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# 3-Keto-9-*O*-substituted Oxime Derivatives of 6-*O*-Methyl Erythromycin A

## Synthesis and *In Vitro* Activity

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A series of 3-keto-9-*O*-substituted oxime derivatives of 6-*O*-methyl erythromycin A were prepared with a novel synthetic route, which include 6 reaction steps—oximation, protection, hydrolysis, oxidation, deprotection and addition. The antibacterial activity of these compounds were tested *in vitro* against both erythromycin-susceptible and erythromycin-resistant organisms. Several of these derivatives showed improved antibacterial activity against some erythromycin-resistant organisms as compared to erythromycin A.

Erythromycin A and related compounds, such as clarithromycin, roxithromycin, azithromycin and so on, have poor efficacy against macrolide-resistant bacteria, because organisms with the inducible or constitutive type of cross-resistance to macrolide, lincosamide and streptogramin B (MLS) antibiotics are prevalent. To address this problem, increased effort has gone into developing new macrolides with better activity against these organisms.

Recently, a series of ketolides, in which the 3-cladinosyl sugar residue was replaced by a ketone functionality, were prepared. These ketolides had good *in vitro* and *in vivo* activity against erythromycin-susceptible Gram-positive organisms, as well as against erythromycin-resistance organisms. The discovery that the cladinose moiety of erythromycin was not absolutely necessary for good antibacterial activity[1] has opened up new areas on the macrolactone ring for SAR exploration. We decided to prepare a compound having an oxime group directly attached to the macrolactone ring at C-9, thus, generating a series of 9-oxime derivatives of the ketolide.

### Chemistry

In this article, we synthesize 3-keto-9-*O*-substituted oxime derivatives of 6-*O*-methyl erythromycin with a novel method. 6-*O*-methyl erythromycin A (**1**) reacted with hydroxylammonium chloride to afford 6-*O*-methyl erythromycin 9-oxime (**2**). **2** reacted with benzyl bromide in the presence of sodium hydride at room temperature to prepared compound (**3**), the benzyl group protected the hydroxyl group at the 2′-position and 9-oxime, and the dimethylamine at the 3′-position at the same time. **3** was treated with 1% hydrochloric acid in methanol to give compound (**4**). **4** was treated with 1-ethyl-3-(3-dimethyl-aminopropyl)-carbodiimide hydrochloride (EDAC) and dimethyl sulfoxide in the presence of pyridinium trifluoroacetate to prepare compound (**5**)[2,3]. These protective groups were removed from **5** *via* catalytic hydrogenation to give compound (**6**).

In this synthetic route, compound **6** is an important intermediate. Alkylating or arylating the 9-oxime hydroxyl group of the compound **6** can give a series of 3-keto-9-*O*-substituted oxime derivatives of 6-*O*-methyl erythromycin A, such as compounds **7**~**9**.

### *In Vitro* Antibacterial Activity

The 3-keto-9-*O*-substituted oxime derivatives of 6-*O*-methyl erythromycin A and the new intermediates were

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Scheme 1. Preparation of 3-keto-9-*O*-substituted oxime derivatives of 6-*O*-methyl erythromycin A.

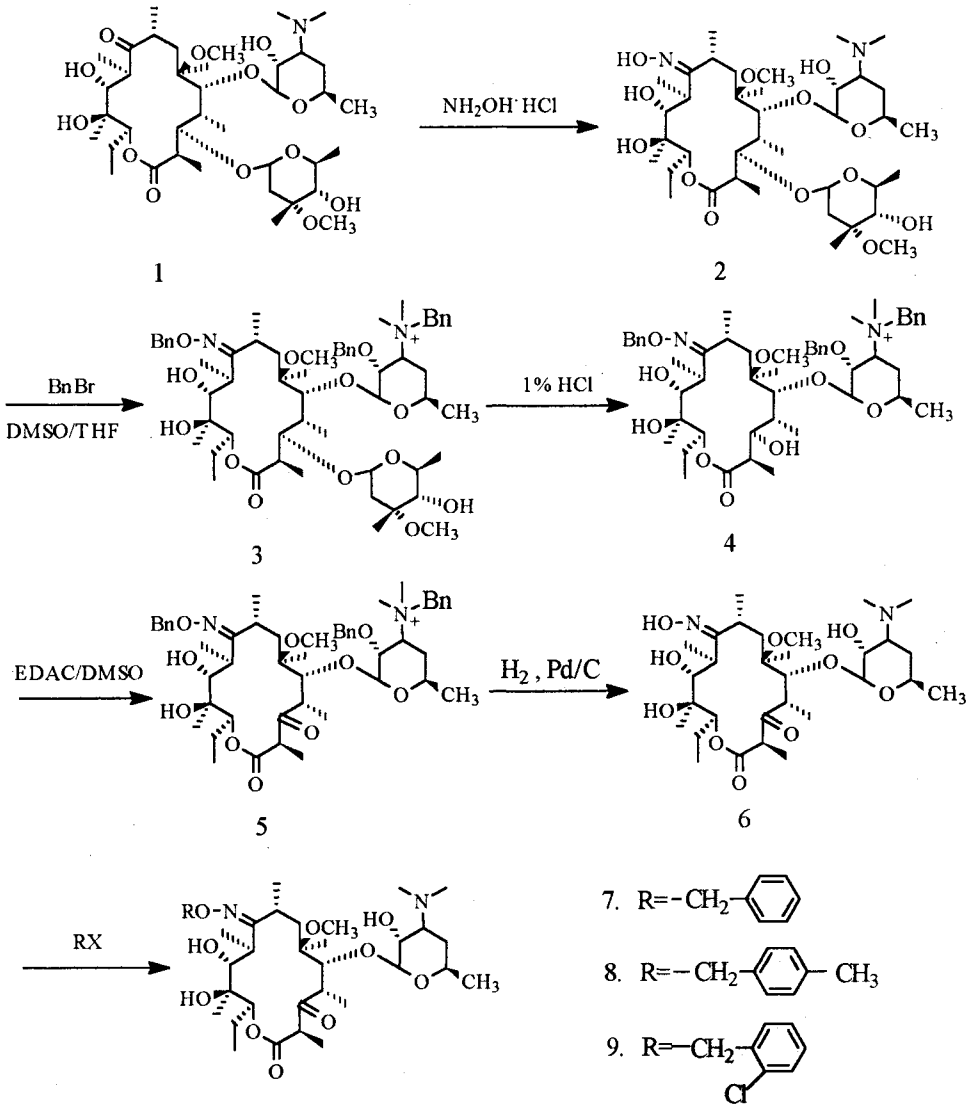


Table 1. *In vitro* activity of 3-keto-9-*O*-substituted oxime derivatives of 6-*O*-methyl erythromycin A and the new intermediates.

| Organism | MIC (μg/ml) | | | | | | |
|---|---|---|---|---|---|---|---|
| | **4** | **5** | **6** | **7** | **8** | **9** | EM |
| *Streptococcus pneumoniae* 100 | 8 | 8 | 0.12 | 64 | 8 | 4 | 0.12 |
| *Enterococcus faecalis* 88 | 16 | 64 | 1 | >128 | 64 | 64 | 1 |
| *Staphylococcus epidermidis* 26069 | 1 | 4 | 0.03 | 4 | 0.25 | 4 | 4 |
| *Streptococcus pneumoniae* 64 | 16 | 32 | >128 | >128 | 64 | 32 | >128 |
| *Staphylococcus aureus* 9525 | 4 | 4 | >128 | >128 | >128 | 32 | >128 |
| *Staphylococcus epidermidis* 9726 | >128 | >128 | >128 | >128 | >128 | >128 | >128 |
| *Escherichia coli* 26 | >128 | >128 | >128 | >128 | >128 | >128 | >128 |

*S. pneumoniae* 100, *E. faecalis* 88 and *S. epidermidis* 26069: erythromycin-susceptible. *S. pneumoniae* 64, *S. aureus* 9525 and *S. epidermidis* 9726: erythromycin-resistant. *E. coli* 26: Gram-negative.

tested *in vitro* against both erythromycin-susceptible and erythromycin-resistant organisms using standard agar dilution methods. *Streptococcus pneumoniae* 100, *Enterococcus faecalis* 88 and *Staphylococcus epidermidis* 26069 are erythromycin-susceptible organisms. *Streptococcus pneumoniae* 64, *Staphylococcus aureus* 9525 and *Staphylococcus epidermidis* 9726 are erythromycin resistant. We also routinely tested against *Escherichia coli* 26 to monitor activity against Gram-negative organisms.

The compounds **6** and **8** show significant activity against *Staphylococcus epidermidis* 26069 compared to erythromycin, but low activity against other erythromycin-susceptible organisms. **4**, **5** and **9** do not show increased activity against erythromycin-susceptible organisms, but they have better activity against erythromycin-resistant organisms than erythromycin A. None of the compounds have activity against Gram-negative organisms.

## Experimental

Melting points were determined on a Reichert micro melting point apparatus and are uncorrected. $^{13}C$ NMR spectra were recorded on a Mercurcy-300 spectrometer in $CDCl_3$ and chemical shifts are reported in ppm relative to $CDCl_3$ (77.00 ppm). Mass spectra were measured with Autospec-Ultima ETOF mass spectrometer.

6-*O*-Methylerythromycin A 9-Oxime (**2**)

To a solution of 6-*O*-methyl erythromycin A (2.5 g, 3.35 mmol) in methanol (13 ml) was added hydroxylammonium chloride (2.4 g, 34.8 mmol) and triethylamine (2.4 ml, 17.3 mmol), and then the mixture was stirred under reflux for 24 hours, the reaction mixture was poured into water, adjusted pH≥9 by adding 20% ammonium hydroxide and extracted with EtOAc. The combined organic layers were washed with $H_2O$ and brine, dried ($MgSO_4$) and evaporated under reduced pressure to give **2** (2.2 g, 2.89 mmol) as a white foam.

mp159~162°C; FAB-MS *m/z* 763 $(M+H)^+$.

2′-*O*,3′-*N*-Dibenzyl-6-*O*-methylerythromycin A 9-(*O*-Benzyl)oxime Bromide (**3**)

To a solution of **2** (2.0 g, 2.62 mmol) in a mixture of DMSO and THF (10 ml : 10 ml) were added benzyl bromide (1.3 ml, 10.9 mmol) and then 60% sodium hydride (0.25 g, 6.25 mmol), and the mixture was stirred at room temperature for 6 hours. The reaction solution, after addition of $H_2O$ (20 ml), was extracted with EtOAc (40 ml×2). The combined organic layers were washed with $H_2O$ and brine, dried ($MgSO_4$) and evaporated under reduced pressure to give the crude product, which was then crystallized from a mixture of ethanol and acetone, and there was obtained **3** (2.84 g, 2.55 mmol) as a white crystal.

mp148~150°C; FAB-MS *m/z* 1033 $(M-Br)^+$.

2′-*O*,3′-*N*-Dibenzyl-9-[(*O*-Benzyl)oxime] of 3-*O*-de(2,6-Dideoxy-3-*C*-methyl-3-*O*-methyl-α-L-ribo-hexopyranosyl)-6-*O*-methylerythromycin Bromide (**4**)

Compound **3** (2.8 g, 2.52 mmol) and 36% hydrochloric acid (1 ml) were suspended in methanol (36 ml) and the mixture was stirred for 7 hours at room temperature. The solution was adjusted to a basic pH by adding 20% ammonium hydroxide and was extracted with EtOAc (100 ml×2) after addition of $H_2O$ (60 ml). The combined organic layers were washed with $H_2O$ and brine, dried ($MgSO_4$) and evaporated under reduced pressure. The residue was crystallized from acetone and petroleum ether to give **4** (1.84 g, 1.93 mmol) as a white crystal.

mp 140~143°C; FAB-MS *m/z* 875 $(M-Br)^+$; $^{13}C$ NMR (300 MHz, $CDCl_3$) $\delta$: 44.33 and 49.83 (3′-$N(CH_3)_2$), 49.51 (6-$OCH_3$), 67.93 ($NCH_2Ph$), 74.86 (2′-$OCH_2Ph$), 75.83 ($=NOCH_2Ph$), 100.50 (1′-C), 127.29~137.67 ($CH_2$-*Ph*), 170.54 (C=O), 174.90 (C=N).

2′-*O*,3′-*N*-Dibenzyl-9-[(*O*-Benzyl)oxime] of 3-*O*-de[(2,6-Dideoxy-3-*C*-methyl-3-*O*-methyl-α-L-ribo-hexopyranosyl)-oxy]-6-*O*-methyl-3-oxo Erythromycin Bromide (**5**)

A mixture of **4** (1.8 g, 1.88 mmol), DMSO (2.7 ml) and 1-ethyl-3-(3-dimethylaminopropyl)-carbodiimide hydrochloride (EDAC) (1.09 g, 5.69 mmol) were combined at room temperature in $CH_2Cl_2$ (11 ml) under a nitrogen atmosphere. To this solution was added pyridinium trifluoroacetate (1.56 g, 5.73 mmol) over a 10-minute period. The reaction mixture was stirred at room temperature for 31 hours. To this solution was added an equal volume of $H_2O$, and the aqueous layer was extracted with EtOAc (20 ml×2) at pH 4.0. The combined organic layers were washed with $H_2O$ and brine, dried ($MgSO_4$) and evaporated under reduced pressure. The residue was crystallized from acetone and petroleum ether to give **5** (1.48 g, 1.55 mmol) as a white crystal.

mp 151~154°C; FAB-MS *m/z* 873 $(M-Br)^+$; $^{13}C$ NMR (500 MHz, $CDCl_3$) $\delta$: 44.36 and 49.69 (3′-$N(CH_3)_2$), 50.94 (6-$OCH_3$), 67.97 ($NCH_2Ph$), 74.93 (2′-$OCH_2Ph$), 75.82 ($=NOCH_2Ph$), 101.88 (1′-C), 169.22 ($C_1$=O), 175.06 (C=N), 204.77 ($C_3$=O).

9-Oxime of 3-*O*-de[(2,6-Dideoxy-3-*C*-methyl-3-*O*-methyl-α-L-ribo-hexopyranosyl)-oxy]-6-*O*-methyl-3-oxo Erythromycin (**6**)

A mixture of **5** (1.4 g, 1.47 mmol), 10% palladium carbon (0.6 g) and ammonium formate (0.23 g, 3.65 mmol) in DMF (14 ml) was stirred vigorously under hydrogen at atmospheric pressure and 50°C for 4 hours. The catalyst was filtered off, and the filtrate was poured into water (15 ml) and extracted with EtOAc (30 ml×2) . The combined organic layers were washed with $H_2O$ and brine, dried ($MgSO_4$) and evaporated under reduced pressure. The residue was dissolved in methanol (14 ml), followed by addition of 10% palladium carbon (0.6 g), ammonium formate (0.21 g, 3.33 mmol) and formic acid (1.2 ml, 31.2 mmol), and then the mixture was stirred under hydrogen at atmospheric pressure and 50°C for 10 hours. The catalyst was filtered off. The filtrate was concentrated and poured into water (15 ml), extracted with EtOAc (20 ml×2) . The combined organic layers were washed with $H_2O$ and brine, dried ($MgSO_4$) and evaporated under reduced pressure. The residue was crystallized from acetone and *n*-hexane to give **6** (0.68 g, 1.13 mmol) as a white crystals.

mp 132~135°C; FAB-MS *m/z* 603 $(M+H)^+$; $^{13}C$ NMR (300 MHz, $CDCl_3$) $\delta$: 40.20 (3′-$N(CH_3)_2$), 49.98 (6-$OCH_3$), 103.43 (1′-C), 169.36 ($C_1$=O), 169.89 (C=N), 205.27 ($C_3$=O).

9-[*O*-(Benzyl)oxime] of 3-*O*-de[(2,6-Dideoxy-3-*C*-methyl-3-*O*-methyl-α-L-ribo-hexopyranosyl)-oxy]-6-*O*-methyl-3-oxo Erythromycin (**7**)

To a solution of **6** (0.20 g, 0.33 mmol) in DMSO-THF (2 ml : 2 ml) were added 82% KOH powder (25 mg, 0.37 mmol), and the mixture was stirred for 10 minutes, then was added benzyl chloride (60 μl, 0.52 mmol), stirred at room temperature for 15 hours. The reaction solution, after addition of $H_2O$ (10 ml), was extracted with EtOAc (15 ml×2). The combined organic layers were washed with $H_2O$ and brine, dried ($MgSO_4$) and evaporated under reduced pressure. The residue was chromatographed on silica eluting with a mixture of acetone, cyclohexane, triethylamine (1 : 5 : 0.1) to obtain **7** (0.12 g, 0.17 mmol) as a white foam.

mp 127~129°C; FAB-MS *m/z* 693 $(M+H)^+$; $^{13}C$ NMR (500 MHz, $CDCl_3$) $\delta$: 41.17 (3′-$N(CH_3)_2$), 49.96 (6-$OCH_3$), 76.58 (=NO*C*$H_2$Ph), 102.99 (1′-C), 127.73~138.04 (−$CH_2$*Ph*), 169.33 ($C_1$=O), 169.33 (C=N), 205.17 ($C_3$=O).

Using the procedure, following derivatives were obtained.

9-[*O*-(4-Methylbenzyl)oxime] of 3-*O*-de[(2,6-Dideoxy-3-*C*-methyl-3-*O*-methyl-α-L-ribo-hexopyranosyl)-oxy]-6-*O*-methyl-3-oxo Erythromycin (**8**)

0.2 g of **6** obtained 0.14 g (0.20 mmol) of **8**.

FAB-MS *m/z* 707 $(M+H)^+$; $^{13}C$ NMR (300 MHz, $CDCl_3$) $\delta$: 41.17 (3′-$N(CH_3)_2$), 50.87 (–Ph–O*C*$H_3$), 77.00 (=NO*C*$H_2$Ph), 102.98 (1′-C), 127.73~138.04 (–$CH_2$*Ph*), 169.33 (C=N), 205.17 ($C_3$=O).

9-[*O*-(2-Chlorobenzyl)oxime] of 3-*O*-de[(2,6-Dideoxy-3-*C*-methyl-3-*O*-methyl-α-L-ribo-hexopyranosyl)-oxy]-6-*O*-methyl-3-oxo Erythromycin (**9**)

0.2 g of **6** obtained 0.11 g (0.18 mmol) of **9**.

FAB-MS *m/z* 727 $(M+H)^+$; $^{13}C$ NMR (300 MHz, $CDCl_3$) $\delta$: 49.96 (6-$OCH_3$), 73.71 (=NO*C*$H_2$Ph), 102.99 (1′-C), 127.73~138.04 (–$CH_2$Ph), 169.36 ($C_1$=O), 169.89 (C=N), 205.17 ($C_3$=O).

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