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Synthesis and antibacterial activity of 4″-*O*-heteroarylcarbamoyl derivatives of macrolide

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ABSTRACT

A series of novel 4″-position modified macrolide derivatives has been synthesized via a facile procedure. Their in vitro antibacterial activities against constitutively erythromycin-resistant strains were evaluated. Among the derivatives tested, compound **8a** which has 11,12-carbamate and 4″-*O*-heteroarylcarbamoyl groups was found to have potent activity against most resistant bacteria.

The mechanism of macrolides binding to the ribosome RNA of bacteria has been getting clearer since the last few years of 20th century. The high-resolution X-ray cocrystal structures of the bacterial ribosome with macrolides have revealed the detailed interaction at atomic level.[1a–d] It demonstrated that the macrolides inhibit bacterial protein synthesis by sterically blocking the passage of nascent polypeptides through the exit tunnel of the ribosome.[2]

The second-generation macrolide antibiotics, such as clarithromycin and azithromycin, have been widely prescribed for upper and lower respiratory tract infection because of their superior antibacterial activity. However, the therapeutic utility of these macrolides has been severely compromised by the emergence of resistant pathogens.[3] Two of the most important mechanisms of macrolide resistance were ribosomal mutation (*erm*) and efflux (*mef*).[4] A great effort has been making to discover novel macrolides to address these status.[5] For example, Telithromycin[6] and Cethromycin,[7] known as ketolide, have been investigated. Telithromycin possesses a 3-keto group and a 11,12-carbamate functionality. In addition, it has a proper aromatic side chain which can interact with nucleotide A752 in domain II of the 23S rRNA. This interaction makes telithromycin show strong activity against major macrolide-resistant strains.[8] It is worthy of notice that ketolide is not the only class of new macrolides for the effective management of macrolide resistance. Many derivatives of nonketolide families have been synthesized by different research groups.[9]

The study of high-resolution X-ray cocrystal structures shows that cladinose group of erythromycin is located at and fits with the cavity formed by G2505, C2610 and C2611 in domain V of the erythromycin binding site.[1a] It has been reported[10] that the induction of methylase in macrolide-resistant bacteria could be dissociated from inhibition of the bacteria by using erythromycin analogs with modification at the 4″-position of the cladinose sugar. Some papers reported[11,12] that introduction of certain groups in 4″-position of the cladinose sugar resulted in decreased inducer activity but had a negligible effect on antibacterial activity. 4″-Modification can alter the relative potency an antibiotic has for induction and inhibition. In these researches, the derivatives modified at 4″-position were obtained, for example, A-60565[10] and CP-544372[11] (Fig. 1). The compound CP-544372, introduction of [2-N-substituted aminoethyl] amino carbonyl chain to 4″-hydroxyl group of cladinose, was identified effective against macrolide-lincosamide-streptogramin B (MLS_B) resistant strains.

According to the result of X-ray cocrystal structure study, Takashima, H. suggested[1a] that the prolonged 4″-aryl alkyl group of CP-544372 reached the chloramphenicol binding site (the peptidyl transferase region) and inhibited peptide formation of bacterial ribosomes. This perspective is very informative for structure-based drug design.[13] We focused on the 4″-position of the cladinosyl moiety as the target. In the case of CP-544372, the length of phenyl side chain was six atoms distance from 4″-*O*. In our work, four aryl and heteroaryl substituted primary amines were chosen (Fig. 2). The length of the 4″-side chains is just six atoms distance from 4″-*O* to aromatic ring. Since VDW interaction and aromatic stacking may increase binding affinity, the aromatic moiety chosen here

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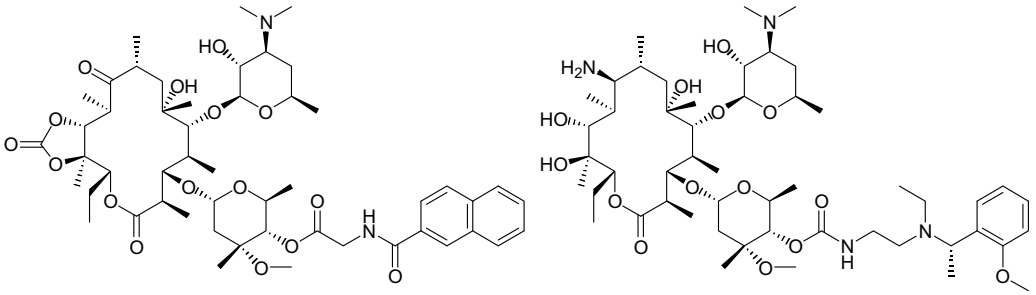


Figure 1. Structures of A-60565 and CP-544372.

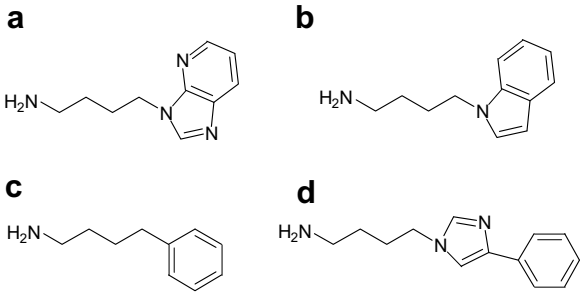
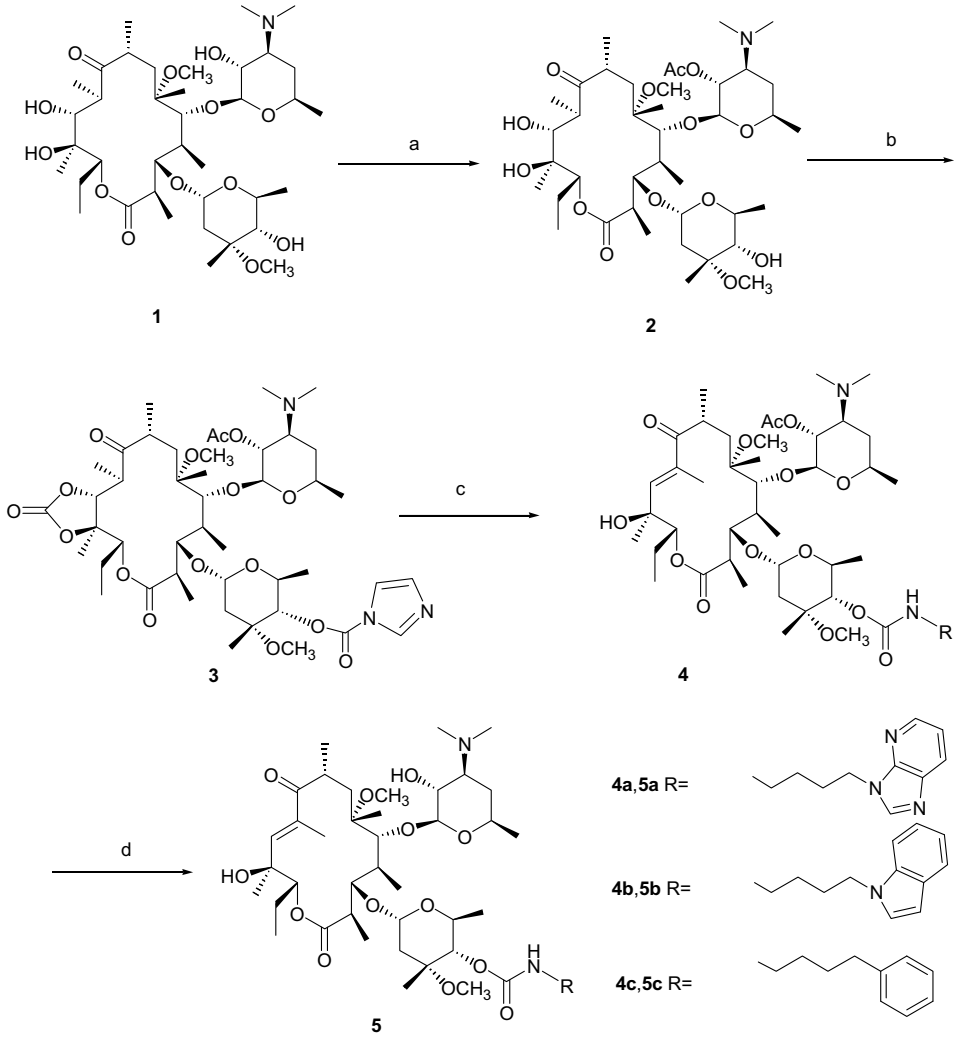


Figure 2. Structures of aryl alkyl side chains.

are phenyl, diaryl or fused bicyclic aryl group with mono, di and tri nitrogen atoms in the heteroaryl systems. These structures have different bulk of molecular and different distribution of hetero atoms. Some of these long anchor groups were proved having significant effect for the antibacterial activity in ketolides.[14]

By substituting of 4″-position with various aryl alkyl side chains, a series of novel 14-membered macrolide derivatives was obtained. The further modification at 11-*N*,12-*O*-carbamate sub-structure was carried out. We hoped that a combination of 4″-*O*-heteroarylcarbamoyl group and 11-*N*,12-*O*-cyclic carbamate anchor group might enhance the antibacterial activity against resistant strains.



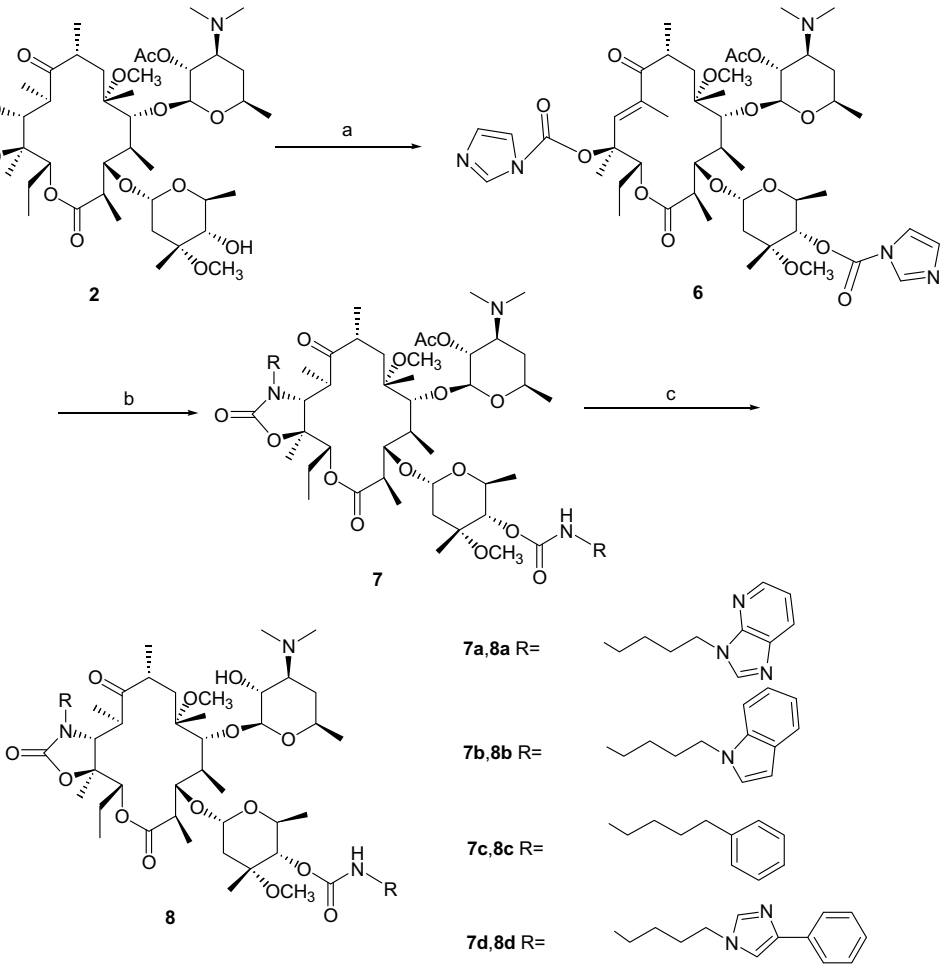
Scheme 1. Reagents and conditions: (a) Et_3N, acetic anhydride, CH_2Cl_2, 5 h, 0 °C, 64%; (b) CDI, NaH, DMF, 0 °C, 1 h, 89%; (c) DBU, RNH_2, DMF, 10 h, 30–36%; (d) MeOH, reflux, 78–85%.

Our approach to get 4″-substituted derivatives is to regioselectively protect 2′-OH of clarithromycin 1 and to modify the 4″-OH. 2′-*O*-Acetyl clarithromycin 2 provided a convenient starting material for the chemical modification leading to the 4″-substituted derivatives.

By using the method of Baker et al.,[6a] **2** was treated with excess 1,1′-carbonyldiimidazole (CDI) and sodium hydride in DMF at 0 °C for 1 h. 11,12-Carbonate 4″-*O*-acylimidazolide **3** was obtained in a yield of 89%. The structure of **3** was confirmed by ^{13}C NMR spectrum in which two carbon peaks of carbonate and carbamate could be found at δ 154.0 and δ 148.5. Compound **4**(**a**–**c**), a series of desired compounds, was prepared by reacting compound **3** with primary arylamines and 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU). Deprotection of the 2′-*O*-acetates **4**(**a**–**c**) in refluxing methanol provided compounds **5**(**a**–**c**) in yields ranging from 78% to 85%. The structures of **5**(**a**–**c**) were determined by ^{13}C NMR and HRMS spectra (Scheme 1).

Compound **2** could be further elaborated to a series of compounds modified at both 4″-position and 11-*N*,12-*O*-cyclic carbamate sub-structure. Agouridas et al. synthesized ketolides with introduction of acylimidazoles by using CDI and sodium hydride.[6b] The desired 11,12-cyclic carbamate ketolides were synthesized by reacted 12-*O*-acylimidazolyl ketolide in acetonitrile with the appropriate amines.[6c] In our case, **2** was treated with excess CDI and sodium hydride in DMF at 25 °C for 10 h. 12-*O*,4″-*O*-Diacylimidazolide **6** was obtained in a yield of 92%. Compound **6** was converted without purification to designed compounds **7**(**a**–**d**) by treatment with various primary arylamines in presence of DBU. ^{13}C NMR study suggested that **7**(**a**–**d**) were got because two carbon peaks of carbamates exist (at about δ 157 and δ 156). Deprotection of the 2′-*O*-acetate compounds **7**(**a**–**d**) in refluxing methanol provided the target compounds **8**(**a**–**d**) in yields ranging from 70% to 80% (Scheme 2). The structures of **8**(**a**–**d**) were confirmed by ^{13}C NMR, ^{1}H NMR and HRMS spectra.

The newly prepared macrolide derivatives, **5a**, **5b**, **8a**, **8b**, and **8d**, together with clarithromycin and azithromycin as references, were tested against a panel of representative respiratory tract pathogens (Table 1). The strains are *Staphylococcus aureus* ATCC29213, 01-430, 01-431, and 01-481 (methicillin-sensitivity staphylococcus aureus (MSSA)), *S. aureus* 01-433, 01-429, 01-483 (methicillin-resistant staphylococcus aureus (MRSA)), *Streptococcus pneumoniae* ATCC49619, 04-050 (erythromycin-susceptible strain (Ery-S)), *S. pneumoniae* 03-436, 03-437, 03-554 (erythromycin-resistance strain (Ery-R)), *Streptococcus pyogenes* 01-469, 03-804, 03-740 (Ery-S), *S. pyogenes* 03-474, 03-475, 03-476, 01-781 (Ery-R), *S. pyogenes* 03-480 (Ery-R encoded by *ermB* gene), *Enterococcus faecalis* 03H065, 03I076 (Ery-S), *E. faecalis* 03A056, 03A080, 03A133 (Ery-R). All the MRSA and Ery-R strains chosen in this test are constitutively resistant strains[15] supplied by the Ministry of Health National Antimicrobial Resistance Investigation Net (MOHNARIN, China). The in vitro antibacterial activity was reported as minimum inhibitory concentrations (MICs), which was determined by the broth microdilution method as recommended by the NCCLS.[16]



Scheme 2. Reagents and conditions: (a) CDI, NaH, DMF, 0 °C, 10 h, 92%; (b) DBU, RNH_2, DMF, 10 h, 20–62%; (c) MeOH, reflux, 70–80%.

Table 1
Antibacterial activity of **5**(**a** and **b**), **8**(**a**,**b**, and **d**)

Pathogens	**5a**	**5b**	**8a**	**8b**	**8d**	Clar	Azi
ATCC 29213	16	16	1	16	4	0.25	1
01-430 MSSA	8	8	1	16	4	0.25	0.5
01-431 MSSA	16	16	1	16	4	0.25	1
01-481 MSSA	16	16	1	16	4	0.25	1
01-433 MRSA	16	16	16	16	16	>256	>256
01-429 MRSA	16	16	16	16	16	>256	>256
01-483 MRSA	16	16	16	16	16	>256	>256
S. pneumoniae							
ATCC 49619	2	0.5	0.125	16	0.25	0.031	0.025
S. pneumoniae							
04-050 (Ery-S)	0.062	0.031	0.062	16	0.25	0.031	0.25
03-436 (Ery-R)	2	8	0.25	16	0.25	128	>256
03-437 (Ery-R)	2	8	0.125	16	0.125	64	256
03-554 (Ery-R)	8	8	0.25	16	0.25	128	256
S. pyogenes							
01-469 (Ery-S)	0.5	0.5	0.125	2	0.5	0.016	0.062
03-804 (Ery-S)	0.5	1	0.125	2	0.5	0.031	0.125
03-740 (Ery-S)	0.5	1	0.125	2	0.5	0.031	0.125
03-474 (Ery-R)	16	16	0.5	8	2	256	>256
03-475 (Ery-R)	16	16	0.5	8	2	256	>256
03-476 (Ery-R)	16	16	0.5	8	2	256	>256
03-480 (Ery-R)	16	16	0.5	8	2	256	>256
01-781 (Ery-R)	16	16	0.5	8	2	256	>256
E. faecalis							
03H065 (Ery-S)	2	4	0.5	4	1	0.125	0.25
03I076 (Ery-S)	2	4	0.5	4	1	0.125	0.25
03A056 (Ery-R)	16	16	16	16	16	>256	>256
03A080 (Ery-R)	16	16	16	16	16	>256	>256
03A133 (Ery-R)	16	16	16	16	16	>256	>256

The tabulated results exhibited that clarithromycin and azithromycin were potent against erythromycin-susceptible strains and inactive against all the resistant strains.

Compounds **5a** and **5b** showed improved activity against all the erythromycin-resistant strains, for example *S. pneumoniae* 03-436, 03-437, and 03-554; *S. pyogenes* 03-474, 03-475, 03-476, and 01-781; as well as *S. pyogenes* 03-480 strain encoded by *ermB* gene. The active against erythromycin-susceptible strains of **5a** and **5b** decreased in a certain extent.

With the presence of anchor groups at both 4″-position and 11-*N*,12-*O*-carbamate of macrolide lactone ring, **8a** and **8d** were active against erythromycin-resistant strains. **8d** showed a greater improvement against erythromycin-resistant strains than **5a and 5b**. But the activity against erythromycin-susceptible strains of **8d** was less than that of the references.

The most active compound **8a** was obtained when the fused bicyclic aryl alkyl side chain (4-(3H-imidazo[4,5-*b*]pyridin-3-yl)-butyl)amine was appended to the macrolide core. The compound **8a** demonstrated more than 500-fold better active than clarithromycin and azithromycin against resistant *S. pneumoniae* strains and resistant *S. pyogenes* strains. To the resistant *S. pyogenes* 03-480 strain encoded by *ermB* gene, **8a** showed also potent activity. Meanwhile, **8a** retained almost the same activity against erythromycin-susceptible strains comparable to one of the references, azithromycin. We believe that the potent activity achieved by **8a** against resistant strains was a result of the secondary interaction between the 11,12-anchor group and the nucleotide A752 in domain II of 23S rRNA.

Compound **8b** was less active against both erythromycin-susceptible strains and erythromycin-resistant strains compared to **8a** and **8d**. The contrast of the antibacterial activity between **8a** and **8b** showed that the difference was caused not only by the position of the aryl alkyl side chains on the macrolide core, but also by the structure of the side chain.

Typical of 4″-substituted macrolides, the compounds **5a, 5b, 8a, 8b, and 8d** showed improved activity against MRSA 01-433, 01-429, and 01-483. The active against MSSA of the 4″-substituted macrolides were, except **8a**, markedly decrease. Compared to azithromycin, **8a** still remained the activity against MSSA 01-430, 01-431, and 01-481. It is worthy of note that all the compounds in this paper showed mild activity against MRSA tested. This seems a result of modification on 4″-position. With increase in MRSA infections worldwide, and expanding an existing antibacterial activity is noteworthy. This work may provide significant opportunities for the design of new macrolide antibiotics having potent activity against MRSA.

For *E. faecalis* strains, the activity of the tested compounds appeared weaker than that of the references against Ery-S strains. 4″-Modified macrolides showed modest activity against Ery-R strains.

As a highlight, all the target compounds could be obtained through few steps procedure from clarithromycin. The improved antibacterial activity against resistant bacteria achieved by these derivatives is the result of the possible interaction of the aryl alkyl group of 4″-position with ribosome RNA bases in the exit tunnel by aromatic stacking or VDW interaction. These studies present a considerable opportunity for the development of new macrolide antibiotics to combat the growing problems of MLS-resistance.

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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.2008.09.022.

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