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Synthesis and antibacterial activity of novel 4″-*O*-arylalkylcarbamoyl and 4″-*O*-((arylalkylamino)-4-oxo-butyl)carbamoyl clarithromycin derivatives

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ABSTRACT

Novel series of novel 4″-*O*-arylalkylcarbamoyl and 4″-*O*-((arylalkylamino)-4-oxo-butyl)carbamoyl clarithromycin derivatives were designed, synthesized and evaluated for their in vitro antibacterial activities. These derivatives retained excellent activity against the erythromycin-susceptible strains and showed significantly improved activity against all of the tested erythromycin-resistant strains. Among them, compound **4c** was the most effective (0.06 μg/mL) against *Streptococcus pneumonia* encoded by the *erm* gene and compound **4a** was had the most potent activity (0.25 μg/mL) against *S. pneumonia* encoded by the *erm* and *mef* genes.

Second-generation macrolides such as clarithromycin[1] (CAM) (Fig. 1) and azithromycin[2] (AZM) have been widely used for the treatment of upper and lower respiratory tract infections, as well as genital infections due to their superior antibacterial activity and pharmacokinetic properties, and fewer gastrointestinal side effects compared with first-generation macrolide erythromycin (EMA). Their mechanism of action has been elucidated that these macrolides reversibly bind to the nucleotide A2058 in domain V of the 23S rRNA in the ribosomal 50S subunit and block protein synthesis.[3] However, the therapeutic utility of macrolides has been severely compromised by the emergence of widespread bacterial resistance which become a serious medical problem worldwide.[4] The most important mechanism of macrolide resistance are mediated by *erm*-encoded methylation of 23S rRNA. Expression of an *erm*-resistant determinant in bacteria results in production of a methyltransferase which modifies the key nucleotide A2058, thereby conferring resistance to macrolides.[5]

To address the problems of the bacterial resistance, third-generation macrolides known as ketolides exemplified by telithromycin and cethromycin have been investigated. The ketolides are characterized by excellent activity against several types of macrolide-resistant organisms and may offer alternative therapy for Gram-positive infections attributable to resistant pathogens.[6] Their mechanism of action has been reported to interact with a secondary ribosomal binding site A752 directly in domain II of the 23S rRNA by the C-11,12 carbamate side chain or the C-6 side chain in the ketolides in addition to the main interaction of the drugs in domain V, which leads to tighter binding to bacterial ribosomes and imparts some activity against *erm*-resistant organisms.[7,8] The tighter binding also inhibits the *mef*-mediated mechanism by creating an influx rate of the ketolide that exceeds its efflux rate.

While significant efforts have gone into the discovery of increasingly potent ketolides, a substantial amount work has also been carried out on the next-generation macrolides to effectively cope with bacterial resistance. These investigations have led to the discovery of C-4″-modified macrolides[9–13] such as A-66332[9] and CP-544372[10] (Fig. 1). In particular, CP-544372 containing a long anchor group at the C-4″ position of 9(*S*)-erythromycylamine, exhibits excellent in vitro and in vivo activity against macrolide-resistant strains encoded by the *erm* gene with competitive binding to chloramphenicol, suggesting that the anchor group can reach the chloramphenicol-binding sites in the peptide tunnel.[14] Its long anchor group is six atom distances from 4″-oxygen atom to the benzene ring. Therefore, the C-4″-modified macrolides display a higher affinity for the ribosomes of resistant bacteria, which is due to the additional interaction mediated by the C-4″ side chain.

The study of high-resolution X-ray co-crystal structures has revealed that the binding sites of clarithromycin, clindamycin and chloramphenicol differ from each other, but they show some overlapping nucleotides in the peptide tunnel.[15] Particularly, the high nucleotide content in the peptide tunnel gives rise to a number

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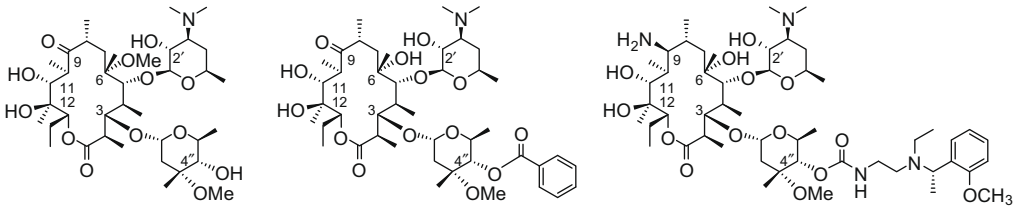


Figure 1. Structures of clarithromycin, A-66332 and CP-544372.

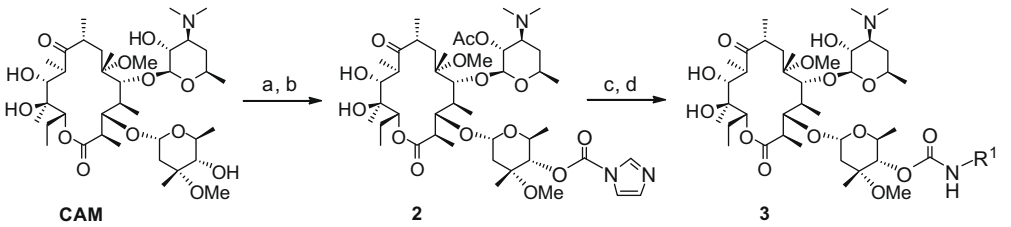
of possible interactions such as hydrogen bonding, π-stacking as well as electrostatic interactions. Therefore, a reasonably long group at the C-4″ position is helpful for the interaction with the nucleotides in the peptide tunnel. To probe the effect of different lengths of C-4″ side chains in antibacterial activity, we designed novel structural series of C-4″-modified clarithromycins with C-4″-prolonged side chains from 4″-oxygen atom to aromatic ring is three, four, eight and nine atom distances, respectively.

The novel series of C-4″-modified clarithromycins were designed and synthesized from CAM as a starting material. Acetylation of the 2′-hydroxyl group of CAM with acetic anhydride (Ac_2O) was followed by transformation of the 4″-hydroxyl group to the acyl imidazole utilizing 1,1′-carbonyldiimidazole (CDI) and triethylamine (Et_3N) to give 4″-*O*-acylimidazolide (**2**). These reactions proceeded very smoothly at room temperature in 79% yield. The 4″-*O*-arylalkylcarbamoyl clarithromycin derivatives (**3a**–**g**) were obtained in yields ranging from 62% to 67% by condensation of **2** with the corresponding arylalkylamines in the presence of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) at 60 °C, followed by selective removal of the 2′-*O*-acetyl group by heating with methanol (Scheme 1). Similarly, the 4″-*O*-((arylalkylamino)-4-oxo-butyl)carbamoyl clarithromycin derivatives (**4a**–**e**) were prepared by condensation of **2** with the corresponding arylalkylcarbamoyl butylamines in the presence of DBU at room temperature and subsequent methanolysis at 55 °C (Scheme 2).The yields were within the range of 69–76%.

The two series of C-4″-modified clarithromycins **3a**–**g** and **4a**–**e** prepared above, as well as EMA, CAM and AZM as references, were tested for in vitro antibacterial activity against four phenotypes of Gram-positive strains. The activities are reported in Table 1 as minimum inhibitory concentrations (MICs) determined using the broth microdilution method. *Streptococcus pneumonia* ATCC49619 are an erythromycin-susceptible strain, and *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.

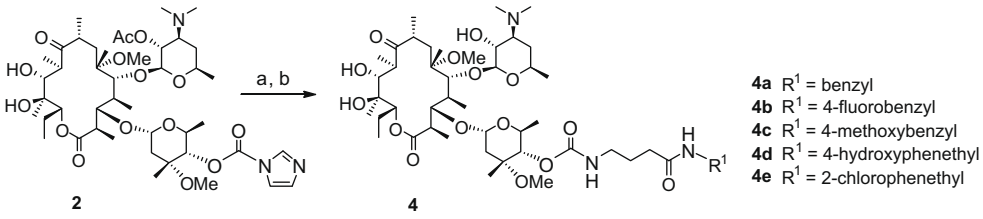
The two series of C-4″-modified clarithromycins showed excellent activity against erythromycin-susceptible *S. pneumonia* ATCC49619. Among them, compounds **3c**, **3d** and **4a**–**e** were found to be the most potent activity (MIC 0.03 μg/mL) comparable to those of EMA, CAM and AZM. As for the activity against the erythromycin-resistant *S. pneumonia*, most of the series **3** showed improved activity in comparison with the references. Among them, compounds **3a**, **3c** and **3g** were the most effective (0.25 μg/mL) against *S. pneumoniae* A22072 encoded by the *mef* gene, and compounds **3a** and **3b** displayed greatly improved activity against *S. pneumoniae* encoded by the *erm* gene or the *erm* and *mef* genes, showing 16-fold and 16-fold higher activity than the parent CAM, respectively. In contrast, the series **4** had much better activity than the series **3** against *S. pneumoniae* encoded by the *erm* gene or the *erm* and *mef* genes, but the both series showed similar activity against *S. pneumoniae* encoded by the *mef* gene. Among all of the tested series, compound **4c** was the most effective (0.06 μg/mL) against *S. pneumoniae* B1encoded by the *erm* gene, showing 267-fold higher activity than the corresponding **3c**, and compound **4a** had the most potent activity (0.25 μg/mL) against *S. pneumoniae* AB11encoded by the *erm* and *mef* genes, showing 32-fold better activity than the corresponding **3a**.

The results described above suggested that introduction of the arylalkyl group at the C-4″ position of CAM not only retains good activity against the erythromycin-susceptible strains, but also shows remarkably improved activity against all of the tested erythromycin-resistant *S. pneumonia*. In particular, introduction of the prolonged 4″-*O*-arylalkyl group further increase activity against *S. pneumoniae* encoded by the *erm* gene, and the *erm* and *mef* genes. The prolonged 4″-*O*-arylalkyl group with eight to nine atom distances from 4″-oxygen atom to aromatic ring might reach the



3a R^1 = benzyl
3b R^1 = 4-fluorobenzyl
3c R^1 = 4-methoxybenzyl
3d R^1 = 4-hydroxyphenethyl
3e R^1 = 2-chlorophenethyl
3f R^1 = phenethyl
3g R^1 = 3,4-methylenedioxyphenethyl

Scheme 1. Reagents and conditions: (a) Ac_2O, Et_3N, CH_2Cl_2, rt, 24 h, 85%; (b) CDI, CH_2Cl_2, rt, 24 h, 93%; (c) R^1NH_2, DBU, DMF, 60 °C, 7 h; (d) CH_3OH, 45 °C, 12 h, 62–67% for two steps.



Scheme 2. Reagents and conditions: (a) corresponding amine hydrochloride, DBU, DMF, rt, 24 h; (b) CH_3OH, 55 °C, 24 h, 69–76% for two steps.

Table 1
In vitro antibacterial activity of 4″-*O*-arylalkylcarbamoyl and 4″-*O*-((arylalkylamino)-4-oxo-butyl)carbamoyl clarithromycin derivatives

Strains/compounds	MICs (μg/mL)														
	3a	**3b**	**3c**	**3d**	**3e**	**3f**	**3g**	**4a**	**4b**	**4c**	**4d**	**4e**	EMA	CAM	AZM
S. pneumoniae ATCC49619[a]	0.25	0.06	0.03	0.03	0.12	0.06	0.06	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03
S. pneumoniae B1[b]	4	4	16	16	128	16	32	2	1	0.06	0.25	32	128	64	128
S. pneumoniae A22072[c]	0.25	0.5	0.25	1	4	0.5	0.25	0.5	0.25	0.25	0.5	1	8	4	4
S. pneumoniae AB11[d]	8	8	32	64	128	32	64	0.25	2	1	1	32	256	128	256

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

chloramphenicol-binding sites and interact with them, resulting in a higher affinity to the resistant ribosome.

In conclusion, two series of novel 4″-*O*-arylalkylcarbamoyl and 4″-*O*-((arylalkylamino)-4-oxo-butyl)carbamoyl clarithromycin derivatives were designed, synthesized and evaluated for their in vitro antibacterial activities. These derivatives retained excellent activity against the erythromycin-susceptible strains and showed significantly improved activity against the erythromycin-resistant strains. Especially, the series **4** displayed much higher activity than the series **3** against *S. pneumoniae* encoded by the *erm* gene or the *erm* and *mef* genes. Among all of the tested series, compound **4c** was the most effective against *S. pneumoniae* encoded by the *erm* gene, and compound **4a** had the most potent activity against *S. pneumoniae* encoded by the *erm* and *mef* genes. It is noteworthy that both compounds have a prolonged 4″-*O*-arylalkyl group with eight to nine atom distances from 4″-oxygen atom to aromatic ring.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.04.051.

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