Chinese Chemical Letters xxx (2013) xxx–xxx

Contents lists available at SciVerse ScienceDirect

Chinese Chemical Letters

journal homepage: www.elsevier.com/locate/cclet

Original article

Synthesis and antibacterial activity of novel 10,11-epoxy acylide erythromycin derivatives

Ying Nie [a], Yin Sun [b], Qi-Dong You [b,*]

[a] Experimental Center of Pharmaceutical Science, China Pharmaceutical University, Nanjing 210009, China
[b] Jiangsu Key Laboratory of Drug Design and Optimization, China Pharmaceutical University, Nanjing 210009, China

ARTICLE INFO

Article history:
Received 21 January 2013
Received in revised form 3 February 2013
Accepted 4 February 2013
Available online xxx

Keywords:
Epoxide acylide erythromycin derivatives
Synthesis
Single-crystal X-ray diffraction
Resistance antibacterial activity

ABSTRACT

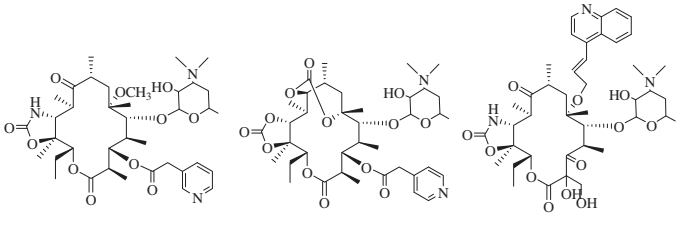
A series of novel acylide derivatives have been synthesized from clarithromycin A *via* a facile procedure. The C-3 modifications involved replacing the natural C-3 cladinosyl group in clarithromycin core with different aryl-piperzine sidechain *via* chemical synthesis. Meanwhile a distinctive intermediate with 10,11-epoxy moiety was obtained. The structure and stereochemistry of this novel structure were confirmed *via* NMR and X-ray crystallography. Potential anti-bacterial activities against both Gram-positive and Gram-negative bacteria were reported. Because of existence of C10,11-epoxide, these derivatives can be used as intermediates for further structural modification.

1. Introduction

Macrolide antibiotics, including erythromycin and clarithromycin, are a class of preferred drug class for the safe and effective treatment of respiratory tract infections. They are potent against several key respiratory pathogens including *Staphylococcus aureaus*, *Streptococcus pnuemoniae*, *etc.* The 14-membered macrolide antibiotic erythromycin is unstable under acidic condition [1,2]. Therefore, the second-generation macrolides have been developed, such as clarithromycin, azithromycin, and roxithromycin. Over the past decade the resistance to these second-generation marolides has emerged. There are two main mechanisms of macrolide resistance. One mechanism is target site modification *via* methylation of the ribosomal target in bacteria. This type of resistance is referred to as the MLS_B phenotype [3,4]. The other mechanism is antibiotic efflux caused by two classes of pumps, ATP-binding-cassette (ABC) transporter superfamily and the major facilitator superfamily (MFS) [3]. To overcome the resistance much attention has been focused on the modification of C-3, C-6, C-9, C-11 and C-12.

Ketolides which are developed in recent structural studies, including Telithromycin and Cethromycin (ABT-773), represent improved activity against erythromycin- and penicillin-resistant isolates of *Streptococcus pneumoniae*. In addition, acylides, in which C3-cladinosyl group of erythromycin A is replaced by an *O*-acyl group, are reported to be active against many bacterial respiratory pathogens, including erythromycin-resistant strains [5].

The acylides, such as FMA0122 **1** and TEA0929 **2**, exhibit markedly enhanced activity against MLS_B-resistant strains [6]. U.S. Patent No. 2002/0103140 describes a new class of 6-*O*-alykl-2-*nor*-2-sustituted ketolide derivatives [6]. In this invention the compound example **3** demonstrates even better antibacterial activity against *Streptococcus pneumoniae* than Cethromycin (ABT-773), in which 2-methyl is replaced by *sec*-l,2-diols. The novel analogs modified with C-2 position exhibit improved aqueous solubility. As reported in the patent, the compound example **3** is prepared through epoxidation of the double bond at C-2 position.



1 **2** **3**

To address the bacterial resistance and solubility problems, we wish to produce a series of 3-*O*-acyl erythromycin derivatives with well hydrophilicity. We have obtained a series of novel acylides in which the aryl-piperzine sidechain is attached to the macrolide

* Corresponding author.
E-mail address: youqidong@gmail.com (Q.-D. You).

1001-8417/$ – see front matter © 2013 Qi-Dong You. Published by Elsevier B.V. on behalf of Chinese Chemical Society. All rights reserved.
http://dx.doi.org/10.1016/j.cclet.2013.02.015

core *via* a C3-carbamate linkage with 10,11-epoxy moiety simultaneously.

2. Experimental

The synthesis route of the target compounds was outlined in Scheme 1. The synthesis of **14** started with commercially available clarithromycin. Clarithromycin was reacted with acetic anhydride in methylene chloride with the presence of pyridine and 4-dimethylamino pyridine (DMAP) to give 2′,4″-diacetyl erythromycin A derivative **4**. Compound **4** was treated by bis(trichloromethanol)carbonate (BTC) under base condition (pyridine) in methylene chloride to produce 11,12-cabamate compound **5**. Treatment of **5** with 1,8-diazabicyclo[5,4,0]undec-7-ene (DBU) in acetone yielded the corresponding enone **6** at room temperature and under refluxing. Compound **6** was protected by a carbobenzyloxy group (Cbz) to afford 3′-*N*-benzyloxycarbonyl erythromycin A derivative **7**. Compound **7** was converted to the target C-3 desugarized compound **8** by diluted hydrochloric acid to remove C3-cladinosyl group. Treatment of compound **8** with metachloroperoxybenzoic acid resulted in epoxidation of the C10,11 alkene. The product isolated was the C10,11-epoxide **9**.

The NMR spectra were in accordance with the C10,11-epoxide acylide structure **9**. The epoxy formation was supported by a singlet at 2.90 ppm in the ^{1}H NMR spectrum, and the carbamate signal at 212 ppm in the ^{13}C NMR spectrum due to the C-9 carbonyl proton. Meanwhile, the signal coupled to the C10,11 double bond at 6.41 ppm in ^{1}H NMR experiment disappeared. MS and NMR data indicate that compound **9** is C10,11-epoxide, and the stereo configuration of the two chiral carbons in the cyclic formation are 10*R*, 11*S* based on the evidence in Fig. 1 from the X-ray crystallography.

Removal of the benzyloxycarbonyl and acetyl group of compound **9** by catalytic hydrogenation furnished *N*-demethyl erythromycin A derivative **10**. Compound **10** underwent reductive *N*-demethylation in ethanol with formaldehyde in the presence of formic acid under mild reflux to afford compound **11**.

Acetylation of **11** with acetic anhydride as before gave **12**. Alcohol **12** was reacted with acryloyl chloride, Et_3N and DMAP in CH_2Cl_2 at 0 °C to afford acrylate ester **13** [7].

Finally, the resulting acrylate ester **13** was reacted with phenyl piperazine derivatives, and was removed the acetyl group to give

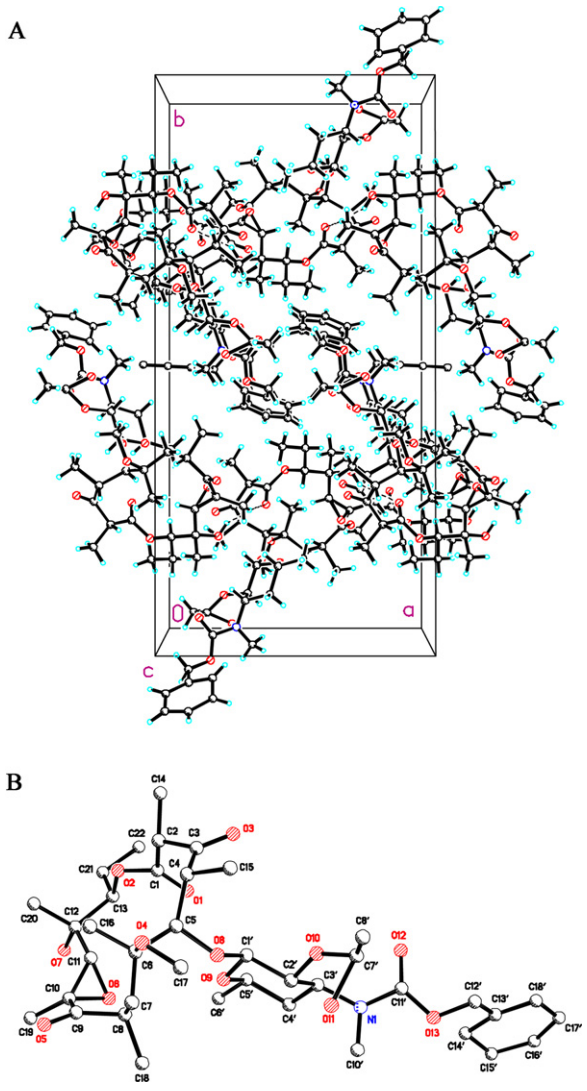
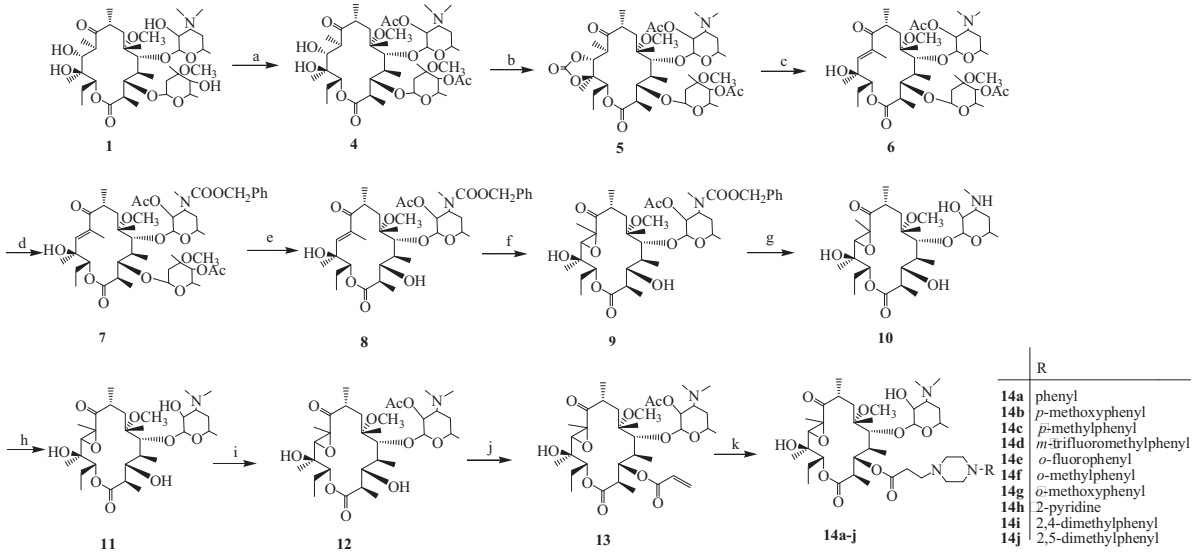


Fig. 1. The single-crystal X-ray diffraction data of compound **9**: (A) Crystal packing of compound **9**; (B) Molecule structure of compound **9** without hydrogen.



Scheme 1. Reagents and conditions: (a) Ac_2O, pyridine, DMAP, CH_2Cl_2, r.t. for 5 h; (b) BTC, pyridine, CH_2Cl_2, 0 °C for 4 h, r.t. for 10 h; (c) DBU, acetone, reflux, 8 h; (d) benzyl chloroformate, $NaHCO_3$, 1,4-dioxane, 55 °C, 3 h; (e) HCl, C_2H_5OH, 40 °C, 2 d; (f) *m*CPBA, CH_2Cl_2, 30 °C, 5 h; (g) H_2, Pd-C, HAc, C_2H_5OH, H_2O, N.T.P., 2 d; (h) HCHO, HCOOH, C_2H_5OH, reflux, 3 h; (i) Ac_2O, TEA, CH_2Cl_2, r.t. for 5 h; (j) acryloyl chloride, Et_3N, DMAP, CH_2Cl_2, r.t. for 6 h; (k) (i) CH_2Cl_2, reflux, 3 d, (ii) CH_3OH, reflux, 2 d.

Please cite this article in press as: Y. Nie, et al., Synthesis and antibacterial activity of novel 10,11-epoxy acylide erythromycin derivatives, Chin. Chem. Lett. (2013), http://dx.doi.org/10.1016/j.cclet.2013.02.015

Table 1
In vitro antibacterial activity of C10,11-epoxide acylide derivatives.

Organism	MIC (μg/mL)						
	14d	**14f**	**14i**	**14j**	Azithromycin	Clarithromycin	Erythromycin
Staphylococcus aureus	32	128	64	128	0.25	0.03125	4
Staphylococcus epidermidis	64	128	64	64	128	128	>128
Staphylococcus aureus MRSA	128	128	64	64	32	4	2
Staphylococcus epidermidis MRSE	64	128	64	64	32	16	32
Escherichia coli	128	128	>128	64	2	32	>128
Salmonella typhi	128	64	128	128	2	16	128
Klebsiella pneumonia	128	128	>128	128	2	32	128
Hemophilus influenzae	>128	64	64	>128	0.03125	1	16
Escherichia coli ESBL	>128	128	>128	>128	32	>128	>128
Klebsiella pneumonia ESBL	4	>128	>128	>128	8	>128	>128

target compounds **14a–j**. The structures of the target compounds synthesized herein were fully confirmed by melting points, ^{1}HNMR, IR, ESI-MS, and elemental analysis [8].

3. Results and discussion

The 10,11-epoxy acylide erythromycin derivatives and the reference compounds, clarithromycin and azithromycin were tested against different representative pathogens. Various pathogens were tested in order to identify the potency of these acylide analogs.

The *in vitro* antibacterial activity is reported as minimum inhibitory concentrations (MICs). As shown in Table 1, most of these compounds are as effective as erythromycin to the Gram-positive bacteria. Meanwhile, several compounds displayed improved activity to Gram-negative bacteria. To readily interpret the structure–activity relationships of the C-3 position substituent, the C6-carbamoyl side chain was initially held constant, while various C3-carbamates were examined. Basic groups were introduced in these C3-carbamate derivatives to increase their water solubility. However, attempts to improve activity by structural modification of acylides with aryl-piperzine sidechains shortening or lengthening the pyridylalkyl chain, as in compounds aryl-piperzine sidechain had a detrimental effect against either Gram-positive bacteria or Gram-negative bacteria compared to clarithronycin, telithromycin, and azithromycin.

4. Conclusion

In summary, we have discovered a novel series of acylide antibiotics that employ a C-3 carbamate for attachment of the arylpiperzine sidechain with 10,11-epoxy moiety. Meanwhile, we synthesized a unique 10,11-epoxy acylide skeleton structure, and verified its spatial configuration through X-ray crystallography. Compounds with various carbamate groups at the C-3 position were studied for their antibacterial activity. Part of these compounds showed improved activity against Gram-negative bacteria compared with erythromycin and clathromycin.

Acknowledgments

This work was supported by Jiangsu Hengrui Pharmaceutical Company. The X-ray crystallography was performed at Shanghai Institute of Organic Chemistry, China Academy of Science.

References

[1] P. Kurath, P. Jones, R. Egan, et al., Acid degradation of erythromycin A and erythromycin B, Experentia 27 (1971) 362.
[2] K. Krowick, A. Zamojski, Chemical modification of erythromycin I. 8,9-anhydro-6^9-hemiketal of A, J. Antibiot. 26 (1973) 569–574.
[3] R. Leclercq, Mechanisms of resistance to macrolides and lincosamides: nature of the resistance elements and their clinical implications, Clin. Infect. Dis. 34 (2002) 482–492.
[4] J.C. Pechere, Macrolide resistance mechanisms in Gram-positive cocci, Int. J. Antimicrob. Agents 18 (2001) S25–S28.
[5] T. Tanikawa, T. Asaka, M. Kashimura, et al., Synthesis and antibacterial activity of a novel series of acylides: 3-O-(3-pyridyl) acetylerythromycin A derivatives, J. Med. Chem. 46 (2003) 2706–2715.
[6] T. Ly, S. Yat, M. Zhenkun, 6-O-alkyl-2-nor-2-substituted ketolide derivatives, U.S. Patent. 0103140 (2002).
[7] A.K. Ghosh, K. Jae-Hun, An enantioselective synthesis of the C1 C9 segment of antitumor macrolide peloruside A, Tetrahedron Lett. 44 (2003) 3967–3969.
[8] Selected characteristic data for the compounds. **9**: Yellow powder; mp 91–93 °C, IR (KBr, cm^{-1}): υ 1706, 1729, 1748; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.28–7.40 (m, 5H, Ph-H), 5.03 (dd, 1 H, J = 2.3 Hz and 9.2 Hz, H_{13}), 4.69 (dd, 1 H, J = 2.3 Hz and 8.2 Hz, $H_{2'}$), 3.10 (s, 3H, C6-OCH_3), 1.88 (s, 3H, C2′-$OCOCH_3$), 1.66 (s, 3H, C10-CH_3); ESI-MS m/z (%): 772 (M+Na$^+$). X-ray analysis: $C_{39}H_{59}NO_{13}$, M_r = 749.90, crystal size 0.586 mm × 0.173 mm × 0.049 mm, orthorhombic in $p2_{1/n}$ with a = 14.024(3), b = 28.998(6), c = 10.915(2) Å, a, b, g = 90°, V = 3185.0(9) Å^3, Dcalu = 1.151 Mg/m^3, and Z = 4, absorption coefficient 0.087 mm^{-1}, computing structure solution SHELXL-97, theta range for data collection 1.61 to 25.50°, limiting indices $-16 \le h \le 10$, $-35 \le k \le 19$, $-13 \le l \le 12$, reflection collected 23690, refinement method full-matrix least-squares, final R indices [$I > 2$ sigma (I)] R_1 = 0.1016, wR_2 = 0.2461, R indices (all data) R_1 = 0.2601, wR_2 = 0.3102, goodness-of-fit on F^2 0.894, largest difference peak 0.360 eÅ^{-3}, largest difference hole −0.236 eÅ^{-3}. **14a**: White crystal; mp 127–129 °C, IR (KBr, cm^{-1}): υ 1165, 1706, 1741; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.09–7.28 (m, 5H, Ph-H), 5.01 (dd, 1 H, J = 2.4 Hz and 10.4 Hz, H_{13}), 3.23 (s, 3H, C6-OCH_3), 3.18 (m, 4H, 2×CH_2 in piperazinyl), 2.65 (m, 6H, 2×CH_2 in piperazinyl and CH_2N), 2.39 (s, 6H, N(CH_3)$_2$), 1.64 (s, 3H, C10-OCH_3); ESI-MS m/z (%): 803 (M+H$^+$). **14b**: White crystal; mp 117–119 °C, IR (KBr, cm^{-1}): υ 1164, 1512, 1741; ^{1}H NMR (300 MHz, $CDCl_3$): δ 6.81–7.27 (m, 4H, Ph-H), 5.01 (dd, 1 H, J = 2.3 Hz and 10.3 Hz, H_{13}), 3.76 (s, 3H, Ar-OCH_3), 3.12 (s, 3H, C6-OCH_3), 3.07 (m, 4H, 2×CH_2 in piperazinyl), 2.68 (m, 6H, 2×CH_2 in piperazinyl and CH_2N), 2.46 (s, 6H, N(CH_3)$_2$), 1.62 (s, 3H, C10-CH_3); ESI-MS m/z (%): 833 (M+H$^+$). **14c**: White crystal; mp 105-106 °C, IR (KBr, cm^{-1}): υ 1164, 1741; ^{1}H NMR (300 MHz, $CDCl_3$): δ 6.81–7.27 (m, 4H, Ph-H), 5.01 (dd, 1 H, J = 2.7 Hz and 10.5 Hz, H_{13}), 3.22 (s, 3H, C6-OCH_3), 3.15 (m, 4H, 2×CH_2 in piperazinyl), 2.63 (m, 6H, 2×CH_2 in piperazinyl and CH_2N), 2.38 (s, 6H, N(CH_3)$_2$), 2.17 (s, 3H, Ar-CH_3), 1.65 (s, 3H, C10-OCH_3); ESI-MS m/z (%): 817 (M+H$^+$). **14d**: White crystal; mp 112–113 °C, IR (KBr, cm^{-1}): υ 1164, 1742; ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.03–7.37 (m, 4H, Ph-H), 5.01 (dd, 1 H, J = 6.5 Hz and 14.0 Hz, H_{13}), 3.24 (s, 3H, C6-OCH_3), 3.11 (m, 4H, 2×CH_2 in piperazinyl), 2.66 (m, 6H, 2×CH_2 in piperazinyl and CH_2N), 2.40 (s, 6H, N(CH_3)$_2$), 1.65 (s, 3H, C10-OCH_3); ESI-MS m/z (%): 871 (M+H$^+$). **14e**: White crystal; mp 114–116 °C, IR (KBr, cm^{-1}): υ 1164, 1742; ^{1}H NMR (300 MHz, $CDCl_3$): δ 6.90–7.45 (m, 4H, Ph-H), 5.00 (dd, 1 H, J = 2.4 Hz and 10.3 Hz, H_{13}), 3.23 (s, 3H, C6-OCH_3), 3.14 (m, 4H, 2×CH_2 in piperazinyl), 2.82 (m, 6H, 2×CH_2 in piperazinyl and CH_2N), 2.29 (s, 6H, N(CH_3)$_2$), 1.64 (s, 3H, C10-OCH_3); ESI-MS m/z (%): 821 (M+H$^+$). **14f**: White crystal; mp 124–125 °C, IR (KBr, cm^{-1}): υ 1163, 1742; ^{1}H NMR (300 MHz, $CDCl_3$): δ 6.95–7.18 (m, 4H, Ph-H), 5.00 (dd, 1 H, J = 2.2 Hz and 10.2 Hz, H_{13}), 3.24 (s, 3H, C6-OCH_3), 3.19 (m, 4H, 2×CH_2 in piperazinyl), 2.83 (m, 6H, 2×CH_2 in piperazinyl and CH_2N), 2.29 (s, 9H, Ph-CH_3 and N(CH_3)$_2$), 1.63 (s, 3H, C10-OCH_3); ESI-MS m/z (%): 817 (M+H$^+$). **14g**: White crystal; mp 134–136 °C, IR (KBr, cm^{-1}): υ 1164, 1741; ^{1}H NMR (300 MHz, $CDCl_3$): δ 6.87–7.02 (m, 4H, Ph-H), 5.00 (dd, 1 H, J = 2.7 Hz and 10.1 Hz, H_{13}), 3.86 (s, 3H, Ph-OCH_3), 3.23 (s, 3H, C6-OCH_3), 3.15 (m, 4H, 2×CH_2 in piperazinyl), 2.69 (m, 6H, 2×CH_2 in piperazinyl and CH_2N), 2.31 (s, 6H, N(CH_3)$_2$), 1.64 (s, 3H, C10-OCH_3); ESI-MS m/z (%): 833 (M+H$^+$). **14h**: White crystal; mp 99–107 °C, IR (KBr, cm^{-1}): υ 1165, 1741; ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.18 (d, 1 H, Pyr-H_6), 7.47 (t, 1 H, Pyr-H_4), 6.63 (t, 2H, Pyr-H_3, Pyr-H_5), 5.00 (dd, 1 H, J = 3.7 Hz and 8.9 Hz, H_{13}), 3.22 (s, 3H, C6-OCH_3), 3.18 (m, 4H, 4H, 2×CH_2 in piperazinyl), 2.61 (m, 6H, 2×CH_2 in piperazinyl and CH_2N), 2.41 (s, 6H, N(CH_3)$_2$), 1.65 (s, 3H, C10-OCH_3); ESI-MS m/z (%): 842 (M+H$^+$). **14i**: White crystal; mp 100–102 °C, IR (KBr, cm^{-1}): υ 1164, 1742; ^{1}H NMR (300 MHz, $CDCl_3$): δ 6.90–6.99 (m, 3H, Ph-H), 5.00 (dd, 1 H, J = 2.7 Hz and 10.1 Hz, H_{13}), 3.23 (s, 3H, C6-OCH_3), 3.11 (m, 4H, 2×CH_2 in piperazinyl), 2.66 (m, 6H, 2×CH_2 in piperazinyl and CH_2N), 2.26 (s, 6H, N(CH_3)$_2$), 2.25 (s, 3H, Ph-CH_3), 2.22 (s, 3H, Ph-CH_3), 1.65 (s, 3H, C10-OCH_3); ESI-MS m/z (%): 832 (M+H$^+$). **14j**: White crystal; mp 114–115 °C, IR (KBr, cm^{-1}): υ 1165, 1741; ^{1}H NMR (300 MHz, $CDCl_3$): δ 6.80–7.07 (m, 3H, Ph-H), 5.00 (dd, 1 H, J = 2.7 Hz and 10.1 Hz, H_{13}), 3.22 (s, 3H, C6-OCH_3), 3.14 (m, 4H, 2×CH_2 in piperazinyl), 2.65 (m, 6H, 2×CH_2 in piperazinyl and CH_2N), 2.31 (s, 6H, N(CH_3)$_2$), 2.24 (s, 3H, Ph-CH_3), 2.21 (s, 3H, Ph-CH_3), 1.65 (s, 3H, C10-OCH_3); ESI-MS m/z (%): 832 (M+H$^+$).

Please cite this article in press as: Y. Nie, et al., Synthesis and antibacterial activity of novel 10,11-epoxy acylide erythromycin derivatives, Chin. Chem. Lett. (2013), http://dx.doi.org/10.1016/j.cclet.2013.02.015