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Synthesis and antibacterial activity of 4″,11-di-*O*-arylalkylcarbamoyl azithromycin derivatives

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ABSTRACT

A series of new 4″,11-di-*O*-arylalkylcarbamoyl azithromycin derivatives were designed, synthesized and evaluated for their in vitro antibacterial activities. Some derivatives exhibited greatly improved activity against erythromycin-resistant bacteria. Among them, compounds **5f** and **5k** were found to have potent activity against erythromycin-resistant *Streptococcus pneumoniae* whose resistance was encoded by the *erm* or *mef* gene.

The first macrolide, erythromycin A, demonstrated a broad-spectrum of antimicrobial activity and was used primarily for respiratory, skin and soft tissue infections. Newer 14-and 15-membered macrolides such as clarithromycin (CLA) and azithromycin (AZI) (Fig. 1), have been developed to address the limitations of erythromycin.[1] Unfortunately, like erythromycin A, both AZI and CLA show poor activity against macrolide-resistant bacteria.[2] The most common mechanisms of resistance are mediated by *erm*-encoded methylation of 23S rRNA or *mef*-encoded efflux. Expression of an *erm* resistance determinant in bacteria leads to the production of a methyltransferase which modifies the key nucleotide, A2058, in the MLS_B (macrolide-lincosamide-streptogramin B) binding site and thereby confers resistance to these macrolides.[3] The ketolides, as exemplified by telithromycin and cethromycin, may offer alternative therapy for Gram-positive infections attributable to resistant pathogens.[4] Their mechanism of action is that the C-11, 12 carbamate side chain or C-6 side chain in the ketolides interacts with nucleotide A752 directly in domain II of the 23S rRNA and inhibits protein synthesis by blocking elongation.[5]

While significant efforts have gone into the discovery of increasingly potent ketolides, a substantial amount work has also been carried out on novel macrolides. These investigations have lead to the discovery of 11-modified and 4″-carbamate macrolides.[2] 11-*O*-substituented clarithromycin derivatives such as EP-1553 (Fig. 1), exhibited excellent activity against macrolide-resistant*Staphylococcus aureus, Streptococcus pneumoniae* and *Streptococcus pyogenes*.[6] Especially, one of the leading 4″-carbamate macrolides, CP-544372 (Fig. 1), demonstrated good in vitro and in vivo activity against macrolide-susceptible and macrolide-resistant organisms.

AZI, the first 15-membered macrolide, has been widely prescribed for the treatment of respiratory tract infections owing to its high efficacy and ideal pharmacokinetic property, but it is inactive against resistant bacteria. The skeleton of AZI is also very similar to that of CLA, except that the lactone ring is expanded around the 9-position.[7] However, there have been few modifications of 15-membered macrolides. To obtain more potent macrolides against resistant strains, a series of new 15-membered macrolide analogues of AZI, including modified products to the hydroxyl group at C-4″ and the diol at C-11 and C-12, were designed and synthesized in this paper.

By substitution of the 4″-position with various arylalkyl side chains, novel 4″-substituted azithromycin derivatives were obtained. The modification at the 11-position was carried out by introducing an arylalkyl or alkyl chain. The combined modification at the 4″-*O*-arylalkylcarbamoyl group and the 11-*O*-arylalkylcarbamoyl moiety was also performed to obtain new derivatives. On the basis of report[8] that 11,12-cyclic carbonate azithromycin was treated with corresponding amine in the presence of pyridine hydrochloride or 1-methyl-1*H*-imidazole to provide 11-carbamate of azithromycin, novel 4″,11-di-*O*-arylcarbamoyl azithromycin derivatives were prepared from 11,12-cyclic carbonate azithromycin 4″-carbamates.

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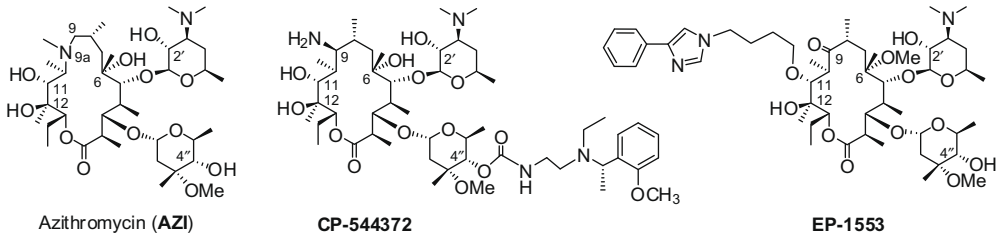
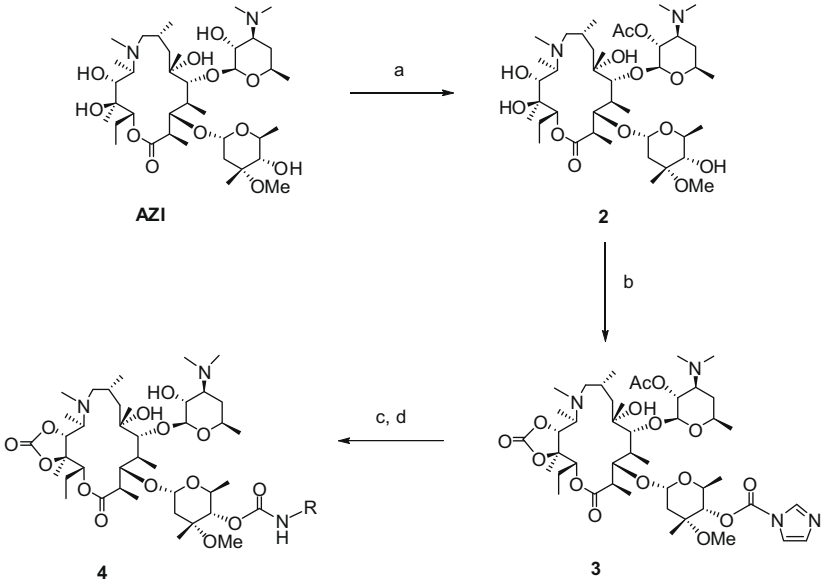
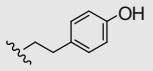
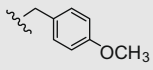
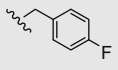
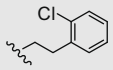


Figure 1. Structures of azithromycin, CP-544372 and EP-1553.



Scheme 1. Synthesis of **4** as precursors of the 4″,11-di-*O*-arylcarbamoyl azithromycin derivatives. Reagents and conditions: (a) acetic anhydride, CH_2Cl_2, Et_3N, rt, 12 h, 92%; (b) CDI, Et_3N, toluene, 75 °C, 24 h, 96%; (c) RNH_2, DMF, DBU, rt,10 h; (d) CH_3OH, 55 °C, 24 h, 73–80% for 2 steps.

Table 1
Minimum inhibitory concentrations (MICs) value of **4a–d**

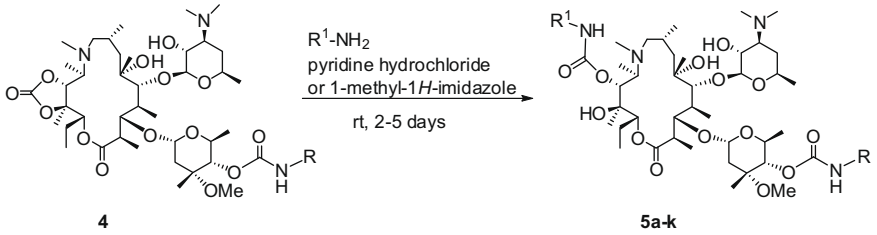
Compound	R	MICs (μg/mL)			
		S. pneumoniae ATCC49619[a]	*S. pneumoniae* B1[b]	*S. pneumoniae* A22072[c]	*S. pneumoniae* AB11[d]
4a		<0.03	8	1	4
4b		<0.03	8	0.25	16
4c		<0.03	8	0.06	4
4d		<0.03	8	0.25	2
AZI		<0.03	128	4	256
CLA		<0.03	64	4	128

[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.

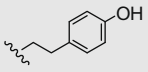
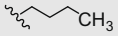
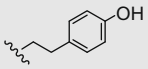
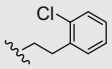
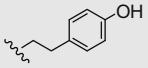
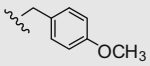
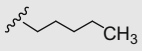
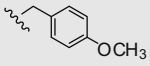
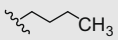
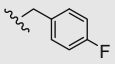
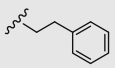
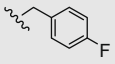
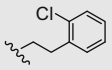
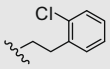
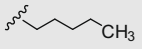
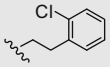
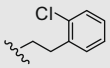
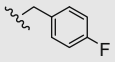
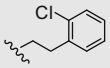
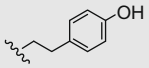
A series of 11,12-cyclic carbonate azithromycin 4″-carbamate derivatives (**4**) were designed as precursors of the 4″,11-di-*O*-arylcarbamoyl azithromycin derivatives. Synthesis of **4** is outlined in Scheme 1. Protection of the 2′-hydroxyl group of AZI with acetic anhydride provided 2′-acetyl azithromycin (**2**) in 92% yield. 11,12-Carbonate 4″-*O*-acylimidazolide **3** was obtained in 96% yield in toluene at 75 °C by treatment of **2** with 1,1′-carbonyldiimidazole (CDI). In contrast, the reaction of **2** with CDI in toluene at room temperature afforded a 4″-*O*-acylimidazolide product.[9] Finally, compounds (**4a–d**) were prepared by coupling **3** with corresponding amines in the presence of 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU), followed by methanolysis. The yields were within the range of 73–80%.

The activity of **4a–d** against four phenotypes of Gram-positive strains were evaluated using the broth microdilution method (Table 1). The strains are *S. pneumoniae* ATCC49619 (erythromycin-susceptible strain), *S. pneumoniae* B1 (erythromycin-resistant strain encoded by the *erm* gene), *S. pneumoniae* A22072 (erythromycin-resistant strain encoded by the *mef* gene) and *S. pneumoniae* AB11 (erythromycin-resistant strain encoded by the *erm* and *mef* genes). All the 4″-carbamate derivatives (**4a–d**) retained excellent activity against erythromycin-susceptible *S. pneumoniae* and showed greatly improved activity against erythromycin-resistant *S. pneumoniae*, compared with AZI and CLA. Among these compounds, the most active compound was **4c**, whose activity against erythromycin-resistant *S. pneumoniae* encoded by the *mef* gene exhibited 16-fold, 4-fold and 4-fold greater than **4a**, **4b** and **4d**, respectively. Compound **4d** possessed the most activity against

Table 2
4″,11-Di-*O*-arylcarbamoyl azithromycin derivatives



4 **5a-k**

Compound	R	R^1	Yield[a] (%)	mp (°C)	R_f[b]
5a			79	104–107	0.44
5b			76	110–113	0.45
5c			78	118–120	0.41
5d			69	115–117	0.50
5e			64	106–109	0.50
5f			65	105–108	0.50
5g			67	104–106	0.50
5h			67	98–101	0.53
5i			66	115–117	0.48
5j			79	119–121	0.43
5k			65	121–124	0.49

[a] Isolated yield.
[b] TLC solvent system: dichloromethane–methanol, 1:5.

erythromycin-resistant *S. pneumoniae* encoded by the *erm* and *mef* genes, as well showing 2-fold, 8-fold and 2-fold better activity than **4a**, **4b** and **4c**, respectively. These results confirm that the 4″-*O*-arylalkylcarbamoyl group can enhance the activity against erythromycin-resistant *S. pneumoniae*.

Compound **4** was readily converted to 4″,11-di-*O*-arylalkylcarbamoyl derivatives (**5a**–**k**) by coupling with the related amines in the presence of pyridine hydrochloride or 1-methyl-1*H*-imidazole at room temperature for 2–5 days in yields ranging from 64% to 79% (Table 2). The structures of **5a**–**k** were identified by IR, ^{1}H NMR and MS spectra.[10,11]

As a consequence, 11 kinds of 4″,11-di-*O*-arylalkylcarbamoyl derivatives were efficiently prepared for in vitro antibacterial testing, using the broth microdilution method (Table 3). All the compounds (**5a**–**k**) showed greatly improved activity (0.25–0.5 μg/mL) against erythromycin-resistant *S. pneumoniae* encoded by the *erm* gene, exhibiting 16–32-fold greater activity than **4** (8 μg/mL). Compared to their precursors (**4**), most of the tested compounds still re-

Table 3
Minimum inhibitory concentrations (MICs) value of **5a–k**

Strain/compound	MICs (μg/mL)												
	AZI	CLA	**5a**	**5b**	**5c**	**5d**	**5e**	**5f**	**5g**	**5h**	**5i**	**5j**	**5k**
S. pneumoniae ATCC49619[a]	<0.03	<0.03	<0.03	<0.03	<0.03	0.06	0.06	<0.03	<0.03	<0.03	<0.03	0.06	<0.03
S. pneumoniae B1[b]	128	64	0.25	0.25	0.25	0.5	0.5	0.25	0.25	0.5	0.25	0.25	0.25
S. pneumoniae A22072[c]	4	4	1	0.25	0.12	1	1	0.06	0.12	0.5	0.25	0.25	0.06
S. pneumoniae AB11[d]	256	128	128	16	128	32	128	32	2	16	4	4	16

[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.

tained potent activity against erythromycin-resistant *S. pneumoniae* encoded by the *mef* gene. In contrast, the tested compounds did not show improved activity against erythromycin-resistant *S. pneumoniae* encoded by the *erm* and *mef* genes. These results suggest that introduction of 11-*O*-arylalkylcarbamoyl or 11-*O*-alkylcarbamoyl group to its precursor **4** can further enhance the activity against erythromycin-resistant *S. pneumoniae* encoded by the *erm* gene, but do not increase the activity against erythromycin-resistant *S. pneumoniae* encoded by the *mef* gene or *erm* and *mef* genes.

In conclusion, a series of new 4″,11-di-*O*-arylalkylcarbamoyl azithromycin derivatives were designed and synthesized. They possessed greatly improved activity against erythromycin-resistant bacteria. Among them, compounds **5f** and **5k** were found to have potent activity against erythromycin-resistant *S. pneumoniae* encoded by the *erm* or *mef* gene. These results suggest that introduction of 11-*O*-arylalkylcarbamoyl or 11-*O*-alkylcarbamoyl group to its precursor **4** which has 4″-*O*-arylalkylcarbamoyl group can further enhance the activity against erythromycin-resistant *S. pneumoniae* encoded by the *erm* gene.

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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.2009.01.092.

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10. Typical experimental procedure; 11-*O*-butylcarbamoyl-4″-*O*-(4-hydroxylphenethyl-carbamoyl)-azithromycin (**5a**). To a solution of **4a** (1.40 g, 1.50 mmol) in *n*-butylamine (5 mL) at room temperature was added pyridine hydrochloride (0.34 g, 3.00 mmol). The resulting solution was allowed to stir for 2–5 days at the same temperature. The reaction was quenched with water (30 mL) and the aqueous layer was extracted with dichloromethane (3 × 15 mL). The combined organic layers were washed with brine and dried over anhydrous Na_2SO_4, filtered, and concentrated in vacuo. The residue was purified by flash chromatography (dichloromethane–methanol, 10:1) to afford 1.21 g (79%) of **5a** as a white solid: mp: 104–107 °C; R_f = 0.44 (dichloromethane–methanol, 5:1); IR (KBr): 3419, 2974, 2934, 2874, 1724, 1615, 1516, 1459, 1382, 1301, 1251, 1170, 1118, 1072, 1049, 1034, 1016 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$) δ (ppm) 7.02–7.01 (m, 4H), 5.05 (m, 1H), 4.98 (d, J = 9.8 Hz, 1H), 4.50–4.46 (m, 3H), 4.36 (m, 1H), 4.26 (m, 2H), 3.57–3.50 (m, 3H), 3.48 (m, 2H), 3.32 (m, 2H), 3.29 (s, 3H), 3.15–3.13 (m, 3H), 2.97 (m, 1H), 2.74 (m, 7H), 2.22 (s, 3H), 2.10 (m, 1H), 2.01 (m, 2H), 1.90 (m, 1H), 1.74 (m, 2H), 1.65 (m, 1H), 1.36 (m, 6H), 1.26 (s, 3H), 1.22 (m, 2H), 1.20–1.17 (m, 6H), 1.14 (d, J = 6.1 Hz, 3H), 0.99–0.95 (m, 12H), 0.92–0.89 (m, 9H); MS (ESI) m/z, 1012.0 $[M+H]^+$, calcd for $C_{52}H_{90}N_4O_{15}$, 1012.3 [M+H].
11. 11-*O*-(4-hydroxyphenethyl-carbamoyl)-4″-*O*-(2-chlorophen-ethyl-carbamoyl)-azithromycin (**5k**). To a solution of **4d** (1.43 g, 1.50 mmol) in 1-methyl-1*H*-imidazole (15 mL) at room temperature was added tyramine (0.41 g, 3.00 mmol). The resulting solution was allowed to stir for 2–5 days at the same temperature. The reaction was quenched with water (30 mL) and the aqueous layer was extracted with dichloromethane (3 × 15 mL). The combined organic layers were washed with brine and dried over anhydrous Na_2SO_4, filtered, and concentrated in vacuo. The residue was purified by flash chromatography (dichloromethane–methanol, 20:1) to afford 1.06 g (65%) of **5k** as a white solid: mp: 121–124 °C; R_f = 0.49 (dichloromethane–methanol, 5:1); IR (KBr): 3429, 2974, 2936, 2854, 1728, 1614, 1594, 1515, 1457, 1376, 1344, 1245, 1170, 1111, 1093, 1073, 1050, 1036, 1015 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$) δ (ppm) 7.28 (m, 1H), 7.21 (m, 3H), 7.09 (m, 2H), 6.84 (m, 2H), 5.00–4.95 (m, 2H), 4.56 (m, 2H), 4.40 (m, 1H), 4.42 (m, 1H), 4.29–4.26 (m, 3H), 3.59–3.56 (m, 4H), 3.55 (m, 4H), 3.30 (s, 3H), 3.00 (m, 3H), 2.74 (m, 6H), 2.60 (m, 1H), 2.37 (d, J = 14.8 Hz, 1H), 2.23 (s, 3H), 2.10 (m, 1H), 2.01 (m, 2H), 1.90 (m, 2H), 1.70–1.62 (m, 3H), 1.47 (m, 2H), 1.26 (s, 3H), 1.20 (m, 2H), 1.25–1.13 (m, 18H), 1.02 (m, 6H), 0.89 (m, 3H); MS (ESI) m/z, 1094.0 $[M+H]^+$, calcd for $C_{56}H_{89}ClN_4O_{15}$, 1094.7 [M+H].