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MACROLIDE-TYPE MOTILIN RECEPTOR AGONISTS: ASSESSMENT OF THE BIOLOGICAL VALUE OF THE 2'- AND 4"-HYDROXYL GROUPS OF ACID-STABLE 8,9-ANHYDROERYTHROMYCIN A 6,9-HEMIACETALS

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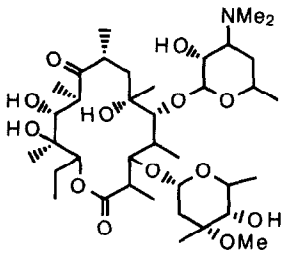
Abstract: In order to evaluate the biological significance of the 2'- and 4"-hydroxyl groups of the acid stable 11-deoxy-12-*O*-methyl-11-oxo-8,9-anhydroerythromycin A 6,9-hemiacetals **5-7**, the 2'- and 4"-deoxy **8-11** were prepared and tested for motilin agonistic activity. It has been shown that the 4"-hydroxyl group is not a major contributor to the bioactivity, while the 2'-hydroxyl group is a mandatory one.

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 enol 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 the motilin agonistic activity of the ketal **3** was lower than that of EMA (**1**).[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.[4] EM-523 (**4**) is currently undergoing clinical trials as a prokinetic agent.[5]

Previous work from our laboratories was presented describing the preparation of 11-deoxy-12-*O*-methyl-11-oxo-8,9-anhydroerythromycin A 6,9-hemiacetals **5-7** designed based on the acid-decomposition mechanism of EMA (**1**).[6] These molecules showed increased acid-stability and oral activity as expected and were thought to have potential for orally active prokinetic agents. It is clearly desirable to further evaluate the biological significance of various structural features of these molecules. The subsequent follow-up study revealed the contribution of the sugar hydroxyl groups to the bioactivity. We now report the preparation and motilin agonistic activity of the 2'- and 4"-deoxy compounds **8-11**.



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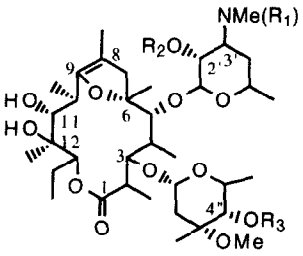
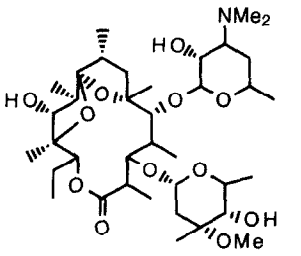
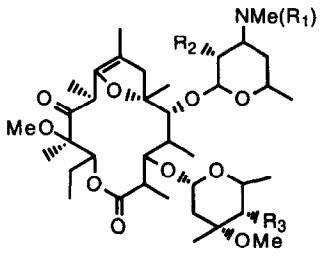


2 R_1=Me, R_2=R_3=H
4 R_1=Et, R_2=R_3=H



3

First, the 2'-hydroxyl group of compound **7** was acetylated with acetic anhydride and pyridine in CH_2Cl_2 at room temperature, followed by treatment with 1,1'-thiocarbonyldiimidazole and 4-dimethylaminopyridine in CH_2Cl_2 at room temperature to produce **12** (yield 58%). Reaction of **12** with triphenyltin hydride and 2,2'-azobisisobutyronitrile (AIBN) in toluene at refluxing temperature and then hydrolysis of the 2'-acetoxy group with sodium bicarbonate in MeOH-H_2O at room temperature gave the deoxy compound **10** (yield 46%).[7] Next, compound **10** was treated with iodine and sodium acetate in MeOH-H_2O at 55 °C to produce the N-methyl compound **13** (yield 21%).[4] Reductive N-methylation of **13** with 10% Pd/C and aq. HCHO in AcOH-EtOH with 1 atm H_2 afforded the N,N-dimethyl compound **8** (yield 74%). Introduction of ethyl group to the 3'-amino group of **13** was effected with ethyl iodide in the presence of N,N-diisopropylethylamine in MeOH at room temperature to give **9** (yield 51%). The 2'-hydroxyl group of **10** was converted to the xanthate **14** (yield 36%) by the usual way (NaH, imidazole, CS_2, MeI, THF) and deoxygenated (Ph_3SnH, AIBN, PhMe) to give the 2'- and 4"-dideoxy compound **11** (yield 50%).[7,8]



5 R_1=Me, R_2=R_3=OH
6 R_1=Et, R_2=R_3=OH
7 R_1=iso-Pr, R_2=R_3=OH
8 R_1=Me, R_2=OH, R_3=H
9 R_1=Et, R_2=OH, R_3=H
10 R_1=iso-Pr, R_2=OH, R_3=H
11 R_1=iso-Pr, R_2=R_3=H
12 R_1=iso-Pr, R_2=OAc, R_3=OC(S)-(1-imidazolyl)
13 R_1=H, R_2=OH, R_3=H
14 R_1=iso-Pr, R_2=OC(S)SMe, R_3=H

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	8.64±0.14	8.41±0.02	7.98±0.22	0.4±0.3	1.2±0.3
9	8.46±0.05	8.42±0.10	8.04±0.11	0.3±0.07	0.2±0.2
10	8.60±0.10	8.75±0.08	8.21±0.06	0.09±0.01	0.3±0.09
11	8.73±0.18		6.68±0.34	>70	
4	8.50±0.06	6.52±0.16	7.32±0.10	0.9±0.3	14.9±4.9
5	8.04±0.04	8.05±0.08	6.93±0.14	2.9±1.5	2.4±0.9
6	8.42±0.12	8.19±0.08	7.36±0.13	1.1±0.7	4.3±1.0
7	8.22±0.06	8.10±0.02	7.41±0.16	1.0±0.4	1.5±0.5
motilin	9.31±0.13		8.34±0.06	0.05±0.005	

[a]Negative logarithm of IC_{50} (M) with ± SEM (n = 3-4). See footnote 9 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 10 for experimental details. [d]Dose to give 100 of motor index (MI), with ± SEM (n = 3-5). See footnote 11 for experimental details.

Motilin agonistic activity of **8-11** was evaluated in comparison with EM-523 (**4**), **5-7**, and motilin (Table I). The 4"-deoxy compounds **8-10** showed increased motilin receptor binding (pIC_{50}) and *in vitro* and *in vivo* smooth muscle contractile (pEC_{50} and MI_{100} (i.v.)) activities compared to EM-523 (**4**) and the corresponding 4"-hydroxyl compounds **5-7** , suggesting that the 4"-hydroxyl group may not always be necessary to elicit the motilin agonistic activity. The *in vitro* and *in vivo* contractile activities of the most active **10** were almost comparable to those of motilin. On the other hand, the 2'- and 4"-dideoxy compound **11** greatly reduced the contractile activities *in vitro* and *in vivo*, though the binding affinity remained almost unchanged. The acid-stability of compounds 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**, as well as **5-7**,[6] was not altered by the acid-treatment, while EM-523 (**4**) showed substantially reduced activity by the same treatment (Table I). These results suggest that the 12-methoxy compounds **8-10** may be acid-stable.

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**, as well as **5-7**,[6] 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).

These 4"-deoxy compounds **8-10**, as well as **5-7**,[6] showed weak or little antibiotic activity (Table II).

In summary, we have been able to show that the 4"-hydroxyl group is not a major contributor to motilin agonistic activity of the 11-deoxy-12-*O*-methyl-11-oxo-8,9-anhydroerythromycin A 6,9-hemiacetals, while the 2'-hydroxyl group is a mandatory one. The 4"-deoxy GM-665 (**10**) and the derivatives **8** and **9** 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	50	1.6	100	>200	100
9	200	100	>200	>200	>200
10	200	100	>200	>200	>200
1	0.39	0.1	0.39	100	6.3
4	100	25	>200	>200	>200
5	6.3	3.1	13	>200	100
6	200	50	>200	>200	>200
7	>200	200	>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.

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(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.

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

(9) 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.

(10) Contractile activity *in vitro* was measured in the rabbit duodenum preparation as previously reported (Satoh, T.; Inatomi, N.; Satoh, H.; Marui, S.; Itoh, Z.; Omura, S. *J. Pharmacol. Exp. Ther.* **1990**, *254*, 940-944). 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) 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.

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