of **19**.

Examination of Prodrugs of Compound 19 As described above, compound **19**, with an amphoteric monocyclic side chain, was selected as a candidate for a novel gastrointestinal prokinetics. Unfortunately, **19** showed only weak gastrointestinal activity by oral administration in further evaluation. It seemed that poor oral absorption of **19** was attributed to its low lipophilicity. On the other hand, the ethyl ester (**13**), its synthetic precursor, had potent gastrointestinal activity after oral administration, but an undesired emetic side effect was observed. We therefore set about examining the other various ester prodrugs of **19**.

We tested for the gastrointestinal prokinetic and emetic action as well as the physicochemical and metabolic stability of the prodrugs. Their results were summarized in Table 11.

Among the double ester type of prodrugs (**57**—**60**), **58** showed the best pharmacological effect, but its physicochem-

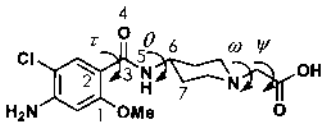


Fig. 1

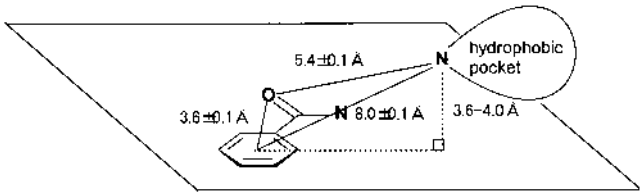


Fig. 2. 5-HT_4 Pharmacophore Model

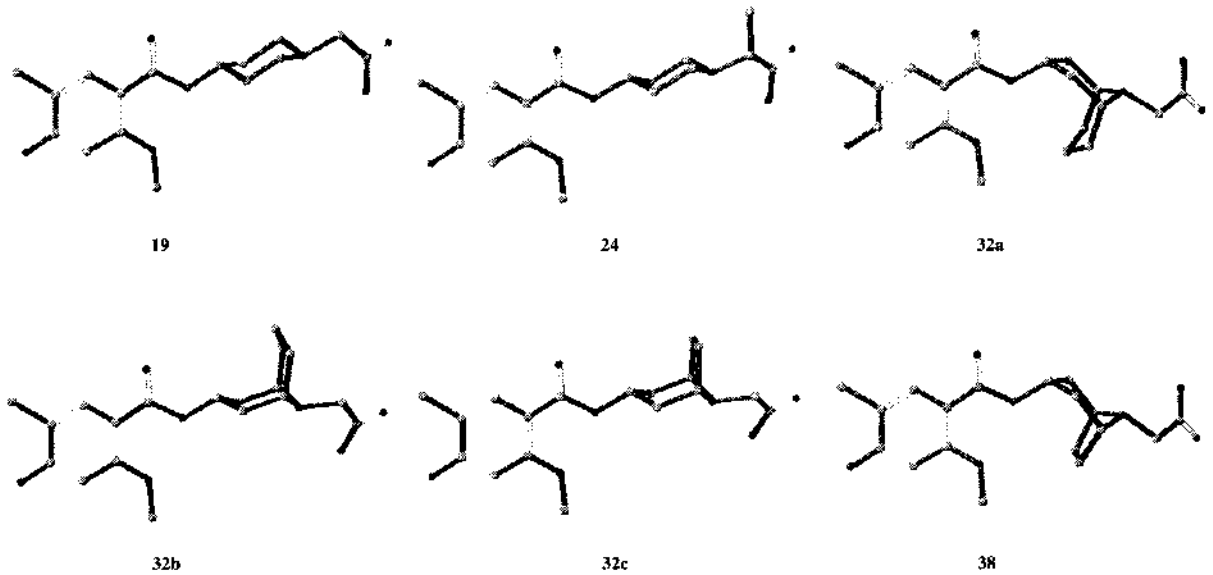


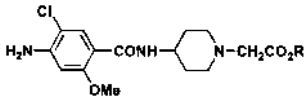
Fig. 3. Presumed Active Conformation of Compounds **19**, **24**, **32a**—**c**, **38** Calculated by the Molecular Modeling Program CAChe (Version 4.1.1)

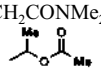
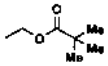
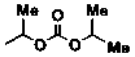
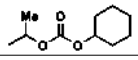
Table 10. Energy and Structural Parameters of the Conformations and 5-HT_4 Agonist Activity

Compd.	Energy (kcal/mol)	E[a] (kcal/mol)	τ[b]	θ[b]	ϖ[b]	ψ[b]	Ar–N (Å)[c]	Ar–O (Å)[d]	O–N (Å)[e]	h (Å)[f]	5-HT_4 agonist activity (pEC_{50})
19	−10.79	0.29	−148.9	89.7	167.3	142.9	7.93	3.51	5.47	1.49	6.11
24	−7.56	0.03	−146.8	108.8	164.9	141.6	7.93	3.51	5.56	1.54	5.64
32a	7.87	0.06	−143.0	−127.7	65.3	−145.3	7.57	3.50	5.75	2.20	5.13
32b	3.88	0.04	−148.1	108.4	164.9	149.8	7.83	3.51	5.65	2.02	4.62
32c	2.50	0.01	−146.5	111.8	175.5	150.2	7.82	3.50	5.69	1.72	5.40
38	4.61	0.16	−145.5	−122.8	69.9	−149.3	7.48	3.50	5.75	1.79	5.32
Pharmacophore model							8.0±0.1	3.6±0.1	5.4±0.1	3.6—4.0	—

a) Increment of energy with respect to the lowest energy conformation. *b*) Torsion angle (°) defined in Fig. 1. *c*) Distance between the centroid of the benzene ring and the basic nitrogen of the amine. *d*) Distance between the centroid of the benzene ring and the oxygen of the carboxyl group. *e*) Distance between the oxygen of the carboxyl group and the basic nitrogen of the amine. *f*) Deviation of the basic nitrogen with respect to the benzene ring.

Table 11. Pharmacological Data for Prodrugs (**13**, **44**—**60**)



Compd.	R	Gastric antrum[a] (1 mg/kg, i.d.)	Ascending colon[b] (1 mg/kg, i.d.)	Vomiting in ferrets (10 mg/kg, p.o.) Response (%)	Stability	
					Physicochemical[c] residual %	Metabolic[d] residual %
13	Et	++	100	100	99	61
44	Me	++	100	75	97	69
45	*n*-Pr	++	80	25	97	49
46	*n*-Bu	++	100	0	97	22
47	*n*-Pentyl	++	80	0	98	2
48	*n*-Hex	++	80	13	99	6
49	iso-Pr	++	80	50	98	80
50	iso-Bu	++	100	13	NT[e]	33
51	iso-Pentyl	++	60	0	100	7
52	CH_2CH_2OMe	++	100	0	96	70
53	Benzyl	++	40	0	100	30
54	CH_2COMe	+	60	0	96	ND[f]
55	CH_2CO_2Et	++	60	0	96	25
56	CH_2CONMe_2	+	40	0	92	97
57		++	80	0	82	ND[f]
58		++	100	0	73	ND[f]
59		++	40	0	85	ND[f]
60		++	60	0	84	7

a, *b*) See footnote *a* and *b* in Table 5. *c*) Physicochemical stability in buffer solution (pH 6.8). *d*) *In vitro* metabolic stability with human liver S9. *e*) Not tested. *f*) Not detected.

ical stability was not so good. Although benzyl ester (**53**) and alkyl esters, having functional groups (**54**—**56**), showed no emetic action, they had only moderate gastrointestinal prokinetic action. Methoxyethyl ester (**52**) showed good pharmacological actions but it was expected to be tolerant to hydrolysis. Among straight or branched alkyl esters (**44**—**51**), *n*-butyl (**46**) and *n*-pentyl (**47**) esters were preferable in terms of pharmacological and pharmacokinetic profiles. We selected the *n*-butyl prodrug (**46**), which exhibited the most potent gastrointestinal prokinetic activity, as a developing candidate.

Compound **46** also did not show the emetic side effect after oral administration in dogs. After oral administration of **46** (10 mg/kg) in rats, only **19** was detected in plasma. Accordingly, **46** was expected to exhibit excellent gastrointestinal prokinetic activity without significant CNS or emetic side effects.

Conclusion

1) The combination of amphoteric-ionization and cyclization of the terminal amine moiety of **1a** caused an increase in gastro- and colon-prokinetic activities and a decrease in dopamine D_2 receptor affinity. This combination seemed to be an effective approach to a selective gastrointestinal prokinetic activity devoid of other pharmacological activity such as CNS side effects, and also to generating marvelous colon-prokinetic activity.

2) Amphoteric-ionization was also effective for reducing the penetration into the brain.

3) The 5-HT_4 receptor agonist activity of **19** was consid-

ered to participate partially in the gastrointestinal prokinetic effect.

4) According to our conformational analyses, the α-methyl group in **24** and the alkylene-bridged chain in *exo*-bicyclic compounds (**32b**, **32c**) were found to be located at the same side of their amide carbonyl groups. These conformations might disturb the interaction in the binding mode between the ligand and 5-HT_4 receptor, leading to their lower gastrointestinal activity.

5) The *n*-butyl ester (**46**) of **19** was found to improve oral availability compared to **19**, without an emetic side effect.

Further studies of the candidate, the ester **46** (Code No: **AU-224**), as a new gastrointestinal prokinetic agent for preclinical evaluation, is now in progress, and the mechanism for the active metabolite **19** is also being examined in detail.

Experimental

Chemistry All melting points were measured on a Yanagimoto melting point apparatus and are uncorrected. Spectral data were obtained using the following apparatus: ^{1}H-NMR spectra with JEOL FX-90Q (90 MHz), JEOL EX-270 (270 MHz) and JEOL A-500 (500 MHz) spectrometers; mass spectra (MS) with JEOL JMS-DX 300 mass spectrometer; IR spectra with Hitachi 270-30 spectrometer. Chemical shifts are expressed as δ (ppm) values with tetramethylsilane (TMS) as an internal standard. Column chromatography was carried out with Kieselgel 60 (Merck) or Aluminium oxide 90 (Merck). Elemental analyses were performed using Yanagimoto MT-3 and MT-5 elemental analysis apparatus, and analytical results were within $\pm$0.4% of theoretical values. TLC was conducted on a 0.25 mm pre-coated silica gel plate ($60F_{254}$, Merck), and spots were detected by inspection under short (254 nm) wavelength UV light, or by the colors developed with iodine. Organic extracts were dried over anhydrous Na_2SO_4. The solvent was evaporated under reduced pressure.

The following known intermediates and reference compounds were prepared essentially according to the literature: 4-amino-5-chloro-*N*-[2-(diethylamino)ethyl]-2-methoxybenzamide (metoclopramide **1a**),[6] *cis*-4-amino-5-chloro-*N*-[1-[3-(4-fluorophenoxy)propyl]-3-methoxy-4-piperidyl]-2-methoxybenzamide (cisapride **1b**),[4] 4-acetylamino-2-methoxybenzoic acid (**25**),[6] *endo*- or *exo*-3-amino-9-benzyl-9-azabicyclo[3.3.1]nonane (**26a**, **b**),[7] *exo*-3-amino-8-benzyl-8-azabicyclo[3.2.1]octane (**26c**),[7] 1-methyl-1*H*-indazole-3-carbonyl chloride (**39d**),[8] 1*H*-indole-3-carbonyl chloride (**39e**),[9] 1-methyl-1*H*-indole-3-carbonyl chloride (**39f**),[9] 4-amino-5-chloro-*N*-(1-methyl-4-piperidyl)-2-methoxybenzamide (**61**),[11] *endo*-4-amino-5-chloro-2-methoxy-*N*-(9-methyl-9-azabicyclo[3.3.1]nonan-3-yl)benzamide (**62**),[7] *exo*-4-amino-5-chloro-2-methoxy-*N*-(9-methyl-9-azabicyclo[3.3.1]nonan-3-yl)benzamide (**63**),[7] *exo*-4-amino-5-chloro-2-methoxy-*N*-(8-methyl-8-azabicyclo[3.2.1]oct-3-yl)benzamide (**64**),[7] *endo*-4-amino-5-chloro-2-methoxy-*N*-(8-methyl-8-azabicyclo[3.2.1]oct-3-yl)benzamide (BRL24682; **65**),[7] *endo*-1-methyl-*N*-(9-methyl-9-azabicyclo[3.3.1]non-3-yl)-1*H*-indazole-3-carboxamide (**66**),[8] *endo*-*N*-(9-methyl-9-azabicyclo[3.3.1]non-3-yl)indole-3-carboxamide (**67**),[7] *endo*-1-methyl-*N*-(9-methyl-9-azabicyclo[3.3.1]non-3-yl)indole-3-carboxamide (**68**).[7]

Method A. *N*-(1-Benzyl-4-piperidyl)trifluoroacetamide (6) A solution of 4-amino-1-benzylpiperidine **5** (25.0 g, 131 mmol) and trifluoroacetic anhydride (20.4 ml, 147 mmol) in CH_2Cl_2 (100 ml) was stirred at room temperature for 30 min. The reaction mixture was concentrated and saturated aqueous $NaHCO_3$ was added to the residue. The mixture was extracted with CH_2Cl_2. The extract was dried and evaporated. The resulting residue was converted to the hydrochloride in the usual way to give the hydrochloride (41.6 g, 98%) of **6** as a colorless crystal, which was recrystallized from AcOEt to give colorless needles, mp 205—207 °C. *Anal.* Calcd For $C_{14}H_{17}F_3N_2O\cdot HCl$: C, 52.10; H, 5.62; N, 8.68. Found: C, 51.89; H, 5.53; N, 8.67. MS *m/z*: 286 (M^+). IR ν (liq) cm^{-1}: 1714. ^{1}H-NMR (DMSO-d_6): 1.25—2.65 (10H, m), 3.35 (1H, br s), 3.55—3.95 (2H, m), 4.13 (3H, s), 4.25—4.80 (1H, m), 7.15—7.80 (3H, m), 8.05—8.35 (1H, m), 8.40—9.40 (1H, m).

***N*-(4-Piperidyl)trifluoroacetamide (7)** A mixture of the hydrochloride of **6** (40.0 g, 124 mmol), 10% Pd–C (5.0 g) and H_2O (50 ml) in MeOH (200 ml) was stirred at 35 °C and 2 atm of H_2 for 3 h. The catalyst was removed by filtration and the filtrate was evaporated. The resulting residue was recrystallized from MeOH to afford the hydrochloride (20.8 g, 72%) of **7** as a colorless crystal, mp 236—240 °C. *Anal.* Calcd For $C_7H_{11}F_3N_2O\cdot HCl$: C, 36.14; H, 5.20; N, 12.04. Found: C, 36.01; H, 5.12; N, 12.25. MS *m/z*: 196 (M^+). IR ν (liq) cm^{-1}: 1720. ^{1}H-NMR (DMSO-d_6): 1.75—1.95 (2H, m), 2.05—2.20 (2H, m), 3.05—3.20 (2H, m), 3.40—3.55 (2H, m), 3.95—4.15 (1H, m).

***N*-(1-Trityl-4-piperidyl)trifluoroacetamide (8)** A mixture of **7** (186.4 g, 950 mmol), trityl chloride (264.8 g, 950 mmol) and Et_3N (145.7 ml, 1.05 mol) in CH_2Cl_2 was stirred at room temperature for 3 h. Water (1000 ml) was poured into the reaction mixture and the mixture was extracted with CH_2Cl_2. The extracts were evaporated and the resulting residue was washed with diisopropyl ether (iso-Pr_2O) to afford 303.5 g (73%) of **8** as a colorless crystal, which was used for the next step without further purification. MS *m/z*: 438 (M^+). IR ν (KBr) cm^{-1}: 1716, 1696. ^{1}H-NMR ($CDCl_3$): 1.35—1.60 (2H, m), 1.70—1.80 (2H, m), 1.90—2.05 (2H, m), 2.95—3.20 (2H, m), 3.60—3.70 (1H, m), 6.05—6.15 (1H, m), 7.15—7.50 (15H, m).

4-Amino-1-tritylpiperidine (9) A mixture of **8** (206.4 g, 471 mmol), KOH (62.13 g, 941 mmol) in H_2O (1 l) and EtOH (1 l) was refluxed for 2.5 h. After cooling, the resulting precipitate was collected by filtration and washed successively with water and *n*-hexane, to afford 151.1 g (94%) of **9** as a colorless crystal, which was used for the next step without further purification. MS *m/z*: 342 (M^+). IR ν (KBr) cm^{-1}: 1596. ^{1}H-NMR ($CDCl_3$): 1.30—1.95 (6H, m), 2.40—2.60 (1H, m), 2.85—3.20 (2H, m), 7.10—7.55 (15H, m).

4-Amino-5-chloro-2-methoxy-*N*-(1-trityl-4-piperidyl)benzamide (11) A mixture of **10** (16.4 g, 81.3 mmol), Et_3N (13.6 ml, 97.7 mmol) and $ClCO_2Et$ (8.55 ml, 89.9 mmol) in anhydrous tetrahydrofuran (THF, 330 ml) was stirred under ice cooling. After 2 h, **9** (30.6 g, 89.3 mmol) was added dropwise to the mixture and stirred at room temperature for 20 h. The insoluble substance was filtered off and the filtrate was evaporated. The resulting residue was dissolved in CH_2Cl_2. The CH_2Cl_2 solution was washed with water, dried and evaporated. The residue was washed with *n*-hexane to afford 39.4 g (92%) of **11** as a colorless crystal, which was used for the next step without further purification. MS *m/z*: 525, 527 (3 : 1, M^+). IR ν (KBr) cm^{-1}: 1646, 1624. ^{1}H-NMR ($CDCl_3$): 1.55—1.80 (2H, m), 1.90—2.20 (2H, m), 2.70—3.40 (2H, m), 3.55—4.10 (3H, m), 4.33 (2H, br s), 6.25 (1H, s), 7.05—7.55 (15H, m), 7.67 (1H, br s), 8.06 (1H, s).

4-Amino-5-chloro-2-methoxy-*N*-(4-piperidyl)benzamide (12) A mixture of **11** (39.4 g, 74.9 mmol) and concentrated HCl (10 ml) in acetone (600 ml) was refluxed for 40 min. After cooling, the resulting precipitate was collected by filtration and washed with acetone to afford the hydrochloride (25.8 g, 78%) of **12**[5] as a colorless crystal, which was recrystallized from EtOH to afford colorless crystals, mp 214—217 °C. *Anal.* Calcd for $C_{13}H_{18}ClN_3O_2\cdot HCl\cdot 1/2H_2O$: C, 47.43; H, 6.12; N, 12.76. Found: C, 47.64; H, 6.39; N, 12.97. MS *m/z*: 283, 285 (3 : 1, M^+). IR ν (KBr) cm^{-1}: 2948, 2812, 1640. ^{1}H-NMR (DMSO-d_6): 1.60—1.85 (2H, m), 1.90—2.15 (2H, m), 2.85—3.10 (2H, m), 3.10—3.35 (2H, m), 3.83 (3H, s), 3.85—4.10 (1H, m), 6.35 (1H, s), 7.62 (1H, s), 7.65—7.80 (1H, m).

Ethyl 4-[(4-Amino-5-chloro-2-methoxybenzoyl)amino]-1-piperidineacetate (13) A mixture of the hydrochloride of **12** (3.20 g, 9.91 mmol), ethyl bromoacetate (1.22 ml, 11.0 mmol) and K_2CO_3 (3.04 g, 22.0 mmol) in DMF (32 ml) was stirred at 60 °C for 2.5 h. After evaporation of the solvent, water was added to the residue and extracted with CH_2Cl_2. The organic layer was washed with water, dried and evaporated to afford yellow oily residue. The residue was converted to the hydrochloride in the usual manner to afford the hydrochloride (3.31 g, 73%) of **13** as a pale yellow crystal, which was recrystallized from EtOH to afford colorless flakes, mp 196.5—198.5 °C. *Anal.* Calcd for $C_{17}H_{24}ClN_3O_4\cdot HCl$: C, 50.25; H, 6.20; N, 10.34. Found: C, 49.98; H, 6.23; N, 10.36. MS *m/z*: 369, 371 (3 : 1, M^+). IR ν (KBr) cm^{-1}: 1758, 1650. ^{1}H-NMR (DMSO-d_6): 1.26 (3H, t, J=7.0 Hz), 1.75—2.20 (4H, m), 3.05—3.70 (4H, m), 3.75—4.20 (1H, m), 3.84 (3H, s), 4.17 (2H, s), 4.24 (2H, q, J=7.0 Hz), 6.54 (1H, s), 7.63 (1H, s), 7.74 (1H, br s).

Compounds **15**—**18** were prepared in a manner similar to that described above and their physicochemical data were summarized in Table 1.

Ethyl 4-[(4-Amino-5-chloro-2-methoxybenzoyl)amino]-1-piperidinepropionate (14) A mixture of **12** (2.79 g, 9.80 mmol) and ethyl acrylate (1.35 ml, 12.5 mmol) in EtOH (28 ml) was refluxed for 4 h. The reaction mixture was concentrated and the residue was washed with iso-Pr_2O to afford pale yellow crystals (3.34 g, 87%), which were recrystallized from a mixture of acetone and Et_2O to give colorless needles, mp 116—117.5 °C. *Anal.* Calcd for $C_{18}H_{26}ClN_3O_4$: C, 56.32; H, 6.83; N, 10.95. Found: C, 56.28; H, 6.78; N, 10.87. MS *m/z*: 383, 385 (3 : 1, M^+). IR ν (KBr) cm^{-1}: 1730, 1628. ^{1}H-NMR (DMSO-d_6): 1.18 (3H, t, J=7.0 Hz), 1.40—1.60 (2H, m), 1.70—1.90 (2H, m), 2.05—2.15 (2H, m), 2.35—2.80 (6H, m), 3.65—

3.85 (1H, m), 3.84 (3H, s), 4.06 (2H, q, J=7.0 Hz), 5.70—5.85 (2H, br s), 6.49 (1H, s), 7.60—7.75 (1H, m), 7.66 (1H, s).

4-[(4-Amino-5-chloro-2-methoxybenzoyl)amino]-1-piperidineacetic Acid (19) A mixture of the hydrochloride of **13** (2.23 g, 5.49 mmol) and 2 N aqueous NaOH solution (9.86 ml) in MeOH (22 ml) was refluxed for 1 h. The reaction mixture was concentrated and the resulting residue was dissolved in a small amount of water. The solution was adjusted to pH 2 with 10% HCl. The resulting precipitate was collected by filtration and washed with water to afford the hydrochloride (1.47 g, 78%) of **19** as a pale brown crystal, which was recrystallized from water to afford pale yellow pillars, mp 248—251 °C (dec.). *Anal.* Calcd for $C_{15}H_{20}ClN_3O_4 \cdot HCl \cdot 1/4H_2O$: C, 47.07; H, 5.66; N 10.98. Found: C, 47.34; H, 5.58; N, 11.08. MS *m/z*: 341, 343 (3 : 1, M^+). IR ν (KBr) cm^{-1}: 1734, 1628. ^{1}H-NMR (DMSO-d_6): 1.75—2.20 (4H, m), 3.05—3.35 (2H, m), 3.35—3.60 (2H, m), 3.85 (3H, s), 3.85—4.10 (1H, m), 3.98 (2H, s), 6.55 (1H, s), 7.63 (1H, s), 7.65—7.80 (1H, m).

Compounds **20**—**24** were prepared in a manner similar to that described above and their physicochemical data were summarized in Table 1.

Method B. ***endo*-4-Acetylamino-*N*-(9-benzyl-9-azabicyclo[3.3.1]non-3-yl)-2-methoxybenzamide (27a)** A mixture of 4-acetylamino-2-methoxybenzoic acid **25** (11.0 g, 52.6 mmol), $ClCO_2Et$ (5.28 ml, 55.2 mmol) and Et_3N (8.43 ml, 60.5 mmol) in THF (210 ml) was stirred for 1.5 h at room temperature. Under ice-cooling, *endo*-3-amino-9-benzyl-9-azabicyclo[3.3.1]-nonane **26a** (12.7 g, 55.2 mmol) was added dropwise to the mixture and the resulting mixture was stirred at room temperature for 2.5 d. The reaction mixture was concentrated and water was added to the residue. The solution was adjusted to pH 9 with 10% aqueous K_2CO_3. The resulting precipitate was collected by filtration, washed successively with water and ethanol and dried to afford 15.7 g (71%) of **27a** as a colorless crystal, which was used in the next step without further purification. MS *m/z*: 421 (M^+). IR ν (KBr) cm^{-1}: 1690, 1624. ^{1}H-NMR (DMSO-d_6): 0.90—1.15 (2H, m), 1.25—1.60 (3H, m), 1.80—2.40 (5H, m), 2.06 (3H, s), 2.95—3.15 (2H, m), 3.81 (2H, s), 3.86 (3H, s), 4.25—4.50 (1H, m), 7.10—7.45 (5H, m), 7.18 (1H, dd, J=8.5, 2 Hz), 7.49 (1H, d, J=2 Hz), 7.60—7.80 (1H, m), 7.72 (1H, d, J=8.5 Hz), 10.04 (1H, s).

Compounds **27b**, **c** were prepared in a manner similar to that described above. **27b**: MS *m/z*: 421 (M^+). IR ν (KBr) cm^{-1}: 1692, 1666. ^{1}H-NMR (DMSO-d_6): 1.35—1.60 (2H, m), 1.55—2.15 (8H, m), 2.07 (3H, s), 2.75—2.95 (2H, m), 3.83 (2H, s), 3.87 (3H, s), 4.55—4.85 (1H, m), 7.10—7.45 (5H, m), 7.18 (1H, dd, J=8.5, 1.5 Hz), 7.50 (1H, d, J=2 Hz), 7.60—7.75 (1H, m), 7.70 (1H, d, J=8.5 Hz), 10.04 (1H, s). **27c**: MS *m/z*: 407 (M^+). IR ν (KBr) cm^{-1}: 1694, 1630. ^{1}H-NMR (DMSO-d_6): 1.50—1.85 (6H, m), 1.90—2.15 (2H, m), 2.06 (3H, s), 3.05—3.35 (2H, m), 3.55 (2H, s), 3.85 (3H, s), 4.05—4.30 (1H, m), 7.10—7.45 (5H, m), 7.17 (1H, dd, J=8.5, 1.5 Hz), 7.48 (1H, d, J=1.5 Hz), 7.60—7.80 (1H, m), 7.70 (1H, d, J=8.5 Hz), 10.04 (1H,s).

***endo*-4-Acetylamino-*N*-(9-azabicyclo[3.3.1]non-3-yl)-2-methoxybenzamide (28a)** A mixture of **27a** (15.5 g, 36.8 mmol), acetic acid (20 ml) and Pearlman's catalyst (2.0 g) in MeOH (180 ml) was stirred at room temperature and an atmospheric pressure of H_2 for 1 h. The catalyst was removed by filtration and the filtrate was evaporated. The resulting residue was dissolved in water and the solution was adjusted to pH 9 with 10% aqueous K_2CO_3. The resulting precipitate was collected by filtration, washed with water and dried to give 11.7 g (95%) of **28a** as a pale yellow crystal, which was recrystallized from 1,2-dichloroethane to afford colorless prisms, mp 196—200 °C. *Anal.* Calcd for $C_{18}H_{25}N_3O_3 \cdot 1/4H_2O$: C, 64.36; H, 7.65; N, 12.51. Found: C, 64.32; H, 7.53; N, 12.47. MS *m/z*: 331 (M^+). IR ν (KBr) cm^{-1}: 1692, 1634. ^{1}H-NMR (DMSO-d_6): 1.10—1.70 (7H, m), 1.80—2.30 (3H, m), 2.06 (3H, s), 3.00—3.40 (2H, m), 3.86 (3H, s), 3.95—4.20 (1H, m), 7.17 (1H, dd, J=8.5, 1.5 Hz), 7.48 (1H, d, J=1.5 Hz), 7.55—7.75 (1H, m), 7.72 (1H, d, J=8.5 Hz), 10.04 (1H, br s).

Compounds **28b**, **c** were prepared in a manner similar to that described above and their physicochemical data were summarized in Table 2.

Ethyl *endo*-3-[(4-Acetylamino-2-methoxybenzoyl)amino]-9-azabicyclo-[3.3.1]nonane-9-acetate (29a) A mixture of **28a** (11.6 g, 34.5 mmol), ethyl bromoacetate (4.60 ml, 41.4 mmol) and K_2CO_3 (5.72 g, 41.4 mmol) in DMF (75 ml) was stirred at 60 °C for 2 h. The reaction mixture was poured into water. The resulting precipitate was collected by filtration and washed with water to afford 13.4 g (91%) of **29a** as a pale yellow crystal, which was recrystallized from benzene to give colorless prisms, mp 85—88 °C. *Anal.* Calcd for $C_{22}H_{31}N_3O_5 \cdot 1/2H_2O$: C, 61.95; H, 7.56; N, 9.85. Found: C, 61.85; H, 7.55; N, 9.86. MS *m/z*: 417 (M^+). IR ν (liq) cm^{-1}: 1732, 1694, 1636. ^{1}H-NMR ($CDCl_3$): 1.00—1.65 (4H, m), 1.26 (3H, t, J=7 Hz), 1.75—2.15 (2H, m), 2.22 (3H, s), 2.40—2.65 (2H, m), 3.05—3.30 (2H, m), 3.47 (2H, s), 3.92 (3H, s), 4.15 (2H, q, J=7 Hz), 4.35—4.60 (1H, m), 6.83 (1H, dd, J=8.5, 2 Hz), 7.65—7.80 (1H, m), 7.83 (1H, d, J=2 Hz), 8.04 (1H, d, J=8.5 Hz), 8.77 (1H, s).

Compounds **29b**, **c** were prepared in a manner similar to that described above and their physicochemical data were summarized in Table 2.

Ethyl *endo*-3-[(4-Acetylamino-5-chloro-2-methoxybenzoyl)amino]-9-azabicyclo[3.3.1]nonnane-9-acetate (30a) The mixture of **29a** (13.0 g, 31.1 mmol) and SO_2Cl_2 (3.42 ml, 34.3 mmol) in CH_2Cl_2 (140 ml) was stirred at room temperature for 1.5 h. The reaction mixture was basified with aqueous $NaHCO_3$. The organic layer was dried and evaporated. The resulting residue was purified by column chromatography [SiO_2, CH_2Cl_2–MeOH (50 : 1)] to afford 11.4 g (81%) of **30a** as a pale yellow amorphous solid. High resolution MS *m/z*: Calcd for $C_{22}H_{30}ClN_3O_5$: 451.1874, 453.1844. Found: 451.1859, 453.1853. MS *m/z*: 451, 453 (M^+, 3 : 1). IR ν (KBr) cm^{-1}: 1750, 1646. ^{1}H-NMR ($CDCl_3$): 1.05—2.20 (8H, m), 1.28 (3H, t, J=7.5 Hz), 2.28 (3H, s), 2.40—2.65 (2H, m), 3.10—3.35 (2H, m), 3.49 (2H, s), 3.98 (3H, s), 4.17 (2H, q, J=7.5 Hz), 7.55—7.70 (1H, m), 7.80 (1H, s), 8.22 (1H, s), 8.32 (1H, s).

Compounds **30b**, **c** were prepared in a manner similar to that described above and physicochemical data were summarized in Table 2.

Ethyl *endo*-3-[(4-Amino-5-chloro-2-methoxybenzoyl)amino]-9-azabicyclo[3.3.1]nonane-9-acetate (31a) A mixture of **30a** (11.0 g, 24.4 mmol) and 20% ethanolic hydrochloride (66 ml) in EtOH (22 ml) was refluxed for 1 h. The reaction mixture was evaporated and the resulting residue was dissolved in water. The solution was adjusted to pH 10 with K_2CO_3. The resulting precipitate was collected by filtration and washed successively with water and iso-Pr_2O to give 8.88 g (89%) of **31a** as a pale brown crystal, which was recrystallized from EtOH to afford colorless needles, mp 163.5—164.5 °C. *Anal.* Calcd for $C_{20}H_{28}ClN_3O_4$: C, 58.60; H, 6.88; N, 10.25. Found: C, 58.39; H, 6.84; N, 10.26. MS *m/z*: 409, 411 (M^+, 3 : 1). IR ν (KBr) cm^{-1}: 1744, 1646. ^{1}H-NMR ($CDCl_3$): 1.00—1.65 (5H, m), 1.27 (3H, t, J=7.5 Hz), 1.75—2.15 (3H, m), 2.35—2.65 (2H, m), 3.05—3.30 (2H, m), 3.47 (2H, s), 3.89 (3H, s), 4.16 (2H, q, J=7.5 Hz), 4.30—4.55 (1H, m), 6.30 (1H, s), 7.40—7.60 (1H, m), 8.10 (1H, s).

Compounds **31b**, **c** were prepared in a manner similar to that described above and physicochemical data were summarized in Table 2.

***endo*-3-[(4-Amino-5-chloro-2-methoxybenzoyl)amino]-9-azabicyclo[3.3.1]nonane-9-acetic Acid (32a)** A mixture of **31a** (8.00 g, 19.5 mmol) and 2 N aqueous NaOH solution (19.5 ml, 39.0 mmol) in MeOH (80 ml) was refluxed for 1 h. The reaction mixture was concentrated and the resulting residue was dissolved in a small amount of water. The solution was adjusted to pH 1 with 10% HCl. The resulting precipitate was collected by filtration, washed with water and iso-Pr_2O, and recrystallized from a mixture of MeOH and iso-Pr_2O to afford the hydrochloride (5.75 g, 68%) of **32a** as a colorless crystalline powder, mp 189—194 °C (dec.). *Anal.* Calcd for $C_{18}H_{24}ClN_3O_4 \cdot HCl \cdot H_2O$: C, 49.55; H, 6.23; N, 9.63. Found: C, 49.38; H, 5.99; N, 9.49. IR ν (KBr) cm^{-1}: 1732, 1626. ^{1}H-NMR (DMSO-d_6): 1.40—1.65 (3H, m), 1.70—1.90 (2H, m), 1.95—2.30 (3H, m), 2.35—2.60 (2H, m), 3.75—3.90 (2H, m), 3.84 (3H, s), 4.17 (2H, s), 4.35—4.55 (1H, m), 6.53 (1H, s), 7.65 (1H, s), 7.55—7.85 (1H, m).

Compounds **32b**, **c** were prepared in a manner similar to that described above and their physicochemical data were summarized in Table 2.

Method C. Methyl *endo*-3-[(4-Acetylamino-5-chloro-2-methoxybenzoyl)amino]-8-azabicyclo[3.2.1]octane-8-carboxylate (34) A mixture of **33**[7] (40.0 g, 0.109 mmol), K_2CO_3 (16.0 g, 0.116 mol) and $ClCO_2Me$ (80.0 ml, 1.04 mol) in chloroform (400 ml) was refluxed for 19 h. The resulting precipitate was filtered off and washed with CH_2Cl_2. The filtrate and washing CH_2Cl_2 were combined and evaporated, and the residue was washed successively with AcOEt and MeOH to give 17.5 g (39%) of **34** as a colorless crystal, which was recrystallized from a mixture of MeOH and CH_2Cl_2 to afford colorless crystalline powder, mp 250—252 °C. *Anal.* Calcd for $C_{19}H_{24}ClN_3O_5 \cdot 1/2H_2O$: C, 54.48; H, 6.02; N, 10.03. Found: C, 54.17; H, 5.77; N, 9.79. MS *m/z*: 409, 411 (M^+, 3 : 1). IR ν (liq) cm^{-1}: 1704, 1682, 1652. ^{1}H-NMR (DMSO-d_6): 1.70—1.85 (2H, m), 1.90—2.15 (6H, m), 2.15 (3H, s), 3.61 (3H, s), 3.90 (3H, s), 4.05—4.25 (3H, m), 7.80 (1H, s), 7.81 (1H, s), 8.25—8.40 (1H, m), 9.45 (1H, br s).

***endo*-4-Acetylamino-*N*-(8-azabicyclo[3.2.1]oct-3-yl)-5-chloro-2-methoxybenzamide (35)** A mixture of **34** (16.3 g, 39.8 mmol) and trimethylsilyl iodide (22.0 ml, 155 mmol) in CH_2Cl_2 (400 ml) was stirred at room temperature for 6 h. Sodium hydrogensulfite solution was added to the reaction mixture. The mixture was stirred at room temperature for 15 min and adjusted to pH 2 with 10% HCl. The aqueous layer was basified with K_2CO_3 and extracted with CH_2Cl_2. The extract was washed with water, dried and evaporated to give a yellow brown oil, which was purified by column chromatography [alumina, CH_2Cl_2–MeOH (50 : 1)] to afford 8.41 g (60%) of

35 as a pale yellow viscous oil. MS *m/z*: 351, 353 (M^+, 3 : 1). IR ν (KBr) cm^{-1}: 1694, 1644. ^{1}H-NMR ($CDCl_3$): 1.65—2.30 (9H, m), 2.28 (3H, s), 3.60—3.75 (2H, m), 4.02 (3H, s), 4.30—4.45 (1H, m), 7.82 (1H, br s), 8.21 (1H, s), 8.34 (1H, s), 8.40—8.50 (1H, m).

Ethyl *endo*-3-[(4-Acetylamino-5-chloro-2-methoxybenzoyl)amino]-8-azabicyclo[3.2.1]octane-8-acetate (36) A mixture of **35** (8.00 g, 22.7 mmol), ethyl bromoacetate (4.18 g, 25.0 mmol) and K_2CO_3 (3.46 g, 25.0 mmol) in DMF (50 ml) was stirred at 60 °C for 2 h. After the addition of water, the reaction mixture was extracted with AcOEt. The extract was washed with water, dried and evaporated. The resulting residue was washed with iso-Pr_2O to give 5.20 g (52%) of **36** as a colorless crystal, which was recrystallized from EtOH to afford colorless prisms, mp 144—145 °C. *Anal.* Calcd for $C_{21}H_{28}ClN_3O_5$: C, 57.60; H, 6.44; N, 9.60. Found: C, 57.45; H, 6.32; N, 9.49. MS *m/z*: 437, 439 (M^+, 3 : 1). IR ν (KBr) cm^{-1}: 1738, 1704, 1640. ^{1}H-NMR ($CDCl_3$): 1.29 (3H, t, *J*=7 Hz), 1.65—2.45 (9H, m), 2.28 (3H, s), 3.26 (2H, s), 3.30—3.50 (2H, m), 4.02 (3H, s), 4.21 (2H, q, *J*=7 Hz), 4.25—4.40 (1H, m), 7.80 (1H, br s), 8.21 (1H, s), 8.34 (1H, s), 8.35—8.50 (1H, m).

Ethyl *endo*-3-[(4-Amino-5-chloro-2-methoxybenzoyl)amino]-8-azabicyclo[3.2.1]octane-8-acetate (37) A mixture of **36** (4.60 g, 10.5 mmol) and 10% ethanolic hydrochloride (30 ml) in EtOH (10 ml) was refluxed for 1 h. The reaction mixture was evaporated and water was added to the resulting residue. The aqueous layer was washed with AcOEt and then adjusted to pH 9 with 10% aqueous K_2CO_3. The resulting crystals were collected by filtration and washed with water and iso-Pr_2O to give 4.09 g (98%) of **37** as a pale yellow crystal, which was recrystallized from EtOH to afford colorless crystals, mp 187—188 °C. *Anal.* Calcd for $C_{19}H_{26}ClN_3O_4$: C, 57.65; H, 6.62; N, 10.61. Found: C, 57.57; H, 6.52; N, 10.42. MS *m/z*: 395, 397 (M^+, 3 : 1). IR ν (KBr) cm^{-1}: 1748, 1640. ^{1}H-NMR ($CDCl_3$): 1.28 (3H, t, *J*=7 Hz), 1.60—2.50 (8H, m), 3.26 (2H, s), 3.25—3.50 (2H, m), 3.93 (3H, s), 4.20 (2H, q, *J*=7 Hz), 4.20—4.40 (1H, m), 4.41 (2H, br s), 6.31 (1H, s), 8.10 (1H, s), 8.20—8.35 (1H, m).

***endo*-3-[(4-Amino-5-chloro-2-methoxybenzoyl)amino]-8-azabicyclo[3.2.1]octane-8-acetic Acid (38)** A mixture of **37** (3.60 g, 9.09 mmol) and 2 N aqueous NaOH solution (9.1 ml) in MeOH (36 ml) was refluxed for 1 h. The reaction mixture was concentrated and the resulting residue was dissolved in a small amount of water. The solution was adjusted to pH 1 with 10% HCl. The resulting precipitate was collected by filtration and washed with water to give crude crystals, which were recrystallized from water to afford the hydrochloride (3.19 g, 81%) of **38** as a pale yellow crystal, mp 254—256 °C (dec.). *Anal.* Calcd for $C_{17}H_{22}ClN_3O_4 \cdot HCl \cdot 3/2H_2O$: C, 47.34; H, 6.08; N, 9.74. Found: C, 47.36; H, 5.96; N, 9.79. MS *m/z*: 367, 369 (M^+, 3 : 1). IR ν (KBr) cm^{-1}: 1736, 1628. ^{1}H-NMR (DMSO-d_6): 1.95—2.60 (8H, m), 3.85 (3H, s), 3.95—4.15 (1H, m), 4.01 (2H, s), 5.90 (2H, br), 6.56 (1H, s), 7.65 (1H, s), 8.05—8.20 (1H, m).

Method D. *endo*-*N*-(9-Benzyl-9-azabicyclo[3.3.1]non-3-yl)-1-methyl-1H-indazole-3-carboxamide (40d) A mixture of *endo*-3-amino-9-benzyl-9-azabicyclo[3.3.1]nonane (13.8 g, 59.9 mmol), Et_3N (6.60 g, 65.2 mmol) and 1-methyl-1H-indazole-3-carbonyl chloride **39d** (10.2 g, 52.2 mmol) in CH_2Cl_2 (150 ml) was stirred at room temperature for 2 h. The reaction mixture was washed with water, dried and evaporated. The resulting residue was purified by column chromatography (SiO_2, $CHCl_3$) and crystallized from iso-Pr_2O to give 14.75 g (73%) of **40d** as a colorless crystal, which was recrystallized from MeOH to afford colorless needles, mp 161—162 °C. *Anal.* Calcd for $C_{24}H_{28}N_4O$: C, 74.20; H, 7.26; N, 14.42. Found: C, 74.15; H, 7.32; N, 14.41. MS *m/z*: 388 (M^+). IR ν (KBr) cm^{-1}: 1672. ^{1}H-NMR ($CDCl_3$): 0.75—2.25 (8H, m), 2.25—2.75 (2H, m), 2.90—3.35 (2H, m), 3.87 (2H, s), 4.09 (3H, s), 4.50—4.95 (1H, m), 6.70—6.95 (1H, m), 7.05—7.65 (8H, m), 8.35—8.55 (1H, m).

Compounds **40e**, **f** were prepared in a manner similar to that described above. **40e**: mp 212—214 °C (CH_2Cl_2–MeOH). *Anal.* Calcd for $C_{24}H_{27}N_3O$: C, 77.18; H, 7.29; N, 11.25. Found: C, 77.08; H, 7.34; N, 11.24. MS *m/z*: 373 (M^+). IR ν (KBr) cm^{-1}: 1620. ^{1}H-NMR (DMSO-d_6): 0.70—2.75 (10H, m), 2.85—3.30 (2H, m), 3.83 (2H, s), 4.05—4.85 (1H, m), 6.90—7.60 (9H, m), 7.99 (1H, d, *J*=3 Hz), 8.10—8.30 (1H, m), 11.38 (1H, br s). **40f**: mp 210—211 °C (EtOH). *Anal.* Calcd for $C_{25}H_{29}N_3O$: C, 77.49; H, 7.54; N, 10.84. Found: C, 77.45; H, 7.57; N, 11.10. MS *m/z*: 387 (M^+). IR ν (KBr) cm^{-1}: 1614. ^{1}H-NMR ($CDCl_3$): 0.80—2.20 (8H, m), 2.30—2.80 (2H, m), 3.00—3.30 (2H, m), 3.76 (3H, s), 3.86 (2H, s), 4.25—5.10 (1H, m), 5.70—5.80 (1H, m), 7.10—7.50 (8H, m), 7.63 (1H, s), 7.80—8.15 (1H, m).

***endo*-*N*-(9-Azabicyclo[3.3.1]non-3-yl)-1-methyl-1H-indazole-3-carboxamide (41d)** A mixture of **40d** (12.9 g, 3.32 mmol), Pearlman's catalyst (2.0 g) and acetic acid (25 ml) in MeOH (225 ml) was stirred at room temperature and atmospheric pressure of H_2 for 3 h. The catalyst was removed by filtration and the filtrate was evaporated. The resulting residue was dissolved in 10% HCl and the insoluble substance was removed by filtration. The filtrate was basified with K_2CO_3 and the precipitate was collected by filtration, washed with water and dried to give 9.90 g (100%) of **41d** as a colorless amorphous solid, which was converted to the hydrochloride in the usual manner. The hydrochloride was recrystallized from EtOH to afford colorless needles, mp >300 °C. *Anal.* Calcd for $C_{17}H_{22}N_4O \cdot HCl$: C, 60.98; H, 6.92; N, 16.73. Found: C, 60.77; H, 7.01; N, 16.55. MS *m/z*: 298 (M^+). IR ν (KBr) cm^{-1}: 1640. ^{1}H-NMR (DMSO-d_6): 1.25—2.65 (10H, m), 3.35 (1H, br s), 3.55—3.95 (2H, m), 4.13 (3H, s), 4.25—4.80 (1H, m), 7.15—7.80 (3H, m), 8.05—8.35 (1H, m), 8.40—9.40 (1H, m).

Compounds **41e**, **f** were prepared in a manner similar to that described above. **41e**: mp 232—236 °C (dec., aqueous EtOH). *Anal.* Calcd for $C_{17}H_{21}N_3O \cdot H_2O$: C, 67.75; H, 7.69; N, 13.94. Found: C, 67.85; H, 7.76; N, 13.86. MS *m/z*: 283 (M^+). IR ν (KBr) cm^{-1}: 1606. ^{1}H-NMR (DMSO-d_6): 0.90—2.35 (10H, m), 2.95—3.55 (2H, m), 3.80—4.50 (1H, m), 6.90—7.55 (4H, m), 7.99 (1H, s), 8.00—8.25 (1H, m), 11.39 (1H, br s). **41f**: mp 218—219 °C (EtOH). *Anal.* Calcd for $C_{18}H_{23}N_3O$: C, 72.70; H, 7.80; N, 14.13. Found: C, 72.54; H, 7.84; N, 13.96. MS *m/z*: 297 (M^+). IR ν (KBr) cm^{-1}: 1614. ^{1}H-NMR ($CDCl_3$): 1.00—2.10 (8H, m), 1.90 (1H, s), 2.10—2.60 (2H, m), 3.20—3.55 (2H, m), 3.80 (1H, s), 4.00—4.60 (1H, m), 5.60—5.95 (1H, m), 7.10—7.50 (3H, m), 7.67 (1H, s), 7.80—8.05 (1H, m).

Ethyl *endo*-3-[[(1-Methyl-1*H*-indazol-3-yl)carbonyl]amino]-9-azabicyclo[3.3.1]nonane-9-acetate (42d) A mixture of **41d** (2.50 g, 8.38 mmol), ethyl bromoacetate (1.54 g, 9.22 mmol) and K_2CO_3 (1.16 g, 8.39 mmol) in DMF (20 ml) was stirred at 70 °C for 4.5 h. After the addition of water, the reaction mixture was extracted with Et_2O. The extract was washed with water, dried and evaporated. The residue was purified by column chromatography [SiO_2, CH_2Cl_2] to afford 2.69 g (84%) of **42d** as a pale yellow viscous oil. High resolution MS *m/z*: Calcd for $C_{21}H_{28}N_4O_3$: 384.2161. Found: 384.2173. MS *m/z*: 384 (M^+). IR ν (KBr) cm^{-1}: 1748, 1662. ^{1}H-NMR ($CDCl_3$): 0.90—2.25 (8H, m), 1.28 (3H, t, *J*=7.5 Hz), 2.25—2.80 (2H, m), 3.05—3.45 (2H, m), 3.49 (2H, s), 4.08 (3H, s), 4.17 (2H, q, *J*=7.5 Hz), 4.20—4.80 (1H, m), 6.65—6.95 (1H, m), 7.10—7.60 (3H, m), 8.20—8.50 (1H, m).

Compounds **42e**, **f** were prepared in a manner similar to that described above and their physicochemical data were summarized in Table 3.

***endo*-3-[[(1-Methyl-1*H*-indazol-3-yl)carbonyl]amino]-9-azabicyclo-[3.3.1]nonane-9-acetic Acid (43d)** A mixture of **42d** (2.00 g, 5.20 mmol) and 2 N aqueous NaOH solution (5.2 ml) in MeOH (20 ml) was refluxed for 1 h. The reaction mixture was evaporated and the resulting residue was dissolved in a small amount of water. The solution was adjusted to pH 2 with 10% aqueous HCl. The resulting precipitate was collected by filtration to afford 1.50 g (67%) of **43d** as a colorless amorphass solid. *Anal.* Calcd for $C_{19}H_{24}N_4O_3 \cdot HCl \cdot 2H_2O$: C, 53.21; H, 6.81; N, 13.06. Found: C, 53.34; H, 6.66; N, 13.04. MS *m/z*: 357 (M^++1). IR ν (KBr) cm^{-1}: 1744, 1652. ^{1}H-NMR (DMSO-d_6): 1.25—2.70 (10H, m), 3.70—4.05 (2H, m), 4.14 (3H, s), 4.18 (2H, s), 4.35—5.05 (1H, m), 7.10—7.85 (3H, m), 8.00—8.50 (2H, m).

Compounds **43e**, **f** were prepared in a manner similar to that described above and their physicochemical data was summarized in Table 3.

Method E. Methyl 4-[(4-Amino-5-chloro-2-methoxybenzoyl)amino]-1-piperidineacetate (44) To a suspension of **19** (5.00 g, 14.6 mmol) in MeOH (75 ml) was added concentrated sulfuric acid (1.80 g). The mixture was refluxed for 16 h. After cooling, the solvent was evaporated to give the residue, which was basified (pH=9) with aqueous K_2CO_3 and extracted with CH_2Cl_2. The extract was washed with saturated aqueous NaCl, dried and evaporated to afford 3.82 g (74%) of **44** as pale brown residue, which was recrystallized from MeOH to afford 2.95 g of pale brown prisms, mp 196—197 °C. *Anal.* Calcd for $C_{16}H_{22}ClN_3O_4$: C, 54.01; H, 6.23; N,11.81. Found: C, 53.80; H, 6.14; N, 11.83. MS *m/z*: 355, 357 (3 : 1, M^+). IR ν (KBr) cm^{-1}: 3400, 3320, 3216, 1756. ^{1}H-NMR (DMSO-d_6): 1.45—1.55 (2H, m), 1.75—1.85 (2H, m), 2.30—2.35 (2H, m), 2.70—2.80 (2H, m), 3.23 (2H, s), 3.62 (3H, s), 3.70—3.85 (1H, m), 3.85 (3H, s), 5.80 (2H, br s), 6.50 (1H, s), 7.67 (1H, s), 7.68 (1H, d, *J*=7.5 Hz).

***n*-Propyl 4-[(4-Amino-5-chloro-2-methoxybenzoyl)amino]-1-piperidineacetate (45)** A solution of *n*-propyl bromide (1.78 g, 14.5 mmol) in DMF (15 ml) was added to a suspension of **19** (4.50 g, 13.2 mmol) and K_2CO_3 (2.00 g, 14.5 mol) in DMF (135 ml) at 60 °C over 1 h. The mixture was stirred at 60 °C for 2 h. After cooling, the reaction mixture was added to water and extracted with toluene. The extract was washed with saturated aqueous NaCl, dried and evaporated to afford colorless residue. The residue was washed with *n*-heptane to afford 3.94 g (78%) of the crude **45**, which was converted to the mesylate in the usual manner. The mesylate of **45** was recrystallized from iso-PrOH to give a colorless crystal, mp 209—210 °C.

Anal. Calcd for $C_{18}H_{26}ClN_3O_4 \cdot CH_4O_3S$: C, 47.54; H, 6.30; N, 8.75. Found: C, 47.47; H, 6.41; N, 8.69. MS *m/z*: 383, 385 (3 : 1, M^+). IR ν (KBr) cm^{-1}: 3408, 3324, 3216, 1754. ^{1}H-NMR (DMSO-d_6): 0.93 (3H, t, J=7 Hz), 1.66 (2H, sextet, J=7 Hz), 1.70—2.00 (2H, m), 2.00—2.10 (2H, m), 2.33 (3H, s), 3.10—3.40 (2H, m), 3.40—3.70 (2H, m), 3.84 (3H, s), 3.90—4.10 (1H, m), 4.17 (2H, br s), 4.22 (2H, br s), 5.82 (2H, br s), 6.52 (1H, s), 7.63 (1H, s), 7.74 (1H, br s), 9.92 (1H, br s).

Compounds **46**—**60** were prepared in a manner similar to that described above and their physicochemical data were summarized in Table 4.

Pharmacology

Gastrointestinal Contractile Activity in Dogs The gastrointestinal motility was measured, according to the method of Z. Itoh *et al*., in dogs of both sexes, weighing 7.9 to 12.0 kg. Under general anesthesia, a strain-gauge force transducer was chronically sutured on the ascending colon 10 cm distal to the cecum in a direction to measure circular muscle contractions. A sailastic cannula for intravenous (i.v.) or intra-duodenal (i.d.) administration of the tested compound was placed into the superior vena cava or duodenum. The administration of the tested compounds to the dogs was performed more than 2 weeks after the surgery. The tested compounds were solved in saline, including 1% lactic acid, and were intravenously or intra-duodenally administered to the animals more than 2 h after feeding. Gastric motility was expressed as a symbol based on a motor index, representing the area surrounded by the construction wave and the base line during a 20 min period. Colonic motility was expressed as a ratio of the number of dogs, showing giant contractions in the ascending colon during 40 min after the administration, *versus* the number of tested dogs.

Dopamine D_2 Binding Assay The test compounds at the concentration of 1—100 μM were tested in binding assays using rat brain synaptic membranes for competition with [^{3}H]Spiperon in the rat striatum at their respective binding sites. Each assay was started by an addition of tissue preparation and terminated by rapid filtration through a Whatman GF/B glass fiber filter under the reduced pressure. The filters were washed three times with 5 ml of ice-cold buffer and transferred to scintillation vials that contained 7 ml of scintillator, and the radioactivity in the filter was counted with a scintillation counter. IC_{50} values (the concentration causing 50 inhibition of ^{3}H-labeled ligand specific binding) of the test compounds were calculated with the equation of Cheng and Prusoff.

5-HT_4 Agonist Activity The esophagus was removed from a male Wister rat, and two preparations, obtained from each animal, was used as a longitudinal preparation. The tissues were mounted in organ baths containing 10 ml of Krebs–Henseleit solution. The bathing medium was maintained at 37 °C, pH 7.4, and was equilibrated with a gas mixture consisting of 95% O_2 and 5% CO_2. An initial tension of 1.0 g was applied and the responses were recorded isometrically with a force-displacement transducer. All preparations were equilibrated for at least 60 min before the start of the experiments. Once the carbachol (10^{-6} M)-induced contractions reached a steady state, drugs were added cumulatively, and relaxation was calculated as a percentage of the initial carbachol-induced force.

Emetic Action in Ferrets Male ferrets, weighing 0.9 to 1.3 kg, and fasted for 16 h, were used. The tested compounds were dissolved with 10% aqueous DMSO solution and were administered orally (p.o.). Then, the incidence of vomiting during the 2 h after the administration was observed.

Physicochemical Stability The tested compounds were dissolved in the pH6.8 buffer solution (Japanese Pharmacopoeia 2nd fluid), and the solution was heated at 37 °C for 3 h. The initial concentration of prodrug was 100 μg/ml. The concentration of prodrug that remained was determined by HPLC.

Metabolic Stability with Human Liver S9 The tested compounds were added to human liver S9 (HBI, 0.1 mg protein/ml), pre-incubated at 37 °C, and the mixture was incubated at 37 °C for 30 min. The initial concentration of prodrug was 1 μmol/l. The concentration of prodrug that remained was determined by HPLC.

Brain Distribution The tested compounds were administrated intravenously to CD-1(ICR) mice (CharlesRiver Japan, male, 10 mg/kg, n=4). Blood and brain were taken 2 min after administration. The concentrations of plasma and brain were determined by HPLC or liquid chromatography/mass spectrometory/mass spectrometory (LC/MS/MS). Brain distribution was estimated from the concentration ratio of each compound in brain to plasma.

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