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Synthesis of two and antibacterial activity of one novel oxime ether derivatives of erythromycin A

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Abstract

The synthesis of novel erythromycin A 9-*O*-(2-ethenesulfony-ethyl)-oxime and erythromycin A 9-*O*-(3-oxo-butyl)-oxime from erythromycin A (EA) by the Michael reaction is described and to describe the effects of transformation of ketone in position 9 of EA to an oxime ether. This transformation occurred in a single step without protecting of any functional moiety of erythromycin oxime and zero waste manner in good yield. The antibacterial screen of EA 9-*O*-(2-ethenesulfony-ethyl)-oxime is also reported.
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1. Introduction

Erythromycin A (EA) is the most widely used and effective macrolide antibiotic against most Gram-positive and some Gram-negative bacteria [1]. EA is an orally administered antibiotic and the main reason for its resorption variability and consequently the low antibiotic level in the serum is its instability in the gastric juice. It is well known that in acidic condition EA gives first an internal enolic ether and secondly an internal ketal by reaction with the ketone in position 9 and hydroxy groups in position 6 and 12. Neither product exhibits antibiotic activity and this ketal formation is irreversible [2] and EA oxime and some of its derivatives are much more stable to mineral acid [3]. The transformation of the ketone in position 9 to an oxime is a possible way of preventing internal ketalization. As a semisynthetic macrolide the discovery of roxitromycin, (9-[*O*[(2-methoxyethoxy)methyl]oxime]-erythromycin), is the result of a rational and scientific process, based on the fact that at least one reason for EA's resorption variability after oral administration was its instability in the gastric juice [3].

This instability is due to the reactivity of the ketone in position 9 in acidic medium and one chemical approach was to mask it by an oxime function. Several derivatives of EA ketone had been described including the hydrazone [4], numerous oxime, erythromycylamine and erythromycylamine-aldehyde condensation products [3–6] and oxime ether derivatives [7,8] but as EA 9-*O*-(2-ethenesulfony-ethyl)-oxime (**3**) and EA 9-*O*-(3-oxo-butyl)-oxime (**4**) were practically unexplored. It has been reported that the reaction of oximes with activated olefins: e.g. reaction of cyclopentanone oxime with ethenesulfonyl-benzene, has provided cyclopentanone *O*-(2-benzenesulfonyl-ethyl)oxime [9] and reaction of propan-2-one oxime with acrylic acid methyl ester provided isopropylaminooxy-propionic acid methylester [10]. This versatile methodology has been applied to synthesis the novel erythromycin oxime ether derivatives.

In this paper, we have considered it of interest to study synthesis and the effects of such modifications on the spectrum of antibacterial activity. We anticipate that this work might lead to the erythromycin oxime ethers analogs with a different synthetic route and different activity profile. Both compounds have been prepared from EA oxime and activated olefins via Michael

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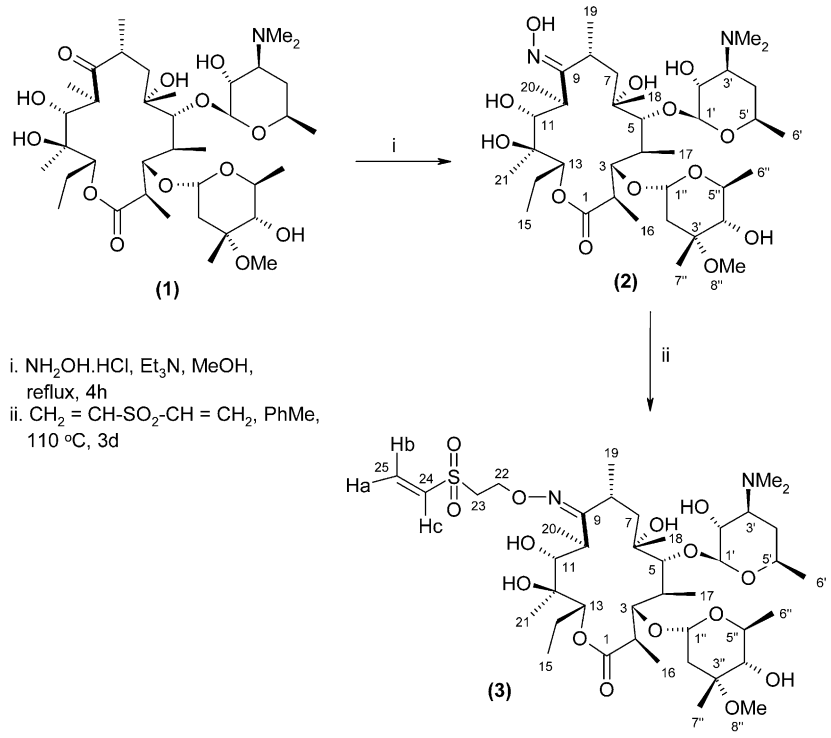
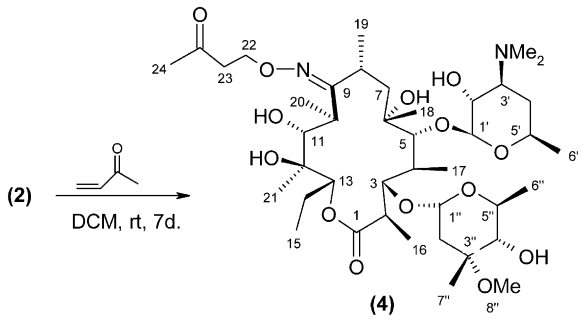


Scheme 1.

reaction (Schemes 1 and 2) and the antibacterial activities of EA 9-*O*-(2-ethenesulfony-ethyl)-oxime (**3**) compared with that of EA (Tables 2a and 2b).

1.1. Synthesis and structure determination (chemistry)

EA oxime (**2**) [12,17] with divinylsulfone (toluene, reflux, 3d) gave *O*-Michael adduct (**3**) in 76% yield. As shown in Scheme 1 compound (**2**) underwent a facile and chemoselective *O*-Michael addition upon treatment with divinylsulfone to afford the *O*-Michael adduct (**3**) in good yield in one pot reaction without protection of any functional moiety of corresponding EA oxime (**2**). The adduct (**3**) was isolated and the structures of it were determined by 600 MHz ^{1}H NMR (Table 1), ^{2}D-COSY studies and FAB MS technique (see Section 3) and also from the literature data of known erythromycin derivatives and by a comparison with previous study [11–13,17]. Such structure is also consistent with the known chemistry of the erythromycins [14,15]. Previous studies shown that direct *O*-alkylation of erythromycin oxime allowed access to *E*-stereoisomers which are more



Scheme 2.

Table 1
^{1}H chemical shifts (ppm) and ^{1}H–^{1}H coupling constants (Hz) for *O*-(vinylysulfone)erythromycin A 9-ethoxime (**3**) at 600 MHz NMR in $CDCl_3$

Position	^{1}H	M[a]	Position	^{1}H	M[a]
1			22	4.41	t
2	2.89	dq	23	3.33	t
3	4.11	bd	24	6.66	m
4	1.98	m	25	6.45(Ha)	d
5	3.52	d		6.16(Hb)	d
6			1′	4.45	d
7	1.90	dd	2′	3.02	m
8	2.38	m	3′	2.55	m
9			4′	1.6	m
10	2.69	dq	5′	3.98	m
11	3.62	d	6′	1.28	d
12			7′, 8′	2.15	s
13	5.07	dd	1″	4.88	bd
14	1.54	m	2″	2.35	dd
15	0.85	t	3″		
16	1.18	d	4″	3.05	dd
17	1.08	d	5″	4.00	dq
18	1.45	s	6″	1.11	bd
19	1.17	d	7″	1.24	s
20	1.29	d	8″	3.30	s
21	1.12	s			

M[a], multiplicity of ^{1}H resonances.

interesting than the *Z*-ones [6–12]. We assume that, under giving conditions and from the spectral data, we obtain same stereoisomers.

Similarly the reaction of (**2**) with methylvinyl ketone (DCM, 25 °C, 7d) gave *O*-(methylketone)erythromycin A 9-ethoxime (**4**) in 69% yield via a facile and chemoselective *O*-Michael addition under mild condition in one pot reaction (Scheme 2). The structure of compound (**4**) was determined by 400 MHz ^{1}H NMR, ^{2}D-COSY studies and FAB MS technique (see Section 3). The ^{1}H NMR spectra of (**4**) in $CDCl_3$ shown the pattern similar to those of (**3**), indicating the C-9 ketoxime to *O*-(methylketone)erythromycin A 9-ethoxime. Further the mass spectra (FAB MS) of *O*-alkyl ketone EA 9-ethoxime derivatives (**4**) exhibited the characteristic protonated molecular ion peaks with the increasing mass units due to the additional alkyl ketone groups (see Section 3). This was also confirmed by a comparison of the results which were previously reported for erythromycin oxime ethers [7–10,17].

It is also interesting to note that in both cases the reactions proceeds chemoselectively in a single step without protection any functional moiety of correspon ding erythromycin oxime (**2**) and underwent a facile and chemoselective *O*-Michael addition upon giving reaction conditions (Schemes 1 and 2).

The antibacterial activity of *O*-(vinylsulfone)erythromycin A 9-ethoxime (**3**) against a range of bacteria has been described. The compound tested has been found active in some cases against Gram-positive bacteria tested (Tables 2a and 2b).

In conclusion, the synthesis of novel *O*-(vinylsulfone)erythromycin A 9-ethoxime (**3**) and *O*-(vinylketone)erythromycin A 9-ethoxime (**4**) were achieved by *O*-Michael addition reaction. This approach also opens up the possibility of constructing other oxime ether derivatives at the C-9 position of the macrolide core of erythromycin. Since the main reason for low antibiotic level of EA in the serum is instantly in the gastric juice and its oxime ether derivatives are much more stable to mineral acids, the result obtained can be considered interesting enough for further investigations.

Table 2a
Antibacterial activity of EA and the compound (**3**)

Test organism	MIC (μg/ml)	
	EA	Comp. **3**
S. aureous ATCC 29213	0.39	12.50
S. epidermidis ATCC 12228	0.19	3.12
S. faecalis ATCC 29212	1.56	50
C. diphtheriae G 12/6	<0.04	0.09
E. coli ATCC 25922	100	>200

2. Experimental

2.1. Material and methods

Nuclear magnetic resonance spectra and decoupling experiments were determined at 400 and 600 MHz on a Bruker AM spectrometer. Chemical shifts are given in parts per million (δ) downfield from tetramethylsilane as internal standard. Spectra were determined in deuteriochloroform. The following abbreviations are used; s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet, br = broad and brs = broad singlet, brd = broad doublet. Flash column chromatography was performed using silica gel 60 (230–400 mesh). Melting points were determined on a Kofler hot stage apparatus and are uncorrected. Mass spectra were recorded at 70 eV on a VG Autospec mass spectrometer. Specific rotations were measured at ambient temperature with an Optical Activity Ltd, AA-1000 polarimeter. All solvents were purified before use. Divinylsulfone and methylvinyl ketone were purchased from Aldrich Chemical Company and ERY were obtained from Sigma.

2.2. EA oxime (*2*) were prepared according to literature procedure [12,17]

The product was obtained as colourless solid in 75% yield. M.p. 170 °C, $[\alpha]_D = -70$ (*c*, 1 g/100 ml, EtOH) *m/z* (%) (FAB): 749 (*M*+1, 4), 591(2), 174(7), 158(100), 116 (49), 98(29) and 72(23). ^{1}H and ^{13}C chemical shifts (ppm) and ^{1}H–^{1}H coupling constants (Hz) have been reported by McGill et al. [12].

2.3. *O*-(Vinylysulfone)erythromycin A 9-ethoxime (*3*)

A solution of EA oxime (**2**) (0.3 g, 0.40 mmol) and divinylsulfone (0.047 g, 0.4 mmol) in dry toluene (40 ml) was stirred and boiled under reflux for 3 days. After cooling the solvent was removed in vacuo and the residue chromatographed on silica eluting with 1:9:90 v/v NH_3/MeOH/CH_2Cl_2 to afford the product (**3**) (0.264 g, 76%) as a pale yellow solid that crystallised from dichloromethane–hexane as colourless prisms, m.p. 118–120 °C. $[\alpha]_D = -64.8$ (*c* 1 g/100 ml, $CHCl_3$). HRMS: 866.4809, $C_{41}H_{74}N_2O_{15}S$: 866.4798, *m/z* (FAB): 867 (*M*+1, 5), 709(3), 174(9), 158(100), 116(38), 98(10), 72(13) and 59(3).

2.4. *O*-(Methylketone)erythromycin A 9-ethoxime (*4*)

A solution of EA oxime (**2**) (0.1 g, 0.134 mmol), and methyl vinyl ketone (56 mg, 0.268 mmol) in dry dichloromethane (20 ml) was stirred under N_2 at room temperature for 7 days. The solvent was removed in vacuo and the residue subjected to column chromatography eluting with 9:1 v/v chloroform/Et_3N to afford

Table 2b
The antibacterial screen of EA-vinylsulfonyl ethoxime (**3**)

Test organism	Comp. (**3**) [a]	EA	Test organism	Comp. (**3**) [a]	EA
B. fragilis B70	4	1	*Pr. vulgaris* H	>64	>64
B. fragilis BC1	8	0.5	*P. aeruginosa* 55528	>64	>64
B. fragilis NCTC10581	8	2	*P. aeruginosa* Badia	>64	>64
E. coli 10418	64	4	*P. aeruginosa* K799 61	64	16
E. coli DCO	>64	64	*P. aeruginosa* 799 wt	>64	64
E. coli DC2	64	4	*S. marscescens* S6	>64	>64
E. coli DCOTEM-1	>64	32	*B. cereus* BRL 1243	8	0.125
E. coli ESS	4	0.125	*B. subtilis* ATCC 6633	4	<0.06
E. coli JT425	>64	64	*E. feacalis I*	4	0.125
E. coli K12/TEM-5	>64	16	*S. aureus carter* 37	8	0.125
E. cloaae N1	>64	>64	*S. aureus* F89	8	0.125
E. cloaae P99	>64	64	*S. aureus oxford*	8	0.125
H. influenzae Q1	64	4	*S. aureus Russell*	8	0.125
H. influenzae WM493	64	4	*S. aureus* V573	>64	>64
K. pneumoniae E70	>64	>64	*S. epidermidis* PHLN20	8	0.125
Morax. Catarhalis 1502	0.125	<0.06	*S. agalactiae Hester*	<0.06	<0.06
Mora. CatarhalisRavasio	1	<0.06	*S. pneumoniae* 1761	0.125	<0.06
Morg. Morganii T361	>64	64	*S. pneumoniae* ERY2	16	4
P. mirabilis C889	>64	>64	*S. pneumoniae* PU 7	0.125	<0.06
			S. ppyogenes CN10	0.125	<0.06

[a] Values are MICs (minimum inhibitory concentrations in microgram/millilitre).

the *O*-Michael adduct (**4**) (0.075 g, 69%), m.p. 154–157 °C, $[\alpha] = -57.5$ (1 g/100 ml, $CHCl_3$), *m/z* (%) (FAB): 819 (M^+, 37), 158(44), 109(31), 83(56) and 55(100). HRMS: 818.5119, $C_{41}H_{74}N_2O_{14}$: 818.5140, δ (400 MHz, partial data): 2.61(s, 3H, MeC=O), 4.43(m, 2H, H22) and 3.66(m, 2H, H24).

The ^1H NMR spectra of (**4**) in $CDCl_3$ shown the pattern similar to those of (**3**), indicating the C-9 ketoxime to *O*-(methylketone)erythromycin A 9-ethoxime. Further the mass spectra (FAB MS) of *O*-alkyl ketone EA 9-ethoxime derivatives (**4**) exhibited the characteristic protonated molecular ion peaks with the increasing mass units due to the additional alkyl ketone groups.

3. In vitro antibacterial activity studies

3.1. Materials and methods

The following organisms were selected for use in the study [16] *Staphylococcus aureus*, ATCC 29213, *Staphylococcus epidemidis* ATCC 12228 *Streptococcus faecalis*, ATCC 29212, *Corynebacterium diphtheriae* G 12/6 and *Escherichia coli* ATCC 25922.

In vitro antibacterial activities of EA and the compound were investigated in duplicate (two separate studies) by macrodilution broth method. This was done according to the National Committee for Clinical Laboratory Standards (NLCLLS) M7-A3 guidelines (*). For this purpose: Stock solutions of the drugs were prepared by dissolving 10-mg drug in appropriate volume of ethanol and adding Mueller-Hinton Broth (MHB, Oxoid). Then the stock solutions were diluted with MHB to obtain final drug concentrations ranging from 400 to 0.08 μg/ml. Bacterial suspensions were prepared from fresh cultures according to 0.5 McFarland Standards and were diluted with MHB to reach a concentration of 1×10^6 CFU/ml. Antibiotic solutions series were inoculated with 1 ml of bacterial suspensions to obtain a final concentration of approximately 5×10^5 CFU/ml. The inoculated tubes were incubated for 16–20 h at 35 °C. *C. diphtheriae* were reincubated for an additional 24 h and were reread at 48 h. Minimum inhibitory concentrations (MIC) were read as the lowest concentration at which there was no visible growth. (*) NLCLLS Method for Dilution Antibacterial Susceptibility Test for Bacteria that Grow Aerobically—Third Edition; Approved Standard NCCLS document M7-A3 NCCLS, 771 East Lancester Avenue, Villanova, PA, 1985.

4. Result and discussion

The in vitro antibacterial activity of *O*-(vinylsulfone)erythromycin A 9-ethoxime (**3**) against *S. aureus*, *S. epidemidis*, *S. faecalis*, *C. diphtheriae*, *E. coli* has been described previously. The compound tested has been found active in some cases against Gram-positive bacteria tested (Table 2a). In general, the transformation of the ketone in position 9 of EA to an oxime resulted in a decrease of in vitro antibacterial activity against the organism tested, but the drop in activity was

not so dramatic except for *S. faecalis* and *E. coli.* Therefore, some more biological assays has also been done and reported in Table 2b [17].

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