Bioorganic & Medicinal Chemistry Letters 20 (2010) 5527–5531

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry Letters

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

A new series of macrolide derivatives with 4″-*O*-saccharide substituents

Peng Xu [a,†], Xiao-zhuo Chen [a,†], Lu Liu [a], Zhi-ping Jin [b], Ping-sheng Lei [a,*]

[a] *Key Laboratory of Bioactivity Substance and Resources Utilization of Chinese Herbal Medicine, Ministry of Education Institute of Materia Medica, Peking Union Medica College & Chinese Academy of Medical Sciences, Beijing 100050, PR China*
[b] *Jingxin Pharmaceutical. Co., Xinchang, Zhejiang 312400, PR China*

ARTICLE INFO

Article history:
Received 6 May 2010
Revised 23 June 2010
Accepted 16 July 2010
Available online 21 July 2010

Keywords:
Macrolide
Synthesis
Resistant pathogen
Glycosylation

ABSTRACT

A series of novel derivatives of macrolide with 4″-*O*-mono- or disaccharides were synthesized. The corresponding glycosyl trichloroacetimidates were used as the donors in the glycosylations. The *in vitro* antibacterial activities of **7a**–**f** and **13**–**16** against a panel of susceptible and resistant pathogens were tested. The modification of 4″-*O*-mono- or disaccharides may lead to the understanding of interaction of the macrolide and the bacterial ribosome.

Macrolide antibiotics have been used clinically to treat respiratory tract infections for over 50 years, since the first agent erythromycin A was discovered. With the intensive emergence of antibacterial resistance against macrolides, great efforts have been made to find new structures potent against resistant pathogens. The most well-known series of third generation macrolides is the ketolides, represented by telithromycin[1] and cethromycin.[2] (Fig. 1) Their structural features, including a 3-keto group, a 11,12-cyclic carbamate, and a tethered hetero-aromatic substituent, play key roles in the observed superior activity against erythromycin-resistant bacterial strains.

It is noteworthy to mention that ketolides are not the only class of new macrolides to overcome macrolide resistance. Many derivatives of nonketolide families have been synthesized by different research groups[3] in the search for compounds with potent antibacterial activity.

Carbohydrates are involved in a broad variety of biological phenomena and are, in particular, associated with tremendous diagnostic and therapeutic potential. Macrolides with 14-membered lactone ring have two monosaccharides, D-desosamine and L-cladinose, in their structures and both components, especially the D-desosamine, are important for the binding of macrolides to ribosomes.

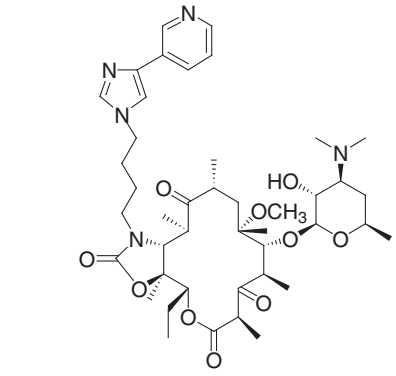
Using saccharide as a component to modify the macrolide core has rarely been reported.[4] A hydroxy group is necessary for introduction of a saccharide fragment and it is generally accepted that modification on 2′-hydroxy group will lead to significant decrease of antibacterial activities.[5] In the case of telithromycin, 11- and 12-positions are appropriate for introducing a cyclic carbamate substructure with a N-tethered aryl–alkyl side chain. 4″-Modified macrolide derivatives have been investigated by some research groups[6–9] and compounds such as **1–3** were found to have potent activity against susceptible and resistant bacterial strains (Fig. 1).

These observations led us to focus on the 4″-position and elongate the saccharide chain at this position, with considerations based on following facts. First, the 4″-OH group shows good reactivity for glycosylation and modification at the 4″-position can be achieved through a few reaction steps. Second, the elongated saccharide fragment has several –OH groups which could be donors or acceptors of hydrogen bond interactions. And last, recent research has indicated that the contributions of van der Waals interactions are more important and significant than electrostatic contributions in the binding of macrolides to the binding pocket.[10] The –OH groups of a 4″-*O*-saccharide could be further modified, for example, with benzoyl or benzylidene acetal groups. These modifications provide more opportunities to have non-bonded atom–atom interactions, such as hydrophobic interactions or aromatic stacking effects between a macrolide and ribosome.

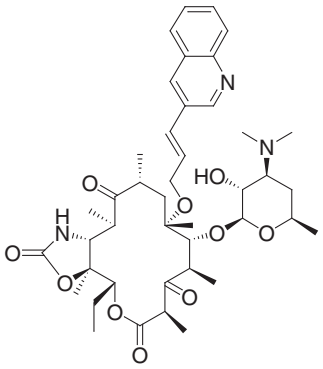
In this study, the synthesis of **7a**–**f** started from commercially available clarithromycin (Scheme 1). Selective acetylation of the 2′-OH of clarithromycin left a remaining 4″-OH as an ideal site at which to attach a saccharide section. We deducted that the steric hindrance of two *cis*-methyl groups made the reactivity of 11-OH lower than that of 4″-OH. Glycosylation of **5** with an appropriately protected glycosyl trichloroacetimidate donor **4a**–**d**[11] (Fig. 2), in the presence of triethylsilyl trifluoromethanesulfonate (TESOTf), produced **6a**–**d** in yields ranging from 25% to 46%. The presence of

* Corresponding author. Tel.: +86 10 63162596; fax: +86 10 63017757.
E-mail addresses: Lei@imm.ac.cn, wangdizhu007@163.com (P. Lei).
† Authors contributed equally to this work.

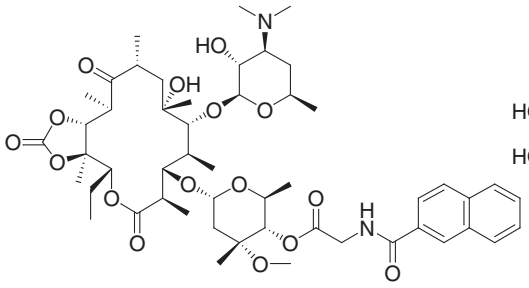
0960-894X/$ - see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmcl.2010.07.072



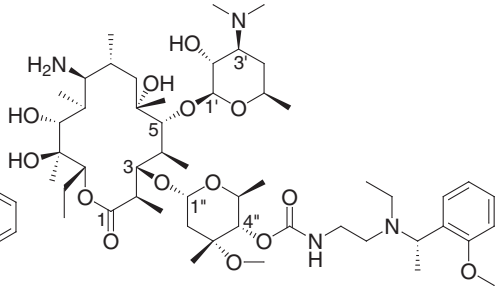
Telithromycin



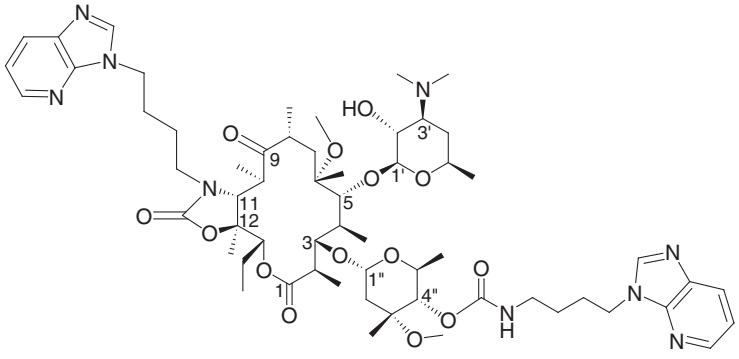
Cethromycin



1 (A-60565)



2 (CP-544372)



3

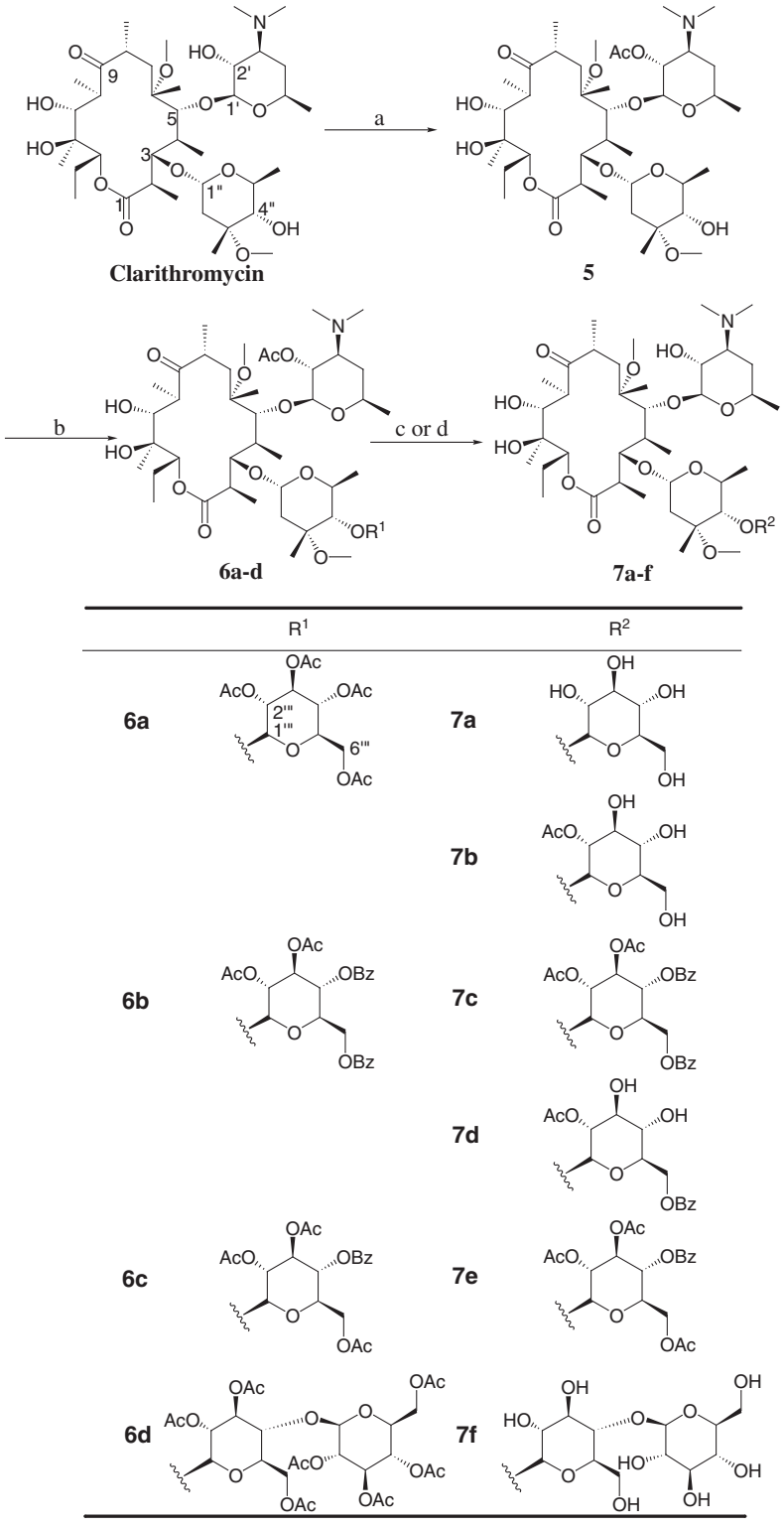
Figure 1. Structures of Telithromycin, cethromycin and compounds **1**–**3**.

the proton signal of 11-OH (about 4.0 ppm) confirmed that the glycosylation had taken place at 4″-position. The acetyl substitution on 2-OH of the donors ensured the β-configuration was generated at the anomeric centre (coupling constant of $J_{1,2}$ = 7.2–8.1 Hz). React **6a** with K_2CO_3 in methanol at 50 °C for 12 h afforded products **7a** and **7b** in a 1:1 ratio. The 2′-OAc of **6b** and **6c** could be selectively removed by refluxing in MeOH overnight. Products **7c** and **7e** were obtained in high yields (85% and 88%, respectively). An attempt to selectively deacetylate **6b** failed using guanidine/EtOH. Compound **7d** with 2‴-OAc and 6‴-OBz was obtained. Considering that the polar surface area is an important factor in determining the biopharmaceutical properties of a lead compound and that a reduced polar surface area will possibly improve the pharmacokinetic profile of macrolide compounds,[12] one or several acyl groups remained on compounds **7b**–**7e**. The complete deacetylation of **6d**, affording **7f**, was accomplished by a reaction in MeOH/K_2CO_3.

The synthesis of **13**–**16** followed a different procedure with **7a**–**f**. A heteroaryl–alkyl side chain was introduced in 11,12-position before glycosylation at 4″-OH. The two fused-heteroaryl side chains attached at 11,12-carbamate substructure were proved to be potent in our previous work.[13] Acylimidazole intermediate **8**[14] was reacted with [4-(3*H*-imidazo[4,5-*b*]pyridin-3-yl)butyl]amine, using 1,8-diazabicyclo[5.4.0] undec-7-ene (DBU) as base, and producing a cyclic carbamate product **9** (Scheme 2). Deprotection of two acetyl groups of **9** was carried out by heating **9** in MeOH in the presence of K_2CO_3. After selective protection of 2′-OH gave **11** in excellent yield. Glycosylation of **11** with donor **4a** or **4e,**[11] using TESOTf as a promoter, produced two macrolide derivatives with saccharide substituted at 4″-position. Removal of the acetyl groups by heating these products in MeOH/K_2CO_3, respectively, gave **13** and **14**.

Followed a same manner as described above, **8** was coupled with [4-(1*H*-imidazo[4,5-*b*]pyridin-1-yl)butyl]amine to give **10**. After protecting group manipulation on **10** yielded **12**. Glycosylation of **12** with **4a** or **4e** followed by deacetylation, **15** and **16** were afforded.

The antibacterial activities of **7a**–**f** and **13**–**16**, with clarithromycin and telithromycin as reference compounds, against both



Scheme 1. Reagents and conditions: (a) Ac_2O, Et_3N, rt, 95%; (b) **4a–4d**, TESOTf, CH_2Cl_2, −15 °C, 25–46%; (c) K_2CO_3, MeOH, 40 °C; (d) guanidine, EtOH, CH_2Cl_2, −15 °C.

erythromycin-susceptible and erythromycin-resistant strains are shown in Table 1. A 6-membered panel of bacteria were employed, including methicillin-susceptible *Staphylococcus aureus* (MSSA) ATCC29213, methicillin-resistant *S. aureus* (MRSA) BK2464, methicillin-resistant *Staphylococcus epidermidis* (MRSE) MRSE-1, erythromycin-resistant *Streptococcus pneumoniae* ERSP-2 (*erm*B and *mef* encoded), and erythromycin-resistant *Streptococcus pyogenes* 8902 (*erm*B encoded) and ERSPy-1(*mef* encoded). The *in vitro* antibacterial activities were reported as minimum inhibitory concentrations (MICs), determined by the broth microdilution method, as recommended by the NCCLS (National Committee of Clinical Laboratory Standard).[15]

Among **7a–f**, regardless of a mono- or disaccharide substitution at the 4″-position and with acyl groups on the saccharide chain or

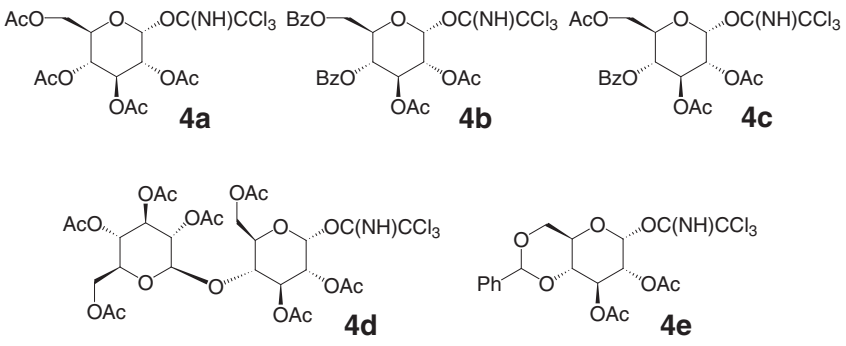
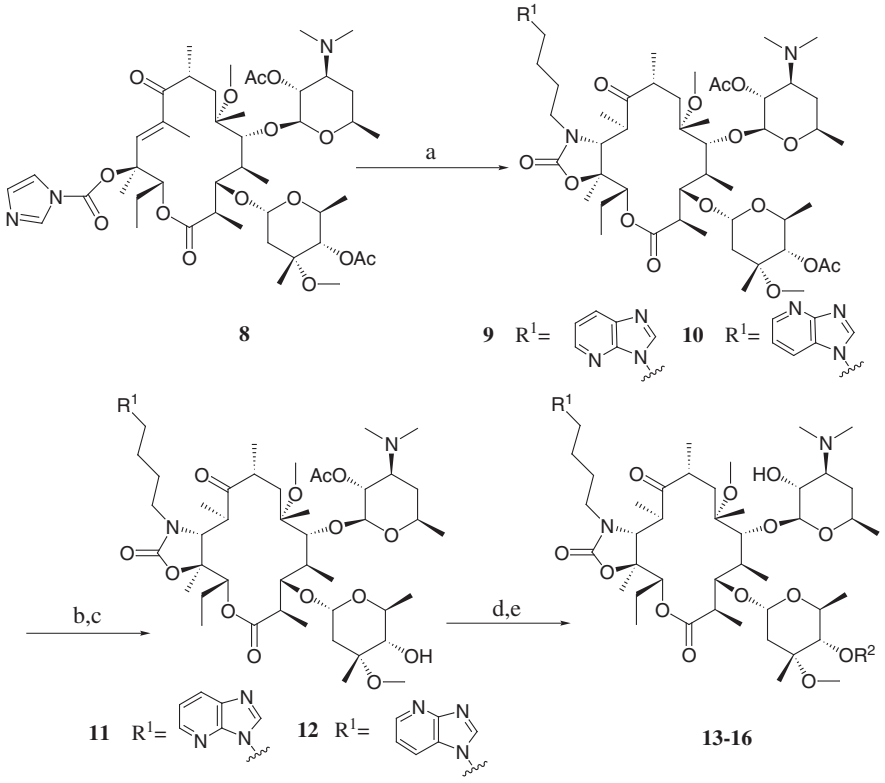
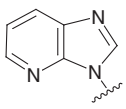
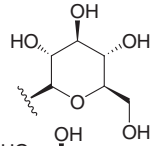
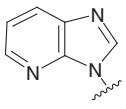
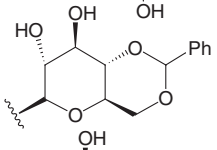
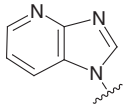
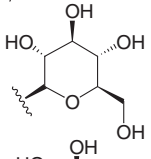
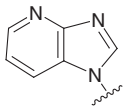
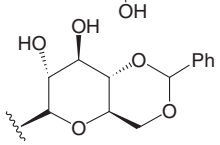


Figure 2. Structures of glycosyl donors **4a**–**e**.



	R^1	R^2
13		
14		
15		
16		

Scheme 2. Reagents and conditions: (a) [4-(3H-imidazo[4,5-*b*]pyridin-3-yl)butyl]amine or [4-(1H-imidazo[4,5-*b*]pyridin-1-yl)butyl]amine, acetonitrile/H_2O (10/1 v:v), 50 °C, 36 h; (b) K_2CO_3, MeOH, 40 °C; (c) Ac_2O, Et_3N, rt; (d) **4a** or **4e**, TESOTf, CH_2Cl_2, −15 °C; (e) K_2CO_3, MeOH, 40 °C.

Table 1
In vitro antibacterial activities of compounds **7a–f** and **13–16**

Pathogens	MIC (μg/mL)					
	S. aureus ATCC 29213	*S. aureus* BK2464	*S. epidermidis* MRSE-1	*S. pneumoniae* ERSP-2	*S. pyogenes* 8902	*S. pyogenes* ERSPy-1
Clarithromycin	0.25	>32	16	16	32	2
Telithromycin	0.125	>32	0.125	0.125	0.25	0.25
7a	>16	>16	16	16	16	16
7b	>16	>16	>16	16	>16	16
7c	>16	>16	16	16	16	8
7d	16	>16	16	8	16	8
7e	16	>16	16	16	16	1
7f	>16	>16	>16	16	>16	>16
13	>16	>16	>16	8	16	16
14	4	>16	4	2	4	0.5
15	>16	>16	>16	8	16	8
16	4	>16	4	1	8	2

not, the compounds were inactive against either susceptible or resistant strains.

Among compounds **13–16**, no improvement in antibacterial activity could be detected when an additional 11,12-carbamate substructure with an aryl–alkyl side chain was introduced. This is different from previous observations of ketolide series[16] and supposed here to be mainly because the conformations of the macrolide core, different from those of ketolides,[17] did not lead the side chain to domain II of the 23S rRNA and, thus, no additional interactions with the ribosome took place.[18] Compared with compound **13**, compound **14** showed at least a 4-fold more potent activity against most pathogens. The same trend was observed when comparing **15–16**, indicating that a 4‴,6‴-*O*-benzylidene acetal moiety contributed to some extent to the enhancement of activity. We speculated that the stronger activities of compounds **14** and **16** than **13** and **15** were due to hydrophobic interactions or aromatic stacking effects between benzylidene groups with bacterial ribosome. The most active compound **14** exhibited 4-fold more active against *S. pyogenes* ERSPy-1 than clarithromycin, 8-fold more active against *S. pneumoniae* ERSP-2 and *S. pyogenes* 8902 than clarithromycin, but still less potent than telithromycin.

In conclusion, a series of novel macrolides derivatives with 4″-*O*-saccharide were synthesized. Derivatives with 4″-*O*-saccharide or with an incorporated 11,12-carbamate aryl–alkyl side chain could not dramatically increase antibacterial activity compared with the parent compound, suggesting that the bulky saccharide structure did not fit well in the binding pocket and may have disturbed the interactions of the macrolide with bacterial ribosome. Even through, this work will enrich the structure types of described macrolide derivatives and provide hints for future structure–activity relationship studies of macrolide antibiotics.

Acknowledgments

This work was supported financially by the National Natural Science Foundation of China (30572275) and National S&T Major Special Project on Major New Drug Innovation (2008ZX09401-004 and 2009ZX09301-003).

Supplementary data

^{1}H NMR, ^{13}C NMR, HRMS data of compounds **7a–7f**, **13–16** and experimental procedures of these compounds are available online version, at doi:10.1016/j.bmcl.2010.07.072.

References and notes

1. Denis, A.; Agouridas, C.; Auger, J. M.; Benedetti, Y.; Bonefoy, A.; Breti, F.; Chantot, J.-F.; Dussaratti, Y.; Fromentin, C.; D'Ambrieres, S. G.; Lachaud, S.; Laurin, P.; Le Martret, O.; Loyau, V.; Tessot, N.; Pejac, J. M.; Perron, S. *Bioorg. Med. Chem. Lett.* **1999**, *9*, 3075.
2. Ma, Z.; Clark, R. F.; Brazzale, A.; Plattner, J.; Or, Y. S. *J. Med. Chem.* **2001**, *44*, 4137.
3. Sarbani, P. *Tetrahedron* **2006**, *62*, 3171.
4. Liang, C.; Yao, S.; Chiu, Y.; Leung, P.; Robert, N.; Seddon, J.; Sears, P.; Hwang, C.; Ichikawa, Y.; Romero, A. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 1307.
5. Asaka, T.; Manaka, A.; Sugiyama, H. *Curr. Top. Med. Chem.* **2003**, *3*, 961.
6. Kobori, T.; Sugimoto, K. Jpn. Kokai Tokkyo Koho. JP 11116592, **1999**.
7. Su, W. G.; Smyth, K. T.; Rainville, J. P.; Kaneko, T.; Sutcliffe, J. A.; Brennan, L. A.; Duignan, J. M.; Girard, A. E.; Finegan, S. M.; Cimochoski, C. R. 38th Intersci. *Conf. Antimicrob. Agents Chemother.* San Diego, CA, **1998**; Abstr. F123.
8. Ma, S.; Ma, R.; Liu, Z.; Ma, C.; Shen, X. *Eur. J. Med. Chem.* **2009**, *44*, 4010.
9. Xu, P.; Liu, L.; Jin, Z.; Wang, G.; Liu, J.; Li, Y.; Lei, P. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 5507.
10. Yam, W. K.; Wahab, H. A. *J. Chem. Inf. Model* **2009**, *49*, 1558.
11. The synthesis methods of **4a–e** were shown in Supplementary data.
12. Miroshnyk, I.; Mirza, S.; Zorky, P. M.; Heinamaki, J.; Yli-Kauhaluoma, J.; Yliruusi, J. *Bioorg. Med. Chem.* **2008**, *16*, 232.
13. Xu, P.; Liu, L.; He, W.; Li, Y.; Liu, J.; Lei, P. *J. Asian Nat. Prod. Res.* **2009**, *11*, 880–897.
14. Tetsuya, T.; Toshifumi, A.; Masato, K.; Keiko, S.; Hiroyuki, S.; Masakazu, S.; Kazuya, K.; Shigeo, M.; Atsushi, N. *J. Med. Chem.* **2003**, *46*, 2706.
15. National Committee for Clinical Laboratory Standards. Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically, 5th ed.; Approved Standard: NCCLS Document M7-A5; 2000, 20(2).
16. Takashi, N.; Tsutomu, I.; Tatsuro, Y.; Koichi, N.; Yukitoshi, N.; Koichi, U.; Toshihiko, H.; Hideaki, M. *Bioorg. Med. Chem.* **2005**, *13*, 6615.
17. Pfister, P.; Jenni, S.; Poehlsgaard, J.; Thomas, A.; Douthwaite, S.; Ban, N.; Bottger, E. C. *J. Mol. Biol.* **2004**, *342*, 1569.
18. George, G. Z.; Michael, W.; Ayman, N.; Lawern, M. V.; Aleksandra, W.; John, M. E.; Alfred, S. G.; Stephen, D.; Daryl, J. H. *Drugs* **2002**, *62*, 1771.