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Original article

Synthesis and antibacterial activity of novel 3-*O*-carbamoyl derivatives of clarithromycin and 11,12-cyclic carbonate azithromycin

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ABSTRACT

Two series of novel 3-*O*-carbamoyl derivatives of clarithromycin and 11,12-cyclic carbonate azithromycin were designed, synthesized and evaluated for their *in vitro* antibacterial activities. Compounds **4j** and **4k** were the most potent activity against erythromycin-susceptible *Staphylococcus aureus*, *Streptococcus pyogenes* and *Streptococcus pneumoniae*, which were comparable to those of clarithromycin and azithromycin. Compounds **4d**, **4h** and **4i** showed potent activity against erythromycin-resistant *S. pneumoniae* encoded by the *mef* gene and compounds **4h** and **4i** displayed greatly improved activity against erythromycin-resistant *S. pneumoniae* encoded by the *erm* gene. Compound **7c** exhibited improved activity against erythromycin-resistant *S. pneumoniae* encoded by the *erm* and *mef* genes.

1. Introduction

Macrolides belong to one of the most commonly used families of clinically important antibiotics, which are applied primarily for the infections caused by Gram-positive bacteria such as *Staphylococcus aureus*, *Streptococcus pneumoniae* and *Streptococcus pyogenes*. They are usually safe and effective for the treatment of upper and lower respiratory tract infections, as well as genital infections [1,2]. First-generation macrolides (e.g. erythromycin A (Fig. 1)) readily lose their antibacterial activity under acidic conditions due to degradation [3]. These degraded products are known to be responsible for undesirable gastrointestinal side effects [4,5]. Second-generation macrolides such as clarithromycin (CAM) and azithromycin (AZM) (Fig. 1) have been developed to address the limitations of erythromycin A, and are widely prescribed owing to their efficacy and safety [6]. However, the therapeutic utility of these macrolides has been severely compromised by the emergence of resistant pathogens [7]. Particularly, the increasing resistance of community-acquired respiratory tract infections to various antimicrobials is a pandemic phenomenon [8]. The commonest mechanism of resistance is mediated by *erm*-encoded methylation of 23S rRNA or *mef*-encoded efflux. Expression of an *erm* resistant determinant in bacteria results in production of a methyltransferase which modifies the key nucleotide, A2058, in the macrolide–lincosamide–streptogramin B (MLS_B) binding site, thereby conferring resistance to macrolides [9–11]. Ketolides such as telithromycin can effectively address bacterial resistance and other issues associated with current macrolide regimens [12,13]. The C-11,12 carbamate side chain or the C-6 side chain in the ketolides may interact with nucleotide A752 directly in domain II of the 23S rRNA in addition to the main interaction of the drugs in domain V. This leads to tighter binding to ribosomes and imparts some activity against methylated ribosomes in some species [14,15].

Emergence of bacterial resistance has also prompted further research directed toward the discovery of new generation macrolides that exhibit greater efficacy and safety, has a broader spectrum of activity, and is particularly effective against resistant pathogens. The study of high-resolution X-ray cocrystal structures have revealed that macrolides bind at the entrance to the peptide tunnel in the 23S rRNA, and the cladinose group in their structures is located at and fits with the cavity formed by G2505, C2610 and C2611 in domain V [16]. On the basis of the results of the X-ray cocrystal structure study, many new derivatives of macrolides for the effective management of erythromycin resistance have been investigated by different research groups [17]. These investigations have led to the discovery of a promising class of macrolide antibiotics, named as acylides [18–21]. TEA0777 [18] and TEA0929 [17] (Fig. 2), for example, showed significantly potent activity against not only erythromycin-susceptible Gram-positive pathogens but also MLS_B-resistant *S. aureus* and efflux-resistant *S. pneumoniae*.

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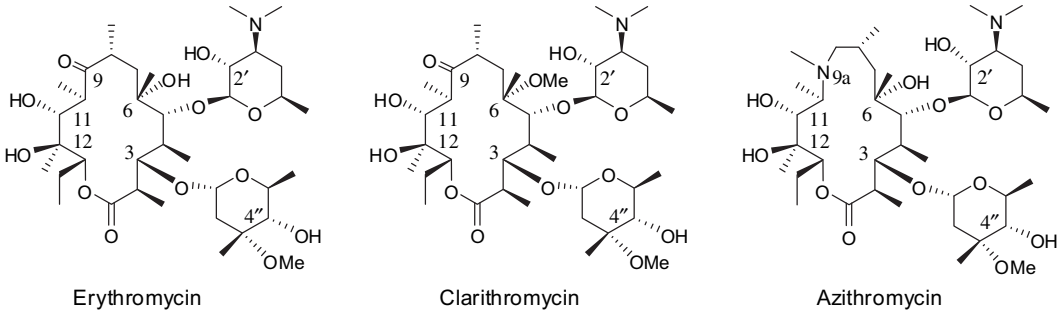


Fig. 1. Structures of erythromycin, clarithromycin and azithromycin.

Acylides therefore are innovative semisynthetic macrolides that have potential as new generation macrolide antibiotics.

The acylides possess a 3-*O*-acyl group, the length of which is two atoms distance from 3-oxygen atom to aromatic ring as well as a five-membered cyclic carbamate attached to the 11,12-position that was crucial to rigidify the conformation of the mother ring [21]. In particular, 3-*O*-arylacetyl group were mostly active in the acylides and display a higher affinity for forming interactions with bacterial ribosomes [19]. However, the 3-*O*-acyl side chains in the acylides are unstable and are easily subjected to hydrolysis under physiological condition. Besides, the acylides have poor activity against erythromycin-resistant *S. pneumoniae* encoded by the *erm* gene. These encouraged us to explore the synthesis of new 3-*O*-modified macrolide derivatives bearing other stable side chains for example 3-*O*-carbamoyl side chain at the 3-*O*-position in place of the 3-*O*-acyl side chain. On the other hand, the cavity formed by G2505, C2610 and C2611 in domain V [16] is very informative for the development of new 3-*O*-modified macrolide derivatives with the activity against erythromycin-resistant strains. Particularly, the high nucleotide content in the cavity gives rise to a number of possible interactions such as hydrogen bonding, π-stacking as well as electrostatic interactions. Therefore, a prolonged anchor groups at the 3-*O*-position may be helpful for the interaction with the binding sites of the nucleotides in the cavity.

On the basis of the consideration detailed above, we designed novel 3-*O*-carbamoyl derivatives of CAM with prolonged 3-*O*-carbamoyl side chains, the length of which is three or four atom distance from 3-oxygen atom to aromatic ring in order to probe the effect of different lengths of 3-*O*-carbamoyl side chains in antibacterial activity. Since hydrogen bonding and π-stacking may increase binding affinity, the substituted aromatic moieties in the structures have different bulk of molecule and different distribution of heteroatoms. In addition, AZM is first 15-membered azalide antibiotic and has beneficial antibacterial and excellent pharmacokinetic profiles. Its skeleton is very similar to that of CAM except that the lactone ring is expanded around the 9-position [17]. In order to investigate the effect of the azalide skeleton in antibacterial activity, we also designed novel 3-*O*-carbamoyl derivatives of 11,12-cyclic carbonate AZM comprising the essential features for addressing bacterial resistance due to efflux and methylation of the ribosome.

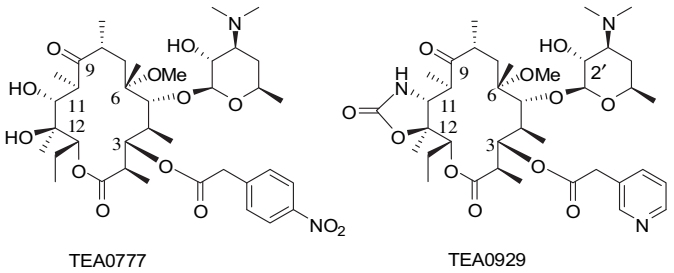


Fig. 2. Structures of TEA0777 and TEA0929.

2. Chemistry

2.1. Synthesis of 3-O-carbamoyl derivatives of CAM

By substituting L-cladinose at the 3-*O*-position with various carbamoyl groups, novel 3-*O*-carbamoyl derivatives of CAM (**4a**–**k**) were prepared in 30–36% yields as shown in Scheme 1. Selective cleavage of the 3-*O*-sugar moiety from CAM with 1 M aqueous hydrochloric acid (HCl) and subsequent protection of the 2′-hydroxyl group with acetic anhydride (Ac_2O) in the presence of triethylamine (Et_3N) gave 2′-acetate (**2**). Treatment of **2** with 1,1′-carbonyldiimidazole (CDI) in the presence of 4-(dimethylamino)-pyridine (DMAP) provided the 3-*O*-acylimidazolide (**3**) as a common intermediate to introduce various functional groups at the 3-*O*-position.

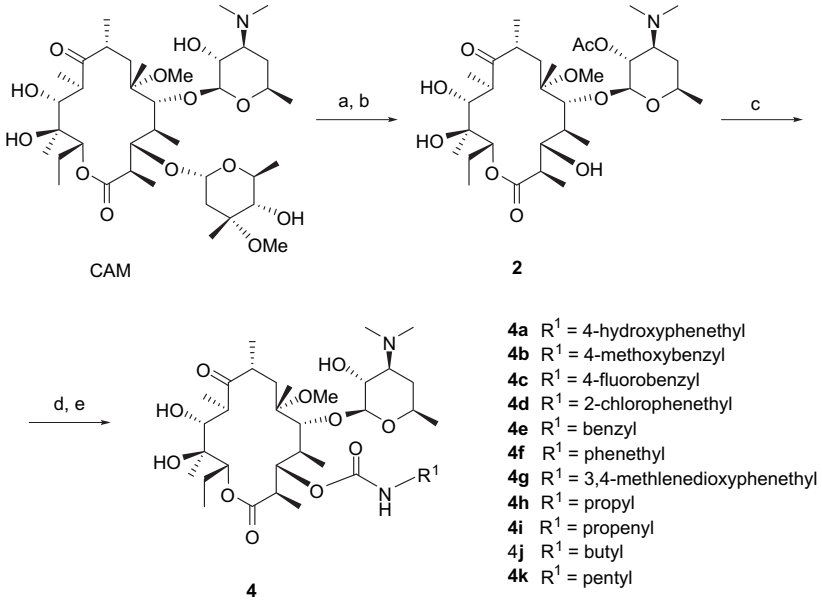
3-*O*-Arylcarbamoyl derivatives of CAM (**4a**–**g**) were obtained in 56–69% yields by condensation of **3** with the corresponding arylamines in the presence of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) in dimethylformamide (DMF), followed by selective removal of the 2′-*O*-acetyl group by heating with methanol. In contrast, 3-*O*-alkylcarbamoyl derivatives of CAM (**4h**–**k**) were prepared by coupling **3** with the corresponding alkylamines in methyl cyanide (CH_3CN) and water at 65 °C, followed by methanolysis. The yields were within the range of 56%–63%.

2.2. Synthesis of 3-O-carbamoyl derivatives of 11,12-cyclic carbonate AZM

Novel 3-*O*-carbamoyl derivatives of 11,12-cyclic carbonate AZM (**7a**–**g**) were obtained in 47–55% yields by substituting L-cladinose at the 3-*O*-position with various carbamoyl groups. The synthetic route is outlined in Scheme 2. Selective removal of the 3-*O*-sugar moiety with 1 M HCl afforded 3-*O*-descladinosyl AZM. Treatment of the 3-*O*-descladinosyl AZM with Ac_2O in the presence of Et_3N in dichloromethane (CH_2Cl_2) gave the 2′-acetate AZM (**5**). Treatment of **5** with CDI in toluene provided the 11,12-cyclic carbonate 3-*O*-acylimidazolide (**6**). Finally, the desired compounds were synthesized by condensation of **6** with the corresponding amines in the presence of DBU in DMF, followed by selective deprotection of the 2′-*O*-acetyl group by heating with methanol.

3. Antibacterial activity

The antibacterial screening of 3-*O*-carbamoyl derivatives of CAM and 11,12-cyclic carbonate AZM prepared above was performed by a standard dilution assay for the determination of

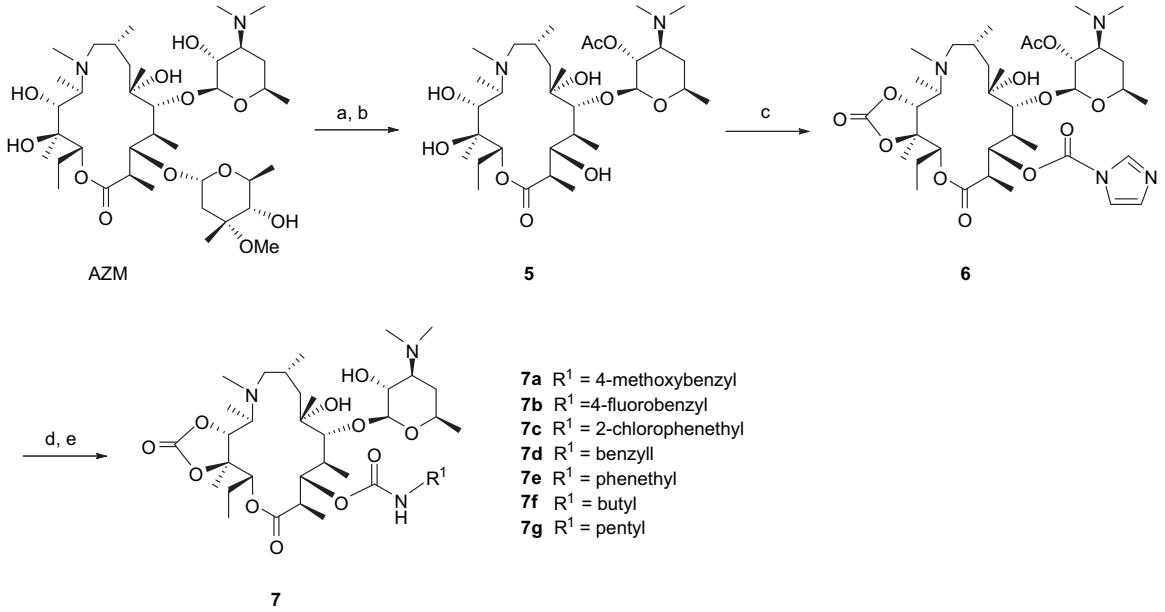


Scheme 1. Reagents and conditions: (a) HCl, EtOH/H_2O, rt, 20 h, 85%; (b) Ac_2O, Et_3N, CH_2Cl_2, rt, 48 h, 90%; (c) CDI, DMAP, CH_2Cl_2, rt, 24 h, 69%; (d) R^1NH_2 (arylamines), DBU, DMF, 55 °C, 12 h or R^1NH_2 (alkylamines), CH_3CN/H_2O, 65 °C, 12 h; (e) CH_3OH, 50 °C, 12 h, 56–69% for 2 steps.

minimal inhibitory concentrations (MICs). MIC values for all compounds were determined in comparison with CAM and AZM on six phenotypes of sensitive and resistant Gram-positive bacterial strains. *S. aureus* ATCC25923, *S. pyogenes* and *S. pneumoniae* ATCC49619 are three erythromycin-susceptible strains. *S. pneumoniae* B1, *S. pneumoniae* A22072 and *S. pneumoniae* AB11 are three erythromycin-resistant strains whose resistance were encoded by the *erm* gene, the *mef* gene, and the *erm* and *mef* genes, respectively.

4. Results and discussion

MIC values for 3-*O*-carbamoyl derivatives of CAM are shown in Table 1. Almost all of the 3-*O*-carbamoyl derivatives of CAM showed excellent activity against erythromycin-susceptible strains and some of them exhibited improved activity against erythromycin-resistant *S. pneumoniae*, compared with CAM, AZM. In marked contrast, compound **2** as precursor of 3-*O*-carbamoyl derivatives of CAM did not show any antibacterial activity. Among the target compounds, compounds **4j** and **4k** were found to be the most potent activity (MIC 0.03 and 0.03 μg/mL) against the erythromycin-susceptible strains tested, which were comparable to those of CAM and AZM. Compounds **4d**, **4h** and **4i** had significant activity (MIC 0.25, 0.25 and 0.25 μg/mL) against erythromycin-resistant *S. pneumoniae* encoded by the *mef* gene, showing 512-fold, 512-fold and 512-fold greater activity than the precursor **2**, respectively. Compounds **4h** and **4i** showed greatly improved activity against



Scheme 2. Reagents and conditions: (a) HCl, MeOH/H_2O, rt, 24 h, 92%; (b) Ac_2O, Et_3N, CH_2Cl_2, rt, 24 h, 90%; (c) CDI, Et_3N, toluene, rt, 48 h, 90%; (d) R^1NH_2, DBU, DMF, rt, 10 h; (e) CH_3OH, 55 °C, 24 h, 63–74% for 2 steps.

Table 1
In vitro antibacterial activity of 3-*O*-carbamoyl-3-*O*-descladinosylclarithromycin derivatives.

Strain/compound	MICs (μg/mL)													
	4a	**4b**	**4c**	**4d**	**4e**	**4f**	**4g**	**4h**	**4i**	**4j**	**4k**	**2**	CAM	AZM
S. aureus ATCC25923[a]	0.12	0.25	0.25	0.25	0. 5	0.25	0.25	0.25	0.25	0.12	0.12	128	0.12	0.25
S. pyogenes[b]	0.25	0.5	0.25	0.25	0. 5	0.25	0.5	0.25	0.25	0.12	0.12	128	0.12	0.12
S. pneumoniae ATCC49619[c]	0.25	0.12	0.06	0.12	0.25	0.5	0.25	0.25	0.12	0.03	0.03	128	0.03	0.03
S. pneumoniae B1[d]	64	256	256	32	256	128	64	2	2	32	32	128	64	128
S. pneumoniae A22072[e]	0.5	4	4	0.25	8	2	1	0.25	0.25	0.5	0. 5	128	4	4
S. pneumoniae AB11[f]	128	256	256	64	256	128	128	4	4	64	32	256	128	256

[a] *S. aureus* ATCC25923: erythromycin-susceptible strain.
[b] *S. pyogenes*: erythromycin-susceptible strain.
[c] *S. pneumoniae* ATCC49619: erythromycin-susceptible strain.
[d] *S. pneumoniae* B1: erythromycin-resistant strain encoded by the *erm* gene.
[e] *S. pneumoniae* A22072: erythromycin-resistant strain encoded by the *mef* gene.
[f] *S. pneumoniae* AB11: erythromycin-resistant strain encoded by the *erm* and *mef* genes.

erythromycin-resistant *S. pneumoniae* encoded by the *erm* gene or the *erm* and *mef* genes, showing 64-fold and 64-fold better activity than AZM or precursor **2**, respectively. The results described above suggested that removal of the L-cladinosyl sugar of CAM leads to a complete loss of antibacterial activity, and introduction of the 3-*O*-carbamoyl side chains at the 3-*O*-position completely restores the activity against erythromycin-susceptible strains and shows greatly improved activity against erythromycin-resistant *S. pneumoniae* encoded by the *mef* gene. These results are consistent with the results with 3-*O*-acylerythromycin A derivatives reported by Tanikawa T. *et al.* [18,19] In addition, compounds **4h** and **4i** increased significantly the activity against erythromycin-resistant *S. pneumoniae* encoded by the *erm* gene, showing 50-fold and 50-fold better activity than TEA0777 (MIC > 100 μg/mL) [18] or TEA0929 (MIC > 100 μg/mL) [19], respectively. This suggested that introduction of the 3-*O*-carbamoyl side chain at the 3-*O*-position in place of the 3-*O*-acyl side chain in acylides may increase the activity against erythromycin-resistant *S. pneumoniae* encoded by the *erm* gene.

For comparison with the 3-*O*-carbamoyl derivatives of CAM, the 3-*O*-carbamoyl derivatives of 11,12-cyclic carbonate AZM (**7a**–**g**) were tested for *in vitro* antibacterial activity against the Gram-positive strains described above. Their activities are summarized in Table 2. The 3-*O*-carbamoyl derivatives of 11,12-cyclic carbonate AZM did not show the activity against erythromycin-susceptible strains. Similarly, Compound **5** as precursor of the 3-*O*-carbamoyl derivatives of 11,12-cyclic carbonate AZM did not show the activity against erythromycin-susceptible strains as well. In contrast, some of the target compounds showed slightly improved activity against erythromycin-resistant *S. pneumoniae*. Especially, compound **7c** exhibited significantly improved activity against erythromycin-resistant *S. pneumoniae* encoded by the *erm* and *mef* genes, showing 32-fold and 32-fold higher activity than AZM and precursor **5**, respectively. These suggested that removal of the L-cladinosyl moiety of AZM results in a complete loss of antibacterial activity and introduction of the 3-*O*-carbamoyl side chains at the 3-*O*-position does not completely restores the activity against the erythromycin-susceptible strains, but it shows improved activity against erythromycin-resistant *S. pneumoniae* encoded by the *erm* and *mef* genes.

Table 2
In vitro antibacterial activity of 3-*O*-carbamoyl-3-*O*-descladinosylazithromycin 11,12-cyclic carbonate derivatives.

Strain/compound	MICs (μg/mL)									
	7a	**7b**	**7c**	**7d**	**7e**	**7f**	**7g**	**5**	**CAM**	**AZM**
S. aureus ATCC25923[a]	128	64	32	64	64	64	64	128	0.12	0.12
S. pyogenes[b]	16	16	8	32	8	8	16	128	0.12	0.12
S. pneumoniae ATCC49619[c]	8	4	4	16	4	8	4	128	0.03	0.03
S. pneumoniae B1[d]	64	64	32	64	64	64	32	128	64	128
S. pneumoniae A22072[e]	128	32	16	128	64	128	64	128	4	4
S. pneumoniae AB11[f]	64	64	8	64	32	32	32	256	128	256

[a] *S. aureus* ATCC25923: erythromycin-susceptible strain.
[b] *S. pyogenes*: erythromycin-susceptible strain.
[c] *S. pneumoniae* ATCC49619: erythromycin-susceptible strain.
[d] *S. pneumoniae* B1: erythromycin-resistant strain encoded by the *erm* gene.
[e] *S. pneumoniae* A22072: erythromycin-resistant strain encoded by the *mef* gene.
[f] *S. pneumoniae* AB11: erythromycin-resistant strain encoded by the *erm* and *mef* genes.

Two series of 3-*O*-carbamoyl macrolide derivatives described above displayed different antibacterial activities owing to differences in their skeleton structures. The 3-*O*-carbamoyl derivatives of CAM are 14-membered macrolide derivatives with the 3-*O*-carbamoyl side chain in place of L-cladinose at the 3-*O*-position. They exhibited not only excellent activity against erythromycin-susceptible strains such as *S. aureus*, *S. pneumoniae* and *S. pyogenes*, but also significant activity against erythromycin-resistant *S. pneumoniae* encoded by the *mef* gene. The results suggested that the 3-*O*-carbamoyl side chain in their structures could interact with the binding sites in the cavity formed by G2505, C2610 and C2611 in domain V, resulting in a higher affinity to bacterial ribosomes. The 3-*O*-carbamoyl derivatives of 11,12-cyclic carbonate AZM are 15-membered azalide derivatives with the 3-*O*-carbamoyl side chain and 11,12-cyclic carbonate group. The 11,12-cylic carbonate group introduced into the azalide derivatives is to overcome efflux resistance on the basis of previous reports [22,23] that the introduction of a cyclic carbonate to the 11,12-position of CAM derivatives enhanced activity against resistant bacteria mediated by *mef*-encoded efflux. However, the 3-*O*-carbamoyl derivatives of 11,12-cyclic carbonate AZM did not show activity against both erythromycin-susceptible strains and erythromycin-resistant *S. pneumoniae* encoded by the *mef* gene. These results led us to presume that the expansion of lactone ring around the C-9a position might change the stereochemistry of the azalide derivatives compared with 3-*O*-carbamoyl derivatives CAM so that their 3-*O*-carbamoyl side chain could not interact with the binding sites in the cavity in domain V and the 11,12-cylic carbonate group could not impart its activity against the *mef*-encoded efflux. Finally, these alterations affect the antibacterial activity of the 3-*O*-carbamoyl derivatives of 11,12-cyclic carbonate AZM.

5. Conclusion

Two series of novel 3-*O*-carbamoyl derivatives of CAM and 11,12-cyclic carbonate AZM were designed, synthesized by introducing the 3-*O*-carbamoyl group instead of L-cladinose at the 3-*O*-position. Almost all of the 3-*O*-carbamoyl derivatives of CAM

showed excellent activity against erythromycin-susceptible bacteria. Among them, compounds **4j** and **4k** were the most potent activity against erythromycin-susceptible *S. aureus*, *S. pyogenes* and *S. pneumoniae*, which were comparable to those of CAM and AZM. In marked contrast, 3-*O*-carbamoyl derivatives of 11,12-cyclic carbonate AZM did not exhibit the activity against the erythromycin-susceptible strains. As for the activity against erythromycin-resistant strains, compounds **4d**, **4h** and **4i** showed potent activity against erythromycin-resistant *S. pneumoniae* encoded by the *mef* gene and displayed greatly improved activity against erythromycin-resistant *S. pneumoniae* encoded by the *erm* gene. Compound **7c** exhibited improved activity against erythromycin-resistant *S. pneumoniae* encoded by the *erm* and *mef* genes. The results suggested that introduction of the 3-*O*-carbamoyl side chains at the 3-*O*-position shows greatly improved activity against erythromycin-resistant *S. pneumoniae* encoded by the *mef* gene and the *erm* gene, and introduction of the 3-*O*-carbamoyl side chain at the 3-*O*-position in place of the 3-*O*-acyl side chain in acylides may increase the activity against erythromycin-resistant *S. pneumoniae* encoded by the *erm* gene.

6. Experimental

All necessary solvents were purified prior to use. Dichloromethane, toluene and *N*, *N*-dimethylformamide were distilled from calcium hydride and stored over 4 Å molecular sieves. Triethylamine were distilled from calcium hydride and stored over sodium hydroxide. Reactions were monitored by thin-layer chromatography (TLC) using 0.25-mm pre-coated silica gel plates. Visualization was accomplished with UV light and aqueous potassium permanganate stain followed by charring on a hot-plate. Flash chromatography was performed with the indicated solvents using silica gel 60 (particle size 0.040–0.063 mm). Yields refer to chromatographically and spectroscopically pure compounds unless otherwise stated. ^{1}H NMR spectra were recorded on Bruker Avance DRX 600 spectrometer at ambient temperature. Infrared spectra were recorded on KBr pellets using Nicolet Nexus 470FT-IR spectrometer. Mass spectra were recorded on API 4000 instrument. The C, H, N analyses were carried out on PE-2400 II elemental analyser. Melting points are uncorrected and were determined on an X-6 melting point apparatus.

6.1. 2′-*O*-Acetyl-3-*O*-descladinosylclarithromycin (**2**)

To a solution of clarithromycin (9.0 g, 12.0 mmol) in absolute ethanol (39 mL) and water (108 mL) at room temperature was added dropwise 1 M HCl (21.6 mL). The resulting solution was allowed to stir for 20 h at the same temperature. And then, the reaction solution was neutralized to pH = 10.5–11.0 with 1 M sodium hydroxide and stirred for 2 h at the room temperature. The resulting precipitate was filtered, washed with cool water, dried to afford 6.01 g (84.8%) of 3-*O*-descladinosylclarithromycin as a white solid: mp 158–160 °C, R_f = 0.53 (dichloromethane/methanol 10:1, v/v).

To a solution of 3-*O*-descladinosylclarithromycin (5.6 g, 9.49 mmol) prepared above in CH_2Cl_2 (40 mL) at room temperature was added Ac_2O (1.8 mL, 18.9 mmol) and Et_3N (5.5 mL, 37.96 mmol). The resulting solution was allowed to stir for 24 h at the same temperature. The reaction was quenched with 5% aqueous $NaHCO_3$ (50 mL) and the aqueous layer was extracted with CH_2Cl_2 (2 × 30 mL). The combined organic layers were washed with brine, dried over anhydrous Na_2SO_4, filtered, and concentrated in vacuo. The residue was crystallized from ethyl acetate-n-hexane (1:20) to afford 5.37 g (89.5%) of **2** as a white solid: mp 132–136 °C, R_f = 0.56 (dichloromethane/methanol 10:1, v/v).

6.2. 2′-*O*-Acetyl-3-*O*-acylimidazolyl-3-*O*-descladinosylclarithromycin (**3**)

To a solution of **2** (4.1 g, 6.49 mmol) in CH_2Cl_2 (40 mL) at room temperature was added DMAP (1.58 g, 12.98 mmol) and CDI (3.16 g, 19.47 mmol). The resulting solution was allowed to stir for 24 h at the same temperature. The reaction was quenched with 5% aqueous $NaHCO_3$ (40 mL) and the aqueous layer was extracted with CH_2Cl_2 (2 × 20 mL) saturated brine (2 × 20 mL). The organic layer were washed with brine (2 × 20 mL), dried over anhydrous Na_2SO_4, filtered, and concentrated in vacuo to afford 3.26 g (69.1%) of **3** as a white solid: mp 124–128 °C, R_f = 0.60 (dichloromethane/methanol 10:1, v/v).

6.3. General methods for the preparation of 3-*O*-arylalkylcarbamoyl-3-*O*-descladinosylclarithromycin derivatives (**4a**–**k**)

Method I To a solution of **3** (1.6 g, 2.2 mmol) in DMF (25 mL) was added DBU (0.48 mL, 3.3 mmol) and corresponding arylamine (11.0 mmol). The resulting solution was stirred for 12 h at 55 °C. The reaction was quenched with water (40 mL) and the aqueous layer was extracted with ethyl acetate (2 × 20 mL). The combined organic layers were washed with 5% aqueous $NaHCO_3$ and brine, and dried over anhydrous Na_2SO_4, filtered. The filtrate was concentrated in vacuo to afford a crude product.

A solution of the above crude product in methanol (15 mL) was heated to 50 °C and stirred for 12 h at the same temperature. After concentrating the reaction solution in vacuo, the residue was purified by flash chromatography (dichloromethane/methanol 7:1, v/v) to afford the desired products **4a**–**g**.

Method II To a solution of **3** (1.6 g, 2.2 mmol) in CH_3CN (20 mL) and water (2 mL) was added the corresponding alkylamine (11.0 mmol). The resulting solution was stirred for 12 h at 65 °C. The reaction was quenched with 0.5 M NaH_2PO_4 (20 mL) and the aqueous layer was extracted with ethyl acetate (3 × 20 mL). The combined organic layers were washed with brine, and dried over anhydrous Na_2SO_4, filtered. The filtrate was concentrated in vacuo to afford a crude product.

A solution of the above crude product in methanol (15 mL) was heated to 50 °C and stirred for 12 h at the same temperature. After concentrating the reaction solution in vacuo, the residue was purified by flash chromatography (dichloromethane/methanol 7:1, v/v) to afford the desired products **4h**–**k**.

6.3.1. 3-*O*-((4-Hydroxyphenethyl)carbamoyl)-3-*O*-descladinosylclarithromycin (**4a**)

White crystals, yield 56.4%, mp 159–162 °C, TLC R_f = 0.31 (dichloromethane/methanol 7:1, v/v); IR (KBr): 3453, 2973, 2938, 2878, 2835, 2785, 1735, 1612, 1586, 1513, 1457, 1404, 1377, 1330, 1246, 1172, 1109, 1074, 1050,1033 cm^{-1}; ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.28 (m, 2H), 6.86 (m, 2H), 5.18 (dd, J = 11.1 Hz, J = 1.78 Hz, 1H), 4.92 (d, J = 11.0 Hz, 1H), 4.31–4.29 (m, 2H), 3.96–3.95 (m, 2H), 3.82 (m, 2H), 3.79 (s, 3H), 3.25 (m, 1H), 3.05 (s, 3H), 3.02–2.99 (m, 2H), 2.36 (s, 6H), 1.83–1.79 (m, 2H), 1.60–1.51 (m, 9H), 1.28–1.22 (m, 5H), 1.17–1.10 (m, 18H), 0.83 (t, 3H); MS (ESI) m/z calcd. for $C_{39}H_{64}N_2O_{12}$ 752.5, found $[M+H]^+$ 753.8; Analysis calculated for $C_{39}H_{64}N_2O_{12}$: C 62.21, H 8.57, N 3.72. Found: C 62.13, H 8.53, N 3.76.

6.3.2. 3-*O*-((4-Methoxybenzyl)carbamoyl)-3-*O*-descladinosylclarithromycin (**4b**)

White crystals, yield 55.8%, mp 108–110 °C, TLC R_f = 0.47 (dichloromethane/methanol 7:1, v/v); IR(KBr): 3475, 2973, 2939, 2879, 2835, 2785, 1735, 1659, 1612, 1586, 1513, 1458, 1404, 1378, 1344, 1330, 1246, 1172, 1139, 1109, 1074, 1050, 1033 cm^{-1}; ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.28–7.26 (m, 2H), 6.86 (m, 2H), 5.46 (m, 1H),

5.18 (dd, $J = 11.1$ Hz, $J = 2.2$ Hz, 1H), 4.91 (d, $J = 11.0$ Hz 1H), 4.31–4.29 (m, 2H), 4.00–3.96 (m, 2H), 3.84–3.81 (m, 1H), 3.81–3.78 (m, 3H), 3.75 (m, 1H), 3.25–3.23 (m, 1H), 3.18–3.16 (m, 1H), 3.05 (s, 3H), 2.98 (m, 2H), 2.84–2.80 (m, 1H), 2.42–2.36 (m, 7H), 2.18 (m, 1H), 1.96–1.92 (m, 2H), 1.83–1.79 (m, 2H), 1.53–1.47 (m, 3H), 1.28–1.25 (m, 4H), 1.26–1.23 (m, 9H), 1.18–1.10 (m, 9H), 0.83 (t, 3H); MS (ESI) *m/z* calcd. for $C_{39}H_{64}N_2O_{12}$ 752.4, found $[M + H]^+$ 753.7; Analysis calculated for $C_{39}H_{64}N_2O_{12}$: C 62.21, H 8.57, N 3.72. Found: C 62.30, H 8.54, N 3.70.

*6.3.3. 3-O-((4-Fluorobenzyl)carbamoyl)-3-O-descladinosyl-clarithromycin (**4c**)*

White crystals, yield 59.4%, mp 115–117 °C, TLC $R_f = 0.50$ (dichloromethane/methanol 7:1, v/v); IR(KBr): 3458, 2974, 2940, 2879, 2834, 2786, 1736, 1606, 1510, 1458, 1404, 1378, 1345, 1330, 1260, 1225, 1171, 1109, 1074, 1051, 1033 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$): δ 7.32–7.30 (m, 2H), 7.02–7.00 (m, 2H), 5.45 (m, 1H), 5.18 (dd, $J = 11.0$ Hz, $J = 1.95$, 1H), 4.92–4.90 (d, $J = 11.0$ Hz, 1H), 4.39–4.34 (m, 2H), 3.97–3.95 (m, 2H), 3.83–3.78 (m, 2H), 3.25 (m, 1H), 3.17–3.14 (m, 1H), 3.05 (s, 3H), 3.01–2.99 (m, 2H), 2.85–2.83 (m, 1H), 2.57–2.54 (m, 1H), 2.19 (s, 6H), 2.19–2.17 (m, 1H), 1.95–1.92 (m, 2H), 1.83–1.79 (m, 3H), 1.54–1.48 (m, 3H), 1.29–1.25 (m, 3H), 1.20–1.09 (m, 18H), 0.83 (t, 3H); MS (ESI) *m/z* calcd. for $C_{38}H_{61}FN_2O_{11}$ 740.4, found $[M + H]^+$ 741.8; Analysis calculated for $C_{38}H_{61}FN_2O_{11}$: C 61.60, H 8.30, N 3.78. Found: C 61.52, H 8.32, N 3.74.

*6.3.4. 3-O-((2-Chlorophenethyl)carbamoyl)-3-O-descladinosyl-clarithromycin (**4d**)*

White crystals, yield 58.2%, mp 118–121 °C, TLC $R_f = 0.47$ (dichloromethane/methanol 7:1, v/v); IR(KBr): 3447, 2974, 2939, 2879, 2834, 2786, 1816, 1736, 1511, 1457, 1404, 1378, 1330, 1245, 1170, 1109, 1074, 1051, 1033 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$): δ 7.36 (d, $J = 2.0$ Hz, 1H), 7.26–7.19 (m, 3H), 5.18 (dd, $J = 11.1$ Hz, $J = 1.98$, 1H), 5.02–4.99 (m, 1H), 4.90 (d, $J = 11.1$ Hz, 1H), 4.13 (m, 1H), 4.03–3.97 (m, 1H), 3.82–3.79 (m, 2H), 3.64–3.60 (m, 1H), 3.35–3.30 (m, 3H), 3.25 (m, 1H), 3.20–3.17 (m, 1H), 3.06 (s, 3H), 3.01–2.98 (m, 3H), 2.83–2.80 (m, 1H), 2.58–2.55 (m, 1H), 2.43–2.41 (m, 1H), 2.31 (s, 6H), 2.18–2.14 (m, 1H), 1.94–1.92 (m, 1H), 1.84–1.80 (m, 2H), 1.65–1.63 (m, 1H), 1.56–1.47 (m, 2H), 1.29 (s, 3H), 1.26–1.23 (m, 3H), 1.18–1.10 (m, 15H), 0.83 (t, 3H); MS (ESI) *m/z* calcd. for $C_{39}H_{63}ClN_2O_{11}$ 771.3, found $[M + H]^+$ 771.9; Analysis calculated for $C_{39}H_{63}ClN_2O_{11}$: C 60.72, H 8.23, N 3.63. Found: C 60.79, H 8.19, N 3.67.

*6.3.5. 3-O-((Benzyl)carbamoyl)-3-O-descladinosylclarithromycin (**4e**)*

Slightly yellow crystals, yield 69.0%, mp 100–103 °C, TLC $R_f = 0.58$ (dichloromethane/methanol 7:1, v/v); IR(KBr): 3462, 2975, 2940, 2880, 2834, 2786, 1817, 1743, 1693, 1458, 1378, 1330, 1264, 1233, 1171, 1109, 1077, 1050, 1033 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$): δ 7.33–7.31 (m, 2H), 7.29 (m, 1H), 7.28 (m, 2H), 5.37 (m, 1H), 5.21–5.16 (m, 3H), 5.07 (m, 1H), 4.93 (m, 1H), 4.84 (m, 1H), 4.39–4.27 (m, 2H), 3.99–3.94 (m, 2H), 3.85–3.81 (m, 1H), 3.78–3.75 (m, 1H), 3.25–3.23 (m, 1H), 3.06 (m, 3H), 3.02–2.99 (m, 1H), 2.56 (m, 1H), 2.31–2.28 (s, 6H), 2.17 (m, 1H), 2.13 (m, 1H), 1.95–1.93 (m, 1H), 1.83–1.79 (m, 2H), 1.57–1.55 (m, 1H), 1.52–1.49 (m, 2H), 1.29–1.25 (m, 3H), 1.23–1.08 (m, 18H), 0.85–0.83 (m, 3H); MS (ESI) *m/z* calcd. for $C_{38}H_{62}N_2O_{11}$ 722.4, found $[M + H]^+$ 723.9; Analysis calculated for $C_{38}H_{62}N_2O_{11}$: C 63.14, H 8.64, N 3.88. Found: C 63.17, H 8.60, N 3.89.

*6.3.6. 3-O-((Phenethyl)carbamoyl)-3-O-descladinosyl-clarithromycin (**4f**)*

Slightly yellow crystals, yield 60.6%, mp 107–109 °C, TLC $R_f = 0.51$ (dichloromethane/methanol 7:1, v/v); IR(KBr): 3453, 2974, 2939, 2879, 2834, 2786, 1737, 1604, 1498, 1456, 1405, 1378, 1330, 1246, 1171, 1109, 1075, 1051, 1033 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$): δ 7.33–7.30 (m, 2H), 7.27 (m, 1H), 7.20 (m, 2H), 5.07 (m, 1H), 4.93 (m, 1H), 4.03–4.01 (d, $J = 14.5$, 1H), 3.97 (m, 1H), 3.82 (m, 2H), 3.69–3.63 (m, 1H), 3.41–3.38 (m, 1H), 3.30–3.25 (m, 2H), 3.24 (m, 1H), 3.06 (s, 3H), 2.83–2.80 (m, 1H), 2.56 (m, 1H), 2.37 (m, 1H), 2.32 (m, 6H), 2.14–2.13 (m, 1H), 1.93 (m, 1H), 1.83–1.79 (m, 2H), 1.57–1.53 (m, 2H), 1.53–1.49 (m, 1H), 1.29–1.28 (m, 4H), 1.25–1.20 (m, 6H), 1.16–1.08 (m, 15H), 0.83 (t, 3H); MS (ESI) *m/z* calcd. for $C_{39}H_{64}N_2O_{11}$ 736.5, found $[M + H]^+$ 738.1; Analysis calculated for $C_{39}H_{64}N_2O_{11}$: C 63.56, H 8.75, N 3.80. Found: C 63.49, H 8.70, N 3.83.

*6.3.7. 3-O-((3,4-Methylenedioxyphenethy)carbamoyl)-3-O-descladinosylclarithromycin (**4g**)*

Slightly yellow crystals, yield 64.8%, mp 110–113 °C, TLC $R_f = 0.54$ (dichloromethane/methanol 7:1, v/v); IR(KBr): 3446, 2973, 2938, 2879, 2785, 1735, 1504, 1490, 1456, 1378, 1331, 1247, 1109, 1075, 1050 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$): δ 6.75 (m, 1H), 6.67 (m, 1H), 6.63 (m, 1H), 5.94 (s, 2H), 5.18 (dd, $J = 11.1$, $J = 1.8$, 1H), 4.91–4.89 (m, 2H), 4.13–4.10 (m, 1H), 4.03 (m, 1H), 3.96 (m, 1H), 3.81–3.77 (m, 2H), 3.62–3.59 (m, 1H), 3.24–3.21 (m, 1H), 3.19–3.17 (m, 2H), 3.06 (s, 3H), 3.00 (m, 1H), 2.82–2.80 (m, 1H), 2.76–2.71 (m, 2H), 2.57–2.54 (m, 1H), 2.31 (s, 6H), 1.94–1.92 (m, 2H), 1.84–1.79 (m, 2H), 1.70–1.65 (m, 3H), 1.52–1.47 (m, 2H), 1.29 (m, 9H), 1.18–1.10 (m, 12H), 0.83 (t, 3H); MS (ESI) *m/z* calcd. for $C_{40}H_{64}N_2O_{13}$ 780.4, found $[M + H]^+$ 781.9; Analysis calculated for $C_{40}H_{64}N_2O_{13}$: C 61.52, H 8.26, N 3.59. Found: C 61.55, H 8.21, N 3.62.

*6.3.8. 3-O-((Propyl)carbamoyl)-3-O-descladinosylclarithromycin (**4h**)*

White crystals, yield 58.8%, mp 217–220 °C, TLC $R_f = 0.49$ (dichloromethane/methanol 7:1, v/v); IR(KBr): 3446, 2972, 2938, 2877, 2786, 1735, 1633, 1521, 1459, 1405, 1378, 1344, 1263, 1171, 1109, 1077, 1052, 1034, 1008 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$): δ 5.25–5.20 (m, 1H), 5.19 (dd, $J = 11.1$, $J = 2.1$, 1H), 4.91 (m, 1H), 4.12 (d, $J = 20.0$, 1H), 3.84 (m, 1H), 3.79 (m, 1H), 3.37 (m, 1H), 3.27–3.25 (m, 1H), 3.24–3.22 (m, 2H), 3.05 (s, 3H), 3.02 (m, 2H), 2.83 (m, 1H), 2.68 (m, 1H), 2.57–2.54 (m, 2H), 2.41 (s, 6H), 2.34 (m, 1H), 2.18 (m, 1H), 1.94 (m, 1H), 1.82 (m, 1H), 1.72 (m, 1H), 1.56–1.51 (m, 4H), 1.28 (s, 4H), 1.27–1.23 (m, 3H), 1.16 (s, 3H), 1.14–1.10 (m, 12H), 0.94 (t, 3H), 0.83 (t, 3H); ^{13}C NMR (600 MHz, $CDCl_3$): δ 220.7, 173.9, 156.6, 103.0, 81.7, 78.3, 77.8, 77.0, 74.2, 70.5, 69.5, 65.7, 50.1, 45.5, 43.2, 42.9, 40.2, 38.7, 37.3, 35.6, 29.2, 23.3, 21.2, 19.3, 18.0, 16.1, 14.9, 12.6, 11.3, 10.5, 9.1; MS (ESI) *m/z* calcd. for $C_{34}H_{62}N_2O_{11}$ 674.4, found $[M + H]^+$ 675.9; Analysis calculated for $C_{34}H_{62}N_2O_{11}$: C 60.51, H 9.26, N 4.15. Found: C 60.58, H 9.22, N 4.12.

*6.3.9. 3-O-((Propenyl)carbamoyl)-3-O-descladinosylclarithromycin (**4i**)*

White crystals, yield 61.8%, mp 219–223 °C, TLC $R_f = 0.52$ (dichloromethane/methanol 7:1, v/v); IR(KBr): 3458, 2974, 2940, 2879, 2834, 2786, 1737, 1645, 1515, 1458, 1405, 1378, 1238, 1171, 1109, 1075, 1051, 1033 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$): δ 5.89–5.83 (m, 1H), 5.23–5.14 (m, 2H), 5.08–5.04 (m, 1H), 4.92–4.90 (m, 1H), 4.06 (d, $J = 7.3$, 1H), 4.00–3.96 (m, 1H), 3.91–3.89 (m, 1H), 3.82–3.80 (m, 2H), 3.72 (m, 1H), 3.39–3.37 (m, 2H), 3.25 (m, 1H), 3.22–3.19 (m, 1H), 3.05 (s, 3H), 3.02–2.99 (m, 1H), 2.86–2.82 (m, 1H), 2.58–2.55 (m, 1H), 2.14 (s, 1H), 2.32 (s, 6H), 2.20–2.18 (m, 1H), 1.96–1.92 (m, 1H), 1.84–1.80 (m, 2H) 1.69–1.67 (m, 2H), 1.56–1.53 (m, 1H), 1.50–1.47 (m, 1H), 1.29 (s, 3H), 1.23 (m, 3H), 1.16–1.07 (m, 15H), 0.83 (t, 3H); MS (ESI) *m/z* calcd. for $C_{34}H_{60}N_2O_{11}$ 672.4, found $[M + H]^+$ 673.8; Analysis calculated for $C_{34}H_{60}N_2O_{11}$: C 60.69, H 8.99, N 4.16. Found: C 60.64, H 9.02, N 4.13.

*6.3.10. 3-O-((Butyl)carbamoyl)-3-O-descladinosylclarithromycin (**4j**)*

White crystals, yield 56.4%, mp 107–110 °C, TLC $R_f = 0.48$ (dichloromethane/methanol 7:1, v/v); IR(KBr): 3460, 2973, 2939, 2877, 2834, 2786, 1737, 1695, 1515, 1458, 1405, 1378, 1345, 1330, 1245, 1171, 1109, 1075, 1052, 1032 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$): δ 5.19–5.17 (dd, $J = 11.1$, $J = 2.2$, 1H), 4.90 (m, 2H), 4.07–4.05 (m, 1H), 3.99–3.96 (m, 1H), 3.84–3.81 (m, 2H), 3.37–3.35 (m, 2H), 3.29–3.20

(m, 2H), 3.09 (m, 1H), 3.05 (s, 3H), 3.01–2.99 (m, 1H), 2.83 (m, 1H), 2.39–2.31 (m, 6H), 2.19–2.17 (m, 2H), 1.95–1.92 (m, 1H), 1.84–1.80 (m, 2H), 1.70–1.68 (m, 3H), 1.56–1.47 (m, 4H), 1.38–1.34 (m, 2H), 1.29 (s, 3H), 1.23 (m, 3H), 1.16–1.08 (m, 15H), 0.93 (t, 3H), 0.83 (t, 3H); MS (ESI) *m/z* calcd. for $C_{35}H_{64}N_2O_{11}$ 688.5, found $[M+H]^+$ 689.7; Analysis calculated for $C_{35}H_{64}N_2O_{11}$: C 61.02, H 9.36, N 4.07. Found: C 60.97, H 9.33, N 4.10.

6.3.11. 3-O-((Pentyl)carbamoyl)-3-O-descladinosylclarithromycin (**4k**)

White crystals, yield 63.0%, mp 105–107 °C, TLC $R_f = 0.48$ (dichloromethane/methanol 7:1, v/v); IR(KBr): 3457, 2972, 2938, 2876, 2786, 1737, 1517, 1458, 1405, 1379, 1331, 1250, 1171, 1110, 1075, 1051, 1033 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$): δ 5.18 (dd, $J = 11.1$, $J = 2.0$, 1H), 4.90–4.87 (m, 2H), 4.05 (d, $J = 7.3$, 1H), 3.96 (s, 1H), 3.82–3.80 (m, 2H), 3.37 (m, 1H), 3.31–3.30 (m, 1H), 3.28–3.25 (m, 1H), 3.20–3.17 (m, 1H), 3.08 (s, 3H), 3.01–2.99 (m, 1H), 2.85–2.80 (m, 1H), 2.57–2.55 (m, 1H), 2.43–2.41 (m, 1H), 2.29 (s, 6H), 2.18–2.16 (m, 1H), 1.95–1.90 (m, 3H), 1.68–1.64 (m, 2H), 1.54–1.50 (m, 4H), 1.38–1.32 (s, 4H), 1.32–1.29 (m, 3H), 1.22 (m, 4H), 1.15–1.09 (m, 15H), 0.90 (t, 3H), 0.83 (t, 3H); ^{13}C NMR (600 MHz, $CDCl_3$) δ 220.8, 173.9, 156.5, 103.1, 81.2, 78.3, 77.9, 77.0, 74.2, 70.5, 69.5, 66.1, 50.1, 45.5, 43.2, 41.2, 40.4, 38.7, 37.3, 35.6, 29.8, 29.0, 28.7, 22.4, 21.2, 19.4, 18.0, 16.1, 15.0, 14.0, 12.6, 10.5, 9.1; MS (ESI) *m/z* calcd. for $C_{36}H_{66}N_2O_{11}$ 702.5, found $[M+H]^+$ 703.9; Analysis calculated for $C_{36}H_{66}N_2O_{11}$: C 61.51, H 9.46, N 3.99. Found: C 61.57, H 9.42, N 4.01.

6.4. 2′-O-Acetyl-3-O-descladinosylazithromycin (**5**)

To a solution of azithromycin (2.0 g, 2.67 mmol) in anhydrous methanol (20 mL) at room temperature was added dropwise 1 M HCl until the reaction mixture was adjusted to pH = 1.0–1.5. The resulting solution was allowed to stir for 24 h at the same temperature. And then the reaction solution was neutralized to pH = 10.5–11.0 with 1 M sodium hydroxide and was stirred for 24 h at the room temperature. The reaction was quenched with 5% $NaHCO_3$ (20 mL) and the aqueous layer was extracted with CH_2Cl_2 (2×10 mL). The combined organic layers were dried over anhydrous Na_2SO_4, filtered, and concentrated in vacuo to afford 1.50 g (92.1%) of 3-*O*-descladinosylazithromycin as a white foam: mp 137–140 °C, $R_f = 0.522$ (dichlormethane/methanol, 10:1, v/v).

To a solution of 3-*O*-descladinosylazithromycin (1.5 g, 2.67 mmol) prepared above in CH_2Cl_2 (20 mL) at room temperature was added Ac_2O (0.5 mL, 5.34 mmol) and Et_3N (1.48 mL, 10.68 mmol). The resulting solution was allowed to stir for 24 h at the same temperature. The reaction was quenched with 5% aqueous $NaHCO_3$ (20 mL) and the aqueous layer was extracted with CH_2Cl_2 (2×10 mL). The combined organic layers were dried over anhydrous Na_2SO_4, filtered, and concentrated in vacuo. The residue was crystallized from acetone–water (2:1) to afford 1.34 g (89.7%) of **5** as a white solid: mp 141–143 °C, $R_f = 0.554$ (dichlormethane/methanol, 10:1, v/v).

6.5. 2′-O-Acetyl-3-O-acylimidazolyl-3-O-descladinosylazithromycin 11,12-cyclic carbonate (**6**)

To a solution of **5** (1.5 g, 2.37 mmol) in toluene (20 mL) was added Et_3N (0.60 mL, 4.33 mmol) and CDI (1.02 g, 5.80 mmol). The resulting solution was stirred for 48 h at the room temperature. The reaction was quenched with saturated $NaHCO_3$ (20 mL) and the aqueous layer was extracted with toluene (2×6 mL). The combined organic layers were dried over anhydrous Na_2SO_4, filtered, and concentrated in vacuo to afford 1.60 g (89.8%) of **6** as a white foam: mp 147–150 °C; $R_f = 0.592$ (dichlormethane/methanol, 10:1, v/v).

6.6. General methods for the preparation of 3-O-arylalkylcarbamoyl-3-O-descladinosylazithromycin 11,12-cyclic carbonate derivatives (**7a**–**g**)

To a solution of **6** (1.6 g, 2.13 mmol) in DMF (15 mL) was added DBU (0.33 mL, 2.25 mmol, 1.0 equiv) and corresponding amine (2.25 mmol, 1.0 equiv). The resulting solution was stirred for 10 h at the room temperature. The reaction was quenched with water (30 mL) and the aqueous layer was extracted with ethyl acetate (3×15 mL). The combined organic layers were washed with brine (3×15 mL), and dried over anhydrous Na_2SO_4, filtered. The filtrate was concentrated in vacuo to afford a crude product.

A solution of the above crude product in methanol (15 mL) was heated to 55 °C and stirred for 20 h at the same temperature. After concentrating the reaction solution in vacuo, the residue was purified by flash chromatography (dichlormethane/methanol, 10:1, v/v) to afford products **7a**–**g**.

6.6.1. 3-O-((4-Methoxybenzyl)carbamoyl)-3-O-descladinosylazithromycin 11,12-cyclic carbonate (**7a**)

White solids, yield 64.9%, mp 113–116 °C; TLC $R_f = 0.634$ (dichlormethane/methanol 10:1, v/v); IR (KBr): 3430, 2972, 2938, 2878, 2835, 1812, 1726, 1612, 1586, 1513, 1459, 1378, 1349, 1378, 1349, 1241, 1213, 1164, 1112, 1071, 1052 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$) δ 7.27–7.24 (m, 2H), 6.87–6.84 (m, 2H), 5.05–5.04 (m, 1H), 4.54 (m, 2H), 4.38–4.36 (m, 1H), 4.26–4.19 (m, 2H), 3.99–3.97 (m, 1H), 3.80 (s, 3H), 3.72–3.70 (m, 1H), 3.64–3.61 (m, 1H), 3.57–3.55 (m, 2H), 3.27–3.23 (m, 1H), 2.96 (m, 1H), 2.88–2.82 (m, 2H), 2.67–2.62 (m, 1H), 2.52–2.49 (m, 2H), 2.25 (m, 9H), 1.98 (m, 3H), 1.92–1.86 (m, 6H), 1.31–1.26 (m, 5H), 1.25–1.23 (m, 7H), 0.94–0.90 (m, 9H); MS (ESI) *m/z* calcd. for $C_{40}H_{65}N_3O_{12}$ 779.5, found $[M+H]^+$ 780.9; Analysis calculated for $C_{40}H_{65}N_3O_{12}$: C 61.60, H 8.40, N 5.39. Found: C 61.55, H 8.39, N 5.35.

6.6.2. 3-O-((4-Fluorobenzyl)carbamoyl)-3-O-descladinosylazithromycin 11,12-cyclic carbonate (**7b**)

White solids, yield 57.5%, mp 106–110 °C; TLC $R_f = 0.612$ (dichlormethane/methanol 10:1, v/v); IR (KBr): 3435, 2973, 2939, 2879, 2790, 1813, 1729, 1606, 1510, 1458, 1378, 1350, 1336, 1240, 1164, 1112, 1051 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$) δ 7.30–7.26 (m, 2H), 7.02–6.98 (m, 2H), 5.11 (m, 1H), 4.50–4.39 (m, 2H), 4.34–4.21 (m, 2H), 4.33–4.21 (m, 2H), 3.68–3.54 (m, 2H), 3.27–3.22 (m, 1H), 2.88–2.82 (m, 1H), 2.69–2.60 (m, 2H), 2.53–2.43 (m, 1H), 2.37 (m, 2H), 2.25 (m, 9H), 2.19 (m, 1H), 1.91–1.85 (m, 2H), 1.68–1.63 (m, 4H), 1.45 (m, 3H), 1.31–1.23 (m, 4H), 1.06–1.01 (m, 9H), 0.94–0.90 (m, 9H); MS (ESI) *m/z* calcd. for $C_{39}H_{62}FN_3O_{11}$ 767.4, found $[M+H]^+$ 768.7; Analysis calculated for $C_{39}H_{62}FN_3O_{11}$: C 61.00, H 8.14, N 5.47. Found: C 60.95, H 8.10, N 5.54.

6.6.3. 3-O-((2-Chlorophenethyl)carbamoyl)-3-O-descladinosylazithromycin 11,12-cyclic carbonate (**7c**)

White solids, yield 55.3%, mp 117–111 °C; TLC $R_f = 0.582$ (dichlormethane/methanol 10:1, v/v); IR (KBr): 3435, 2972, 2934, 2877, 1812, 1737, 1637, 1509, 1457, 1379, 1351, 1327, 1239, 1166, 1113, 1048 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$) δ 7.26–7.19 (m, 3H), 5.04 (m, 1H), 4.60 (s, 1H), 4.55 (m, 1H), 4.40 (d, $J = 4.2$ Hz, 1H), 3.95 (m, 1H), 3.82–3.80 (m, 1H), 3.66 (m, 2H), 3.56 (m, 1H), 3.36–3.35 (m, 2H), 3.00–2.95 (m, 3H), 2.83 (m, 4H), 2.68–2.60 (m, 1H), 2.50–2.47 (m, 1H), 2.25 (m, 6H), 2.19 (m, 2H), 1.89 (m, 2H), 1.66 (m, 2H), 1.45–1.42 (m, 5H), 1.23 (m, 1H), 1.07–1.00 (m, 12H), 0.94–0.89 (m, 9H); MS (ESI) *m/z* calcd. for $C_{40}H_{64}ClN_3O_{11}$ 797.4, found $[M+H]^+$ 798.7; Analysis calculated for $C_{40}H_{64}ClN_3O_{11}$: C 61.17, H 8.08, N 5.26. Found: C 61.13, H 8.09, N 5.22.

*6.6.4. 3-O-((Benzyl)carbamoyl)-3-O-descladinosylazithromycin 11,12-cyclic carbonate (**7d**)*

White solids, yield 55.3%, mp 126–130 °C; TLC $R_f = 0.605$ (dichlormethane/methanol 10:1, v/v); IR (KBr): 3430, 2972, 2936, 2878, 2789, 1812, 1714, 1636, 1497, 1456, 1380, 1350, 1238, 1213, 1165, 1113, 1051 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$) δ 7.33–7.24 (m, 5H), 5.13 (m, 1H), 4.53 (m, 1H), 4.47–4.45 (m, 1H), 4.38 (m, 1H), 4.34–4.28 (m, 2H), 3.71 (d, $J = 10.4$ Hz, 2H), 3.51–3.47 (m, 2H), 3.27–3.22 (m, 2H), 2.84–2.82 (m, 1H), 2.67–2.63 (m, 1H), 2.50–2.45 (m, 1H), 2.28–2.20 (m, 7H), 2.25–2.23 (m, 6H), 2.01 (m, 1H), 1.89 (m, 1H), 1.75 (m, 1H), 1.67–1.63 (m, 2H), 1.46 (m, 2H), 1.25–1.23 (m, 4H), 1.05–1.01 (m, 9H), 0.94–0.88 (m, 9H); MS (ESI) *m/z* calcd. for $C_{39}H_{63}N_3O_{11}$ 749.5, found $[M+H]^+$ 750.5; Analysis calculated for $C_{39}H_{63}N_3O_{11}$: C 62.46, H 8.47, N 5.60. Found: C 62.40, H 8.49, N 5.57.

*6.6.5. 3-O-((Phenethyl)carbamoyl)-3-O-descladinosylazithromycin 11,12-cyclic carbonate (**7e**)*

White solids, yield 59.0%, mp 106–108 °C; TLC $R_f = 0.605$ (dichlormethane/methanol, 10:1, v/v); IR (KBr): 3415, 2972, 2934, 2878, 1810, 1734, 1632, 1497, 1457, 1378, 1328, 1241, 1166, 1110, 1050 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$) δ 7.31–7.29 (m, 2H), 7.26–7.21 (m, 3H), 5.03 (m, 1H), 4.62–4.60 (m, 2H), 4.44 (m, 1H), 3.65–3.55 (m, 2H), 3.52–3.44 (m, 2H), 3.33 (m,1H), 3.28–3.25 (m, 3H), 2.84 (m, 4H), 2.49 (m, 1H), 2.39–2.37 (m, 1H), 2.32–2.20 (m, 10H), 1.91–1.86 (m, 1H), 1.77–1.67 (m, 4H), 1.48–1.43 (m, 4H), 1.30–1.24 (m, 4H), 1.04–1.00 (m, 9H), 0.96–0.89 (m, 9H); MS (ESI) *m/z* calcd. for $C_{40}H_{65}N_3O_{11}$ 763.5, found $[M+H]^+$ 764.8; Analysis calculated for $C_{40}H_{65}N_3O_{11}$: C 62.89, H 8.38, N 5.50. Found: C 62.80, H 8.42, N 5.55.

*6.6.6. 3-O-((Butyl)carbamoyl)-3-O-descladinosylazithromycin 11,12-cyclic carbonate (**7f**)*

White solids, yield 64.8%, mp 121–124 °C; TLC $R_f = 0.634$ (dichlormethane/methanol, 10:1, v/v); IR (KBr): 3414, 2971, 2937, 2874, 2790, 1813, 1714, 1639, 1508, 1458, 1380, 1350, 1335, 1239, 1213, 1164, 1113, 1051 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$) δ 4.92 (dd, $J = 9.2$ Hz, $J = 3.2$ Hz, 1H), 4.49 (m, 2H), 4.43 (m, 1H), 3.69–3.65 (m, 2H), 3.56–3.47 (m, 2H), 3.29–3.24 (m, 1H), 3.20–3.13 (m, 2H), 3.10–3.03 (m, 1H), 2.88–2.81 (m, 2H), 2.55–2.49 (m, 2H), 2.28 (m, 9H), 2.17 (s, 1H), 1.90 (m, 1H), 1.70–1.68 (m, 1H), 1.50 (m, 3H), 1.46 (m, 6H), 1.30 (m, 3H), 1.24(m, 1H), 1.06–1.01 (m, 12H), 0.95–0.90 (m, 12H); MS (ESI) *m/z* calcd. for $C_{36}H_{65}N_3O_{11}$ 715.5, found $[M+H]^+$ 716.5; Analysis calculated for $C_{36}H_{65}N_3O_{11}$: C 60.40, H 9.15, N 5.87. Found: C 60.35, H 9.12, N 5.84.

*6.6.7. 3-O-((Pentyl)carbamoyl)-3-O-descladinosylazithromycin 11,12-cyclic carbonate (**7g**)*

White solids, yield 59.0%, mp 111–115 °C; TLC $R_f = 0.634$ (dichlormethane/methanol, 10:1, v/v); IR (KBr): 3415, 2935, 1813, 1714, 1636, 1508, 1458, 1379, 1350, 1335, 1278, 1238, 1212, 1164, 1113, 1051 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$) δ 4.93–4.90 (m, 1H), 4.48–4.46 (m, 2H), 4.31–4.27 (m, 1H), 3.68–3.65 (m,2H), 3.54–3.51 (m, 2H), 3.27–3.23 (m, 1H), 3.19–3.14 (m, 2H), 3.08–3.02 (m, 2H), 2.86–2.83 (m, 2H), 2.67–2.63 (m, 1H), 2.53–2.49 (m, 1H), 2.26 (m, 9H), 2.19–2.17 (m, 1H), 1.96–1.90 (m, 1H), 1.71 (m, 1H), 1.53–1.50 (m, 6H), 1.46 (m, 3H), 1.32–1.28 (m, 2H), 1.25–1.20 (m, 5H), 1.09–1.01 (m, 9H), 0.95–0.88 (m, 12H); MS (ESI) *m/z* calcd. for $C_{37}H_{67}N_3O_{11}$ 729.5, found $[M+H]^+$ 730.8; Analysis calculated for $C_{37}H_{67}N_3O_{11}$: C 60.88, H 9.25, N 5.76 Found: C 60.85, H 9.22, N 5.80.

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Appendix. Supplemental data

Supplemental data associated with this article can be found in online version at doi:10.1016/j.ejmech.2009.11.032.

References

[1] J.A. Washington II, W.R. Wilson, Mayo Clin. Proc 60 (1985) 189–203.
[2] J.A. Washington II, W.R. Wilson, Mayo Clin. Proc 60 (1985) 271–278.
[3] P.J. Atkins, T.O. Herbert, N.B. Jones, Int. J. Pharm. 30 (1986) 199–207.
[4] Z. Itoh, K. Nakaya, H. Suzuki, H. Aria, K. Wakabayashi, Am. J. Physiol. 247 (1984) G688–G694.
[5] S. Omura, K. Tsuzuki, T. Sunazuka, S. Marui, H. Toyoda, N. Inatomi, Z. Itoh, J. Med. Chem 30 (1987) 1943–1948.
[6] G.G. Zhanel, M. Dueck, D.J. Hoban, L.M. Vercaigne, J.M. Embil, A.S. Gin, J.A. Karlowsky, Drugs 61 (2001) 443–498.
[7] L. Katz, D.T.W. Chu, J.J. Plattner, J. Med. Chem 39 (1996) 3853–3874.
[8] D. Felmingham, R.N. Grüneberg, J. Antimicrob. Chemother 45 (2000) 191–203.
[9] C.J. Lai, B. Weisblum, Proc. Natl. Acad. Sci. USA 68 (1971) 856–860.
[10] B. Weisblum, Antimicrob. Agents Chemother. 39 (1995) 577–585.
[11] G.G. Zhanel, M. Walters, A. Noreddin, L.M. Vercaigne, A. Wierzbowski, J.M. Embil, A.S. Gin, S. Douthwaite, D.J. Hobanl, Drugs 62 (2002) 1771–1804.
[12] R.P. Bax, R. Anderson, J. Crew, P. Fletcher, T. Johnson, E. Kaplan, B. Knaus, K. Kristinsson, M. Malek, L. Strandberg, Nat. Med. 4 (1998) 545–546.
[13] A. Bryskier, Clin. Infect. Dis. 27 (1998) 865–883.
[14] L.H. Hansen, P. Mauvais, S. Douthwaite, Mol. Microbiol. 31 (1999) 623–631.
[15] W.S. Champney, C.L. Tober, Curr. Microbiol. 42 (2001) 203–210.
[16] F. Schlunzen, R. Zarivach, J. Harms, A. Bashan, A. Tocilj, R. Albrecht, A. Yonath, F. Franceschi, Nature 413 (2001) 814–821.
[17] S. Pal, Tetrahedron 62 (2006) 3171–3200.
[18] T. Tanikawa, T. Asaka, M. Kashimura, J. Med. Chem. 44 (2001) 4027–4030.
[19] T. Tanikawa, T. Asaka, M. Kashimura, K. Suzuki, H. Sugiyama, M. Sato, K. Kameo, S. Morimoto, A. Nishida, J. Med. Chem. 46 (2003) 2706–2715.
[20] B. Zhu, B.A. Marinelli, D. Abbanat, B.D. Foleno, T.C. Henninger, K. Bush, M.J. Macielag, Bioorg. Med. Chem. Lett. 16 (2006) 1054–1059.
[21] P. Xu, L. Liu, Z. Jin, P. Lei, Bioorg. Med. Chem. Lett. 17 (2007) 3330–3334.
[22] P.B. Fernandes, W.R. Baker, L.A. Freiberg, D.J. Hardy, E.J. McDonald, Antimicrob. Agents Chemother. 33 (1989) 78–81.
[23] W.S. Champney, C.L. Tober, Curr. Microbiol. 38 (1999) 342–348.