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Design, synthesis, and anti-*Helicobacter pylori* activity of erythromycin A (*E*)-9-oxime ether derivatives

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Abstract—The synthesis and anti-*Helicobacter pylori* (*H. pylori*) activity evaluation of a new series of erythromycin A (*E*)-9-oxime ether derivatives are described. These compounds exhibited comparable in vitro anti-*H. pylori* activity and improved acid stability compared to the reference compound clarithromycin.

The Gram-negative bacterium *Helicobacter pylori* can infect the stomach in childhood and cause lifelong chronic gastritis, which can lead to peptic ulcer disease and gastric cancer.[1] Although a number of novel and potent anti-*H. pylori* agents including antibiotics, proton-pump inhibitors, and H_2-receptor antagonists have been developed, their application to the infection is very limited. Only a small number of multi-drug therapies such as proton-pump inhibitors or H_2-receptor antagonists with antibiotics have been used for eradication of *H. pylori*.[2,3] Dual treatments combining clarithromycin, a potent anti-*H. pylori* agent derived from erythromycin, with other agents were most popular because of their powerful efficacy in eradicating *H. pylori*.[4] The major problem associated with clarithromycin, however, is its acid instability, leading to degradation in the stomach.[5] Another erythromycin-derived antibiotic roxithromycin is known to have good pharmacokinetic character owing to its stability under gastric acid condition. From the structural points of view, clarithromycin has a methoxy group at the C-6 position, and roxithromycin has an oxime methoxyethoxymethyl (MEM) ether group at the C-9 position. These two functional groups are known to affect their conformation and increase their stability in acidic solution.[6] Based on these structural information, a new series of analogues **I** and **II** that combines the structural characteristics of both clarithromycin and roxithromycin was designed (Fig. 1). It was expected that the compounds **I** and **II** would show synergistic effects such as comparable anti-*H. pylori* activity to and higher acid stability than clarithromycin.

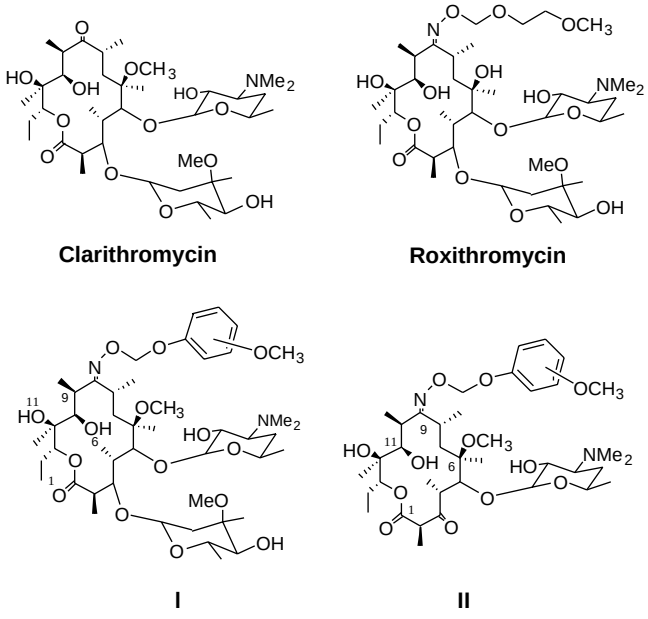

Figure 1. Erythromycin A derivatives.

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Herein, the synthesis and anti-*H. pylori* activity of new erythromycin A (*E*)-oxime ether derivatives represented by the structures **I** and **II**, and their stability under acidic condition are described.

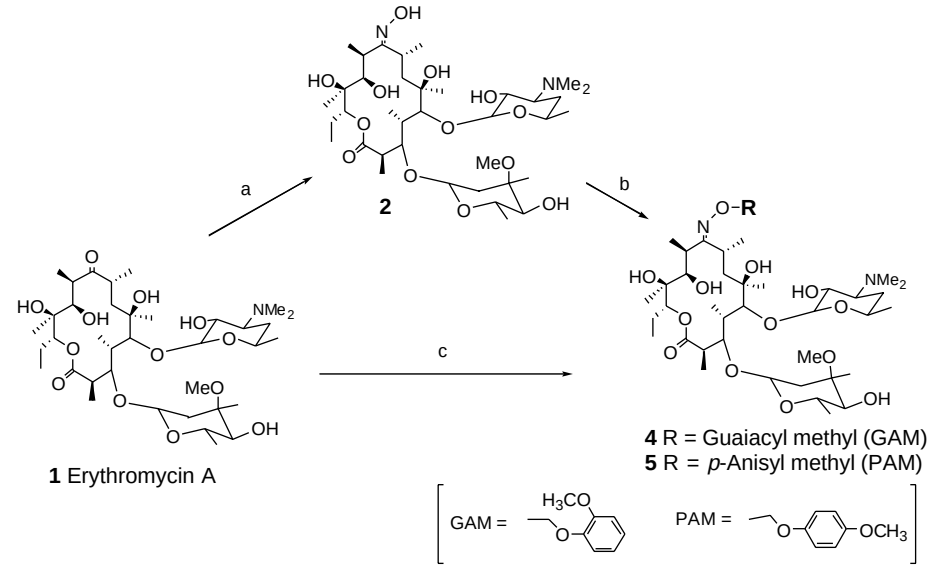
Erythromycin A (*E*)-oxime ether derivatives were synthesized via several stages. The first stage was the 9-oxime ether formation to yield the compounds **4** and **5** (Scheme 1). It could be performed via two reaction routes. One was the direct condensation of the corresponding alkoxyamines **8** and **9** with the 9-carbonyl group of the erythromycin A, and the other was the formation of the known compound oxime **2**[7] followed by the oxime ether formation with the corresponding alkyl chlorides **3**. The alkyl chlorides **3** and the alkoxyamines **8** and **9** could easily be prepared from dimethoxybenzenes by general synthetic methods illustrated in Scheme 2.

The second stage was the selective methylation of 6-hydroxy group (Scheme 3). After silylation of the hydroxy groups on the sugar rings of the compounds **4** and **5**, the resulting compounds **10** and **11** were subjected to methylation using methyl iodide and potassium hydroxide in a mixed solvent of DMSO and THF. A number of previous studies on selective methylation had already been reported discussing the difficulty in controlling such reactions.[8] Upon tuning the equivalents of the reagents, the best result was obtained by using 2.0 equiv potassium hydroxide and 2.5 equiv methyl iodide at 0 °C (Table 1). Removal of the trimethylsilyl group with ethanolic formic acid gave 6-O-methylated compounds **14** and **15** in good yields.

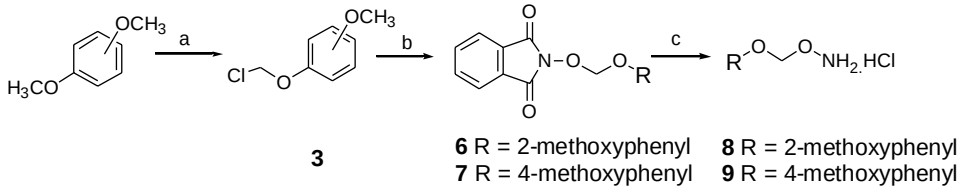
Further reaction of **14** and **15** was carried out to produce ketolide derivatives **20** and **21** (Scheme 3). Hydrolysis of the cladinose with 2 N hydrochloric acid followed by acetylation of the 2′-hydroxy group with acetic anhydride gave 3-hydroxy intermediates **16** and **17**. Oxidation of the 3-hydroxy group was efficiently performed with John's reagent, and deprotection of the 2′-acetyl group with methanol gave the corresponding ketolides **20** (51%) and **21** (55%). All new compounds were purified by flash column chromatography and characterized by ^{1}H NMR, ^{13}C NMR, and MS.[9]

The erythromycin A (*E*)-9-oxime ether derivatives **4**, **5**, **14**, **15**, **20**, and **21** were tested for in vitro antimicrobial activity against *H. pylori*.[10] The activity results are summarized in Table 2.

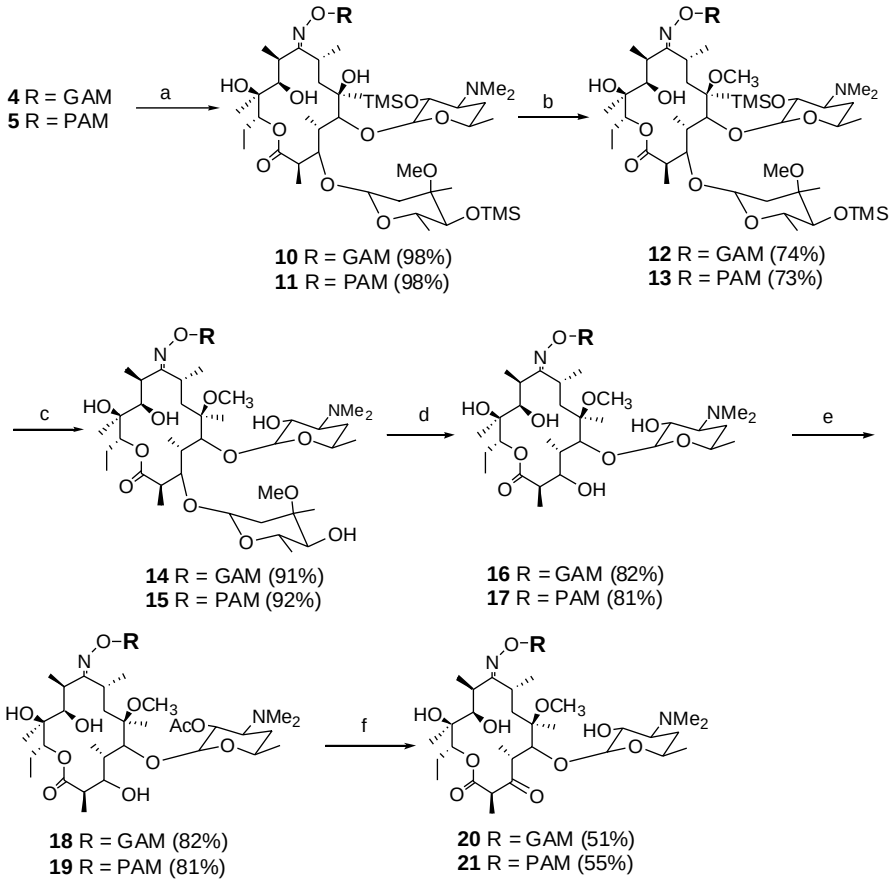
All of the compounds obtained by introducing guaiacylmethyl (GAM) or *p*-anisylmethyl (PAM) moiety at the 9-oxime group and a methyl group at the 6-hydroxy group exhibited potent in vitro activity with the MICs ranging from 0.02 to 0.04 μg/ml, which were equivalent



Scheme 1. Reagents and conditions: (a) hydroxylamine hydrochloride, TEA, MeOH, reflux, 68%; (b) **3** (GAM-Cl or PAM-Cl), K_2CO_3, DMF/ether (1/3), 88%; (c) **8** (GAM-ONH_2) or **9** (PAM-ONH_2), MeOH, 43%.



Scheme 2. Reagents and conditions: (a) PCl_5, benzoyl peroxide, CCl_4, reflux, 82%; (b) *N*-hydroxysuccinimide, K_2CO_3, DMF, 66%; (c) i—hydrazine, EtOH, reflux; ii—HCl, 63%.



Scheme 3. Reagents and conditions: (a) HMDS, NH_4Cl, DMF, 50 °C; (b) methyl iodide, KOH, DMSO/THF (1/1); (c) formic acid, 50% aq EtOH; (d) 2 N HCl, rt; (e) Ac_2O, K_2CO_3, acetone; (f) John's reagent, acetone, rt.

Table 1. Optimization of selective methylation conditions for the compounds **10** and **11**

Reactant	KOH (equiv)	CH_3I (equiv)	Temp (°C)	Time (h)	Area % of HPLC[a]		
					6-OH	6-OCH_3	Di-OCH_3
10	1.1	1.3	−5	2.0	88.6	11.4	—
10	1.1	1.3	0	2.0	86.8	13.1	—
10	1.1	1.3	5	2.0	81.3	16.7	2.0
10	1.1	2.0	0	5.0	15.4	28.4	59.5
10	1.5	2.0	0	4.0	19.3	80.6	
10	2.0	2.5	0	2.0	0.5	94.5	—
11	2.0	2.5	0	0.5	14.2	85.8	
11	2.0	2.5	0	2.0	7.6	90.3	2.1

[a] Waters 2487 Model. Detector; UV = 210 nm. Eluent; MeOH/H_2O (0.04% ethanolamine) = 96/4.

Table 2. MICs of the compounds against *Helicobacter pylori* NCTC10638

Compound	*H. pylori* NCTC10638 MIC (μg/ml)
4	0.03
5	0.02
14	0.02
15	0.03
20	0.04
21	0.02
Clarithromycin	0.02

to the reference clarithromycin (CAM). The inhibitory activity of the compounds against *H. pylori* was little affected by the position of the methoxy group on the phenyl ring.

The acid stability of the new erythromycin A (*E*)-9-oxime ether derivatives was examined at pH 1 by HPLC analysis.[11] The results are summarized in Table 3.

Except for the oxime compounds **4** and **5**, all the other compounds were more stable than the reference CAM. The compounds **14** and **15** remained intact 15 times more than CAM after 1 h at pH 1. The ketolide derivatives **20** and **21** were far more stable than CAM. Over 60% of the compounds remained unaffected after 3 h. The excellent acid stability of the ketolides could be

Table 3. HPLC area percent of residual compounds at pH 1

Time (h)	Area % of HPLC[a]						
	4	**5**	**14**	**15**	**20**	**21**	CAM
SM[b]	92.32	96.33	96.11	81.94	95.75	96.21	92.39
0[c]	82.42	68.17	67.00	74.03	80.91	82.46	81.95
0.5	29.21	23.63	56.04	56.59	73.53	76.84	29.92
1.0	2.60	6.51	46.09	46.61	69.31	69.27	3.12
1.5	1.00	—	26.01	28.31	65.43	65.17	1.10
3.0	1.00	—	15.98	16.17	60.78	61.03	—

[a] Eluent: MeOH (650)/.067 M KH_2PO_4 (350) pH 4.0; column: Shiseido, CAPCELL PAK, RP-^{18}C, 5 μm, 4.6 × 250 mm; UV detector wavelength: 210 nm, 245 nm.
[b] SM means the purity of the compounds checked by HPLC.
[c] Checked right after the complete dissolution of the samples in pH 1 buffer solution (0.2 M KCl/0.2 M HCl).

understood by the absence of the acid-labile 6-cladinose moiety in their structures.

In summary, a new series of erythromycin A 9-(*E*)-oxime ether derivatives **4**, **5**, **14**, **15**, **20**, and **21** was designed and synthesized. The compounds demonstrated potent anti-*H. pylori* activity and good stability under acidic condition. This series of compounds, especially the ketolides **20** and **21**, seems to be a good starting point for the development of novel and effective anti-*H. pylori* agents.

Acknowledgment

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9. Spectral data for **5**: ^{1}H NMR ($CDCl_3$, 300 MHz): δ 0.84 (t, J = 7.24 Hz, 3H, 15-CH_3), 0.97 (d, J = 6.88 Hz, 3H, 19-CH_3), 1.07–1.27 (m, 25H), 1.34 (s, 3H, 18-CH_3), 1.55 (m, 5H), 1.99 (m, 2H), 2.77 (s, 6H, $N(CH_3)_2$), 2.34 (m, 2H, 2″-Hex), 2.65 (dd, J = 13.6 Hz, 6.48 Hz, 1H, 4″-H), 2.88 (m, 1H, 3′-H), 3.01 (t, J = 9.14 Hz, 1H), 7.3 (s, 1H), 3.21 (dd, J = 10.1 Hz, 7.3 Hz, 1H, 2′-H), 3.31 (s, 3H, 3″-OCH_3), 3.40–3.50 (m, 2H, 5-H, 8-H), 3.62 (m, 1H, 5′-H), 3.77 (s, 3H), 3.98 (m, 2H, 5″-H, 3-H), 4.25 (s, 1H, 11-H), 4.37 (d, 1H, J = 7.21 Hz, 1′-H), 4.89 (d, 1H, J = 7.50 Hz, 1″H), 5.15 (dd, 2H, J = 10.8 Hz, 1.59 Hz, 13-CH_2), 5.52 (dd, 2H, J = 27.9 Hz, 7.19 Hz, ph-O-CH_2O), 6.83 (m, 3H), 6.95 (m, 2H). ^{13}C NMR ($CDCl_3$, 75.5 MHz): δ 9.52, 11.04, 14.93, 16.53, 16.62, 18.97, 21.50, 21.77, 21.88, 27.17, 27.51, 29.02, 33.59, 35.43, 37.95, 39.32, 40.65, 65.79, 65.86, 69.19. 70.58, 71.33, 73.04, 74.66, 75.31, 77.23, 80.44, 83.70, 95.86, 96.67, 103.42, 115.00, 115.05, 118.82, 150.80, 155.47, 174.31, 175.57. MS (FAB) 886^+ (M^++1).
Spectral data for **15**: ^{1}H NMR ($CDCl_3$, 300 MHz): δ 0.74 (m, 6H), 1.19 (m, 40H), 1.74 (m, 2H), 2.12 (s, 6H, $N(CH_3)_2$), 2.41 (m, 1H), 2.78 (s, 3H, 6-OCH_3), 3.30 (m, 2H), 3.47 (m, 3H), 3.60 (s, 3H, ph-OCH_3), 3.84 (m, 1H), 4.18 (m, 1H), 4.25 (m, 1H), 4.76 (br s, 1H), 4.93 (d, J = 10.4 Hz, 1H), 5.37 (m, 2H), 6.64 (m, 4H). ^{13}C NMR ($CDCl_3$, 75.5 MHz): δ 8.20, 9.76, 14.17, 14.60, 17.72, 19.19, 19.66, 21.00, 21.30, 26.23, 27.72, 32.34, 33.99, 36.51, 38.10, 39.39, 44.16, 48.60, 49.97, 54.75, 64.62, 64.73, 66.90, 67.76, 68.92, 70.11, 71.78, 73.10, 75.93, 77.07, 77.41, 77.71, 79.41, 95.13, 101.84, 113.72, 116.94, 150.36, 153.86, 171.75, 174.80. MS (FAB): 972^+ (M^+).
Spectral data for **21**: ^{1}H NMR ($CDCl_3$, 300 MHz): δ 0.82 (t, J = 7.07 Hz, 3H), 0.92 (d, J = 6.71 Hz, 3H), 1.13 (d, J = 6.68 Hz, 3H), 1.22 (m, 25H), 2.01 (m, 1H), 2.26 (s, 6H, $N(CH_3)_2$), 2.52 (s, 3H, 6-OCH_3), 2.57 (d, J = 9.89 Hz, 2H), 3.15 (m, 2H), 3.53 (m, 3H), 3.75 (s, 3H, ph-OCH_3), 3.81 (m, 2H), 4.26 (m, 3H), 5.15 (d, J = 9.91 Hz, 1H), 5.52 (dd, J = 20.52 Hz, 7.58 Hz, 2H), 6.77 (dd, J = 37.87 Hz, 8.81 Hz, 4H). ^{13}C NMR ($CDCl_3$, 75.5 MHz):δ 11.19, 14.82, 15.00, 15.52, 16.73, 18.95, 20.18, 21.76, 22.01, 27.57, 28.77, 33.78, 38.47, 40.77, 47.00, 50.16, 51.42, 56.17, 66.35, 69.96, 70.45, 70.83, 74.26, 78.19, 78.56, 95.71, 103.95, 114.96, 118.02, 151.65, 155.22, 169.90, 172.55, 205.96. MS (FAB): 739^+ (M^+).
10. The strain is *H. pylori* NCTC10630 obtained from the Seoul National University medical center.
11. Percent of remaining compounds after stirring the corresponding compound at pH 1 was analyzed. The sample (1 mg) was dissolved in 1 ml of pH 1 buffer solution (0.2 M KCl/0.2 M HCl) and stirred. After proper time periods, 10 μl aliquots were sampled, filtered through a membrane filter, and injected for HPLC analysis. Elution solvent was 0.067 M phosphate buffer solution in methanol, which was adjusted to pH 4.0. Reverse-phase ^{18}C column was used and the dual detector wavelength was 210 and 245 nm.