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A novel series of 11-*O*-carbamoyl-3-*O*-descladinosyl clarithromycin derivatives bearing 1,2,3-triazole group: design, synthesis and antibacterial evaluation

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Running title: 11-*O*-Carbamoyl-3-*O*-descladinosyl derivatives

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Abstract

A series of novel 11-*O*-carbamoyl-3-*O*-descladinosyl clarithromycin derivatives bearing the 1,2,3-triazole group were designed, synthesized, and evaluated for their in vitro antibacterial activity. The antibacterial results indicated that most of the target compounds not only increased their activity against resistant bacterial strains, but also partially retained the activity against sensitive bacterial strains compared with clarithromycin. Among them, **13d** had the best antibacterial activity against resistant strains, including *Streptococcus pneumoniae* B1 expressing the *erm*B gene (16 μg/mL), *Streptococcus pneumoniae* AB11 expressing the *mef*A and *erm*B genes (16 μg/mL) and *Streptococcus pyogenes* R1 (16 μg/mL), showing more than 16, 8 and 16-fold higher activity than that of CAM, respectively. Moreover, **13d** and **13g** exhibited the best antibacterial activity against sensitive bacterial strains, including *Staphylococcus aureus* ATCC25923 (4 μg/mL) and *Bacillus Subtilis* ATCC9372 (1 μg/mL). The MBC results showed that the most promising compounds **13d** and **13g** exhibited antibacterial activity through bacteriostatic mechanism, while the time-kill kinetic experiment revealed bactericidal kinetics of **13g** from microscopic point of view. In vitro antibacterial experiments and molecular docking results further confirmed that it was feasible to our initial design strategy by modifying the C-3 and C-11 positions of clarithromycin to increase the activity against resistant bacteria.

Keywords: 11-*O*-Carbamoyl-3-*O*-descladinosyl derivatives; Antibacterial activity; Bacterial resistance; Design and synthesis.

The term "macrolide" was first proposed by R. B. Woodward in 1957.[1] Commonly used macrolides contain a 14-, 15- or 16-membered macrolide ring and glycosyl groups, which are linked by glycoside bonds.[2] They are widely used in clinic due to their strong antibacterial activity, broad antibacterial spectrum and remarkable curative effect. The antibacterial mechanism of macrolide antibiotics is to prevent the extension of nascent polypeptide chains and to further inhibit the synthesis of proteins by binding with the peptide exit tunnel of peptidyl-transferase center (PTC) which is located at the large subunit (50S) of bacterial ribosome.[3] Erythromycin A (EMA) as a representative of first-generation macrolides (Fig. 1) is clinically safe and effective in the treatment of upper and lower respiratory tract infections, and is especially suitable for the patients who are resistant or allergic to β-lactam antibiotics.[4] In view of the degradation of EMA under acidic conditions leading to poor bioavailability and gastrointestinal side effects, second-generation macrolides, represented by clarithromycin (CAM) and azithromycin (AZM), with improved antibacterial activity, higher bioavailability and fewer gastrointestinal side effects, have been obtained further development and widespread application.[5]

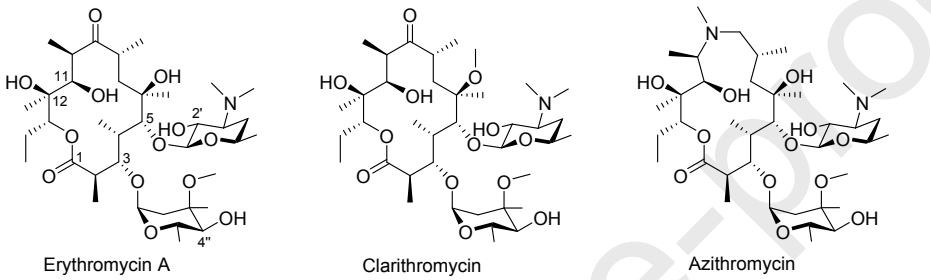


Fig. 1. Structures of erythromycin A, clarithromycin and azithromycin

However, with the increased clinical application of macrolide antibiotics, the problem of bacterial resistance to them has become quite serious.[6] The two main resistance mechanisms of macrolide-resistant pathogens are ribosomal methylation modification and efflux pump active efflux. The former is a methyltransferase encoded by the *erm* gene that dimethylates the nucleotide A2058 of the 23S rRNA in the bacterial ribosome, weakening the interaction between the macrolide antibiotic and bacterial ribosome while the latter is an efflux transporter encoded by the *mef* gene that vents the macrolide out of the bacterial cell.[7, 8]

The emergence and prevalence of resistant pathogens has led to severe limitations in the effectiveness of macrolide antibiotics. An X-ray cocrystal structure research stated clearly that the 3-*O*-descladinosyl moiety at the C-3 position of macrolides is not essential for its antibacterial activity, but it can induce bacterial *mef* gene expression and produce efflux resistance.[9] Therefore, third-generation macrolide antibiotics with anti-drug-resistant bacterial activity, represented by ketolides, have been developed. Among them, telithromycin (Fig. 2) was approved by FDA in 2004, it binds to wild bacterial ribosomes more tightly than CAM and AZM, and exhibits strong activity against erythromycin-resistant *Streptococcus pneumoniae* and *Haemophilus influenza*.[10, 11] The clinical candidate drug cethromycin exhibits remarkable activity against multidrug-resistant respiratory pathogens, effectively treating community-acquired pneumonia (CAP) caused by macrolide-lincosamide-streptogramin B (MLSB) resistant bacteria.[12] Solithromycin, also in clinical research, shows strong efficacy against a variety of resistant bacteria including methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant *Enterococci* (VRE).[13, 14] Seiple et al.[15] established a pathway for the total synthesis of macrolide antibiotics and ascertained that ketolides bearing 1,2,3-triazole side chains were extremely effective against common macrolide-resistant strains. In addition to ketolides, other chemical modifications can also improve the antibacterial activity of

macrolides. For instance, EP-1553, an 11-*O*-substituted clarithromycin derivative, exhibits fairly good activity against sensitive and resistant *Streptococcus pyogenes*, *S. aureus* and *S. pneumoniae*.[16, 17]

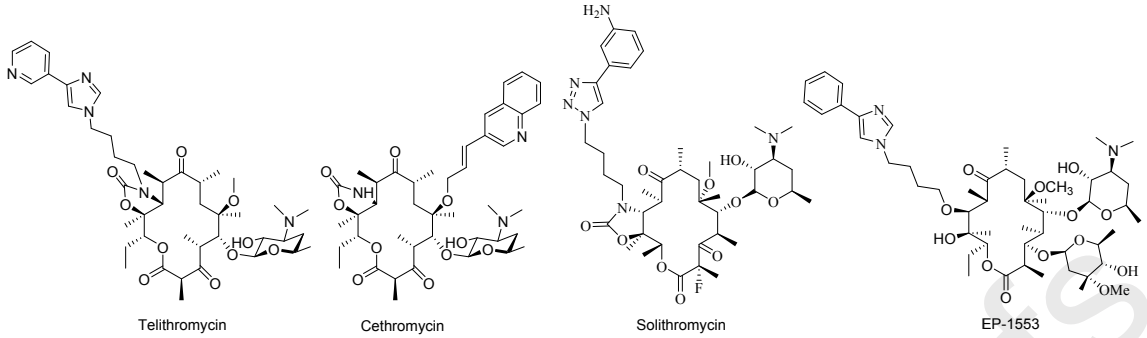
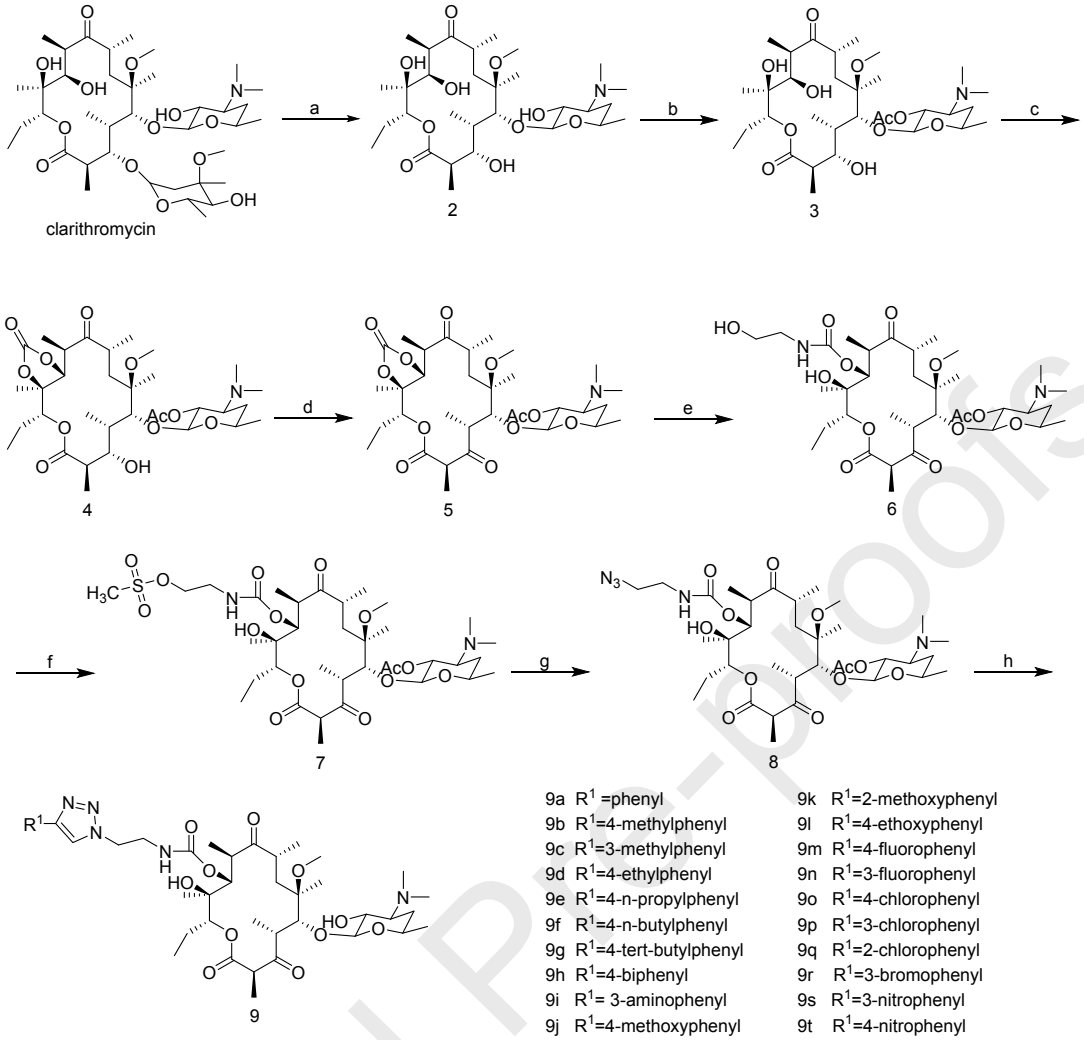


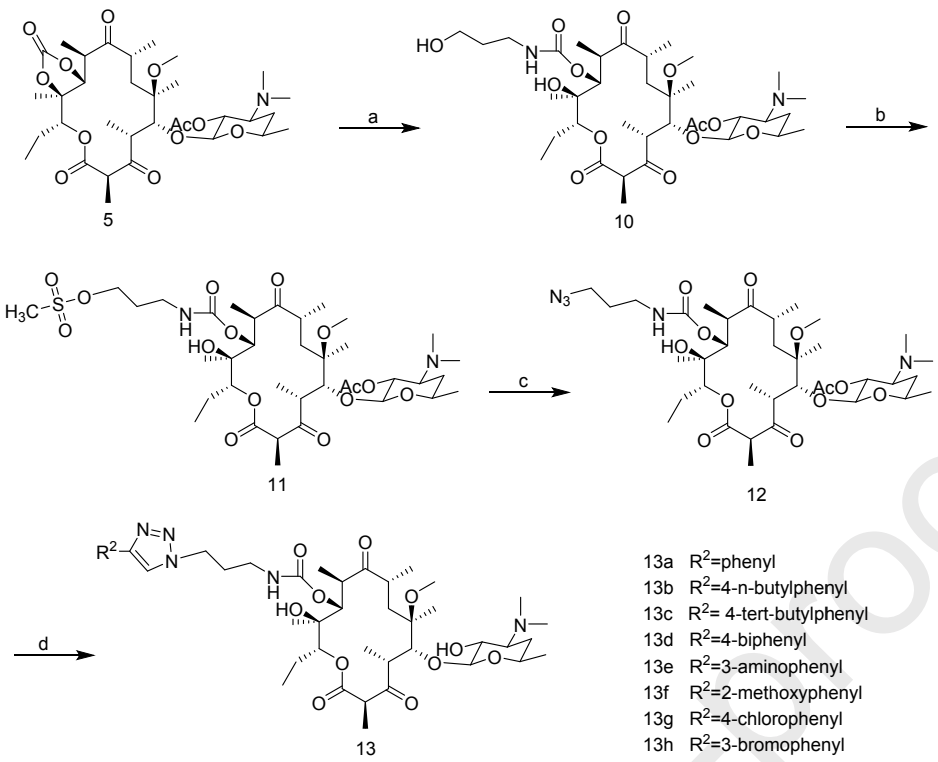
Fig. 2. Structures of telithromycin, cethromycin, solithromycin and EP-1553

Based on the above-mentioned causes of macrolide resistance and current research, we have proposed a new modification strategy for the second-generation macrolide antibiotic CAM. Firstly, we retained the 2'-OH which interacts with A2058 and A2059 in domain V of the bacterial ribosomal 23S rRNA, thereby producing antibacterial activity against susceptible bacteria.[18] Secondly, substitution of a ketone group for the cladinose moiety at the C-3 position with the purpose of reducing the effect of active efflux mediated by the *mef* gene.[19] Thirdly, we noticed that there are few literature reports on the chemical modification of the C-11 position of CAM. Moreover, our previous work[20, 21] also have confirmed that introduction of aralkyl side chain at the C-11 position of CAM can increase the anti-resistant bacteria activity. thus, we designed 11-*O*-carbamoyl-3-*O*-descladinosyl clarithromycin derivatives with potent activity against A2058 and A2059 methylated modified resistant strains through introducing a carbamate side chain bearing 1,2,3-triazole with appropriate length, flexibility and spatial extension to the C-11 position of CAM, which would generate new binding site with the nucleotide residue U790 of bacterial ribosome by hydrogen bond interaction, π-π stacking interaction and hydrophobic interaction.[22-24]

The synthetic methods for 11-*O*-carbamoyl-3-*O*-descladinosyl clarithromycin derivatives **9a-9t** and **13a-h** are shown in Scheme 1 and 2, respectively. CAM was treated with diluted hydrochloric acid (HCl) to produce the 3-descladinosylclarithromycin **2**.[25] Using triethylamine as acid binding agent, the 2'-OH of **2** was selectively protected by acetic anhydride (Ac_2O) in dichloromethane (DCM) to generated the acetyl product **3** due to the reactivity of the 2'-OH is stronger than that of the 3-OH.[26] Then, cyclization of the 11-OH and the 12-OH of **3** to 11,12-cyclic carbonate by bis(triehloromethyl)carbonate (BTC) in the presence of pyridine gave intermediate **4**. [27] Subsequently, the 3-OH was oxidized by pyridinium chlorochromate (PCC) to obtain ketolide **5**.[28] Catalyzed by pyridine hydrochloride, **6** was afforded by treating **5** with ethanolamine. The terminal hydroxyl group on the C-11 side chain of **6** was esterified with methanesulfonyl chloride (MsCl) to obtain mesylate intermediate **7**. Intermediate **7** was treated with sodium azide (NaN_3) in the mixed solvent of water and N,N-dimethylformamide (DMF) at 60°C to produce the key intermediate **8**. After that, intermediate **8** clicked reaction with the corresponding phenylacetylene catalyzed by cuprous iodide (CuI) in toluene solution[29] and followed by deprotection in methanol (MeOH) to afford target compounds **9a-9t** (Scheme 1).[30] The synthetic method for another series of target compounds **13a-h** is shown in Scheme 2. Intermediate **5** reacted with 3-aminopropanol to afford **10** in the presence of pyridine hydrochloride at 15°C. The subsequent synthetic method for target compounds was same as that of target compounds **9a-9t**. The structures of all the target compounds were confirmed by ^{1}H NMR and MS spectra.



Scheme 1. Synthesis of 11-*O*-carbamoyl-3-*O*-descladinosyl clarithromycin derivatives (**9a–9t**). Reagents and conditions: (a) HCl, H_2O, rt, 5 h, 83%; (b) Ac_2O, Et_3N, DCM, 0 °C~rt, 36 h, 94%; (c) pyridine, BTC, DCM, 0 °C~rt, 20 h, 99%; (d) PCC, DCM, rt, 24 h, 88%; (e) ethanolamine, pyridine hydrochloride, 15 °C, 2 h, 37%; (f) MsCl, Et_3N, DCM, 0~25 °C, 2 h, 91%; (g) NaN_3, DMF, H_2O, 60 °C, 12 h, 76%; (h) i) phenylacetylene, CuI, toluene, 90 °C, 24 h; ii) CH_3OH, 55 °C, 24 h, 56~68%.



Scheme 2. Synthesis of 11-*O*-carbamoyl-3-*O*-descladinosyl clarithromycin derivatives (**13a–13h**). Reagents and conditions: (a) 3-aminopropanol, pyridine hydrochloride, 15 °C, 2 h, 42%; (f) MsCl, Et_3N, DCM, 0~25 °C, 2 h, 89%; (g) NaN_3, DMF, H_2O, 60 °C, 12 h, 78%; (h) i) phenylacetylene, CuI, toluene, 90 °C, 24 h; ii) CH_3OH, 55 °C, 24 h, 56~65%.

The in vitro antibacterial activity of target compounds were determined by the standard broth microdilution procedures recommended by NCCLS. The tested strains included four susceptible strains of *S. aureus* ATCC25923 (erythromycin-susceptible strain), *Bacillus subtilis* ATCC9372 (penicillin-susceptible strain), *Escherichia coli* ATCC25922 (penicillin-susceptible strain) and *Pseudomonas aeruginosa* ATCC27853 (penicillin-susceptible strain), and four resistant strains of *S. aureus* ATCC43300 (methicillin-resistant strain), *S. pyogenes* R1 (erythromycin-resistant strain isolated clinically), *S. pneumoniae* B1 (erythromycin-resistant strain expressing the *erm*B gene), and *S. pneumoniae* AB11 (erythromycin-resistant strain expressing the *erm*B and *mef*A genes). The minimal inhibitory concentration (MIC) values for the above target compounds as well as CAM and AZM as the references were tested against a panel of sensitive and resistant bacteria strains as shown in Table 1.

Table 1

In vitro antibacterial activity of 11-*O*-carbamoyl-3-*O*-descladinosyl clarithromycin derivatives

Compound	Minimum inhibitory concentration (MIC) (μg/mL)							
	S. aureus ATCC25923[a]	*E. coli* ATCC25922[b]	*B. Subtilis* ATCC9372[c]	*P. aeruginosa* ATCC27853[d]	*S. pneumoniae* AB11[e]	*S. pneumoniae* B1[f]	*S. pyogenes* R1[g]	*S. aureus* ATCC43300[h]
CLM	0.25	>128	0.25	32	128	>256	256	>256
AZM	0.25	>128	0.25	4	256	>256	256	>256
13a	8	>128	8	128	64	128	64	256
13b	16	>128	2	128	32	32	16	64
13c	16	>128	16	128	64	64	32	128
13d	4	>128	1	128	16	16	16	64
13e	16	>128	4	128	32	128	128	>256
13f	8	>128	2	128	32	256	64	>256
13g	4	>128	1	128	16	64	32	128
13h	8	>128	2	128	32	64	32	128
9a	32	>128	8	128	256	256	256	>256
9b	32	>128	8	128	256	>256	256	>256
9c	32	>128	8	128	256	>256	256	>256
9d	32	>128	4	128	256	>256	128	>256
9e	32	>128	8	128	128	256	64	256
9f	16	>128	4	128	64	128	32	256
9g	32	>128	8	128	128	128	64	256

9h	32	>128	4	128	64	128	32	128
9i	32	>128	32	128	256	>256	256	>256
9j	16	>128	4	128	256	>256	256	>256
9k	16	>128	4	128	>256	>256	256	>256
9l	16	>128	16	128	256	>256	128	>256
9m	16	>128	4	128	128	256	64	>256
9n	8	>128	4	128	128	256	128	256
9o	8	>128	4	128	128	128	64	256
9p	8	>128	4	128	128	128	64	256
9q	8	>128	4	128	128	128	64	256
9r	16	>128	8	128	128	256	128	>256
9s	8	>128	4	128	128	256	64	>256
9t	16	>128	4	128	256	256	64	>256

[a]*S. aureus* ATCC25923: erythromycin-susceptible strain;

[b]*E. coli* ATCC25922: penicillin-susceptible strain;

[c]*B. Subtilis* ATCC9372: penicillin-susceptible strain;

[d]*P.aeruginosa* ATCC27853: penicillin-susceptible strain;

[e]*S. pneumoniae* AB11: erythromycin-resistant strain expressing the *erm*B and *mef*A genes;

[f]*S. pneumonia* B1: erythromycin-resistant strain expressing the *erm*B gene;

[g]*S. pyogenes* R1: erythromycin-resistant strain isolated clinically;

[h]*S. aureus* ATCC43300: methicillin-resistant strain.

Compared to the references of CAM and AZM, the 11-*O*-carbamoyl-3-*O*-descladinosyl clarithromycin derivatives universally showed greatly improved activity against *S. pneumoniae* B1, *S. pneumoniae* AB11, *S. pyogenes* R1 and *S. aureus* ATCC43300, but slightly weaker activity against *S. aureus* ATCC25923 and *B. subtilis* ATCC9372 than the references. Unfortunately, none of them displayed improved activity against *E. coli* ATCC25922 and *P. aeruginosa* ATCC27853. Among them, **13d** showed significantly improved activity (16 μg/mL) against *S. pneumoniae* B1, which was >16-fold more active than that of CAM and AZM (>256 and >256 μg/mL), while **13b**, **13d**, **13e**, **13f**, **13g** and **13h** exhibited the most effective activity against *S. pneumoniae* AB11 with MIC values of 16-32 μg/mL, showing 4 to 8-fold better than CAM (128 μg/mL) and 8 to 16-fold better than AZM (256 μg/mL), respectively. In addition, **9f**, **9h**, **13b**, **13c**, **13d**, **13g** and **13h** displayed the best antibacterial activity with MIC values of 16-32 μg/mL against *S. pyogenes* R1, 8 to 16-fold better than CAM and AZM (256 and 256 μg/mL). In the inhibition of *S. aureus* ATCC43300, the most active **13b** and **13d** showed an MIC value of 64 mg/mL, >4-fold better than CAM and AZM (>256 μg/mL). Furthermore, **13d** and **13g** with the best anti-sensitive bacteria activity, had an MIC value of 4 and 1 μg/mL against *S. aureus* ATCC25923 and *B. subtilis* ATCC9372, respectively. Besides, many compounds demonstrated antibacterial activity with MIC values of 8-32 μg/mL and 2-8 μg/mL against *S. aureus* ATCC25923 and *B. subtilis* ATCC9372, respectively.

In general, the target compounds exhibited greatly improved activity against resistant bacteria and slightly weak activity against susceptible bacteria in comparison with CAM and AZM. Furthermore, most of target compounds showed favorable activity against Gram-positive bacteria but weak activity against Gram-negative bacteria, probably because the target compounds are difficult to pass through the cell wall of Gram-negative bacteria. The results indicate that simultaneous modification of the C-3 and C-11 positions of CAM can greatly enhance the activity against resistant strains and partially retain the activity against sensitive strains. We believe that the C-11 side chain can directly interact with the nucleotide A752 and U790, and the alteration of the length of the C-11 side chain and the different substituents on the terminal benzene ring of the C-11 side chain lead to the change of interaction force, thereby result in the difference of antibacterial activity for the target compounds.

Preliminary structure-activity relationship (SAR) studies on the 11-*O*-carbamoyl-3-*O*-descladinosyl clarithromycin derivatives indicated that when the length between the carbon atom at the C-11 position and the 1,2,3-triazole moiety was extended from 5 atoms to 6 atoms (**9a** and **13a**, **9f** and **13b**, **9g** and **13c**, **9h** and **13d**, **9i** and **13e**, **9k** and **13f**, **9o** and **13g**, **9r** and **13h**), the activity against sensitive bacteria increased by 2 to 8-fold and the activity against resistant bacteria increased by 2 to 4-fold, which suggests that the 1,2,3-triazole side chain with the appropriate length may be more susceptible to interaction with the U790 of 23S rRNA. The electron-donating groups on the terminal benzene ring of the 1,2,3-triazole side chain (**9a-9d** and **9i-9l**) result in loss of activity against the drug-resistant strains, which indicates that these compounds only retain partial interaction with sensitive bacteria, without new binding force with the ribosomes of the drug-resistant bacteria. Aryl substituents (**9h** and **13d**) and electron-withdrawing groups (**9m-9t**, **13g** and **13h**) on the benzene ring are beneficial to the antibacterial activity. In contrast, weak electron-donating groups on the benzene ring (**9e-9g** and **13b** and **13c**) are detrimental to the antibacterial activity. However, new binding force is generated in space due to the flexibility of their alkyl substituent, resulting in improved activity. In particular, linear alkane groups (**9f** and **13b**) are more active than branched alkane groups (**9g** and **13c**). Position of the substitutes on the benzene ring has no prominent effect on the activity (**9j-9k**, **9m-9q** and **9s-9t**). For halogen-substituted compounds, the chlorine atom substitution is superior to the fluorine and bromine

substitution (**9m-9r** and **13g-13h**) in the activity. Therefore, we speculated that the appropriate length of the 1,2,3-triazole side chain can reach the chloramphenicol binding site in the bacterial ribosome, and the different substituents on the benzene ring affect the binding ability of the target compounds to the binding site, thereby inhibiting the formation of bacterial peptide.

To investigate whether the antibacterial activity of these target compounds was bactericidal or bacteriostatic in nature, we determined the minimum bactericidal concentration (MBC) values of **13d** and **13g** against susceptible bacteria (*B. Subtilis* ATCC9372) and resistant bacteria (*S. pneumoniae* AB11) (Table 2). By comparing their MBC values with their corresponding MIC values, we discovered that **13d** and **13g** exhibiting bacteriostatic action against *B. Subtilis* ATCC9372 with MBC/MIC ratio of 128 and 32, respectively. Similarly, **13d** and **13g** were observed as bacteriostatic agents against *S. pneumoniae* AB11 with the MBC/MIC ratio of 16 and 8, respectively. The above results reveal that the test compounds exert an antibacterial effect against sensitive and resistant bacterial strains by bacteriostatic action.

Table 2

Comparison of MIC and MBC values for **13d** and **13g** in *B. Subtilis* ATCC9372 and *S. pneumoniae* AB11.

Compound	MBC(μg/mL)	MIC(μg/mL)	MBC/MIC
B. subtilis ATCC9372[a]			
13d	128	1	128
13g	32	1	32
CAM	128	0.25	512
S. pneumoniae AB11[b]			
13d	256	16	16
13g	128	16	8
CAM	128	128	1

[a]*B. Subtilis* ATCC9372: penicillin-susceptible strain;

[b]*S. pneumoniae* AB11: erythromycin-resistant strain expressing the *erm*B and *mef*A genes.

In order to further examine the antibacterial potency of the target compounds, time-kill kinetic experiment of **13g** against *S. pneumoniae* AB11 was tested. The growth of bacteria was observed when it was incubated without **13g** or with 0.5 MIC of **13g** as shown in Fig. 3. On the contrary, **13g** exhibited certain bactericidal effect (0-9 h) and bacteriostatic effect (9-24 h) at the concentrations of 1, 2 and 4 MIC. Time-kill kinetic experiment further proved that **13g** was a concentration-dependent bacteriostatic agent from a microscopic point of view.

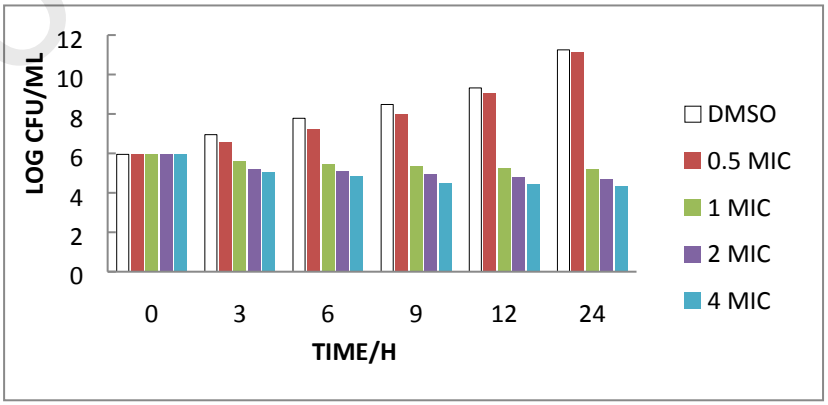
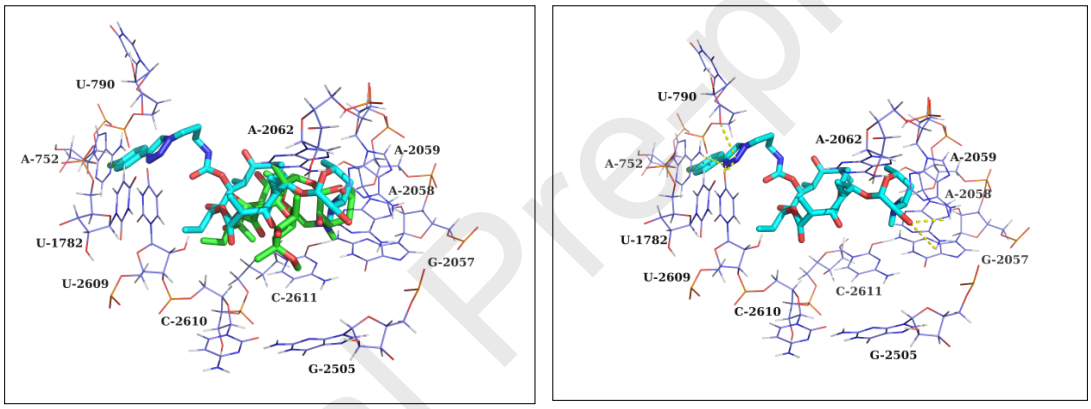


Fig. 3. Time-kill kinetics of compound 13g against *S. pneumoniae* AB11.

By analyzing the activity data, we found that these target compounds had good antibacterial activity, and especially their anti-resistant bacterial activity showed obvious improvement compared with that of CAM and AZM, indicating the combined modifications of the C-3 and the C-11 positions of CAM are instrumental in activity against multiple drug-resistant bacterial strains. It is worth noting that these compounds have significantly improved activity against *S. pneumoniae* B1 and *S. pneumoniae* AB11, which confirms the correctness of our design mentality. We believe that removal of the cladinose group at the C-3 position of CAM and oxidation of the resulting hydroxyl group to ketone, and further introduction of the C-11 carbamate side chains containing 1,2,3-triazole group not only weaken the active efflux of the resistant bacteria but also produce additional affinity for the bacterial ribosome, thereby effectively inhibiting the type of *erm* and *mef* resistant bacteria. Docking model (Fig. 4) performed through Le-dock further indicated that **13g** has similar binding mode to CAM when it binds to *E. coli* ribosome (PDB code, 4v7s), preserving the interaction between 2'-OH and A2058, which explains why the compound displays the activity against susceptible bacteria. What's more, the 1,2,3-triazole side chain at the C-11 position of **13g** forms hydrogen bonds with the A752 and U790 nucleotide residues of 23S rRNA. Meanwhile, **13g** also interacts with the pocket formed by G2505, C2610 and C2611 in domain V, thereby increasing the activity against resistant bacteria.



a b

Fig. 4. (a) The docking model of compound **13g** (blue) and clarithromycin (green) in *E. coli* ribosome (PDB code, 4v7s). Both are located in approximately the same spatial area in *E. coli* ribosome. (b) Model of compound **13g** docked into *E. coli* ribosome (PDB code, 4v7s). In the model, the predicted hydrogen bonds between compound **13g** and the nucleotide residues are indicated by dotted lines.

In summary, a novel series of 11-*O*-carbamoyl-3-*O*-descladinosyl clarithromycin derivatives bearing the 1,2,3-triazole group were designed, synthesized and evaluated for their in vitro antimicrobial activity against various drug-susceptible and -resistant bacterial strains. The antibacterial results indicated that the target compounds greatly enhanced the activity against resistant bacterial strains and partially retained the activity against sensitive bacterial strains. Among them, **13d** had the best antibacterial activity against resistant strains, including *S. pneumoniae* B1 (16 μg/mL), *S. pneumoniae* AB11 (16 μg/mL) and *S. pyogenes* R1 (16 μg/mL), showing more than 16, 8 and 16-fold higher activity than that of CAM, respectively. Moreover, **13d** and **13g** exhibited the best antibacterial activity against sensitive bacterial strains, including *S. aureus* ATCC25923 (4 μg/mL) and *B. Subtilis* ATCC9372 (1 μg/mL). On the other hand, measurement of MBC showed that the most promising compounds **13d** and **13g** exhibited antibacterial activity through bacteriostatic mechanism, while the time-kill kinetic experiment revealed bactericidal kinetics of **13g** from microscopic point of view. In vitro antibacterial experiments and molecular docking results further confirmed that it was feasible to

our initial design strategy by modifying the C-3 and C-11 positions of CAM to enhance the activity against resistant bacteria.

Conflict of interest

The authors declare that this study was carried out only with public funding. There is no funding or no agreement with commercial for profit firms.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://

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10-hydroxyl ketolide derivatives. *Bioorganic & Medicinal Chemistry Letters.* 2013; 23: 3452-3457.

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Figure captions

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

Fig. 2. Structures of telithromycin, cethromycin, solithromycin and EP-1553.

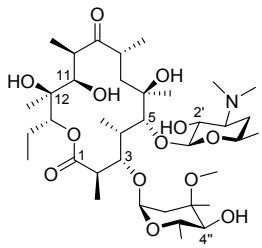
Scheme 1. Synthesis of 11-*O*-carbamoyl-3-*O*-descladinosyl clarithromycin derivatives (**9a–9t**). Reagents and conditions: (a) HCl, H_2O, rt, 5 h, 83%; (b) Ac_2O, Et_3N, DCM, 0 °C~rt, 36 h, 94%; (c) pyridine, BTC, DCM, 0 °C~rt, 20 h, 99%; (d) PCC, DCM, rt, 24 h, 88%; (e) ethanolamine, pyridine hydrochloride, 15 °C, 2 h, 37%; (f) MsCl, Et_3N, DCM, 0~25 °C, 2 h, 91%; (g) NaN_3, DMF, H_2O, 60 °C, 12 h, 76%; (h) i) phenylacetylene, CuI, toluene, 90 °C, 24 h; ii) CH_3OH, 55 °C, 24 h, 56~68%.

Scheme 2. Synthesis of 11-*O*-carbamoyl-3-*O*-descladinosyl clarithromycin derivatives (**13a–13h**). Reagents and conditions: (a) 3-aminopropanol, pyridine hydrochloride, 15 °C, 2 h, 42%; (f) MsCl, Et_3N, DCM, 0~25 °C, 2 h, 89%; (g) NaN_3, DMF, H_2O, 60 °C, 12 h, 78%; (h) i) phenylacetylene, CuI, toluene, 90 °C, 24 h; ii) CH_3OH, 55 °C, 24 h, 56~65%.

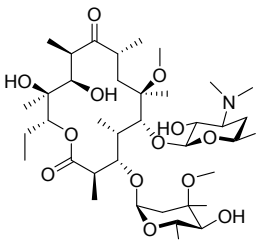
Fig. 3. Time-kill kinetics of compound **13g** against *S. pneumoniae* AB11.

Fig. 4. (a) The docking model of compound **13g** (blue) and clarithromycin (green) in *E. coli* ribosome (PDB code, 4v7s). Both are located in approximately the same spatial area in *E. coli* ribosome. (b) Model of compound **13g** docked into *E. coli* ribosome (PDB code, 4v7s). In the model, the predicted hydrogen bonds between compound **13g** and the nucleotide residues are indicated by dotted lines.

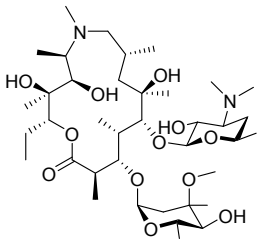
Fig. 1



Erythromycin A

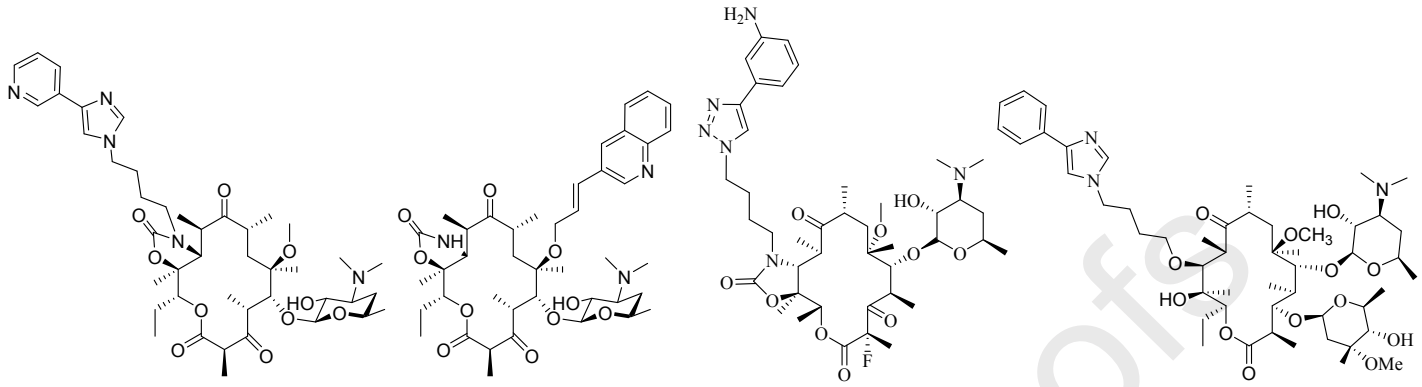


Clarithromycin

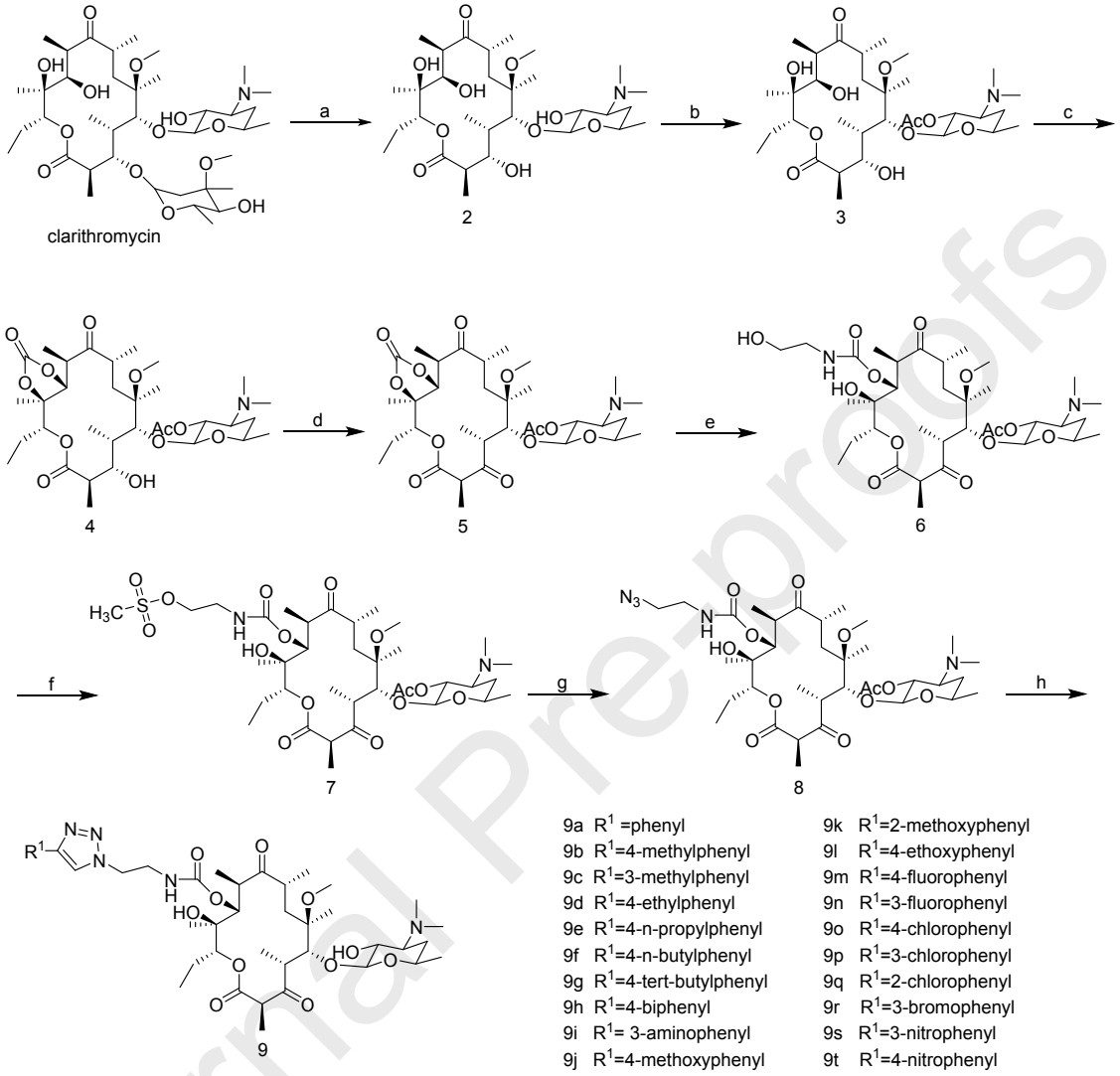


Azithromycin

Fig. 2



Scheme 1



9a R^1 =phenyl
9b R^1=4-methylphenyl
9c R^1=3-methylphenyl
9d R^1=4-ethylphenyl
9e R^1=4-n-propylphenyl
9f R^1=4-n-butylphenyl
9g R^1=4-tert-butylphenyl
9h R^1=4-biphenyl
9i R^1= 3-aminophenyl
9j R^1=4-methoxyphenyl
9k R^1=2-methoxyphenyl
9l R^1=4-ethoxyphenyl
9m R^1=4-fluorophenyl
9n R^1=3-fluorophenyl
9o R^1=4-chlorophenyl
9p R^1=3-chlorophenyl
9q R^1=2-chlorophenyl
9r R^1=3-bromophenyl
9s R^1=3-nitrophenyl
9t R^1=4-nitrophenyl

Scheme 2

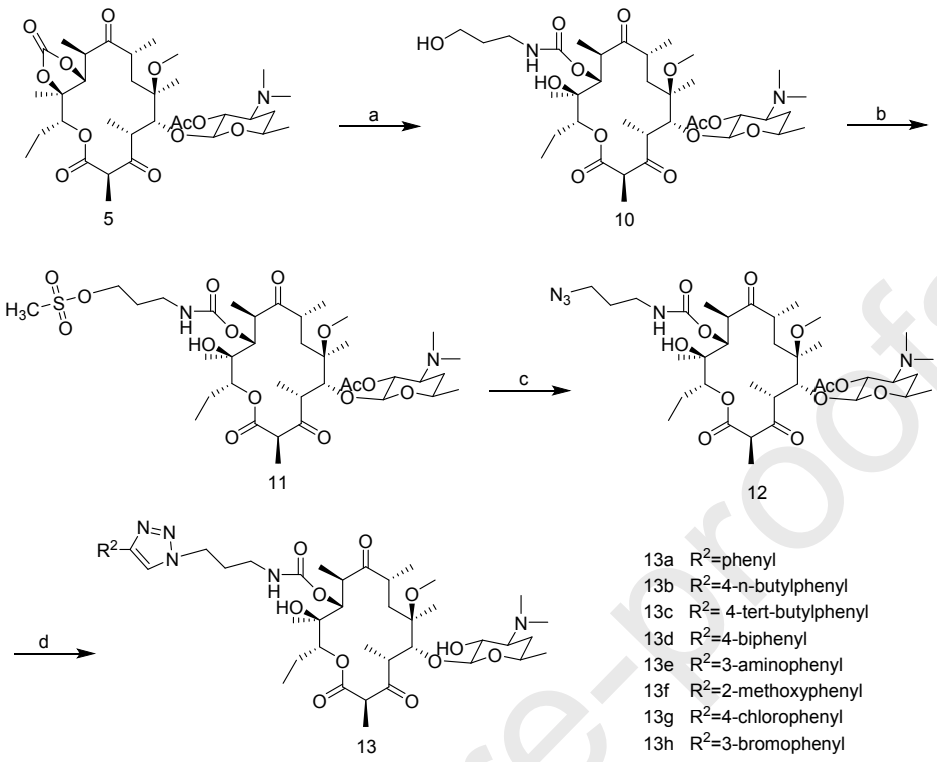


Fig. 3

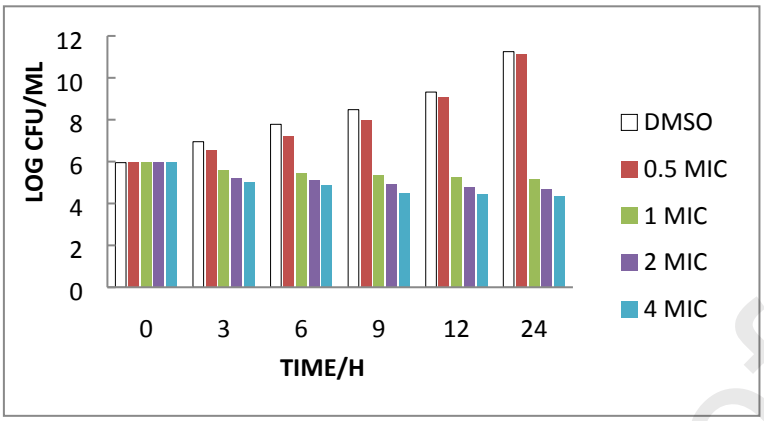
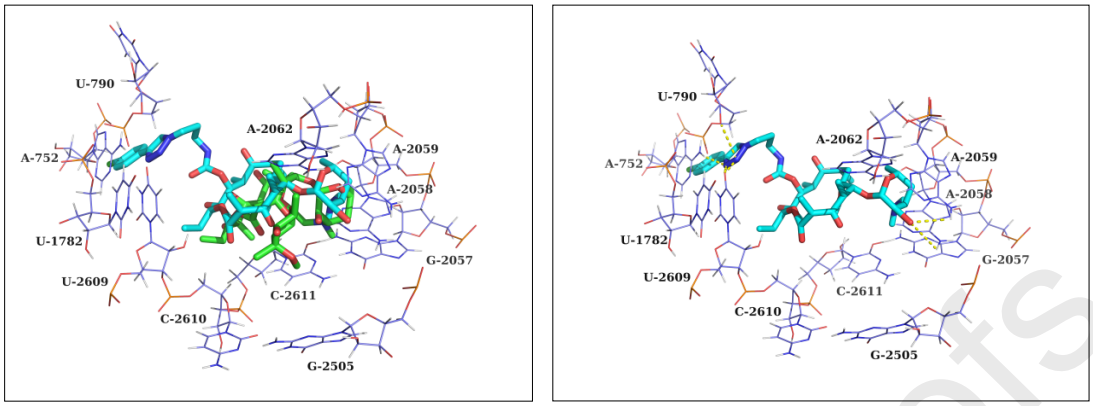


Fig. 4



a b

Table 1

In vitro antibacterial activity of 11-*O*-carbamoyl-3-*O*-descladinosyl clarithromycin derivatives

Compound	Minimum inhibitory concentration (MIC) (μg/mL)							
	S. aureus ATCC25923[a]	*E. coli* ATCC25922[b]	*B. Subtilis* ATCC9372[c]	*P. aeruginosa* ATCC27853[d]	*S. pneumoniae* AB11[e]	*S. pneumoniae* B1[f]	*S. pyogenes* R1[g]	*S. aureus* ATCC43300[h]
CLM	0.25	>128	0.25	32	128	>256	256	>256
AZM	0.25	>128	0.25	4	256	>256	256	>256
13a	8	>128	8	128	64	128	64	256
13b	16	>128	2	128	32	32	16	64
13c	16	>128	16	128	64	64	32	128
13d	4	>128	1	128	16	16	16	64
13e	16	>128	4	128	32	128	128	>256
13f	8	>128	2	128	32	256	64	>256
13g	4	>128	1	128	16	64	32	128
13h	8	>128	2	128	32	64	32	128
9a	32	>128	8	128	256	256	256	>256
9b	32	>128	8	128	256	>256	256	>256
9c	32	>128	8	128	256	>256	256	>256
9d	32	>128	4	128	256	>256	128	>256
9e	32	>128	8	128	128	256	64	256
9f	16	>128	4	128	64	128	32	256
9g	32	>128	8	128	128	128	64	256

9h	32	>128	4	128	64	128	32	128
9i	32	>128	32	128	256	>256	256	>256
9j	16	>128	4	128	256	>256	256	>256
9k	16	>128	4	128	>256	>256	256	>256
9l	16	>128	16	128	256	>256	128	>256
9m	16	>128	4	128	128	256	64	>256
9n	8	>128	4	128	128	256	128	256
9o	8	>128	4	128	128	128	64	256
9p	8	>128	4	128	128	128	64	256
9q	8	>128	4	128	128	128	64	256
9r	16	>128	8	128	128	256	128	>256
9s	8	>128	4	128	128	256	64	>256
9t	16	>128	4	128	256	256	64	>256

[a]*S. aureus* ATCC25923: erythromycin-susceptible strain;

[b]*E. coli* ATCC25922: penicillin-susceptible strain;

[c]*B. Subtilis* ATCC9372: penicillin-susceptible strain;

[d]*P.aeruginosa* ATCC27853: penicillin-susceptible strain;

[e]*S. pneumoniae* AB11: erythromycin-resistant strain expressing the *erm*B and *mef*A genes;

[f]*S. pneumonia* B1: erythromycin-resistant strain expressing the *erm*B gene;

[g]*S. pyogenes* R1: erythromycin-resistant strain isolated clinically;

[h]*S. aureus* ATCC43300: methicillin-resistant strain.

Table 2

Comparison of MIC and MBC values for **13d** and **13g** in *B. Subtilis* ATCC9372 and *S. pneumoniae* AB11.

Compound	MBC(μg/mL)	MIC(μg/mL)	MBC/MIC
B. subtilis ATCC9372[a]			
13d	128	1	128
13g	32	1	32
CAM	128	0.25	512
S. pneumoniae AB11[b]			
13d	256	16	16
13g	128	16	8
CAM	128	128	1

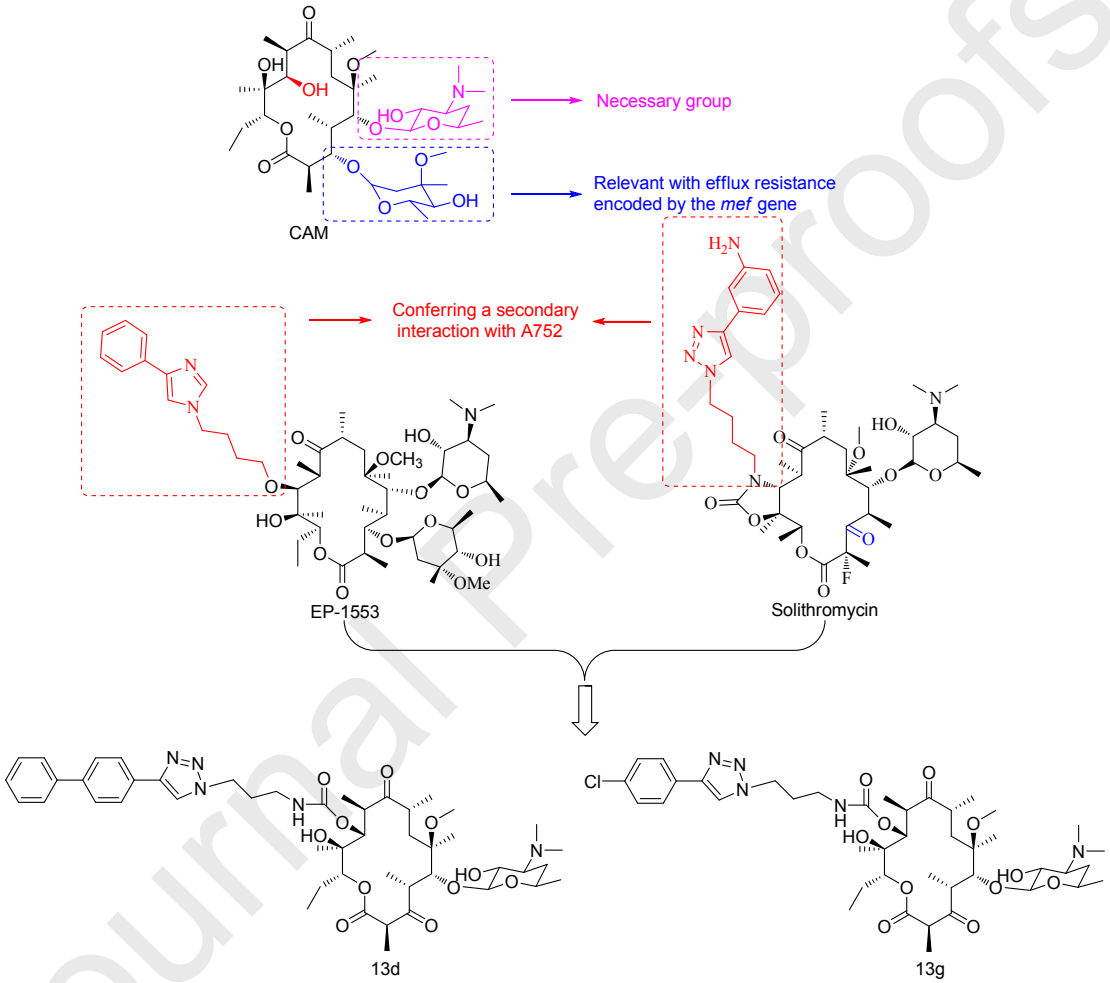
[a]*B. Subtilis* ATCC9372: penicillin-susceptible strain;

[b]*S. pneumoniae* AB11: erythromycin-resistant strain expressing the *erm*B and *mef*A genes.

Graphical abstract:

A novel series of 11-*O*-carbamoyl-3-*O*-descladinosyl clarithromycin derivatives bearing 1,2,3-triazole group: design, synthesis and antibacterial evaluation

Yuetai Teng, Yinhui Qin, Di Song, Xingbang Liu, Yingang Ma, Panpan Zhang, Shutao Ma*



11-*O*-carbamoyl-3-*O*-descladinosyl clarithromycin derivatives

Research Highlights

> Novel 11-*O*-carbamoyl-3-*O*-descladinosyl clarithromycin derivatives were synthesized and evaluated. > Some of them showed effective activity against both susceptible and resistant strains. > Compound **13d** and **13g** exhibited prominent antibacterial activity.