Drugs of the Future 2002, 27(3): 255-272

CCC: 0377-8282/2002

Review Article

Design, SAR and pharmacology of GM-611, the first acid-stable nonpeptide motilin receptor agonist

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Introduction

Pathological symptoms of the digestive tract, such as heartburn, loss of appetite, eructation, nausea, vomiting, epigastric pain and perceived gastric swelling, are referred to as indefinite epigastric complaints. These complaints have been suggested to be caused by motility disorders in the upper digestive tract (esophagus, stomach and small intestine). Stomach ulcers, duodenal ulcers and stomach surgery are well-known causes of peristaltic abnormalities in the upper digestive tract, but these problems have also been observed with many other types of illness. In particular, attention has recently focused on a disorder termed nonulcer dyspepsias (NUDs). NUDs are symptoms in which indefinite epigastric complaints occur, despite the absence of identifiable organic illness; no signs of digestive tract disease sufficient to account for the symptoms can be found, in spite of detailed examinations. In recent years, methods for measuring motility in the digestive tract have been developed, and peristaltic abnormalities in the upper digestive tract have also been observed in NUD patients. As a result, it has become clear that the main cause of indefinite epigastric complaints in NUDs is the delay in gastric emptying after eating. Furthermore, peristaltic abnormalities have been widely observed in diabetics and are thought to be due to a decrease in the rate of stomach emptying caused by peripheral and autonomic nerve disorders. In diabetes, these problems can result in digestive tract complications which are a major concern.

Drugs for treating the above symptoms are termed gastroprokinetic agents (1). These drugs stimulate gastric motility, reducing the delay in gastric emptying and thus improving the symptoms. The prokinetic agents currently on the market can be classified into two main groups on the basis of their mechanism of action, namely, serotonin agonists such as cisapride, and dopamine antagonists such as metoclopramide and domperidone. These drugs are widely used for treating abnormalities of the digestive tract, especially indefinite epigastric complaints caused by decreased peristalsis. However, dopamine antagonists have the disadvantage of causing neuroendocrine side effects and/or extrapyramidal dyskinetic reactions (seen especially after metoclopramide). Cisapride has also been reported to have a potential risk of causing fatal arrhythmia, especially when used in combination with certain other medications or by patients with particular underlying medical conditions (2). Furthermore, peristalsis stimulated by these drugs is different from naturally occurring peristalsis, which propagates from the upper to the lower part of the digestive tract. As a result of this difference, side effects such as diarrhea and vomiting frequently occur.

Motilin is a gastrointestinal peptide hormone whose physiological role in humans is the induction of well-coordinated contractions throughout the gastrointestinal tract in the fasted state, namely, interdigestive migrating contractions. Exogenous motilin increases gastrointestinal motility in animals, including humans, thus improving the

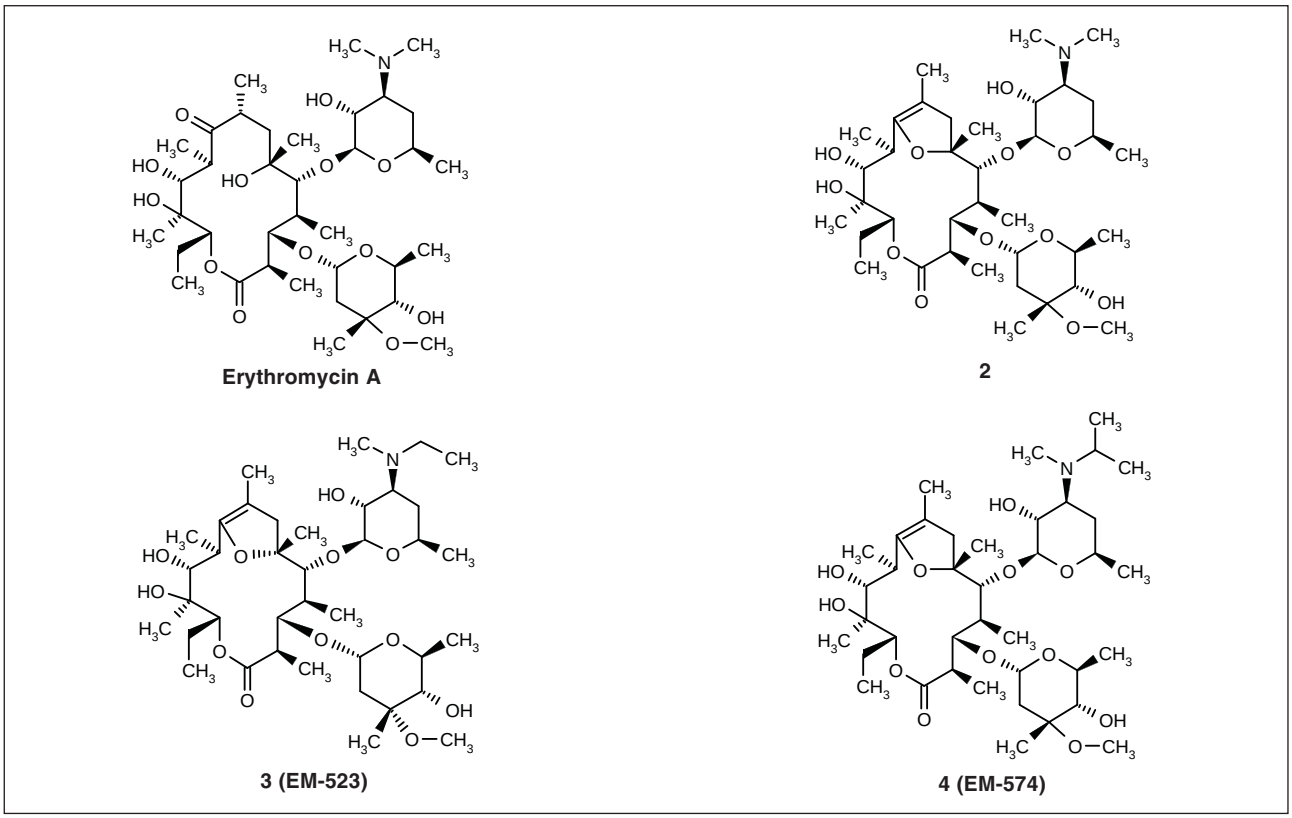


Fig. 1. Erythromycin A and the enol ether derivatives **2-4** having more potent prokinetic activity and decreased antibacterial activity.

delay in gastric emptying associated with many types of illness (3). However, motilin is a peptide composed of 22 amino acid residues, and development for oral use has been difficult. Erythromycin A (EMA; **1**, Fig. 1) and its derivatives were recently shown to mimic the effect of motilin (3). EMA is a typical macrolide-type antibiotic which has been widely used for decades as an antibacterial drug. However, its gastrointestinal side effects are well known. Research led to the discovery that EMA is a motilin agonist, and that it stimulates smooth muscle motilin receptors which are located in several sites of the mammalian gastrointestinal tract, including humans (3). It was also shown that EMA, like motilin, increases gastrointestinal motility. Furthermore, in clinical studies EMA was found to improve gastrointestinal complications associated with such diseases as diabetes.

The receptor for motilin was recently isolated from human stomach, identified and cloned (4). Both EMA and motilin interacted with the cloned receptor, providing a molecular basis for its effects on the human gastrointestinal tract. However, EMA has several disadvantages for use as a prokinetic agent. It has antibacterial properties. It is acid-unstable and, therefore, is readily degraded by gastric acids when administered orally. It has low activity, which means that large doses (several hundred mg/day) are necessary.

Omura and Itoh found that conversion of the 9-ketone of the macrolactone ring system of EMA to an enol ether, such as **2**, led to an increase in gastrointestinal motor stimulating activity (10-fold that of EMA) with a concomitant decrease in antibacterial activity (5). Based on these findings, they studied the structure-activity relationships (SAR) of **2**, especially by substitution on the 3'-position of the basic sugar, and selected the *N*-ethyl (**3**, EM-523) and *N*-isopropyl (**4**, EM-574) compounds, with higher prokinetic activity and no antibacterial potency, for further investigation and clinical trials (Fig. 1) (6, 7).

Design of acid-stable erythromycin A enol ethers

During a search for more promising prokinetic EMA derivatives, we observed that compounds **2-4** were labile to acid and would be easily degraded by gastric acid when administered orally. We then focused our research on acid-stable and orally active EMA derivatives with higher prokinetic activity and no antibacterial properties.

Under acidic conditions, EMA is first degraded to an enol ether **2** and then to a ketal **5** by reaction of the 9-ketone group with hydroxyl groups at positions 6 and 12 (Fig. 2) (8, 9). Although the enol ether **2** showed higher i.v. prokinetic activity and decreased antibacterial properties as compared to EMA, it was labile to acid as well as EMA (6). The ketal **5**, on the other hand, was confirmed to be acid-stable but possessed only low prokinetic activity (see Table I) (6). In an attempt to avoid the conversion

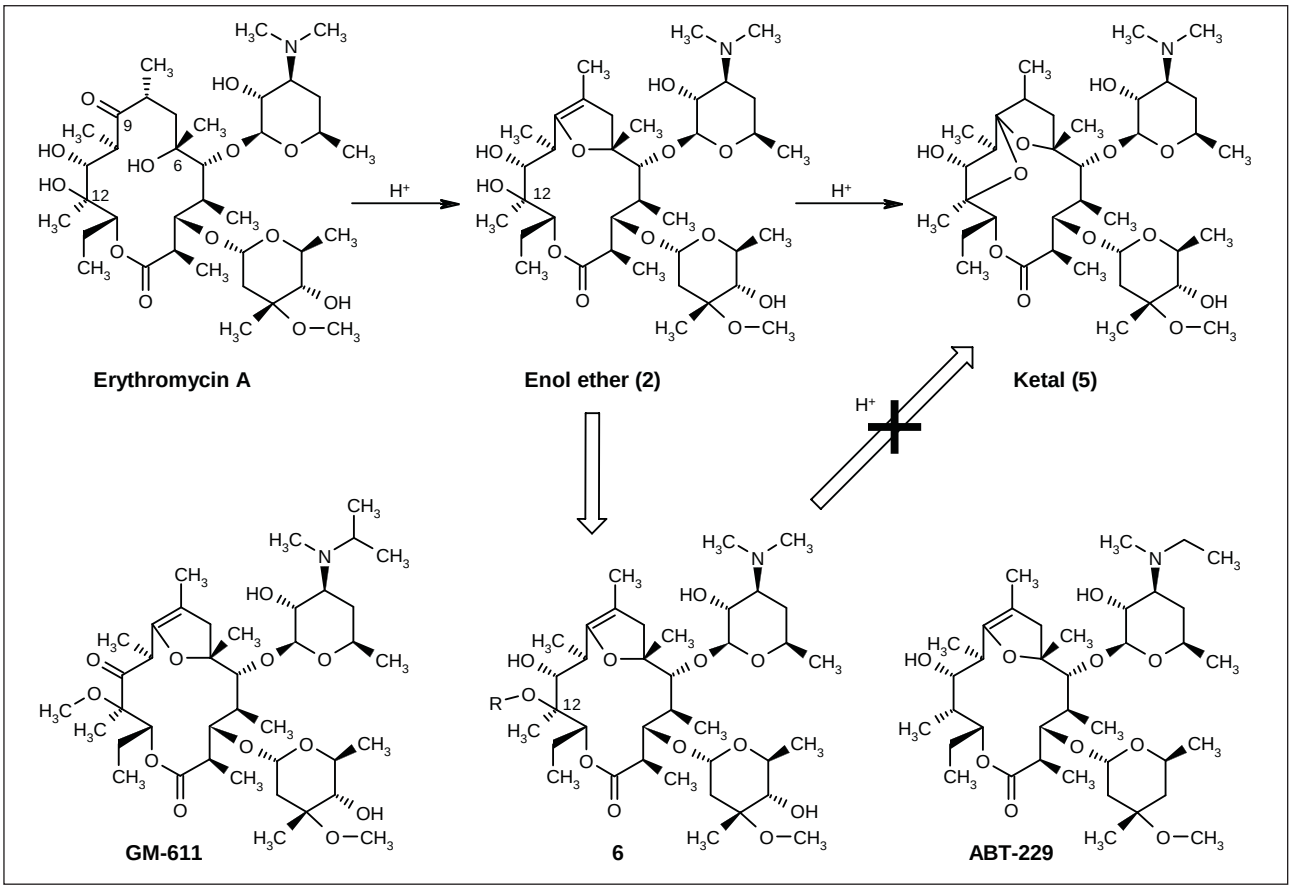


Fig. 2. Acid degradation mechanism of erythromycin A, design of acid-stable EMA enol ether derivatives **6** and GM-611, and structure of ABT-229.

of **2** to **5**, we performed chemical modification of **2**. It was hypothesized that masking the 12-OH group of **2** using stable protecting groups would result in compounds stable to acid which would not be converted to the ketal **5**. We selected an alkyl group as the 12-OH protecting group. One of the proposed compounds was 12-*O*-alkyl (compound **6**). An SAR study of **6** led to the identification of GM-611, the first candidate for further development and clinical trials (10-14) (Fig. 2).

In order to obtain an acid-stable EMA enol ether derivative, a group from Abbott synthesized 12- and 4''-dideoxy compound ABT-229. However, ABT-229 was labile to acid and dramatically decreased the *in vitro* contractile activity measured in the rabbit duodenum preparation when treated with acidic solution below pH 3 for 30 min before testing. For example, only one-tenth of the contractile activity remained in pH 2.74 and it was almost completely lost in pH 1.20. The acid-decomposition mechanism of ABT-229 was apparently different from that of enol ethers such as **2-4**, since it lacked the 12-hydroxyl group which is essential for forming the ketal (Fig. 2) (15).

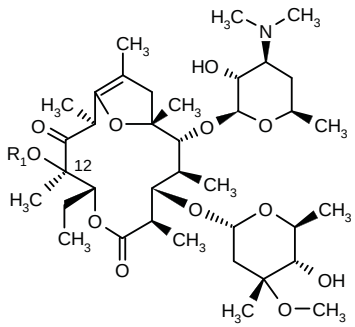
Chemistry and SAR

A critical step for the synthesis of 12-*O*-alkyl-11-hydroxyl compounds was to find an effective method for protecting the 11-hydroxyl group in order to avoid concomitant alkylation of the 11-hydroxyl and translactonization under alkylation and basic conditions. We studied two methods: the conventional protection-deprotection of the 11-hydroxyl group and the conversion of the 11-hydroxyl group to the 11-oxo, which would be reverted to the 11-hydroxyl group after selective alkylation of the 12-hydroxyl group. Because preliminary experiments with the first method were not successful, we focused our investigations on the second method via the 11-ketone intermediates.

12-O-Alkyl-11-oxo compounds

The 11-hydroxyl group of enol ether **7** was oxidized to the 11-ketone **8** with dimethylsulfoxide, trifluoroacetic anhydride and triethylamine. The 2'- and 4''-hydroxyl protecting groups were deprotected with sodium bicarbonate in MeOH-H_2O and subsequently the *N*-methyl group was

Table I: Motilin receptor binding and contractile activities of EMA derivatives.



Compd	R_1	pIC_{50}[a]	*In vitro* pIC_{50} (HCl)[a,b]	pEC_{50}[c]	*In vivo* MI_{100}[d] (μg/kg i.v.)
12	CH_3	8.04 ± 0.04	8.05 ± 0.08	6.93 ± 0.14	2.9 ± 1.5
13	CH_3CH_2	8.30 ± 0.10		7.58 ± 0.15	6.2 ± 4.4
14	$CH_3CH_2CH_2$	7.88 ± 0.05		6.57 ± 0.27	>70
15	$C_6H_5CH_2$	7.61 ± 0.18		6.46 ± 0.13	>70
1 (EMA)		7.36 ± 0.13	7.15 ± 0.11	6.50 ± 0.10	32.3 ± 12.8
2		8.47 ± 0.18	6.65 ± 0.19	7.38 ± 0.15	1.0 ± 0.3
3 (EM-523)		8.50 ± 0.06	6.52 ± 0.16	7.32 ± 0.10	0.9 ± 0.3
4 (EM-574)		8.60 ± 0.13	7.05 ± 0.17	8.03 ± 0.12	0.3 ± 0.1
5		6.81 ± 0.12	6.77 ± 0.11	<5.0	>70
Motilin		9.31 ± 0.13		8.34 ± 0.06	0.05 ± 0.005
Cisapride					366 ± 169

[a]Negative logarithm of IC_{50} (M) with ± SEM (n = 3-4). [b]Measured after treatment with hydrochloric acid solution (pH 2.5). [c]Negative logarithm of EC_{50} (M) with ± SEM (n = 3-6). [d]Dose to give 100 of motor index (MI) with ± SEM (n = 3-5). (From refs. 26, 27, 29 and 30.)

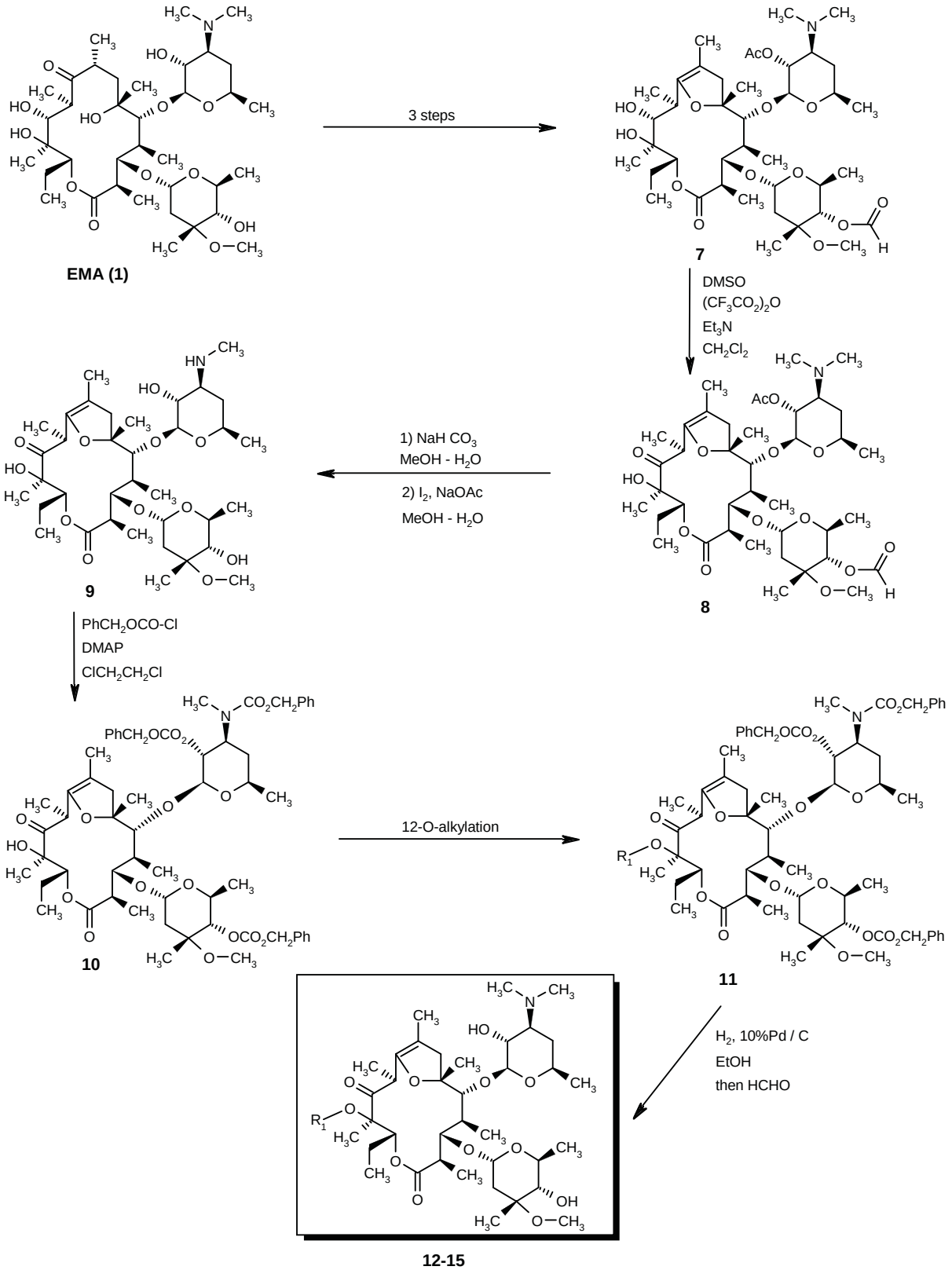
removed by treatment with iodine and sodium acetate in MeOH-H_2O to obtain **9**. The *N*-monomethyl compound **9** was then treated with carbobenzoxy chloride in the presence of 4-dimethylaminopyridine (DMAP), leading to compound **10**. Alkylation of the 12-hydroxyl group of **10** with alkyl halide and sodium hydride to afford **11**, followed by reductive deprotection of the carbobenzoxy group and reductive methylation of 3'-amino group gave the 12-*O*-alkyl-11-oxo compounds **12-15** (Scheme 1 and Table I). Reduction of the 11-oxo group of **12** under normal conditions ($NaBH_4$ or $NaBH_3CN$ in MeOH) to obtain the corresponding 11-hydroxyl compound was unsuccessful.

In vitro and *in vivo* screening for motilin agonist activity of 12-*O*-alkyl-11-oxo compounds **12-15** showed that the 12-*O*-methyl compound **12** had almost comparable potency to the 11,12-dihydroxy compound **2** and would be more stable to acid than **2** (Table I). This finding further indicated that the 11-hydroxyl group of the EMA derivative **2**, as well as the 12-hydroxyl group as shown earlier by Abbott researchers (15), is not necessarily essential for motilin agonist activity. Because the methyl group was the most potent among the 12-*O*-alkyl groups studied, especially for *in vivo* activity, compound **12** was selected as a lead for further research.

An alternative synthetic method for **12** was developed for bulk supply for chemical modification. The 12-hydroxy-11-oxo compound **8** was methylated with methyl iodide and sodium hydride to give **16** and subsequently hydrolyzed with sodium bicarbonate in MeOH-H_2O to afford **12**. Compound **12** was selectively demethylated by reaction with iodine and sodium acetate in MeOH-H_2O to give the *N*-monomethyl compound **17**. Modification of the 3'-amino group of **17** using several methods (5-7, 10, 13) afforded the *N*-substituted compounds **18-50** (Scheme 2 and Tables II-IV).

Modification of the hydroxyl groups of the sugar residues was also studied (Scheme 3 and Table V) (11, 13). The 2'-hydroxyl group of compound **12** was protected by acetylation and then the protected compound was exposed to oxidation, followed by hydrolytic deprotection, to give the 4''-oxo compound **51**, which also led to the 4''-oxime and 4''-amino compounds **52** and **53**. Deoxygenation of the 4''-hydroxyl group of compounds **12**, **18** and **20** was carried out (11, 13, 15, 16) via corresponding thiocarbonyl intermediates **54-56**, to afford compounds **57-59**. The 2'-hydroxyl group of the *N*-isopropyl compound **59** was further removed by a similar method to give the 2'- and 4''-dideoxy compound **60** (11).

Scheme 1: Synthesis of 12-*O*-alkyl-11-oxo compounds



Scheme 2: Modification of the 3'-amino group

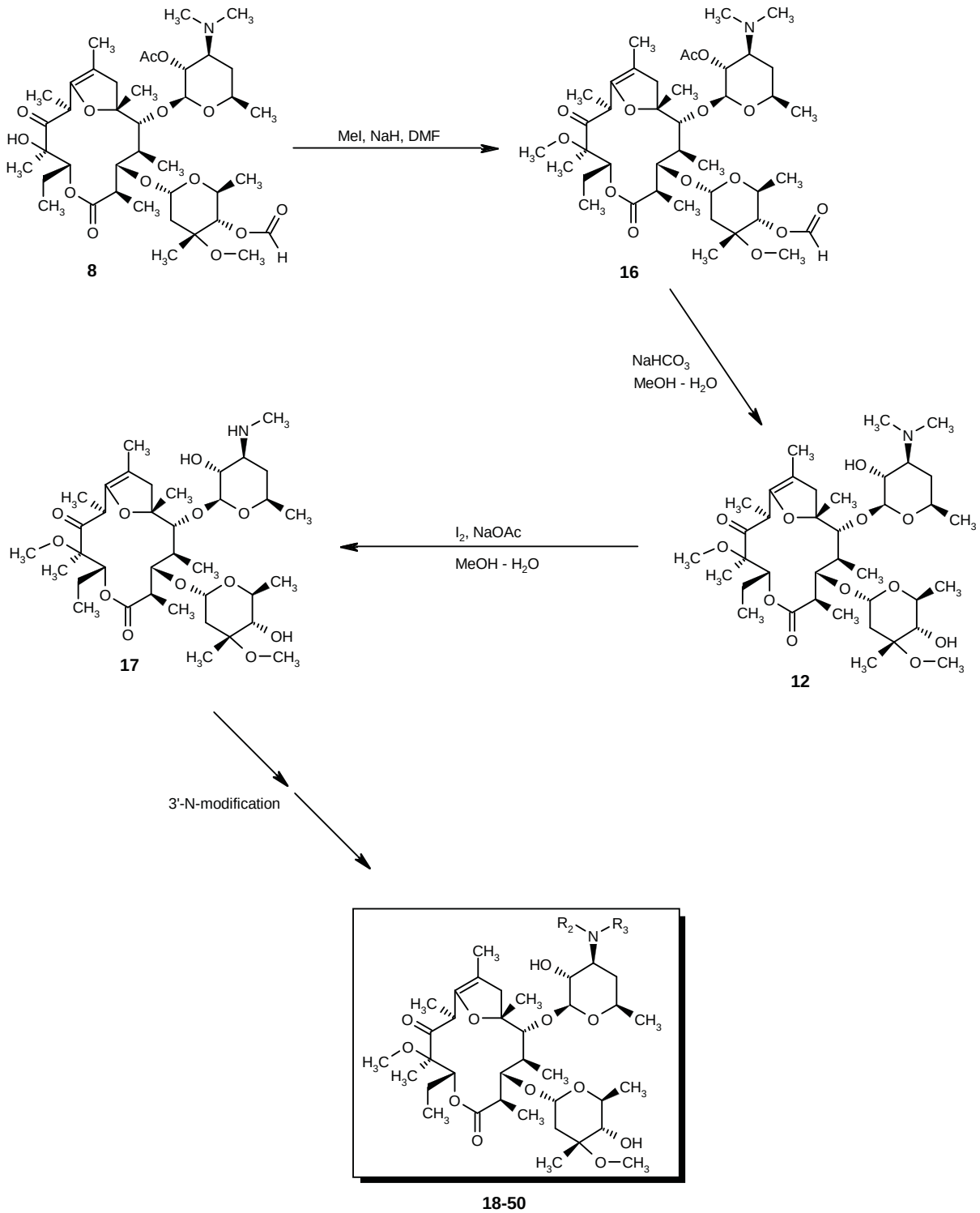
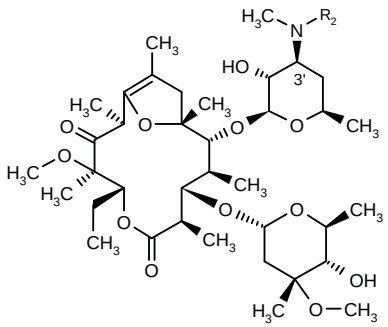


Table II: Motilin receptor binding and contractile activities of EMA derivatives.



Compd	R_2	pIC_{50}[a]	*In vitro* pIC_{50} (HCl)[a,b]	pEC_{50}[c]	*In vivo* MI_{100}[d] (μg/kg i.v.)
12	CH_3	8.04 ± 0.04	8.05 ± 0.08	6.93 ± 0.14	2.9 ± 1.5
18	CH_2CH_3	8.42 ± 0.12	8.19 ± 0.08	7.36 ± 0.13	1.1 ± 0.7
19	$CH_2CH_2CH_3$	8.22 ± 0.06		6.52 ± 0.10	17.9 ± 13.9
20	$CH(CH_3)_3$	8.22 ± 0.06	8.10 ± 0.02	7.41 ± 0.16	1.0 ± 0.4
21	$CH(CH_3)CH_2CH_3$	8.51 ± 0.18		7.80 ± 0.09	4.5 ± 1.5
22	$CH_2CH{=}CH_2$	8.24 ± 0.08		7.25 ± 0.26	17.6 ± 5.0
23	$CH_2C{\equiv}CH$	7.89 ± 0.08		6.21 ± 0.01	18.8 ± 9.9
24	$CH(CH_3)CH{=}CH_2$	8.13 ± 0.22		7.02 ± 0.21	>70
25	$CH_2C(CH_3)_3$	7.18 ± 0.07		6.43 ± 0.09	>70
26	$CH(CH_2CH_3)_2$	8.18 ± 0.12		7.38 ± 0.16	6.5 ± 4.7
27	$CH_2CH(CH_3)_2$	7.81 ± 0.13		7.36 ± 0.08	13.3 ± 6.3

[a-d]See footnotes in Table I.

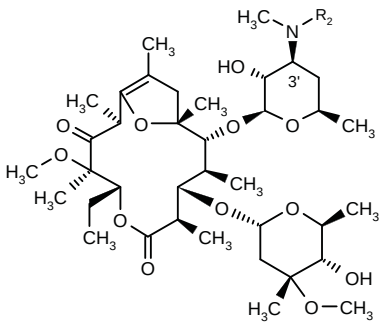
Motilin agonist activity of the compounds was assessed by motilin receptor binding (pIC_{50}) and *in vitro* and *in vivo* smooth muscle contractile (pEC_{50} and MI_{100} (i.v.)) activity in comparison with EMA (**1**), the 11,12-dihydroxy compounds **2-4** and the ketal **5**. Acid stability was evaluated by treatment with hydrochloric acid solution (pH 2.5) at room temperature for 2 h, followed by assaying the solution for motilin receptor binding (Tables I-V).

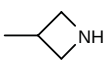
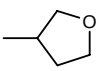
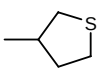
In vitro studies under our experimental conditions did not give clear SARs. On the other hand, results from an *in vivo* study in which compounds were intravenously administered confirmed the results of Omura and Itoh (5-7), namely, that among the 3'-*N*-substituted amino compounds synthesized, the *N*-isopropyl derivative **20** was the most active and the quaternary ammonium type derivatives **49** and **50** exhibited more potent activity compared to the corresponding tertiary amino derivatives such as **12** and **23**.

Modification of the 2'- and 4''-hydroxyl groups gave interesting results (Table V). Conversion of the 4''-hydroxyl group of **12** into an oxo (**51**), oxime (**52**) or amino group (**53**) significantly diminished the i.v. *in vivo* activity, although the *in vitro* activity was not significantly changed. On the other hand, deoxygenation of the 4''-hydroxyl group increased the potency, especially for screening of the *in vitro* and *in vivo* contractile activity as seen with compounds **57-59**, which were in agreement with the results of the Abbott group (15). The *in vitro* and *in vivo* contractile activities of the most active compound **59** were almost comparable to those of motilin. Further elimination of the 2'-hydroxyl group of **59** to afford **60**, however, resulted in decreased *in vitro* contractile activity and loss of *in vivo* potency. Interestingly, motilin receptor binding affinity of **60** remained almost unchanged, indicating that compound **60** may be a partial motilin receptor agonist. Based on these results, we hypothesized that only the 2'-hydroxyl group may be necessary for motilin agonist activity of erythromycins.

In vitro acid stability screening showed that the binding affinity of all the 12-methoxy-11-oxo compounds tested was not altered by acid treatment, while the 12-hydroxyl compounds **2**, **3** (EM-523) and **4** (EM-574) showed substantially reduced activity under the same treatment (Tables I-V). These results suggested that the 12-methoxy compounds may be acid-stable. In order to investigate the acid stability in more detail, **20** (GM-611) was treated with buffer solutions under several pH conditions at 37 °C and compared with **3** (EM-523). The 11-hydroxy compound **3** was immediately and almost completely degraded within 15 min in pH <3.0, while the

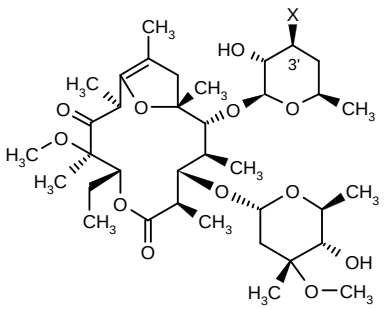
Table III: Motilin receptor binding and contractile activities of EMA derivatives.

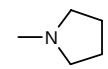
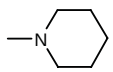
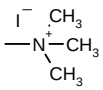
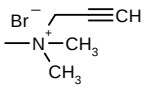


		In vitro			*In vivo*
Compd	R_2	pIC_{50}[a]	pIC_{50} (HCl)[a,b]	pEC_{50}[c]	MI_{100}[d] (µg/kg i.v.)
28	CH_2CH_2OH	8.23 ± 0.09		7.32 ± 0.18	1.1 ± 0.4
29	$CH_2CH_2NH_2$	8.21 ± 0.09		7.49 ± 0.13	2.8 ± 0.7
30	CH_2CH_2CN	7.52 ± 0.09		<5.0	>70
31	CH_2CH_2F	8.26 ± 0.01		7.24 ± 0.26	10.1 ± 2.5
32	CH_2CF_3	8.22 ± 0.12		7.66 ± 0.05	2.3 ± 0.8
33	$CH(CH_2F)_2$	7.17 ± 0.15		6.57 ± 0.12	>70
34	$CH_2CHOHCH_3$	8.13 ± 0.16		6.85 ± 0.42	23.1 ± 7.7
35	$CH_2C(O)CH_3$	6.28 ± 0.11		5.14 ± 0.04	23.1 ± 4.4
36	$CH_2C(O)NH_2$	7.67 ± 0.07		6.96 ± 0.16	>70
37		8.30 ± 0.18		7.24 ± 0.08	>70
38		7.35 ± 0.17		5.69 ± 0.09	>70
39		7.59 ± 0.07		5.93 ± 0.10	16.9 ± 7.6
40		7.94 ± 0.05		6.44 ± 0.18	13.0 ± 5.2
41		7.63 ± 0.03		6.57 ± 0.19	>70
42		7.43 ± 0.02		5.87 ± 0.18	>70

[a-d]See footnotes in Table I.

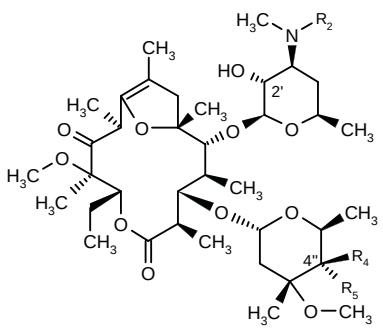
Table IV: Motilin receptor binding and contractile activities of EMA derivatives.



Compd	X	*In vitro* pIC_{50}[a]	*In vitro* pIC_{50} (HCl)[a,b]	pEC_{50}[c]	*In vivo* MI_{100}[d] (µg/kg i.v.)
43	NH_2	8.24 ± 0.04		6.48 ± 0.26	>70
17	$NH(CH_3)$	8.41 ± 0.12		7.29 ± 0.14	>70
44	$NH(CH_2CH_3)$	8.49 ± 0.11		7.26 ± 0.12	29.1 ± 11.1
45	$NH[CH(CH_3)_2]$	8.29 ± 0.09		<5.0	>70
46	$N(CH_2CH_3)_2$	8.17 ± 0.06		7.40 ± 0.20	12.6 ± 2.6
47		8.16 ± 0.18		7.07 ± 0.08	28.6 ± 23.4
48		7.89 ± 0.13		7.07 ± 0.07	12.5 ± 5.2
49		8.19 ± 0.09		7.71 ± 0.13	0.2 ± 0.1
50		8.19 ± 0.10	7.88 ± 0.22	7.94 ± 0.05	0.3 ± 0.2

[a-d]See footnotes in Table I.

Table V: Motilin receptor binding and contractile activities of EMA derivatives.



Compd	R_2	R_4R_5	*In vitro* pIC_{50}[a]	*In vitro* pIC_{50} (HCl)[a,b]	pEC_{50}[c]	*In vivo* MI_{100}[d] (µg/kg i.v.)
12	CH_3	H, OH	8.04 ± 0.04	8.05 ± 0.08	6.93 ± 0.14	2.9 ± 1.5
51	CH_3	-O-	8.17 ± 0.08		6.78 ± 0.23	38.7 ± 4.9
52	CH_3	-N(OH)-	7.59 ± 0.07		6.20 ± 0.14	>70
53	CH_3	H,NH_2	7.83 ± 0.08		6.43 ± 0.07	24.2 ± 2.4
57	CH_3	H,H	8.64 ± 0.14	8.41 ± 0.02	7.98 ± 0.22	0.4 ± 0.3
58	CH_2CH_3	H,H	8.46 ± 0.05	8.42 ± 0.10	8.04 ± 0.11	0.3 ± 0.07
59	$CH(CH_3)_2$	H,H	8.60 ± 0.10	8.75 ± 0.08	8.21 ± 0.06	0.09 ± 0.01
60	2'-deoxy of **59**		8.73 ± 0.18		6.68 ± 0.34	>70

[a-d]See footnotes in Table I.

Scheme 3: Modification of the 2'- and 4''-hydroxyl groups

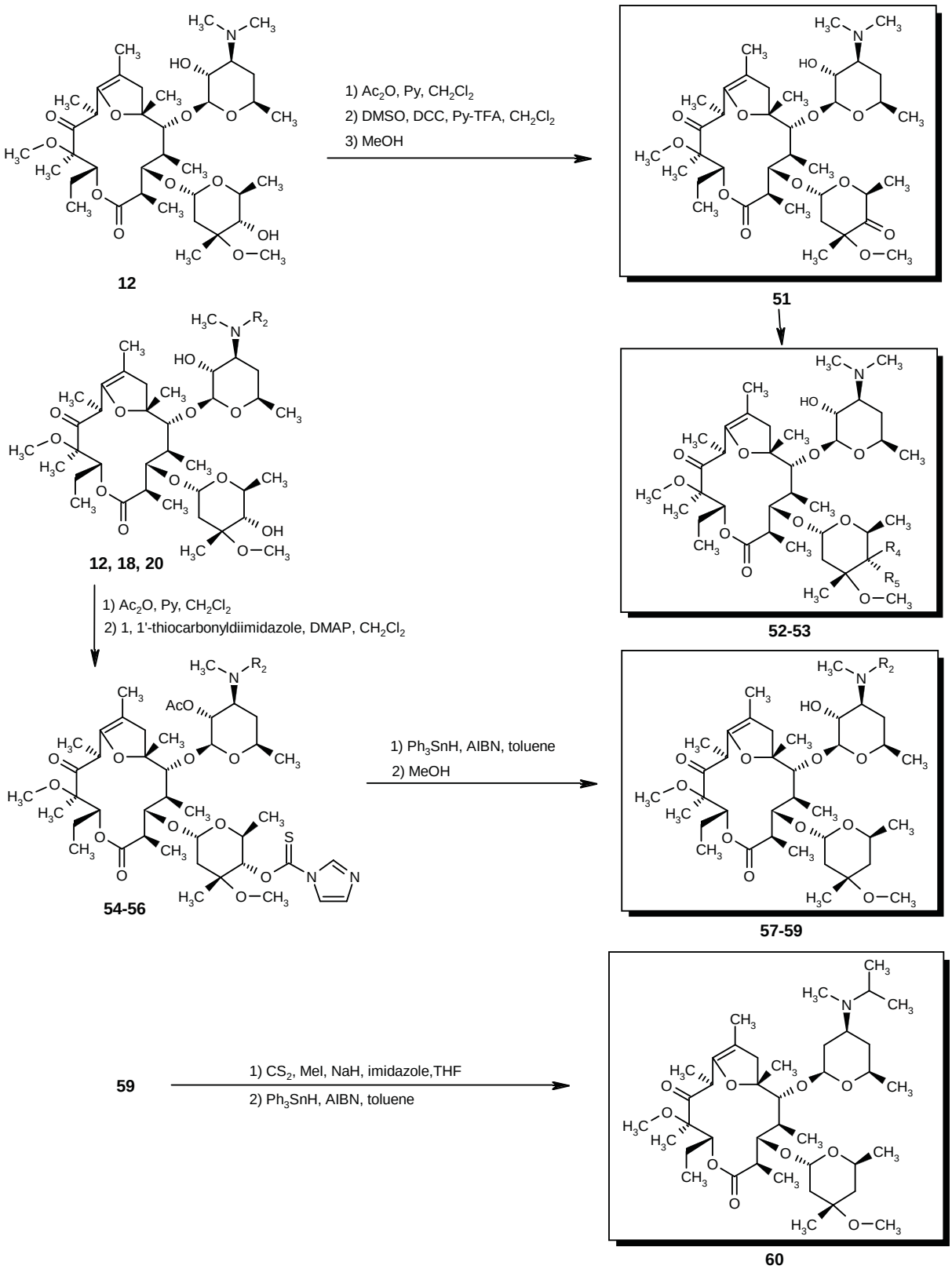
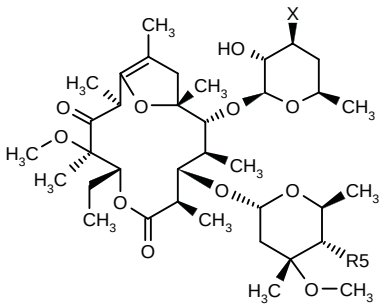
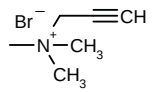


Table VI: In vivo *contractile activity.*



Compd	X	R^5	MI_{100}[d] (µg/kg i.v.)	MI_{100}[d] (µg/kg i.g.)	i.g./i.v.
12	$-N(CH_3)_2$	OH	2.9 ± 1.5	2.4 ± 0.9	0.8
18	$-N(CH_3)CH_2CH_3$	OH	1.1 ± 0.7	4.3 ± 1.0	3.9
20	$-N(CH_3)[CH(CH_3)]_2$	OH	1.0 ± 0.4	1.5 ± 0.5	1.5
50		OH	0.3 ± 0.2	8.7 ± 4.3	29.0
57	$-N(CH_3)_2$	H	0.4 ± 0.3	1.2 ± 0.3	3.0
58	$-N(CH_3)CH_2CH_3$	H	0.3 ± 0.07	0.2 ± 0.2	0.7
59	$-N(CH_3)[CH(CH_3)_2]$	H	0.09 ± 0.01	0.3 ± 0.09	3.3
3 (EM-523)			0.9 ± 0.3	14.9 ± 4.9	16.6
Cisapride			366 ± 169	1080 ± 377	3.0

[d]See footnote in Table I.

11-methoxy compound **20** was slowly degraded and remained unchanged approximately 40% in pH 2.2 even after 6 h (10). The increased stability to acid of the 12-*O*-methyl-11-oxo derivatives is an advantage when the compounds are administered orally. Compounds **12**, **18**, **20** and, **57-59**, administered intragastrically (i.g.), exhibited almost the same degree of *in vivo* activity as those administered i.v., whereas **3** (EM-523) required more than a 10-fold dose to elicit similar *in vivo* activity when given i.g. compared to i.v. administration (Table VI) (10, 11, 13). The quaternary ammonium compound **50** also showed relatively weak *in vivo* activity when administered i.g. The lower oral activity of **3** and **50** in comparison with their i.v. activity may be attributed to their low bioavailability due to acid instability of **3** and possible low oral absorption of **50**. Cisapride, a prokinetic agent, showed relatively weak *in vivo* activity in this model.

*12-*O*-Methyl-11-hydroxy compounds*

While we were performing intensive SAR studies of 12-*O*-alkyl-11-oxo EMA derivatives, we were also investigating a method for synthesizing acid-stable 12-*O*-alkyl-11-hydroxyl derivatives. We finally selected a benzyl group as a suitable protecting group of the 11-hydroxyl group. Compound **61** was treated with acetic acid to yield the enol ether **62**, of which 4''-hydroxyl group was subsequently protected by carbobenzoxylation to give **63**. Compound **63** was allowed to react with benzyl bromide and sodium hydride to protect the 11-hydroxyl group. The resulting 11-*O*-benzyl compound **64** was methylated with methyl iodide and sodium hydride, followed by reductive deprotection of carbobenzoxy group to afford **65**. Compound **65** then afforded the *N*-alkyl derivatives **66-68**. The 11-*O*-benzyl group of **66-68** was successfully removed by reduction with 10% Pd/C and 1 atm H_2 in the presence of trifluoroacetic acid in methanol to obtain the desired 11-hydroxyl-12-*O*-methyl compounds **69-71** (Scheme 4 and Table VII) (12).

Motilin agonist activity of the 11-hydroxyl-12-methoxy compounds **69-71** was almost comparable to the corresponding 11-oxo compounds **12**, **18** and **20,** again indicating that the 11-hydroxyl group of erythromycins is not always required for their motilin agonist activity (12). Compounds **69-71** appeared to be acid-stable since their binding affinity was only slightly altered by the acid treatment, as expected.

Most of the 12-*O*-methyl EMA enol ether derivatives showed weak or little antibiotic activity (Table VIII).

Pharmacology of GM-611

The pharmacology of GM-611 was assessed by motilin receptor binding activity, contractile activity in

Scheme 4: Synthesis of 12-*O*-methyl-11-hydroxyl compounds

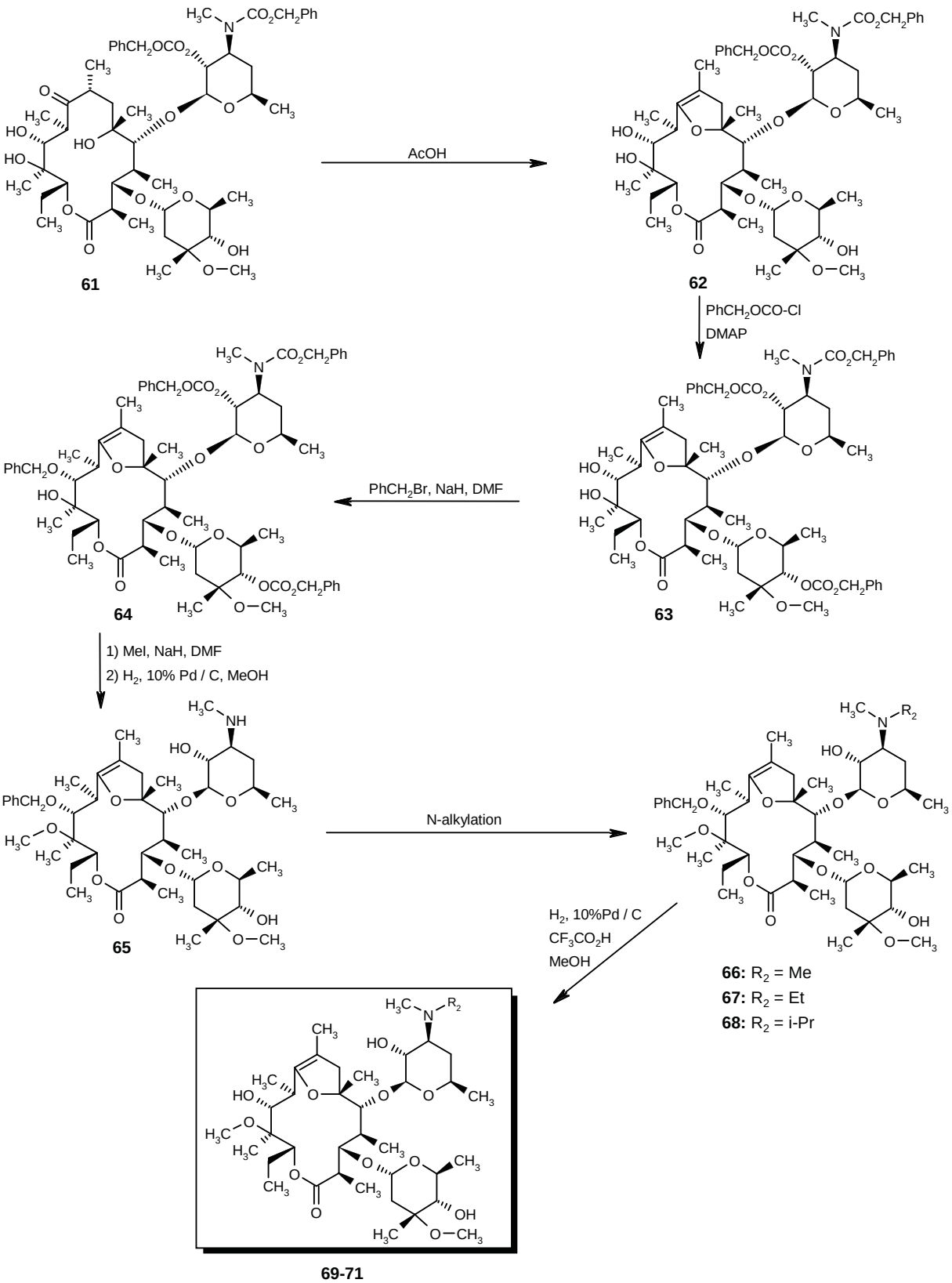
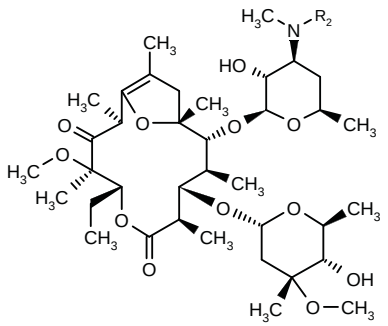


Table VII: Motilin receptor binding and contractile activities of EMA derivatives.



Compd	R_2	*In vitro* pIC_{50}[a]	*In vitro* pIC_{50} (HCl)[a,b]	*In vitro* pEC_{50}[c]	*In vivo* MI_{100}[d] (μg/kg i.v.)	*In vivo* MI_{100}[d] (μg/kg i.g.)
69	CH_3	8.12 ± 0.05	7.40 ± 0.08	7.71 ± 0.11		
70	CH_2CH_3	8.33 ± 0.11	8.08 ± 0.23	7.64 ± 0.11		
71	$CH(CH_3)_2$	8.16 ± 0.07	7.82 ± 0.02	7.89 ± 0.07	0.44 ± 0.12	1.2 ± 0.57

[a-d]See footnotes in Table I.

Table VIII: Antibacterial activity (MIC[a]; μM/ml) of EMA derivatives.

Compd	*B. subtilis* ATCC 6633	*S. pneumoniae* No. 12	*S. aureus* 209P	*E. coli* NIHJ JC-2	*K. pneumoniae* IFO 3512
1 (EMA)	0.39	0.098	0.39	100	6.3
3 (EM-523)	100	25	>200	>200	>200
12	6.3	3.1	13	>200	100
18	200	50	>200	>200	>200
20 (GM-611)	>200	200	>200	>200	>200
50	100	100	100	>400	>400
57	50	1.6	100	>200	100
58	200	100	>200	>200	>200
59	200	100	>200	>200	>200
69	6.3	1.6	25	>200	200
70	>200	50	>200	>200	>200
71	>200	200	>200	>200	>200

[a]Minimum inhibitory concentration (MIC) was estimated by agar dilution method.

isolated tissues, acid stability, antibacterial activity and the effects on gastric motility and gastric emptying in dogs and nonhuman primates (17-25).

Motilin receptor binding activity

A binding assay for motilin receptors was performed according to the procedure first described by Bormans *et al.* (26). In this assay, homogenates of smooth muscle tissue from the rabbit small intestine were incubated at 25 °C for 120 min with 25 pM ^{125}I-pMTL (porcine motilin) and either GM-611 or unlabeled pMTL. After incubation, the radioactivity bound to the tissue was determined with a γ-counter. Specific binding was defined as the difference between total binding and nonspecific binding. GM-611 and pMTL displaced ^{125}I-pMTL bound to the homogenates of the smooth muscle tissue of the rabbit small intestine in a concentration-dependent manner. The displacement curves of GM-611 and pMTL were parallel. The IC_{50} values (mean ± SE, n = 5) were 4.9 ± 0.9 and 0.8 ± 0.1 nM for GM-611 and pMTL, respectively. The Hill coefficient values (mean ± SE, n = 5) of GM-611 and pMTL, obtained from a logistic equation, were 0.95 ± 0.08 and 0.97 ± 0.11, respectively. These results indicated that there was no significant binding cooperation of GM-611 and pMTL to the motilin receptor.

GM-611 and pMTL also inhibited the specific binding of ^{125}I-pMTL to homogenates of monkey duodenum smooth muscle tissue, with IC_{50} values (mean ± SE, n = 5) of 13.3 ± 4.3 and 2.6 ± 1.2 nM, respectively.

In contrast to the motilin receptor, GM-611 had no affinity (IC_{50} >10 μM) for adrenergic (α_1, α_2, β_1, β_2 and β_3), muscarinic (M_1, M_2 and M_3), histamine (H_1, H_2 and H_3), dopamine (D_1 and D_2), serotonin (5-HT_1, 5-HT_2, 5-HT_3 and 5-HT_4) or cholecystokinin (CCK-A and CCK-B) receptors.

In vitro *contractile activity*

In vitro contractile activity was evaluated using isolated segments of the rabbit duodenum as previously reported (27). GM-611 (1-1000 nM) and pMTL (0.1-100 nM) induced concentration-dependent contractions in the duodenal segment, with EC_{50} values (mean ± SE) of 14.9 ± 2.5 (n = 6) and 4.6 ± 0.5 nM (n = 10), respectively. The concentration-dependent contractile responses for GM-611 and pMTL were competitively inhibited by pretreatment with a selective motilin antagonist, GM-109 (28) at concentrations of 0.1-3 μM. The pA_2 values (negative logarithm of molar concentration of antagonist causing a 2-fold shift to the right of the concentration response curve) for GM-109 inhibition of GM-611 and pMTL were 7.43 ± 0.1 and 7.37 ± 0.11, respectively.

The contractile responses by GM-611 and pMTL were not influenced by pretreatment with atropine (1 μM), a muscarinic receptor antagonist, hexamethonium (1 μM), a nicotinic receptor antagonist, naloxone (10 μM), an opiate receptor antagonist, tropisetron (1 μM), a 5-HT_3 and 5-HT_4 receptor antagonist, or tetrodotoxin (1 μM), a sodium channel blocker. These results suggest that rabbit small intestinal contractions evoked by GM-611 are directly mediated through motilin receptors on smooth muscle cells, rather than by the intramural nervous system or by muscarinic, nicotinic, 5-HT_3, 5-HT_4 or opiate receptors. These results are consistent with the receptor binding studies.

The contractile responses of the duodenum induced by GM-611, as well as motilin, were significantly suppressed by removing calcium from the media or by verapamil (1 μM), a calcium channel blocker. These findings suggest that the contractile actions of GM-611 and pMTL depend largely on extracellular calcium, and that the release of calcium from intracellular calcium stores has a relatively minor role.

Acid stability

The acid stability of GM-611 was also evaluated by assessing *in vitro* contractile activity, in addition to motilin receptor binding and physicochemical studies. GM-611 (30 nM) was dissolved in 0.9% saline and the pH was adjusted to 1.20, 2.75 or 4.30 using dilute hydrochloric acid. GM-611 in 0.9% saline at pH 7.4 was used as a control. The solutions were then incubated at 37 °C for 30 min. Following incubation, the contractile activity of the GM-611 solutions was determined in the isolated rabbit duodenal segment assay (27). The results are expressed as a percentage of contractile activity induced by 100 μM acetylcholine. The contractile activity of GM-611 was similar to control values at each pH range, demonstrating that GM-611 would be acid-stable. These results were consistent with those of the receptor binding and physicochemical experiments described previously.

Antibacterial activity

The antibacterial activity of GM-611 was estimated by determining the minimum inhibitory concentration (MIC) against Gram-positive and Gram-negative bacteria. As compared to EMA, GM-611 showed little antibacterial activity against *B. subtilis*, *S. pneumoniae*, *S. aureus*, *E. coli* and *K. pneumoniae* (Table VIII).

In vivo *contractile activity*

The gastrointestinal contractile activity in conscious dogs was monitored by means of chronically implanted force transducers sutured onto the gastric antrum (stomach) and small intestines to record circular muscle contractions (29, 30). The pattern of gastrointestinal motility differs in the fasting and digestive states. In the digestive state, relatively weak contractions occur regularly, whereas in the fasting state, bursts of strong contractions (MMC or migrating motor complex) occur periodically in the stomach and migrate to the lower small intestine. These experiments were, therefore, carried out in both the fasting and digestive states. To measure motility quantitatively, the signals from the gastric antrum were automatically digitalized and recorded. The area of contractions induced by drug administration was then calculated and used as the motor index (MI). In the fasting state, the MI of naturally occurring MMC was designated as 100% and contractile responses of GM-611 and pMTL were expressed as a percentage of MMC. A value below 100% would indicate a decrease in the duration of contractile activity while a value greater than 100% would indicate an increase in the duration of contractile activity. GM-611 was given i.v. or p.o. 10-20 min after termination of naturally occurring MMC in the stomach. In the digestive state, the MI during the 40 min before drug administration was defined as 100%, and the percentage changes in gastric motility induced by GM-611 was calculated every 20 min. GM-611 was administered intravenously or orally 2 h after feeding (31).

GM-611 (0.3-30 μg/kg, i.v.) induced strong contractions in the stomach in the fasting state, and the contractions migrated caudally along the small intestine. This pattern of contractions was similar to that of motilin. Gastrointestinal contractions induced by GM-611 or pMTL were completely suppressed by i.v. infusion of

GM-109 (10 mg/kg/h), a motilin receptor antagonist. These results suggest that GM-611 induced gastrointestinal contractions in dogs via the motilin receptor.

The gastric MI was measured in fasted dogs receiving i.v. or oral GM-611 at doses of 0.3-30 μg/kg or i.v. pMTL at doses of 0.01-1 μg/kg. At a dose of 1 μg/kg the gastric MI of pMTL and GM-611 was approximately 100%. There was a dose-dependent increase in the MI as the dose of GM-611 increased to 30 μg/kg. This study showed that i.v. or oral GM-611 stimulates gastric contractile activity at doses as low as 1 μg/kg.

In the digestive state, as compared to the fasting state, steady contractile activity without motor quiescence was observed in the stomach. GM-611 given i.v. (3-100 μg/kg) 2 h after feeding increased the amplitude of the gastric contractions. Duodenal motility was found to be well coordinated with gastric motility. As seen in the fasting study, the gastric MI increased in a dose-dependent manner following i.v. or oral administration of GM-611. Following i.v. administration of GM-611 (3-100 μg/kg), the peak increase of motility was observed 20 min after drug administration and motility then decreased to the predose level at 60 min postadministration. Oral GM-611 (300-3000 μg/kg) also increased gastric motility in the digestive state, with peak activity occurring 40-60 min after drug administration. The MI remained above predose levels throughout the entire observation period of 120 min. The minimally effective doses of GM-611 that increased the MI in the digestive state were 10 and 1000 μg/kg for the i.v. and oral doses, respectively.

Gastric emptying in dogs

The paracetamol absorption technique was used to measure the gastric emptying rate. Paracetamol is not absorbed from the stomach but is absorbed rapidly from the duodenum (32). Thus, the blood concentration of paracetamol correlates with the gastric emptying rate. GM-611 was administered to dogs by i.v. injection (0.05, 0.1 or 0.2 mg/kg) 5 min after feeding or by oral administration (0.25, 0.5 or 1.0 mg/kg) 15 min before feeding of a test meal. After feeding of a test meal containing 30 mg/kg of paracetamol, blood samples were collected every 15 min and plasma concentrations were measured by HPLC. The peak plasma concentration of paracetamol was observed at 45 min after feeding of the test meal. The paracetamol plasma concentration *versus* time curves were used to calculate the AUC from 0-45 min after feeding of the test meal. This AUC was then used to estimate the index of gastric emptying. Gastric emptying measured by this method was significantly accelerated by the administration of GM-611 at doses as low as 0.1 mg/kg (i.v.) and 0.5 mg/kg (p.o.).

Studies in delayed gastric emptying dog models

The effects of GM-611 on gastric emptying were investigated in delayed gastric emptying models of clonidine (α_2-adrenergic agonist) or truncal vagotomy in dogs. Gastric emptying was measured by the paracetamol method described above. Clonidine was injected s.c. immediately after ingestion of a test meal containing paracetamol (30 mg/kg). Gastric emptying was significantly delayed by clonidine (0.03 and 0.1 mg/kg). GM-611 was administered i.v. (0.4 mg/kg) 5 min after or p.o. (1.0 mg/kg) 15 min before ingestion of a test meal. GM-611 significantly accelerated gastric emptying in the clonidine (0.03 mg/kg s.c.)-induced delayed gastric emptying model.

The anterior and posterior vagal trunks were cut at the supradiaphragm in dogs. After truncal vagotomy, MMC originating from the gastric antrum disappeared, although irregular MMC were still observed in the duodenum in the fasting state. The gastric emptying rate was significantly decreased after truncal vagotomy. Orally administered GM-611 (0.25 and 0.5 mg/kg) significantly accelerated gastric emptying in the vagotomy model.

In vivo *studies in monkeys*

Animal experiments on the effects of motilin and its agonists have been performed mainly in rabbits and dogs. There are only a few reports on the activity of motilin in other animals since only a few species respond to motilin in both *in vivo* and *in vitro* experiments. In these studies the effects of GM-611 on gastrointestinal motility and gastric emptying rate were examined in conscious rhesus monkeys. The gastrointestinal contractile activity was monitored by means of chronically implanted force transducers in the stomach and duodenum. In the fasting state, strong contractions occurred at constant intervals in the gastric antrum and migrated through the small intestine. In the stomach, these strong contractions lasted about 25 min and then stopped abruptly. This pattern was similar to the MMC measured in humans and dogs.

GM-611 or pMTL was administered intravenously at doses of 8-240 and 0.3-8 μg/kg, respectively. The gastric MI was estimated as previously described. Both GM-611 and pMTL induced gastric contractions in a dose-dependent manner. The MI of pMTL in monkeys was similar to that of pMTL in dogs. GM-611, however, was less active in monkeys than in dogs, in which the effective dose was 24 μg/kg. The gastric emptying rate of a liquid test meal was measured by the paracetamol method. GM-611 was given i.v. 5 min after or p.o. 30 min before the test meal. Both i.v. and oral GM-611 accelerated the rate of gastric emptying. The minimally effective dose was approximately 0.08 and 8.1 mg/kg by the i.v. and oral routes of delivery, respectively.

The pharmacological activity of GM-611 is summarized in Table IX.

Clinical applications

Basically, GM-611 is expected to have the same clinical applications as erythromycin A (EMA) and its

Table IX: Summary of pharmacology of GM-611 in rabbits, dogs and monkeys.

Species		
Rabbit	Motilin receptor binding assay (IC_{50}, nM)	4.9
	In vitro contractile activity in the duodenum (EC_{50}, nM)	14.9
Dog	*In vivo* contractile activity in the fasting state	
	Effective dose, μg/kg i.v.	~1
	Effective dose, μg/kg p.o.	~1
	In vivo contractile activity in the digestive state	
	Effective dose, μg/kg i.v.	~10
	Effective dose, μg/kg p.o.	~1000
	Gastric emptying	
	Effective dose, mg/kg i.v.	0.1
	Effective dose, mg/kg p.o.	0.5
	Gastric emptying in clonidine-induced delayed gastric emptying model	
	Effective dose, mg/kg i.v.	0.4
	Effective dose, mg/kg p.o.	1.0
	Gastric emptying in vagotomy-induced delayed gastric emptying model	
	Effective dose, mg/kg p.o.	0.25
Monkey	Motilin receptor binding assay (IC_{50}, nM)	13.3
	In vivo contractile activity in the fasting state	
	Effective dose, μg/kg i.v.	~24
	Gastric emptying	
	Effective dose, mg/kg i.v.	0.08
	Effective dose, mg/kg, p.o.	8.1

derivatives EM-523 and EM-574 (3). However, GM-611 appears to have some advantages over these compounds in that it is acid-stable, especially when orally administered to patients with delayed gastric motility. Some reports demonstrated that EMA was effective in patients with diabetic, idiopathic or postoperative gastroparesis by improving the delayed gastric emptying or motility. The efficacy of motilin agonists is generally thought to be more pronounced than existing gastroprokinetic agents, including metoclopramide, domperidone and cisapride. Another possible application is gastroesophageal reflux disease (GERD), although more data for this indication are needed.

Some papers suggested the presence of motilin receptors on colonic smooth muscle, indicating potential therapeutic application of motilin agonists in idiopathic constipation and irritable bowel syndrome (33, 34). Preliminary studies showed that EMA increased stool frequency and decreased colonic transit time when orally administered to healthy volunteers and patients with idiopathic constipation (33, 35). However, others reported that intravenously injected EMA failed to effectively stimulate colonic motility, except for the lowest dose investigated, in chronically constipated patients (36). These controversial results on EMA may be, at least in part, attributable to its antibacterial activity, low motilin agonist potency or overdoses. Thus, further research on newly developed EMA derivatives with higher activity and without antibacterial activity is encouraged.

Itoh *et al*. (37, 38) found that motilin stimulates the release of insulin and pancreatic polypeptides from the pancreas through the vagal-cholinergic muscarinic pathways in fasted dogs. They then investigated whether EMA also stimulates insulin secretion and consequently improves glycemic control in patients with type 2 (non-insulin-dependent) diabetes mellitus (39). Intravenously administered EMA stimulated insulin secretion and decreased blood glucose concentrations in both diabetic patients and normal subjects. The response was more pronounced in diabetic patients. Oral EMA (1200 mg/day t.i.d. for 1 week) significantly decreased fasting blood glucose concentrations and increased basal insulin secretion in diabetic patients. In the glucose tolerance test on the last day of treatment, EMA significantly augmented insulin secretion and decreased blood glucose levels. After 4 weeks of treatment with oral EMA (600 mg/day t.i.d.), significant improvements were observed in fasting blood glucose and HbA1c concentrations. The enhancement of basal and intravenous glucose-stimulated insulin secretion by EMA was especially evident in the first 20-30 min. These results suggest that EMA has the potential to become a new class of insulinotropic agent, because type 2 diabetic patients generally have a characteristic defect in initial insulin secretion and EMA improves the early insulin secretion. It is well known that early insulin secretion is very important for maintaining postprandial glucose levels within limits, preventing enhanced blood glucose and thus increased (but delayed) insulin response which might lead to diabetic complications. The short duration of action of EMA may also minimize the occurrence of severe hypoglycemia. Moreover, the potent gastroprokinetic activity of EMA could synergistically improve glycemic control in diabetic patients with delayed gastric emptying. However, studies to determine the effects of

EMA on the long-term control of diabetes should be done using EMA derivatives without antibacterial activity, due to the potential for the emergence of resistant bacterial strains during long-term use.

GM-611 is undergoing phase IIb clinical trials for the treatment of gastroparesis. Other possible applications, including gastroesophageal reflux disease and constipation, are also being investigated.

Summary

Based on the acid-decomposition mechanism of EMA, the acid-stable EMA derivative GM-611 was designed and selected as a candidate for further development and clinical trials. In binding studies, GM-611 as well as porcine motilin (pMTL) displaced ^{125}I-pMTL bound to homogenates of rabbit and monkey duodenal smooth muscle tissues, with similar displacement curves. In *in vitro* experiments, GM-611 induced concentration-dependent contractions in isolated longitudinal segments of rabbit duodenum. The contractile responses due to GM-611 were competitively inhibited by pretreatment with a selective motilin antagonist, GM-109, but not by pretreatment with atropine, tetrodotoxin, naloxone, hexamethonium or tropisetron. GM-611 was stable to acid and showed little antibacterial activity. GM-611 had potent motilin agonist activity and gastric emptying effects in normal animals as well as in delayed gastric emptying animal models in monkeys and dogs when given i.v. or p.o. GM-611 is in phase IIb clinical trials for the treatment of gastroparesis. Applications in other gastrointestinal disorders are also being studied.

Acknowledgements

The authors thank all of our colleagues Drs. T. Sato, K. Tsuzuki, N. Taka, K. Yogo, M. Ikuta, K. Ozaki, H. Kuboniwa, H. Onoda, M. Akima, H. Nabata, M. Kotoh, H. Sudo, M. Onoma, K. Kamei, and T. Dan for their invaluable contributions to this research program.

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