Pergamon

Bioorganic & Medicinal Chemistry Letters, Vol. 4, No. 11, pp. 1347-1352, 1994

0960-894X/94 $7.00+0.00

0960-894X(94)00154-5

POTENT, ACID-STABLE AND ORALLY ACTIVE MACROLIDE-TYPE MOTILIN RECEPTOR AGONISTS, GM-611 AND THE DERIVATIVES

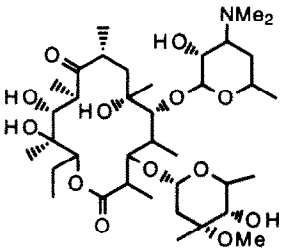
Hiroshi Koga,* Tsutomu Sato, Koichi Tsuzuki, Harumi Onoda, Hitoshi Kuboniwa, and Hisanori Takanashi

Fuji-Gotemba Research Laboratories, Chugai Pharmaceutical Co., Ltd., 1-135, Komakado, Gotemba, Shizuoka, 412, Japan

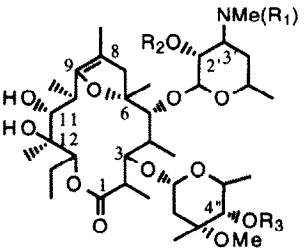
Abstract: Based on the acid decomposition mechanism of erythromycin A, 11-deoxy-12-*O*-methyl-11-oxo-8,9-anhydroerythromycin A 6,9-hemiacetals were designed and synthesized. GM-611 (**11**) and the derivatives **8** and **10** were acid-stable and showed potent *in vitro* and *in vivo* motilin agonistic activities, and these compounds were thought to be promising orally active prokinetic agents.

Motilin is a gastrointestinal peptide hormone of which physiological role is the induction of well-coordinated contractions through the gastrointestinal tract in the fasted state, called interdigestive migrating contractions. It has been recently shown that erythromycin A (**1**, EMA), a macrolide antibiotic, mimics the effects of motilin, and stimulates smooth muscle motilin receptors which are located at several levels of the mammalian gastrointestinal tract including human.[1] Clinical trials have suggested that EMA (**1**) is a promising prokinetic agent.[2] However, its instability to acid, antimicrobial activity, and low gastrokinetic activity appear to be serious drawbacks, especially when administered orally. It is known that under acidic conditions EMA (**1**) gives first an internal enolic ether **2** and then an internal ketal **3** by reaction of the 9-ketone group with hydroxyl groups in positions 6 and 12.[3] This ketal formation is irreversible and gastrointestinal smooth muscle contractile activity of the ketal **3** was lower than that of EMA (**1**) (Table I).[4] Since the intermediate **2**, however, exhibited higher motilin agonistic activity than EMA (**1**), structure-activity relationship study of **2** has been undertaken and led to EM-523 (**4**). EM-523 (**4**) was more active than EMA (**1**) and showed activity comparable to **2**, and was devoid of antibiotic activity while it remained labile to acid (Tables I and II, and Figure 1).[4] EM-523 (**4**) is currently undergoing clinical trials as a prokinetic agent.[5]

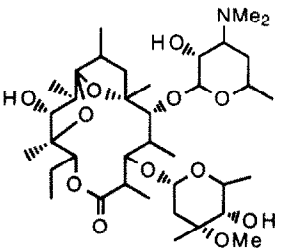
An obvious way to prevent this internal ketalization should be to mask the enol ether or the 12-hydroxyl group. Hydrogenation of the enol ethers (*e.g.*, **2** and **4**) gave the corresponding 8,9-dihydro compounds in low yields with many synthetic problems.[4,6] Although these compounds showed increased stability under acidic conditions as expected, the gastrointestinal smooth muscle contractile activities diminished.[4] We attempted to protect the 12-hydroxyl group by *O*-alkylation and found that the 12-*O*-methyl derivatives exhibited increased acid-stability and oral activity. We describe herein the motilin agonistic activity and acid-stability of 11-deoxy-12-*O*-methyl-11-oxo-8,9-anhydroerythromycin A 6,9-hemiacetals.



1

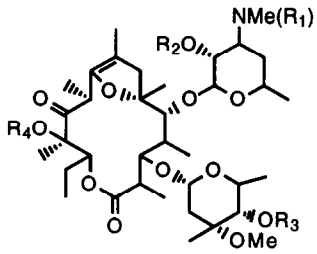


2 R_1=Me, R_2=R_3=H
4 R_1=Et, R_2=R_3=H
5 R_1=Me, R_2=Ac, R_3=CHO



3

First, in order to avoid concomitant methylation of the 11-hydroxyl group and translactonization under methylation and basic conditions,[4,7] known enol ether **5**[8] whose 2'- and 4"-hydroxyl groups were protected, was subjected to oxidation with dimethylsulfoxide, N,N'-dicyclohexylcarbodiimide, and pyridinium trifluoroacetate in CH_2Cl_2 at room temperature to give the 11-oxo compound **6** in 67% yield. Methylation of 12-hydroxyl group of **6** with methyl iodide and sodium hydride in N,N-dimethylformamide at 0 °C, followed by deprotection of the 2'- and 4"-hydroxyl protecting groups with sodium bicarbonate in MeOH-H_2O at room temperature led to 12-methoxy-11-oxo compound **8** *via* **7** in 54% yield from **6**. Reduction of 11-oxo group of **8** under usual conditions ($NaBH_4$ or $NaBH_3CN$ in MeOH) to obtain the corresponding 11-hydroxyl compound was unsuccessful. Selective demethylation of 3'-dimethylamino group of **8** proceeded smoothly by reaction with iodine and sodium acetate in MeOH-H_2O at 50 °C to give **9** in 74% yield.[4] Finally, introduction of ethyl and isopropyl groups to 3'-amino group of **9** was effected with ethyl and isopropyl iodides, respectively, in the presence of N,N-diisopropylethylamine in MeOH at 40-60 °C to afford N-ethyl and N-isopropyl derivatives, **10** and GM-611 (**11**) in 63 and 61% yields, respectively.[9]



6 R_1=Me, R_2=Ac R_3=CHO, R_4=H
7 R_1=R_4=Me, R_2=Ac, R_3=CHO
8 R_1=R_4=Me, R_2=R_3=H
9 R_1=R_2=R_3=H, R_4=Me
10 R_1=Et, R_2=R_3=H, R_4=Me
11 R_1=iso-Pr, R_2=R_3=H, R_4=Me

Table I. Motilin Receptor Binding and Contractile Activities of EMA Derivatives

	in vitro			*in vivo*	
compd	pIC_{50}[a]	pIC_{50}(HCl)[a,b]	pEC_{50}[c]	MI_{100} (i.v., μg/kg)[d]	MI_{100} (i.g., μg/kg)[d]
8	8.04±0.04	8.05±0.08	6.93±0.14	2.9±1.5	2.4±0.9
10	8.42±0.12	8.19±0.08	7.36±0.13	1.1±0.7	4.3±1.0
11	8.22±0.06	8.10±0.02	7.41±0.16	1.0±0.4	1.5±0.5
1	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	6.81±0.12	6.77±0.11	<5.0	>70	
4	8.50±0.06	6.52±0.16	7.32±0.10	0.9±0.3	14.9±4.9

[a]Negative logarithm of IC_{50} (M) with ± SEM (n = 3-4). See footnote 13 for experimental details. [b]Measured after treatment with hydrochloric acid solution (pH 2.5). [c]Negative logarithm of EC_{50} (M) with ± SEM (n = 3-6). See footnote 14 for experimental details. [d]Dose to give 100 of motor index (MI), with ± SEM (n = 3-5). See footnote 15 for experimental details.

Motilin agonistic activity of **8**, **10**, and **11** was tested in comparison with EM-523 (**4**) (Table I). Compounds **8**, **10**, and **11** showed motilin receptor binding (pIC_{50}) and *in vitro* and *in vivo* smooth muscle contractile (pEC_{50} and MI_{100} (i.v.)) activities almost comparable to or slightly less than EM-523 (**4**), suggesting that the 11- and 12-hydroxyl groups of **4** may not always be necessary to elicit the motilin agonistic activity.[4] The acid-stability of **8**, **10**, and **11** was evaluated by treatment with hydrochloric acid solution (pH 2.5) at room temperature for 2 hr, followed by assaying the solution for the motilin receptor binding. The binding affinity of **8**, **10**, and **11** was not altered by the acid-treatment, while **4** showed substantially reduced activity by the same treatment (Table I). These results suggest that the 12-methoxy

compounds **8**, **10**, and **11** may be acid-stable. The acid-stability of GM-611 (**11**) was also confirmed by comparing with that of EM-523 (**4**) under several pH conditions at 37 °C. EM-523 (**4**) was immediately and almost completely degraded within 15 min below pH 3.0, while GM-611 (**11**) was slowly degraded and there remained unchanged approximately 40% in pH 2.2 even after 6 hr (Figure 1).

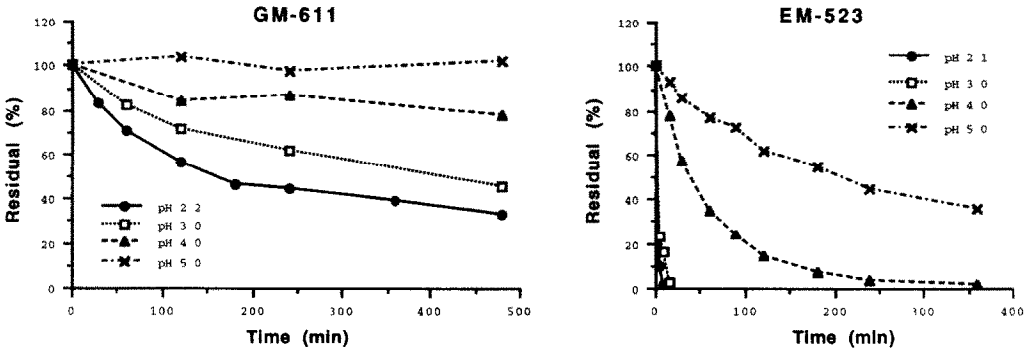


Figure 1. Stability of GM-611 (**11**) and EM-523 (**4**) in Acidic Solution at 37 °C[10]

The increased stability to acid of the 12-*O*-methyl-11-oxo derivatives seems to be of great advantage when administered orally. Compounds **8**, **10**, and **11** administered intragastrically (i.g.) exhibited almost the same degree of *in vivo* activities as those administered i.v., whereas EM-523 (**4**) required more than ten-fold dose to elicit similar *in vivo* activity when given i.g. compared to i.v. administration (Table I).

Compound **11** was inactive at concentrations up to 100 μM in binding studies at cholecystokinin CCK-A, CCK-B, serotonin 5-HT_{1a}, 5-HT_2, 5-HT_3, and dopamine D_2 receptors, and the *in vitro* contractile activity of **11**, as well as motilin and EM-523 (**4**),[11] was not affected by atropin (10^{-6} M).[12,14]

These 12-*O*-methyl-11-oxo derivatives **8**, **10**, and **11** showed weak or little antibiotic activity (Table II).

In conclusion, GM-611 (**11**) and the derivatives **8** and **10** are a novel series of potent, acid-stable and orally active macrolide-type motilin agonists. These biological profiles identify these compounds as potential candidates for useful prokinetic agents.

Table II. Antimicrobial Activity (MIC) of EMA Derivatives

compd	antibacterial activity: MIC,[a] μM/ml				
	B. subtilis ATCC 6633	*S. pneumoniae* No. 12	*S. aureus* 209P	*E. coli* NIHJ JC-2	*K. pneumoniae* IFO 3512
8	6.3	3.1	13	>200	100
10	200	50	>200	>200	>200
11	>200	200	>200	>200	>200
1	0.39	0.1	0.39	100	6.3
4	100	25	>200	>200	>200

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

References and Notes

(1) (a) Kondo, Y.; Torii, K.; Omura, S.; Itoh, Z. *Biochem. Biophys. Res. Commun.* **1988**, *150*, 877-882. (b) Peeters, T.; Matthijs, G.; Depoortere, I.; Cachet, T.; Hoogmartens, J.; Vantrappen, G. *Am. J. Physiol.* **1989**, *257*, G470-G474. (c) Itoh, Z.; Nakaya, M.; Suzuki, T.; Arai, H.; Wakabayashi, K. *Am. J. Physiol.* **1984**, *247*, G688-G694. (d) Tomomasa, T.; Kuroume, T.; Arai, H.; Wakabayashi, K.; Itoh, Z. *Dig. Dis. Sci.* **1986**, *31*, 157-161.

(2) Drenth, J. P. H.; Engels, L. G. J. B. *Drugs* **1992**, *44*, 537-553.

(3) (a) Kurath, P.; Jones, P. H.; Egan, R. S.; Perun, T. J. *Experientia* **1971**, *27*, 362. (b) Atkins, P. J.; Herbert, T. O.; Jones, N. B. *Int. J. Pharmaceut.* **1986**, *30*, 199-207.

(4) (a) Omura, S.; Tsuzuki, K.; Sunazuka, T.; Marui, S.; Toyoda, H. *J. Med. Chem.* **1987**, *30*, 1941-1943. (b) Tsuzuki, K.; Sunazuka, T.; Marui, S.; Toyoda, H.; Omura, S.; Inatomi, N.; Itoh, Z. *Chem. Pharm. Bull.* **1989**, *37*, 2687-2700.

(5) *Drugs Fut.* **1991**, *16*, 110-112.

(6) Klein, L. L.; Freiberg, L. A.; Kurath, P.; Henry, R. F. *J. Org. Chem.* **1993**, *58*, 3209-3212.

(7) Kirst, H. A.; Wind, J. A.; Paschal, J. W. *J. Org. Chem.* **1987**, *52*, 4359-4362.

(8) Tadanier, J.; Martin, J. R.; Egan, R. S.; Goldstein, A. W.; Stanaszek, R. S.; Hirner, E.; Fischer, F. *J. Org. Chem.* **1974**, *39*, 2495-2501.

(9) All new compounds were characterized by ^{1}H and ^{13}C NMR and high-resolution mass spectroscopy.

(10) GM-611 (**11**) and EM-523 (**4**) were dissolved in 50% acetonitrile at concentrations of 1 mg/ml, respectively. These solutions were diluted to 10 times with McIlvaine buffers of several pH and incubated at 37 °C. Samples were withdrawn at appropriate intervals, and were neutralized with

sodium hydroxide solution. Then the residual concentrations were determined by the high performance liquid chromatography (HPLC) method. The HPLC conditions were as follows; detector, ultraviolet absorption spectra; detector wavelength, 205 nm; column temperature, 40 °C; flow rate, 1 ml/min; column, A-212 (C8) (YMC CO., Ltd.) for GM-611 and A-312 (C18) (YMC CO., Ltd.) for EM-523; mobile phase, 0.05 M phosphate buffer (adjusted to pH 6.0) and acetonitrile (2:3) for GM-611 and 0.05 M phosphate buffer (adjusted to pH 6) and acetonitrile (1:1) for EM-523.

(11) Satoh, T.; Inatomi, N.; Satoh, H.; Marui, S.; Itoh, Z.; Omura, S. *J. Pharmacol. Exp. Ther.* **1990**, *254*, 940-944.

(12) Unpublished results.

(13) Motilin receptor binding studies were performed as previously described (Bormans, V.; Peeters, T. L.; Vantrappen, G. *Regul. Pept.* **1988**, *15*, 143-153). Briefly, the homogenate of rabbit small intestinal smooth muscle tissue was incubated with iodinated porcine motilin (Otsuka Pharmaceutical Co., specific activity >22.2 MBq/μg, final concentration 25 pM) in Tris-buffer for 120 min. The reaction was stopped by adding cold incubation buffer and membrane-bound motilin was separated by centrifugation. All data were corrected for nonspecific binding. Displacement studies were performed by adding increasing amount of compound and IC_{50} value of each compound was determined. Each compound was dissolved in DMSO or hydrochloric acid solution (pH 2.5), and then left for 2 hr at room temperature before experiments.

(14) Contractile activity *in vitro* was measured in the rabbit duodenum preparation as previously reported.[11] Muscle strips (5 x 20 mm) from rabbit duodenum were mounted along their longitudinal axes in organ baths containing Krebs' solution kept at 28 °C and bubbled continuously with 5% CO_2 and 95% O_2. Isotonic contractions of strips were recorded by means of isotonic transducers, which were prelorded with 1 g. Each compound was added cumulatively to the organ bath and contractions were expressed as percentage of that induced by acetylcholine (10^{-4} M), and EC_{50} value was determined. The maximum contractile responses of compounds tested were almost the same as that of motilin.[11] Atropin (10^{-6} M) was added to the organ bath 5 min before challenging with each compound.

(15) Contractile activity *in vivo* was measured by means of chronically implanted force transducers on the serosa of the gastrointestinal tract positioned to record circular muscle contraction in the gastric antrum and the small intestine in fasted concious dogs (Itoh, Z.; Takeuchi, S.; Aizawa, I.; Takayanagi, R. *Am. J. Dig. Dis.* **1978**, *23*, 229-238). Each compound was administered intravenously (i.v.) or intragastrically (i.g.) about 15 min after the termination of interdigestive contractions in the stomach. To measure motility quantitatively, the area of contractions of the stomach induced by compound was calculated by a personal computer and used as the motor index (MI) (Inatomi, N.; Satoh, H.; Maki, Y.; Hashimoto, N.; Itoh, Z.; Omura, S. *J. Pharmacol. Exp. Ther.* **1989**, *251*, 707-712). The dose of each compound to give 100 of MI was determined.

(*Received in USA* 5 *November* 1993; *accepted* 25 *April* 1994)