Bioorganic & Medicinal Chemistry Letters 19 (2009) 3426–3429

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

journal homepage: www.elsevier.com/locate/bmcl

Discovery of novel motilin antagonists: Conversion of tetrapeptide leads to orally available peptidomimetics

Naoki Taka [a,*], Hiroharu Matsuoka [a], Tsutomu Sato [a], Hitoshi Yoshino [a], Ikuhiro Imaoka [a], Haruhiko Sato [a], Ken-ichiro Kotake [b], Yoshikazu Kumagai [b], Kenshi Kamei [c], Ken-ichi Ozaki [d], Atsuko Higashida [e], Toshio Kuroki [f]

[a] Chemistry Research Dept. 1, Fuji-Gotemba Research Laboratories, Chugai Pharmaceutical Co. Ltd, 1-135 Komakado Gotemba City, Shizuoka 412-8513, Japan
[b] Pharmaceutical Technology Dept., Fuji-Gotemba Research Laboratories, Chugai Pharmaceutical Co. Ltd, 1-135 Komakado Gotemba City, Shizuoka 412-8513, Japan
[c] CMC Planning and Coordination Dept., Fuji-Gotemba Research Laboratories, Chugai Pharmaceutical Co. Ltd, 1-135 Komakado Gotemba City, Shizuoka 412-8513, Japan
[d] Pharmaceutical Research Dept. 1, Fuji-Gotemba Research Laboratories, Chugai Pharmaceutical Co. Ltd, 1-135 Komakado Gotemba City, Shizuoka 412-8513, Japan
[e] Discovery Platform Technology Dept., Fuji-Gotemba Research Laboratories, Chugai Pharmaceutical Co. Ltd, 1-135 Komakado Gotemba City, Shizuoka 412-8513, Japan
[f] Research Planning and Coordination Dept., Fuji-Gotemba Research Laboratories, Chugai Pharmaceutical Co. Ltd, 1-135 Komakado Gotemba City, Shizuoka 412-8513, Japan

ARTICLE INFO

Article history:
Received 28 November 2008
Revised 7 May 2009
Accepted 11 May 2009
Available online 20 May 2009

Keywords:
Motilin
Motilin receptor antagonist
GM-109
Peptidomimetics

ABSTRACT

We successfully discovered peptidomimetic motilin antagonists (**17c** and **17d**) through the improvement of physicochemical properties of a tetrapeptide antagonist (**2**). Furthermore, with oral administration and based on motilin antagonistic activity, both compounds suppressed motilin-induced colonic and gastric motility in conscious dogs.

Irritable bowel syndrome (IBS) is a gastrointestinal (GI) disorder characterized by altered bowel function and abdominal pain in the absence of detectable structural abnormalities[1] A variety of drugs have been used to alleviate the major manifestations of IBS; however, no single drug has proven consistently effective in treating IBS. Motilin, a 22-amino acid peptide intestinal hormone, has been reported to stimulate contractile activity of the gastrointestinal tract and to have some clinical relevance to some gastrointestinal diseases, such as IBS[2] and functional dyspepsia (FD).[3] Taking advantage of this finding, we started motilin derivatization. Previously, we reported a macrocyclic motilin agonist, GM-611 (**1**) (Chart 1),[4] which is currently under clinical study in the US as an anti-diabetic gastroparesis. Our next focus is the discovery of motilin antagonists,[5] with an anti-IBS or FD agent as the target. We have developed an SAR on the peptidic motilin antagonist and identified critical amino acid residues for the interaction with a motilin receptor. From this research, a cyclic tetrapeptide, GM-109 (**2**) has been identified as a potent motilin antagonist.[6] However, **2** had a poor PK profile, an oral bioavailability of 0%, and did not exhibit any pharmacological effects after oral administration. Recently, non-peptide motilin antagonist **3** was disclosed.[7] However; there have been no reports of the oral activities of motilin antagonists to date. Here we report on the chemical modifica-

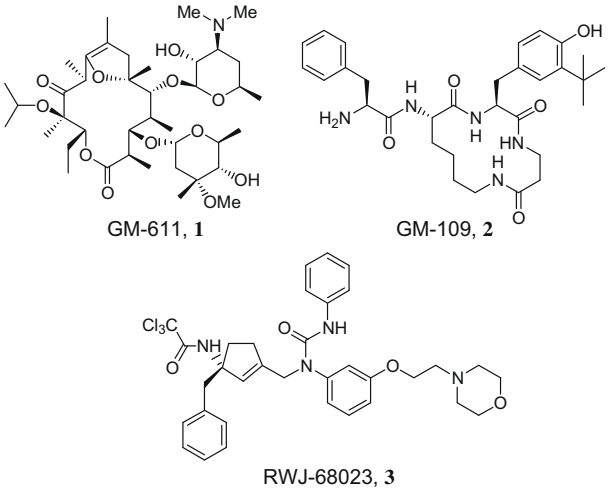


Chart 1. Structures of motilin agonist and antagonist.

* Corresponding author.
E-mail address: takanok@chugai-pharm.co.jp (N. Taka).

0960-894X/$ - see front matter © 2009 Published by Elsevier Ltd.
doi:10.1016/j.bmcl.2009.05.059

tions of GM-109 resulting in orally active agents and their biological evaluations.

In order to convert **2** to an oral agent, the physicochemical drawbacks, such as high MW, too many hydrogen bonding acceptors/donors, and metabolically unstable peptide bonds, need to be improved.[8]

We first focused on downsizing the molecular weight of **2**. We assumed that the macrocyclic backbone of **2** was unnecessary for motilin receptor binding because the Phe and Thy(*t*-Bu) residues are thought to play a critical role in motilin receptor binding and that the backbone plays an important role in maintaining the active conformation of the two resides. Therefore, we first prepared tripeptide derivatives without the backbone.

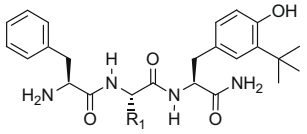
As shown in Scheme 1, Fmoc-Thy(*t*-Bu) **6** was initially prepared from Thy-OMe **4**. The reaction of **4** with *tert*-butyl acetate in the presence of $HClO_4$ gave **5** in moderate yield. Hydrolysis of **5** and subsequent Fmoc protection afforded Fmoc-Thy(*t*-Bu) **6** in 61% yield in two steps. The tripeptide **9** series was synthesized using a solid-phase reaction method. Fmoc-resin was de-protected by piperidine, which was then coupled with Thy(*t*-Bu) **6,** using BOP, to afford **7**. Next, **7** was de-protected and coupled with Fmoc-protected amino acid (AA) to give di-peptide **8**. Coupling dipeptide **8** with N-terminal AA and deprotection gave tripeptide **9**.

The results of motilin receptor binding activities and motilin antagonistic activities of **9a–f** are shown in Table 1.

Although **9a**, of which central AA is Gly, did not exhibit motilin receptor binding activity, **9b–f** showed various extents of antagonistic activities. Importantly, **9c**, **9d, 9e**, and **9f** exhibited motilin antagonistic activity comparable to that of **2** (GM-109). The data suggested that the relatively large R_1 group kept the tripeptide in active conformation and supports our hypothesis.

Next we focused on stabilization against metabolism through the modification of **9c**, which has the smallest molecular weight among the potent antagonists **9c–f**. Stabilization included design and synthesize derivatives bearing cleavage-resistant amide bonds

Table 1
Results of motilin receptor binding and motilin antagonistic action assay for **9**

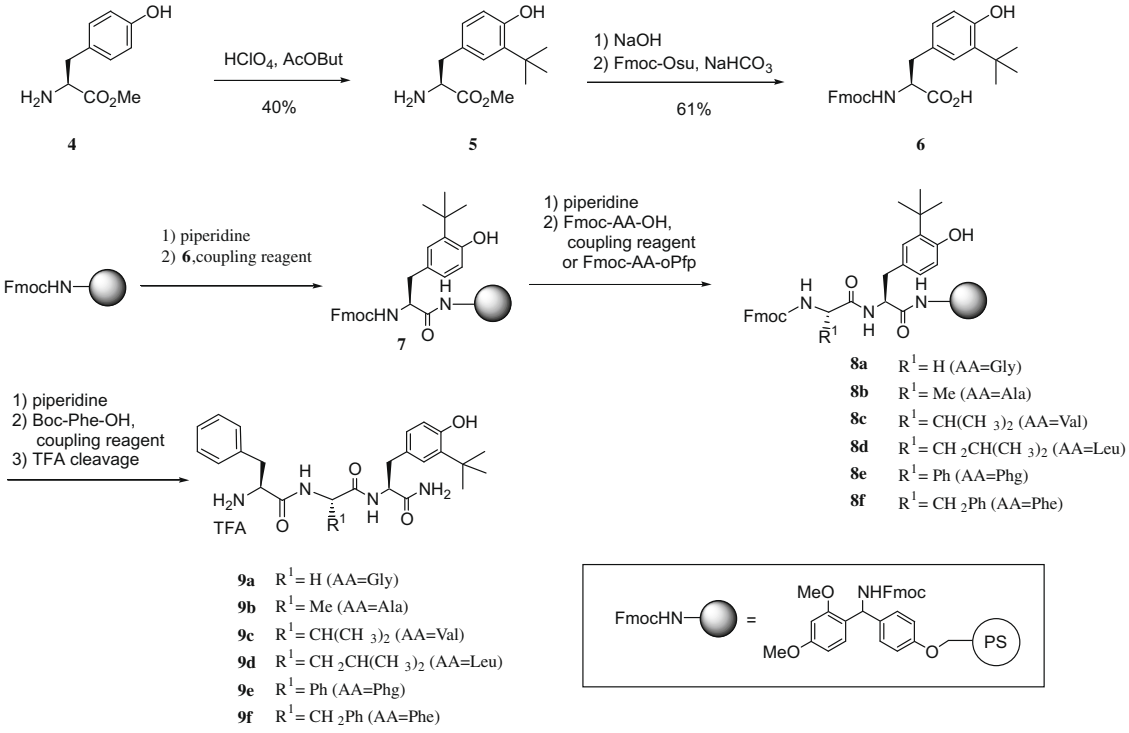


Compound	R^1	IC_{50}[a] (nM)	pA_2[b]
9a	H	—	—
9b	CH_3	185	<6
9c	$CH(CH_3)_2$	46	6.9
9d	$CH_2CH(CH_3)_2$	27	7.3
9e	Ph	12	7.8
9f	CH_2Ph	6	7.2
GM-109		12	7.4

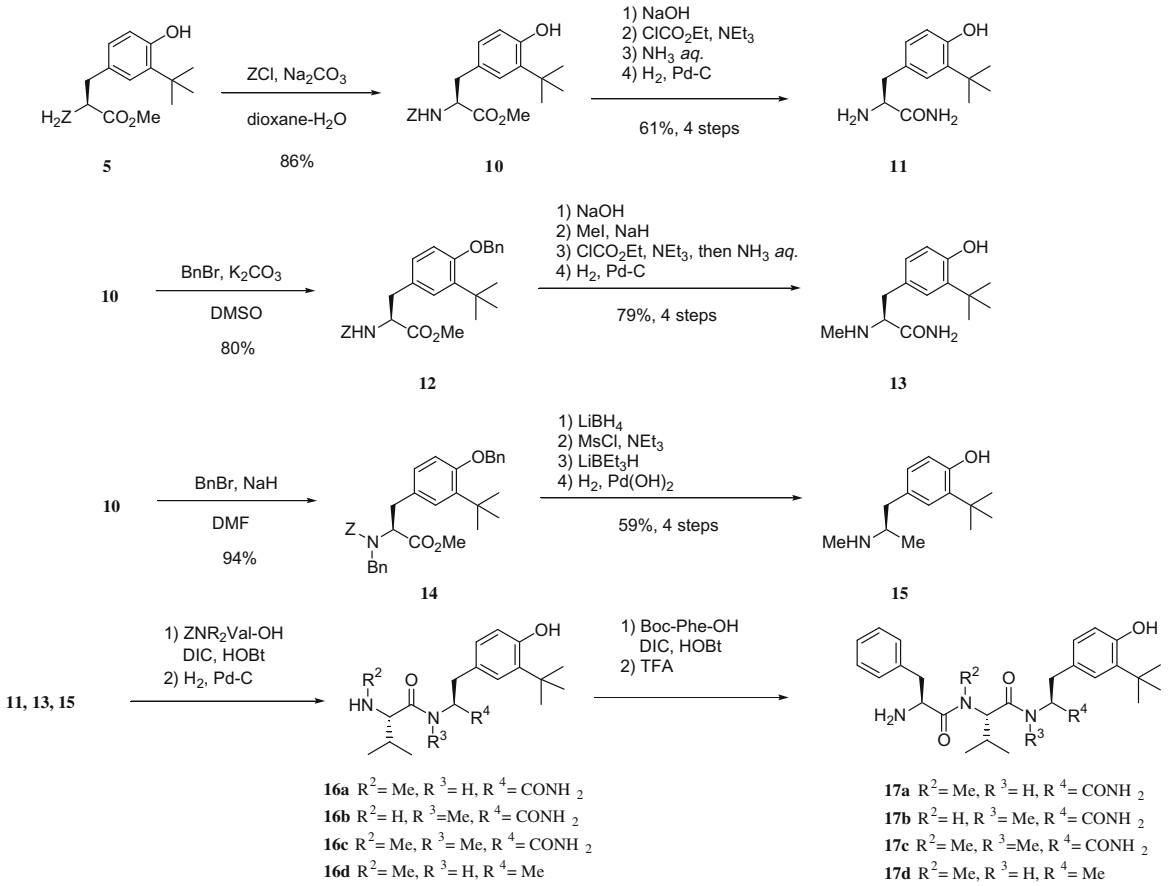
[a] IC_{50}, expressing MTL receptor binding activity, was the concentration necessary to displace 50% of the binding of ^{125}I-motilin (rabbit duodenum homogenate).[9]
[b] pA_2, expressing MTL antagonist activity, is the negative logarithm of the molar concentration of compound causing a twofold shift in the concentration–response curve for motilin (rabbit duodenum specimen).[10]

by means of N-methylation at amide bond or replacement of terminal tyrosine moiety with non-amino acid forms, **17a–d**. Syntheses of target molecules **17a–d** are outlined in Scheme 2.

An amide intermediate **11** was prepared from tyrosine derivative **5**. First, Z-protection of Thy(*t*-Bu) **5** gave **10**, and amidation of **10** using a mixed anhydride method and deprotection gave the amide **11**. Meanwhile, *N*-methyl tyrosine intermediate **13** was prepared from **10**. Initially, the phenol moiety of **10** was protected by the benzyl group to give ether **12**. Subsequent hydrolysis of **12** afforded a carboxylic acid, which was then converted to the corresponding *N*-methyl amide from treatment of MeI and NaH. During this methylation, no methyl ester was obtained. Next, amidation of the *N*-methyl amide followed by deprotection by Pd/C gave N-methylated Thy(*t*-Bu)-NH_2 **13.** Phenethyl amine **15** was



Scheme 1.



Scheme 2.

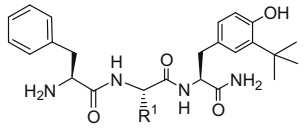
prepared as follows. Double benzyl protection of **10** obtained **14** in 94% yield. Reduction of the ester group using lithium borohydride gave the corresponding alcohol, which was methansulfonylated and converted into a methyl group using Super-Hydride®. Deprotection of the benzyl group afforded **15** in 59% yield in four steps. Coupling of each intermediates **11**, **13**, and **15** with Z-protected valine derivative in the presence of HOBT and DIC afforded dipeptide derivatives, then Z group of the dipeptides were removed by Pd/C–H_2 to give **16a-d**. Finally, **16a–d** were coupled with Boc-Phe and the following deprotection yielded the target compounds **17a–d**.

The results of the motilin receptor binding activities and motilin antagonistic activities are shown in Table 2.

Compounds **17a–d** showed potent motilin binding activity and motilin antagonistic activity. In particular, **17c** and **17d**, bearing a phenetyl moiety, were the potent antagonists, with more potent motilin antagonistic activity in pA_2 value than that of GM-109. Although **17**a was the most potent binder, its antagonistic activity was relatively weaker, compared with that of **17d**. This reduced activity may have been due to insufficient metabolic stability in the ex vivo assay.

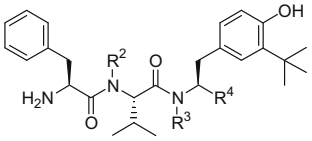
Compounds **17a–d** were further evaluated on metabolic stability and permeability, using rat liver S-9 fraction and Caco-2 cells, respectively (Table 3). Compound **17a**, which had an N-methylated amide bond at the valine moiety, showed considerable metabolic stability, but it did not show permeability. Interestingly, **17b**, a mono-methylated derivative like **17a** but showed neither the stability nor permeability, suggesting a methylation site is critical

Table 2
Results of motilin receptor binding and motilin antagonistic action assay for **9c** and **17**



Compound	R^2	R^3	R^4	IC_{50} (nM)	pA_2
9c	H	H	$CONH_2$	46	6.9
17a	Me	H	$CONH_2$	1.1	7.9
17b	H	Me	$CONH_2$	24	7.2
17c	Me	Me	$CONH_2$	4.3	8.6
17d	Me	H	Me	1.9	8.4
GM-109				12	7.4

Table 3
Metabolic stability and permeability of **17a–d**



Compd.	R^2	R^3	R^4	Metabolic stability[a]	Permeability[b]	BA (%)[c]
17a	Me	H	$CONH_2$	69	0	6
17b	H	Me	$CONH_2$	7	0	n.t.
17c	Me	Me	$CONH_2$	80	2.31	30
17d	Me	H	Me	100	12.1	65

[a] Metabolic stability was expressed as residual % value using rat liver S-9 after incubation for 30 min at 37 °C.
[b] Permeability was expressed as apparent permeability value (Papp: 10^{-6} cm/s.) using Caco-2 cells (apical pH 6.8).
[c] BA was expressed as bioavailability in rat at 10 mg/kg.

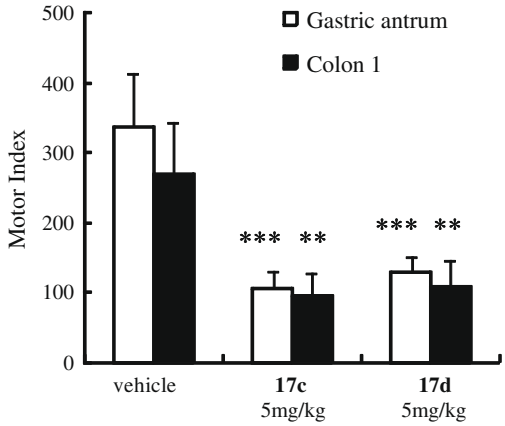


Figure 1. In vivo MTL antagonistic activities of **17c** and **17d.**[10] Each column represents the mean ± SE from 6 dogs, $^{**}P < 0.01$ and $^{***}P < 0.001$ compared with the vehicle group by student's *t*-test.

for stability. Importantly, **17c** and **17d** showed satisfactory stability and permeability. The drastic improvements were thought to be due to the conversion of the peptide bond to the usual amide bond and a reduction in hydrogen bonding donors/acceptors. In a rat PK study, oral bioavailability results for **17c** and **17d** were 30% and 65%, respectively. These findings encouraged us to conduct an in vivo pharmacological study with oral administration. Figure 1 shows the results of the pharmacological evaluation of **17c** and **17d** in dog.

Compounds **17c** and **17d** orally suppressed motilin-induced colonic and gastric motility in conscious dogs. The data strongly suggest that **17c** and **17d** exhibit motilin receptor antagonist behavior and have the desired profile of a candidate anti-IBS or anti-FD drug.

In summary, we successfully modified the peptidic antagonist GM109 and generated peptidomimetic antagonists **17c** and **17d**. We confirmed that N-methylation at the peptide bond and replacement of the amino acid with a non-amino acid, such as a phenetyl group, effectively improved the ADME properties. Compounds **17c** and **17d** exhibited good bioavailability, and thus potent antagonists even in vivo.

References and notes

1. Camilleri, M. *Pharmaocogenomics* **2005**, *6*, 491.
2. (a) Fukudo, S.; Suzuki, J. *Tohoku J. Exp. Med.* **1987**, *151*, 373; (b) Sjolund, K.; Ekman, R.; Lindgren, S.; Rehfeld, J. F. *Scan. J. Gastroenterol.* **1996**, *31*, 1110; (c) Simren, M.; Abrahamsson, H.; Bjornsson, E. S. *Gut* **2001**, *48*, 20; (d) Simren, M.; Bjornsson, E. S.; Abrahamsson, H. *Neurogastroenterol. Motil.* **2005**, *17*, 51; (e) Chovet, M. *Curr. Opin. Chem. Biol.* **2000**, *4*, 428.
3. (a) Malagelada, J. R. *Scan. J. Gastroenterol. Suppl.* **1991**, *182*, 29; (b) Kamerling, I. M. C.; Van Haarst, A. D.; Burggraaf, J.; deKam, M.; Biemond, I.; Jones, R.; Cohen, A. F.; Masclee, A. A. M. *Dig. Dis. Sci.* **2002**, *47*, 1732; (c) Cuomo, R.; Vandaele, P.; Coulie, B.; Peeters, T. *Am. J. Gastroenterol.* **2005**, *100*, 1.
4. (a) Koga, H.; Takanashi, H.; Itoh, Z.; Omura, S. *Drug Future* **2002**, *27*, 255; (b) Haramura, M.; Okamachi, A.; Tsuzuki, K.; Yogo, K.; Ikuta, M.; Kozono, T.; Takanashi, H.; Murayama, E. *Chem. Pharm. Bull.* **2001**, *49*, 40; (c) Peeters, T. L. *Curr. Opin. Investig. Drugs* **2001**, *2*, 555.
5. (a) Takanashi, H.; Yogo, K.; Ozaki, K.; Ikuta, M.; Akima, M.; Koga, H.; Nabata, H. *J. Pharmacol. Exp. Ther.* **1995**, *273*, 624; (b) Marsault, E.; Hoveyda, H. R.; Peterson, M. L.; Saint-Louis, C.; Landry, A.; Vezina, M.; Ouellet, L.; Wang, Z.; Ramaseshan, M.; Beaubien, S.; Benakli, K.; Beauchemin, S.; Deziel, R.; Peeters, T. L.; Fraser, G. L. *J. Med. Chem.* **2006**, *49*, 7190; (c) Peeters, T. L.; Depoortere, I.; Macielag, M. J.; Dharanipragara, R.; Marvin, M. S.; Florance, J. R.; Galdes, A. *Biochem. Biophys. Res. Commun.* **1994**, *198*, 411; (d) Poitras, P.; Miller, P.; Gagnon, D.; St-Pierre, S. *Biochem. Biophys. Res. Commun.* **1994**, *205*, 449.
6. Takanashi, H.; Yogo, K.; Ozaki, K.; Ikuta, M.; Akima, M.; Koga, H.; Nabata, H. *J. Pharmacol. Exp. Ther.* **1995**, *273*, 624.
7. (a) Kamerling, L. M. C.; van Haarst, A. D.; burggraaf, J.; Schoemaker, R. C.; De Kam, M. L.; Heinzerling, H.; Cohen, A. F.; Masclee, A. A. *Br. J. Clin. Pharmacol.* **2004**, *57*, 393; (b) Beavers, M. P.; Gunnet, J. W.; Hageman, W.; Miller, W.; Moore, J. B.; Zhou, L.; Chen, R. H. K.; Xiang, A.; Urbanski, M.; Combs, D. W.; Mayo, K. H.; Demarest, K. Ts. *Drug Des. Discovery* **2001**, *17*, 243; (c) Johnson, S. G.; Gunnet, J. W.; Moore, J. B.; Miller, W.; Wines, P.; Rivero, R. A.; Combs, D.; Demarest, K. T. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 3362.
8. Lipinski, C. A.; Lombardo, F.; Dominy, B. W.; Feeney, P. J. *Adv. Drug Delivery Rev.* **1997**, *23*, 3.
9. Motilin receptor-binding assay was performed according to the procedure introduced by Depoortere et al. with a slight modification. A portion of the colonic smooth muscle homogenate was incubated at 25 °C with 25 pM [^{125}I] motilin. After incubation for 120 min, the reaction was stopped by adding 2 mL of ice-cold buffer. Bound and free ligands were separated by centrifugation at 1500*g* for 5 min. The pellet was then washed with ice-cold buffer, and radioactivity determined using a gamma counter. IC_{50}, determined as the concentration to displace 50% of the binding of ^{125}I-motilin, expressed the MTL receptor binding activity. Rabbit upper small intestine longitudinal muscle strips were mounted in an organ bath containing 10 mL of modified Krebs' solution kept at 28 °C to prevent excessive spontaneous contraction, the solution gassed with a mixture of 95% O_2 and 5% CO_2, and each strip loaded with a 1.0 g weight. Before each experiment, the strips were repeatedly stimulated with 100 μM acetylcholine until a reproducible response was obtained. Contractile activity was measured using an isotonic transducer and recorded using a pen recorder while increasing the concentration of motilin (0.1–1000 nM) in the bath solution. Next, the same experiment was repeated with compounds added in the bath solution 15 min before addition of the motilin was started. EC_{50} was defined as the molar concentration of compound causing a twofold shift to the right of the motilin concentration–response curve.
10. In the interdigestive state, **17c** or **17d** (3 or 10 mg/kg) or vehicle (3% gum arabic) was administered into the stomach via the chronically implanted silicon tube approximately 15 min after the end of the phase III contractions of the MMC in the gastric antrum. Thirty minutes later, motilin (3 μg/kg) was intravenously administered into the vena cava via the silicon tube. Quantitative analysis of gastric and colonic contractile activities was performed by calculating the absolute motility indices from the area between the contractile wave and the baseline. The motility index induced by intravenous motilin injection (3 μg/kg) was designated as 100% (control) in each animal, and values obtained in the presence of **17c**, **17d**, or vehicle were calculated as a percentage of the control.