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Synthesis and antibacterial activity of novel 4″-*O*-(1-aralkyl-1,2,3-triazol-4-methyl-carbamoyl) azithromycin analogs

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ABSTRACT

Three novel structural series of 4″-*O*-(1-aralkyl-1,2,3-triazol-4-methyl-carbamoyl) azithromycin analogs were designed, synthesized and evaluated for their *in vitro* antibacterial activity. All the target compounds exhibited excellent activity against erythromycin-susceptible *Streptococcus pyogenes*, and significantly improved activity against three phenotypes of erythromycin-resistant *Streptococcus pneumoniae* compared with clarithromycin and azithromycin. Among the three series of azithromycin analogs, the novel series of 11,4″-disubstituted azithromycin analogs **9a**–**k** exhibited the most effective and balanced activity against susceptible and resistant bacteria. Among them, compound **9j** showed the most potent activity against *Staphylococcus aureus* ATCC25923 (0.008 μg/mL) and *Streptococcus pyogenes* R2 (1 μg/mL). Besides, all the 11,4″-disubstituted azithromycin analogs **9a**–**k** except **9f** shared the identical activity with the MIC value <0.002 μg/mL against *Streptococcus pyogenes* S2. Furthermore, compounds **9g**, **9h**, **9j** and **9k** displayed significantly improved activity compared with the references against all the three phenotypes of resistant *S. pneumoniae*. Particularly, compound **9k** was the most effective (0.06, 0.03 and 0.125 μg/mL) against all the erythromycin-resistant *S. pneumoniae* expressing the *erm* gene, the *mef* gene and the *erm* and *mef* genes, exhibiting 2133, 133 and 2048-fold more potent activity than azithromycin, respectively.

Macrolide antibiotics as a safe and effective agents have been widely used for the treatment of upper and lower respiratory tract infections in the early 1950s.[1,2] Erythromycin A (EMA), the first generation macrolide, has been used clinically for more than 60 years, but it readily loses its antibacterial activity due to acid-mediated decomposition, resulting in poor bioavailability and undesirable gastrointestinal (GI) side effects.[3] Second generation macrolides exemplified by clarithromycin (CAM) and azithromycin (AZM) (Fig. 1) have been developed to overcome the acid instability of erythromycin A in late 1980s, which not only prevented the formation of degradation products, but also exhibited more favorable pharmacokinetic profiles and better GI tolerability than first generation macrolides.[4,5]

With the widespread use of the antibiotics, especially misuse, the increasing incidence of bacterial resistance to macrolides is becoming a major threat to successful treatment of infectious diseases.[6] The molecular mechanisms of macrolide resistance are diverse, but two major mechanisms of resistance are ribosome methylation resistance encoded by the *erm* gene and drug efflux pump encoded by the *mef* gene.[7] Expression of an *erm*-resistant determinant in bacteria leads to 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, while efflux pump encoded by the *mef* gene results in pumping macrolides out of cell, thus preventing them from reaching the binding sites.[8,9] Consequently, it's urgent to develop newer generation macrolides that exhibit significant activity against resistant pathogens to control the infectious disease. The third generation macrolides known as ketolides such as telithromycin and cethromycin were introduced in 1990s. They exhibit excellent antibacterial activity against several macrolide-resistant strains and may offer alternative therapy for Gram-positive infections attributable to resistant pathogens.[10] The C-11,12 carbamate side chain or the C-6 side chain in the ketolides may interact with secondary ribosomal binding site A752 directly in domain II of the 23S rRNA in addition to the main interaction with the nucleotide A2058 in domain V in bacterial ribosomes. This

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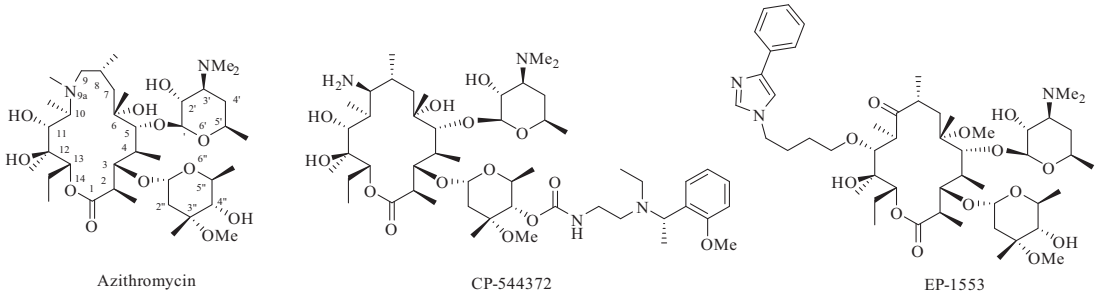


Fig. 1. Structures of azithromycin, CP-544372 and EP-1553.

interaction results in tighter binding to the bacterial ribosomes and imparts some activity against methylated ribosomes in some species.[11–13]

Owing to the successful development of ketolide antibiotics, especially telithromycin and cethromycin, numerous chemical modifications of macrolides have been carried out to discover more potent antimicrobial agents. CP-544372 (Fig. 1) containing a long C-4″ anchor group with six atoms from 4″-oxygen atom to the terminal benzene ring as a representative of C-4″ modified carbamate macrolides shows good *in vitro* and *in vivo* activity against MLS_B-resistant strains with competitive binding to chloramphenicol, suggesting that the anchor group reaches the peptidyl transferase center (PTC) region, the chloramphenicol-binding site.[14,15] Besides, EP-1553 (Fig. 1), as a representative of another structural series of 11-carbamate macrolides was reported, which exhibited excellent activity against macrolide-resistant bacteria.[9,14]

Structure–activity relationships (SARs) of these mentioned macrolide derivatives have provided us with the information toward an understanding of the essential structural features needed for antibacterial activity. Specifically, it has been recognized that arylalkyl groups attached to the C-4″ position of the cladinose sugar in the structure are essential for overcoming MLS_B resistance, and their lengths are important for conferring potent activity against resistant bacteria, whereas the introduction of 11,12-cyclic carbonate ring or 11-*O*-arylalkylcarbamoyl side chains could strengthen the interaction with A752.[16,17] Consequently, an optimized strategy to overcome macrolide-resistant bacteria would be the design of macrolide analogs with two side chains which would interact with A752 in domain II of the 23S rRNA and nucleotide binding sites between the PTC and the macrolide roadblock, respectively. Based on this, we have reported a series of novel 11, 12-cyclic carbonate-4″-carbamate and 11,4″-di-*O*-arylcarbamoyl azithromycin derivatives.[9,16,18] Among them, Compounds **7k**, **28** and **30** (Fig. 2), as a representative of the above respective series, exhibited remarkably improved activity against erythromycin-resistant *S. pneumoniae* expressing the *erm* gene, the *mef* gene, and the *erm* and *mef* gene compared to EMA, CAM or AZM. Here we described a derivatization stream followed in order to investigate the influence of linker features on biological properties. Therefore, we introduced novel 4″-*O*-carbamoyl side chains containing 1,2,3-triazol group connecting 4″-oxygen atom and terminal groups together to probe the effect of the different 4″-*O*-carbamoyl side chains on antibacterial activity. On the basis of the considerations detailed above, we designed, synthesized three novel structural series of 4″-*O*-(1-aralkyl-1,2,3-triazol-4-methyl-carbamoyl)azithromycin analogs to address macrolide resistance caused by efflux and methylation of the ribosome. The terminal groups employed to the C-11 and C-4″ side chains were a variety of substituted aromatic groups, which could be beneficial for gaining a favorable binding affinity for the bacterial ribosome through hydrogen bonding, π-stacking or van der Waals (VDW) interaction.

All the side chains (aminomethyl triazoles **A_1-A_{15}**) were prepared according to methods outlined in Scheme 1. The reaction of corresponding halide with NaN_3 gave aromatic azides in the presence of ethanol-water. Then aromatic azides were coupled with propargyl amine to give corresponding aminomethyl triazoles (**A_1-A_{15}**).

The synthetic method for 4″-*O*-(1-aralkyl-1,2,3-triazol-4-methyl-carbamoyl) azithromycin analogs (**4a**–**j**) is shown in

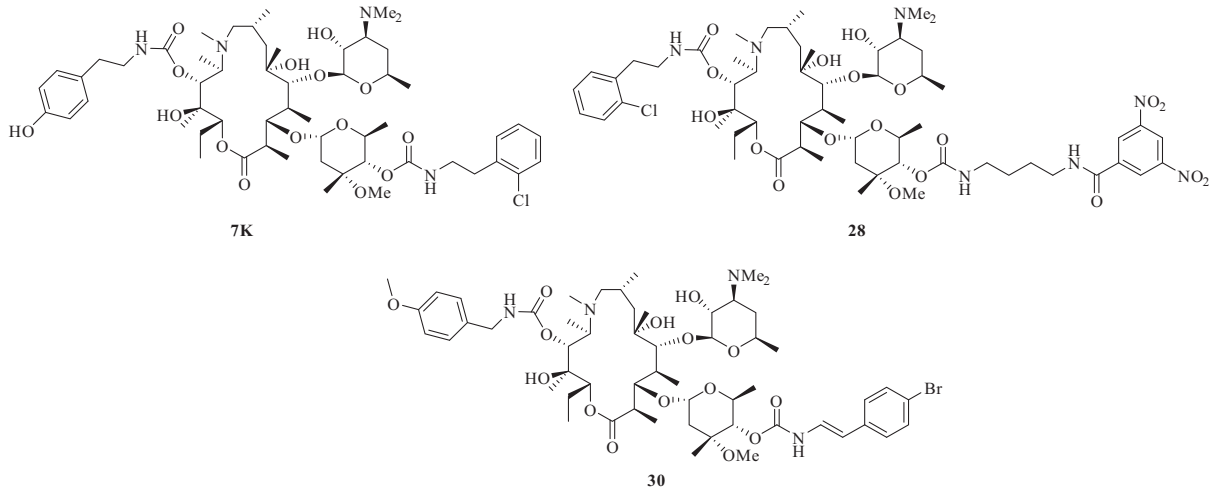
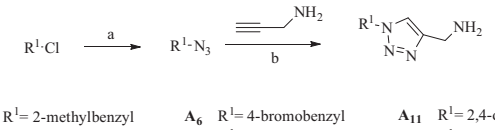


Fig. 2. Structures of **7k**, **28** and **30**.



A_1	R^1= 2-methylbenzyl	A_6	R^1= 4-bromobenzyl	A_{11}	R^1= 2,4-dichlorobenzyl
A_2	R^1= 3-methylbenzyl	A_7	R^1= 2-fluorobenzyl	A_{12}	R^1= 2-chlorobenzyl
A_3	R^1= 4-methylbenzyl	A_8	R^1= 3-fluorobenzyl	A_{13}	R^1= 3-chlorobenzyl
A_4	R^1= 4-nitrobenzyl	A_9	R^1= 4-fluorobenzyl	A_{14}	R^1= 4-chlorobenzyl
A5	R^1= benzyl	A_{10}	R^1= 2,6-dichlorobenzyl	A_{15}	R^1= cyclohexyl

Scheme 1. Synthesis of aminomethyl triazoles. Reagents and conditions: a) NaN_3, EtOH, H_20, reflux, 75–89%; b) Propargyl amine, $CuSO_4$, Sodium ascorbate, tBuOH, H_2O, rt, 46–63%.

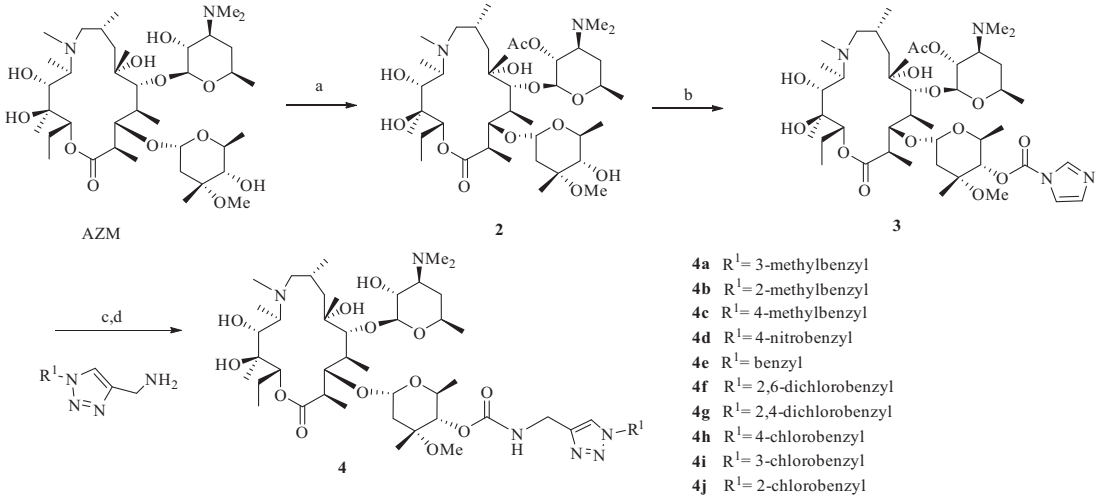
Scheme 2. AZM was used as the starting material for the synthesis of the target compounds. It was first coupled with acetic anhydride catalyzed by triethylamine at room temperature for 24 h to generate the compound **2**, which was treated with 1,1-carbonyldiimidazole (CDI) in toluene at 55 °C to give the important intermediate **3**. Finally, 4″-carbamate azithromycin analogs (**4a**–**j**) were prepared by coupling **3** with corresponding aminomethyl triazoles in the presence of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU), followed by methanolysis.

The desired target compounds, 11,12-cyclic carbonate-4″-*O*-(1-aralkyl-1,2,3-triazol-4-methyl-carbamoyl) azithromycin analogs (**8a**–**o**) and 11,4″-di-*O*-arylalkylcarbamoyl azithromycin analogs (**9a**–**k**), were synthesized as shown in Scheme 3. Compound **2** was treated with 1,1-carbonyldiimidazole (CDI) in toluene at 55 °C to generate the important intermediate 14″-*O*-acylimidazolyl-11,12-cyclic carbonate **5**. Then compound **6** was obtained by coupling the intermediate **5** with propargyl amine catalyzed by 1,8-Diazabicyclo [5,4,0]-undec-7-ene (DBU), followed by deprotection of the acetyl group in methanol. Subsequently, compound **7** were reacted with the corresponding aromatic azides to give the desired target compounds **8a**–**o**. Then corresponding 11,12-cyclic carbonate-4″-*O*-(1-aralkyl-1,2,3-triazol-4-methyl-carbamoyl) azithromycin analogs was readily converted to novel 11,4″-di-*O*-arylalkylcarbamoyl azithromycin analogs (**9a–k**) by coupling with the corresponding amines in the presence of pyridine hydrochloride at room temperature in yields ranging from 68% to 86%.

All the target compounds, as well as CAM and AZM as references, were tested for *in vitro* antibacterial activity against eight phenotypes of Gram-positive strains and two phenotypes of Gram-negative strains. Their activities are reported as minimum inhibitory concentrations (MICs). The sensitive and resistant Gram-positive bacterial strains contain: *S. aureus* ATCC25923 (erythromycin-susceptible strain), *S. pneumoniae* ATCC49619 (erythromycin-susceptible strain), *S. pyogenes* S2 (erythromycin-susceptible strain isolated clinically), *S. aureus* ATCC29213 (methicillin-resistant strain), *S. pneumoniae* B1 (erythromycin-resistant strain encoded by the *erm* gene), *S. pneumoniae* A22072 (erythromycin-resistant strain encoded by the *mef* gene), *S. pneumoniae* AB11 (erythromycin-resistant strain encoded by the *erm* and *mef* genes), *S. pyogenes* R2 (erythromycin-resistant strain isolated clinically). Two phenotypes of Gram-negative strains are *E. coli* ATCC25922, *P. aeruginosa* ATCC27853.

MIC values for 4″-*O*-(1-aralkyl-1,2,3-triazol-4-methyl-carbamoyl) azithromycin analogs (**4a**–**j**) are presented in Table 1. Most of the above analogs retained comparable activity compared with AZM and CAM against erythromycin-susceptible *S. pyogenes* S2, and all of them showed improved activity against erythromycin-resistant *S. pyogenes* R2 and the three phenotypes of resistant *S. pneumoniae*. In particular, compound **4g** displayed the most potent activity against not only erythromycin-resistant *S. pyogenes* S2 (0.016 μg/mL) but also three phenotypes of erythromycin-resistant *S. pneumoniae* B1, *S. pneumoniae* A22072 and *S. pneumoniae* AB11 (0.5, 0.25 and 0.25 μg/mL), showing 12.5, 256, 16 and 1024-fold enhanced activity than AZM, respectively, which suggested that introduction of the 1-aralkyl-1,2,3-triazol-4-methyl-carbamoyl side chain into the C-4″ position of AZM could enhance antibacterial activity against erythromycin-resistant bacteria.

Compared with their precursors 4″-*O*-(1-aralkyl-1,2,3-triazol-4-methyl-carbamoyl) azithromycin analogs, almost all of the 11,12-cyclic carbonate-4″-*O*-(1-aralkyl-1,2,3-triazol-4-methyl-carbamoyl) azithromycin analogs (**8a**–**o**) showed compromised or similar activity against all the tested strains, which suggested that the introduction of 11,12-cyclic carbonate moiety failed to confer lower MIC values against erythromycin-susceptible or erythromycin-resistant strains. However, the compounds in this series still showed potent activity against erythromycin-susceptible *S. pneumoniae* ATCC49619 (0.5–1 μg/mL) and *S. pyogenes* S2 (0.008–0.25 μg/mL), and showed improved activity against all the three phenotypes of resistant *S. pneumoniae* compared with AZM and CAM. Among them, compounds **8a**, **8e** and **8g** displayed the most potent activity against *S. pneumoniae* ATCC49619 (0.5 μg/mL), and compound **8d** (0.008 μg/mL) exhibited better activity against



4a R^1= 3-methylbenzyl
4b R^1= 2-methylbenzyl
4c R^1= 4-methylbenzyl
4d R^1= 4-nitrobenzyl
4e R^1= benzyl
4f R^1= 2,6-dichlorobenzyl
4g R^1= 2,4-dichlorobenzyl
4h R^1= 4-chlorobenzyl
4i R^1= 3-chlorobenzyl
4j R^1= 2-chlorobenzyl

Scheme 2. Synthesis of 4″-*O*-(1-aralkyl-1,2,3-triazol-4-methyl-carbamoyl) azithromycin analogs (**4a**–**j**). Reagents and conditions: a) Ac_2O, DCM, Et_3N, rt, 24 h, 92%; b) CDI, Et_3N, toluene, 55 °C, 40 h, 95%; c) DMF, DBU, rt, 12 h, 82–93%; d) CH_3OH, 55 °C, 12 h, 90–96%.

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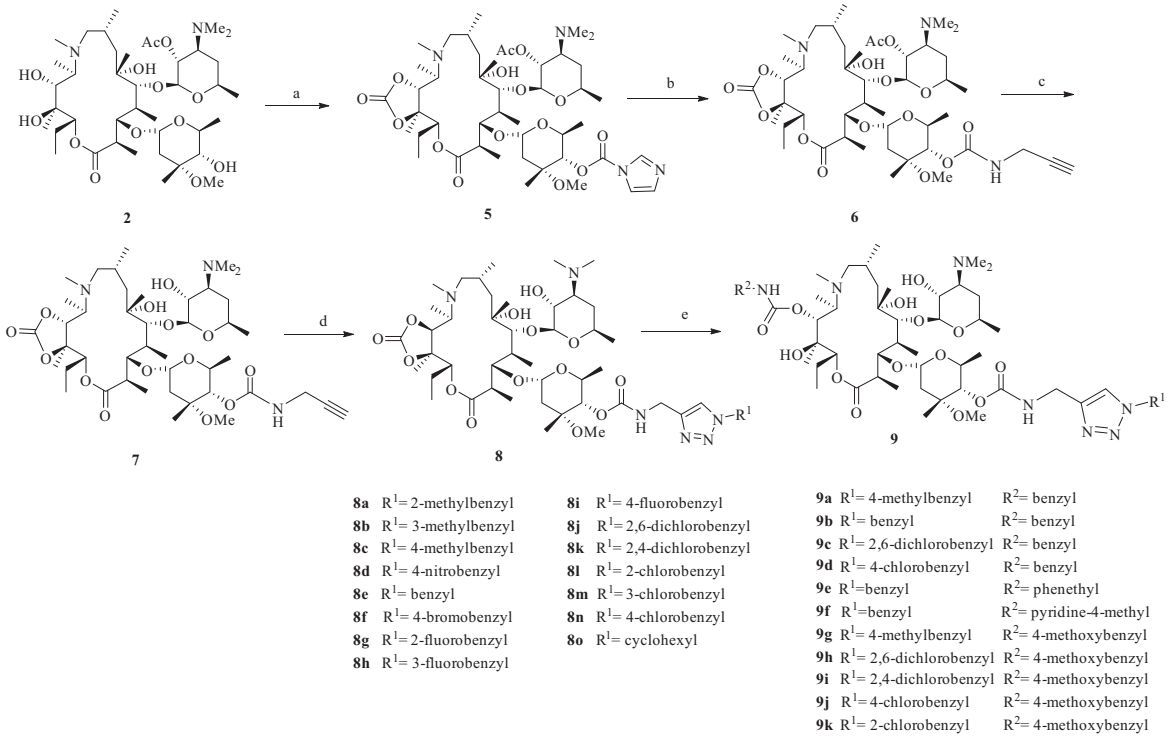

Scheme 3. Synthesis of 11,12-cyclic carbonate-4″-*O*-(1-aralkyl-1,2,3-triazol-4-methyl-carbamoyl) azithromycin analogs (**8a–o**) and 11,4″-di-*O*-arylalkylcarbamoyl azithromycin analogs (**9a–k**). Reagents and conditions: a) CDI, Et_3N, toluene, 55 °C, 40 h, 93%; b) propargylamine, DMF, DBU, rt, 20 h, 96%; c) CH_3OH, 55 °C, 12 h, 70%; d) R^1N_3, $CuSO_4$, sodium ascorbate, tBuOH, H_2O, 8 h, 77–89%; e) pyridine hydrochloride, R^2NH_2, rt, 2–5 days, 68–86%.

Table 1
4″-*O*-(1-Aralkyl-1,2,3-triazol-4-methyl-carbamoyl) azithromycin analogs with their *in vitro* antibacterial activity against susceptible and resistant strains (μg/mL).

Compounds	*S. aureus* ATCC25923[a]	*S. pneumoniae* ATCC49619[b]	*S. aureus* ATCC29213[c]	*S. pyogenes* S2[d]	*S. pyogenes* R2[e]	*S. pneumoniae* B1[f]	*S. pneumoniae* A22072[g]	*S. pneumoniae* AB11[h]	*E. coli* ATCC25922	*P. aeruginosa* ATCC27853
4a	1	0.5	16	0.03	32	1	1	1	32	32
4b	1	0.25	16	0.03	64	0.5	0.5	0.5	16	64
4c	1	0.5	16	0.03	64	0.5	0.5	1	32	64
4d	1	1	16	0.03	64	1	2	1	32	32
4e	1	0.5	8	0.06	32	2	1	4	32	32
4f	1	0.5	16	0.03	32	0.5	0.5	0.5	32	32
4g	1	0.25	16	0.016	64	0.5	0.25	0.25	64	32
4h	1	0.5	16	0.03	64	2	1	0.5	64	32
4i	1	0. 25	8	0.06	64	0.5	0.25	1	16	32
4j	1	0.25	8	0.03	64	1	0.25	0.5	128	32
CAM	0.12	0.125	0.25	0.03	128	128	4	128	64	32
AZM	0.25	0.125	1	0.03	128	128	4	256	16	8

[a] *S. aureus* ATCC25923: erythromycin-susceptible strain.
[b] *S. pneumoniae* ATCC49619: erythromycin-susceptible strain.
[c] *S. aureus* ATCC29213: methicillin-resistant strain.
[d] *S. pyogenes* S2: erythromycin-susceptible strain isolated clinically.
[e] *S. pyogenes* R2: erythromycin-resistant strain isolated clinically.
[f] *S. pneumoniae* B1: erythromycin-resistant strain encoded by the *erm*B gene.
[g] *S. pneumoniae* A22072: erythromycin-resistant strain encoded by the *mef*A gene.
[h] *S. pneumoniae* AB11: erythromycin-resistant strain encoded by the *erm*B and *mef*A genes.

erythromycin-susceptible *S. pyogenes* S2 than the references. Particularly, compound **8j** possessed the most potent activity against three phenotypes of erythromycin-resistant *S. pneumoniae* B1, *S. pneumoniae* A22072 and *S. pneumoniae* AB11 (1, 0.25 and 0.25 μg/mL), showing 128, 16 and 1024-fold enhanced activity than AZM, respectively (Table 2). As for Gram-negative organisms, all of the target compounds displayed weaker activity against *E. coli* ATCC25922 and *P. aeruginosa* ATCC27853 than the references and a few of the target compounds such as compounds **8d** and **8n** maintained the activity of CAM against *E. coli* ATCC25922.

MIC values for 11,4″-di-*O*-arylalkylcarbamoyl azithromycin analogs (**9a–k**) are shown in Table 2. Compared with their precursors 11,12-cyclic carbonate-4″-*O*-(1-aralkyl-1,2,3-triazol-4-methyl-carbamoyl) azithromycin analogs (**8a–o**), almost all of the 11,4″-di-*O*-arylalkylcarbamoyl azithromycin analogs (**9a–k**) showed improved activity against all the tested strains. Moreover, all the analogs **9a–k** showed remarkablely improved activity compared with AZM and CAM against erythromycin-susceptible *S. pyogenes* S2 and all the three phenotypes of resistant *S. pneumoniae*. Especially, for erythromycin-susceptible *S. pyogenes* S2, all the

Table 2
11,12-Cyclic carbonate-4″-*O*-(1-aralkyl-1,2,3-triazol-4-methyl-carbamoyl) azithromycin analogs and 11,4″-di-*O*-arylalkylcarbamoyl azithromycin analogs with their *in vitro* antibacterial activity against susceptible and resistant strains (μg/mL).

Compounds	*S. aureus* ATCC25923[a]	*S. pneumoniae* ATCC49619[b]	*S. aureus* ATCC29213[c]	*S. pyogenes* S2[d]	*S. pyogenes* R2[e]	*S. pneumoniae* B1[f]	*S. pneumoniae* A22072[g]	*S. pneumoniae* AB11[h]	*E. coli* ATCC25922	*P. aeruginosa* ATCC27853
8a	4	0.5	32	0.06	64	4	2	2	128	64
8b	2	1	32	0.25	64	4	2	2	128	64
8c	4	1	64	0.25	64	2	1	1	128	64
8d	4	1	32	0.008	128	4	4	0.5	64	64
8e	4	0.5	32	0.06	128	8	8	4	128	64
8f	2	1	32	0.06	32	4	1	1	128	64
8g	4	0.5	32	0.125	128	2	1	0.25	128	64
8h	4	1	32	0.125	128	4	4	0.5	128	64
8i	4	1	32	0.125	32	8	1	4	128	64
8j	4	1	32	0.125	16	1	0.25	0.25	128	64
8k	4	1	32	0.125	32	4	1	2	128	64
8l	4	1	32	0.06	16	8	2	4	128	64
8m	4	1	32	0.06	16	4	0.5	1	128	64
8n	4	1	32	0.125	64	8	1	4	64	64
8o	16	1	64	0.25	32	8	1	4	128	64
9a	0.5	0.125	16	<0.002	32	0.25	0.125	0.25	32	32
9b	0.016	0.125	16	<0.002	32	0.25	0.25	0.25	16	32
9c	0.25	0.125	16	<0.002	64	0.25	0.25	0.25	32	32
9d	0.25	0.25	16	<0.002	32	0.25	0.125	0.25	32	32
9e	0.25	0.125	16	<0.002	32	0.25	0.125	0.25	16	8
9f	1	0.5	32	0.03	32	0.125	0.06	0.25	32	64
9g	0.06	0.125	32	<0.002	8	0.125	0.03	0.25	16	64
9h	0.25	0.06	16	<0.002	1	0.125	0.03	0.25	16	16
9i	0.25	0.06	16	<0.002	8	0.125	0.125	0.25	16	16
9j	0.008	0.125	16	<0.002	1	0.125	0.06	0.125	16	8
9k	0.03	0.125	16	<0.002	8	0.06	0.03	0.125	32	8
CAM	0.12	0.125	0.25	0.03	128	128	4	128	64	32
AZM	0.25	0.125	1	0.03	128	128	4	256	16	8

[a] *S. aureus* ATCC25923: erythromycin-susceptible strain.
[b] *S. pneumoniae* ATCC49619: erythromycin-susceptible strain.
[c] *S. aureus* ATCC29213: methicillin-resistant strain.
[d] *S. pyogenes* S2: erythromycin-susceptible strain isolated clinically.
[e] *S. pyogenes* R2: erythromycin-resistant strain isolated clinically.
[f] *S. pneumoniae* B1: erythromycin-resistant strain encoded by the *erm*B gene.
[g] *S. pneumoniae* A22072: erythromycin-resistant strain encoded by the *mef*A gene.
[h] *S. pneumoniae* AB11: erythromycin-resistant strain encoded by the *erm*B and *mef*A genes.

target compounds except compound **9f** exhibited the MIC values of <0.002 μg/mL. Besides, compounds **9g**–**k** exhibited not only 16–500-fold, 8–16-fold, >30-fold better activity than their precursors **8c**, **8j**–**k**, **8h** and **8l** against *S. aureus* ATCC25923, *S. pneumoniae* ATCC49619 and *S. pyogenes* S2, but also presented 1024–2133-fold, 32–133-fold and 1024–2048-fold improved activity compared with the references against erythromycin-resistant *S. pneumoniae* expressing the *erm* gene, the *mef* gene, and the *erm* and *mef* genes. Among them, compounds **9j** (0.008 μg/mL) and **9b** (0.016 μg/mL) exhibited the most potent activity better than other 11,4″-di-*O*-arylalkylcarbamoyl azithromycin analogs in this series against erythromycin-susceptible *S. aureus* ATCC25923, and compounds **9h** and **9i** were the most effective (0.06 μg/mL) against erythromycin-susceptible *S. pneumoniae* ATCC49619. Besides, compound **9j** displayed greatly improved activity against erythromycin-resistant *S. pyogenes* R2 with the MIC value of 1 μg/mL, showing 128-fold improved activity better than CAM or AZM. In addition, almost all of the target compounds **9a**–**k** showed improved activity against erythromycin-resistant *S. pneumoniae* encoded by the *erm* gene, the *mef* gene, and the *erm* and *mef* genes in comparison with their precursors **8c**, **8e**, **8h**, **8l** and **8j**–**k**. These results clearly indicated that introduction of an arylalkyl group into the C-11 position of the 4″-*O*-(1-aralkyl-1,2,3-triazol-4-methyl-carbamoyl) azithromycin analogs dramatically enhanced their antibacterial activity against erythromycin-resistant bacteria. Among them, compounds **9g**, **9h**, **9j** and **9k** displayed favorable activity against all the three phenotypes of resistant *S. pneumoniae*. Particularly, compound **9k** with the terminal 4-methoxybenzyl group on its C-11 side chain was the most effective (0.06, 0.03 and 0.125 μg/mL) against erythromycin-resistant *S. pneumoniae* B1, *S. pneumoniae* A22072 and *S. pneumoniae* AB11, exhibiting 2133, 133 and 2048-fold enhanced activity better than AZM, respectively. Moreover, 11,4″-di-*O*-arylalkylcarbamoyl azithromycin analogs (**9a**–**k**) exhibited better activity against *E. coli* ATCC25922 and *P. aeruginosa* ATCC27853 than their precursors, which suggested that introduction of an arylalkyl group into the 11-position of 4″-*O*-arylalkylcarbamoyl azithromycin analogs might enhance their antibacterial activity against Gram-negative organisms.

From the data mentioned above we can summarized their SARs as follows. Introduction of the novel arylalkylcarbamoyl side chains containing 1,2,3-triazol group into the C-4″ position of AZM could greatly improve activity against erythromycin-resistant *S. pneumoniae* compared with AZM, which suggested that prolonged anchor group at the C-4″ position might increase the affinity for the new binding sites of the nucleotides in the peptide tunnel, further enhancing antibacterial activity against resistant strains. However, introduction of 11,12-cyclic carbonate ring into the C-4″ modified azithromycin analogs (**4a**–**j**) was not beneficial for retaining or enhancing antibacterial activity, which might be related to the absence of the 11-hydroxyl group as a hydrogen bond donor. In remarkable contrast, introduction of the arylalkylcarbamoyl side chains into the C-11 position of the C-4″ modified azithromycin analogs (**4a**–**j**) showed significantly improved activity against erythromycin-resistant *S. pneumoniae* in comparison with the C-4″ modified azithromycin analogs (**4a**–**j**). This led us

to presume that the C-11 arylalkyl side chains could interact with a new binding site of nucleotide in domain II of the 23S rRNA, resulting in tighter binding to the ribosomes in macrolide-resistant bacteria. Additionally, in the series of the 11,4″-disubstituted azithromycin analogs (**9a**–**k**) with the most effective and balanced activity against susceptible and resistant bacteria, the compounds bearing the same C-4″ side chain showed significantly different antibacterial activity, for example, 11-*O*-4-methoxybenzyl analogs (**9g**, **9h** and **9j**) had much better activity than 11-*O*-benzyl analogs (**9a**, **9c** and **9d**), which indicated that the terminal 11-*O*-4-methoxybenzyl group might easily interact with the secondary ribosomal binding site A752 through hydrogen bonding, π-stacking or van der Waals (VDW) interaction, thereby further enhancing affinity for the ribosomes.

On the whole, although three novel series of azithromycin analogs had different modification sites, almost all of the compounds displayed excellent activity against the susceptible *S. pyogenes* and improved activity compared with AZM and CAM against all the three phenotypes of resistant *S. pneumoniae*. In the series of 4″-*O*-(1-aralkyl-1,2,3-triazol-4-methyl-carbamoyl) azithromycin analogs (**4a**–**j**), almost all of the target compounds retained excellent activity against erythromycin-susceptible *S. pyogenes* S2 and showed improved activity against erythromycin-resistant *S. pyogenes* and *S. pneumoniae*. These results suggested that introduction of the 1-aralkyl-1,2,3-triazol-4-methyl-carbamoyl group into the 4″-position of 15-membered macrolides was beneficial for retaining or enhancing activity of compounds against some erythromycin-susceptible and erythromycin-resistant strains. Compared with the parent 4″-*O*-(1-aralkyl-1,2,3-triazol-4-methyl-carbamoyl) azithromycin analogs, most of the 11,12-cyclic carbonate-4″-*O*-(1-aralkyl-1,2,3-triazol-4-methyl-carbamoyl) azithromycin analogs (**8a**–**o**) showed compromised activity against all the tested strains. Furthermore, 11,4″-di-*O*-arylalkylcarbamoyl azithromycin analogs (**9a**–**k**) showed greatly improved activity against all the tested strains in comparison with their precursors 11,12-cyclic carbonate-4″-*O*-(1-aralkyl-1,2,3-triazol-4-methyl-carbamoyl) azithromycin analogs. This suggested that introduction of an arylalkyl group into the C-11 position of the 4″-*O*-(1-aralkyl-1,2,3-triazol-4-methyl-carbamoyl) azithromycin analogs dramatically enhanced their antibacterial activity against some susceptible or resistant strains. Among three series of azithromycin analogs, the novel series of 11,4″-di-*O*-arylalkylcarbamoyl azithromycin analogs **9a**–**k** exhibited potent and balanced activity against susceptible and resistant bacteria. In particular, compounds **9g**, **9h**, **9j** and **9k** showed favorable activity against susceptible bacteria and resistant bacteria.

In summary, three novel structural series of 4″-carbamate, 11,12-cyclic carbonate-4″-carbamate and 11,4″-di-*O*-arylcarbamoyl analogs of azithromycin were designed, synthesized and evaluated for their *in vitro* antibacterial activity against various phenotypes of Gram-positive and Gram-negative bacterial species. A majority of the target compounds showed potent activity against erythromycin-susceptible and erythromycin-resistant *S. pyogenes* comparable to CAM and AZM, while all the target compounds exhibited significantly improved activity compared with the references against the three phenotypes of resistant *S. pneumoniae*. Among three series of azithromycin analogs, the novel series of 11,4″-di-*O*-arylalkylcarbamoyl azithromycin analogs **9a**–**k** exhibited the most effective and balanced activity against susceptible and resistant bacteria. Among them, compound **9j** exhibited the most significantly enhanced activity against *S. aureus* ATCC25923 (0.008 μg/mL), *S. pyogenes* R2 (1 μg/mL), showing 31-fold and 128-fold better activity than AZM, respectively. Moreover, all the azithromycin analogs **9g**–**k** except **9f** shared the identical activity with the MIC value <0.002 μg/mL against *S. pyogenes* S2. In addition, compounds **9g**, **9h**, **9j** and **9k** displayed significantly improved activity compared with the references against all the three phenotypes of resistant *S. pneumoniae*. Particularly, compound **9k** was the most effective (0.06, 0.03 and 0.125 μg/mL) against erythromycin-resistant *S. pneumoniae* B1, *S. pneumoniae* A22072 and *S. pneumoniae* AB11, exhibiting 2133, 133 and 2048-fold more potent activity than AZM, respectively. More importantly, the results suggested that the modification of both C-11 and 4″-*O* positions of azithromycin could dramatically enhance the antibacterial activity.

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A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2017.06.044.

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