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MACROLIDE-TYPE MOTILIN RECEPTOR AGONISTS: ACID-STABLE 12-*O*-METHYL-8,9-ANHYDROERYTHROMYCIN A 6,9-HEMIACETALS

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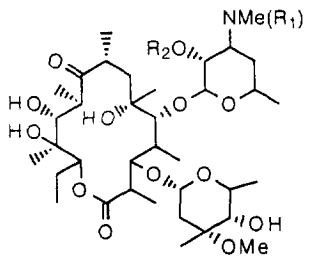
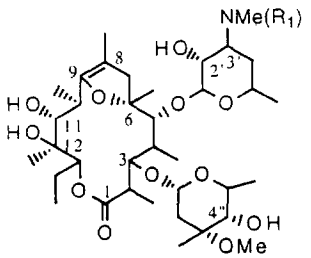
Abstract: Based on the acid decomposition mechanism of erythromycin A, 12-*O*-methyl-8,9-anhydroerythromycin A 6,9-hemiacetals were designed and synthesized. These compounds were acid-stable and showed potent *in vitro* and *in vivo* motilin agonistic activities, and were thought to be promising orally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

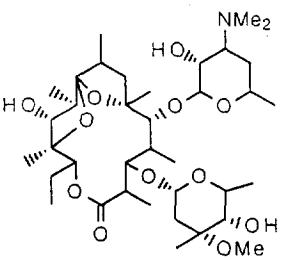
Motilin is a gastrointestinal peptide hormone that induces contractions of the gastrointestinal tract. It has been recently shown that erythromycin A (**1**, EMA), a macrolide antibiotic, is a motilin receptor agonist.[1] Thus, although EMA (**1**) is expected to be a promising prokinetic agent,[2] its instability to acid, antimicrobial activity, and low gastrokinetic activity appear to be serious drawbacks for its application to clinical trials. 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).[4] EM-523 (**4**) is currently undergoing clinical trials as a prokinetic agent.[5]



1 R_1=Me, R_2=H
8 R_1=R_2=$COOCH_2Ph$

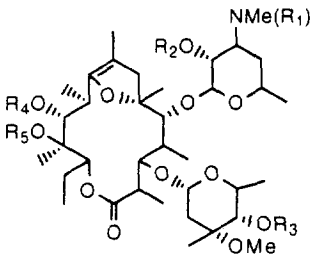


2 R_1=Me
4 R_1=Et



3

We previously designed acid-stable motilin receptor agonists 11-deoxy-12-*O*-methyl-11-oxo-8,9-anhydroerythromycin A 6,9-hemiacetals, based on the acid decomposition mechanism of EMA (**1**).[6] Herein we wish to report the design, synthesis, and biological activity of the 11-hydroxy-12-*O*-methyl analogues **5-7**.



5 R_1=R_5=Me, R_2=R_3=R_4=H
6 R_1=Et, R_2=R_3=R_4=H, R_5=Me
7 R_1=iso-Pr, R_2=R_3=R_4=H, R_5=Me
9 R_1=R_2=$COOCH_2Ph$, R_3=R_4=R_5=H
10 R_1=R_2=R_3=$COOCH_2Ph$, R_4=R_5=H
11 R_1=R_2=R_3=$COOCH_2Ph$, R_4=CH_2Ph, R_5=H
12 R_1=R_2=R_3=$COOCH_2Ph$, R_4=CH_2Ph, R_5=Me
13 R_1=R_2=R_3=H, R_4=CH_2Ph, R_5=Me
14 R_1=R_5=Me, R_2=R_3=H, R_4=CH_2Ph
15 R_1=Et, R_2=R_3=H, R_4=CH_2Ph, R_5=Me
16 R_1=iso-Pr, R_2=R_3=H, R_4=CH_2Ph, R_5=Me

Compounds **5-7** were prepared via the 11-*O*-benzyl protected intermediates, in order to avoid concomitant methylation of the 11-hydroxyl group and translactonization under methylation and basic conditions.[4,7] Compound **8**[8] was treated with acetic acid at room temperature to give the enol ether **9** in 99% yield. The 4"- and 11-hydroxyl groups of **9** were successively protected by the reaction with benzyloxycarbonyl chloride in the presence of 4-dimethylaminopyridine, followed by the reaction with benzyl bromide and NaH in *N,N*-dimethylformamide (DMF) to afford **11** (41%) through **10** (83%). Methylation of 12-hydroxyl group of **11** with MeI and NaH in DMF gave the 12-*O*-methyl compound **12** in 53% yield. Reduction of **12** with 10% Pd/C and 1 atm H_2 in MeOH produced **13** in 90% yield. Reductive *N*-methylation ($NaBH_3CN$, aq. HCHO, MeOH), *N*-ethylation (EtI, $(iso\text{-}Pr)_2NEt$, MeOH), and *N*-isopropylation (iso-PrI, $(iso\text{-}Pr)_2NEt$, MeOH) of **13** gave the *N*-alkylated compounds **14** (76%), **15** (53%), and **16** (80%), respectively. The 11-*O*-benzyl group of **14-16** was successfully removed by reduction with 10% Pd/C and 1 atm H_2 in the presence of trifluoroacetic acid in MeOH to obtain the desired products **5** (55%), **6** (73%), and **7** (67%).[9]

Motilin agonistic activity of **5-7** was tested in comparison with EM-523 (**4**) (Table I). Compounds **5-7** 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 EM-523 (**4**), suggesting that the 12-hydroxyl group of **4** may not always be necessary to elicit the motilin agonistic activity.[4] The acid-stability of **5-7** 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 **5-7** was little altered by the acid-treatment as expected, while **4** showed substantially reduced activity by the same treatment (Table I). These results suggest that the 12-methoxy compounds **5-7** may be acid-stable. The increased stability to acid of the 12-*O*-methyl derivatives seems to be of great advantage when administered orally. Compound **7** when administered intragastrically (i.g.) was 10-fold more potent than EM-523 (**4**) (Table I).

These 12-*O*-methyl derivatives **5-7** showed weak or little antibiotic activity (Table II).

Thus, compound **7** (GM-652) and the derivatives **5** and **6** are a novel class of potent, acid-stable and orally active macrolide-type motilin agonists. These biological profiles identify the compound as a potential candidate for useful prokinetic agent.

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]
5	8.12±0.05	7.40±0.08	7.71±0.11		
6	8.33±0.11	8.08±0.23	7.64±0.11		
7	8.16±0.07	7.82±0.02	7.89±0.07	0.44±0.12	1.2±0.57
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 10 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 11 for experimental details. [d]Dose to give 100 of motor index (MI), with ± SEM (n = 3-5). See footnote 12 for experimental details.

Table II. Antimicrobial Activity (MIC) of EMA Derivatives

	antibacterial activity: MIC,[a] μM				
compd	*B. subtilis* ATCC 6633	*S. pneumoniae* No. 12	*S. aureus* 209P	*E. coli* NIHJ JC-2	*K. pneumoniae* IFO 3512
5	6.3	1.6	25	>200	200
6	>200	50	>200	>200	>200
7	>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

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(9) All new compounds were characterized by ^{1}H and ^{13}C NMR and FAB-MS.

(10) 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.

(11) 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.

(12) 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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