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Synthesis of 2-Imidazolidinylidene Propanedinitrile Derivatives as Stimulators of Gastrointestinal Motility—III

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Abstract—Recently, we reported that a ranitidine derivative **2** (fumarate: KW-5092), which had a 2-imidazolidinylidene propanedinitrile moiety (A), showed potent gastrointestinal motility enhancing activity. We have also found that introduction of substituents such as benzyl or 4-fluorobenzyl (i.e., giving **3** or **4**) at the *N*-3 position of the moiety (A) significantly increased this activity. In this study, novel 2-imidazolidinylidene propanedinitrile derivatives possessing a thioether **5–15** were prepared and evaluated for *in vitro* assays; acetylcholinesterase (AChE) inhibitory activity and potentiating action on electrically induced contractions of guinea pig ileum. Compound **5**, in which a nitrogen atom of compound **2** was replaced by a sulfur atom, was more potent than **2** in these tests. Also, in a series of thioether derivatives, introduction of substituents at the *N*-3 position of the 2-imidazolidinylidene propanedinitrile moiety markedly influenced both activities. In particular, compounds **12** and **13**, which showed an excellent potency during *in vitro* study (AChE IC_{50} = 3.6 and 2.7 nM; ES. EC_{30} = 2.1 and 2.5 nM, respectively), were found to be more active in the enhancement of gastrointestinal motility in anesthetized rabbits than their corresponding parent compounds **3** and **4**, respectively. In addition, compounds **12** and **13** showed lower affinity for the histamine H_2-receptor than ranitidine. Therefore, these compounds may be potent and selective stimulators of gastrointestinal motility.

Introduction

It is now well established that numerous clinical syndromes of digestive diseases may be related to gastrointestinal motor abnormalities. The disturbance of the motility is said to be associated with dysphagia, postoperative ileus, gastric stasis, achalasia, vomiting, abdominal pain, paralytic ileus, constipation, etc. A number of gastrointestinal motility enhancing agents, such as metoclopramide [acetylcholine (ACh) release enhancer and D_2-receptor antagonist], neostigmine [acetylcholinesterase (AChE) inhibitor], domperidone (D_2-receptor antagonist), have been used to treat these symptoms of gastrointestinal disease.[1–5]

Recently, ranitidine (**1**), a histamine H_2-receptor antagonist, has been reported to enhance gastric emptying and gastric motility both in animals[6–8] and in man[9] by inhibition of AChE[10–13] and enhancement of ACh release from the cholinergic nerves.[14–16] To obtain potent stimulators of gastrointestinal motility, we synthesized and evaluated novel ranitidine derivatives, possessing a 2-imidazolidinylidene propanedinitrile moiety (A) in place of a 2-nitro-1,1-ethenediamine moiety.[17] These compounds were much more potent with respect to AChE inhibitory activity and potentiating action on electrically evoked contractions of the isolated guinea pig ileum than ranitidine. Besides, we have found that introduction of a variety of substituents R^1 on the basic nitrogen atom (B) or R^2 at the *N*-3 position of the 2-imidazolidinylidene propanedinitrile moiety [i.e., a nitrogen atom (C)] remarkably influenced both activities.[18,19] Among these derivatives, compounds **2–4** (Chart 1) enhanced motility of the gastric antrum and the colon in anesthetized rabbits together with a negligible histamine H_2-receptor blocking property. Therefore, it is suggested that the 2-imidazolidinylidene propanedinitrile moiety is responsible for the potent gastrointestinal motility stimulatory activity of these compounds. Conversely, introduction of methyl, benzyl or acetyl function on the nitrogen atom (B) produced a significant decrease in AChE inhibitory activity and potentiating action on electrically induced contractions of ileum strips.[19] One possible explanation for the decrease in these activities is that a proton on the nitrogen atom (B) may play a role in conferring 'potent' AChE inhibition and enhancement of ileum contractions. In view of these observations, we were very interested in pharmacological activities of 2-imidazolidinylidene propanedinitrile derivatives possessing thioether as illustrated in the general formula (I) in Chart 1.

This paper describes the synthesis of novel thioether containing 2-imidazolidinylidene propanedinitrile derivatives **5–15**, their AChE inhibitory activities, enhancing action on gastrointestinal motility and histamine H_2-receptor blocking properties. In our

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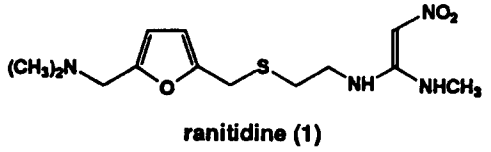
pharmacological assays, ranitidine (1), compounds **2–4**, metoclopramide and neostigmine were used as reference compounds.

Chemistry

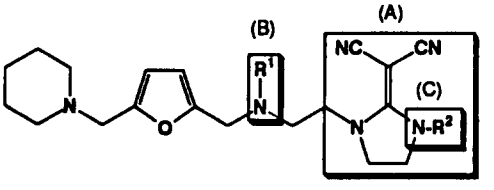
Compounds **5–7**, **10** and **12–15** were obtained according to a previously reported method with a slight modification (Scheme 1, method A).[20] Nucleophilic substitution of a *p*-toluenesulfonyloxy group in compound **16**[17] with a thiol **18**[20] in the presence of K_2CO_3 in tetrahydrofuran (THF) afforded compound **5**. Conversely, alkylation or benzylation of *p*-toluenesulfonate **16** with appropriate alkyl or benzyl halides **19** yielded the *N*-3 substituted 2-imidazolidinylidene propanedinitrile derivative **17**. Target compounds **6**, **7**, **10** and **12–15** were synthesized from the intermediate **17** in the same manner as that for the compound **5**, or by reaction with thioanion derived from disulfide **20**. Because reaction of *p*-toluenesulfonate **16** with 2-bromopropane, 1-bromo-2-methylpropane or cyclohexylmethyl bromide did not yield the corresponding *N*-3 substituted propanedinitrile **17**, compounds **8**, **9** and **11** were obtained by use of an intramolecular cyclization as illustrated in Scheme 2, method B.[18,19] The compounds synthesized are listed in Table 1.

Pharmacological Results and Discussion

The compounds prepared were first evaluated for AChE inhibitory activity and *in vitro* enhancing activity of gastrointestinal motility. Moreover, compounds that showed significant activities in both tests were subjected to histamine H_2-receptor binding assays and gastrointestinal motility enhancing activity in anesthetized rabbits.



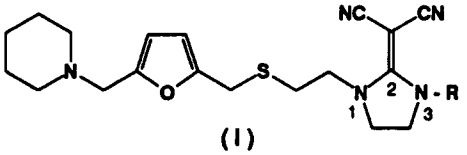
ranitidine (1)



2; R^1, R^2 = H

3; R^1 = H, R^2 = Bzl

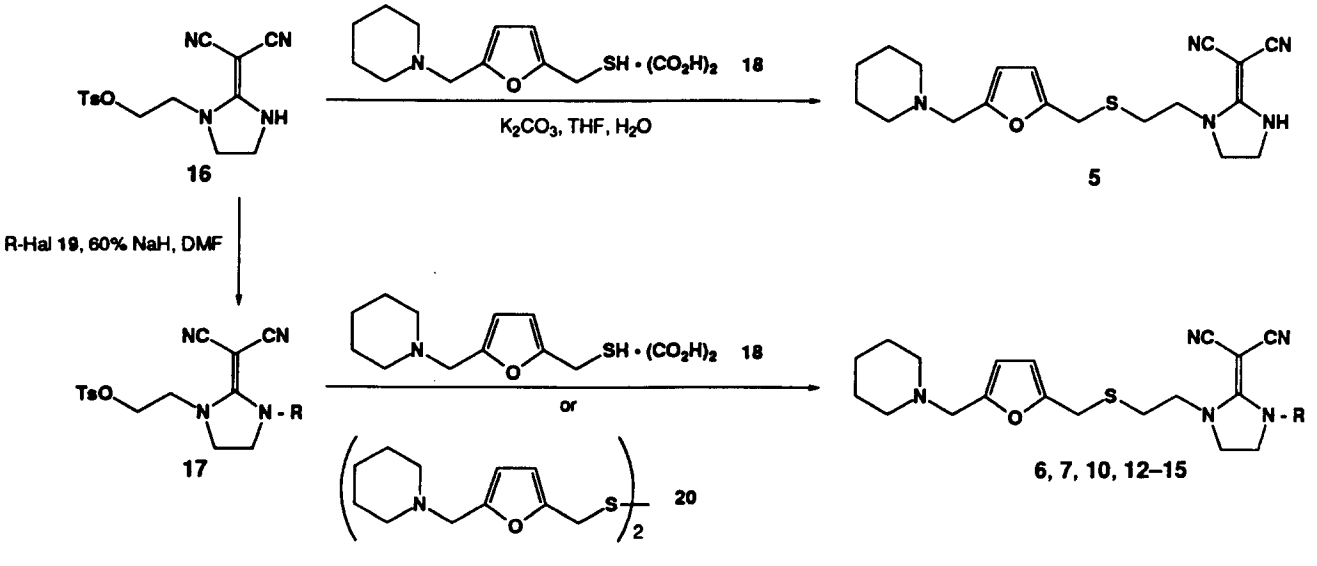
4; R^1 = H, R^2 = 4-F-Bzl



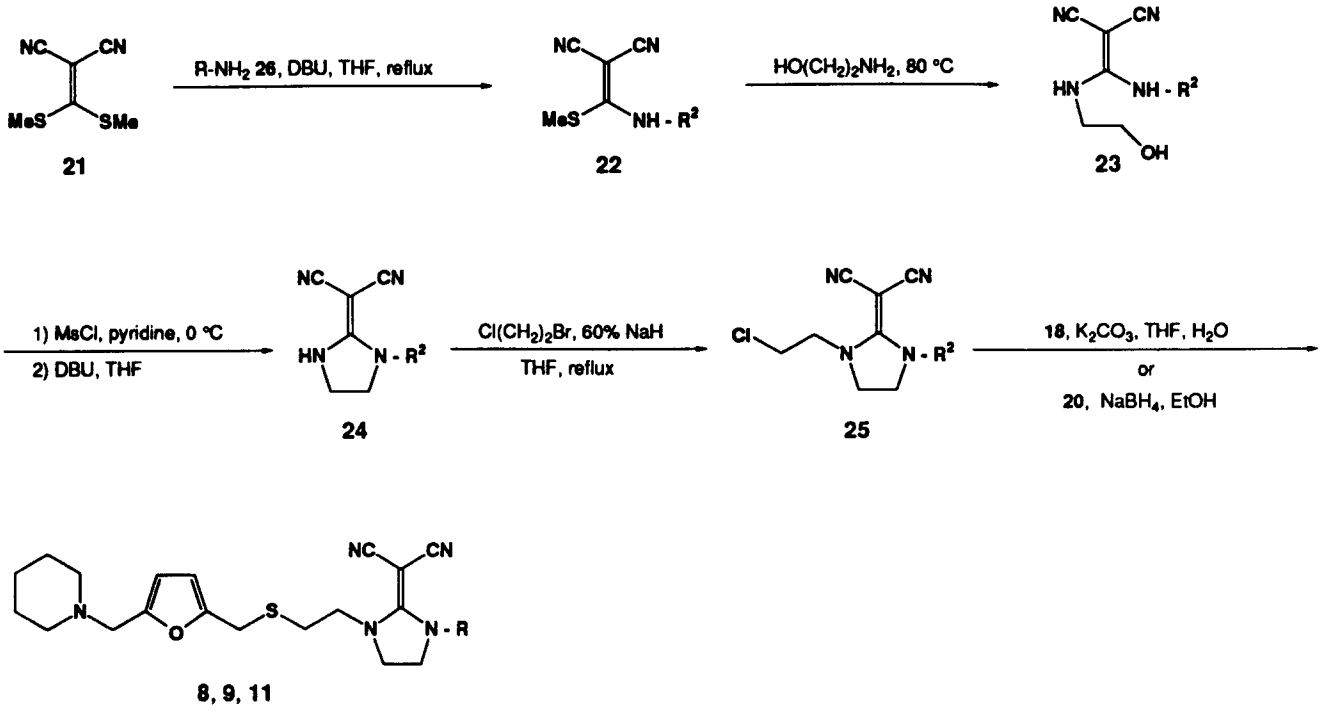
(I)

(b) R-Hal **19**, 60% NaH, DMF;

Chart 1.

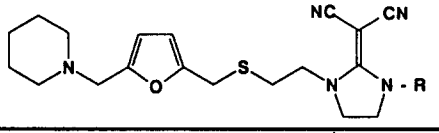


Scheme 1: method A.



Scheme 2: method B.

Table 1. Physiological and pharmacological data for imidazolidinylidene propanedinitrile derivatives **5–15**



No	R	mp, °C	Recryst solvent[a]	Formula[b]	Method[c]	AChE IC_{50} (nM)[d]	ES. EC_{30} (nM)[e]
5	H	117–119	EA-PE	$C_{19}H_{25}N_5OS$	A	18 ± 2.1	10.0
6	CH3	133–134	AC	$C_{20}H_{27}N_5OS \cdot C_2H_2O_4$[f]	A	6.5 ± 0.12	4.7
7	C_2H_5	139.5–140	AC	$C_{21}H_{29}N_5OS \cdot C_2H_2O_4$[f]	A	2.7 ± 0.12	2.5
8	$(CH_3)_2CH$	142.5–143.5	AC	$C_{22}H_{31}N_5OS \cdot C_2H_2O_4$[f]	B	1.7 ± 0.13	–[g]
9	$(CH_3)_2CHCH_2$	124–125	AC	$C_{23}H_{33}N_5OS \cdot C_2H_2O_4$[f]	B	3.3 ± 0.65	3.6
10	$(CH_3)_2C{=}CHCH_2$	134.5–136	AC	$C_{24}H_{33}N_5OS \cdot C_2H_2O_4$[f]	A	14 ± 0.9	–[g]
11	c-$C_6H_{11}CH_2$	125.5–128	AC	$C_{26}H_{37}N_5OS \cdot C_2H_2O_4$[f]	B	8.5 ± 1.7	5.1
12	$C_6H_5CH_2$	150.5–151.5	AC	$C_{26}H_{31}N_5OS \cdot C_2H_2O_4$[f]	A	3.6 ± 0.78	2.1
13	4-F$C_6H_4CH_2$	137–138	AC	$C_{26}H_{30}FN_5OS \cdot C_2H_2O_4$[f]	A	2.7 ± 0.09	2.5
14	3,4-$(CH_3O)_2$-$C_6H_3CH_2$	150.5–151.5	AC	$C_{28}H_{35}N_5O_3S \cdot C_2H_2O_4$[f]	A	1.7 ± 0.2	3.1
15	$C_6H_5CH{=}CHCH_2$	143–147.5	AC	$C_{28}H_{33}N_5OS \cdot C_2H_2O_4$[f]	A	4.4 ± 0.66	5.3
1	(ranitidine)					650 ± 38	1800
2h						30 ± 0.33	16
3i						6.7 ± 0.1	9.5
4i						6.8 ± 1.2	11
metoclopramide						21000 ± 1300	430
neostigmine						22 ± 1.5	–[g]

[a]EA = AcOEt, PE = i-Pr_2O, AC = acetone; [b]All compounds were analysed for C,H and N; analytical results were shown in the Experimental Section; [c]See the Experimental Section; [d]The IC_{50} values are means ± S.E. of three separate experiments done with four different concentrations; [e]Electrical stimulation: The mean concentration of three experiments producing a 30% potentiation of the electrically stimulated contractions of the isolated guinea pig ileum; [f]Oxalic acid; [g]Although these compounds potentiated the electrical stimulation, the maximum contraction was not achieved to 30% See text; [h]See reference 17; [i]See reference 19.

The AChE inhibitory activity was measured by the photometric method of Ellman *et al.*[21] The inhibitory activity was expressed as the IC_{50} value. The *in vitro* gastrointestinal motility enhancing activity was determined by the potentiating action on electrically evoked contractions of the isolated guinea pig ileum.[11,12,17] The results were represented by the EC_{30} value, i.e., the concentration of the tested compounds producing a 30% potentiation of the contractions induced by electrical stimulation. These results are summarized in Table 1.

In our biological assays, ranitidine showed AChE inhibitory activity and potentiating activity on electrically induced contractions (IC_{50} = 650 nM, EC_{30} = 1800 nM, respectively). Metoclopramide was 4 times more potent in potentiating activity than ranitidine and inactive in AChE inhibition. Neostigmine was active in AChE inhibition and it potentiated electrically stimulated ileal contractions at low concentrations (1–30 nM). However, the maximum contraction of neostigmine was not achieved to 30% because it induced remarkable elevation of the baseline tonus. Recently, neostigmine was reported to produce only an uncoordinated antroduodenal hypermotility in patients with marked gastric hypomotility.[9] Consequently, compounds which showed such a baseline tonus elevation would be inferred not to be favorable gastrointestinal motility stimulators and were not evaluated any further, despite being potent in AChE inhibition. As previously described, compounds **2–4** were more potent than ranitidine and metoclopramide in both inhibiting AChE and potentiating electrically evoked contractions.[17,19]

Compound **5** (R = H) was slightly more active than the parent compound **2** in both assays. Introduction of alkyl functions at the *N*-3 position of 2-imidazolidinylidene propanedinitrile moiety of compound **5** further increased both activities. For example, compound **7** was found to be 6 and 4 times more potent than compound **5** in inhibiting AChE (IC_{50} = 2.7 nM) and in potentiating action on the ileal contraction (EC_{30} = 2.5 nM), respectively. The *N*-3 substituted analogs except compounds **8** and **10** showed more potent enhancing activity of electrically stimulated contractions than all of the reference compounds. Compounds **8** and **10** outwardly showed a decrease in the potentiating action on the twitch response owing to a base line tonus elevation similar to neostigmine. While compound **10** (R = 2-methyl-2-butenyl) was least potent among the other *N*-3 substituted derivatives in AChE inhibition (IC_{50} = 14 nM), **8** (R = *iso*-propyl) was one of the most active compounds tested in this assay (IC_{50} = 1.7 nM). Therefore, it is suggested that not potency in AChE inhibition but other unknown factors may produce the unfavorable elevation of base line tonus. As for the kinds of the alkyl functions, the increasing order in potentiating effect on electrically elicited contractions was cyclohexylmethyl (**11**) < methyl (**6**) < *iso*-butyl (**9**) < ethyl (**7**) group. Also, compounds **12–15**, having benzyl groups as the substituent R were active in both activities in the nanomolar order. Thus these compounds were almost equipotent to *N*-3 alkylated derivatives and more potent than all of the reference compounds in both assays.

These results reveal that a proton on the nitrogen atom (B) (see Chart 1) is not crucial for 'potent' AChE inhibition and enhancement of guinea pig ileum contractions. Instead, the 2-imidazolidinylidene propanedinitrile (A) and the substituent R introduced at the *N*-3 position (C) may play a significant role for those activities. The difference in three dimensional structure or mechanism of AChE inhibition between ranitidine and the 2-imidazolidinylidene propanedinitrile derivatives are now investigating.

On the basis of the results obtained from both *in vitro* tests, compounds **5–7**, **12** and **13** were evaluated by the histamine H_2-receptor binding assay (Table 2). This assay was carried out by the method of Gajtkowski *et al.*[22] Interestingly, all of the tested thioether derivatives showed lower binding affinity for the histamine H_2-receptor by a factor of about 10 in comparison to ranitidine. Considering that a 2-[[[5-(piperidinomethyl)-2-furanyl]methyl]thio]ethyl part of the tested compounds is one of the common structures of histamine H_2-receptor antagonists,[23] the 2-imidazolidinylidene propanedinitrile moiety also seems to be responsible for decreasing binding affinity of these novel ranitidine analogs to the receptor and increasing gastrointestinal motility.

Table 2. Histamine H_2-receptor binding of 2-imidazolidinylidene propanedinitrile derivatives

No.	H_2-Receptor binding[a] 10^{-5} M	10^{-4} M
5	2	61
6	17	73
7	46	64
12	32	78
13	48	75
1 (ranitidine)	75	99
2	18	49

[a]Mean percent inhibition of two experiments of [^{3}H]tiotidine binding to the histamine H_2-receptor in guinea pig cortex.

We selected compounds **12** and **13** for further evaluation of their gastrointestinal motor stimulating activity in anesthetized rabbits. This assay was carried out by the method described previously.[17,19] Figure 1 shows that intravenous administration of compounds **12** and **13** (1 mg kg^{-1}) rapidly enhanced the motor activity of both the gastric antrum and the descending colon with slight, transient decrease in the systemic blood pressure and this potentiating response continued for over 30 min. Furthermore, these effects of **12** and **13** on the gastrointestinal motility were found to be greater than those of their corresponding parent compounds **3** and **4** (only a result of compound **4** is represented in Fig. 1).

In summary, thioether containing 2-imidazolidinylidene propanedinitrile derivatives showed a potent AChE inhibitory activity and potentiating action on electrically stimulated contractions. Introduction of various alkyl and benzyl groups at the *N*-3 position of the 2-imidazolidinylidene propanedinitrile moiety further enhanced both activities. The results of the histamine H_2-receptor binding assay suggested that the 2-imidazolidinylidene propanedinitrile moiety may play a significant role in decreasing binding affinity of the tested compounds for this receptor. In particular, compounds **12** and **13** strongly enhanced motility of both the gastric antrum and the colon in anesthetized

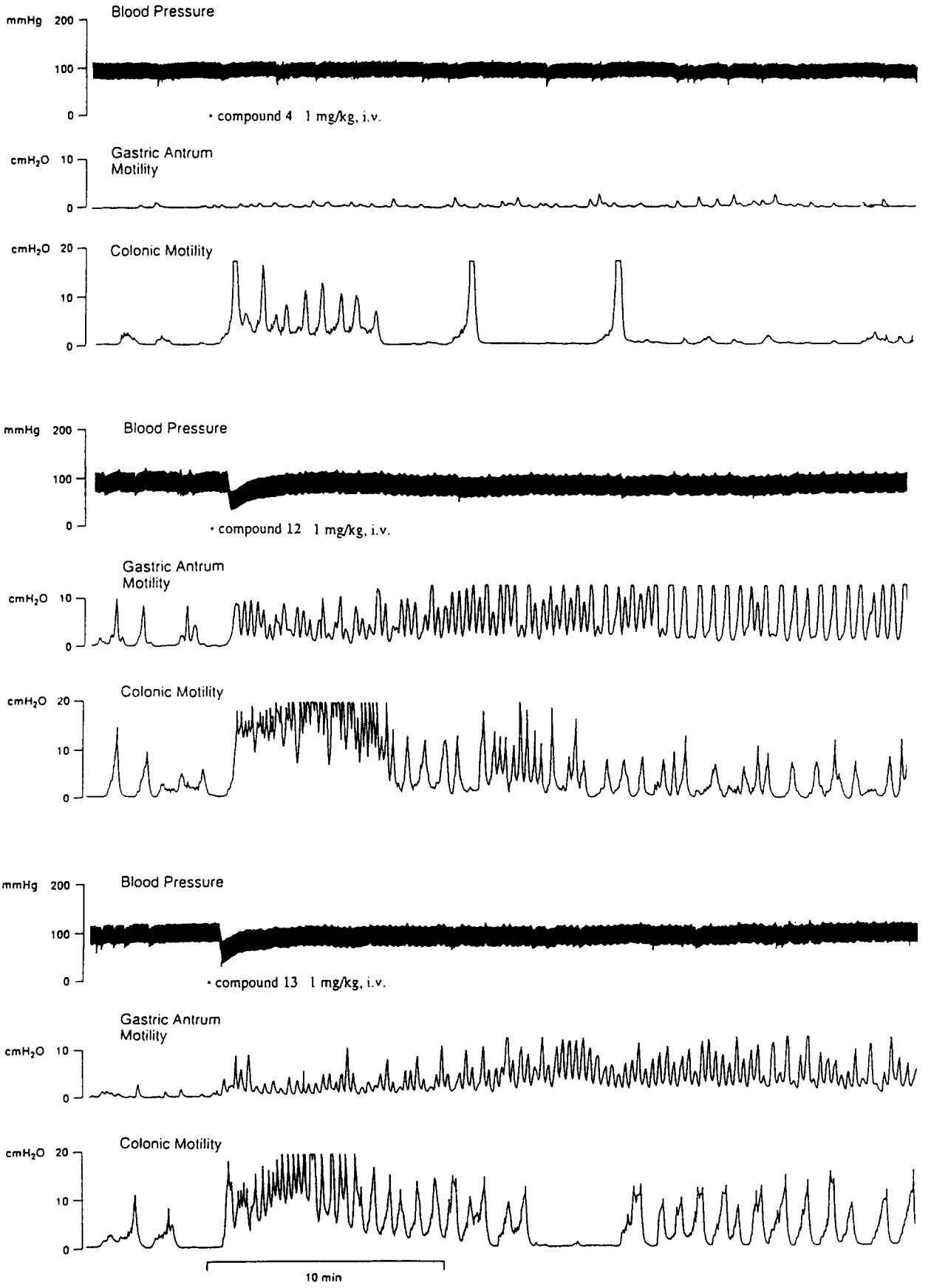


Figure 1. Effects of compounds **4**, **12** and **13** on gastrointestinal motor activity in anesthetized rabbits.

rabbits. Therefore, these compounds may be used for digestive diseases caused by the disturbance of gastrointestinal motility. Further pharmacological evaluation of these analogs is now in progress.

Experimental

Chemistry

All melting points were determined on a Yanako micromelting point apparatus and are uncorrected. IR spectra were recorded on a JASCO IR-400 spectrometer and electron-impact mass spectra (EIMS) were recorded on a JEOL JMS D-300 or a JMS DA-500 spectrometer. ^{1}H NMR spectra were measured at 90 MHz with a Hitachi R-90H spectrometer and at 270 MHz with a JEOL JNM GX-270 spectrometer. Chemical shifts are expressed as δ (ppm) values with tetramethylsilane as the internal standard (NMR abbreviations: *s* = singlet, *d* = doublet, *t* = triplet, *q* = quartet, *m* = multiplet, *br* = broad). Organic extracts were dried over anhydrous $MgSO_4$. The solvent was evaporated under reduced pressure. Merck Kieselgel 60 was used for column chromatography.

Method A-1. {1-[2-[[[5-(Piperidinomethyl)-2-furanyl]methyl]thio]ethyl]-2-imidazolidinylidene}propanedinitrile (5).[20] To a stirred solution of thiol **18** (3.5 g, 11.6 mmol) and *p*-toluenesulfonate **16** (3.85 g, 11.6 mmol) in THF (60 mL) and H_2O (40 mL) was added K_2CO_3 (6.42 g, 46.5 mmol) under a nitrogen atmosphere. The resulting mixture was stirred at room temperature for 6 days. After evaporation of the solvents, the residue was partitioned between CH_2Cl_2 and brine. The organic layer was dried, and concentrated to afford an oil, which was chromatographed on silica gel with ethyl acetate–MeOH (10:1) to give a crude solid. This solid was recrystallized from ethyl acetate–*i*-Pr_2O to give 2.46 g (57%) of **5** as white needles: EIMS m/z 371 (M^+); ^{1}H NMR ($CDCl_3$) δ 6.15 (1H, *d*, *J* = 3.1 Hz), 6.06 (1H, *d*, *J* = 3.1 Hz), 5.90 (1H, *br s*), 3.76 (2H, *s*), 3.71 (6H, *m*), 3.46 (2H, *s*), 2.81 (2H, *t*, *J* = 6.2 Hz), 2.40 (4H, *m*), 1.51 (6H, *m*); IR (KBr) 2200, 2176 (both CN) cm^{-1}; Calcd for $C_{19}H_{25}N_5OS$: C, 61.43%, H, 6.78%, N, 18.85%; Found C, 61.58%, H, 6.99%, N, 18.90%.

Method A-2. {1-[2-[[[5-(Piperidinomethyl)-2-furanyl]methyl]thio]ethyl]-3-methyl-2-imidazolidinylidene}propanedinitrile (6)

Step 1. To a stirred solution of **16** (8.0 g, 24.1 mmol) in DMF (100 mL) was added portionwise 60% NaH (1.16 g, 29 mmol) while cooling under ice. After the mixture was stirred at 4 °C for 30 min, MeI (1.8 mL, 28.9 mmol) was added. The resulting mixture was stirred at room temperature for a further 30 min. The solvent was evaporated to dryness and the resulting residue was partitioned between CH_2Cl_2 and brine. The organic layer was dried and concentrated to yield a crude solid of {1-[2-[(*p*-toluenesulfonyl)oxy]ethyl]-3-methyl-2-imidazolidinylidene}propanedinitrile (**17**; R = methyl). The crude solid was recrystallized from ethyl acetate–*i*-Pr_2O to give 4.68 g (56%) of a pure compound: mp 124–126 °C; ^{1}H NMR ($CDCl_3$) δ 7.74 (2H, *d*, *J* = 9.1 Hz), 7.31 (2H, *d*, *J* = 9.1 Hz), 4.27 (2H, *t*, *J* = 6.7 Hz), 3.73 (6H, *m*), 3.14 (3H, *s*), 2.43 (3H, *s*); Calcd for $C_{16}H_{18}N_4O_3S$: C, 55.48%, H, 5.24%, N, 16.17%; Found C, 55.59%, H, 5.37%, N, 15.93%.

Step 2. To a stirred solution of [5, 5'-bis(piperidinomethyl)]furfuryldisulfide **20** (1.5 g, 3.57 mmol) in EtOH (30 mL) was added dropwise a solution of $NaBH_4$ (0.3 g, 7.9 mmol) in EtOH (5 mL). After the resulting mixture was heated under reflux for 30 min, compound **17** (R = methyl: 2.2 g, 6.35 mmol) was added to the mixture. The resulting solution was further heated for 4 h, and then the solvent was evaporated to dryness. The residue was dissolved in CH_2Cl_2 and washed with diluted HCl, saturated aqueous $NaHCO_3$ solution and brine. The organic layer was dried and concentrated to give a crude oil, which was chromatographed on silica gel with $CHCl_3$–MeOH (30:1) to yield 2.01 g (82%) of **6** as a pale yellow solid. This was converted to the oxalate in acetone in the usual manner: EIMS m/z 385 (M^+); ^{1}H NMR ($CDCl_3$; free base form) δ 6.18 (1H, *d*, *J* = 3.3 Hz), 6.07 (1H, *d*, *J* = 3.3 Hz), 3.77 (2H, *s*), 3.64 (6H, *m*), 3.47 (2H, *s*), 3.18 (3H, *s*), 2.82 (2H, *t*, *J* = 6.8 Hz), 2.41 (4H, *m*), 1.52 (6H, *m*); IR (KBr) 2200, 2160 (both CN) cm^{-1}; Calcd for $C_{20}H_{27}N_5OS{\cdot}C_2H_2O_4$: C, 55.56%, H, 6.15%, N, 14.73%; Found C, 55.65%, H, 6.40%, N, 14.78%. Similarly, compounds **7**, **10** and **12**–**15** were obtained.

{1-[2-[[[5-(Piperidinomethyl)-2-furanyl]methyl]thio]ethyl]-3-ethyl-2-imidazolidinylidene}propanedinitrile (7). EIMS m/z 399 (M^+); ^{1}H NMR ($CDCl_3$; free base form) δ 6.19 (1H, *d*, *J* = 3.2 Hz), 6.09 (1H, *d*, *J* = 3.2 Hz), 3.77 (2H, *s*), 3.64 (6H, *m*), 3.59 (2H, *q*), 3.47 (2H, *s*), 2.82 (2H, *t*, *J* = 6.9 Hz), 2.43 (4H, *m*), 1.52 (6H, *m*), 1.26 (3H, *t*); IR (KBr) 2200, 2160 (both CN) cm^{-1}; Calcd for $C_{21}H_{29}N_5OS{\cdot}C_2H_2O_4$: C, 56.42%, H, 6.38%, N, 14.30%; Found C, 56.43%, H, 6.59%, N, 13.92%.

{1-[2-[[[5-(Piperidinomethyl)-2-furanyl]methyl]thio]ethyl]-3-[4-(2-methyl-2-butenyl)-2-imidazolidinylidene}propanedinitrile (10). EIMS m/z 439 (M^+); ^{1}H NMR ($CDCl_3$; free base form) δ 6.18 (1H, *d*, *J* = 3.1 Hz), 6.10 (1H, *d*, *J* = 3.1 Hz), 5.16 (1H, *m*), 4.12 (2H, *br d*, *J* = 6.1 Hz), 3.77 (2H, *s*), 3.56 (6H, *m*), 3.47 (2H, *s*), 2.82 (2H, *t*, *J* = 6.8 Hz), 2.39 (4H, *m*), 1.74 (6H, *br d*, *J* = 5.9 Hz), 1.48 (6H, *m*); IR (KBr) 2200, 2160 (both CN) cm^{-1}; Calcd for $C_{24}H_{33}N_5OS{\cdot}C_2H_2O_4$: C, 58.96%, H, 6.66%, N, 13.22%; Found C, 58.74%, H, 6.75%, N, 13.11%.

{1-[2-[[[5-(Piperidinomethyl)-2-furanyl]methyl]thio]ethyl]-3-benzyl-2-imidazolidinylidene}propanedinitrile (12). EIMS m/z 461 (M^+); ^{1}H NMR ($CDCl_3$; free base form) δ 7.37 (5H, *m*), 6.18 (1H, *d*, *J* = 3.1 Hz), 6.11 (1H, *d*, *J* = 3.1 Hz), 4.76 (2H, *s*), 3.78 (2H, *s*), 3.62 (6H, *m*), 3.46 (2H, *s*), 2.85 (2H, *t*, *J* = 6.9 Hz), 2.40 (4H, *m*), 1.49 (6H, *m*); IR (KBr) 2200, 2160 (both CN) cm^{-1}; Calcd for $C_{26}H_{31}N_5OS{\cdot}C_2H_2O_4$: C, 60.96%, H, 6.03%, N, 12.70%; Found C, 60.70%, H, 6.17%, N, 12.56%.

{1-[2-[[[5-(Piperidinomethyl)-2-furanyl]methyl]thio]-ethyl]-3-(4-fluorobenzyl)-2-imidazolidinylidene}propanedinitrile (13). EIMS m/z 479 (M^+); ^1H NMR ($CDCl_3$; free base form) δ 7.11 (4H, *m*), 6.16 (1H, *d*, *J* = 3.1 Hz), 6.03 (1H, *d*, *J* = 3.1 Hz), 4.74 (2H, *s*), 3.78 (2H, *s*), 3.61 (6H, *m*), 3.46 (2H, *s*), 2.86 (2H, *t*, *J* = 6.9 Hz), 2.38 (4H, *m*), 1.47 (6H, *m*); IR (KBr) 2200, 2160 (both CN) cm^{-1}; Calcd for $C_{26}H_{30}FN_5OS \cdot C_2H_2O_4$: C, 59.04%, H, 5.66%, N, 12.29%; Found C, 59.05%, H, 5.79%, N, 12.36%.

{1-[2-[[[5-(Piperidinomethyl)-2-furanyl]methyl]thio]-ethyl]-3-(3,4-dimethoxybenzyl)-2-imidazolidinylidene}-propanedinitrile (14). EIMS m/z 521 (M^+); ^1H NMR ($CDCl_3$; free base form) δ 6.85 (3H, *m*), 6.19 (1H, *d*, *J* = 3.3 Hz), 6.09 (1H, *d*, *J* = 3.3 Hz), 4.68 (2H, *s*), 3.87 (6H, *s*), 3.78 (2H, *s*), 3.64 (6H, *m*), 3.45 (2H, s), 2.83 (2H, *t*, *J* = 6.9 Hz), 2.39 (4H, m), 1.47 (6H, m); IR (KBr) 2200, 2160 (both CN) cm^{-1}; Calcd for $C_{28}H_{35}N_5O_3S \cdot C_2H_2O_4$: C, 58.90%, H, 6.10%, N, 11.45%; Found C, 58.61%, H, 6.25%, N, 11.23%.

{1-[2-[[[5-(Piperidinomethyl)-2-furanyl]methyl]thio]-ethyl]-3-(1-phenyl-1-propenyl)-2-imidazolidinylidene}-propanedinitrile (15). EIMS m/z 487 (M^+); ^1H NMR ($CDCl_3$; free base form) δ 7.29 (5H, *m*), 6.50 (1H, *d*, *J* = 15.2 Hz), 6.0–6.4 (3H, *m*), 4.28 (2H, *d*, *J* = 6.1 Hz), 3.76 (2H, *s*), 3.66 (6H, *m*), 3.44 (2H, *s*), 2.85 (2H, *t*, *J* = 6.8 Hz), 2.43 (4H, *m*), 1.54 (6H, *m*); IR (KBr) 2200, 2160 (both CN) cm^{-1}; Calcd for $C_{28}H_{33}N_5OS \cdot C_2H_2O_4$: C, 62.37%, H, 6.11%, N, 12.12%; Found C, 62.24%, H, 6.21%, N, 12.06%.

Method B. {1-[2-[[[5-(Piperidinomethyl)-2-furanyl]-methyl]thio]ethyl]-3-(2-propyl)-2-imidazolidinylidene}-propanedinitrile (8)

Step 1. A mixture of **21** (7.0 g, 41.1 mmol), *i*-propylamine (3.6 mL, 42.3 mmol) and $CHCl_3$ (100 mL) was stirred at room temperature for 1.5 h. The reaction mixture was concentrated to dryness, and the resulting crude solid was triturated with *i*-Pr_2O to yield 6.7 g (90%) of [(methylthio)(2-propylamino)methylene]-propanedinitrile (**22**: R = *i*-propyl) as a pale orange solid: mp 124–126 °C; ^1H NMR ($CDCl_3$) δ 7.74 (2H, *d*, *J* = 9.1 Hz), 7.31 (2H, *d*, *J* = 9.1 Hz), 4.27 (2H, *t*, *J* = 6.7 Hz), 3.73 (6H, *m*), 3.14 (3H, *s*), 2.43 (3H, *s*); Calcd for $C_8H_{11}N_3S$: C, 53.01%, H, 6.12%, N, 23.18%; Found C, 53.33%, H, 6.41%, N, 23.45%.

Step 2. A mixture of **22** (R = *i*-propyl: 6.5 g, 35.9 mmol) and ethanolamine (2.2 g, 36.0 mmol) was allowed to stand at 70 °C for 5 h under reduced pressure. After water was added to the reaction mixture, the resulting precipitates were collected by filtration, washed with EtOH to give 4.3 g (62%) of [[(2-hydroxy)ethylamino](2-propylamino)methylene]propanedinitrile (**23**: R = *i*-propyl): mp 124–126 °C; ^1H NMR (DMSO-d_6) δ 7.30 (1H, *br s*), 7.22 (1H, *br s*), 5.24 (1H, *br t*), 4.01 (1H, *m*), 3.57 (2H, *m*), 3.38 (2H, *m*), 1.26 (6H, *d*, *J* = 6.4 Hz); Calcd for $C_9H_{14}N_4O$: C, 55.65%, H, 7.27%, N, 28.84%; Found C, 55.39%, H, 7.34%, N, 29.01%.

Step 3. To a stirred solution of **23** (R = *i*-propyl: 4.25 g, 21.9 mmol) in anhydrous pyridine (50 mL) was added dropwise methanesulfonyl chloride (2.6 mL, 33.6 mmol) while cooling under ice. After the reaction mixture was stirred for 30 min, the solvent was evaporated. The resulting residue was dissolved in THF (100 mL) and DMF (10 mL). After the solution was cooled in an ice-bath, 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU; 5.0 g, 32.8 mmol) was added to the cooled solution. The mixture was stirred at 4 °C for 3 h, and then the solvent was evaporated to dryness. The resulting residue was dissolved in $CHCl_3$ and washed with diluted HCl, saturated aqueous $NaHCO_3$ solution and brine. The organic layer was dried and concentrated to yield a crude solid of [1-(2-propyl)-2-imidazolidinylidene] propanedinitrile (**24**: R = *i*-propyl). This was recrystallized from ethyl acetate–*i*-Pr_2O to afford 3.56 g (92%) of pure **24** as white needles: mp 124–126 °C; ^1H NMR ($CDCl_3$) δ 5.91 (1H, *br s*), 4.70 (1H, *m*), 3.63 (4H, *m*), 1.27 (6H, *d*, *J* = 6.5 Hz); Calcd for $C_9H_{12}N_4$: C, 61.34%, H, 6.86%, N, 31.79%; Found C, 61.06%, H, 6.59%, N, 31.66%.

Step 4. To a stirred solution of **24** (R = *i*-propyl: 3.18 g, 18.0 mmol) in THF (40 mL) was added portionwise 60% NaH (1.1 g, 27.5 mmol) under ice-cooling. After the reaction mixture was stirred at 4 °C for 0.5 h, 1-bromo-2-chloroethane (7.5 mL, 90.1 mmol) was added. The mixture was further stirred at refluxing temperature for 11 h. To the resulting mixture was added ice-water, and the solvent was evaporated. The resulting residue was partitioned between CH_2Cl_2 and brine. The organic layer was dried and concentrated to afford a crude solid, which was triturated with *i*-Pr_2O to give 4.07 g (94%) of [1-(2-chloroethyl)-3-(2-propyl)-2-imidazolidinylidene]propanedinitrile (**25**: R = *i*-propyl) as a white solid: mp 124–126 °C; ^1H NMR ($CDCl_3$) δ 4.63 (1H, *m*), 3.96 (4H, *m*), 3.68 (4H, *m*), 1.29 (6H, *d*, *J* = 6.4 Hz); Calcd for $C_{11}H_{15}ClN_4$: C, 55.34%, H, 6.33%, N, 23.47%; Found C, 55.42%, H, 6.35%, N, 23.61%.

Compound **8** was obtained in 93% yield by use of *step 2* in *method A-2* in a manner similar to the synthesis of compound **6**. Free base of compound **8** was converted to the oxalate in acetone in the usual manner: EIMS m/z 413 (M^+); ^1H NMR ($CDCl_3$; free base form) δ 6.18 (1H, *d*, *J* = 3.1 Hz), 6.10 (1H, *d*, *J* = 3.1 Hz), 4.60 (1H, *m*), 3.77 (2H, *s*), 3.59 (6H, *m*), 3.47 (2H, *s*), 2.82 (2H, *t*, *J* = 6.8 Hz), 2.36 (4H, *m*), 1.48 (6H, *m*), 1.24 (6H, *d*, *J* = 6.6 Hz); IR (KBr) 2200, 2165 (both CN) cm^{-1}; Calcd for $C_{22}H_{31}N_5OS \cdot C_2H_2O_4$: C, 57.24%, H, 6.60%, N, 13.91%; Found C, 57.19%, H, 6.58%, N, 13.62%. Similarly, compounds **9** and **11** were synthesized.

{1-[2-[[[5-(Piperidinomethyl)-2-furanyl]methyl]thio]-ethyl]-3-(2-methyl-1-propyl)-2-imidazolidinylidene}propanedinitrile (9). EIMS m/z 388 (M^+); ^1H NMR ($CDCl_3$; free base form) δ 6.18 (1H, *d*, *J* = 3.2 Hz), 6.10 (1H, *d*, *J* = 3.2 Hz), 3.77 (2H, *s*), 3.63 (6H, *m*), 3.47 (2H, *s*), 3.35 (2H, *d*, *J* = 7.5 Hz), 2.83 (2H, *t*, *J* = 6.6 Hz), 2.44 (4H, *m*), 2.05 (1H, *m*), 1.51 (6H, *m*), 0.97 (6H, *d*, *J* = 6.6 Hz); IR (KBr) 2200, 2160 (both CN) cm^{-1}; Calcd

for $C_{23}H_{33}N_5OS \cdot C_2H_2O_4$: C, 58.01%, H, 6.82%, N, 13.53%; Found C, 57.73%, H, 6.84%, N, 13.35%.

[1-[2-[[[5-(Piperidinomethyl)-2-furanyl]methyl]thio]-ethyl]-3-cyclohexylmethyl-2-imidazolidinylidene]propanedinitrile (11). EIMS m/z 467 (M^+); ^1H NMR ($CDCl_3$; free base form) δ 6.19 (1H, *d*, *J* = 2.9 Hz), 6.10 (1H, *d*, *J* = 2.9 Hz), 3.77 (2H, *s*), 3.63 (6H, *m*), 3.47 (2H, *s*), 3.35 (2H, *d*, *J* = 7.3 Hz), 2.83 (2H, *t*, *J* = 6.9 Hz), 2.42 (4H, *m*), 1.0–2.0 (17H, *m*); IR (KBr) 2200, 2160 (both CN) cm^{-1}; Calcd for $C_{26}H_{37}N_5OS \cdot C_2H_2O_4$: C, 60.30%, H, 7.05%, N, 12.56%; Found C, 60.40%, H, 7.01%, N, 12.49%.

Pharmacological methods

Inhibition of acetylcholinesterase. AChE inhibitory activity was measured at 25 °C and pH 8.0 by the photometric method of Ellman *et al.*[21] using acetylthiocholine (ATCh) as a substrate. In the standard procedure, to 50 μL aliquots of rat brain AChE (equal to 2.5 mg wet tissue) in 0.1 M potassium phosphate buffer (pH 8.0, 2.65 mL) was added 0.1 mL of 5,5'-dithiobis(2-nitrobenzoic acid) (DTNB) in a buffer (final concentration; 0.3 mM). A volume of 0.1 mL of inhibitor in buffer or buffer alone was then added to the enzyme. The samples were preincubated at 25 °C for 5 min prior to the addition of 0.1 mL of ATCh to start the hydrolysis. The variations in optical absorbance at 412 nm were measured at 60 s intervals for 5 min by means of a Hitachi U-3210 spectrophotometer.

Gastrointestinal motility enhancing sctivity in vitro*: effect on electrically evoked contractions.* The ileum was isolated from male Hartley guinea pigs, weighing 250–400 g. Ileal strips, 20–30 mm long, were suspended vertically in an organ bath containing warmed Tyrode's solution (37 ± 1 °C), and a gaseous mixture of 95% O_2 and 5% CO_2 was passed through the solution. The muscle strips were initially loaded at 1 g tension and their mechanical activities were measured by isotonic transducer (Nihon Kohden). To excite the neuronal components in the intestinal wall, the preparations were stimulated electrically by single rectangular pulses (1 msec duration, 0.1 Hz frequency, supramaximal voltage) through a pair of platinum electrodes. The electrical stimulation caused the reproducible twitch response which was abolished by tetrodotoxin and atropine, indicating the cholinergic nature of the contraction. After a stabilization of the twitch response (30 min), the test compounds were dissolved or suspended in physiological saline and were added cumulatively to the organ bath, and the effects on the electrically induced contraction were examined. The activity of the test compounds were represented as EC_{30} values i.e., the concentration of the compounds producing 30% potentiation of electrically induced contraction.

Histamine H_2-receptor binding assay. The test compounds at concentrations of between 10^{-5} and 10^{-4} M were tested in binding assays using guinea pig cerebral cortex for competition with 2 nM [^{3}H]tiotidine.[22] Nonspecific binding was determined by the addition of 5 mM histamine. Samples were incubated at 25 °C for 30 min. The assay was terminated by rapid filtration through Whatman GF/C glass-filters under reduced pressure. The filters were washed three times with 5 mL of 50 mM sodium–potassium phosphate buffer (pH 7.4) and transferred to scintillation vials with Scintisol EX-H. The radioactivities in the filters were counted using a Packard 2200CA scintillation counter.

Gastrointestinal motility enhancing activity in vivo*: effect of compounds 12 and 13 on gastrointestinal motility in anesthetized rabbits.* Male rabbits (Japan White strain), weighing 2.3–3.3 kg, were anesthetized with urethane (1.3 g kg^{-1}, ip). After anesthetization, the trachea was cannulated to preserve the respiration. Polyethylene cannulas were inserted into the left carotid artery for measurement of the systemic blood pressure through a pressure transducer and into the right ear vein for the systemic administration of drugs. The abdominal cavity was opened by midline incision, and the gastric antrum and the descending colon were exposed. To measure the gastrointestinal motility, intraluminal pressure changes were detected by rubber balloons, inserted in the gastric antrum and the descending colon. Each balloon was filled with distilled water (water pressure applied to the balloon was usually set at 10 cm H_2O) and connected to a pressure transducer equipped with an ink-writing polygraph. After the operation procedures were complete, the animals were allowed to equilibrate for 60 min at which time steady contractile activity and blood pressure were established. Following this, a dose of 1 mg kg^{-1} of each compound (**12** and **13**) was dissolved in physiological saline and administered intravenously into an ear vein at 60 min intervals and the effects on the gastrointestinal motility and blood pressure were examined and monitored, respectively.

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References

1. Scarpignato, C. In: *Drugs in Gastroenterology*; Chapter 1, Braga, P. C.; Gusland, M.; Tittobello, A., Eds; Ravan Press; New York, 1991.

2. Gidda, J. S.; Monkovic, I. *Annu. Rep. Med. Chem.* **1985**, *20*, 117.

3. King, F. D.; Sanger, G. J. *Annu. Rep. Med. Chem.* **1988**, *23*, 201.

4. Brogden, R. N.; Carmine, A. A.; Heel, R. C.; Speight, T. M.; Avery, G. S. *Drugs* **1982**, *24*, 360.

5. Albibi, R.; McCallum, R. W. *Ann. Intern. Med.* **1983**, *98*, 86.

6. Bertaccini, G.; Scarpignato, C. *Br. J. Pharmacol.* **1982**, *77*, 443.

7. Bertaccini, G.; Poli, E.; Adami, M.; Coruzzi, G. *Agents and Actions* **1983**, *13*, 157.

8. Fioramonti, J.; Soldani, G.; Honde, G.; Bueno, L. *Agents and Actions* **1984**, *15*, 260.

9. Bortolotti, M.; Cucchiara, S.; Brunelli, F.; Sarti, P.; Samimi, M.; Mazza, M.; Del Campo, L.; Barbara, L. *Gastroenterology* **1992**, *102*, Part 2, A428.

10. Hansen, W. E.; Bertl, S. *The Lancet* **1983**, *29*, 235.

11. Galli, A.; Mantovani, P.; Pepeu, G. *Biochem. Pharmacol.* **1984**, *33*, 1845.

12. Kounenis, G.; Koutsoviti-Paradopolou, M.; Elezoglou, V. *J. Pharmacobio. Dyn.* **1986**, *9*, 941.

13. Mehta, S. M.; Bhalara, D. D.; Goyal, R. K. *Agents and Actions* **1987**, *21*, 38.

14. Bertaccini, G.; Coruzzi, G. *Agents and Actions* **1982**, *12*, 168.

15. Yoshida, N.; Karasawa, T.; Kadokawa, T. *Arch. int. Pharmacodyn.* **1988**, *295*, 245.

16. Poli, E.; Coruzzi, G.; Bertaccini, G. *Agents and Actions* **1990**, *30*, 191.

17. Sasho, S.; Obase, H.; Ichikawa, S.; Kitazawa, T.; Nonaka, H.; Yoshizaki, R.; Ishii, A.; Shuto, K. *J. Med. Chem.* **1993**, *36*, 572.

18. Sasho, S.; Obase, H.; Ichikawa, S.; Yoshizaki, R.; Ishii, A.; Shuto, K. *Bioorg. Med. Chem. Lett.*. **1994**, *4*, 615.

19. Sasho, S.; Obase, H.; Harakawa, H.; Ichikawa, S.; Kitazawa, T.; Kishibayashi, N.; Yokoyama, T.; Nonaka, H.; Yoshizaki, R.; Ishii, A.; Shuto, K. *Bioorg. Med. Chem.* **1994**, *2*, 1107.

20. Price, B. J.; Clitherow, J. W.; Bradshaw, J. Ger. Offen. 2 734 070, 1976; *Chem. Abstr.* **1978**, *88*, 190580b, 741.

21. Ellman, G. L.; Courtney, K. D.; Andres Jr, V.; Featherstone, R. M. *Biochem. Pharmacol.* **1961**, *7*, 88.

22. Gajtkowski, G. A.; Norris, D. B.; Rising, T. J.; Wood, T. P. *Nature* **1983**, *304*, 65.

23. For a recent review of histamine H_2-receptor antagonists, see: Bauer, R. F.; Collins, P. W.; Jones, P. H. *Annu. Rep. Med. Chem.* **1987**, *22*, 191.

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