Available online at www.sciencedirect.com
SCIENCE DIRECT®
Bioorganic & Medicinal Chemistry 12 (2004) 3807–3813
Bioorganic & Medicinal Chemistry

Synthesis and antimicrobial activity of erythromycin-A oxime analogs ☆

Deepa Pandey,[a] S. B. Katti,[a] W. Haq[a,*] and C. K. M. Tripathi[b]

[a]*Division of Medicinal Chemistry, Central Drug Research Institute, Lucknow 226001, India*
[b]*Division of Fermentation Technology, Central Drug Research Institute, Lucknow 226001, India*

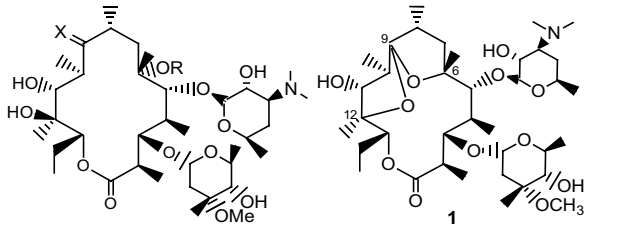
Received 5 April 2004; accepted 8 May 2004
Available online 2 June 2004

Abstract—A series of erythromycin-A oxime ether as well as esters have been synthesized. Ether derivatives were synthesized through the epoxy ether intermediate of erythromycin-9-oxime, followed by opening of the epoxy linkage through various amines, whereas esters have been prepared through DCC mediated protocol. These derivatives have been evaluated for antibacterial activity and found to be as active as erythromycin-A.
© 2004 Elsevier Ltd. All rights reserved.

1. Introduction

Macrolide antibiotics like erythromycin A being an old member of oral antibiotic cover broad spectrum of antibacterial activity against gram positive bacterial species, responsible for upper and lower respiratory tract infections. Apart from these therapeutic utilities macrolides are also used as prokinitic agents[1] and in the treatment of inflammatory disorders like asthma.[2] The 14 membered macrolide antibiotic however loses its antibacterial activity under acidic condition of stomach by degradation resulting in poor bioavailability of drug and undesirable side effects like increase in gastrointestinal motility.[3] To overcome the acid instability problem numerous chemical modifications of erythromycin A have been investigated giving rise to second generation macrolide like clarithromycin (CAM),[4] roxithromycin[5] and azithromycin[6] in the early 1990s.

Since the degradative pathway involves interaction between the hydroxyl group at C-6 and C-12 with the carbonyl group at C-9, firstly converting into internal enolic ether and secondly antibacterially inactive 6,9,9,12-spiroketal.[7] (**1**) Modifications at these positions have resulted in some acid stable macrolide like clarithromycin.[4]

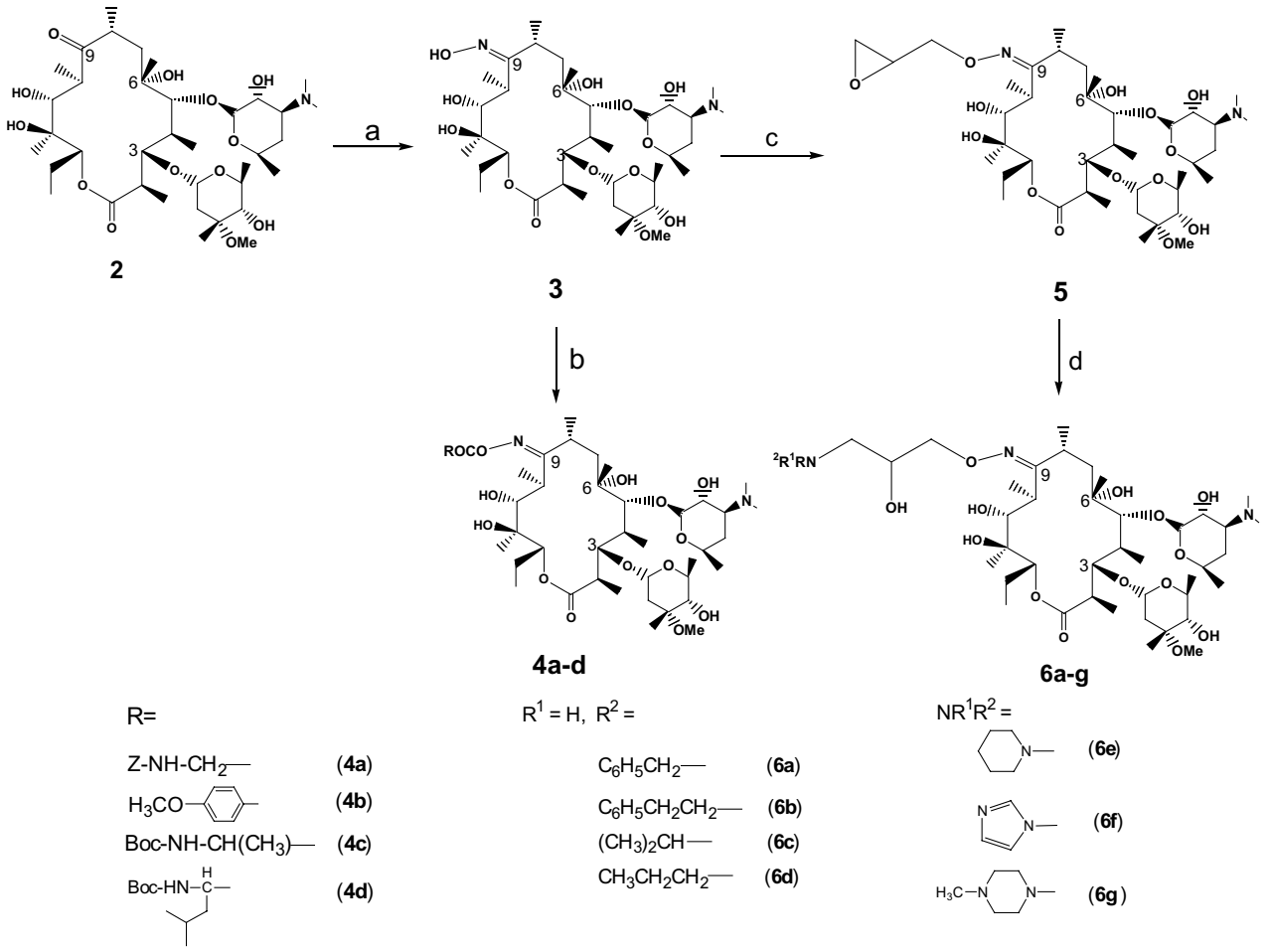


The transformation of the carbonyl group in position 9 to an oxime is a possible way of preventing internal ketalization. The methoxy ethoxy methyl ether derivative of erythromycin otherwise known as roxithromycin exhibits enhanced activity against the resistant strain as well as it eliminates the acid instability problem.[5] Some other chemical approaches focused on the modification of C-9 ketone moiety, involve several derivatives of erythromycin including the hydrazone,[8] numerous oxime, erythromycylamine and erythromycylamine aldehyde condensation products,[9] and oxime ether derivative.[10] It was also demonstrated earlier that the introduction of supplementary amino group in the macrolide for example, azalides and some 9-amino oximes, was beneficial for the antibacterial activity.[11]

Inspired by these facts we have recently developed a methodology to make some modifications at C-9 position of erythromycin to get new macrolide with higher

Keywords: Macrolide; Erythromycin; Antibacterial; Aminoalcohol ether derivatives.
☆ CDRI Communication No.: 6519.
* Corresponding author. Tel.: +91-0522-2212411x4373; fax: +91-0522-2223405; e-mail: haq_w59@yahoo.com

0968-0896/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2004.05.011



Scheme 1. Reagents and conditions: (a) $NH_2OH{\cdot}HCl$, pyridine, rt, 80%; (b) aminoacid/carboxylic acid, DCC, 52–70%; (c) epichlorohydrine, K_2CO_3, acetone, reflux; (d) $R^1R^2NH{\cdot}CHCl_3$, rt, 20–52%.

antibacterial activity and/or improved pharmacokinetics. We intended to explore the effect of amino alcohol ethers as well as 9-oxime esters on the antibacterial activity of erythromycin. For the purpose we have prepared the desired amino alcohol ether derivatives in a three step process starting from erythromycin, first converting it into the 9-oxime then it was transformed into its epoxy ether derivative in a single step by using epichlorohydrin. Finally the epoxy linkage has been opened through various amines to get the desired amino alcohol ether derivatives of erythromycin-A oxime. In this series we have examined the effect of heterocyclic amine and aromatic amine since they are found to be beneficial for the antibacterial activity,[12] as well as simple aliphatic amines. Further we have also synthesized the corresponding ester derivatives of erythromycin-9-oxime, since the oxime esters have proved to be effective prodrug.[13] Accordingly we have prepared a few oxime esters using DCC protocol (Scheme 1).

2. Chemistry

The desired 9-oxime amino alcohol ether derivatives were synthesized as follows. Treatment of erythromycin with 10 equiv of hydroxylamine hydrochloride at room temperature afforded the erythromycin-9-oxime (**3**) in ~80% yield. This was further treated with (±) epichlorohydrin to furnish the epoxy ether (**5**), an unstable product, which could not be purified to homogeneity. However the epoxy ether formation was confirmed by TLC where the oxime spot was disappeared completely. The epoxy ether (**5**) characterized by IR and mass spectral data. The presence of a weak peak at 1282 cm^{-1} in the IR spectra and a molecular ion peak at 805 in FABMS conformed the formation of epoxy ether (**5**). The 9-oxime amino alcohol derivatives (**6a**–**g**) were prepared by the treatment of (**5**), in situ with various amines at room temperature. For the synthesis of erythromycin-9-oxime ester derivative (**4a**–**d**) the corresponding oxime was treated with various amino acids and carboxylic acids in 1.5 equiv using DCC as coupling agent (1.5 equiv) at 0 °C to get the desired compounds (Tables 1 and 2).

3. Results and discussion

In the present series we have attempted to enhance the antibacterial activity of erythromycin by incorporating amino alcohol moiety to the macrolide skeleton, as well as we have synthesized some erythromycin-9-oxime ester derivatives. The antibacterial activity of erythromycin-9-oxime analogs is shown in Table 3. These compounds

Table 1. Erythromycin-9-oxime ester derivatives

Compound	RCO–	Yield (%)
4a	*Z*-Gly–	65
4b	*p*-Methoxy benzoyl	51.2
4c	Boc–Ala–	70
4d	Boc–Leu–	53.1

Table 2. Amino alcohol functionalised erythromycin-A-9-oxime ethers

Compound	NR^1R_2	Yield[a]
6a	Benzylamino	50
6b	Phenethylamino	52
6c	Isopropylamino	36
6d	*n*-Propylamino	39
6e	Piperidinyl	45
6f	Imidazolyl	20
6g	1-Methyl piperazinyl	36

[a]The yields are calculated on the basis of erythromycin-9-oxime (**3**).

were tested in vitro for their antibacterial activity using standard broth dilution assay against *S. aureus*, *B. subtilis*, *K. pneumoniae* and *E. coli* strains taking erythromycin as reference.[14] The antibacterial activity of erythromycin-9-oxime esters (**4a**–**d**) was found to be at par with erythromycin. All the amino alcohol ether derivatives exhibited increased MIC values or in other words activity lower than erythromycin itself except the results obtained for *E. coli* where the derivatives have shown better activity. Compounds **4a** and **4b** exhibited highest activity (MIC value 1.56). All the analogues tested had enhanced activity with MIC value (6.250–12.5) μg/mL) against gram-negative *E. coli* ATCC1053 strain except **6f** than parent compound erythromycin-A (25 μg/mL). All the compounds in the series have shown moderate antibacterial activity against gram-positive organism. Compound **6e** exhibited highest activity against *S. aureus* ATCC9144 with MIC 0.781 μg/mL being only one dilution factor less potent than the parent compound erythromycin. Compound **6d** found to be the least active with their MIC value 3.125 against both the *S. aureus* strains. The compounds **6e**–**g** having heterocyclic amine substitution exhibited mixed activity profile against the bacterial strains showing the lack of correlation in the activity profile. Finally the introduction of aromatic functionality in the macrolide skeleton via the compound **6a** and **6b** also resulted in the loss of antibacterial activity by two dilution factors (1.562 μg/mL) than the parent erythromycin. Therefore the introduction of amino alcohol moiety in the macrolide skeleton not resulted in the enhancement of antibacterial activity.

4. Conclusion

In summary, the synthesis of a novel series of erythromycin-A-9-oxime analogs has been described. These derivatives have exhibited promising antibacterial activity towards *E. coli* strain, as well as moderate antibacterial activity against the other bacterial strains, tested. Better activity of the new derivatives against *E. coli* is an important finding because pathogenic *E. coli* like Enterobacteriaceae, a potential hazard to human health, are highly resistant to erythromycin.[15] Recently azithromycin and telethromycin have been shown superior activity compared to erythromycin.[15] The present results provide a new lead for further structure–function studies of macrolide. This report comprises a simple synthesis of macrolide derivatives without protection of other functional groups in good yields. Currently we are exploring modification at this key position to obtain macrolides with promising antibacterial activity.

5. Experimental

^{1}H NMR spectra was obtained with a DRX-300 Bruker FT-NMR spectrophotometer taking $(CH_3)_4Si$ (TMS) as an internal standard. ^{13}C NMR spectra was recorded on DPX-200 Bruker FT-NMR (50 MHz) spectrometer. The chemical shifts are reported as parts per million (ppm). IR spectra were obtained with a FT-IR Perkin–Elmer (model). Mass spectra were taken from JEOL-SX-102 instrument using fast atom bombardment (FAB positive) technique. Melting points (mp) were determined on a Complab melting point apparatus and are

Table 3. In vitro antibacterial activity of ery-9-oxime analogs (MIC μg/mL)

Compound	Minimum inhibitory concentration (g/mL)				
	S. aureus		*B. subtilis*	*K. pneumoniae*	*E. coli*
	ATCC9144	ATCC12598	ATCC6633	ATCC13883	ATCC1053
2 (Ery-A)	0.390	0.390	0.195	0.049	25.0
4a	0.390	0.390	0.390	0.195	1.562
4b	0.390	0.780	0.195	0.97	1.562
4c	0.390	0.780	—	—	—
4d	0.390	0.780	—	—	—
6a	1.562	1.562	1.562	0.781	6.250
6b	1.562	1.562	1.562	0.195	12.5
6c	1.562	3.125	12.5	3.125	25.0
6d	3.125	3.125	12.5	1.562	12.5
6e	0.781	1.562	6.25	0.781	12.5
6f	1.562	1.562	6.25	1.562	50.0
6g	3.125	1.562	1.562	0.390	12.5

uncorrected. The C, H, N analyses were carried out on CARLO-ERBA EA1108 elemental analyser. Thin-layer chromatography (TLC) was performed on readymade silica gel plates (Merck) by means of solvent systems indicated below. Column chromatography separations were obtained on Silica gel (230–400 mesh).

5.1. Erythromycin-9-oxime 3

Erythromycin (0.730 g, 1 mM) was taken in pyridine (5 mL) and hydroxylamine hydrochloride (10 mM) was added to this solution. The reaction mixture was stirred at room temperature for 72 h. Pyridine was evaporated under reduced pressure and the residue was taken in CH_2Cl_2. The organic layer was washed with brine and dried over sodium sulfate. Solvent was removed under reduced pressure and the residue was precipitated by addition of hexane. The compound isolated white amorphous solid. Yield 0.7 g, mp 157–164 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.66 (m, 1H) C_2–H, 1.38 (m, 3H) 2-Me, 3.50–3.58 (m, 1H) C_3–H, 2.07 (dq, 1H) C_4–H, 1.27 (m, 3H) 4-Me, 3.51 (d, 1H) C_5–H, 1.33 (s, 3H) 6-Me, 1.56 (dd, 1H) and 1.90–1.96 (m, 1H) C_7–H, 2.58 (m, 1H) C_8–H, 1.08 (m, 3H) 8-Me, 3.01 (m, 1H) C_{10}–H, 1.13 (m, 3H) 10-Me, 3.86 (d, 1H) C_{11}–H, 1.47 (s, 3H) 12-Me, 5.18 (dd, 1H) C_{13}–H, 1.49 (m, 1H) and 1.90–1.96 (m, 1H) C_{14}–H, 0.85 (t, 3H) CH_3CH_2, 4.38 (d, 1H) C_1'–H, 3.24 (dd, 1H) C_2'–H, 2.74 (m, 1H) C_3'–H, 2.28 (s, 6H) C_3'–NMe_2, 1.20–1.28 (m, 1H) C_4'–H, 3.50–3.58 (m, 1H) C_5'–H, 1.26 (m, 3H) C_5'–Me, 4.98 (dd, 1H) 1″H, 1.59 (dd, 1H) $2''H_{ax}$, 2.38 (dd, 1H) $2H''_{eq}$, 3.04 (dd, 1H) 4″H, 4.04 (dq, 1H) 5″H, 1.31 (d, 1H) 6″H, 1.25 (s, 1H) 7″H, 3.32 (s, 3H) 3″-OCH_3; ^{13}C NMR ($CDCl_3$): δ 176.5 (C-1), 46 (C-2), 16.5 (C_2–Me), 79.2 (C-3), 35.9 (C-4), 9.8 (C–4Me), 83.5 (C-5), 78.0 (C-6), 27.5 (C–6Me), 37.2 (C-7), 45.5 (C-8), 19.2 (C–8Me), 168.2 (C-9), 38.9 (C-10), 12.3 (C-10Me), 68.9 (C-11), 73.8 (C-12), 17.1 (C-12Me), 75 (C-13), 21.5 (C-14), 12.4 (C-15), 104 (C-1′), 72.3 (C-2′), 65.1 (C-3′), 40.2 (C-3′NMe_2), 29.3 (C-4′), 68.9 (C-5′), 23.2 (C–5′Me), 96.5 (C-1″), 35.1 (C-2″), 72.3 (C-3″), 78.0 (C-4″); Mass (FAB) 749 $(M+H)^+$.

5.2. Erythromycin-9-oxime esters 4(a–d)

General procedure for all the compounds: To a solution of erythromycin-9-oxime 200 mg (0.25 mM) in 10 mL dichloromethane was added the carboxylic acid, (0.25 mM). The reaction mixture was cooled at 0 °C then dicyclohexylcarbodiimide (60 mg, 0.25 mM) is added under stirring and stirred for additional 3 h at room temperature. Dicyclohexylurea was removed by filtration and the filtrate was evaporated to dryness in vacuo. The residue was taken in ethyl acetate and washed with 5% $NaHCO_3$ and brine, dried on anhydrous $MgSO_4$ evaporated under reduced pressure and precipitated through hexane. To remove the traces of DCU the resulting compound was treated with cold tetrahydrofuran and the solid separated by filtration. The filtrate was evaporated under reduced pressure to obtain the title compound as white solid. Yield (52–70%).

Compound **4a**: Yield 65%, ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.38 (m, 3H) 2-Me, 2.07 (m, 1H) C_4–H, 1.27 (m, 3H) 4-Me, 3.51 (d, 1H) C_5–H, 1.33 (s, 3H) 6-Me, 1.56 (dd, 1H) and 1.90–1.96 (m, 1H) C_7–H, 2.58 (m, 1H) C_8–H, 1.08 (m, 3H) 8-Me, 3.01 (m, 1H) C_{10}–H, 1.13 (m, 3H) 10-Me, 1.47 (s, 3H) 12-Me, 1.50 (m, 1H) and 1.90–1.96 (m, 1H) C_{14}–H, 0.85 (t, 3H) CH_3CH_2, 4.38 (d, 1H) C_1'–H, 3.24 (m, 1H) C_2'–H, 2.74 (m, 1H) C_3'–H, 2.28 (s, 6H) C_3'–NMe_2, 1.20–1.28 (m, 1H) C_4'–H, 3.50–3.58 (m, 1H) C_5'–H, 1.26 (m, 3H) C_5'–Me, 4.99 (dd, 1H) 1″H, 3.04 (dd, 1H) 4″H, 4.04 (m, 1H) 5″H, 1.25 (s, 1H) 7″H, 3.32 (s, 3H) 3″-OCH_3, 7.20 (s, 5H) C_6H_5, 5.05 (s, 2H) $PhCH_2$ 3.94 (s, 2H) NH–CH_2; ^{13}C NMR (50 MHz, $CDCl_3$): δ 176.4 (C-1), 46 (C-2), 16.5 (C_2–Me), 79.2 (C-3), 35.9 (C-4), 9.8 (C–4Me), 83.5 (C-5), 78.0 (C-6), 27.5 (C–6Me), 37.2 (C-7), 45.5 (C-8), 19.2 (C–8Me), 168.2 (C-9), 38.9 (C-10),12.3 (C–10Me), 68.9 (C-11), 73.8 (C-12), 17.1 (C–12Me), 75 (C-13), 21.5 (C-14), 12.4 (C-15), 104 (C-1′), 72.3 (C-2′), 65.1 (C-3′), 40.2 (C–3′NMe_2), 29.3 (C-4′), 68.9 (C-5′), 23.2 (C–5′Me), 96.5 (C-1″), 35.1(C-2″), 78.0 (C-4″), 128.7–128.9 (5C of C_6H_5), 137.4 (1C of C_6 H_5), 68.6 ($PhCH_2$), 157.2 (CO–NH), 46.2 ($NHCH_2$), 170.8 (CH_2CO); Mass (FAB) 940 $(M+H)^+$.

Compound **4b**: Yield 51.2%, ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.38 (m, 3H) 2-Me, 2.07 (m, 1H) C_4–H, 1.27 (m, 3H) 4-Me, 3.51 (d, 1H) C_5–H, 1.33 (s, 3H) 6-Me, 1.56 (dd, 1H) and 1.90–1.96 (m, 1H) C_7–H, 2.58 (m, 1H) C_8–H, 1.08 (m, 3H) 8-Me, 3.01 (m, 1H) C_{10}–H, 1.13 (m, 3H) 10-Me, 1.47 (s, 3H) 12-Me, 1.49 (m, 1H) and 1.90–1.96 (m, 1H) C_{14}–H, 0.85 (t, 3H) CH_3CH_2, 4.38 (d, 1H) C_1'–H, 3.24 (m, 1H) C_2'–H, 2.74 (m, 1H) C_3'–H, 2.28 (s, 6H) C_3'–NMe_2, 1.20–1.28 (m, 1H) C_4'–H, 3.50–3.58 (m, 1H) C_5'–H, 1.26 (m, 3H) C_5'–Me, 4.98 (dd, 1H) 1″H, 3.04 (dd, 1H) 4″H, 4.04 (m, 1H) 5″H, 1.25 (s, 1H) 7″H, 3.32 (s, 3H) 3″-OCH_3, 6.88 (d, 2H) and 7.96 (d, 2H) C_6H_4, 3.83 (s, 3H) OCH_3; Mass (FAB) 883 $(M+H)^+$. Anal. Calcd $C_{45}H_{74}N_2O_{15}$C 61.20%, H 8.45%, N 3.17%. Found: C 61.28%, H 8.49%, 3.37N%.

Compound **4c**: Yield 70%, ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.38 (m, 3H) 2-Me, 2.07 (m, 1H) C_4–H, 1.27 (m, 3H) 4-Me, 3.51 (d, 1H) C_5–H, 1.33 (s, 3H) 6-Me, 1.56 (dd, 1H) and 1.90–1.96 (m, 1H) C_7–H, 2.58 (m, 1H) C_8–H, 1.08 (m, 3H) 8-Me, 3.01 (m, 1H) C_{10}–H, 1.13 (m, 3H) 10-Me, 1.47 (s, 3H) 12-Me, 1.49 (m, 1H) and 1.90–1.96 (m, 1H) C_{14}–H, 0.85 (t, 3H) CH_3CH_2, 4.38 (d, 1H) C_1'–H, 3.24 (m, 1H) C_2'–H, 2.74 (m, 1H) C_3'–H, 2.28 (s, 6H) C_3'–NMe_2, 1.20–1.28 (m, 1H) C_4'–H, 3.50–3.58 (m, 1H) C_5'–H, 1.26 (m, 3H) C_5'–Me, 4.98 (dd, 1H) 1″H, 3.04 (dd, 1H) 4″H, 4.04 (m, 1H) 5″H, 1.25 (s, 1H) 7″H, 3.32 (s, 3H) 3″-OCH_3 0.88 (s, 9H) Me_3C, 1.45 (d, 3H) CH_3–CH; Mass (ESMS) 921 $(M+H)^+$. Anal. Calcd $C_{45}H_{81}N_3O_{16}$C 58.74%, H 8.87%, N 4.57%. Found: C 58.22%, H 8.98%, N 4.58%.

Compound **4d**: Yield 53.1%, ^{1}H NMR (200 MHz, $CDCl_3$): δ 1.38 (m, 3H) 2-Me, 2.07 (m, 1H) C_4–H, 1.27 (m, 3H) 4-Me, 3.51 (d, 1H) C_5–H, 1.33 (s, 3H) 6-Me, 1.56 (dd, 1H) and 1.90–1.96 (m, 1H) C_7–H, 2.58 (m, 1H) C_8–H, 1.08 (m, 3H) 8-Me, 3.01 (m, 1H) C_{10}–H, 1.13 (m, 3H) 10-Me, 1.47 (s, 3H) 12-Me, 1.49 (m, 1H) and 1.90–1.97 (m, 1H) C_{14}–H, 0.85 (t, 3H) CH_3CH_2, 4.38 (d, 1H)

C_1'–H, 3.24 (m, 1H) C_2'–H, 2.74 (m, 1H) C_3'–H, 2.28 (s, 6H) C_3'–NMe_2, 1.20–1.28 (m, 1H) C_4'–H, 3.50–3.58 (m, 1H) C_5'–H, 1.26 (m, 3H) C_5'–Me, 5.0 (dd, 1H) 1″H, 3.04 (dd, 1H) 4″H, 4.04 (m, 1H) 5″H, 1.25 (s, 1H) 7″H, 3.32 (s, 3H) 3″-OCH_3, 0.83 (d, 6H) $(CH_3)_2C$, 0.88 (s, 9H) Me_3C, 1.5 (m, 1H) $(CH_3)_2CH$, 1.8 (m, 2H) $CHCH_2$, 4.45 (m, 1H) CHCO; Mass (FAB) 963 $(M+H)^+$. Anal. Calcd $C_{48}H_{87}N_3O_{16}$: C 59.92%, H 9.11%, N 4.37%. Found: C 59.99%, H 8.98%, N 4.38%.

5.3. Erythromycin-9-[O-(2,3-epoxypropyl)]oxime 5

750 mg (1.002 mM) of erythromycin-9-oxime was taken in 40 mL dry acetone to this mixture added 0.5 mL (9.8 mM) of epichlorohydrin and 1 gm anhydrous K_2CO_3. The reaction mixture was refluxed for 6–7 h. Concentrated under reduced pressure to evaporate acetone. The residue was dissolved in EtOAc, washed three times with $NaHCO_3$ and brine, dried over $MgSO_4$, evaporated at reduced pressure, and precipitated by hexane to get (**5**) as, pale yellow solid. The intermediate being an unstable product could not be purified and it was characterized by mass spectroscopy. IR(KBr) 3448, 2972, 1735, 1590, 1461, 1375, 1282, 1163, 1105, 1085, 1054 cm^{-1}. Mass (FAB) 805 (M^+).

5.4. Erythromycin-9-oxime ether derivatives 6(a–g)

General procedure for all the compounds: **5** was taken 800 mg (0.99 mM) in chloroform (8 mL) and was added the corresponding amine (2 mM), the resulting mixture was stirred for 1 h. To the reaction mixture was added two additional equivalents of the amine. This reaction mixture was stirred for 72 h at rt. The reaction mixture was concentrated under reduced pressure, it was added 150 mL of EtOAc, the organic layer was washed three times with brine, dried over Na_2SO_4, filtered, evaporated at reduced pressure to get **6(a–g)** as brown yellow oil. Resulting compound was purified by Silica gel column chromatography using different solvent systems to furnish **6(a–g)** as yellow oil, further purified through crystallised from chloroform–hexane to afford brown yellow powder in 20–52% yield.

5.5. Erythromycin-9-[O-(3-benzylamino-2 hydroxy)propyl]oxime 6a

Purified by silica gel column chromatography ($CHCl_3$–MeOH–NH_4OH (94.4:5:0.6)). Yield 50%, mp 104–106 °C; ^1H NMR (300 MHz, $CDCl_3$): δ 1.38 (m, 3H) 2-Me, 2.07 (m, 1H) C_4–H, 1.27 (m, 3H) 4-Me, 3.51 (d, 1H) C_5–H, 1.33 (s, 3H) 6-Me, 1.56 (dd, 1H) and 1.90–1.96 (m, 1H) C_7–H, 2.58 (m, 1H) C_8–H, 1.08 (m, 3H) 8-Me, 3.01 (m, 1H) C_{10}–H, 1.13 (m, 3H) 10-Me, 1.47 (s, 3H) 12-Me, 1.49 (m, 1H) and 1.90–1.96 (m, 1H) C_{14}–H, 0.85 (t, 3H) CH_3CH_2, 4.38 (d, 1H) C_1'–H, 3.24 (m, 1H) C_2'–H, 2.74 (m, 1H) C_3'–H, 2.28 (s, 6H) C_3'–NMe_2, 1.20–1.28 (m, 1H) C_4'–H, 3.50–3.58 (m, 1H) C_5'–H, 1.26 (m, 3H) C_5'–Me, 4.98 (dd, 1H) 1″H, 3.04 (dd, 1H) 4″H, 4.04 (m, 1H) 5″H, 1.25 (s, 1H) 7″H, 3.32 (s, 3H) 3″-OCH_3, 7.18–7.39 (m, 5H) C_6H_5, 3.80 (s, 2H) $PhCH_2$, 3.70 (d, 2H) O–CH_2, 3.54 (m, 1H) CH–OH, 2.70 (m, 2H) CH_2–NH; ^{13}C NMR (50 MHz, $CDCl_3$): δ 176.5 (C-1), 46 (C-2), 16.5 (C_2–Me), 79.2 (C-3), 35.9 (C-4), 9.8 (C–4Me), 83.5 (C-5), 78.0 (C-6), 27.5 (C–6Me), 37.2 (C-7),45.5 (C-8), 19.2 (C–8Me), 168.2 (C-9), 38.9 (C-10),12.3 (C–10Me), 68.9 (C-11), 73.8 (C-12), 17.1 (C–12Me), 75 (C-13), 21.5 (C-14), 12.4 (C-15), 104 (C-1′), 72.3 (C-2′), 65.1 (C-3′), 40.2 (C–3′NMe_2), 29.3 (C-4′), 68.9 (C-5′), 23.2 (C–5′Me), 96.5 (C-1″), 35.1 (C-2″), 78.0 (C-4″), 126.8–128.4 (5C of C_6H_5), 137.3 (1C of C_6H_5), 54.3 ($PhCH_2$), 74.1 (CH_2–N–O), 67.0 (CH_2OH), 55.5 (CH–NH), MS (FAB): 913 $(M+H)^+$. Anal. Calcd $C_{47}H_{81}N_3O_{14}$: C 62.18%, H 9.13%, N 4.53%. Found: C 61.84, H 8.47%, N 5.10%.

5.6. Erythromycin-9-[O-(3-(2-phenylethylamino)propyl)2-hydroxy]oxime 6b

Purified by silica gel column chromatography ($CHCl_3$–MeOH–NH_4OH (93.4:6:0.6)). Yield 52%, ^1H NMR (300 MHz, $CDCl_3$): δ 2.66 (m, 1H) C_2–H, 1.39 (m, 3H) 2-Me, 3.50–3.58 (m, 1H) C_3–H, 2.03 (m, 1H) C_4–H, 1.27 (m, 3H) 4-Me, 3.51 (d, 1H) C_5–H, 1.33 (s, 3H) 6-Me, 1.56 (m, 1H) and 1.90–1.95 (m, 1H) C_7–H, 1.08 (m, 3H) 8-Me, 3.01 (m, 1H) C_{10}–H, 1.13 (m, 3H) 10-Me, 3.86 (d, 1H) C_{11}–H, 1.47 (s, 3H) 12-Me, 1.49 (m, 1H) and 1.90–1.96 (m, 1H) C_{14}–H, 0.85 (t, 3H) CH_3CH_2, 4.38 (d, 1H) C_1'–H, 3.24 (dd, 1H) C_2'–H, 2.74 (m, 1H) C_3'–H, 2.28 (s, 6H) C_3'–NMe_2, 1.20–1.28 (m, 1H) C_4'–H, 3.50–3.58 (m, 1H) C_5'–H, 1.26 (m, 3H) C_5'–Me, 4.98 (dd, 1H) 1″H, 1.59 (m, 1H) and 2.38 (m, 1H) 2″CH_2, 3.02 (m, 1H) 4″H, 1.25 (s, 1H) 7″H, 3.32 (s, 3H) 3″-OCH_3, 7.18–7.38 (m, 5H) C_6H_5, 2.71 (s, 2H) $PhCH_2$, 2.90 (m, 2H) $PhCH_2CH_2$–NH, 3.70 (d, 2H) O–CH_2, 3.54 (m, 1H) CH–OH, 2.70 (m, 2H) CH_2–NH, ^{13}C NMR (50 MHz, $CDCl_3$): δ 176.4 (C-1), 45.8 (C-2), 16.5 (C_2–Me), 79.3 (C-3), 35.9 (C-4), 9.8 (C–4Me), 83.5 (C-5), 78.0 (C-6), 27.5 (C–6Me), 37.1 (C-7), 45.5 (C-8), 19.2 (C–8Me), 168.2 (C-9), 38.9 (C-10),12.4 (C–10Me), 68.8 (C-11), 73.8 (C-12), 17.2 (C–12Me), 75 (C-13), 21.5 (C-14), 12.4 (C-15), 104 (C-1′), 72.3 (C-2′), 65 (C-3′), 40.2 (C-3′NMe_2), 29.3 (C-4′), 68.9 (C-5′), 23.2 (C–5′Me), 96.5 (C-1″), 35.1 (C-2″), 78.0 (C-4″), 126.6–128.4 (5C of C_6H_5), 138.3 (1C of C_6H_5), 37.7 ($C_6H_5CH_2$), 52.1 ($PhCH_2CH_2$), (CH_2–N–O), 67.0 (CH_2OH), 55.5 (CH–NH), 76.5 (O–CH_2, 72.1 (CH–OH), 53.1 (CH_2–NH); MS (FAB): 927 $(M+H)^+$. Anal. Calcd $C_{48}H_{83}N_3O_{14}$: C 62.53%, H 9.21%, N 4.46%. Found: C 61.41%, H 9.13%, N 4.13%.

5.7. Erothromycin-9-[O-(3-(1-methylethyl)amino-2-hydroxyl)propyl]oxime 6c

Purified by silica gel column chromatography ($CHCl_3$–MeOH–NH_4OH (94.8:5:0.2)). Yield 36%, mp 155-156 °C, ^1H NMR (300 MHz, $CDCl_3$): δ 2.66 (m, 1H) C_2–H, 1.39 (m, 3H) 2-Me, 3.50–3.58 (m, 1H) C_3–H, 2.03 (m, 1H) C_4–H, 1.27 (m, 3H) 4-Me, 3.51 (d, 1H) C_5–H, 1.33 (s, 3H) 6-Me, 1.56 (m, 1H) and 1.90–1.95 (m, 1H) C_7–H, 1.07 (m, 3H) 8-Me, 3.01 (m, 1H) C_{10}–H, 1.13 (m,

3H) 10-Me, 3.86 (d, 1H) C_{11}–H, 1.47 (s, 3H) 12-Me, 1.49 (m, 1H) and 1.90–1.96 (m, 1H) C_{14}–H, 0.85 (t, 3H) CH_3CH$_2$, 4.38 (d, 1H) C_1'–H, 3.24 (dd, 1H) C_2'–H, 2.74 (m, 1H) C_3'–H, 2.28 (s, 6H) C_3'–NMe_2, 1.20–1.28 (m, 1H) C_4'–H, 3.51–3.58 (m, 1H) C_5'–H, 1.27 (m, 3H) C_5'–Me, 4.98 (dd, 1H) 1″H, 1.59 (m, 1H) and 2.38 (m, 1H) 2″CH$_2$, 3.02 (m, 1H) 4″H, 1.25 (s, 1H) 7″H, 3.32 (s, 3H) 3″-OCH$_3$, 1.05 (d, 6H) 2×CH$_3$, 2.90 (m, 1H) CH$_3$–*CH*–CH$_3$, 3.80 (d, 2H) O–CH$_2$, 3.70 (m, 2H) CH–OH, 2.72 (m, 2H) CH$_2$–NH; MS (FAB): 865 $(M+H)^+$. Anal. Calcd $C_{43}H_{81}N_3O_{14}$: C 59.77%, H 9.45%, N 4.86%. Found: C 59.76%, H 8.96%, N 4.77%.

5.8. Erythromycin-9-[O-(3-propylamino-2-hydroxy)propyl]oxime 6d

Purified by silica gel column chromatography (EtOAc–MeOH–NH_4OH (94.8:5:0.2)). Yield 39%, mp 160–162 °C; ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.66 (m, 1H) C_2–H, 1.39 (m, 3H) 2-Me, 3.50–3.58 (m, 1H) C_3–H, 2.03 (m, 1H) C_4–H, 1.27 (m, 3H) 4-Me, 3.51 (d, 1H) C_5–H, 1.33 (s, 3H) 6-Me, 1.56 (m, 1H) and 1.90–1.95 (m, 1H) C_7–H, 1.08 (m, 3H) 8-Me, 3.01 (m, 1H) C_{10}–H, 1.13 (m, 3H) 10-Me, 3.86 (d, 1H) C_{11}–H, 1.47 (s, 3H) 12-Me, 1.49 (m, 1H) and 1.90–1.96 (m, 1H) C_{14}–H, 0.85 (t, 3H) CH_3CH$_2$, 4.38 (d, 1H) C_1'–H, 3.24 (dd, 1H) C_2'–H, 2.74 (m, 1H) C_3'–H, 2.28 (s, 6H) C_3'–NMe_2, 1.20–1.28 (m, 1H) C_4'–H, 3.50–3.58 (m, 1H) C_5'–H, 1.26 (m, 3H) C_5'–Me, 4.98 (dd, 1H) 1″H, 1.59 (m, 1H) and 2.38 (m, 1H) 2″CH$_2$, 3.02 (m, 1H) 4″H, 1.25 (s, 1H) 7″H, 3.32 (s, 3H) 3″-OCH$_3$, 0.80 (t, 3H) CH$_3$–C, 1.45 (m, 2H) *CH*$_2$–CH$_3$, 2.40 (m, 2H) CH$_2$–NH, 3.80 (d, 2H) CH$_2$–O, 3.51 (m, 1H) CH–OH, 2.70 (m, 2H) CH$_2$–N; MS (FAB): 865 $(M+H)^+$. Anal. Calcd for $C_{43}H_{81}N_3O_{14}\cdot 2H_2O$: C 57.73%, H 9.33%, N 4.66%. Found: C 57.78%, H 9.32%, N 4.68%.

5.9. Erythromycin-9-[O-(3-piperidinyl-2-hydroxy)propyl]oxime 6e

Purified by silica gel column chromatography ($CHCl_3$–MeOH–NH_4OH (94.8:5:0.2)). Yield 45%, ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.66 (m, 1H) C_2–H, 1.39 (m, 3H) 2-Me, 3.50–3.58 (m, 1H) C_3–H, 2.03 (m, 1H) C_4–H, 1.27 (m, 3H) 4-Me, 3.51 (d, 1H) C_5–H, 1.33 (s, 3H) 6-Me, 1.56 (m, 1H) and 1.90–1.95 (m, 1H) C_7–H, 1.08 (m, 3H) 8-Me, 3.01 (m, 1H) C_{10}–H, 1.13 (m, 3H) 10-Me, 3.86 (d, 1H) C_{11}–H, 1.47 (s, 3H) 12-Me, 1.49 (m, 1H) and 1.90–1.96 (m, 1H) C_{14}–H, 0.85 (t, 3H) CH_3CH$_2$, 4.38 (d, 1H) C_1'–H, 3.24 (dd, 1H) C_2'–H, 2.74 (m, 1H) C_3'–H, 2.28 (s, 6H) C_3'–NMe_2, 1.20–1.28 (m, 1H) C_4'–H, 3.50–3.58 (m, 1H) C_5'–H, 1.26 (m, 3H) C_5'–Me, 4.98 (dd, 1H) 1″H, 1.59 (m, 1H) and 2.38 (m, 1H) 2″CH$_2$, 3.02 (m, 1H) 4″H, 1.25 (s, 1H) 7″H, 3.32 (s, 3H) 3″-OCH$_3$, 1.50 (br s, 6H) (CH$_2$)$_3$–piperidine, 2.28 (t, 4H) –N(CH$_2$)$_2$–piperidine, 3.68 (d, 1H) CH$_2$–O, 3.54 (m, 1H) CH-OH, 2.51 (d, 2H) CH$_2$N; MS (FAB): 891 $(M+H)^+$. Anal. Calcd for $C_{45}H_{83}N_3O_{14}\cdot H_2O$: C 59.85%, H 9.61%, N 4.55%. Found: C 59.87%, H 9.81%, N 4.55%.

5.10. Erythromycin-9-[O-(3-imidazolyl-2-hydroxy)propyl]-oxime 6f

Purified by silica gel column chromatography ($CHCl_3$–MeOH–NH_4OH (94.8:5:0.2)). Yield 20%, ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.66 (m, 1H) C_2–H, 1.39 (m, 3H) 2-Me, 3.50–3.58 (m, 1H) C_3–H, 2.03 (m, 1H) C_4–H, 1.27 (m, 3H) 4-Me, 3.51 (d, 1H) C_5–H, 1.33 (s, 3H) 6-Me, 1.56 (m, 1H) and 1.90–1.95 (m, 1H) C_7–H, 1.08 (m, 3H) 8-Me, 3.01 (m, 1H) C_{10}–H, 1.13 (m, 3H) 10-Me, 3.86 (d, 1H) C_{11}–H, 1.47 (s, 3H) 12-Me, 1.49 (m, 1H) and 1.90–1.96 (m, 1H) C_{14}–H, 0.85 (t, 3H) CH_3CH$_2$, 4.38 (d, 1H) C_1'–H, 3.24 (dd, 1H) C_2'–H, 2.74 (m, 1H) C_3'–H, 2.28 (s, 6H) C_3'–NMe_2, 1.20–1.28 (m, 1H) C_4'–H, 3.50–3.58 (m, 1H) C_5'–H, 1.26 (m, 3H) C_5'–Me, 4.98 (dd, 1H) 1″H, 1.59 (m, 1H) and 2.38 (m, 1H) 2″CH$_2$, 3.02 (m, 1H) 4″H, 1.25 (s, 1H) 7″H, 3.32 (s, 3H) 3″-OCH$_3$, 7.5 (s, 1H), 2-H (imidazolyl), 6.97 (s, 2H), 4-H and 5-H (imidazolyl), 3.68 (d, 2H) O–CH$_2$, 3.10 (m, 1H) CH–OH, 3.9 (d, 2H) CH$_2$–N; MS (FAB): 874 $(M+H)^+$. Anal. Calcd for $C_{43}H_{76}N_4O_{14}$: C 59.15%, H 8.77%, N 6.42%. Found: C 59.03%, H 8.85%, N 6.33%.

5.11. Erythromycin-9-[O-(3-(1-methylpiperazinyl)2-hydroxyl)propyl]oxime 6g

Purified by silica gel column chromatography (EtOAc–MeOH–NH_4OH (93.8:6:0.2)). Yield 36%, ^{1}H NMR (300 MHz, $CDCl_3$): δ 2.66 (m, 1H) C_2–H, 1.39 (m, 3H) 2-Me, 3.50–3.58 (m, 1H) C_3–H, 2.03 (m, 1H) C_4–H, 1.27 (m, 3H) 4-Me, 3.51 (d, 1H) C_5–H, 1.33 (s, 3H) 6-Me, 1.56 (m, 1H) and 1.90–1.95 (m, 1H) C_7–H, 1.08 (m, 3H) 8-Me, 3.01 (m, 1H) C_{10}–H, 1.13 (m, 3H) 10-Me, 3.86 (d, 1H) C_{11}–H, 1.47 (s, 3H) 12-Me, 1.49 (m, 1H) and 1.90–1.96 (m, 1H) C_{14}–H, 0.85 (t, 3H) CH_3CH$_2$, 4.38 (d, 1H) C_1'–H, 3.24 (dd, 1H) C_2'–H, 2.74 (m, 1H) C_3'–H, 2.28 (s, 6H) C_3'–NMe_2, 1.20–1.28 (m, 1H) C_4'–H, 3.50–3.58 (m, 1H) C_5'–H, 1.26 (m, 3H) C_5'–Me, 4.98 (dd, 1H) 1″H, 1.59 (m, 1H) and 2.38 (m, 1H) 2″CH$_2$, 3.02 (m, 1H) 4″H, 1.25 (s, 1H) 7″H, 3.32 (s, 3H) 3″-OCH$_3$, 2.49 (s, 8H) 4×CH$_2$–N, 2.17 (s, 3H) CH$_3$–N, 3.60 (d, 2H) O–CH$_2$, 3.51 (m, 1H) CH–OH, 2.56 (d, 2H) CH$_2$–N; MS (FAB): 906 $(M+H)^+$. Anal. Calcd for $C_{45}N_{84}N_4O_{14}$: C 59.71%, H 9.35%, N 6.19%. Found: C 59.69, H 9.39%, N 6.16%.

Acknowledgements

The author thank the Director, CDRI for the support and the SIAF for the spectral data.

References and notes

1. Omura, S.; Tsuzuki, K.; Sunazuka, T.; Toyota, H.; Takahashi, I.; Itoh, Z. *J. Antibiot.* **1985**, *38*, 1631.
2. (a) Konno, S.; Asano, K.; Kurokawa, M.; Ikeda, K.; Okamoto, K.; Adachi, M. *Int. Arch. Allergy Imm.* **1994**, *105*, 308; (b) Miyatake, H.; Suzuki, K.; Taki, F.; Satake, T. *Arznei.-Forschung. Drug Res.* **1991**, *41*, 552; (c) Takiz-

awa, H.; Desaki, M.; Ohtoshi, T.; Okazaki, H.; Sato, M.; Akiyama, N.; Shoji, S.; Hiramatsu, K.; Ito, K. *Biochem. Biophys. Res. Commun.* **1995**, *210*, 781.
3. (a) Chu, D. T. W. *Exp. Opin. Invest. Drugs* **1995**, *4*, 65; (b) Lartey, P. A.; Perun, T. In *Studies in Natural Products Chemistry*; Pehman, A., Ed.; Elsevier: Amsterdam, 1993; 13, p 155.
4. Morimoto, S.; Takahasi, Y.; Watanabe, Y.; Omura, S. *J. Antibiot.* **1984**, *37*, 187.
5. (a) Chantot, J. F.; Gase, J. C.; D'Ambrieres and Lutz, A. 23rd Interscience Conference on Antimicrobial Agents Chemotherapy, No. 447, P 165, Las Vegas, October 24–26, **1983**; (b) Chantot, J. F.; Bryskier, A.; Gase, J. C. *J. Antibiot.* **1986**, *39*, 660.
6. Reestima, J. A.; Girard, A. E.; Schekly, W.; Manonsos, M.; Anderson, M. R.; Bright, G. M.; Borovoy, R. J.; Brenan, L. A.; Mason, R. *Antimicrob. Agent Chemother.* **1987**, *31*, 1939.
7. (a) Kurath, P.; Jones, P.; Egan, R.; Perun, T. *Experentia* **1971**, *27*, 362; (b) Itoh, Z.; Nakaya, M.; Suzuki, T.; Hiral, H.; Wakabayashi, K. *Am. J. Physiol.* **1984**, *10*, G688.
8. Wildsmith, E. *Tetrahedron Lett.* **1972**, *1*, 29.
9. (a) Massey, E. H.; Kitchel, E. H.; Martin, L. D.; Gerzon, K. *J. Med. Chem.* **1974**, *17*, 105; (b) Massey, E. H.; Kitchel, E. H.; Martin, L. D.; Gerzon, K.; Murphy, W. *Tetrahedron Lett.* **1970**, *2*, 157; (c) Lartey, P. A.; Deninno, S. L.; Faghih, R.; Hardy, D. J.; Clement, J. J.; Plattner, J. J. *J. Antibiot.* **1992**, *45*, 380; (d) Hunt, E.; Knowles, D. J. C.; Shillingford, C.; Zomaya, I. I. *J. Antibiot.* **1998**, *40*, 1644.
10. (a) Brain, E. G.; Forrest, A. K.; Hunt, E.; Shillingford, C.; Wilson, J. M. *J. Antibiot.* **1989**, *41*, 1817; (b) Nishimoto, A.; Narita, K.; Ohimoto, S.; Takahashi, Y.; Yoshizami, S.; Yoshida, T.; Kado, N.; Okezaki, E.; Kato, H. *Chem. Pharm. Bull.* **2001**, *49*, 1120; (c) Gask, J. C.; D'Ambrieres, G. S.; Lutz, A.; Chantot, J. F. *J. Antibiot.* **1991**, *44*, 313.
11. Agoridas, C.; Denis, A.; Auger, J. M.; Benedetti, A.; Bonnefoy, A.; Bretin, F.; Chantot, J. F.; Fromentin, C.; Gouin D' Ambrieres, S.; Lachand, S.; Laurin, P.; Le martret, O.; Loyau, V.; Tessot, N. *J. Med. Chem.* **1998**, *41*, 4080.
12. Denis, A.; Agoridas, C.; Auger, J.-M.; Benedetti, Y.; Bonnefoy, A.; Bretin, F.; Chantot, J. F.; Dussarat, A.; Fromentin, C.; Gouin D' Ambrieres, S.; Lachand, S.; Laurin, P.; Le Martret, O.; Loyau, V.; Tessot, N.; Pejac, J.-M.; Perron, S. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 3075.
13. Weller, T.; Alif, L.; Berensini, M.; Blackburn, B.; Bunting, S.; Havardy, P.; Hurzeler-Muller, M.; Knopp, D.; Levet-Trafit, B.; Lipari, M. T.; Modi, N. B.; Muller, M.; Refino, C. J.; Schmitt, M.; Schonholzer, P.; Weiss, S.; Steiner, B. *J. Med. Chem.* **1996**, *39*, 3139.
14. Edward Harrison, F.; Bruce Cropp, C. *Appl. Micorbiol.* **1961**, *13*, 212.
15. (a) Ohara, T.; Kojio, S.; Taneike, I.; Nakagawa, S.; Gondaira, F.; Tamura, Y.; Gejyo, F.; Zhang, H. M.; Yamamoto, T. *Antimicrob. Agents Chemother.* **2002**, *46*, 3478; (b) Nakagawa, S.; Kojio, S.; Taneike, I.; Iwakura, N.; Tamura, Y.; Kushiya, K.; Gondaira, F.; Yamamoto, T. *Biochem. Biophys. Res. Commun.* **2003**, *310*, 1194.