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PII: S0223-5234(19)30255-7

DOI: https://doi.org/10.1016/j.ejmech.2019.03.037

Reference: EJMECH 11207

To appear in: *European Journal of Medicinal Chemistry*

Received Date: 18 November 2018

Revised Date: 14 March 2019

Accepted Date: 15 March 2019

Please cite this article as: X.-M. Li, W. Lv, S.-Y. Guo, Y.-X. Li, B.-Z. Fan, M. Cushman, F.-S. Kong, J. Zhang, J.-H. Liang, Synthesis and structure-bactericidal activity relationships of non-ketolides: 9-Oxime clarithromycin 11,12-cyclic carbonate featured with three-to eight-atom-length spacers at 3-OH, *European Journal of Medicinal Chemistry* (2019), doi: https://doi.org/10.1016/j.ejmech.2019.03.037.

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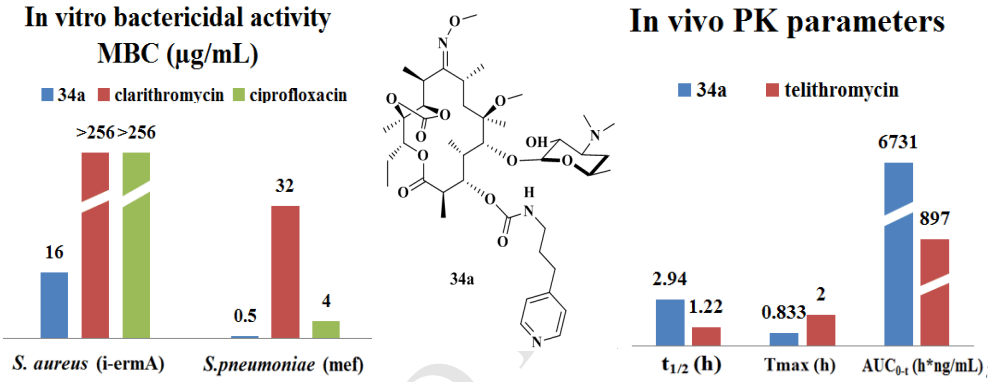

Synthesis and Structure-bactericidal Activity Relationships of Non-ketolides: 9-Oxime Clarithromycin 11,12-Cyclic Carbonate Featured with Three- to Eight-atom-length Spacers at 3-OH

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Abstract: In general, potent non-ketolide versions of erythromycin possessed conformationally constricted two- or three-atom-length sidechains at 3-OH. Novel 14-membered non-ketolides possessing long spacers beyond three-atom length were evaluated for antibacterial activity. The most potent one is **34a**, featuring a five-atom-length flexible linker from of a pyridine ring to the aglycone. Conversion of the pyridine of **34a** to other aryl groups, changing the linker's length of **34a** to longer or shorter ones, and variation of the linker flexibility to a rigid olefin or alkyne led to decreased antibacterial activity. The hybrids of macrolides and quinolones **28b**, **31** and **34b** possessing various sidechains, unlike their 15-membered counterparts, were ineffective compared to **34a**. Similar to the marketed ketolide telithromycin, the non-ketolide **34a** proved to be a time-dependent bactericidal agent, but it exhibited superior in vivo pharmacokinetic properties such as longer half-life, higher plasma concentration, lower clearance and shorter time to reach the highest drug concentration relative to telithromycin. Molecular docking suggested **34a** might π - π interact with the bacterial ribosomal RNA base G2505Er. This study suggested that the bacteriostatic agent erythromycin can be structurally modified to afford a new bactericidal chemotype that targets the ribosome and is superior to ciprofloxacin with regard to its minimum bactericidal concentration.

Keywords: Erythromycin; macrolide; quinolone; ribosome; bactericidal activity; resistant bacteria

1. Introduction

Erythromycin, consisting of a 14-membered ring to which two sugars are attached (Fig. 1), has been widely used for curing upper and lower respiratory tract infections since the 1950s. One of the two sugars, 5-*O*-desosamine, hydrogen bonds to the ribosomal RNA base A2058, and plays a crucial role in the bacteriostatic activity by inhibiting the release of nascent peptides from the bacterial ribosome. Inevitably, clinically isolated pathogens acquire *erm* (erythromycin ribosomal methylation)*/mef* (macrolide efflux)-mediated resistance to erythromycin. [1] Expression of an *erm* gene leads to irreversible mono- or dimethylation at a nitrogen atom of the A2058, which disrupts its ability to act as a hydrogen bond donor toward a hydroxyl oxygen of the 5-*O*-desosamine of the drug. As a result, erythromycin cannot bind as firmly as before and is easily flushed away when nascent peptides pass through the binding sites of erythromycin. In addition, a *mef* gene produces efflux pump proteins that can export drug molecules outside the cell. Thus, the intracellular concentration of erythromycin is reduced below the therapeutic level when it encounters the ribosome. The second-generation erythromycins, clarithromycin and azithromycin (Fig. 1), are also ineffective against these *erm/mef*-encoded bacteria. Meanwhile, more and more bacteria are increasingly resistant to multiple drugs including methicillin, ciprofloxacin, and erythromycin, which possess different antibacterial mechanisms. Therefore, strenuous efforts are presently being made to structurally modify the clinically used antibiotics in order to restore their activities against resistant pathogens. [2, 3]

<Figure 1>

It was found that the other sugar, namely 3-*O*-cladinose, is responsible for the inducible expression of *erm* and *mef* genes. [4] Removal of 3-*O*-cladinose, followed by oxidation of the resulting 3-OH, led to the so-called ketolides. Telithromycin is the first marketed third-generation erythromycin, and several other ketolides are currently in late phases of clinical trials, such as solithromycin, modithromycin and cethromycin, as illustrated in Fig. 2. [5, 6] Recent studies on high-resolution X-ray

crystal structures of erythromycin derivatives in complex with bacterial ribosomes have clarified the fact that the cladinose does not directly interact with rRNA, while the aryl groups anchored the end of the newly introduced sidechains of telithromycin and solithromycin afford an additional π - π stacking interaction with a base pair A752/U2609 of the ribosomal RNA. [7-9]

<Figure 2>

In contrast, non-ketolides are less explored and thus far none of them have entered clinical trials. In particular, the length of the spacers were mainly investigated within a three-atom distance from the 3-oxygen to distal aryl groups. [10-20] Among them, 3-*O*-acylide featured with a pyridyl acetyl (α-methoxyimino) group [12, 19], 3-*O*-alkylide featured with a 1-propenyl pyrimidine [17], and 3-*O*-carbamolide featured with an (*R*)-pyrrolidin-2-yl pyridine [18] (compounds A-D as shown Fig. 3) were found with high potency against erythromycin-resistant pathogens. These potent non-ketolides have been designed to share common structural characteristics, *i.e.* the spacers are conformationally restricted so that they can orient and approach a novel binding site, the base pair C2620/G2505Er (*Escherichia coli* numbering). [18, 19, 21, 22] On the other hand, the linkers' length, derived from 8a-aza-lactam, a fifteen-membered macrolide, has been studied with an extension to eight atoms. [23, 24] It was found that a hybrid of macrolide-quinolone named macrolone (Fig. 4), possessing a seven-atom-length spacer, was extremely active. [24] However, 15-membered macrolides generally have different structure-activity relationships (SAR) than 14-membered scaffolds. [25] For these reasons, an extensive investigation involving the extension of the three-atom-length spacers attached to the 3-OH of clarithromycin, accompanied with various configurations, would be highly desirable.

<Figure 3>

<Figure 4>

Erythromycin is bacteriostatic and is a complete inhibitor of protein synthesis. However, the ketolides such as telithromycin, lacking the bulky cladinose, supplied the room allowing for the passage of the nascent peptides. Their inability to globally

arrest nascent peptide biosynthesis results in a fatal imbalance of protein production within the cells, which was attributed to the cause of their bactericidal activity against some bacterial species. [26] Thus far, less information has been reported on the bactericidal activity of the non-ketolides. [22] Over half of the clinical antibiotics target the ribosome, and few of them are bactericidal agents. [27] Therefore, a study on the structure-bactericidal activity relationships of non-ketolides is important to unveil the unexplored bactericidal mechanisms associated with their effects on bacterial ribosomes.

2. Results and Discussion

2.1 Chemistry

As incorporation of an 11,12-cyclic carbonate and a 9-oxime are beneficial for enhancement of antibacterial activity, [28] we took 9-oxime clarithromycin 11,12-cyclic carbonate as the framework, and then endowed it with 3- to 8-atom-length linkers from the 3-oxygen to distal heteroaryl groups in order to reveal the structure-activity relationships.

As mentioned above, the rigidity of the spacers within a three-atom linker length is critical for the antimicrobial activity of the potent non-ketolides (Fig. 3). We first prepared **3a - 3i** possessing a propyne linker terminated by a variety of mono aryl groups from **1** by propargylation to afford **2** and subsequent Sonogashira reaction (Scheme 1). The starting material **1** was prepared in high yield from commercially available erythromycin 9-oxime in seven steps. [29] Direct hydrolysis of **1** produced **4** as a comparator of **3a - 3i**. Further derivatization at the 9-allyl oxime (**5**, *i.e* 2'-*O*-acetate of **3b**) by Heck reaction led to the 3-OH **6b**, instead of the desired **6a**, due to unexpected dealkylation of the original sidechain at 3-O (Scheme 2). The same compound **6b** was also obtained when using **2** as a precursor for the Heck reaction. In other words, the 3-*O*-propargyl group attached to 3-O is labile under the Heck reaction conditions.

<Scheme 1>

<Scheme 2>

Next, we attempted to install two separate aryl groups at the end of the 3-sidechain of the aglycone in a linear array to probe the potential binding sites. Thus, the related reagents **8a** and **8b** were prepared from the acid **7**, as depicted in Scheme 3. A methyl group regioselectively replaced the acetyl group of the 9-oxime **9**, which was prepared according to the reference [30], in the presence of a base (Scheme 4). The resulting product **10** reacted with bis(trichloromethyl)carbonate (BTC) to afford the 11,12-carbonate **11**. Then, **12** was obtained by propargylation at the 3-O position. Sonogashira coupling of **12** followed by methanolysis of the 2'-acetate produced the target compounds **13a - 13e**.

<Scheme 3>

<Scheme 4>

To achieve the goal of elongation of the spacers, treatment of **11** with 3-chloropropionyl chloride and triethylamine in MeCN followed by methanolysis afforded the key intermediate **14** by a modified procedure (Scheme 5). [31] Michael addition of 3-aminopropyne (or allylamine hydrochloride) to **14** in MeOH gave **15** (or **17**), which was then converted to the unsaturated compound **16** (or **18**) by Sonogashira (or Heck) coupling reaction, as shown in Scheme 5. It should be noted that hydrogenation of **16** and **18** in methanol resulted in the removal of the sidechains with formation of the 3-OH groups due to methanolysis of the esters.

<Scheme 5>

To further compare the effects of the flexibility and the length of the sidechains on antibacterial activity, a series of amino-capped saturated spacers **23a - 23e** and **27** were prepared by employing the Cbz (benzyloxycarbonyl)-protected aminopropyne (or aminobutyne) **21** (or **25**) as starting materials, incorporation of aryl groups in **22** (or **26**), and deprotection under 2-3 atm H_2, as illustrated in Scheme 6. Following the Michael addition procedure, **23a - 23d** reacted with **14**, resulting in the seven-atom-length spacer in compounds **28a - 28d** (Scheme 7), while **27** in reaction with **14** yielded the eight-atom-length spacer in compound **29** (Scheme 7). It was

previously reported that installation of **30** into a 15-membered 8a-lactam conferred high efficacy, [24] so a 14-membered counterpart **31** was synthesized by Michael addition of **30** to **14** (Scheme 7).

<Scheme 6>

<Scheme 7>

The length of the spacers was then shortened to five or six atoms by replacement of the acryloyl group with a substituted carbamate (Scheme 8). Compound **11** was subjected to CDI (carbonyl diimidazole) and DMAP (4-dimethylaminopyridine) in DCM (dichloromethane), and then addition of aminopropyne led to the propargylcarbamate **32**. Methanolysis followed by Sonogashira reaction with the corresponding ArBr or ArI produced **33a - 33b** with the rigid linkers. Further hydrogenation yielded **34a - 34b** with the flexible linkers. Compound **34e** was obtained from **11**, which was successively treated with CDI, **23e**, and MeOH (Scheme 8). Similarly, **35**, which is analogous to **34a** but with elongation with a methylene unit in the spacer, was prepared by treatment of **11** with CDI, **27**, and MeOH (Scheme 8).

<Scheme 8>

2.2 Structure-activity relationships of non-ketolides featured with three- to eight-atom-length spacers at 3-OH

The compounds **3a - 3i**, **4**, **13a - 13e**, **15 - 18**, **28a - 28d**, **29**, **31**, **33a**, **34a - 34b**, **34e**, and **35** were tested against a panel of erythromycin-resistant and erythromycin-susceptible clinical isolates and ATCC stains, including Gram-positive bacteria (*Staphylococcus aureus*, *Streptococcus pneumoniae*, *Streptococcus pyogenes* and *Staphylococcus epidermidis*) and Gram-negative bacteria (*Haemophilus influenzae* and *Moraxella catarrhalis*) by the standard method of two-fold broth dilution. [22] Clarithromycin, azithromycin, telithromycin and ciprofloxacin were used as reference compounds.

The antibacterial assays indicated that introduction of 3-*O*-3-substituted pyridyl-2-propargyl (**3a - 3i**) resulted in 2 - 4 fold improvement of activity in comparison with their 3-OH analog **4** (See Table 1). However, varying the mono aryl

groups did not improve activity against susceptible bacteria relative to the controls, clarithromycin and azithromycin. Among **3a - 3i**, **3b** was slightly more active than the others. To further improve the potency, we initially attempted to synthesize a **3b** derivative, *i.e.* compound **6a**, which features of two sidechains. Unfortunately, it was not possible to synthesize it because the 3-*O*-propargyl group of **5** proved to be labile in the subsequent Heck reaction.

<Table 1>

We substituted 9-methyl oxime for 9-allyl oxime and speculated that two aryl groups bridged by zero- to two-atom-length intervals (**13a -13e**) might be suitable for probing potential rRNA binding sites. Unfortunately, **13a -13e** generally had higher minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs) compared to the controls against most of the tested strains, as presented in Table 2. Unlike **13a -13b** and **13d**, **13c** and **13e** sharing the hydrophobic phenyl group instead of hydrophilic heteroaryl groups were weakly active against the constitutively resistant *S. aureus* (MIC: 32 μg/mL), which was not susceptible to telithromycin and clarithromycin (MICs: >256 μg/mL). Moreover, **13c** and **13e** (MBCs: 8-16 μg/mL) had bactericidal activity comparable to clarithromycin against efflux-resistant *S. pneumonia* PU09 and inducibly resistant *S. pyogenes* 01-968.

<Table 2>

Next, we used the 3-OH to attach an acryloyl group and constructed 7-atom to 8-atom-length rigid or flexible spacers terminated by a variety of heteroaryl groups. The antibacterial data are listed in Table 3. The propyl linker in **28a** was superior to the propargyl **16** and the propenyl **18**, as emphasized by the 16-fold greater potency against inducibly resistant *S. pyogenes* 01-968 (MIC: 0.5 μg/mL). The relative potencies of **16**, **18** and **28a** indicated that the rigidity of the spacers at 3-O does not improve antimicrobial activity. Meanwhile, introduction of a pyridyl group to the unsubstituted rigid linkers (**15** and **17**) diminished the efficacy of the resulting compounds **16** and **18**, which confirmed that rigid linkers cannot be well accommodated by the pocket formed by the bacterial ribosomal RNA bases.

Therefore, we kept the flexible linkers constant and then examined the influence of various aromatic groups that were anchored at the end of the linkers on activities (**28a** - **28d**). Of the four compounds, **28c** and **28d** were weakly active against multidrug-resistant *S. aureus* PU20 (MICs: **28c** and **28d**, 64 μg/mL vs telithromycin, > 256 μg/mL vs ciprofloxacin, 256 μg/mL). However, the pyridine **28a** showed a well-balanced antibacterial profile. For example, the quinolone **28b**, the phenylimidazole **28c**, and the tetrahydroquinoline **28d** were less active than **28a** against inducibly resistant *S. pyogenes* 01-968 (MICs: 8 - 32 μg/mL vs. 0.5 μg/mL of **28a**). Elongating the linker of **28a** by one more methylene led to the homologue **29**, which generally retained the activities of **28a** with the exception of the activity against inducibly resistant *S. pyogenes* 01-968 (MICs: **29**, 64 μg/mL vs **28a**, 0.5 μg/mL) and the activity against inducibly resistant *S. aureus* PU32 (MICs: 128 μg/mL of **29** vs 32 μg/mL of **28a**). It was reported that when **30** was introduced to a 15-membered 8a-lactam, the resulting macrolone (compound E as shown in Fig. 4) had amazingly high potency. [24] For the purpose of comparison, the 14-membered counterpart **31** was prepared. However, the desired macrolone **31**, with an ethylenediamine spacer, was inferior to the pyridine **28a** and the quinolone **28b**, both possessing a propylamine linker. This decreased activity of **31**, contrary to compound E, can be attributed to the different macrocyclic conformations of 14-membered macrolides relative to 15-membered macrolides.

Finally, a shrinkage of linker length to five or six atoms was evaluated with compounds **33**, **34**, and **35**. The same trend was observed when comparing the rigid and less potent **33a** to the flexible but highly potent **34a**. Moreover, **34a** significantly decreased the MIC values, in comparison with the parent clarithromycin, against the clinically resistant isolates except for *H. influenzae*. Conversion of the terminal pyridine **34a** to the quinolone **34b** or the aminopyridine **34e** significantly decreased the potency. Remarkably decreased activity was observed when the sidechain was extended by a methylene unit (**34a** vs **35**).

The non-ketolide **34a** exhibited at least 32-fold higher bactericidal activity

against *mef*-encoded resistant *S. pneumoniae* PU09, *erm*-mediated resistant *S. pyogenes* 01-968, and *erm*-encoded resistant *S. aureus* PU32, in comparison with the parent clarithromycin. Actually, **34a** acquired equal and even superior bactericidal activity in comparison to telithromycin against susceptible *S. pneumoniae* ATCC49619 (MICs: 0.125 μg/mL of **34a** vs 0.125 μg/mL of telithromycin) and resistant *S. pneumoniae* PU09 (MICs: 0.5 μg/mL of **34a** vs 4 μg/mL of telithromycin). It was previously speculated that the ketolide telithromycin, unlike erythromycin, lacks the bulky 3-*O*-cladinose so that it supplied the nascent peptides with more space for the bypass. [27] This structural difference is viewed as an explanation for the bactericidal activity of telithromycin. However, we proved that introduction of a pyridylpropyl carbamoyl moiety at 3-OH could enable the non-ketolides to acquire bactericidal activity against the erythromycin-resistant pathogens.

<Table 3>

2.3 Molecular modelling

The molecular modelling was based on the X-ray crystal structure of a carbamolide in complex with the *Deinococcus radiodurans* 50S ribosomal subunit (PDB ID 4IO9). [18] The molecular docking as presented in Fig. 5 suggested the most potent compound **34a** might interact with the bacterial ribosomal base G2484Dr (G2505, *Escherichia coli* numbering) via π - π stacking. Meanwhile, a weaker lone pair - π contact contributed by the pyridine of **34a** and perpendicular U2590 was observed. In contrast, a little less potent **33a** having a rigid linker posed similarly to flexible **34a**, but the nitrogen atom of the pyridine of **33a** rotated by 120º relative to that of **34a**. The considerably less potent **35** oriented its pyridine ring away from the position close to G2484 due to the longer sidechain. The molecular modelling may provide a rational explanation of the observed structure-activity relationships.

It was previously reported that the non-ketolides with shorter and conformationally restricted spacers at the 3-postion targeted rRNA base G2484Dr (G2505Ec). [18, 19, 29] This study suggested the possibility of a structurally different chemotype **34a** with a prolonged and flexible linker interacting at the same binding

site G2484Dr (G2505Ec).

<Figure 5>

2.4 Bactericidal curves

According to time- or concentration-killing bacteria relationships, antibiotics can be divided into a time-dependent group, a concentration-dependent group, and a mixture of the two groups. For example, macrolides are generally time-dependent while quinolones are concentration-dependent. Bactericidal curves indicated that both **34a** and telithromycin are time-dependent agents because different concentrations exerted little impact on bactericidal effects (Fig. 6).

Telithromycin possessed bactericidal effects (reducing CFU by no less than three log units) against erythromycin-susceptible *S. pneumoniae* ATCC49619 at all MICs. In contrast, **34a** reduced two log units at 1×MIC, but it was bactericidal at the higher MICs. With regard to efflux-resistant *S. pneumoniae* PU09, telithromycin and **34a** showed nearly the same bactericidal curve at various MICs. For erm-mediated *S. pyogenes* 01-968, **34a** presented bactericidal effects at the time point of 12 h, but the reduced number of colonies bounced up to ≤ three log units at 24 h. A similar trend was observed for telithromycin at 1×MIC.

<Figure 6>

2.5 In vivo pharmacokinetic (PK) properties and cytochrome P450 (CYP) inhibition

Pharmacokinetic testing in male SD rats via oral administration suggested **34a** exhibited both longer half-life and higher plasma concentration than telithromycin (Figure S1 in Supplementary Material). Encouragingly, each of the PK properties of **34a** is better than telithromycin (Table 4), such as 7-fold higher AUC (area under curve), 7-fold slower systematic plasma clearance (CL), 2.4-fold longer half-life ($t_{1/2}$), 1.4-fold longer mean residence time (MRT), 5-fold higher C_{max} (the highest drug concentration observed in plasma) and 0.4-fold shorter T_{max} (time at which C_{max} is observed). Because the antibacterial effects of time-dependent drugs heavily depend on the time when the blood concentration is beyond the MIC, the PK properties of **34a** are favorable to increase the exposure time of pathogens to bactericidal

concentrations.

Unfortunately, **34a** is a medium inhibitor of human liver CYP 3A4 (IC50: 2.5 μM; telithromycin, 11.8 μM; Table 4 and Figure S2 in Supplementary Material). As CYP 3A4 is responsible for metabolism of half of the marketed drugs, [32] the chemical use of **34a** might lead to drug-drug interaction. Therefore, further structural modification of **34a** will be required to find safer non-ketolide candidates while retaining good PK properties.

<Table 4>

3. Conclusion

A series of novel erythromycin derivatives were designed that have variable linker chain lengths and rigidities. The SAR indicated that flexile spacers confer greater activities when they were examined within a range of 3-atom to 8-atom lengths. Among the designed non-ketolides, **34a** featured with a 5-atom-length saturated spacer anchored with a pyridyl group is the most potent. Variation of the rigidity and length of the spacers, and conversion of the terminal pyridine to various quinolones and other aryl groups, led to decreased activity.

Surprisingly, **34a** is even more active than the reference ciprofloxacin against inducibly resistant *S. aureus* PU32 (a strain that is resistant to methicillin, ciprofloxacin and erythromycin), susceptible *S. pneumoniae* ATCC49619, and efflux-resistant *S. pneumoniae* PU09 as revealed by an 8- to 16-fold improvement of MBC values. Bactericidal curves indicated that **34a**, similar to telithromycin, is a time-dependent antibiotic rather than concentration-dependent one. In vivo tests in SD rats via oral administration suggested **34a** has much superior PK properties over telithromycin. This study suggested erythromycin can be structurally modified to a non-ketolide version, serving as a probe for study on less-explored underlying bactericidal mechanisms associated with bacterial ribosomes.

4. Experimental Section

4.1 MIC and MBC assays

Each strain of bacteria was purified by plate activation before the experiment, and fresh bacteria were used in the assays. The final concentration of bacterial solution was about 5 ×10^5 CFU/ml. Standard ATCC strains and positive antibiotics were used as quality control in each experiment where the MIC values should be within the suggested MIC ranges, and bacterial solution without antibiotics was used as growth control. *Staphylococcus* and *Moraxella catarrhalis* were incubated in CAMHB medium at 35 □ for 20 hours. *Streptococcus* was incubated in CAMHB medium with 5% horse serum at 35 □ for 20 - 24 hours. *Haemophilus* was incubated in HTM broth medium with 1% the reagent SR158 at 35 □ for 20 - 24 hours. MICs were determined from 0.002/0.032 to 64/256 μg/mL by the standard two-fold broth dilution method recommended by the Clinical and Laboratory Standards Institute. [33] The samples from 1 - 8 fold MIC were transported to the culture medium and subcultured at 35 □ for 24 h in the absence of antibiotics. Then, the lowest concentrations where the number of colonies was reduced to > 3Log_{10} were read as MBCs.

4.2 Docking methods

The crystal structure of the *Deinococcus radiodurans* 50S ribosomal subunit in complex with a carbamolide was retrieved from the Protein Data Bank (code: 4IO9). Only residues within 30 Å of the carbamolide were kept to simplify the calculation. The 9-carbonyl group was changed to an oxime group. The carbamate nitrogen on the 11-position was changed to an oxygen. The carbamate side chain on the 3-position was removed. The docking calculation was performed using the GOLD 3.0.1 program through the genetic algorithm. The carbamate sidechains of compounds **33a**, **34a** and **35** were docked into the receptor binding site through a covalent attachment to the oxygen on the 3-position. Each sidechain was docked 10 times and the best docking solution according to the GoldScore fitness function was chosen as the binding conformation.

4.3 Bactericidal curves testing

A certain concentration of tested compounds (1×MIC, 2×MIC, 4×MIC, 8×MIC or

16×MIC) and bacterial solution (*Streptococcus pneumoniae* or *Streptococcus pyogenes*, about 1×10^6 CFU/mL) were added to the CAMHB medium containing 5% horse serum. The mixture was incubated at 35 □ and the samples were taken at the time points of 1, 2, 4, 6, 8, 12, and 24 h. The samples were diluted according to the turbidity observed by the naked eyes. Three- to four-fold dilutions were made and subcultured at 35 °C for 20 - 24 h. The number of colonies was counted and a bactericidal curve was drawn in which the number of colonies (log) changes with time (h).

4.4 In vivo PK testing and CYP inhibition assays

Telithromycin and **34a** were formulated in saline solution containing 1% DMF/ 20% hydroxypropyl-β-cyclodextrin (w/v). [34] In vivo PK data were obtained in 2-3 male SD rats by oral administration of a single dose of 20 mg/Kg. The blood samples were collected at the time points of 0.25, 0.5, 1, 2, 4, 6, 8 and 24 h. The blood samples were centrifuged at 2500 g for 5 minutes at 4 °C. Quantitative analysis of drug concentrations was performed by LC-MS/MS. The pharmacokinetic parameters for the tested compounds such as half-life ($t_{1/2}$), the highest drug concentration observed in plasma (C_{max}), time at which C_{max} is observed (T_{max}), systematic plasma clearance (CL), mean residence time (MRT) and the area under curve (AUC) were calculated by WinNonlin software (Version 5.2).

A mixture of the human liver microsome CYP 3A4 (final concentration: 0.03 mg/mL), the substrate (midazolam, final concentration: 5.00 μM) and 0.1 M potassium phosphate buffer (pH 7.4) with 12 mM $MgCl_2$ (final concentration: 0.72 mg/mL) was warmed at 37 ºC for 5 min. The tested compounds (0 - 25.00 μM ; or ketoconazole as a positive inhibitor, 0 - 0.25 μM) and 25 mM NADPH solution (final concentration: 1.5 mM) were added and the mixture was incubated at 37 ºC for 10 min. The metabolites were detected by HPLC (ACQUITY UPLC C18, 1.7 μm, 50 x 2.1 mm I.D., Waters; gradient acetonitrile/water as eluents at a flow rate of 0.5 mL/min) and a triple quadrupole mass spectrometer (API 5500). The peak area ratio of metabolite/internal standard was determined for each metabolite. The IC50 values

were calculated using commercial software Graphpad Prism (Version 7).

4.5 Synthetic procedures

All solvents and reagents were obtained from commercial sources (InnoChem; J&K scientific) and used without further purification unless otherwise noted. Column chromatography was performed on silica gel (100-200 or 200-300 mesh). ^{1}H and ^{13}C spectra were taken in $CDCl_3$ on Bruker Ascend 400, ARX 500 or Ascend 700 MHz spectrometers with tetramethylsilane (TMS) as an internal standard. High resolution mass spectra (HRMS) were obtained with Agilent Q-TOF 6520 LC/MS or Bruker Apex IV FT. The purities of the target compounds for antibacterial evaluation were examined by high-performance TLC under the conditions of different eluents and visualization, and further confirmed by NMR spectra by comparing integral calculation of the number of hydrogen atoms of impurities.

*4.5.1. 3-O-Descladinosyl-3-O-acryloyl-6-O-methylerythromycin A 9-O-methyl oxime-11, 12-cyclic carbonate (**14**)*

To a solution of **11** (0.7235 g, 1.05 mmol) and triethylamine (1.468 mL, 10.53 mmol) in acetonitrile (15 mL) was added dropwise a solution of 3-chloropropionyl chloride (0.288 ml, 2.95 mmol) in acetonitrile (15 mL). The reaction mixture was stirred at 80 □ for 3 h and then the solvent was removed under reduced pressure. The mixture was extracted with ethyl acetate and the organic layer was washed successively with water and brine. The organic solvent was removed in vacuum and dried to afford 2'-OAc-**14** (0.9762 g, 1.32 mmol, 125.7%).

A solution of 2'-OAc-**14** (0.9762 mg, 1.32 mmol) in MeOH (20 mL) was stirred at 65 □ for 3 h. The organic solvent was removed in vacuum and dried to afford **14** (0.7905 g, 1.13 mmol, 85.6%) as a dark yellow solid.

*4.5.2. 3-O-Descladinosyl-3-O-[3'-propargylamino-propionyl]-6-O-methylerythromycin A 9-O-methyl oxime-11, 12-cyclic carbonate (**15**)*

To a solution of **14** (0.7905 g, 1.13 mmol) and ethyldiisopropylamine (0.985 mL,

5.9 mmol) in MeOH (15 mL) was added 3-aminopropyne (0.23 mL, 3.54 mmol). The reaction mixture was stirred at 60 □ for 5 h and then the solvent was removed under reduced pressure. The mixture was extracted with dichloromethane and the organic phase was washed successively with water and brine. The organic solvent was removed in vacuum. The residue was purified by column chromatography on silica gel ($CH_2Cl_2/C_2H_5OH/NH_3•H_2O$, 10:0.2:0.1) to afford **15** (0.4926 g, 0.65 mmol, 57.5%).

HRMS (ESI) $(M+H)^+$ *m/z* 754.4484, Calcd for $C_{38}H_{64}N_3O_{12}$ 754.4485. ^{1}H NMR ($CDCl_3$, 400 MHz), δ: 5.16 (dt, *J* = 10.8, 1.7 Hz, 1 H, H-13), 5.07 (d, *J* = 11.0 Hz, 1 H, H-3), 4.88 (s, 1 H, H-11), 3.95 (d, *J* = 7.2 Hz, 1 H, H-1'), 3.82 (s, 3 H, 9-*O*-C**H**$_3$), 3.75-3.69 (m, 1 H, H-8), 3.67 (d, *J* = 3.3 Hz, 1 H, H-5), 3.45-3.42 (m, 2 H, 3-*O*-C**H**$_2$C≡C), 3.41-3.37 (m, 1 H, H-5'), 3.16 (dd, *J* = 10.1, 7.2 Hz, 1 H, H-2'), 3.02 (s, 3 H, 6-OC**H**$_3$), 2.98 (t, *J* = 6.4 Hz, 2 H, -N-C**H**$_2$), 2.95-2.85 (m, 1 H, H-2), 2.63 (t, *J* = 6.4 Hz, 2 H, 3-*O*-CO-C**H**$_2$-), 2.52-2.4 (m, 2 H, H-10, H-3'), 2.28 (s, 6 H, -N(CH_3)$_2$), 2.22 (q, *J* = 2.1 Hz, 1 H, H-propargyl), 2.14-2.06 (m, 1 H, H-4), 1.96-1.85 (m, 1 H, H-14eq), 1.68-1.62 (m, 1 H, H-4'eq), 1.60-1.53 (m, 1 H, H-14ax), 1.49 (s, 3 H, 12-CH_3), 1.45-1.37 (m, 1 H, H-7a), 1.34 (s, 3 H, 6-CH_3), 1.32-1.27 (m, 1 H, H-7b), 1.22 (t, *J* = 6.3 Hz, 6 H, 5'-CH_3, 10-CH_3), 1.14-1.08 (m, 6 H, 2-CH_3, 4-CH_3), 0.93 (d, *J* = 7.0 Hz, 3 H, 8-CH_3), 0.86 (t, *J* = 7.3 Hz, 3 H, 15-CH_3); ^{13}C NMR ($CDCl_3$, 175 MHz), δ: 173.9, 172.3, 164.5, 154.7, 103.8, 84.9, 83.2, 81.8, 78.5, 78.0, 75.7, 71.6, 70.4, 69.6, 65.9, 61.4, 49.8, 43.8, 43.2, 40.3, 38.2, 37.3, 36.5, 34.8, 32.7, 28.4, 25.6, 22.2, 21.2, 19.4, 18.8, 15.6, 15.3, 13.0, 10.2, 8.9.

4.5.3.

*3-O-Descladinosyl-3-O-[3'-(3''-(3'''-pyridyl)propargylamino)-propionyl]-6-O-methyle rythromycin A 9-O-methyl oxime-11, 12-cyclic carbonate (**16**)*

To a solution of **15** (0.4926 g, 0.65 mmol), CuI (12 mg, 0.063 mmol), $(PPh_3)_2PdCl_2$ (23 mg, 0.033 mmol) and triethylamine (0.136 mL, 0.975 mmol) in acetonitrile (15 mL) was added 3-bromopyridine (0.189 mL, 1.95 mmol). The

reaction mixture was recharged with argon and stirred at 80 □ for 3 h in a sealed tube. The mixture was extracted with ethyl acetate and the organic layer was washed successively with water and brine. The organic solvent was removed in vacuum. The residue was purified by column chromatography on silica gel ($CH_2Cl_2/C_2H_5OH/NH_3•H_2O$, 10:0.2:0.1) to afford **16** (0.135 g, 0.162 mmol, 24.9%).

HRMS (ESI) $(M+H)^+$ *m/z* 831.4760, Calcd for $C_{43}H_{67}N_4O_{12}$ 831.4750. ^{1}H NMR ($CDCl_3$, 400 MHz), δ: 8.64 (s, 1 H, pyridine), 8.53 (d, *J* = 4.7 Hz, 1 H, pyridine), 7.74-7.65 (m, 1 H, pyridine), 7.24 (dd, *J* = 7.9, 5.1 Hz, 1 H, pyridine), 5.16 (dt, J_1 = 10.9 Hz, J_2 = 1.9 Hz, 1 H, H-13), 5.08 (d, *J* = 11.0 Hz, 1 H, H-3), 4.88 (s, 1 H, H-11), 3.95 (dd, *J* = 7.3, 1.4 Hz, 1 H, H-1'), 3.82 (s, 3 H, 9-*O*-C**H**$_3$), 3.75-3.64 (m, 4 H, H-5, H-8, 3-*O*-C**H**$_2$C≡C), 3.45-3.35 (m, 1 H, H-5'), 3.16 (dd, *J* = 10.4, 6.8 Hz, 1 H, H-2'), 3.10-3.00 (m, 5 H, 6-*O*-C**H**$_3$, -N-C**H**$_2$), 2.93-2.83 (m, 1 H, H-2), 2.67 (t, *J* = 6.3 Hz, 2 H, 3-*O*-CO-C**H**$_2$-), 2.53-2.40 (m, 2 H, , H-10, H-3'), 2.27 (s, 6 H, -N(CH_3)$_2$), 2.15-2.07 (m, 1 H, H-4), 1.95-1.85 (m, 1 H, H-14eq), 1.65-1.55 (m, 2 H, H-4'eq, H-14ax), 1.49 (s, 3 H, 12-CH_3), 1.44-1.37 (m, 1 H, H-7a), 1.34 (s, 3 H, 6-CH_3), 1.32-1.27 (m, 1 H, H-7b), 1.23 (d, *J* = 6.8 Hz, 3 H, 5'-CH_3), 1.17 (d, *J* = 6.0 Hz, 3 H, 10-CH_3), 1.14-1.05 (m, 6 H, 2-CH_3, 4-CH_3), 0.93 (d, *J* = 7.0 Hz, 3 H, 8-CH_3), 0.86 (t, *J* = 7.3 Hz, 3 H, 15-CH_3); ^{13}C NMR ($CDCl_3$, 175 MHz), δ: 173.9, 172.4, 164.5, 154.7, 152.3, 148.6, 138.5, 123.0, 120.2, 103.8, 90.8, 84.9, 83.2, 81.9, 80.4, 78.5, 78.0, 75.7, 70.3, 69.6, 66.0, 61.4, 49.8, 44.0, 43.2, 40.3, 39.1, 37.3, 36.5, 34.8, 32.7, 28.4, 25.6, 22.2, 21.2, 19.4, 18.8, 15.6, 15.3, 13.0, 10.2, 8.9.

*4.5.4. 3-O-Descladinosyl-3-O-[3'-allylamino-propionyl]-6-O-methylerythromycin A 9-O-methyl oxime-11, 12-cyclic carbonate (**17**)*

To a solution of **14** (1.0147 g, 1.45 mmol) and ethyldiisopropylamine (1.26 mL, 7.24 mmol) in MeOH (20 mL) was added allylamine hydrochloride (0.41 g, 4.35 mmol). The reaction mixture was stirred at 60 □ for 5 h and then the solvent was removed under reduced pressure. The mixture was extracted with dichloromethane

and the organic phase was washed successively with water and brine. The organic solvent was removed in vacuum. The residue was purified by column chromatography on silica gel ($CH_2Cl_2/C_2H_5OH/NH_3•H_2O$, 10:0.3:0.1) to afford **17** (0.058 g, 0.077 mmol, 5.3%).

HRMS (ESI) $(M+H)^+$ *m/z* 756.4626, Calcd for $C_{38}H_{66}N_3O_{12}$ 756.4641. ^{1}H NMR ($CDCl_3$, 400 MHz), δ: 5.88 (ddt, *J* = 16.6, 11.4, 5.9 Hz, 1 H, allyl), 5.25-5.15 (m, 2 H, allyl), 5.09 (t, *J* = 11.8 Hz, 2 H, H-3, H-13), 4.88 (s, 1 H, H-11), 3.94 (d, *J* = 7.2 Hz, 1 H, H-1'), 3.82 (s, 3 H, 9-*O*-C**H**$_3$), 3.75-3.62 (m, 2 H, H-5, H-8), 3.45-3.35 (m, 1 H, H-5'), 3.27 (d, *J* = 6.0 Hz, 2 H, CH_2=CH-C**H**$_2$), 3.16 (dd, *J* = 10.0, 7.3 Hz, 1H, H-2'), 3.02 (s, 3 H, 6-*O*-C**H**$_3$), 2.95-2.83 (m, 3 H, -N-C**H**$_2$, H-2), 2.63 (t, *J* = 6.5 Hz, 2 H, 3-*O*-CO-C**H**$_2$-), 2.51-2.40 (m, 2 H, H-10, H-3'), 2.27 (s, 6 H, -N(CH_3)$_2$), 2.15-2.05 (m, 1 H, H-4), 1.95-1.85 (m, 1 H, H-14eq), 1.65-1.55 (m, 2 H, H-4'eq, H-14ax), 1.49 (s, 3 H, 12-CH_3), 1.44-1.36 (m, 1 H, H-7a), 1.34 (s, 3 H, 6-CH_3), 1.32-1.27 (m, 1 H, H-7b), 1.22 (t, *J* = 7.3 Hz, 6 H, 5'-CH_3, 10-CH_3), 1.10 (dd, *J* = 7.0, 3.2 Hz, 6 H, 2-CH_3, 4-CH_3), 0.93 (d, *J* = 7.0 Hz, 3 H, 8-CH_3), 0.86 (t, *J* = 7.3 Hz, 3 H, 15-CH_3); ^{13}C NMR ($CDCl_3$, 175 MHz), δ: 173.9, 172.5, 164.5, 154.7, 136.5, 116.2, 103.7, 85.0, 83.2, 81.6, 78.5, 77.9, 75.7, 70.4, 69.6, 65.9, 61.4, 52.4, 49.9, 44.3, 43.2, 40.3, 37.3, 36.5, 35.0, 32.7, 28.4, 25.6, 22.2, 21.2, 19.4, 18.8, 15.6, 15.3, 13.0, 10.2, 8.9.

4.5.5.

*3-O-Descladinosyl-3-O-[3'-(3''-(3'''-pyridyl)allylamino)-propionyl]-6-O-methylerythromycin A 9-O-methyl oxime-11, 12-cyclic carbonate (**18**)*

To a solution of **17** (0.5611 g, 0.742 mmol), palladium acetate (33 mg, 0.148 mmol) and tri(*o*-tolyl)phosphine (90 mg, 0.297 mmol) in acetonitrile (10 mL) were added 3-bromopyridine (0.215 mL, 2.23 mmol) and triethylamine (0.206 mL, 1.48 mmol). The reaction mixture was flushed with argon and sealed in a pressure tube. The reaction mixture was stirred at 60 ºC for 1 h and thereafter at 90 ºC for 24 h. The reaction mixture was extracted with ethyl acetate, washed with water and brine and concentrated in vacuum. The crude mixture was purified by column chromatography

on silica gel ($CH_2Cl_2/C_2H_5OH/NH_3•H_2O$, 10:0.5:0.1) to afford **18** (0.188 g, 0.226 mmol, 30.5 %).

HRMS (ESI) $(M+H)^+$ *m/z* 833.4907, Calcd for $C_{43}H_{69}N_4O_{12}$ 833.4907. ^{1}H NMR ($CDCl_3$, 700 MHz), δ: 8.60 (s, 1 H, H-pyridine), 8.53 (d, *J* = 4.7 Hz, 1 H, H-pyridine), 7.70 (d, *J* = 7.8 Hz, 1 H, H-pyridine), 7.26 (dd, *J* = 7.9, 4.8 Hz, 1 H, H-pyridine), 6.62-6.31 (m, 2 H, H-allyl), 5.18 (dd, *J* = 10.6, 2.5 Hz, 1 H, H-13), 5.09 (d, *J* = 11.0 Hz, 1 H, H-3), 4.89 (s, 1 H, H-11), 3.96 (d, *J* = 7.2 Hz, 1 H, H-1'), 3.83 (s, 3 H, 9-*O*-C**H**$_3$), 3.74-3.64 (m, 2 H, H-5, H-8), 3.44-3.36 (m, 1 H, H-5'), 3.21-3.14 (m, 1H, H-2'), 3.03-2.95 (m, 5 H, 6-*O*-C**H**$_3$, -NH-C**H**$_2$), 2.93-2.85 (m, 1 H, H-2), 2.69 (s, 2 H, 3-*O*-CO-C**H**$_2$-), 2.53-2.45 (m, 2 H, , H-10, H-3'), 2.34-2.22 (m, 6 H, -N(CH_3)$_2$), 2.15-2.09 (m, 1 H, H-4), 1.95-1.87 (m, 1 H, H-14eq), 1.60-1.54 (m, 2 H, H-4'eq, H-14ax), 1.50 (s, 3 H, 12-CH_3), 1.34 (s, 3 H, 6-CH_3), 1.33-1.26 (m, 2 H, H-7a, H-7b), 1.24 (d, *J* = 6.8 Hz, 3 H, 10-CH_3), 1.21-1.19 (m, 3 H, 5'-CH_3), 1.15-1.07 (m, 6 H, 2-CH_3, 4-CH_3), 0.95 (d, *J* = 7.0 Hz, 3 H, 8-CH_3), 0.87 (t, *J* = 7.4 Hz, 3 H, 15-CH_3); ^{13}C NMR ($CDCl_3$, 175 MHz), δ: 173.9, 172.2, 164.5, 154.6, 148.2, 132.8, 123.5, 123.4, 123.3, 103.7, 84.9, 83.2, 81.7, 78.5, 78.1, 75.7, 70.5, 69.6, 66.1, 61.4, 51.5, 49.9, 44.4, 43.1, 40.3, 37.2, 36.5, 34.9, 32.7, 28.5, 25.6, 22.2, 21.1, 19.4, 18.8, 15.6, 15.3, 13.0, 10.2, 8.9.

4.5.6.

*3-O-Descladinosyl-3-O-[3'-(3''-(3'''-pyridyl)-propylamino)propionyl]-6-O-methyleryt hromycin A 9-O-methyl oxime-11, 12-cyclic carbonate (**28a**)*

To a solution of **14** (0.356 g, 0.509 mmol) and ethyldiisopropylamine (0.444 mL, 2.545 mmol) in MeOH (20 mL) was added **23a** (0.2079 g, 1.53 mmol). The reaction mixture was stirred at 60 □ for 5 h and then the solvent was removed under reduced pressure. The mixture was extracted with dichloromethane and the organic phase was washed successively with water and brine. The organic solvent was removed in vacuum. The residue was purified by column chromatography on silica gel ($CH_2Cl_2/C_2H_5OH/NH_3•H_2O$, 10:0.2:0.1) to afford **28a** (0.112 g, 0.134 mmol, 26.3%).

HRMS (ESI) $(M+H)^+$ *m/z* 835.5055, Calcd for $C_{43}H_{71}N_4O_{12}$ 835.5063. ^1H NMR ($CDCl_3$, 400 MHz), δ: 8.45 (s, 2 H, H-pyridine), 7.50 (d, *J* = 7.7 Hz, 1 H, H-pyridine), 7.21 (dd, *J* = 8.0, 4.6 Hz, 1 H, H-pyridine), 5.16 (d, *J* = 10.7 Hz, 1 H, H-13), 5.07 (d, *J* = 11.0 Hz, 1 H, H-3), 4.88 (s, 1 H, H-11), 3.93 (d, *J* = 7.1 Hz, 1 H, H-1'), 3.82 (s, 3 H, 9-*O*-C**H**$_3$), 3.76-3.62 (m, 2 H, H-5, H-8), 3.42-3.30 (m, 1 H, H-5'), 3.16 (t, *J* = 8.7 Hz, 1H, H-2'), 3.02 (s, 3 H, 6-*O*-C**H**$_3$), 2.95-2.83 (m, 3 H, H-2, -CO-C**H**$_2$-CH_2NH-), 2.70-2.59 (m, 6 H, -CO-CH_2-C**H**$_2$NH-, -NH-C**H**$_2$-CH_2-C**H**$_2$-), 2.52-2.35 (m, 2 H, , H-10, H-3'), 2.26 (s, 6 H, -N$(CH_3)_2$), 2.15-2.05 (m, 1 H, H-4), 1.95-1.75 (m, 3 H, H-14eq, -NH-CH_2-C**H**$_2$-CH_2-), 1.65-1.55 (m, 2 H, H-4'eq, H-14ax), 1.49 (s, 3 H, 12-CH_3), 1.44-1.38 (m, 1 H, H-7a), 1.34 (s, 3 H, 6-CH_3), 1.32-1.27 (m, 1 H, H-7b), 1.25-1.17 (m, 7 H, 5'-CH_3, 10-CH_3, H-4'ax), 1.14-1.06 (m, 6 H, 2-CH_3, 4-CH_3), 0.93 (d, *J* = 7.0 Hz, 3 H, 8-CH_3), 0.86 (t, *J* = 7.4 Hz, 3 H, 15-CH_3); ^{13}C NMR ($CDCl_3$, 100 MHz), δ: 173.9, 172.5, 164.5, 154.7, 149.9, 147.5, 137.1, 135.7, 123.3, 103.7, 85.0, 83.2, 81.5, 78.5, 77.9, 75.7, 70.4, 69.6, 66.0, 61.4, 49.9, 49.1, 45.0, 43.2, 40.3, 37.3, 36.5, 35.0, 32.7, 31.4, 30.6, 28.4, 25.6, 22.2, 21.2, 19.4, 18.8, 15.6, 15.3, 13.0, 10.2, 8.9.

4.5.7.

*3-O-Descladinosyl-3-O-[3'-(3''-(3'''-carboxy-1'''-cyclopropyl-1''',4'''-dihydro-4'''-oxo-quinol-6'''-yl)-propylamino)propionyl]-6-O-methylerythromycin A 9-O-methyl oxime-11, 12-cyclic carbonate (**28b**)*

To a solution of **14** (0.22 g, 0.315 mmol) and ethyldiisopropylamine (0.27 mL, 1.55 mmol) in MeOH (20 mL) was added **23b** (0.455 g, 1.59 mmol). The reaction mixture was stirred at 60 □ for 6 h. Following the work-up for **28a**, **28b** was obtained by column chromatography on silica gel (CH_2Cl_2/C_2H_5OH/NH_3•H_2O, 10:0.6:0.1) (88 mg, 0.089 mmol, 28.3%).

HRMS (ESI) $(M+H)^+$ *m/z* 985.5364, Calcd for $C_{51}H_{77}N_4O_{15}$ 985.5380. ^1H NMR ($CDCl_3$, 400 MHz), δ: 8.86 (s, 1 H, H-aryl), 8.30 (s, 1 H, H-aryl), 8.03 (d, *J* = 8.7 Hz, 1 H, H-aryl), 7.71 (d, *J* = 8.7 Hz, 1 H, H-aryl), 5.15 (d, *J* = 8.4 Hz, 1 H, H-13), 5.05 (d, *J* = 10.3 Hz, 1 H, H-3), 4.87 (s, 1 H, H-11), 3.94 (d, *J* = 7.4

Hz, 1 H, H-1'), 3.82 (s, 3 H, 9-*O*-C**H**$_3$), 3.75-3.56 (m, 3 H, H-5, H-8, H-cyclopropyl), 3.41-3.32 (m, 1 H, H-5'), 3.16 (t, *J* = 8.8 Hz, 1H, H-2'), 3.01 (s, 3 H, 6-*O*-C**H**$_3$), 2.95-2.56 (m, 9 H, H-2, 3-*O*-CO-C**H**$_2$-C**H**$_2$-, -NH-C**H**$_2$-CH$_2$-C**H**$_2$-), 2.52-2.35 (m, 2 H, , H-10, H-3'), 2.25 (s, 6 H, -N(CH$_3$)$_2$), 2.15-1.81 (m, 4 H, H-4, -NH-CH$_2$-C**H**$_2$-CH$_2$-, H-14eq), 1.69-1.54 (m, 2 H, H-4'eq, H-14ax), 1.49 (s, 3 H, 12-CH$_3$), 1.45-1.38 (m, 3 H, H-7a, H-cyclopropyl), 1.34 (s, 3 H, 6-CH$_3$), 1.33-1.27 (m, 2 H, H-cyclopropyl), 1.25-1.17 (m, 6 H, 5'-CH$_3$, 10-CH$_3$), 1.13-1.05 (m, 6 H, 2-CH$_3$, 4-CH$_3$), 0.94 (d, *J* = 7.0 Hz, 3 H, 8-CH$_3$), 0.85 (q, *J* = 7.6 Hz, 3 H, 15-CH$_3$); ^{13}C NMR (CDCl$_3$, 175 MHz), δ: 178.5, 174.0, 172.5, 167.1, 164.6, 154.7, 147.7, 140.9, 139.5, 134.7, 128.4, 126.0, 125.7, 117.4, 108.5, 103.7, 85.0, 83.2, 81.5, 78.5, 77.8, 75.7, 70.4, 69.7, 66.0, 61.4, 49.9, 49.1, 45.0, 43.1, 40.3, 37.2, 36.5, 35.4, 35.0, 33.0, 32.7, 31.4, 28.4, 25.6, 22.1, 21.2, 19.4, 18.8, 15.6, 15.3, 13.0, 10.2, 8.9, 8.2.

4.5.8.

*3-O-Descladinosyl-3-O-[3'-(3''-(4'''-(1''''-imidazolyl)phen-1'''-yl)-propylamino)propionyl]-6-O-methylerythromycin A 9-O-methyl oxime-11, 12-cyclic carbonate (**28c**)*

To a solution of **14** (0.181 g, 0.259 mmol) and ethyldiisopropylamine (0.226 mL, 1.3 mmol) in MeOH (20 mL) was added **23c** (0.1568 g, 0.78 mmol). The reaction mixture was stirred at 60 □ for 5 h. Following the work-up for **28a**, **28c** was obtained by column chromatography on silica gel ($CH_2Cl_2/C_2H_5OH/NH_3•H_2O$, 10:0.3:0.1) (103.7 mg, 0.115 mmol, 44.4%).

HRMS (ESI) $(M+H)^+$ *m/z* 900.5335, Calcd for $C_{47}H_{74}N_5O_{12}$ 900.5328. ^{1}H NMR (CDCl$_3$, 400 MHz), δ: 7.82 (s, 1 H, H-phenyl), 7.34-7.27 (m, 5 H, H-phenyl, H-imidazolyl), 7.19 (s, 1 H, H-imidazolyl), 5.16 (d, *J* = 10.6 Hz, 1 H, H-13), 5.07 (d, *J* = 11.0 Hz, 1 H, H-3), 4.88 (s, 1 H, H-11), 3.94 (dd, *J* = 7.4, 2.1 Hz, 1 H, H-1'), 3.82 (s, 3 H, 9-*O*-C**H**$_3$), 3.75-3.63 (m, 2 H, H-5, H-8), 3.42-3.33 (m, 1 H, H-5'), 3.17 (t, *J* = 8.9 Hz, 1H, H-2'), 3.02 (s, 3 H, 6-*O*-C**H**$_3$), 2.98-2.85 (m, 3 H, H-2, 3-*O*-CO-CH$_2$-C**H**$_2$-), 2.74-2.61 (m, 6 H, 3-*O*-CO-C**H**$_2$-CH$_2$-, -NH-C**H**$_2$-CH$_2$-C**H**$_2$-), 2.52-2.38 (m, 2 H, , H-10, H-3'),

2.26 (s, 6 H, -N(CH_3)$_2$), 2.15-2.07 (m, 1 H, H-4), 1.96-1.80 (m, 3 H, H-14eq, -NH-CH_2-C**H**$_2$-CH_2-), 1.66-1.54 (m, 2 H, H-4'eq, H-14ax), 1.49 (s, 3 H, 12-CH_3), 1.46-1.39 (m, 1 H, H-7a), 1.34 (s, 3 H, 6-CH_3), 1.31-1.27 (m, 1 H, H-7b), 1.25-1.18 (m, 7 H, 5'-CH_3, 10-CH_3, H-4'ax), 1.14-1.07 (m, 6 H, 2-CH_3, 4-CH_3), 0.94 (d, *J* = 7.0 Hz, 3 H, 8-CH_3), 0.86 (dt, *J* = 8.5, 4.1 Hz, 3 H, 15-CH_3); ^{13}C NMR ($CDCl_3$, 100 MHz), δ: 174.0, 172.5, 164.5, 154.7, 141.5, 135.6, 135.4, 130.3, 129.7, 121.6, 118.3, 103.7, 85.0, 83.2, 81.5, 78.5, 77.9, 75.7, 70.4, 69.6, 66.0, 61.4, 49.9, 49.3, 45.1, 43.2, 40.3, 37.3, 36.5, 34.9, 33.0, 32.7, 31.6, 28.4, 25.6, 22.2, 21.2, 19.4, 18.8, 15.6, 15.3, 13.0, 10.2, 8.9.

4.5.9.

*3-O-Descladinosyl-3-O-[3'-(3''-(1''',2''',3''',4'''-tetrahydroisoquinol-6'''-yl)-propylamino)propionyl]-6-O-methylerythromycin A 9-O-methyl oxime-11, 12-cyclic carbonate **(28d)***

To a solution of **14** (0.154 g, 0.22 mmol) and ethyldiisopropylamine (0.19 mL, 1.1 mmol) in MeOH (15 mL) was added **23d** (0.123 g, 0.66 mmol). The reaction mixture was stirred at 60 □ for 5.5 h. Following the work-up for **28a**, **28d** was obtained by column chromatography on silica gel (CH_2Cl_2/C_2H_5OH/NH_3•H_2O, 10:0.3:0.1) (94 mg, 0.106 mmol, 48.2%).

HRMS (ESI) $(M+H)^+$ *m/z* 889.5548, Calcd for $C_{47}H_{77}N_4O_{12}$ 889.5533. ^{1}H NMR ($CDCl_3$, 400 MHz), δ: 6.78-6.72 (m, 2 H, H-phenyl), 6.40 (d, *J* = 7.9 Hz, 1 H, H-phenyl), 5.16 (d, *J* = 10.7 Hz, 1 H, H-13), 5.06 (d, *J* = 10.9 Hz, 1 H, H-3), 4.88 (s, 1 H, H-11), 3.93 (d, *J* = 6.7 Hz, 1 H, H-1'), 3.82 (s, 3 H, 9-*O*-C**H**$_3$), 3.75-3.65 (m, 2 H, H-5, H-8), 3.42-3.33 (m, 1 H, H-5'), 3.27 (t, *J* = 5.5 Hz, 2 H, H-piperidine), 3.16 (t, *J* = 8.8 Hz, 1H, H-2'), 3.02 (s, 3 H, 6-*O*-C**H**$_3$), 2.93-2.83 (m, 3 H, H-2, 3-*O*-CO-CH_2-C**H**$_2$-), 2.73 (t, *J* = 6.7 Hz, 2 H, H-piperidine), 2.67-2.57 (m, 4 H, 3-*O*-CO-C**H**$_2$-CH_2-, -NH-CH_2-CH_2-C**H**$_2$-), 2.52-2.36 (m, 4 H, , H-10, H-3', -NH-C**H**$_2$-CH_2-CH_2-), 2.26 (s, 6 H, -N(CH_3)$_2$), 2.12-2.07 (m, 1 H, H-4), 1.96-1.87 (m, 3 H, H-14eq, H-piperidine), 1.77-1.55 (m, 4 H, -NH-CH_2-C**H**$_2$-CH_2-, H-4'eq, H-14ax), 1.49 (s, 3 H, 12-CH_3), 1.45-1.37 (m, 1

H, H-7a), 1.34 (s, 3 H, 6-CH_3), 1.31-1.27 (m, 1 H, H-7b), 1.25-1.17 (m, 7 H, 5'-CH_3, 10-CH_3, H-4'ax), 1.12-1.07 (m, 6 H, 2-CH_3, 4-CH_3), 0.93 (d, *J* = 7.0 Hz, 3 H, 8-CH_3), 0.86 (t, *J* = 7.4 Hz, 3 H, 15-CH_3); ^{13}C NMR ($CDCl_3$, 100 MHz), δ: 174.0, 172.5, 164.5, 154.7, 142.9, 130.4, 129.4, 126.6, 121.5, 114.4, 103.7, 85.0, 83.2, 81.6, 78.5, 77.8, 75.7, 70.4, 69.6, 65.9, 61.4, 49.9, 49.6, 45.1, 43.2, 42.1, 40.3, 37.3, 36.5, 35.0, 32.7, 32.0, 28.4, 27.0, 25.6, 22.4, 22.2, 21.2, 19.4, 18.8, 15.6, 15.3, 13.0, 10.2, 8.9.

4.5.10.

*3-O-Descladinosyl-3-O-[3'-(4''-(3'''-pyridyl)butylamino)propionyl]-6-O-methylerythromycin A 9-O-methyl oxime-11, 12-cyclic carbonate (**29**)*

To a solution of **14** (0.36 g, 0.515 mmol) and ethyldiisopropylamine (0.45 mL, 2.58 mmol) in MeOH (20 mL) was added **27** (0.2342 g, 1.56 mmol). The reaction mixture was stirred at 60 □ for 5 h. Following the work-up for **28a**, **29** was obtained by column chromatography on silica gel (CH_2Cl_2/C_2H_5OH/NH_3•H_2O, 10:0.4:0.1) (121 mg, 0.143 mmol, 27.8%).

HRMS (ESI) $(M+H)^+$ *m/z* 849.5213, Calcd for $C_{44}H_{73}N_4O_{12}$ 849.5220. ^{1}H NMR ($CDCl_3$, 400 MHz), δ: 8.43 (s, 2 H, H-pyridyl), 7.49 (d, *J* = 7.8 Hz, 1 H, H-pyridyl), 7.20 (t, *J* = 6.4 Hz, 1 H, H-pyridyl), 5.16 (d, *J* = 10.6 Hz, 1 H, H-13), 5.06 (d, *J* = 10.9 Hz, 1 H, H-3), 4.88 (s, 1 H, H-11), 3.93 (d, *J* = 7.2 Hz, 1 H, H-1'), 3.82 (s, 3 H, 9-*O*-C**H**$_3$), 3.75-3.63 (m, 2 H, H-5, H-8), 3.40-3.33 (m, 1 H, H-5'), 3.16 (t, *J* = 8.8 Hz, 1H, H-2'), 3.02 (s, 3 H, 6-*O*-C**H**$_3$), 2.95-2.85 (m, 3 H, H-2, 3-*O*-CO-CH_2-C**H**$_2$-), 2.67-2.57 (m, 6 H, 3-*O*-CO-C**H**$_2$-CH_2-, -NH-C**H**$_2$-CH_2-CH_2-C**H**$_2$), 2.52-2.36 (m, 2 H, H-10, H-3'), 2.26 (s, 6 H, -N(CH_3)$_2$), 2.14-2.06 (m, 1 H, H-4), 1.95-1.84 (m, 1 H, H-14eq), 1.72-1.53 (m, 6 H, -NH-CH_2-C**H**$_2$-C**H**$_2$-CH_2, H-4'eq, H-14ax), 1.49 (s, 3 H, 12-CH_3), 1.45-1.36 (m, 1 H, H-7a), 1.34 (s, 3 H, 6-CH_3), 1.32-1.27 (m, 1 H, H-7b), 1.25-1.15 (m, 7 H, 5'-CH_3, 10-CH_3, H-4'ax), 1.13-1.05 (m, 6 H, 2-CH_3, 4-CH_3), 0.93 (d, *J* = 7.0 Hz, 3 H, 8-CH_3), 0.86 (t, *J* = 7.4 Hz, 3 H, 15-CH_3); ^{13}C NMR ($CDCl_3$, 175 MHz), δ: 174.0, 172.5, 164.5, 154.7, 150.0, 147.4, 137.4, 135.8, 123.3, 103.7, 85.0, 83.2, 81.5, 78.5, 77.8, 75.7, 70.3, 69.6, 65.9, 61.4, 49.9, 49.7, 45.1, 43.1, 40.3, 37.2, 36.5, 35.0, 32.9, 32.7,

29.6, 28.8, 28.4, 25.6, 22.1, 21.2, 19.4, 18.8, 15.6, 15.3, 13.0, 10.2, 8.9.

4.5.11.

*3-O-Descladinosyl-3-O-[3'-(2''-(3'''-carboxy-7'''-chloro-1'''-cyclopropyl-1''',4'''-dihydro-4'''-oxo-quinol-6'''-ylamino)ethylamino)propionyl]-6-O-methylerythromycin A 9-O-methyl oxime-11, 12-cyclic carbonate (**31**)*

To a solution of **14** (0.5 g, 0.68 mmol) and ethyldiisopropylamine (0.59 mL, 3.4 mmol) in MeOH (20 mL) was added **30** (0.613 g, 2 mmol). The reaction mixture was stirred at 60 □ for 5 h. Following the work-up for **28a**, **31** was obtained by column chromatography on silica gel (CH_2Cl_2/MeOH/NH_3•H_2O, 10:3:0.5) (64 mg, 0.063 mmol, 9.3%).

HRMS (ESI) $(M+H)^+$ *m/z* 1020.4926, Calcd for $C_{50}H_{75}ClN_5O_{15}$ 1020.4943. ^{1}H NMR ($CDCl_3$, 700 MHz), δ: 8.74 (s, 1 H, H-quinolyl), 8.04 (s, 1 H, H-quinolyl),7.52 (s, 1 H, H-quinolyl), 5.36 (s, 1 H, -NH-CH_2-CH_2-N**H**-), 5.15 (d, *J* = 10.6 Hz, 1 H, H-13), 5.07 (d, *J* = 11.0 Hz, 1 H, H-3), 4.87 (s, 1 H, H-11), 3.96 (d, *J* = 11.9 Hz, 1 H, H-1'), 3.83 (s, 3 H, 9-*O*-C**H**$_3$), 3.75-3.63 (m, 2 H, H-5, H-8), 3.56 (s, 1 H, H-cyclopropyl), 3.49-3.33 (m, 3 H, H-5', -NH-CH_2-C**H**$_2$-NH-), 3.24 (dd, *J* = 10.0, 6.8 Hz, 1H, H-2'), 3.11-2.96 (m, 4 H, -NH-C**H**$_2$-CH_2-NH-, -CO-C**H**$_2$-CH_2-NH-), 3.02 (s, 3 H, 6-*O*-C**H**$_3$), 2.97-2.82 (m, 1 H, H-2), 2.75-2.64 (m, 2 H, -CO-C**H**$_2$-CH_2-NH-), 2.52-2.44 (m, 2 H, , H-10, H-3'), 2.39 (s, 6 H, -N(CH_3)$_2$), 2.14-2.06 (m, 1 H, H-4), 1.95-1.85 (m, 1 H, H-14eq), 1.76-1.56 (m, 2 H, H-4'eq, H-14ax), 1.49 (s, 3 H, 12-CH_3), 1.45-1.37 (m, 3 H, H-7a, H-cyclopropyl), 1.34 (s, 3 H, 6-CH_3), 1.27-1.18 (m, 10 H, H-7b, H-4'ax, 5'-CH_3, 10-CH_3, H-cyclopropyl), 1.13-1.02 (m, 6 H, 2-CH_3, 4-CH_3), 0.94 (d, *J* = 7.0 Hz, 3 H, 8-CH_3), 0.85 (t, *J* = 7.3 Hz, 3 H, 15-CH_3); ^{13}C NMR ($CDCl_3$, 175 MHz), δ: 177.5, 173.9, 172.4, 167.3, 164.5, 154.7, 145.9, 143.0, 132.6, 127.7, 126.3, 118.1, 107.2, 104.4, 103.8, 85.0, 83.1, 82.2, 78.5, 78.1, 75.8, 70.2, 69.2, 65.9, 61.4, 49.9, 47.3, 44.3, 43.1, 40.1, 40.0, 37.2, 36.6, 35.4, 32.7, 29.7, 25.6, 22.2, 21.1, 19.4, 18.8, 15.6, 15.3, 13.0, 10.2, 9.0, 8.1.

4.5.12. 3-O-Descladinosyl-3-O-[N-propargylcarbamoyl]-6-O-methylerythromycin A

*9-O-methyl oxime-11, 12-cyclic carbonate (**32**)*

To a solution of **11** (1.0613 g, 1.55 mmol) in CH_2Cl_2 (20 mL) at room temperature were added DMAP (0.378 g, 3.09 mmol) and CDI (0.752 g, 4.64 mmol). The resulting solution was allowed to stirred for 20 h at the same temperature. The reaction was quenched with saturated $NaHCO_3$ (30 mL) and the aqueous layer was extracted with CH_2Cl_2 (2 × 20 mL) saturated brine (2 × 20 mL). The organic layer were washed with saturated NH_4Cl (3 × 20 mL) and brine (3 × 20 mL), and then the organic solvent was removed in vacuum to afford an intermediate **3**-*O*-imidazolylcarbonyl-**11** (1.0745 g, 1.38 mmol, 89.0%).

To a solution of the intermediate **3**-*O*-imidazolylcarbonyl-**11** (1.0745 g, 1.38 mmol) in DMF (10 mL) at 0 □ were added DBU (0.267 mL, 1.89 mmol) and propargylamine (0.13 mL, 1.89 mmol). The resulting solution was stirred for 48 h at room temperature. The reaction was quenched with water (20 mL) and the aqueous layer was extracted with CH_2Cl_2 (3 × 15 mL). The combined organic layers were washed with brine and the organic solvent was removed in vacuum to afford **32** (0.7521 g, 0.983 mmol, 71.2%).

4.5.13.

*3-O-Descladinosyl-3-O-[N-(3'-(3"-pyridyl)propargyl)carbamoyl]-6-O-methylerythromycin A 9-O-methyl oxime-11, 12-cyclic carbonate (**33a**)*

To a solution of **32** (0.7521 g, 0.983 mmol), CuI (18.7 mg, 0.0982 mmol), $(PPh_3)_2PdCl_2$ (34.5 mg, 0.049 mmol) and triethylamine (0.205 mL, 1.47 mmol) in acetonitrile (20 mL) was added 3-bromopyridine (0.284 mL, 2.95 mmol). The reaction mixture was recharged with argon and stirred at 80 □ for 3 h in a sealed tube. The mixture was extracted with ethyl acetate and the organic layer was washed successively with water and brine. The organic solvent was removed in vacuum and dried to afford 2'-OAc-**33a** (0.5567 mg, 0.66 mmol, 67.1%).

A solution of 2'-OAc-**33a** (0.5567 mg, 0.66 mmol) in MeOH (20 mL) was stirred at 65 □ for 3 h. The organic solvent was removed in vacuum and the residue was purified by column chromatography on silica gel ($CH_2Cl_2/C_2H_5OH/NH_3•H_2O$,

10:0.2:0.1) to afford **33a** (0.2538 mg, 0.316 mmol, 47.9%).

HRMS (ESI) $(M+H)^+$ *m/z* 803.4451, Calcd for $C_{41}H_{63}N_4O_{12}$ 803.4437. ^{1}H NMR ($CDCl_3$, 400 MHz), δ: 8.63 (s, 1 H, H-pyridyl), 8.56 (d, *J* = 4.7 Hz, 1 H, H-pyridyl), 7.69 (d, *J* = 7.9 Hz, 1 H, H-pyridyl), 7.29-7.24 (m, 1 H, H-pyridyl), 5.40 (s, 1 H, 3-*O*-CO-N**H**-CH_2-), 5.17 (dt, *J* = 10.8, 3.3 Hz, 1 H, H-13), 4.90 (d, *J* = 11.0 Hz, 1 H, H-3), 4.86 (s, 1 H, H-11), 4.39-4.15 (m, 2 H, 3-*O*-CO-NH-C**H**$_2$-), 4.04 (d, *J* = 7.3 Hz, 1 H, H-1'), 3.82 (s, 3 H, 9-*O*-C**H**$_3$), 3.77-3.65 (m, 2 H, H-5, H-8), 3.44-3.36 (m, 1 H, H-5'), 3.17 (dd, *J* = 10.1, 7.3 Hz, 1H, H-2'), 3.03 (s, 3 H, 6-*O*-C**H**$_3$), 2.92-2.83 (m, 1 H, H-2), 2.52-2.42 (m, 2 H, , H-10, H-3'), 2.20 (s, 6 H, -N(CH_3)$_2$), 2.14-2.06 (m, 1 H, H-4), 1.97-1.86 (m, 1 H, H-14eq), 1.71-1.53 (m, 2 H, H-4'eq, H-14ax), 1.49 (s, 3 H, 12-CH_3), 1.43-1.37 (m, 1 H, H-7a), 1.32 (s, 3 H, 6-CH_3), 1.28-1.25 (m, 1 H, H-7b), 1.23 (d, *J* = 6.7 Hz, 3 H, 10-CH_3), 1.20-1.13 (m, 6 H, 5'-CH_3, 2-CH_3), 1.10 (d, *J* = 7.5 Hz, 3 H, 4-CH_3), 0.93 (d, *J* = 7.0 Hz, 3 H, 8-CH_3), 0.86 (t, *J* = 7.3 Hz, 3 H, 15-CH_3); ^{13}C NMR ($CDCl_3$, 175 MHz), δ: 173.9, 164.5, 156.0, 154.7, 152.4, 149.1, 138.7, 123.1, 119.5, 103.5, 88.5, 85.0, 83.2, 81.8, 80.1, 79.3, 78.4, 75.6, 70.5, 69.4, 66.0, 61.4, 49.8, 43.4, 40.3, 40.2, 37.3, 36.0, 32.7, 31.6, 28.5, 25.6, 22.2, 21.1, 19.3, 18.8, 15.6, 15.0, 13.0, 10.2, 8.9.

4.5.14.

*3-O-Descladinosyl-3-O-[N-(3'-(1''-cyclopropyl-1'',4''-dihydro-4''-oxo-3''-carboxyquinol-6''-yl)propargyl)carbamoyl]-6-O-methylerythromycin A 9-O-methyl oxime-11, 12-cyclic carbonate (**33b**)*

A solution of 1-cyclopropyl-1,4-dihydro-6-iodo-4-oxo-3-quinolinecarboxylic acid (0.278 g, 0.784 mmol), CuI (7.46 mg, 0.039 mmol) and triethylamine (10 mL) in acetonitrile (10 mL) was stirred at room temperature for 20 min. Then **32** (0.3 g, 0.392 mmol) and $(PPh_3)_2PdCl_2$ (13.8 mg, 0.0196 mmol) were added and the mixture was recharged with argon and stirred at 50 □ for 24 h in a sealed tube. Following the work-up for **33a**, **33b** was obtained by column chromatography on silica gel (CH_2Cl_2/MeOH/NH_3.H_2O, 10:3:0.5) (23.4 mg, 0.025 mmol, 26.3%).

HRMS (ESI) $(M+H)^+$ *m/z* 953.4761, Calcd for $C_{49}H_{69}N_4O_{15}$ 953.4754.

4.5.15.

*3-O-Descladinosyl-3-O-[N-(3'-(3''-pyridyl)propyl)carbamoyl]-6-O-methylerythromycin A 9-O-methyl oxime-11, 12-cyclic carbonate (**34a**)*

To a solution **33a** (0.2538 g, 0.316 mmol) in MeOH (10 mL) were added $HCOONH_4$ (1.0152 g, 16.09 mmol), HCOOH (0.179 mL, 4.74 mmol), and 10% Pd-C (0.0254 g). The reaction mixture was flushed with hydrogen to 1 atm. The reaction mixture was stirred at 65 □ for 24 h. 10 % Pd-C was removed by filtration before the solvent was evaporated. The residue was extracted with CH_2Cl_2 and the organic layer was washed successively with water and brine. The organic solvent was removed in vacuum and the residue was purified by column chromatography on silica gel ($CH_2Cl_2/C_2H_5OH/NH_3•H_2O$, 10:0.6:0.1) to afford **34a** (45mg, 0.056 mmol, 17.7%).

HRMS (ESI) $(M+H)^+$ *m/z* 807.4746, Calcd for $C_{41}H_{67}N_4O_{12}$ 807.4750. ^{1}H NMR ($CDCl_3$, 400 MHz), δ: 8.49-8.43 (m, 2 H, H-pyridyl), 7.5 (d, *J* = 7.9 Hz, 1 H, H-pyridyl), 7.23 (dd, *J* = 7.8, 4.9 Hz, 1 H, H-pyridyl), 5.17 (d, *J* = 10.7 Hz, 1 H, H-13), 5.12 (t, *J* = 6.1 Hz, 1 H, -N**H**-), 4.91-4.84 (m, 2 H, H-3, H-11), 3.99 (d, *J* = 7.2 Hz, 1 H, H-1'), 3.82 (s, 3 H, 9-*O*-C**H**$_3$), 3.76-3.64 (m, 2 H, H-5, H-8), 3.38-3.11 (m, 4 H, H-5', H-2', -NH-C**H**$_2$-CH_2-CH_2), 3.02 (s, 3 H, 6-*O*-C**H**$_3$), 2.90-2.82 (m, 1 H, H-2), 2.67 (t, *J* = 7.8 Hz, 2 H, -NH-CH_2-CH_2-C**H**$_2$-), 2.52-2.37 (m, 2 H, , H-10, H-3'), 2.27 (s, 6 H, -N(CH_3)$_2$), 2.12-2.04 (m, 1 H, H-4), 1.92-1.82 (m, 3 H, H-14eq, -NH-CH_2-C**H**$_2$-CH_2-), 1.62-1.53 (m, 2 H, H-4'eq, H-14ax), 1.49 (s, 3 H, 12-CH_3), 1.44-1.36 (m, 1 H, H-7a), 1.32 (s, 3 H, 6-CH_3), 1.27-1.21 (m, 5 H, H-7b, H-4'ax, 10-CH_3), 1.20-1.13 (m, 6 H, 5'-CH_3, 2-CH_3), 1.07 (d, *J* = 7.4 Hz, 3 H, 4-CH_3), 0.93 (d, *J* = 7.0 Hz, 3 H, 8-CH_3), 0.86 (t, *J* = 7.3 Hz, 3 H, 15-CH_3); ^{13}C NMR ($CDCl_3$, 175 MHz), δ: 174.0, 164.5, 156.5, 154.7, 149.7, 147.7, 136.5, 135.7, 123.5, 103.4, 85.0, 83.2, 81.6, 78.4, 78.3, 75.5, 70.4, 69.4, 66.1, 61.4, 49.8, 43.4, 40.6, 40.3, 37.3, 36.0, 32.7, 31.8, 30.2, 28.6, 25.6, 22.2, 21.1, 19.3, 18.9, 15.6, 14.9, 13.0, 10.2, 9.0.

4.5.16.

*3-O-Descladinosyl-3-O-[N-(3'-(3''-carboxy-1''-cyclopropyl-1'',4''-dihydro-4''-oxo-quinol-6''-yl)propyl)carbamoyl]-6-O-methylerythromycin A 9-O-methyl oxime-11, 12-cyclic carbonate (**34b**)*

To a solution **33b** (0.063 g, 0.066 mmol) in MeOH (10 mL) were added $HCOONH_4$ (0.252 g, 3.99 mmol), HCOOH (0.037 mL, 0.99 mmol), and 10% Pd-C (60 mg). The reaction mixture was flushed with hydrogen to 1 atm. The reaction mixture was stirred at 65 □ for 24 h. Following the work-up for **34a**, **34b** was obtained by column chromatography on silica gel (CH_2Cl_2/MeOH/NH_3•H_2O, 10:3:0.5) (16.7 mg, 0.017 mmol, 25.8%).

HRMS (ESI) $(M+H)^+$ *m/z* 957.5063, Calcd for $C_{49}H_{73}N_4O_{15}$ 957.5067. ^{1}H NMR ($CDCl_3$, 400 MHz), δ: 8.87 (s, 1 H, H-quinolyl), 8.30 (s, 1 H, H-quinolyl), 8.05 (d, *J* = 8.7 Hz, 1 H, H-quinolyl), 7.70 (d, *J* = 8.7 Hz, 1 H, H-quinolyl), 5.25-5.13 (m, 2 H, H-13, -N**H**-), 4.91-4.81 (m, 2 H, H-3, H-11), 4.01 (d, *J* = 7.4 Hz, 1 H, H-1'), 3.82 (s, 3 H, 9-*O*-C**H**$_3$), 3.75-3.58 (m, 3 H, H-5, H-8, H-cyclopropyl), 3.42-3.09 (m, 2 H, H-5', H-2'), 3.02 (s, 3 H, 6-*O*-C**H**$_3$), 2.91-2.81 (m, 3 H, H-2, -NH-CH_2-CH_2-C**H**$_2$), 2.52-2.38 (m, 2 H, , H-10, H-3'), 2.26 (s, 6 H, -$N(CH_3)_2$), 2.12-2.02 (m, 1 H, H-4), 1.99-1.83 (m, 3 H, H-14eq, -NH-CH_2-C**H**$_2$-CH_2-), 1.65-1.55 (m, 2 H, H-4'eq, H-14ax), 1.49 (s, 3 H, 12-CH_3), 1.44-1.36 (m, 3 H, H-7a, H-cyclopropyl), 1.32 (s, 3 H, 6-CH_3), 1.28-1.19 (m, 6 H, H-cyclopropyl, H-7b, 10-CH_3), 1.18-1.14 (m, 6 H, 5'-CH_3, 2-CH_3), 1.08 (d, *J* = 7.6 Hz, 3 H, 4-CH_3), 0.93 (d, *J* = 7.0 Hz, 3 H, 8-CH_3), 0.86 (t, *J* = 7.5 Hz, 3 H, 15-CH_3); ^{13}C NMR ($CDCl_3$, 175 MHz), δ: 178.5, 174.1, 167.0, 164.6, 156.5, 154.7, 147.9, 140.1, 139.6, 134.7, 128.4, 126.0, 125.6, 117.6, 108.6, 103.4, 85.1, 83.2, 81,5, 78.4, 75.5, 70.5, 69.5, 66.0, 61.4, 49.8, 43.4, 40.4, 40.3, 37.3, 36.0, 35.4, 32.7, 32.4, 31.6, 29.7, 25.6, 22.2, 21.1, 19.3, 18.9, 15.6, 14.9, 13.0, 10.2, 8.9, 8.3.

4.5.17.

*3-O-Descladinosyl-3-O-[N-(3'-(3''-aminopyrid-5''-yl)propyl)carbamoyl]-6-O-methylerythromycin A 9-O-methyl oxime-11, 12-cyclic carbonate (**34e**)*

To a solution of **11** (1.1483 g, 1.67 mmol) in CH_2Cl_2 (20 mL) at room temperature were added DMAP (0.41 g, 3.34 mmol) and CDI (0.803 g, 5.01 mmol). The resulting solution was allowed to stir for 20 h at the same temperature. The reaction was quenched with saturated $NaHCO_3$ (30 mL) and the aqueous layer was extracted with CH_2Cl_2 (2 × 20 mL). The organic layer were washed with saturated NH_4Cl (3 × 20 mL) and brine (3 × 20 mL). The organic solvent was removed in vacuum to afford an intermediate **3-*O*-**imidazolylcarbonyl-**11** (1.2674 g, 1.63 mmol, 97.6%).

To a solution of the intermediate **3-*O*-**imidazolylcarbonyl-**11** (0.2 g, 0.257 mmol) in DMF(15 mL) at 0 □ were added DBU (0.054 mL, 0.383 mmol) and **23e** (0.0706 g, 0.467 mmol). The resulting solution was stirred for 48 h at room temperature. The reaction was quenched with water (20 mL) and the aqueous layer was extracted with CH_2Cl_2 (3 × 15 mL). The combined organic layers were washed with brine and the organic solvent was removed in vacuum and dried to afford 2'-OAc-**34e** (75 mg, 0.087 mmol, 33.9%).

A solution of 2'-OAc-**34e** (75 mg, 0.087 mmol) in MeOH (10 mL) was stirred at 65 □ for 3 h. The organic solvent was removed in vacuum and the residue was purified by column chromatography on silica gel (CH_2Cl_2/C_2H_5OH/NH_3•H_2O, 10:0.5:0.1) to afford **34e** (5.8 mg, 0.007 mmol, 8.0%).

HRMS (ESI) $(M+H)^+$ *m/z* 822.4867, Calcd for $C_{41}H_{68}N_5O_{12}$ 822.4859. ^{1}H NMR ($CDCl_3$, 400 MHz), δ: 7.93 (s, 1 H, H-pyridine), 7.84 (s, 1 H, H-pyridine), 6.81 (s, 1 H, H-pyridine), 5.44-5.29 (m, 1 H, -NH-), 5.17 (d, *J* = 10.6 Hz, 1 H, H-13), 4.91-4.82 (m, 2 H, H-3, H-11), 4.01 (d, *J* = 7.5 Hz, 1 H, H-1'), 3.82 (s, 3 H, 9-*O*-C**H**$_3$), 3.75-3.63 (m, 2 H, H-5, H-8), 3.36-3.14 (m, 4 H, H-5', H-2', -NH-C**H**$_2$-CH_2-CH_2-), 3.02 (s, 3 H, 6-*O*-C**H**$_3$), 2.92-2.82 (m, 1 H, H-2), 2.61-2.46 (m, 3 H, H-10, -NH-CH_2-CH_2-C**H**$_2$-), 2.40-2.25 (m, 7 H, -N(CH_3)$_2$, H-3'), 2.11-2.02 (m, 1 H, H-4), 1.94-1.79 (m, 3 H, H-14eq, -NH-CH_2-C**H**$_2$-CH_2-), 1.66-1.55 (m, 2 H, H-4'eq, H-14ax), 1.49 (s, 3 H, 12-CH_3), 1.44-1.36 (m, 1 H, H-7a), 1.32 (s, 3 H, 6-CH_3), 1.28-1.12 (m, 10 H, 5'-CH_3, 10-CH_3,

H-4'aq, 2-CH_3), 1.08 (d, *J* = 7.4 Hz,3 H, 4-CH_3), 0.93 (d, *J* = 7.1 Hz, 3 H, 8-CH_3), 0.86 (t, *J* = 7.7 Hz, 3 H, 15-CH_3).

4.5.18.

*3-O-Descladinosyl-3-O-[N-(4'-(3''-pyridyl)butyl)carbamoyl]-6-O-methylerythromycin A 9-O-methyl oxime-11, 12-cyclic carbonate (**35**)*

To a solution of **11** (1.1483 g, 1.67 mmol) in CH_2Cl_2 (20 mL) at room temperature were added DMAP (0.41 g, 3.34 mmol) and CDI (0.803 g, 5.01 mmol). The resulting solution was allowed to stirred for 20 h at the same temperature. The reaction was quenched with saturated $NaHCO_3$ (30 mL) and the aqueous layer was extracted with CH_2Cl_2 (2 × 20 mL). The organic layer were washed with saturated NH_4Cl (3 × 20 mL) and brine (3 × 20 mL). Then the organic solvent was removed in vacuum to afford an intermediate **3-***O***-**imidazolylcarbonyl-**11** (1.2674 g, 1.63 mmol, 97.6%).

To a solution of the intermediate **3-***O***-**imidazolylcarbonyl-**11** (0.5714 g, 0.735 mmol) in DMF(20 mL) at 0 □ were added DBU (0.16 mL, 1.13 mmol) and **27** (0.1712 g, 1.14 mmol). The resulting solution was stirred for 48 h at room temperature. The reaction was quenched with water (20 mL) and the aqueous layer was extracted with CH_2Cl_2 (3 × 15 mL). The combined organic layers were washed with brine, and the organic solvent was removed in vacuum and dried to afford 2'-OAc-**35** (0.3126 mg, 0.362 mmol, 49.3%).

A solution of 2'-OAc-**35** (0.3126 mg, 0.362 mmol) in MeOH (20 mL) was stirred at 65 □ for 3 h. The organic solvent was removed in vacuum and the residue was purified by column chromatography on silica gel (CH_2Cl_2/C_2H_5OH/NH_3•H_2O, 10:0.6:0.1) to afford **35** (113 mg, 0.138 mmol, 38.1%).

HRMS (ESI) $(M+H)^+$ *m/z* 821.4899, Calcd for $C_{42}H_{69}N_4O_{12}$ 821.4907. ^{1}H NMR ($CDCl_3$, 400 MHz), δ: 8.47-8.40 (m, 2 H, H- pyridyl), 7.49 (d, *J* = 7.9 Hz, 1 H, H-pyridyl), 7.25-7.18 (m, 1 H, H-pyridyl), 5.20-5.10 (m, 2 H, H-13, -NH-), 4.90-4.82 (m, 1 H, H-3, H-11), 4.01 (d, *J* = 7.4 Hz, 1 H, H-1'), 3.82 (s, 3 H, 9-*O*-C**H**$_3$), 3.75-3.65 (m, 2 H, H-5, H-8), 3.41-3.31 (m, 2 H, H-5',

-NH-C**H**$_2$-CH$_2$-CH$_2$-CH$_2$-), 3.19 (t, *J* = 8.8 Hz, 1H, H-2'), 3.13-3.06 (m, 1 H, -NH-C**H**$_2$-CH$_2$-CH$_2$-CH$_2$-), 3.02 (s, 3 H, 6-*O*-C**H**$_3$), 2.89-2.82 (m, 1 H, H-2), 2.65 (t, *J* = 7.6 Hz, 2 H, -NH-CH$_2$-CH$_2$-CH$_2$-C**H**$_2$-), 2.52-2.39 (m, 2 H, H-10, H-3'), 2.29 (s, 6 H, -N(CH$_3$)$_2$), 2.12-1.85 (m, 2 H, H-4, H-14eq), 1.72-1.54 (m, 6 H, H-4'ax, H-14ax, -NH-CH$_2$-C**H**$_2$-C**H**$_2$-CH$_2$-), 1.49 (s, 3 H, 12-CH$_3$), 1.44-1.35 (m, 1 H, H-7a), 1.32 (s, 3 H, 6-CH$_3$), 1.28-1.26 (m, 1 H, H-7b), 1.24-1.19 (m, 6 H, 5'-CH$_3$, 10-CH$_3$), 1.13 (d, *J* = 6.7 Hz, 3 H, 2-CH$_3$), 1.07 (d, *J* = 7.4 Hz, 3 H, 4-CH$_3$), 0.93 (d, *J* = 7.1 Hz, 3 H, 8-CH$_3$), 0.86 (t, *J* = 7.4 Hz, 3 H, 15-CH$_3$); ^{13}C NMR (CDCl$_3$, 100 MHz), δ: 174.1, 164.7, 156.5, 154.7, 149.8, 147.5, 137.1, 135.8, 123.4, 103.2, 85.1, 83.2, 81.3, 78.3, 78.2, 70.4, 69.4, 66.1, 61.4, 49.8, 43.4, 40.8, 40.3, 37.3, 36.0, 32.7, 32.5, 29.6, 28.7, 28.2, 25.6, 22.2, 21.2, 19.3, 18.9, 15.6, 14.9, 13.0, 10.2, 8.9.

Supporting information

Pharmacokinetic curves and CYP 3A4 inhibition curves for **34a** and telithromycin, synthetic procedures for all the intermediates, target compounds and reagents, and NMR spectra of **34a**. This material is available free of charge via the internet at http://dx.doi.org/10.1016/j.ejmech.2019.

Acknowledgements

This work is financially supported by the National Natural Science Foundation of China (81673335; 21878022). The authors thank to Prof. Yun Li at Peking University and Dr. He Wang at Peking Union Medical College Hospital for their antibacterial assays. The authors also appreciate the spectral data supporting from the Analysis & Testing Center, Beijing Institute of Technology.

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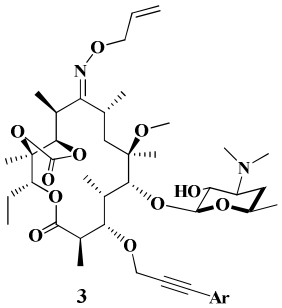
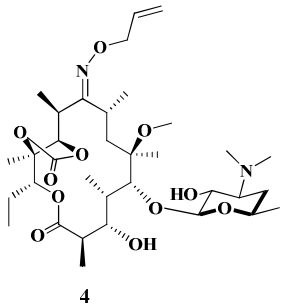
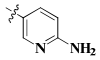
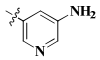
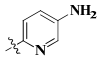
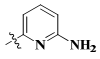
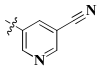
Table 1. *In vitro* antibacterial activity of **3a-3i** and **4** against erythromycin-susceptible and -resistant pathogens.



3

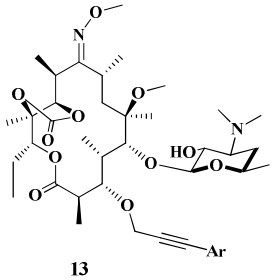


4

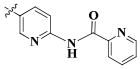
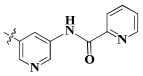
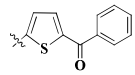
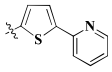
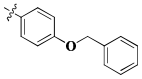
Compd.	(MIC, μg/mL)									
	S. pneumoniae			*S. pyogenes*		*S. aureus*				*S. epidermedis*
	ATCC 49619 EryS [a]	*PU09 mef*	*PU27 c-erm* [b]	*A2 c-erm*	*A3 EryS*	*ATCC 29213 EryS*	*PU32 i-ermA* [c]	*PU20 c-ermC*	*PU64 i-ermA*	*E1*
CLA [d]	0.064	4	4	128	≤0.032	0.25	>256	>256	>256	>256
AZI [e]	0.25	16	64	128	0.25	2	>256	>256	>256	>256
TEL [f]	≤0.032	1	≤0.032	32	≤0.032	0.125	0.125	>256	0.125	0.125
Ar										
3a 	4	4	64	64	4	64	128	256	256	256
3b 	2	4	64	64	4	32	256	256	256	256
3c 	4	8	128	128	4	64	256	256	128	256
3d 	4	8	32	64	4	64	256	256	64	256
3e 	4	8	64	128	4	64	128	>256	>256	>256
3f 	4	4	64	64	4	64	256	256	128	256
3g 	4	8	64	128	4	64	256	256	256	256
3i 	4	8	64	128	4	64	128	128	128	256
4	8	16	128	128	16	128	>256	>256	>256	>256

[a] EryS: erythromycin-susceptible.
[b] c-erm: constitutively resistant bacteria encoded erm genes.
[c] i-erm: inducibly resistant bacteria encoded erm genes.
[d] CLA: clarithromycin.
[e] AZI: azithromycin.
[f] TEL: telithromycin.

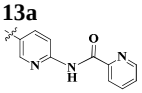
Table 2. *In vitro* antibacterial activity of **13a-13e** against erythromycin-susceptible and -resistant pathogens.

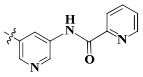
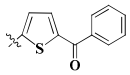
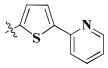
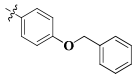


13

Compd.	(MIC and MBC, μg/mL)								
	S. pneumonia ATCC49619 EryS[a]			*S. pneumonia PU09 mef*			*S. pyogenes 01-968 i-ermA*[b]		
	MIC	MBC	MBC/MIC	MIC	MBC	MBC/MIC	MIC	MBC	MBC/MIC
CLA[c]	0.062	0.125	2	4	8	2	1	>8	>8
TEL[d]	0.031	0.031	1	0.25	1	4	0.062	0.5	8
13a 	4	16	4	4	16	4	16	16	1
13b 	4	16	4	4	16	4	16	32	2
13c 	8	16	2	4	8	2	4	8	2
13d 	4	16	4	8	16	2	8	64	8
13e 	8	32	4	8	16	2	4	8	2

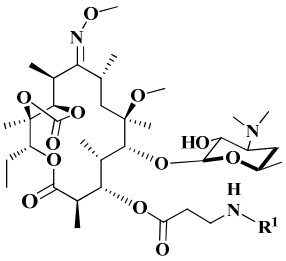
Continued

Compd.	(MIC and MBC, μg/mL)								
	S. aureus PU20 c-ermC[e]*(MRSA*[f]*)*			*S. aureus PU32 i-ermA(MRSA)*			*M. catarrhalis 13L332 EryS*		
	MIC	MBC	MBC/MIC	MIC	MBC	MBC/MIC	MIC	MBC	MBC/MIC
CLA[c]	>256	>256	—	32	>256	>8	0.031	0.125	4
TEL[d]	>256	>256	—	0.25	>2	>8	0.125	0.5	4
13a 	128	>256	—	>256	>256	—	8	8	1
13b	256	>256	—	>256	>256	—	8	16	2

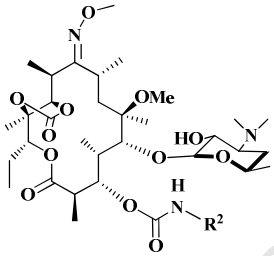
									
13c 	32	>256	>8	>256	>256	—	8	16	2
13d 	256	256	1	>256	>256	—	4	8	2
13e 	32	256	8	>256	>256	—	8	8	1

[a] EryS: erythromycin-susceptible.
[b] i-erm: inducibly resistant bacteria encoded erm genes.
[c] CLA: clarithromycin.
[d] TEL: telithromycin.
[e] c-erm: constitutively resistant bacteria encoded erm genes.

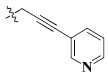
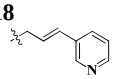
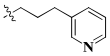
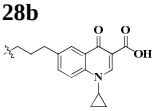
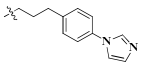
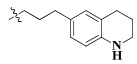
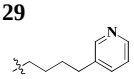
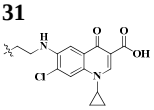
Table 3. *In vitro* antibacterial activity of **15-18**, **28-29**, **31** and **33-35** against erythromycin-susceptible and -resistant pathogens.

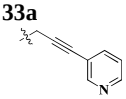
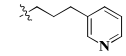
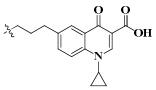
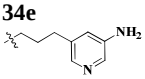
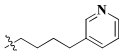


15-18, 28-29, 31

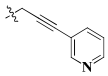
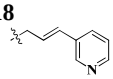
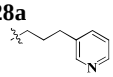
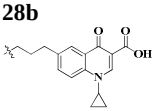


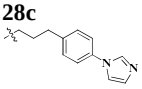
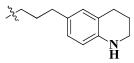
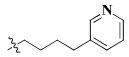
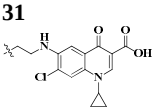
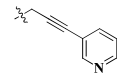
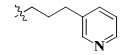
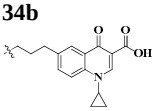
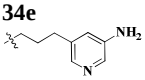
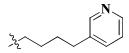
33-35

Compd.	(MIC and MBC, μg/mL)								
	S. pneumonia ATCC49619 EryS[a]			*S. pneumonia PU09 mef*			*H. influenzae ATCC49247 AziS*[b]		
	MIC	MBC	MBC/MIC	MIC	MBC	MBC/MIC	MIC	MBC	MBC/MIC
CLA[c]	0.06	0.25	4	4	32	8	4	8	2
TEL[d]	0.03	0.125	4	0.5	4	8	—	—	—
CIP[e]	0.5	2	4	2	4	2	0.004	0.004	1
R^1									
15 	0.13	1	8	2	4	2	128	128	1
16 	1	4	4	8	16	2	256	256	1
17 	1	2	2	4	8	2	256	256	1
18 	1	2	2	2	2	1	256	256	1
28a 	1	8	8	2	4	2	256	256	1
28b 	0.5	1	2	4	4	1	128	256	2
28c 	2	4	2	4	8	2	256	256	1
28d 	2	2	1	8	8	1	128	128	1
29 	1	4	4	2	4	2	128	256	2
31 	2	4	2	8	8	1	128	128	1

R^2									
33a 	0.03	0.062	2	0.5	2	4	>256	—	—
34a 	0.06	0.125	2	0.5	0.5	1	64	128	2
34b 	1	2	2	2	8	4	256	256	1
34e 	0.25	0.5	2	4	8	2	256	256	1
35 	0.5	2	4	8	8	1	256	256	1

Continued

Compd.	(MIC and MBC, μg/mL)								
	S. pyogenes 01-968 i-ermA[f]			*S. aureus PU32 i-ermA(MRSA*[g]*)*			*S. aureus PU20 c-ermC*[h]*(MRSA)*		
	MIC	MBC	MBC/MIC	MIC	MBC	MBC/MIC	MIC	MBC	MBC/MIC
CLA[c]	8	>64	>8	32	>256	>8	>256	—	—
TEL[d]	0.031	0.13	4	0.25	1	4	>256	—	—
CIP[e]	0.5	0.5	1	64	>256	>4	256	>256	—
R^1									
15 	0.5	>4	>8	16	>128	>8	256	>256	—
16 	8	>64	>8	64	>256	>4	256	>256	—
17 	1	>8	>8	32	>256	>8	>256	—	—
18 	8	>64	>8	128	>256	—	>256	—	—
28a 	0.5	>4	>8	32	>256	>8	256	>256	—
28b 	32	32	1	128	>256	—	128	>256	—

28c 	16	32	2	64	128	2	64	>256	>4
28d 	8	64	8	128	128	1	64	128	2
29 	64	128	2	128	>256	—	256	256	1
31 	16	32	2	256	>256	—	>256	—	—
R^2									
33a 	1	4	4	16	>128	>8	>256	—	—
34a 	0.25	2	8	8	16	2	>256	—	—
34b 	4	8	2	32	>256	>8	256	>256	—
34e 	—	—	—	—	—	—	—	—	—
35 	128	128	1	32	>256	>8	>256	—	—

[a] EryS: erythromycin-susceptible.
[b] AziS: azithromycin-susceptible.
[c] CLA: clarithromycin.
[d] TEL: telithromycin.
[e] CIP: ciprofloxacin.
[f] i-erm: inducibly resistant bacteria encoded erm genes.
[g] MRSA: methicillin-resistant *Staphylococcus aureus*.
[h] c-erm: constitutively resistant bacteria encoded erm genes.

Table 4. *In vivo* PK parameters in male SD rats and human liver microsome CYP inhibition.

	PO administration [a]								CYP 3A4 Inhibition
	n	AUC_{inf} h*ng/mL	AUC_{0-t} h*ng/mL	CL mL/h/kg	$t_{1/2}$ h	MRT h	T_{max} h	C_{max} ng/mL	IC50 [b] μM
34a	3	6756±1248	6731±1231	3034±600	2.94±0.368	4.53±0.621	0.833±0.289	1029±115	2.50
Telithromycin	2	927±117	897±132	21758±2748	1.22±0.26	3.27±0.47	2.00±0.00	204±20.50	11.80
Ketoconazole[c]	_ _	—	—	—	—	—	—	—	0.06

[a]PO administration: Doses for compounds **34a** and telithromycin: 20 mg/kg PO; n: number of rats included to estimate mean ± s.d. values; AUC_{inf}: concentration-time curve extrapolated to infinity; AUC_{0-t}: total area under the curve; CL: systemic plasma clearance; $t_{1/2}$: half-life; MRT: mean residence time; C_{max}: the highest drug concentration observed in plasma; T_{max}: time at which C_{max} is observed;
[b]IC50: Half maximal inhibitory concentration;
[c]Ketoconazole: Positive inhibitor of CYP 3A4.

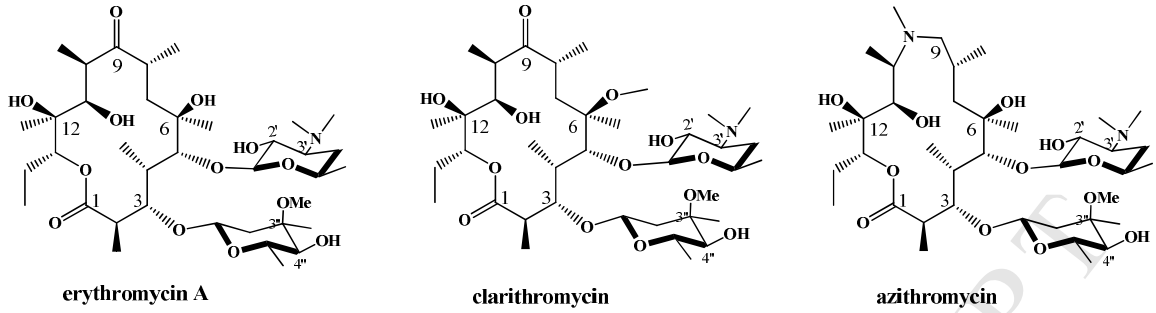


Figure 1. Structures of the erythromycin A, clarithromycin, and azithromycin.

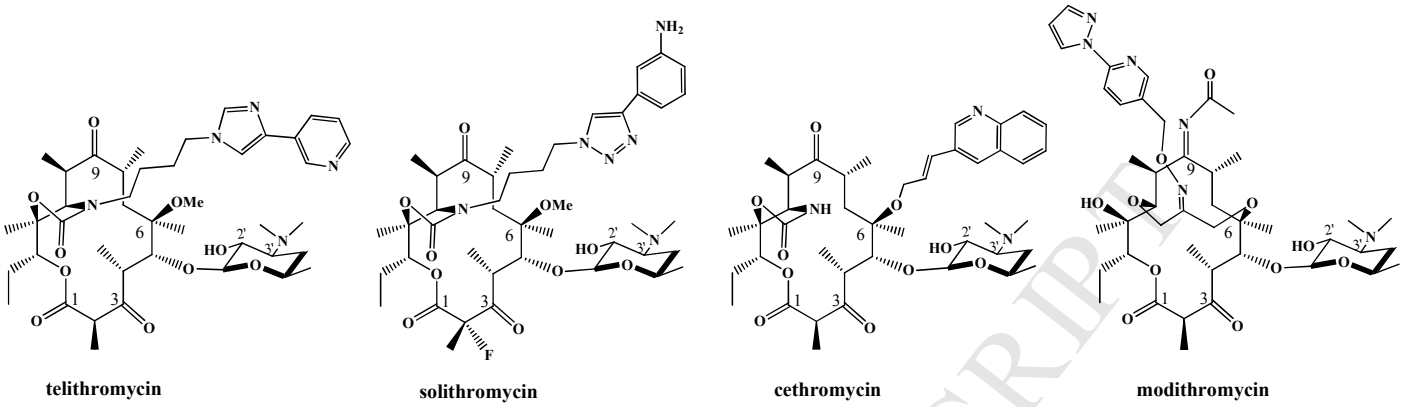


Figure 2. Structures of telithromycin, solithromycin, cethromycin and modithromycin.

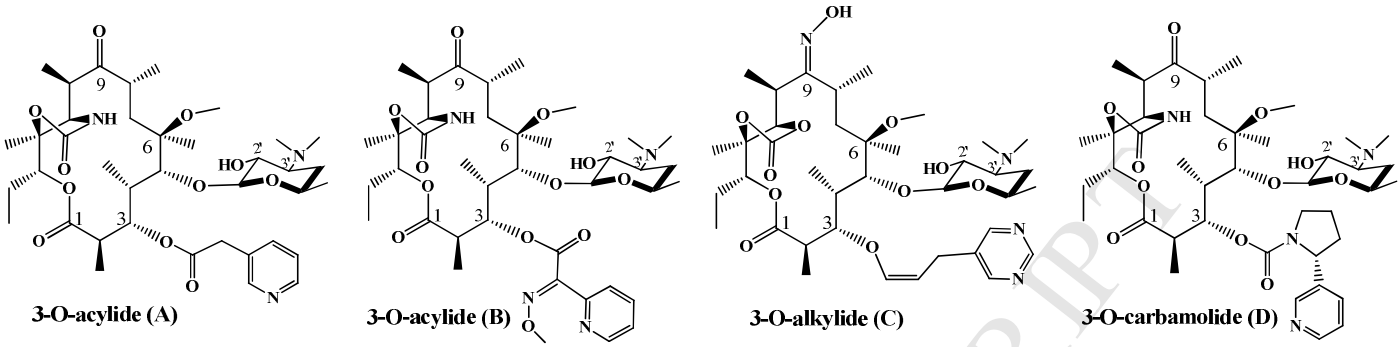


Figure 3. Structures of an acylide, an alkylide, and a carbamolide.

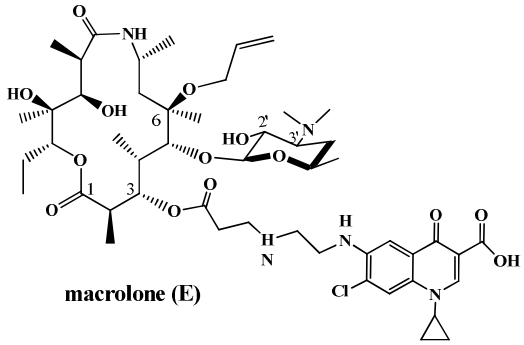


Figure 4. Structure of a macrolone.

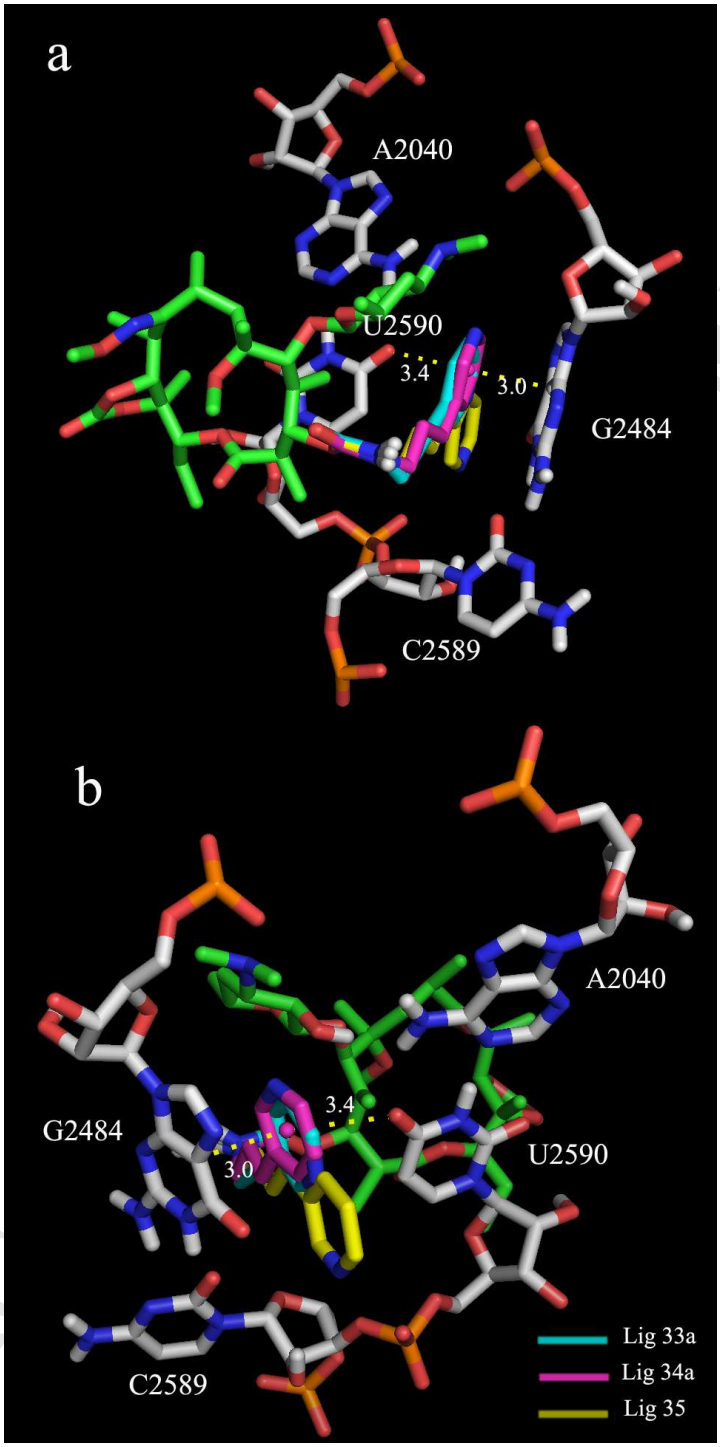
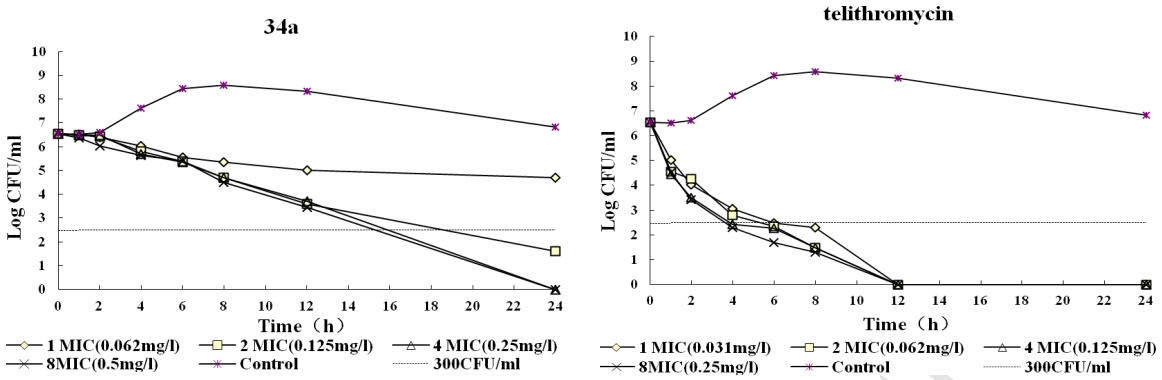
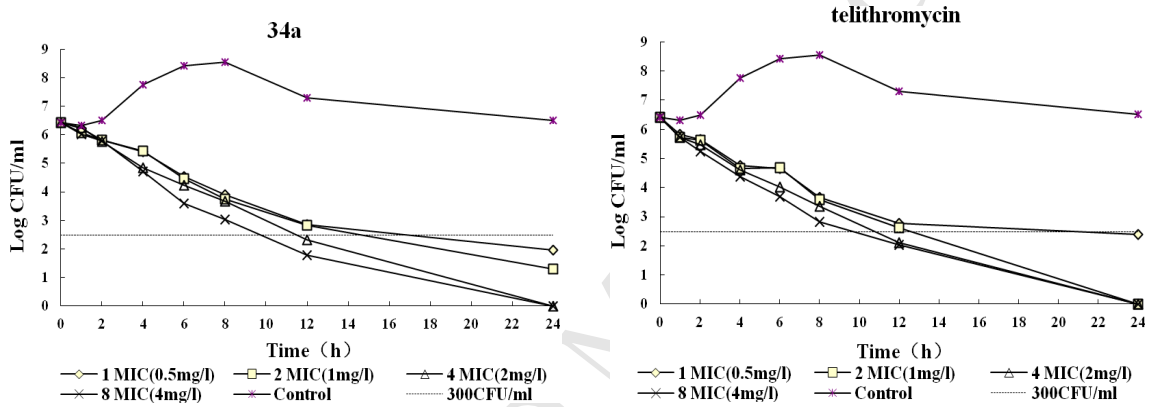


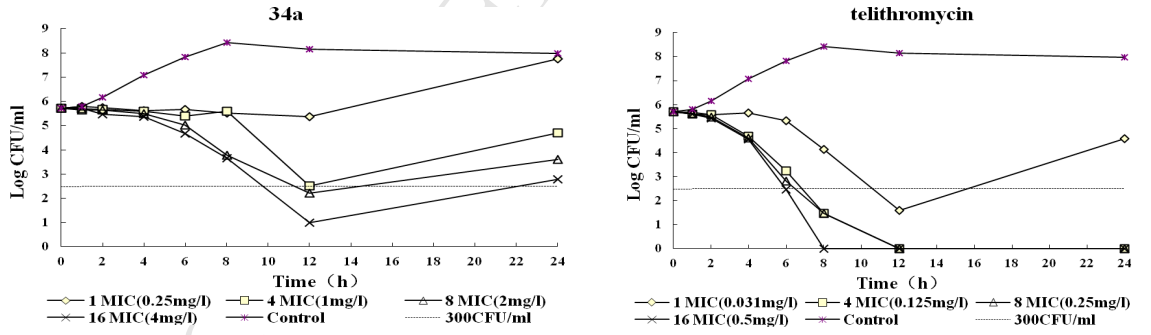
Figure 5. Proposed molecular modelling for **33a**, **34a** and **35** based on the crystal structure of a carbamolide complex with the *Deinococcus radiodurans* 50S subunit (PDB ID 4IO9). The π - π interaction between **34a** and G2484 is denoted with a yellow dash line which collects their centroids. The lone pair - π interaction between **34a** and U2590 is denoted with a yellow dash line which collects the centroid of **34a** and the oxygen atom of U2590. The figures displayed in (a) and (b) are different views of the same overlapped ligand structures (the ligand structure is green except the overlapping sidechains are color coded as described in the figure by the software Pymol 1.6).



a) Bactericidal curves of **34a** and telithromycin against erythromycin-susceptible *S. pneumoniae* ATCC49619.

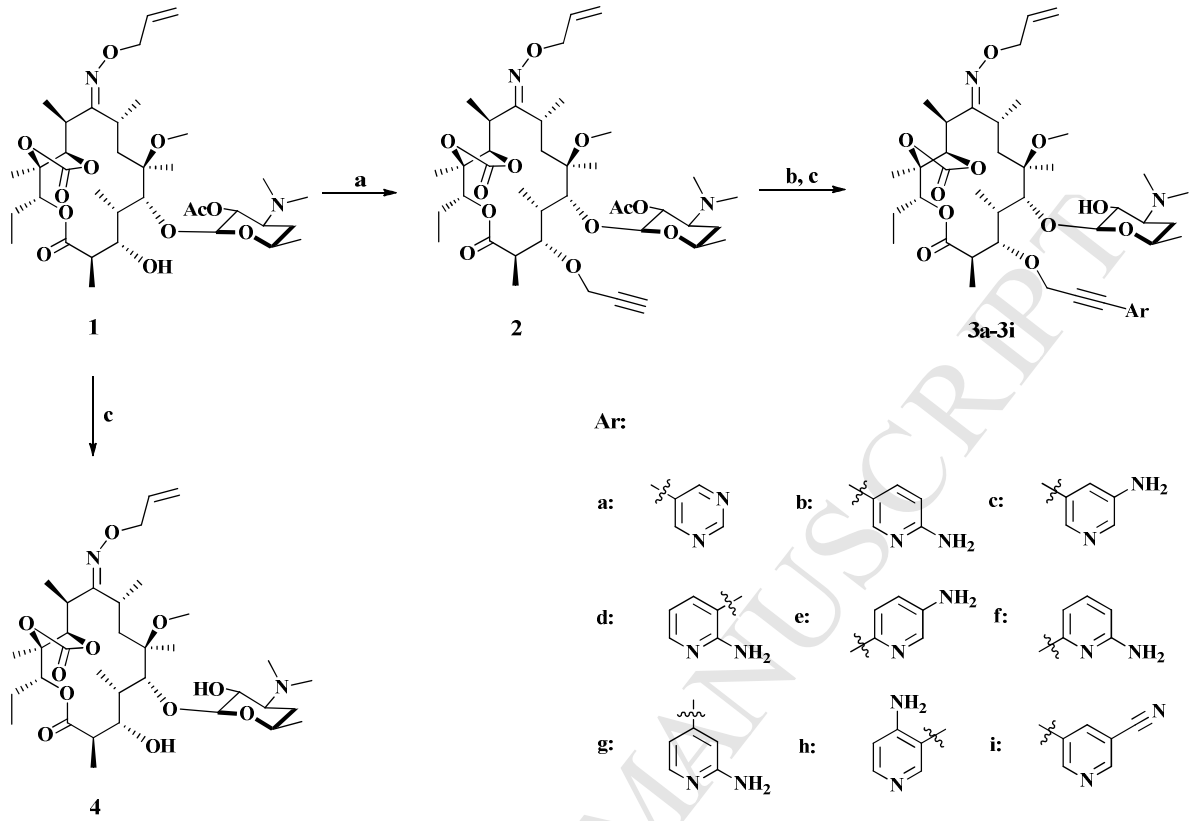


b) Bactericidal curves of **34a** and telithromycin against mef-mediated resistant *S. pneumoniae* PU09.

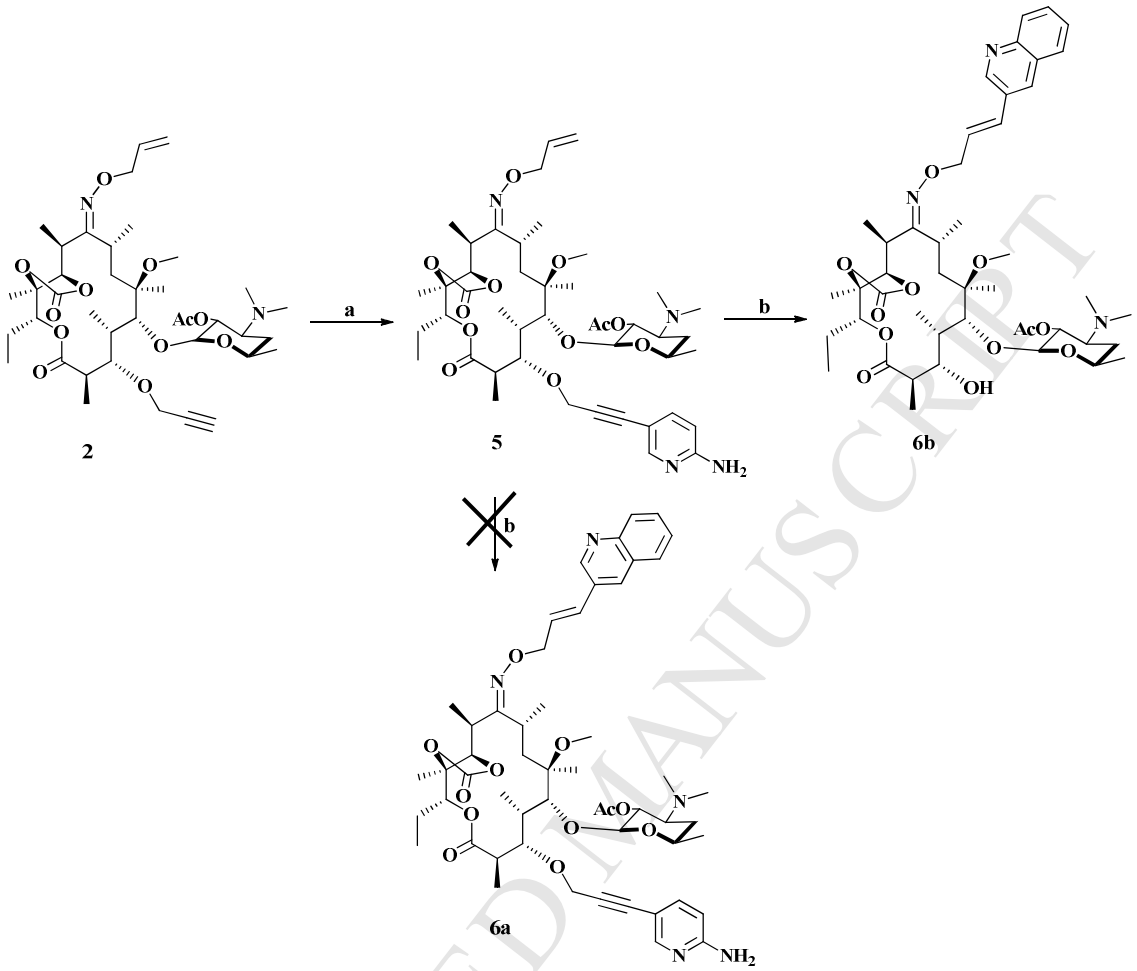


c) Bactericidal curves of **34a** and telithromycin against erm-mediated resistant *S. pyogenes* 01-968.

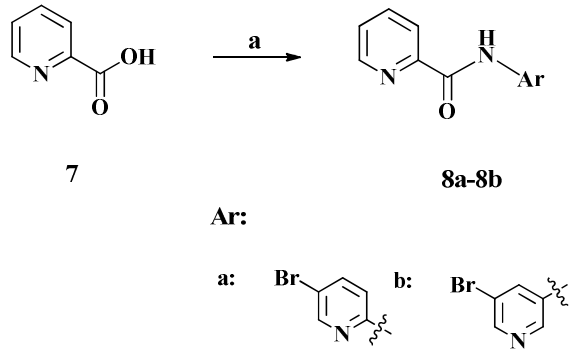
Figure 6. Bactericidal curves of **34a** and telithromycin against erythromycin-susceptible and resistant strains (300 CFU/mL is detection limit) .



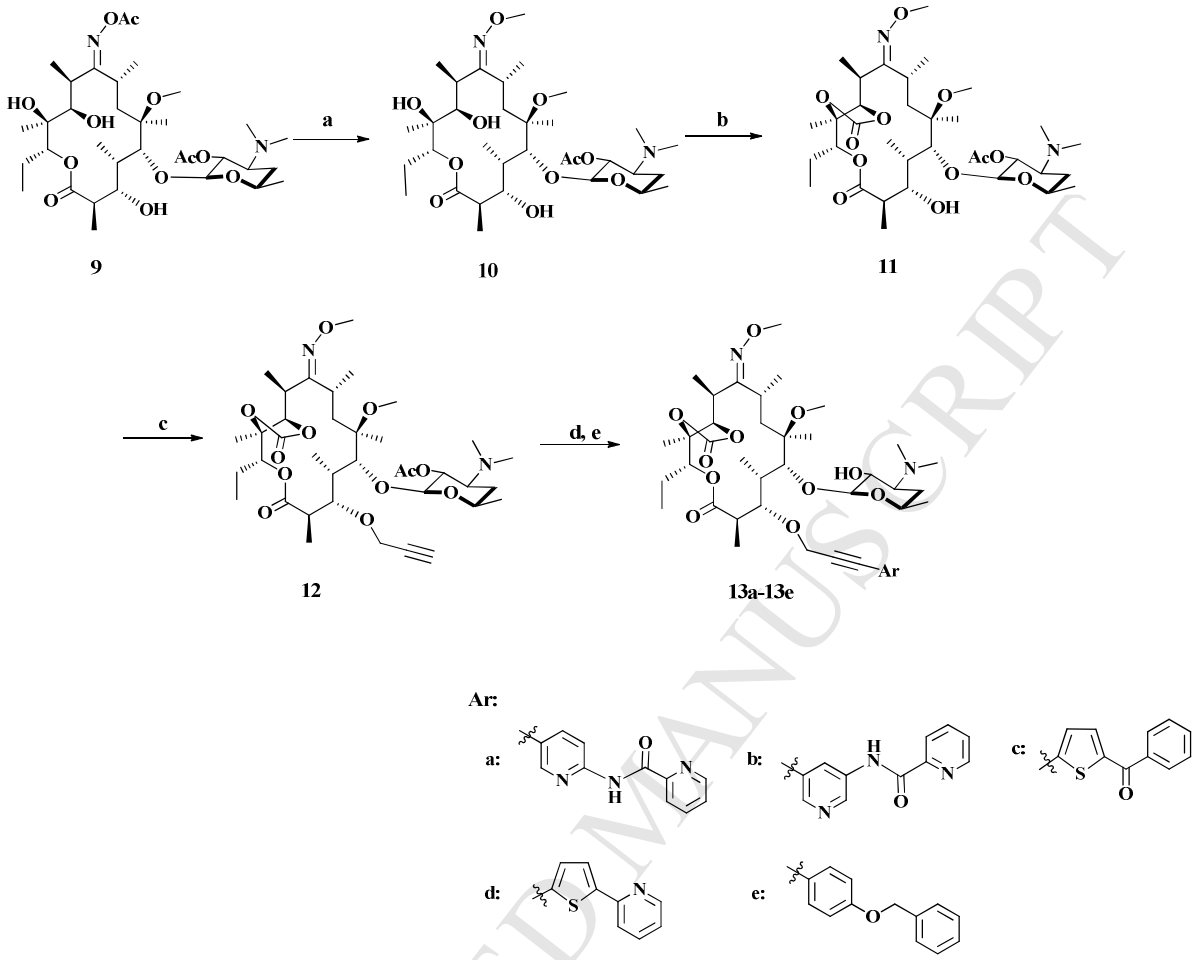
Scheme 1. The synthesis of compounds **3a-3i** and **4**. Reagents and conditions: (a) KO*t*Bu, propargyl bromide, DMF, rt, 1 h; (b) ArBr, $(PPh_3)_2PdCl_2$, CuI, Et_3N, CH_3CN, 80 □, 4 h; (c) MeOH, 65 ºC, 3 h.



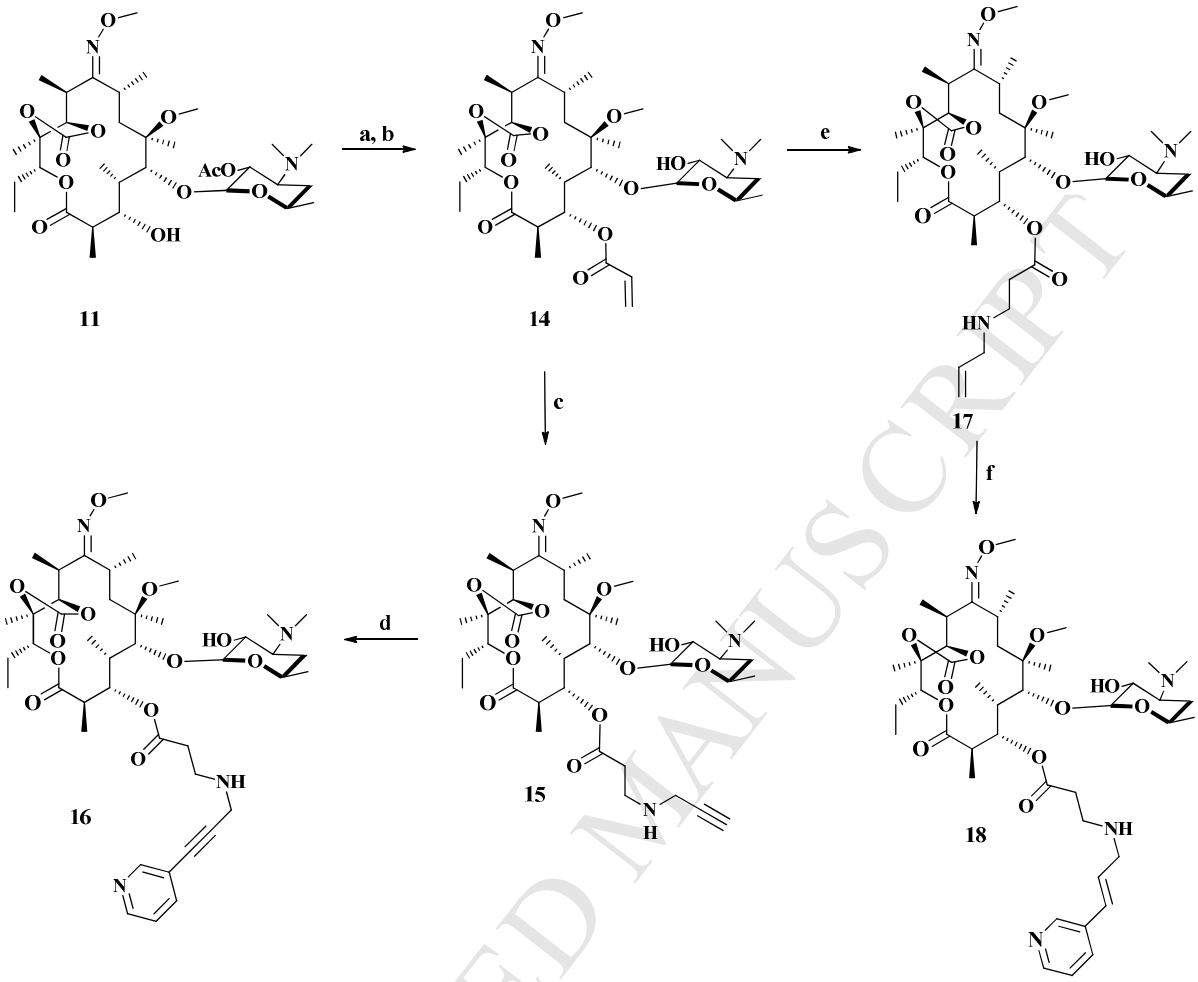
Scheme 2. The synthesis of compound **6b**. Reagents and conditions: (a) 2-amino-5-bromopyridine, $(PPh_3)_2PdCl_2$, CuI, Et_3N, CH_3CN, 80 □, 3 h; (b) ArBr, $Pd(OAc)_2$, P(*o*-tolyl)$_3$, Et_3N, CH_3CN, 60 ºC for 1 h, then 90 ºC for 24 h.



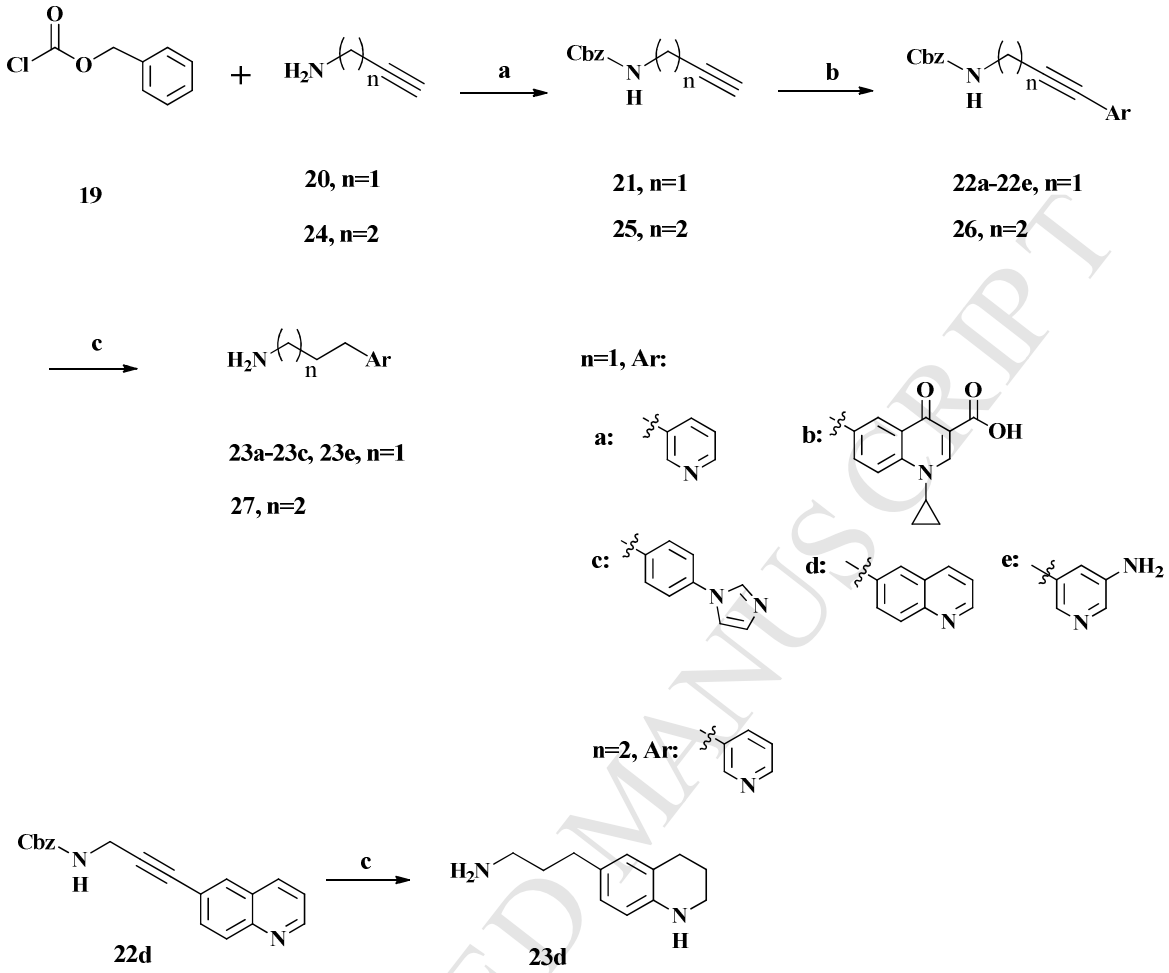
Scheme 3. The synthesis of compounds **8a** and **8b**. Reagents and conditions: (a) i: pivaloyl chloride, Et_3N, DCM, -15 ºC for 45 min; ii: 2-amino-5-bromopyridine or 3-amino-5-bromopyridine, DMAP, rt, 4.5 h.



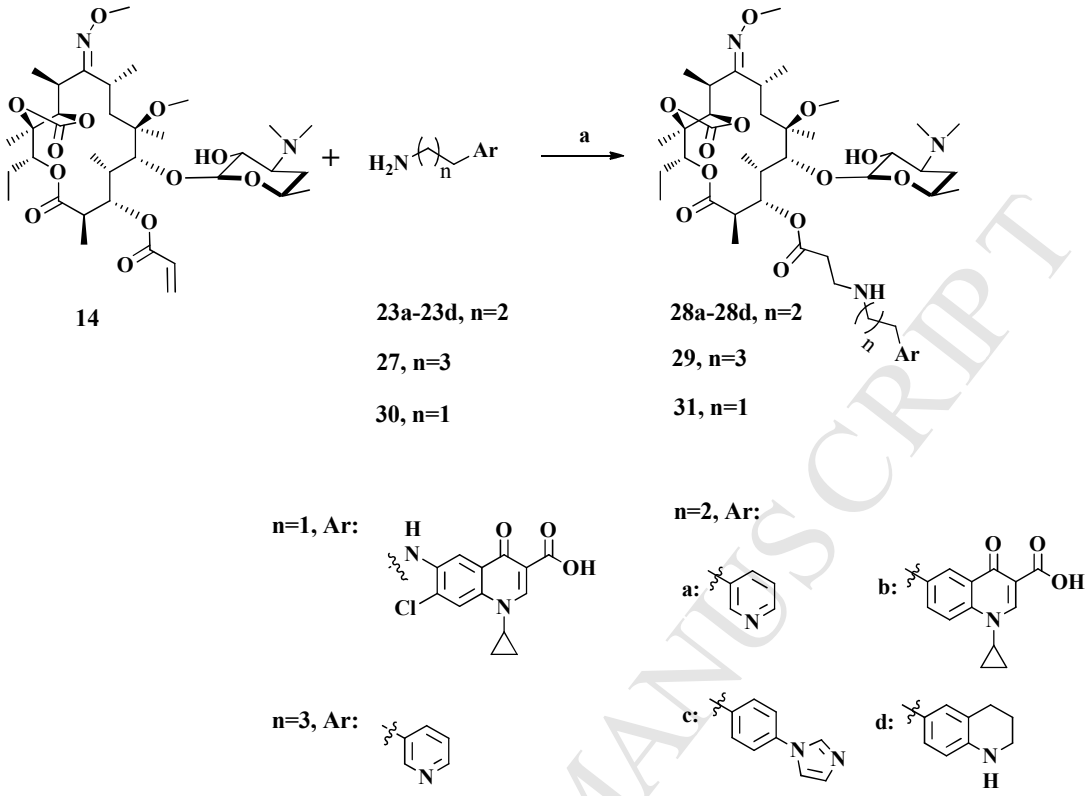
Scheme 4. The synthesis of compounds **13a-13e**. Reagents and conditions: (a) KO*t*Bu, CH_3I, CH_3CN, 1 h; (b) pyridine, bis(trichloromethyl)carbonate, DCM, -5 ℃ for 4 h, then rt for 18-20 h; (c) KO*t*Bu, propargyl bromide, DMF, rt, 2 h; (d) ArBr (including **8a-8b** and other commercially available regents), $(PPh_3)_2PdCl_2$, CuI, Et_3N, CH_3CN, 80 □, 4 h; (e) MeOH, 65 ℃, 3 h.



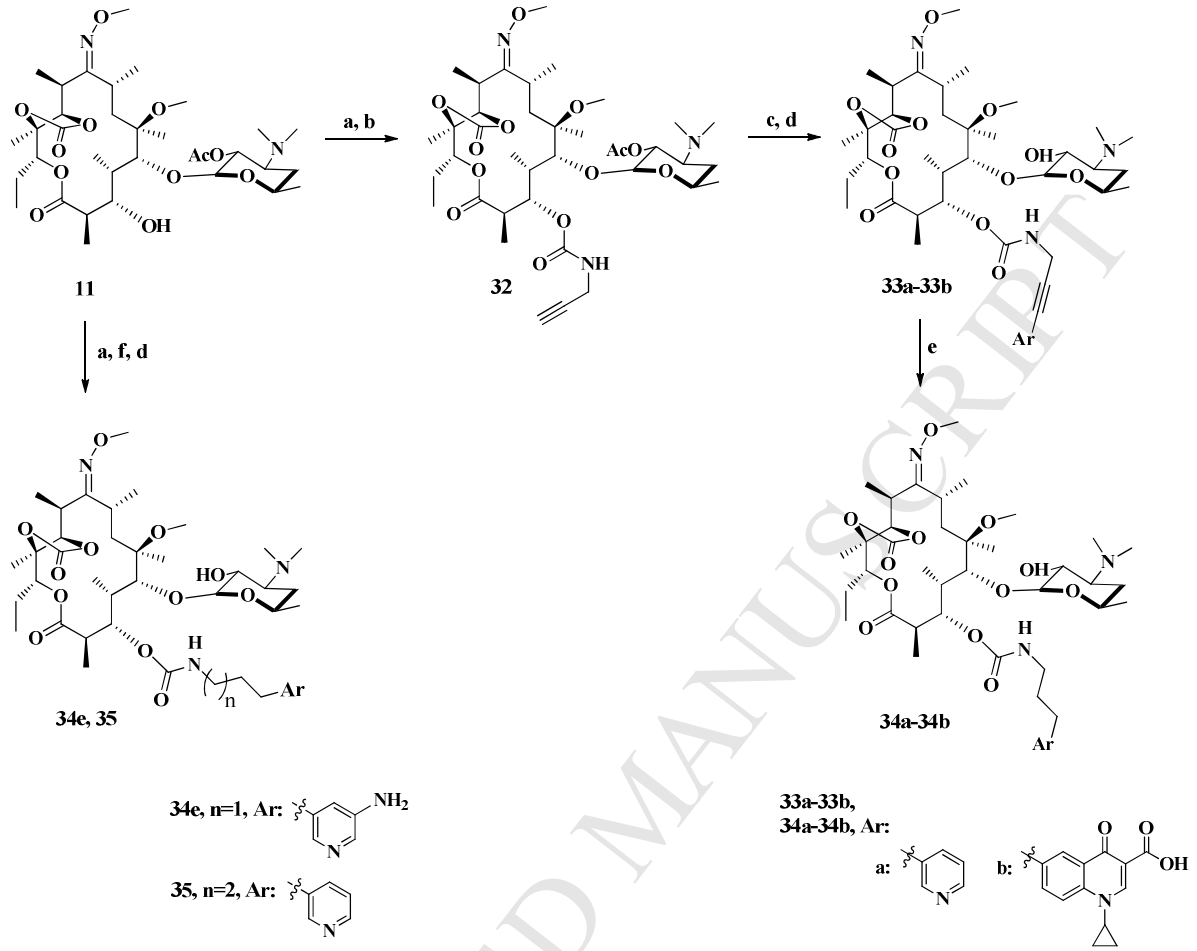
Scheme 5. The synthesis of compounds **15-18**. Reagents and conditions: (a) 3-chloropropionyl chloride, Et_3N, CH_3CN, 80 ºC, 3-5 h; (b) MeOH, 65 ºC, 3 h; (c) 3-aminopropyne, ethyldiisopropylamine, MeOH, 60 ºC, 6 h; (d) 3-bromopyridine, $(PPh_3)_2PdCl_2$, CuI, Et_3N, CH_3CN, 80 ºC, 3 h; (e) allylamine hydrochloride, ethyldiisopropylamine, MeOH, 60 ºC, 6 h; (f) 3-bromopyridine, palladium acetate, tri(*o*-tolyl)phosphine, Et_3N, CH_3CN, 60 ºC for 1 h, then 90 ºC for 24 h.



Scheme 6. The synthesis of compounds **23a-23e** and **27**. Reagents and conditions: (a) $NaHCO_3$, $EtOH/H_2O$ (1:1), 0 ºC to 25 ºC, overnight; (b) ArBr (ArI), $(PPh_3)_2PdCl_2$, CuI, Et_3N, CH_3CN, 80 ºC, 3 h; (c) i: ammonium formate, formic acid, 10% Pd-C, MeOH, 2 atm (or 3 atm) H_2, 60 ºC for 5 h, then rt over a night; ii: 40% aq. NaOH.



Scheme 7. The synthesis of compounds **28a-28d**, **29** and **31**. Reagents and conditions: (a) ethyldiisopropylamine, MeOH, 60 ºC, 5-7 h.



Scheme 8. The synthesis of compounds **34a-34b, 34e** and **35**. Reagents and conditions: (a) DMAP, CDI, DCM, rt, 20 h; (b) 3-aminopropyne, DBU, DMF, 0 ºC to 25 ºC, 48 h; (c) 3-bromopyridine or 1-cyclopropyl-1,4-dihydro-6-iodo-4-oxo-3-quinolylcarboxylic acid, $(PPh_3)_2PdCl_2$, CuI, Et_3N, 80 ºC, CH_3CN, 3 h; (d) MeOH, 60 ºC, 3 h; (e) ammonium formate, formic acid, 10% Pd-C, MeOH, H_2, 60 ºC over a night; (f) **23e** or **27**, DBU, DMF, 0 ºC to 25 ºC, 48 h.

Highlights:

1. SAR of non-ketolides with prolonged spacers at 3-OH was illuminated.

2. **34a** is a time-dependent bactericidal agent with high antibacterial potency.

3. **34a** might be a new chemotype interacting with the bacterial rRNA base G2505Ec.

4. **34a** is superior to analogous macrolones **28b**, **31** and **34b**.

5. **34a** has superior pharmacokinetic properties over telithromycin in SD rats.