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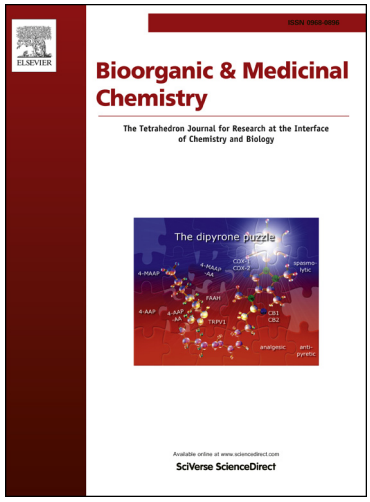


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Dražen Pavlović, Stjepan Mutak

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# Synthesis and Antibacterial Evaluation of Novel 4“-Glycyl Linked Quinolyl-Azithromycins with Potent Activity against Macrolide-Resistant Pathogens

*Dražen Pavlović*[*] *and Stjepan Mutak*

PLIVA Research Institute, Prilaz baruna Filipovića 29, 10000 Zagreb, Croatia



[*] Corresponding author. Present address: Siget 18c, 10000 Zagreb, Croatia, Tel.: +385 91 5975 380. E-mail dpavlovic@hotmail.fr

Abbreviations: $MLS_B$, macrolide-lincosamide-streptogramin B antibiotics; HBTU, O-(Benzotriazol-1-yl)-N, N, N’, N’-tetramethyluronium hexafluorophosphate; DIPEA, diisopropylethylamine; SAR, structure-activity relationships.

**Abstract**

A new azithromycin-based series of antibacterial macrolones is reported, which features the use of a 4”-ester linked glycin for tethering the quinolone side chain to the macrolide scaffold. Among the analogues prepared, compounds **9e** and **22f** with a quinolon-6-yl moiety were found to have potent and well-balanced activity against clinically important respiratory tract pathogens, including erythromycin-susceptible and $MLS_B$ resistant strains of *S. pneumoniae*, *S. pyogenes*, and *H. influenzae*. In addition, potential lead compounds **9e** and **22f** demonstrated outstanding levels of activity against *M. catarrhalis* and inducibly $MLS_B$ resistant *S. aureus*. The best member of this series **22f** rivals or exceeds, in potency, some of the most active ketolide antibacterial agents known today, such as telithromycin and cethromycin.

Keywords

Macrolide antibiotics, C-4”-substituted azithromycins, quinolones, macrolide resistance

## 1. Introduction

The development of bacterial resistance to currently available antibacterial agents is a growing global health problem.[1] To address the drug resistance problem, tremendous efforts have been devoted to the modification of existing classes of antibacterial agents to provide new analogues with improved attributes. As a consequence, a new generation of macrolide antibacterial agents, so called ketolides, have been developed particularly to combat respiratory tract pathogens that have acquired resistance to macrolides.[2] The ketolides are semi-synthetic derivatives of the 14-membered macrolide erythromycin A in which L-cladinose from the C-3 position of the macrolide ring was replaced with a ketone functional group.[3] The most active ketolides additionally contain an 11,12 cyclic carbamate ring or γ-lactone ring in place of the two hydroxyl groups of erythromycin A and an arylalkyl or arylallyl side chain, resulting in enhanced *in vitro* activity especially against constitutively $MLS_B$ resistant respiratory pathogens.

Telithromycin (**1**, Figure 1) is the first member of the ketolide class of antibacterials which has been approved for clinical use and subsequently launched on the market in the EU in 2001 and the US in 2004,[4] while two additional ketolides, modithromycin,[5] and solithromycin[6] are presently in Phase II and Phase III clinical trials in Japan and the US, respectively.[7] However, due to a serious hepatoxicity issue **1** had been subsequently restricted[8], while clinical development of structurally similar ketolide, solithromycin had

been seriously compromised until this issue is fully resolved.[9,10] In addition, we have recently demonstrated that the C-11,C-12 cyclic carbamate present in telithromycin and cethromycin (**2**, Figure 1; ABT-773)[11] can be replaced by a properly functionalized C-11,C-12 α-amino-γ-lactone ring. This type of modification resulted in a novel lead series exemplified by the prototype ketolide antibiotic GW773546X (**3**) (Figure 1).[12,13]

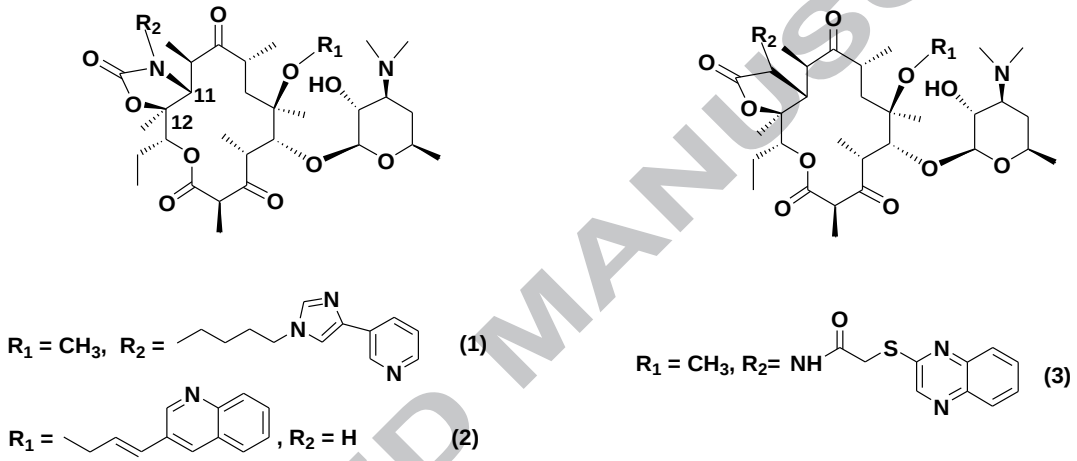



**Figure 1.** Representative examples of ketolides derived from 14-membered macrolides. Structures of telithromycin (**1**), cethromycin (**2**), and GW773546X (**3**)

The ketolide series is not necessarily the only class of macrolides that can effectively address the resistance problems. Numerous other design concepts have come forward in response to the growing need for new macrolide antibiotics with improved antibacterial activity against resistant pathogens. Coupling of two or more different pharmacophore substructures in a single molecule, aimed at obtaining hybrids with a possible cooperative effect and dual mode of action, is an approach which has been investigated by many research

groups, often with success.[14-17] In this connection, the recognition ability of nucleobases has been used to design hybrids with a wide variety of molecules such as porphyrins,[18,19] β-lactam antibiotics,[20] steroids,[21] or peptides.[22,23] Some of these conjugates have found applications in fields such as molecular recognition, inhibition of gene expression, or anticancer and antivirus therapies. Recently, one of the more novel attempts involved the successful coupling of a suitable quinolone and certain cephalosporins.[24-26] In a similar approach, quinolone-sulfonamide, quinolone-trimethoprim, and quinolone-oxazolidinone hybrids have been synthesized and evaluated for their potential to act as inhibitors with a balanced dual mode of action.[27-29]

The 15-membered ring macrolide antibiotics – for example, azithromycin[30] – are an important series within the macrolide class of antibiotics since they offer some advantages over 14-membered macrolides derived from erythromycin A. These advantages include better gastrointestinal tolerance, safety profile and pharmacokinetics coupled with activity against Gram-negative pathogens. However, one of the most important disadvantages of azithromycin in comparison to the novel generations of macrolide antibiotics is its inactivity against most macrolide-resistant respiratory pathogens, especially those that confer resistance by ribosome methylation (*erm*) and efflux by macrolide pumps (*mef*).

In the search of compounds likely to overcome the problem of macrolide resistance a new class of 15-membered ring macrolide antibacterial agents, so called “macrolones”, was

developed in Pliva Research Institute in the early 2000's. The term macrolone stands for a hybrid composed of macrocyclic lactone to which a quinolone moiety is attached via suitable linker of different structure and length (Figure 2). Considering the two pharmacophore substructures: macrolides and quinolones, we wondered if combining the structural elements of the macrolides and quinolones to produce the macrolones, would incorporate the beneficial antibacterial activities of both classes.

In our previous work[31], we have synthesized a range of 15-membered macrolone analogues, which has provided us with the information toward an understanding of the essential structural features needed for antibacterial activity. Specifically, it was recognized that the peripheral modification of the macrolide core, particularly at the C-4" position of cladinose, with quinolone pharmacophore substructures could significantly enhance antibacterial activity against a variety of erythromycin-susceptible and $MLS_B$-resistant Gram-positive pathogens as well as fastidious Gram-negative pathogens.[32] However, contrary to our expectations of their dual mode of action, these studies have also shown that the C-4" substituted macrolones actually act as protein inhibitors in agreement with their exclusive macrolide mode of action. In addition, this approach seems to be very promising in the development of leads for drug discovery applications, as the antibacterial activity of several new C-4" substituted macrolones exceeds that of the parent compounds. Interestingly, the antibacterial activity of some of these new synthesized C-4" substituted azithromycin

derivatives rivaled the action of some of the most effective and most advanced representatives of ketolide class of antibacterial agents[33-35] against all key respiratory pathogens.

## 2. Results and discussion

### *2.1. Chemistry*

In order to expand the structure-activity relationships and more thoroughly define the scope of activity, and select the promising candidates for preclinical development we felt that synthesis of additional 4”-substituted azithromycin analogues could even further facilitate the construction of compound libraries for biological screening, and therefore, considered such an endeavor. We recognized that the C-4” hydroxyl group of azithromycin offered the possibility of alternative modes of attaching the side chain other than the ether linkage. As it had previously been demonstrated that the secondary C-4” hydroxyl group could be relatively easily alkylated with activated alkylating agent, such as allyl-t-butylcarbonate, we reasoned that a series of macrolones having the side chain attached through a C-4” glycyl ester linkage should be synthetically feasible. Our strategy was to functionalize the C-4” position of azithromycin with a suitable small linker sidearm which contain an appropriate functionality at the terminal position. Basically, it was anticipated that BOC protected glycin could be used as starting material to regioselectively acylate the C-4” hydroxyl group of azithromycin. Subsequent deprotection of BOC protecting group should afford 4”-glycyl azithromycin that

could be used as a key intermediate for further derivatization *via* amide coupling or reductive amination. In this article, we report the successful implementation of this strategy resulting in a series of 4”-glycyl tethered azithromycin macrolones with excellent *in vitro* activity. A typical design leading to novel C-4”-glycyl linked macrolides tethered to quinolone subunit by different linkers is shown in Figure 2.

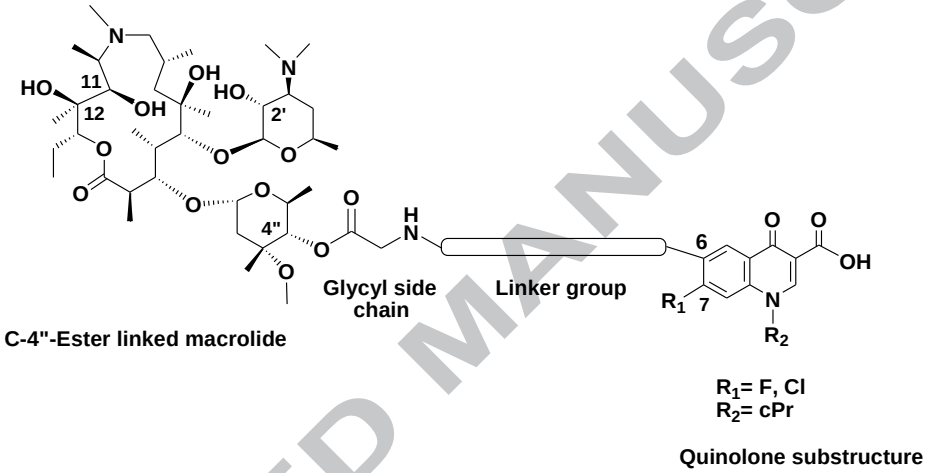
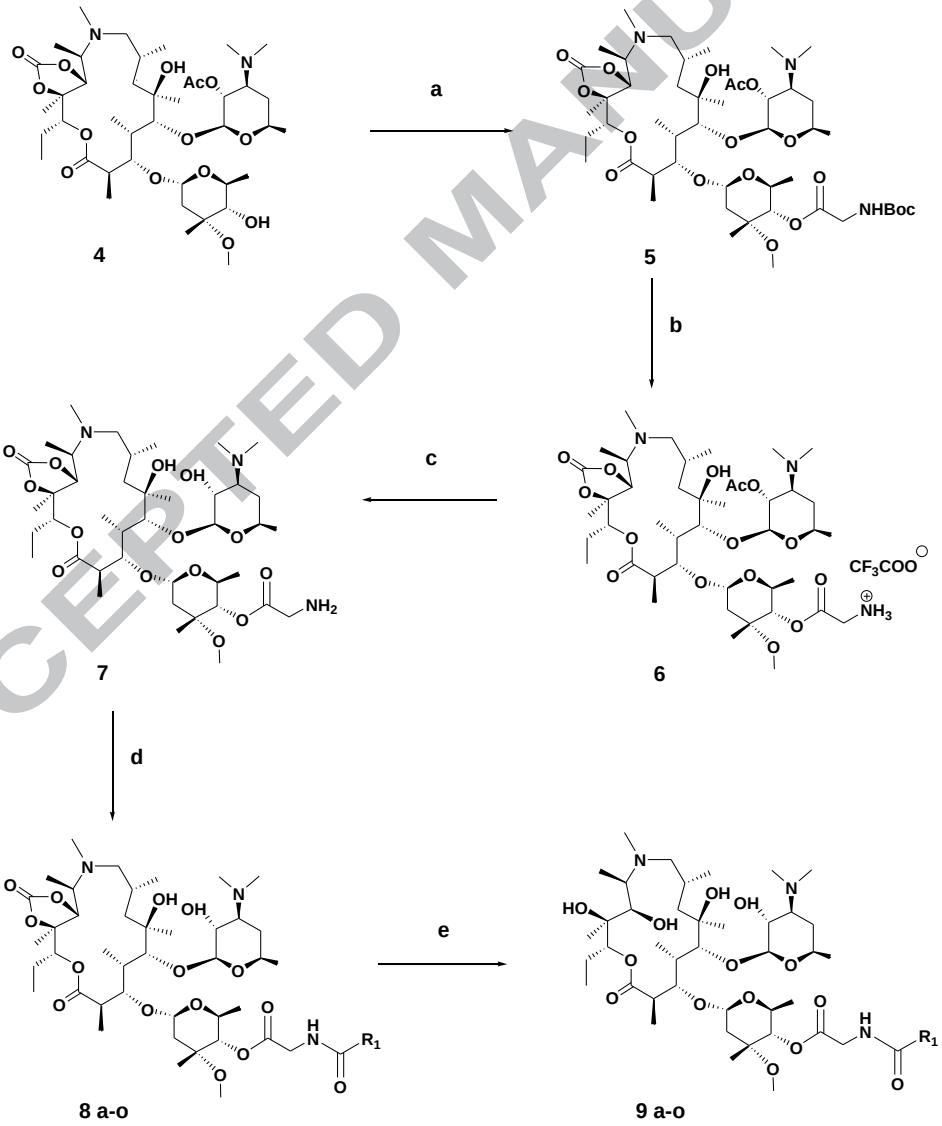



**Figure 2.** Design leading to novel C-4”-glycyl linked macrolides

In order to selectively acylate the hydroxyl group at the C-4”-position, a regioselective protection strategy had to be employed. This approach involved selective acylation of the C-4” hydroxyl group using BOC-protected glycin. Thus, commercially available azithromycin was first protected as its 2’-O-acetyl-11,12-cyclic carbonate derivative **4** according to a modified literature procedure (Scheme 1).[36] Selective acylation with BOC glycin in the presence of a catalytic amount of DMAP followed by subsequent deprotection of the BOC protecting group with trifluoroacetic acid gave the expected trifluoroacetate salt of 2’-O-acetyl-4”-O-glycyl azithromycin-11,12-cyclic carbonate **6** in 75% isolated yield. Deprotection of the 2’-acetyl

group was readily accomplished by stirring with aqueous ammonia in methanol at room temperature to give 4”-glycyl azithromycin **7** in essentially quantitative yield. The structure of **7** was confirmed via $^{1}H$ and $^{13}C$ NMR spectroscopy. This compound served as a key intermediate for the preparation of a series of amide analogues according to Scheme 1.

**Scheme 1.** Synthesis of C-4”-ester linked macrolides by HBTU mediated coupling of key intermediate **7** with selected carboxylic acids (Chart 1)[a]

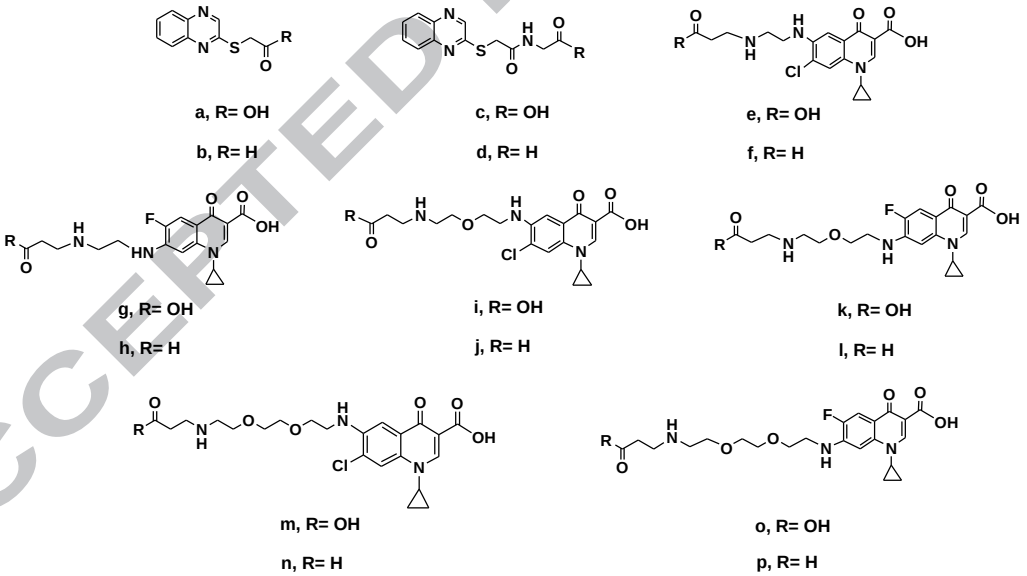



[a]Reagents and conditions: a) BocGly, EDC·HCl, DMAP, $CH_2Cl_2$, rt, 85%; b) TFA, $CH_2Cl_2$,

0°C to rt, 95%; c) aq. $NH_3$, MeOH, rt, 90%; d) $R_1CO_2H$, HBTU, DIPEA, DMF, rt, 70-90%; e) LiOH, THF/$H_2O$, rt, 85-95%.

HBTU mediated coupling of **7** with C-6 and C-7 substituted quinolone carboxylic acids (Chart 1) in the presence of DIPEA cleanly afforded the desired amides **8a-o** in yields ranging from 70-90%. Deprotection of cyclic carbonates **8a-o** with LiOH in aqueous THF finally gave azithromycin derivatives **9a-o** which were purified by flash column chromatography.

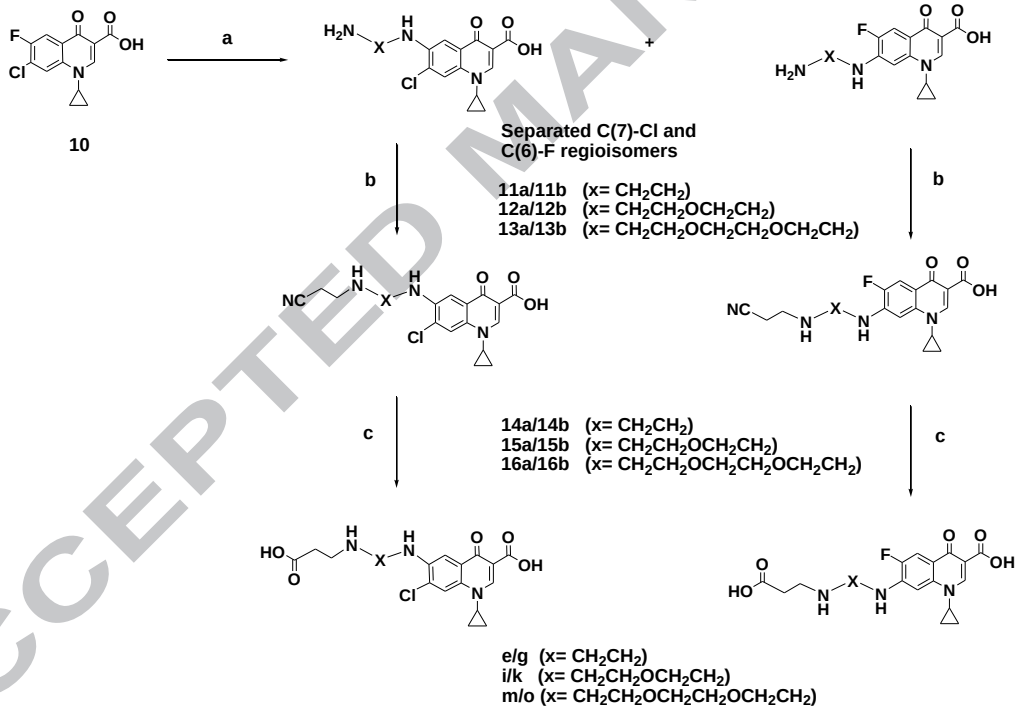
**Chart 1.** Quinoxalines and quinolones used as the $R_1$ substituents in HBTU coupling and reductive amination of **7**




Carboxylic acid **a** used for HBTU coupling reaction was commercially available while the acid **c** was synthesized according to our published procedure.[13] On the other hand, the synthesis of carboxylic acids **e**, **g**, **i**, **k**, **m**, and **o** was effected by treatment of the

commercially available 6-fluoro-7-chloro-1-cyclo-propyl-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (**10**) with appropriate amine and $K_2CO_3$ in refluxing 1-methyl-2-pyrolidone, followed by Michael addition of the resulting separated[37] C(7)-chloro and C(6)-fluoro regioisomers **11a-13a** and **11b-13b** to acrylonitrile according to a modified published procedure (Scheme 2).[38] Michael addition proceeded in a large excess of acrylonitrile and a catalytic amount of DBU (0.5 molar equiv.) at 70°C for 15 h.

**Scheme 2.** Synthesis of C-6 and C-7 substituted quinolone carboxylic acids (**e-o**)[a]



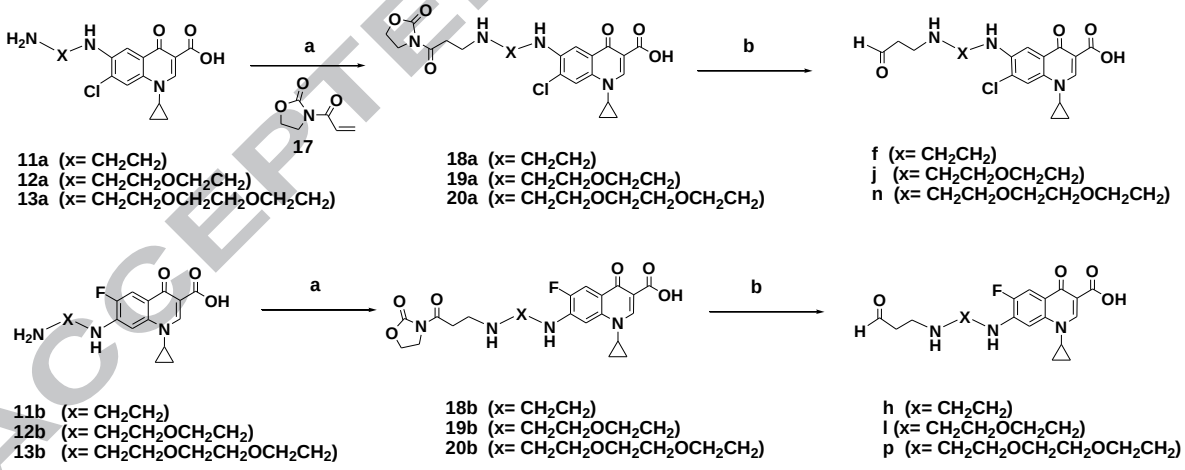

[a]Reagents and conditions: a) ethylenediamine or 2-(2-aminoethoxy)ethanamine or 2-(2-(2-aminoethoxy)ethoxy)ethanamine, $K_2CO_3$, 1-methyl-2-pyrolidone, 100°C, 8 h, 25-40%; b) acrylonitrile, DBU, 70°C, 15 h, 60-70%; c) $H_2SO_4/H_2O$ (1:1), 70°C, 5 h, 100%.

The regioisomeric nitriles **14a-16a** and **14b-16b** thus obtained were then subjected to acidic

hydrolysis in aqueous $H_2SO_4$ (1:1) at 70°C to afford the corresponding carboxylic acids **e-m** and **g-o** in essentially quantitative yield after acidic workup at pH=3.

The synthesis of aldehydes used for reductive amination is illustrated in Scheme 3. Treatment of the regioisomerically pure quinolone amines **11a-13a** and **11b-13b** with 5 equiv. of 3-acryloyl oxazolidin-2-one **17**[39] in i-PrOH at 70°C produced a series of Michael addition adducts **18a-20a** and **18b-20b** which were immediately used in the next step without further purification. Subsequent reduction of the oxazolidinones **18a-20a** and **18b-20b** with diisobutylaluminum hydride at -78°C in THF afforded the desired aldehydes **f-n** and **h-p** in acceptable yields (60-80%).

**Scheme 3.** Synthesis of C-6 and C-7 substituted quinolone aldehydes (**f-p**)[a]



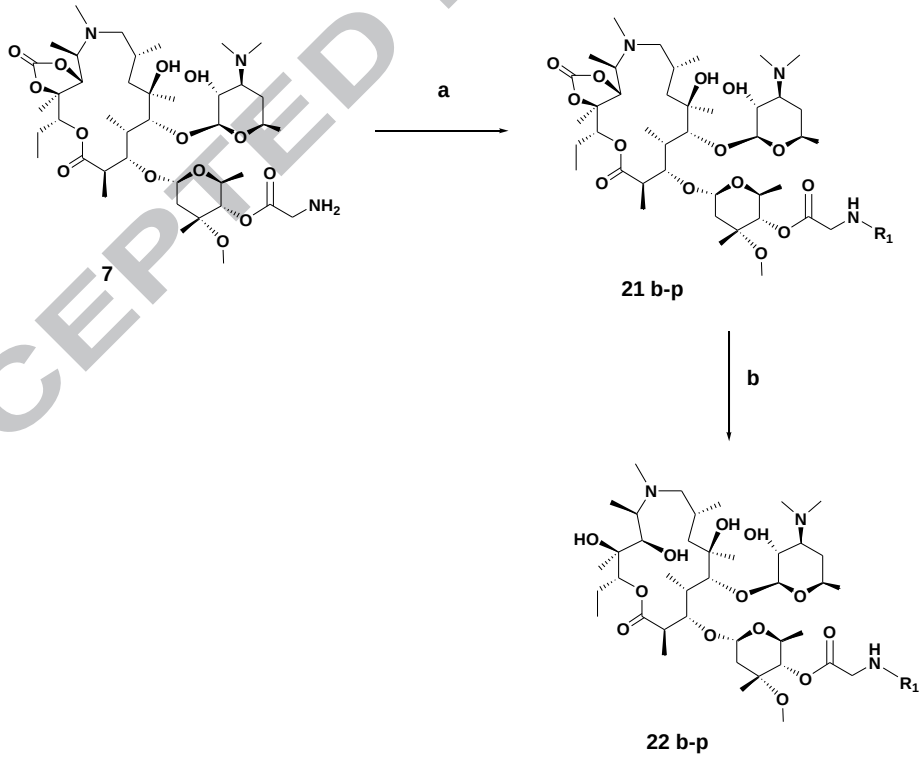

[a]Reagents and conditions: a) 3-acryloyl oxazolidin-2-one (**17**), DIPEA, i-PrOH, 70°C, 16 h, 65-85%; b) DIBAL-H, THF, -78°C, 3 h, 60-80%.

Additionally, an alternate route had to be developed to synthesize aldehydes **b** and **d**. Thus,

the known methyl esters[13] of carboxylic acids **a** and **c** were subjected to DIBAL-H reduction at -78°C in THF to afford the corresponding aldehydes **b** and **d** in 68% and 75% isolated yield, respectively.

Next we focused our attention on the effects of modification of the linker-glycyl side chain connection moiety on the antibacterial activity. From 4”-O-glycyl azithromycin 11,12-cyclic carbonate (**7**), another series of azithromycin analogues was prepared by reductive amination with aldehydes (Chart 1), as outlined in Scheme 4.

**Scheme 4.** Synthesis of C-4”-ester linked macrolides by reductive amination of amine **7** with selected aldehydes (Chart 1)[a]




[a]Reagents and conditions: a) $R_1CHO$, $NaBH(OAc)_3$, AcOH, MeOH, rt, 20 h, 60-90%; b) LiOH, $THF/H_2O$, rt, 15 h, 85-95%.

Reductive amination[40] of **7** with C-6 and C-7 substituted quinolone aldehydes (Chart 1) using sodium triacetoxyborohydride proceeded smoothly to afford the corresponding azithromycin analogues **21b-p** in generally acceptable isolated yields ranging from 60-90%. Subsequent deprotection of cyclic carbonates **21b-p** was carried out with LiOH in aqueous THF to afford azithromycin derivatives **22b-p** in 85-95% isolated yield.

### 2.2. Antibacterial activity

The antibacterial activity of the C-4"substituted azithromycins was tested against a panel of representative pathogens selected from the Pliva Research Institute culture collection. The *in vitro* antibacterial activities are reported as minimum inhibitory concentrations (MICs) that were determined by the agar microdilution method according to NCCLS standards.[41] Table 1 shows the *in vitro* activity of the azithromycin analogues and the reference compounds, azithromycin, telithromycin (**1**), cethromycin (**2**), and ciprofloxacin.

The results tabulated in Table 1 show that although azithromycin is very potent against erythromycin susceptible strains it is only weakly active against efflux resistance (*mef*) and inactive against $MLS_B$ resistance. On the other hand, telithromycin (**1**) and cethromycin (**2**) showed activity against all key resistant pathogens tested except constitutively $MLS_B$ resistant *S. aureus*. In general, the macrolones were all inactive against constitutively $MLS_B$-resistant strain of *Staphylococcus aureus* (MICs > 64 μg/mL). They were, however, in few cases (**9e** and

**22f**), about one dilution more potent than azithromycin against *H. influenzae* strain. In contrast to 4"-ether macrolones[32], most of these compounds were active against inducibly resistant *S. aureus* strain. The most interesting feature of these new compounds was their effectiveness against efflux resistant staphylococci and pneumococci as well as the activity of selected analogues against inducibly and constitutively $MLS_B$-resistant pneumoccoci.

**Table 1.** *In Vitro* Antibacterial Activity of C-4"-Ester Linked Macrolides against Selected Pathogens[a,b]

| Compound | *S. aureus* | | | | *S. pneumoniae* | | | *S. pyogenes* | | | | *M. cat.*[c] | *H. inf.*[d] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Ery-S | $iMLS_B$ | $cMLS_B$ | M | Ery-S | $cMLS_B$ | M | Ery-S | $iMLS_B$ | $cMLS_B$ | M | | |
| **7** | 32 | >64 | >64 | >64 | ≤0.125 | >64 | 32 | ≤0.125 | 32 | >64 | >64 | >64 | >64 |
| **9a** | 4 | 1 | >64 | 8 | ≤0.125 | 16 | 0.25 | ≤0.125 | 8 | 32 | 0.5 | 16 | 8 |
| **9c** | 1 | 0.5 | >64 | 2 | ≤0.125 | 16 | ≤0.125 | 0.25 | 4 | 16 | 0.25 | 4 | 4 |
| **9e** | ≤0.125 | 1 | >64 | 0.5 | ≤0.125 | 0.5 | 0.25 | ≤0.125 | 0.25 | 0.5 | 0.25 | 0.125 | 0.5 |
| **9g** | 0.25 | 8 | >64 | 2 | ≤0.125 | 4 | 1 | ≤0.125 | 1 | 4 | 2 | 1 | 4 |
| **9i** | ≤0.125 | 2 | >64 | 1 | ≤0.125 | 1 | 0.5 | ≤0.125 | 0.5 | 2 | 1 | 0.5 | 2 |
| **9k** | 0.25 | 4 | >64 | 4 | ≤0.125 | 8 | 4 | ≤0.125 | 4 | 16 | 8 | 1 | 4 |
| **9m** | ≤0.125 | 4 | >64 | 0.5 | ≤0.125 | 4 | 1 | ≤0.125 | 1 | 8 | 2 | 1 | 4 |
| **9o** | 0.5 | 32 | >64 | 16 | ≤0.125 | 8 | 4 | ≤0.125 | 4 | 32 | 16 | 2 | 8 |
| **22b** | 2 | 0.5 | >64 | 2 | ≤0.125 | 16 | ≤0.125 | ≤0.125 | 4 | 16 | 0.25 | 1 | 4 |
| **22d** | 8 | 0.25 | >64 | 1 | ≤0.125 | 8 | ≤0.125 | ≤0.125 | 1 | 8 | ≤0.125 | 8 | 8 |
| **22f** | ≤0.125 | 0.25 | >64 | 0.25 | ≤0.125 | ≤0.125 | ≤0.125 | ≤0.125 | ≤0.125 | ≤0.125 | ≤0.125 | ≤0.125 | 0.5 |
| **22h** | ≤0.125 | 1 | >64 | 0.5 | ≤0.125 | 0.5 | ≤0.125 | ≤0.125 | 0.25 | 1 | 0.5 | 0.25 | 1 |
| **22j** | ≤0.125 | 0.5 | >64 | 0.5 | ≤0.125 | 0.25 | 0.25 | ≤0.125 | 0.25 | 1 | 0.5 | 0.25 | 2 |
| **22l** | 0.25 | 2 | >64 | 1 | ≤0.125 | 2 | 0.25 | ≤0.125 | 0.5 | 4 | 2 | 1 | 2 |
| **22n** | ≤0.125 | 1 | >64 | 1 | ≤0.125 | 1 | 0.5 | ≤0.125 | 0.25 | 2 | 1 | 0.5 | 2 |
| **22p** | ≤0.125 | 4 | >64 | 2 | ≤0.125 | 2 | 1 | ≤0.125 | 0.5 | 4 | 1 | 2 | 4 |
| **1** | ≤0.125 | 0.5 | >64 | 0.25 | ≤0.125 | ≤0.125 | 0.5 | ≤0.125 | ≤0.125 | 8 | 0.25 | ≤0.125 | 1 |
| **2** | ≤0.125 | 0.25 | >64 | 0.25 | ≤0.125 | ≤0.125 | ≤0.125 | ≤0.125 | ≤0.125 | 1 | ≤0.125 | ≤0.125 | 1 |
| **Azi** | 1 | >64 | >64 | 32 | ≤0.125 | >64 | 8 | ≤0.125 | >64 | >64 | 16 | 0.25 | 1 |
| **Cipro** | ≤0.125 | 16 | ≤0.125 | ≤0.125 | ≤0.125 | 0.25 | 0.25 | 2 | 0.25 | 0.5 | ≤0.125 | ≤0.125 | ≤0.125 |

[a] Minimum inhibitory concentration (MIC) values are given in μg/mL. [b]All quinolones (**a-p**) were inactive (MIC's> 64 μg/mL) against $cMLS_B$ *S. aureus* strain. [c]*M. catarrhalis*. [d]*H. influenzae*. Ery-S: erythromycin-susceptible strains; $iMLS_B$: inducible resistance to $MLS_B$ antibiotics; $cMLS_B$: constitutive $MLS_B$ resistance; M: efflux mediated macrolide resistance. Azi: azithromycin. Cipro: ciprofloxacin.

### 2.2.1. Structure–activity relationships of 4"-glycyl linked azithromycin macrolones

The key intermediate used for further derivatization, 4"-glycyl azithromycin **7** was basically inactive against all the strains tested except erythromycin-sensitive *S. pneumoniae* and *S. pyogenes* (MIC≤ 0.125 μg/mL). The presence of a quinoxaline ring in the macrolones **9a**, **9c**, **22b**, and **22d** resulted in marked increases in activity against inducibly $MLS_B$ resistant *S. aureus* and *S. pyogenes* as well as constitutively $MLS_B$ resistant strains of *S. pneumoniae* and *S. pyogenes.* Moreover, the compounds were active against both efflux-resistant *S. pneumoniae* and *S. pyogenes*.

By analogy to 4"-ether macrolone series developed previously[32], it was anticipated that attaching a quinolone side chain to **7** would lead to an additional improvement in activity, particularly against inducibly and constitutively $MLS_B$ resistant *S. pneumoniae* and *S. pyogenes*. Initially, a series of quinolone-substituted compounds **9e-o** was prepared to identify the optimum linker between the quinolone ring and the C-4" position of azithromycin. It was found that compounds with the shorter sidechains such as **9e** and **9g** were superior to those with the longer sidechains as in **9i**, **9k**, **9m**, and **9o**. In particular, **9e** and **9g** showed good activity against constitutively $MLS_B$ resistant strains of *S. pneumoniae*

and *S. pyogenes* and the inducibly resistant *S. aureus* strain, whereas **9i**, **9k**, **9m**, and **9o** were basically less potent against all of these strains. All six of the compounds exhibited improved activity against both the erythromycin-susceptible and the efflux-resistant *S. pneumoniae* strains, when compared to 4"-glycyl azithromycin **7**. As with telithromycin, none of the compounds showed measurable activity against the constitutively resistant *S. aureus*. Overall, the macrolone **9e** appeared to have the best antibacterial profile, being the most potent within the series of 6 amide analogues against *S. aureus*, *H. influenzae*, and constitutively $MLS_B$ resistant *S. pneumoniae* and *S. pyogenes*.

Having identified the linker in macrolone **9e** as optimal, we synthesized a series of macrolones in which amide bond connecting the macrolide and quinolone pharmacophores was replaced with an amine bond as in compounds **22f**, **22j**, **22n**, **22h**, **22l**, and **22p**. The 4"-glycyl amine series (**22f-p**) demonstrated much higher levels of *in vitro* activity in comparison to the glycyl amide series (**9e-o**) as the MIC's were consistently lower. Thus, the activity of the macrolone with the longest side chain **22n** was 2 to 4-fold better to its amide counterpart **9m**, with the exception of 2-fold lower potency against the efflux resistant *S. aureus* strain. The antibacterial activity of the macrolone **22j** was in general 2 to 4-fold better in comparison to **9i**, while the macrolone with the shortest side chain **22f** also showed 2 to 4-fold better activity when compared to **9e** against most of the strains tested. In fact, compound **22f** *in vitro* profile compares favorably with telithromycin and cethromycin.

In general, the structure-activity relationships of both series were quite similar to that of C-4" ether macrolones developed previously with the exception of being more potent against inducibly and efflux resistant strains of *S. aureus*.[32] The compounds most active against constitutively resistant streptococcal strains (**9e**, **22f**) are linked through the C-6 position of the quinolone nucleus and the C-4"-position of azithromycin by a spacer of ten atoms in length. In an amide series, antibacterial results show a linker-length-dependent activity which peaked with compound **9e**, a macrolide analogue having a ten carbon-heteroatom linker separating the quinolone ring from the C-4" ester oxygen on the cladinose ring. Compounds **9i** and **9m**, analogues having longer carbon-heteroatom linkers than those of **9e**, show a reduction in antibacterial activity with an increase in the linker length. In a similar fashion compounds within an amine series also show gradual reduction in activity, the analogue with the shortest, ten carbon-heteroatom linker in the series **22f** being the most active, following by the macrolones **22j** and **22n** having 13 and 16 carbon-heteroatom spacers, respectively. The antibacterial activity of the C-6 substituted quinolones within an amide series **9e, 9i,** and **9m** were in general 2-8-fold better than that of the corresponding C-7 substituted analogues **9g, 9k,** and **9o**, respectively, against most of the strains tested. In the same way, the antibacterial activity of the C-6 substituted amine analogues **22f**, **22j**, and **22n** were found to be superior to their corresponding C-7 substituted counterparts, **22h**, **22l**, and **22p**, respectively. In addition, potential lead compounds **9e** and **22f** demonstrated outstanding

levels of activity against *H. influenzae* and constitutively $MLS_B$ resistant *S. pyogenes*. In particular, **9e** and **22f** were more potent than telithromycin against *H. influenzae* strain (MICs 0.5 μg/mL) and $cMLS_B$ *S. pyogenes* (MICs of 0.125).

## 3. Conclusion

In summary, a novel series of azithromycin derivatives with potent activity against key respiratory pathogens, including those resistant to macrolide antibiotics, has been identified. These compounds are characterized by having C(6) or C(7)-substituted quinolonyl moiety tethered to the C-4”-position of the azithromycin skeleton through an ester bond. Biological evaluation of these analogues revealed several highly potent compounds effective against both erythromycin-susceptible and $MLS_B$-constitutively resistant pathogens. In general, amide-linked quinolone analogues (**9e-o**) were not as active as macrolones (**22f-p**) incorporating quinolone substructures linked via an amine group. On the other hand, compounds containing the quinoxaline ring showed weak activity against constitutively $MLS_B$ resistant *S. pneumoniae* and *S. pyogenes*. However, they did show excellent activity against inducibly $MLS_B$ resistant *S. aureus* and both *mef*-resistant *S. pneumoniae* and *S. pyogenes*.

## 4. Experimental section

### *4.1. General experimental methods*

Proton ($^{1}H$) and carbon ($^{13}C$) nuclear magnetic resonance spectra were recorded on either a Bruker 300 or a Bruker 500 MHz spectrometer. Chemical shifts (δ) were recorded in parts per million (ppm) relative to tetramethylsilane as an internal standard. Low-resolution electron impact mass spectra (MS) were recorded on a Varian MAT CH5 spectrometer. Fast atom bombardment (FAB) mass spectra were run on a Finnigan MAT 312 double focusing mass spectrometer, operating at an accelerating voltage of 3kV. The samples were ionized by bombardment with xenon atoms produced by a saddle-field ion source from Ion Tech operating with a tube current of 2 mA at energy of 6 keV. Electrospay positive ion mass spectra were acquired using a Micromass Q-Tof 2 hybrid quadrupole time-of-flight mass spectrometer, equipped with a Z-spray interface, over a mass range of 100-2000 Da, with a scan time of 1.5 s and an interscan delay of 0.1 s in a continuum mode. Reserpine was used as the external mass calibrant lock mass ($[M+H]^+$= 609.2812 Da). The elemental composition was calculated using a MassLynx v4.1 for the $[M+H]^+$ and the mass error quoted within ±5 ppm range. Thin-layer chromatography (TLC) was performed with Merck silica gel 60 F254 0.2-mm plates. The plates were visualized by using an acid-based stain, prepared from p-anisaldehyde (5 mL), concentrated sulfuric acid (5 mL), and glacial acetic acid (0.5 mL) in 95% ethanol (90 mL) and warming on a hot plate. Flash chromatography was carried out using Merck silica gel 60 (230-400 mesh). Solvent systems are reported as volume percent mixtures. Concentration *in vacuo* refers to the removal of solvent using a Büchi rotary evaporator and an aspirator pump.

All chromatography solvents were reagent grade. Tetrahydrofuran (THF) was distilled from sodium benzophenone ketyl, and dichloromethane was distilled from calcium hydride. All other reagents were purified by literature procedures. All reactions were performed under an inert atmosphere of dry argon. The course of the reaction was followed by chromatography on a thin layer (TLC) of silica gel (Merck 60 $F_{254}$) in solvent systems methylene chloride-methanol-ammonium hydroxide 25% (90:9:1.5, system A), (90:9:0.5), system A1) or methylene chloride-acetone (8:2, system B) (7:3, System C) unless otherwise stated. The separation of the reaction products and the purification of the products for the purpose of spectral analyses were performed on a silica gel column (Merck 60, 230-400 mesh, or 60-230 mesh in solvent systems A, B or C unless otherwise stated. Purity of tested compounds was determined by elemental analysis.

The structure of all novel compounds was confirmed by HRMS, and/or NMR spectroscopic methods. Complete and unambiguous assignments for all $^{1}H$ and $^{13}C$ resonances could be achieved on the basis of chemical shift considerations, coupling information (APT $^{13}C$ NMR spectra), and COSY, HSQC, and HMBC spectra.

Reagents were generally purchased in the highest purity available and used without further purification except otherwise stated. Azithromycin[30], telithromycin (**1**)[3] and cethromycin (**2**)[11] were synthesized in-house according to literature procedures and their *in vitro* evaluation were performed to allow direct comparison with 15-membered azalides presented in this paper.

*4.2. Determination of Minimum Inhibitory Concentration (MICs)*

The MICs of all antibiotics were determined by the agar microdilution method as recommended by National Committee of Clinical Laboratory Standards (NCCLS) guidelines. Differences in MIC values were considered significant only when the dilution is more than a factor of 2 apart. Zone sizes were measured with a Fisher Zone Reader, and antibiotic concentrations were calculated from the standard curve for the appropriate compound.

*4.3. Experimental Procedures*

*4.3.1. 2'-O-Acetyl-4"-O-tert-butyloxycarbonylglycyl-azithromycin-11,12-cyclic carbonate (**5**)*

To a solution of 2'-O-acetyl azithromycin-11,12-cyclic carbonate **4** (817.0 mg, 1.0 mmol, for $^1$H NMR spectrum of **4** see the Supporting Information, page S3) in dry dichloromethane (40 mL) was added Bocglycin (437.5 mg, 2.5 mmol), EDC HCl (639.0 mg, 3.35 mmol), and 4-DMAP (102 mg, 0.85 mmol) under argon atmosphere. The resulting mixture was stirred at room temperature for 20 hours. After evaporation to dryness, the residue was dissolved in ethyl acetate (150 mL), washed sequentially with saturated aqueous solution of $NaHCO_3$ (2x100 mL) and saturated aqueous NaCl solution (2x100 mL), and dried over anhydrous magnesium sulfate. The solvent was then evaporated *in vacuo* and the residue purified by flash chromatography on silica gel column (eluens: 90/10 $CH_2Cl_2$/MeOH) to yield 720.9 mg (74%) of **5** as a colorless solid. HRMS (ES) calcd for $C_{48}H_{83}N_3O_{17}$ $(M+H)^+$ 974.5722, found 974.5715. $^1$H NMR (500 MHz, $CDCl_3$) δ 8.00 (bs, 1H, tert-BuOCO*NH*), 4.85 (d, 1H, H-1"),

4.80 (dd, 1H, H-13), 4.75 (dd, 1H, H-2'), 4.65 (d, 1H, H-4"), 4.42 (d, 1H, H-1'), 4.30 (m, 1H, H-11), 4.25 - 4.10 (m, 2H, H-5", H-3), 3.93 (m, 2H, tert-BuOCONH*$CH_2$*), 3.70 (m, 1H, H-5'), 3.45 (m, 1H, H-5), 3.28 (s, 3H, 3"-OMe), 3.13 (m, 1H, H-10), 2.70 (m, 1H, H-2), 2.50-2.30 (m, 2H, H-3', H-9a), 2.25-2.20 (m, 2H, H-2"b, H-9b), 2.15 (s, 6H, 3'-$NMe_2$), 2.13 (s, 3H, 9-NMe), 2.10 (s, 3H, 2'-OCO*Me*), 2.01 (m, 1H, H-9b), 1.92-1.85 (m, 3H, H-4, H-7b, H-8), 1.75-1.60 (m, 2H, H-14b, H-14a), 1.56-1.49 (m, 2H, H-4'b, H-2"a), 1.48 (s, 3H, 12-Me), 1.45 (m, 1H, H-7a), 1.40 (s, 9H, *tert-Bu*OCONH), 1.23 (d, 3H, 5"-Me), 1.19-1.14 (m, 6H, 6-Me, 3"-Me), 1.12 (d, 3H, 2-Me), 1.10 (m, 1H, H-4'a), 1.05 (d, 3H, 5'-Me), 1.00-0.93 (m, 6H, 4-Me, 10-Me), 0.88 (d, 3H, 8-Me), 0.85 (t, 3H, 15-Me); $^{13}$C NMR (125 MHz, $CDCl_3$) δ 177.7 (C-1), 154.3 (tert-BuOCONH), 152.4 (11,12-C=O), 171.8 (4"-C=O), 170.0 (2'-OCOMe), 102.2 (C-1'), 94.7 (C-1"), 85.9 (C-4"), 85.2 (C-11), 84.5 (C-12), 82.3 (C-5), 77.0 (C-3), 75.1 (C-13), 79.2 (tert-BuOCONH, s, C), 73.5 (C-6), 72.9 (C-3"), 70.2 (2C; C-2', C-5'), 66.5 (C-9), 64.6 (C-3'), 59.8 (C-5"), 59.5 (C-10), 47.4 (3"-OMe), 44.6 (C-2), 41.8 (C-4), 41.1 (C-7), 40.4 (3'-$NMe_2$), 34.5 (C-2"), 34.0 (9a-NMe), 30.4 (C-4'), 28.4 (tert-BuOCONH, q, $CH_3$), 27.2 (6-Me), 25.2 (C-8), 22.6 (8-Me), 21.8 (5'-Me), 21.5 (C-14), 21.0 (3"-Me), 18.8 (5"-Me), 14.7 (2-Me), 13.5 (12-Me), 10.6 (C-15), 9.2 (4-Me), 5.6 (10-Me). Anal. Calcd. For $C_{48}H_{83}N_3O_{17}$: C, 59.18; H, 8.59; N, 4.31 Found: C, 59.35; H, 8.81; N, 4.10.

*4.3.2. 2'-O-Acetyl-4"-O-glycyl-azithromycin-11,12-cyclic carbonate trifluoroacetate salt (**6**)*

To a stirred solution of 2'-O-Acetyl-4"-O-Bocglycyl-azithromycin **5** (974.2 mg, 1.0 mmol) in

dichloromethane (20 mL) cooled in an ice-water bath was added trifluoroacetic acid (456.1 mg, 4.0 mmol). The mixture was allowed to warm to room temperature for 6 hours followed by evaporation of the volatile materials under reduced pressure. The residue was crystallized from diethyl ether/dichloromethane mixture (1:1) and filtered to afford trifluoroacetate salt **6** (859.6 mg, 87%) of sufficient purity to be used in subsequent reaction step without further purification. HRMS (ES) calcd for $C_{45}H_{76}F_3N_3O_{17}$ $(M+H)^+$ 988.5127, found 988.5144. Anal. Calcd. For $C_{45}H_{76}F_3N_3O_{17}$: C, 54.70; H, 7.75; N, 4.25 Found: C, 55.34; H, 7.81; N, 4.11.

*4.3.3. 4"-O-glycyl-azithromycin-11,12-cyclic carbonate (**7**)*

To a solution of **6** (889.3 mg, 0.9 mmol) in 20 mL of methanol was added 10 mL of aqueous $NH_3$ (25%). The solution was stirred at room temperature for 18 h. The solvent was removed in vacuo, water (100 mL) was added, and the pH was adjusted to 8 with concentrated aqueous ammonium hydroxide. The solution was extracted with ethyl acetate (2x100 mL), and the organic extracts were dried over $K_2CO_3$ to give 850.0 mg of white foam. The product was purified by flash column chromatography eluting with methylene chloride-methanol-ammonium hydroxide 25% (90:9:0.5) solvent system, to afford 688.9 mg (92%) of the title compound **7** as colorless foam. HRMS (ES) calcd for $C_{41}H_{73}N_3O_{14}$ $(M+H)^+$ 832.5093, found 832.5105. $^1$H NMR (500 MHz, $CDCl_3$) δ: 5.20 (dd, 1H, H-1"), 4.71(m, 1H, H-13), 4.65 (m, 1H, H-4"), 4.57 (d, 1H, H-1'), 4.25 (m, 1H, H-3), 3.81 (m, 1H, H-5"), 3.70 (s, 1H, H-11), 3.66 (m, 1H, H-5'), 3.63 (d, 1H, H-5), 3.32 (s, 3H, 3"OMe), 3.23 (dd, 1H, H-2'), 2.76 (m, 1H,

H-2), 2.75 (m, 2H, $\underline{CH_2}NH_2$), 2.71 (m, 1H, H-10), 2.55 (m, 1H, H-3'), 2.55 (m, 1H, H-9a), 2.39 (d, 1H, H-2"a), 2.32 (s, 3H, 9-NMe), 2.31 (s, 6H, 3'-$NMe_2$), 2.07 (m, 1H, H-9b), 2.04 (m, 1H, H-8), 2.01 (m, 1H, H-4), 1.91 (m, 1H, H-14a), 1.78 (d, 1H, H-7a), 1.68 (m, 1H, H-4'a), 1.61 (dd, 1H, H-2"b), 1.47 (m, 1H, H-14b), 1.31 (s, 3H, 6-Me), 1.29 (m, 1H, H-7b), 1.26 (m, 1H, H-4'b), 1.21 (d, 3H, 2-Me), 1.19 (d, 3H, 5"-Me), 1.16 (d, 3H, 5'-Me), 1.15 (s, 3H, 3"-Me), 1.11 (s, 3H, 12-Me), 1.09 (d, 3H, 10-Me), 1.06 (d, 3H, 4-Me), 0.91 (d, 3H, 8-Me), 0.89 (t, 3H, 15-Me). $^{13}$C NMR (125 MHz, $CDCl_3$) δ: 178.8 (C-1), 171.9 (O$\underline{CO}CH_2NH_2$), 152.4 (11,12-C=O), 102.3 (C-1'), 94.6 (C-1"), 83.1 (C-5), 78.7 (C-4"), 77.6 (C-3), 77.3 (C-13), 74.2 (C-11), 73.5 (C-6), 73.3 (C-12), 72.9 (C-3"), 70.8 (C-2'), 70.0 (C-9), 68.2 (C-5'), 67.7 (C-5"), 65.6 (C-3'), 62.4 (C-10), 53.3 ($\underline{CH_2}NH_2$), 49.3 (3"OMe), 45.1 (C-2), 42.1 (C-7), 42.0 (C-4), 40.2 (3'-$NMe_2$), 36.2 (9N-Me), 34.9 (C-2"), 28.8 (C-4'), 27.5 (6-Me), 26.7 (C-8), 22.8 (5'-Me), 21.9 (8-Me), 21.4 (C-14), 21.2 (3"-Me), 17.7 (5"-Me), 16.1 (12-Me), 14.4 (2-Me), 11.2 (15-Me), 8.9 (4-Me), 7.3 (10-Me). Anal. Calcd. For $C_{41}H_{73}N_3O_{14}$: C, 59.19; H, 8.84; N, 5.05. Found: C, 59.37; H, 8.90; N, 4.75. For $^1$H and COSY NMR spectra of **7** in $CD_3OD$ see the Supporting Information S4-S5.

*4.3.4. HBTU coupling. General procedure.*

*4.3.4.1. 4"-O-(glycyl-(6-(2-(2-carboxyethylamino)ethylamino)-7-chloro-1-cyclopropyl-1,4-dihydro-4-oxoquinolin-6-yl-3-carboxy)-azithromycin-11,12-cyclic carbonate (***8e***)*

DIPEA (201.5 μL, 1.4 mol. equiv.) was added dropwise via syringe at 0°C to a solution of 6-(2-(2-carboxyethylamino)ethylamino)-7-chloro-1-cyclopropyl-1,4-dihydro-4-oxoquinoline-3-carboxylic acid **e** (98.5 mg, 0.25 mmol) and HBTU (92.8 mg, 0.25 mmol) in dry DMF (3.0 mL). The mixture was stirred for 15 min before 4"-O-glycyl-azithromycin-11,12-cyclic carbonate **7** (149.8.3 mg, 0.18 mmol) was added over a period of 30 min. The reaction mixture was stirred at room temperature overnight (20 h), and then diluted with water (40 mL). The aqueous phase was extracted twice with EtOAc (2x50 mL), and the combined organic phases were washed sequentially with saturated aqueous $NaHCO_3$ (40 mL), and brine (40 mL). Drying with $Na_2SO_4$ and evaporation afforded 157.5 mg (70%) of the title compound **8e** as a colorless solid. HRMS (ES) calcd for $C_{59}H_{91}ClN_6O_{18}$ (M+H) 1207.6078, found 1207.6080. $^1$H NMR (500 MHz, $CDCl_3$) δ: 8.70 (s, 1H), 8.03 (s, 1H), 7.59 (s, 1H), 6.35 (bs, 1H, CONH), 5.25 (dd, 1H, H-1"), 4.73(m, 1H, H-13), 4.60 (m, 1H, H-4"), 4.55 (d, 1H, H-1'), 4.24 (m, 1H, H-3), 4.16 (m, 2H, $COCH_2NHCO$), 3.80 (m, 1H, H-5"), 3.69 (s, 1H, H-11), 3.65 (m, 1H, H-5'), 3.60 (d, 1H, H-5), 3.50 (m, 1H, cyclopropyl CH), 3.33 (s, 3H, 3"OMe), 3.24 (dd, 1H, H-2'), 3.18 (t, 2H), 2.95 (t, 2H), 2.80 (t, 2H), 2.75 (m, 1H, H-2), 2.70 (m, 1H, H-10), 2.59 (m, 1H, H-3'), 2.53 (m, 1H, H-9a), 2.38 (d, 1H, H-2"a), 2.37 (t, 2H), 2.34 (s, 3H, 9-NMe), 2.30 (s, 6H, 3'-$NMe_2$), 2.09 (m, 1H, H-9b), 2.06 (m, 1H, H-8), 2.02 (m, 1H, H-4), 1.95 (m, 1H, H-14a), 1.77 (d, 1H, H-7a), 1.68 (m, 1H, H-4'a), 1.60 (dd, 1H, H-2"b), 1.45 (m, 1H, H-14b), 1.43 (m, 2H, cyclopropyl $CH_2$), 1.32 (s, 3H, 6-Me), 1.29 (m, 1H, H-7b), 1.25 (m, 1H, H-4'b), 1.22 (d,

3H, 2-Me), 1.20 (m, 2H, cyclopropyl $CH_2$), 1.17 (m, 3H, 5”-Me), 1.15 (m, 3H, 5’-Me), 1.13 (s, 3H, 3”-Me), 1.11 (s, 3H, 12-Me), 1.08 (d, 3H, 10-Me), 1.05 (d, 3H, 4-Me), 0.92 (d, 3H, 8-Me), 0.88 (t, 3H, 15-Me). $^{13}$C NMR (125 MHz, $CDCl_3$) δ: 177.7 (4”’-CO), 177.0 (C-1), 173.5 (CONH), 172.4 (4”-O<u>CO</u>$CH_2NH_2$), 166.5 (COOH), 153.3 (CO cyclic carbonate), 149.0, 138.9, 134.8, 129.0, 128.0, 119.7, 118.8, 110.5 (C-Ar), 102.5 (C-1’), 94.3 (C-1”), 83.8 (C-5), 79.1(C-4”), 77.5 (C-3), 76.9 (C-13), 74.5 (C-6), 73.9 (C-12), 72.3 (C-11), 72.1 (C-3”), 70.4 (C-2’), 69.6 (C-9), 68.4 (C-5’), 66.5 (C-5”), 65.4 (C-3’), 62.2 (C-10), 53.1, 49.4 (3”OMe), 49.2, 47.9, 45.9, 45.0 (C-2), 42.1 (C-4), 41.4 (C-7), 40.1 (3’-$NMe_2$), 37.9, 36.1, 36.0 (9N-Me), 34.1 (C-2”), 28.7 (C-4’), 27.3 (6-Me), 25.2 (C-8), 21.8 (8-Me), 21.5 (3”-Me), 21.0 (5’-Me), 20.5 (C-14), 18.7 (5”-Me), 16.5 (12-Me), 14.8 (2-Me), 11.5 (15-Me), 9.7, 8.8 (4-Me), 7.3 (10-Me). Anal. Calcd. For $C_{59}H_{91}ClN_6O_{18}$: C, 58.67; H, 7.59; N, 6.96 Found: C, 58.92; H, 7.73; N, 6.79.

According to the above general procedure following 4”-O-ester linked azithromycin-11,12-cyclic carbonates were also prepared: **8a**, **8c**, **8g**, **8i**, **8k**, **8m** and **8o**. These compounds were immediately used in the next step without further purification. For NMR spectra of **8a** see the Supporting Information, pages S6-S10.

*4.3.5. LiOH hydrolysis of cyclic carbonates* **8a-8o**. *General procedure.*

*4.3.5.1. 4”-O-(glycyl-(6-(2-(2-carboxyethylamino)ethylamino)-7-chloro-1-cyclopropyl-1,4-dihydro-4-oxoquinolin-6-yl-3-carboxy)-azithromycin (***9e***)*

To a solution of 4”-O-glycyl-(6-(2-(2-carboxyethylamino)ethylamino)-7-chloro-1-cyclo-propyl-1,4-dihydro-4-oxoquinolin-6-yl-3-carboxy-azithromycin-11,12-cyclic carbonate **8e** (140.0 mg, 0.11 mmol) in THF-water mixture (1:1, 10.0 mL) was added LiOH (125.2 mg, 3.0 mmol), and the resulting reaction mixture was stirred at room temperature for 12 h. The solvent was removed under reduced pressure, and the solid was azeotroped with toluene (5 x 5 mL) and finally dried under vacuum. The acid salt was dissolved in water and the resulting solution was made acidic by dropwise addition of 2M aqueous HCl. The precipitate was filtered off to give 117.0 mg (87%) of the title product as colorless solid. HRMS (ES) calcd for $C_{58}H_{93}ClN_6O_{17}$ $(M+H)^+$ 1181.6286, found 1181.6293. $^1$H NMR (500 MHz, $CDCl_3$) δ: 8.65 (s, 1H), 8.07 (s, 1H), 7.51 (s, 1H), 6.38 (bs, 1H, CONH), 5.29 (dd, 1H), 4.70(m, 1H), 4.62 (m, 1H), 4.53 (d, 1H), 4.30 (m, 1H), 4.15 (m, 2H), 3.75 (m, 1H), 3.70 (s, 1H), 3.64 (m, 1H), 3.58 (d, 1H), 3.47 (m, 1H), 3.30 (s, 3H), 3.22 (dd, 1H), 3.15 (t, 2H), 2.93 (t, 2H), 2.78 (t, 2H), 2.75-2.70 (m, 2H), 2.60-2.52 (m, 2H), 2.39 (d, 1H), 2.35 (t, 2H), 2.31 (s, 3H, 9-NMe), 2.28 (s, 6H, 3’-$NMe_2$), 2.10-2.05 (m, 2H), 2.00-1.94 (m, 2H), 1.74 (d, 1H), 1.65 (m, 1H), 1.60 (dd, 1H), 1.44 (m, 1H), 1.40 (m, 2H, cyclopropyl $CH_2$), 1.35 (s, 3H, 6-Me), 1.30-1.25 (m, 2H), 1.23 (d, 3H, 2-Me), 1.19 (m, 2H, cyclopropyl $CH_2$), 1.18 (m, 3H), 1.16 (m, 3H), 1.13 (s, 3H, 3”-Me), 1.10 (s, 3H, 12-Me), 1.09 (d, 3H, 10-Me), 1.04 (d, 3H, 4-Me), 0.93 (d, 3H, 8-Me), 0.89 (t, 3H, 15-Me). $^{13}$C NMR (125 MHz, $CDCl_3$) δ: 177.9 (4”’-CO), 177.8 (C-1), 173.7 (CONH), 172.1 (4”-O<u>CO</u>$CH_2NH_2$), 166.8 (COOH), 149.5, 139.3, 135.4, 129.5, 128.1, 119.5,

118.3, 110.7 (C-Ar), 102.8 (C-1'), 94.5 (C-1"), 83.3 (C-5), 78.9 (C-4"), 77.7 (C-3), 77.5 (C-13), 74.1 (C-6), 73.7 (C-12), 73.4 (C-11), 73.0 (C-3"), 70.8 (C-2'), 70.0 (C-9), 68.7 (C-5'), 67.1 (C-5"), 65.8 (C-3'), 62.6 (C-10), 52.5, 49.7 (3"OMe), 49.0, 47.3, 46.5, 45.4 (C-2), 42.6 (C-4), 42.2 (C-7), 40.4 (3'-$NMe_2$), 37.3, 36.5, 36.1 (9N-Me), 34.6 (C-2"), 28.9 (C-4'), 27.6 (6-Me), 26.9 (C-8), 21.9 (8-Me), 21.6 (3"-Me), 21.4 (5'-Me), 21.3 (C-14), 18.2 (5"-Me), 16.3 (12-Me), 14.5 (2-Me), 11.2 (15-Me), 9.5, 8.9 (4-Me), 7.1 (10-Me). Anal. Calcd. For $C_{58}H_{93}ClN_6O_{17}$: C, 58.94; H, 7.93; N, 7.11. Found: C, 58.90; H, 7.86; N, 6.80.

*4.3.5.2. Spectral data for compounds* **9a-9o**

The following compounds were also prepared by using the same general procedure:

**9a**: Yield= 72% (for 2 step sequence starting from **7**). HRMS (ES) calcd for $C_{50}H_{81}N_5O_{14}S$ $(M+H)^+$ 1008.5501, found 1008.5505. $^1$H NMR (500 MHz, $CDCl_3$) δ: 8.71 (m, 1H), 8.15 (m, 1H), 8.03 (m, 1H), 7.74 (m, 1H), 7.65 (m, 1H), 6.35 (bs, 1H, CONH), 5.29 (dd, 1H), 4.72-4.60(m, 2H), 4.50 (d, 1H), 4.32 (m, 1H), 4.16 (m, 2H), 3.82 (m, 2H), 3.74 (m, 1H), 3.68 (s, 1H), 3.64 (m, 1H), 3.58 (d, 1H), 3.30 (s, 3H), 3.22 (dd, 1H), 2.75-2.68 (m, 2H), 2.62-2.50 (m, 2H), 2.39 (d, 1H), 2.32 (s, 3H, 9-NMe), 2.30 (s, 6H, 3'-$NMe_2$), 2.11-2.04 (m, 2H), 2.02-1.92 (m, 2H), 1.75 (d, 1H), 1.65 (m, 1H), 1.59 (dd, 1H), 1.40 (m, 1H), 1.34 (s, 3H, 6-Me), 1.30-1.24 (m, 2H), 1.20 (d, 3H, 2-Me), 1.18 (m, 3H), 1.16 (m, 3H), 1.14 (s, 3H, 3"-Me), 1.12 (s, 3H, 12-Me), 1.10 (d, 3H, 10-Me), 1.05 (d, 3H, 4-Me), 0.95 (d, 3H, 8-Me), 0.87 (t, 3H, 15-Me). $^{13}$C NMR (125 MHz, $CDCl_3$) δ: 178.4, 173.5, 172.1, 154.1, 144.3, 142.1,

140.1, 130.5, 129.1, 128.6, 128.3, 101.8, 95.3, 82.9, 78.0, 77.9, 77.0, 74.1, 73.8, 73.2, 72.1, 71.3, 70.5, 69.1, 67.9, 65.3, 62.8, 51.9, 49.6, 44.7, 43.1, 41.8, 40.8, 40.0, 36.5, 34.3, 29.1, 28.0, 26.6, 22.9, 21.7, 21.5, 21.1, 18.6, 16.8, 14.6, 11.4, 9.1, 7.6. Anal. Calcd. For $C_{50}H_{81}N_5O_{14}S$: C, 59.56; H, 8.10; N, 6.95. Found: C, 59.78; H, 8.25; N, 6.78.

**9c**: Yield= 79% (for 2 step sequence starting from **7**). HRMS (ES) calcd for $C_{52}H_{84}N_6O_{15}S$ (M+H) 1065.5715, found 1065.5728. $^1$H NMR (500 MHz, $CDCl_3$) δ: 8.75 (m, 1H), 8.20 (m, 1H), 8.11 (m, 1H), 7.79 (m, 1H), 7.67 (m, 1H), 6.38 (bs, 1H, CONH), 5.31 (dd, 1H), 4.73-4.31(m, 4H), 4.19 (m, 2H), 4.10 (m, 2H), 3.80-3.72 (m, 2H), 3.67 (s, 1H), 3.60 (m, 1H), 3.55 (d, 1H), 3.32 (s, 3H), 3.20 (dd, 1H), 2.76-2.52 (m, 4H), 2.37 (d, 1H), 2.30 (s, 3H, 9-NMe), 2.27 (s, 6H, 3'-$NMe_2$), 2.12-1.90 (m, 4H), 1.78 (d, 1H), 1.67 (m, 1H), 1.55 (dd, 1H), 1.42 (m, 1H), 1.35 (s, 3H, 6-Me), 1.31-1.23 (m, 2H), 1.19 (d, 3H, 2-Me), 1.17-1.15 (m, 6H), 1.11 (s, 3H, 3"-Me), 1.10 (s, 3H, 12-Me), 1.07 (d, 3H, 10-Me), 1.05 (d, 3H, 4-Me), 0.98 (d, 3H, 8-Me), 0.89 (t, 3H, 15-Me). $^{13}$C NMR (125 MHz, $CDCl_3$) δ: 177.9, 173.2, 172.5, 172.0, 153.9, 143.8, 142.4, 140.6, 130.2, 129.1, 128.3, 127.6, 102.1, 95.9, 82.5, 78.2, 77.8, 77.1, 75.0, 74.1, 73.5, 72.4, 71.0, 70.3, 69.9, 67.6, 65.6, 62.3, 56.2, 51.2, 49.2, 44.9, 43.8, 41.6, 40.8, 39.8, 35.9, 34.6, 29.4, 28.9, 27.3, 23.1, 22.2, 21.8, 20.8, 18.9, 17.1, 15.2, 11.7, 9.6, 7.4. Anal. Calcd. For $C_{52}H_{84}N_6O_{15}S$: C, 58.63; H, 7.95; N, 7.89. Found: C, 58.81; H, 8.12; N, 7.75.

**9g**: Yield= 59% (for 2 step sequence starting from **7**). HRMS (ES) calcd for $C_{58}H_{93}FN_6O_{17}$

$(M+H)^+$ 1165.6581, found 1165.6583. $^1$H NMR (500 MHz, $CDCl_3$) δ: 8.60 (s, 1H), 8.15 (s, 1H), 7.68 (s, 1H), 6.52 (bs, 1H, CONH), 5.35 (dd, 1H), 4.76-4.63(m, 2H), 4.53 (d, 1H), 4.36 (m, 1H), 4.18-3.82 (m, 3H), 3.75 (s, 1H), 3.65-3.52 (m, 3H), 3.38 (s, 3H), 3.29 (t, 2H), 3.20 (dd, 1H), 3.10 (t, 2H), 2.81 (t, 2H), 2.77-2.53 (m, 4H), 2.44 (d, 1H), 2.36 (t, 2H), 2.33 (s, 3H, 9-NMe), 2.30 (s, 6H, 3'-$NMe_2$), 2.15-1.95 (m, 4H), 1.76 (d, 1H), 1.68-1.46 (m, 3H), 1.40 (m, 2H, cyclopropyl $CH_2$), 1.35 (s, 3H, 6-Me), 1.30-1.18 (m, 7H), 1.16-1.12 (m, 6H), 1.10 (s, 3H, 3"-Me), 1.08 (s, 3H, 12-Me), 1.04 (d, 3H, 10-Me), 1.02 (d, 3H, 4-Me), 0.99 (d, 3H, 8-Me), 0.88 (t, 3H, 15-Me). $^{13}$C NMR (125 MHz, $CDCl_3$) δ: 178.2, 177.3, 174.1, 172.8, 167.5, 146.8, 144.2, 137.6, 129.8, 127.8, 121.4, 119.3, 110.1, 101.6, 95.1, 84.1, 79.2, 78.4, 76.3, 74.1, 73.2, 73.0, 72.3, 71.4, 70.4, 68.5, 67.5, 66.1, 62.2, 52.9, 50.1, 49.6, 48.2, 46.9, 44.7, 42.9, 42.0, 41.0, 37.0, 36.9, 35.0, 34.8, 29.1, 28.6, 25.7, 21.5, 21.2, 21.0, 20.9, 18.1, 16.9, 15.1, 11.8, 9.7, 9.0, 7.1. Anal. Calcd. For $C_{58}H_{93}FN_6O_{17}$: C, 59.78; H, 8.04; N, 7.21. Found: C, 59.85; H, 8.15; N, 7.10.

**9i**: Yield= 68% (for 2 step sequence starting from **7**). HRMS (ES) calcd for $C_{60}H_{97}ClN_6O_{18}$ $(M+H)^+$ 1225.6548, found 1225.6540. $^1$H NMR (500 MHz, $CDCl_3$) δ: 8.72 (s, 1H), 8.28 (s, 1H), 7.76 (s, 1H), 6.59 (bs, 1H, CONH), 5.41 (dd, 1H), 4.75-4.60(m, 2H), 4.50 (d, 1H), 4.38 (m, 1H), 4.15 (m, 2H), 3.76 (m, 1H), 3.73 (s, 1H), 3.68 (m, 1H), 3.63 (m, 2H), 3.60 (d, 1H), 3.50-3.38 (m, 3H), 3.30 (s, 3H), 3.25 (dd, 1H), 3.20 (m, 2H), 2.85 (m, 2H), 2.75-2.70 (m, 4H), 2.60-2.50 (m, 2H), 2.40 (d, 1H), 2.38 (m, 2H), 2.31 (s, 3H, 9-NMe), 2.28 (s, 6H, 3'-$NMe_2$),

2.15-2.05 (m, 2H), 2.02-1.94 (m, 2H), 1.78 (d, 1H), 1.66 (m, 1H), 1.63 (dd, 1H), 1.48 (m, 1H), 1.42 (m, 2H, cyclopropyl $CH_2$), 1.38 (s, 3H, 6-Me), 1.33-1.20 (m, 5H), 1.17 (m, 2H, cyclopropyl $CH_2$), 1.15-1.13 (m, 6H), 1.11 (s, 3H, 3''-Me), 1.08 (s, 3H, 12-Me), 1.06 (d, 3H, 10-Me), 1.02 (d, 3H, 4-Me), 0.98 (d, 3H, 8-Me), 0.89 (t, 3H, 15-Me). $^{13}$C NMR (125 MHz, $CDCl_3$) δ: 178.3, 177.0, 173.2 , 171.8, 166.3, 148.9, 138.1, 134.8, 129.0, 128.7, 120.1, 118.9, 110.4, 101.5, 95.1, 82.7, 78.2, 77.0, 76.1, 74.4, 73.9, 72.8, 71.5, 71.0, 70.7, 70.3, 69.5, 68.2, 66.8, 65.4, 61.4, 51.8, 49.3, 48.2, 47.0, 46.1, 45.1, 42.0, 41.8, 41.0, 37.8, 36.1, 36.0, 34.4, 28.3, 27.1, 26.5, 22.1, 21.5, 21.1, 20.8, 18.5, 15.7, 14.8, 10.8, 9.4, 8.6, 7.9. Anal. Calcd. For $C_{60}H_{97}ClN_6O_{18}$: C, 58.78; H, 7.98; N, 6.86. Found: C, 58.99; H, 8.20; N, 6.79.

**9k**: Yield= 76% (for 2 step sequence starting from **7**). HRMS (ES) calcd for $C_{60}H_{97}FN_6O_{18}$ $(M+H)^+$ 1209.6843, found 1209.6864. $^1$H NMR (500 MHz, $CDCl_3$) δ: 8.68 (s, 1H), 8.23 (s, 1H), 7.78 (s, 1H), 6.60 (bs, 1H, CONH), 5.45 (dd, 1H), 4.75-4.35(m, 4H), 4.16 (m, 2H), 3.76 (s, 1H), 3.72-3.62 (m, 4H), 3.60-3.35 (m, 4H), 3.28 (s, 3H), 3.24 (dd, 1H), 3.21-2.72 (m, 8H), 2.62-2.52 (m, 2H), 2.43 (d, 1H), 2.35 (m, 2H), 2.31 (s, 3H, 9-NMe), 2.29 (s, 6H, 3'-$NMe_2$), 2.16-1.92 (m, 4H), 1.79 (d, 1H), 1.65 (m, 1H), 1.62 (dd, 1H), 1.50-1.41 (m, 3H), 1.36 (s, 3H, 6-Me), 1.32-1.12 (m, 13H), 1.10 (s, 3H, 3''-Me), 1.08 (s, 3H, 12-Me), 1.05 (d, 3H, 10-Me), 1.03 (d, 3H, 4-Me), 0.91 (d, 3H, 8-Me), 0.88 (t, 3H, 15-Me). $^{13}$C NMR (125 MHz, $CDCl_3$) δ: 177.8, 177.6, 173.7 , 171.1, 166.0, 146.3, 143.8, 137.1, 129.4, 128.2, 121.6, 119.4, 109.3, 101.9, 95.5, 81.4, 78.8, 77.5, 75.5, 74.1, 73.3, 72.4, 71.9, 71.2, 70.5, 70.0, 69.1, 68.8,

67.3, 66.4, 62.1, 52.7, 50.8, 48.8, 47.6, 46.4, 44.8, 42.3, 41.0, 40.6, 37.6, 36.2, 35.5, 33.4, 28.6, 27.1, 25.9, 22.7, 21.3, 20.9, 19.9, 18.4, 15.5, 14.6, 10.5, 9.8, 8.4, 7.5. Anal. Calcd. For $C_{60}H_{97}FN_6O_{18}$: C, 59.58; H, 8.08; N, 6.95. Found: C, 59.79; H, 8.31; N, 6.78.

**9m**: Yield= 74% (for 2 step sequence starting from **7**). HRMS (ES) calcd for $C_{62}H_{101}ClN_6O_{19}$ ($MH^+$) 1269.6810, found 1269.6821. $^1$H NMR (500 MHz, $CDCl_3$) δ: 8.78 (s, 1H), 8.39 (s, 1H), 7.56 (s, 1H), 6.58 (bs, 1H, CONH), 5.40 (dd, 1H), 4.73-4.35(m, 4H), 4.15 (m, 2H), 3.76-3.67 (m, 3H), 3.58 (d, 1H), 3.55-3.42 (m, 8H), 3.31 (s, 3H), 3.27 (dd, 1H), 3.23 (m, 2H), 2.83-2.72 (m, 4H), 2.62-2.51 (m, 2H), 2.42 (d, 1H), 2.33 (s, 3H, 9-NMe), 2.29 (m, 2H), 2.27 (s, 6H, 3'-$NMe_2$), 2.16-1.95 (m, 4H), 1.78 (d, 1H), 1.68 (m, 1H), 1.64 (dd, 1H), 1.46 (m, 1H), 1.40 (m, 2H, cyclopropyl $CH_2$), 1.36 (s, 3H, 6-Me), 1.31-1.19 (m, 5H), 1.16 (m, 2H, cyclopropyl $CH_2$), 1.14-1.12 (m, 6H), 1.11 (s, 3H, 3"-Me), 1.08 (s, 3H, 12-Me), 1.05 (d, 3H, 10-Me), 1.03 (d, 3H, 4-Me), 0.99 (d, 3H, 8-Me), 0.88 (t, 3H, 15-Me). $^{13}$C NMR (125 MHz, $CDCl_3$) δ: 178.9, 177.5, 172.9 , 171.4, 165.9, 149.1, 138.8, 135.4, 129.6, 128.4, 121.3, 119.5, 110.7, 101.8, 95.7, 82.3, 78.9, 76.8, 75.6, 74.7, 73.5, 72.1, 71.9, 71.2, 71.0, 70.5, 70.2, 70.0, 69.2, 68.1, 67.1, 65.8, 60.9, 52.5, 49.8, 48.1, 47.0, 46.3, 44.8, 42.5, 42.0, 41.5, 38.1, 37.2, 36.5, 34.9, 28.7, 27.0, 26.2, 22.6, 21.8, 21.0, 20.5, 18.8, 15.9, 14.6, 11.2, 9.8, 8.8, 8.1. Anal. Calcd. For $C_{62}H_{101}ClN_6O_{19}$: C, 58.64; H, 8.02; N, 6.62. Found: C, 58.88; H, 8.11; N, 6.50.

**9o**: Yield= 81% (for 2 step sequence starting from **7**). HRMS (ES) calcd for $C_{62}H_{101}FN_6O_{19}$ $(M+H)^+$ 1253.7106, found 1253.7134. $^1$H NMR (500 MHz, $CDCl_3$) δ: 8.58 (s, 1H), 8.26 (s,

1H), 7.43 (s, 1H), 6.42 (bs, 1H, CONH), 5.45 (dd, 1H), 4.75-4.33(m, 4H), 4.14 (m, 2H), 3.75-3.66 (m, 3H), 3.56 (d, 1H), 3.53-3.40 (m, 8H), 3.31 (s, 3H), 3.26 (dd, 1H), 3.22-2.71 (m, 6H), 2.60-2.49 (m, 2H), 2.41 (d, 1H), 2.31 (s, 3H, 9-NMe), 2.28 (m, 2H), 2.27 (s, 6H, 3'-$NMe_2$), 2.15-1.67 (m, 6H), 1.64 (dd, 1H), 1.45-1.39 (m, 3H), 1.35 (s, 3H, 6-Me), 1.30-1.15 (m, 7H), 1.14-1.10 (m, 6H), 1.08 (s, 3H, 3"-Me), 1.07 (s, 3H, 12-Me), 1.04 (d, 3H, 10-Me), 1.01 (d, 3H, 4-Me), 0.97 (d, 3H, 8-Me), 0.89 (t, 3H, 15-Me). $^{13}$C NMR (125 MHz, $CDCl_3$) δ: 178.1, 177.3, 174.1, 171.9, 166.5, 146.8, 143.4, 137.4, 130.1, 129.0, 121.1, 120.2, 109.9, 102.2, 96.1, 82.5, 79.1, 77.3, 75.0, 74.2, 73.5, 72.2, 71.7, 71.4, 71.0, 70.6, 70.1, 69.9, 69.0, 68.3, 67.9, 67.4, 61.4, 54.3, 51.3, 48.5, 47.2, 46.5, 45.3, 43.2, 41.9, 41.1, 38.4, 36.7, 35.9, 33.9, 29.2, 27.8, 26.6, 23.2, 21.7, 20.8, 19.8, 18.7, 15.9, 14.9, 11.4, 9.7, 8.4, 7.8. Anal. Calcd. For $C_{62}H_{101}FN_6O_{19}$: C, 59.41; H, 8.12; N, 6.70. Found: C, 59.63; H, 8.22; N, 6.68.

*4.3.6. Reductive amination. General procedure.*

*4.3.6.1. 4"-O-(glycyl-(6-(2-(2-propylamino)ethylamino)-7-chloro-1-cyclopropyl-1,4-dihydro-4-oxoquinolin-6-yl-3-carboxy)-azithromycin-11,12-cyclic carbonate* (**21f**)

To a magnetically stirred solution of 6-(2-(2-formylethylamino)ethylamino)-7-chloro-1-cyclopropyl-1,4-dihydro-4-oxoquinoline-3-carboxylic acid **f** (831.2 mg, 2.2 mmol) in 10 mL of methanol was added 4"-O-glycyl-azithromycin-11,12-cyclic carbonate **7** (915.2 mg, 1.1 mmol). After being stirred at room temperature for 30 min, the solution was treated with 0.12 mL (2.2 mmol) of HOAc and cooled to 0°C. $NaBH(OAc)_3$ (466.0 mg, 2.2 mmol) dissolved in

MeOH (3 mL) was then added over a period of 10 min. Stirring at room temperature was continued for additional 20 h after which HPLC analysis showed completion of the reaction. The reaction mixture was worked up and the crude product was chromatographed on silica gel to furnish 882.7 mg (65%) of the title compound. HRMS (ES) calcd for $C_{59}H_{93}ClN_6O_{17}$ $(M+H)^+$ 1193.6286, found 1193.6298. $^1$H NMR (500 MHz, $CDCl_3$) δ: 8.68 (s, 1H), 8.07 (s, 1H), 7.55 (s, 1H), 5.31 (dd, 1H, H-1”), 4.71(m, 1H, H-13), 4.62 (m, 1H, H-4”), 4.53 (d, 1H, H-1’), 4.22 (m, 1H, H-3), 3.78 (m, 1H, H-5”), 3.68 (s, 1H, H-11), 3.64 (m, 1H, H-5’), 3.58 (d, 1H, H-5), 3.51 (m, 2H, CO$\underline{CH_2}$NH), 3.45 (m, 1H, cyclopropyl $\underline{CH}$), 3.31 (s, 3H, 3”OMe), 3.25 (dd, 1H, H-2’), 3.18 (m, 2H), 2.78 (m, 2H), 2.74 (m, 1H, H-2), 2.65 (m, 1H, H-10), 2.58 (m, 1H, H-3’), 2.55 (m, 4H), 2.50 (m, 1H, H-9a), 2.35 (d, 1H, H-2”a), 2.33 (s, 3H, 9-NMe), 2.31 (s, 6H, 3’-$NMe_2$), 2.10 (m, 1H, H-9b), 2.05-2.01 (m, 2H, H-8, H-4), 1.93 (m, 1H, H-14a), 1.75 (d, 1H, H-7a), 1.67 (m, 1H, H-4’a), 1.58 (dd, 1H, H-2”b), 1.53 (m, 2H), 1.43 (m, 1H, H-14b), 1.38 (m, 2H, cyclopropyl $CH_2$), 1.30 (s, 3H, 6-Me), 1.27-1.24 (m, 2H, H-7b, H-4’b), 1.22 (d, 3H, 2-Me), 1.19 (m, 2H, cyclopropyl $CH_2$), 1.17-1.14 (m, 6H, 5”-Me, 5’-Me), 1.12 (s, 3H, 3”-Me), 1.10 (s, 3H, 12-Me), 1.06 (d, 3H, 10-Me), 1.02 (d, 3H, 4-Me), 0.94 (d, 3H, 8-Me), 0.89 (t, 3H, 15-Me). $^{13}$C NMR (125 MHz, $CDCl_3$) δ: 177.7 (4”’-CO), 177.0 (C-1), 172.4 (4”-O$\underline{CO}CH_2NH_2$), 166.5 (COOH), 153.3 (CO cyclic carbonate), 149.0, 138.9, 134.8, 129.0, 128.0, 119.7, 118.8, 110.5 (C-Ar), 102.5 (C-1’), 94.3 (C-1”), 83.8 (C-5), 79.1(C-4”), 77.5 (C-3), 76.9 (C-13), 74.5 (C-6), 73.9 (C-12), 72.3 (C-11), 72.1 (C-3”), 70.4 (C-2’), 69.6

(C-9), 68.4 (C-5'), 66.5 (C-5"), 65.4 (C-3'), 62.2 (C-10), 49.9, 49.4 (3"OMe), 47.9, 46.7, 45.9, 45.0 (C-2), 42.1 (C-4), 41.4 (C-7), 40.1 (3'-$NMe_2$), 37.9, 36.1, 36.0 (9N-Me), 34.1 (C-2"), 29.5, 28.7 (C-4'), 27.3 (6-Me), 25.2 (C-8), 21.8 (8-Me), 21.5 (3"-Me), 21.0 (5'-Me), 20.5 (C-14), 18.7 (5"-Me), 16.5 (12-Me), 14.8 (2-Me), 11.5 (15-Me), 9.7, 9.6, 8.8 (4-Me), 7.3 (10-Me). Anal. Calcd. For $C_{59}H_{93}ClN_6O_{17}$: C, 59.36; H, 7.85; N, 7.04. Found: C, 59.39; H, 7.89; N, 6.89.

The compounds **21b, 21d, 21h, 21j, 21l, 21n** and **21p** were prepared according to the same general procedure as for the synthesis of **21f** and were used without further purification in the next step. For NMR spectra of **21h, 21j, 21l,** and **21n** see the Supporting Information, pages S21-S40.

*4.3.7. LiOH hydrolysis of cyclic carbonates* **21b-21p**. *General procedure.*

*4.3.7.1. 4"-O-(glycyl-(6-(2-(2-propylamino)ethylamino)-7-chloro-1-cyclopropyl-1,4-dihydro-4-oxoquinolin-6-yl-3-carboxy)-azithromycin* (**22f**)

To a solution of 4"-O-(glycyl-(6-(2-(2-propylamino)ethylamino)-7-chloro-1-cyclopropyl-1,4-dihydro-4-oxoquinolin-6-yl-3-carboxy)-azithromycin-11,12-cyclic carbonate **21f** (800.0 mg, 0.65 mmol) in THF-water mixture (1:1, 7.0 mL) was added LiOH (167.0 mg, 4.0 mmol), and the resulting reaction mixture was stirred at room temperature for 15 h. The solvent was removed under reduced pressure, and the solid was azeotroped with toluene (4 x 10 mL) and finally dried under vacuum. The acid salt was dissolved in water and the resulting solution

was made acidic by dropwise addition of 2M aqueous HCl. The precipitate was filtered off to give 667.8 mg (85%) of the title product as colorless solid. HRMS (ES) calcd for $C_{58}H_{95}ClN_6O_{16}$ $(M+H)^+$ 1167.6493, found 1167.6506. $^1$H NMR (500 MHz, $CDCl_3$) δ: 8.72 (s, 1H), 8.23 (s, 1H), 7.34 (s, 1H), 5.35 (dd, 1H, H-1”), 4.70-4.60 (m, 2H, H-13, H-4”), 4.53 (d, 1H, H-1’), 4.21-3.76 (m, 2H, H-3, H-5”), 3.65 (s, 1H, H-11), 3.64-3.56 (m, 2H, H-5’, H-5), 3.49 (m, 2H, CO$\underline{CH_2}$NH), 3.40 (m, 1H, cyclopropyl $\underline{CH}$), 3.30 (s, 3H, 3”OMe), 3.27 (dd, 1H, H-2’), 3.17-2.77 (m, 4H), 2.73-2.57 (m, 3H, H-2, H-10, H-3’), 2.54 (m, 4H), 2.47 (m, 1H, H-9a), 2.38 (m, 1H, H-2”a), 2.33 (s, 3H, 9-NMe), 2.30 (s, 6H, 3’-$NMe_2$), 2.12 (m, 1H, H-9b), 2.05-1.92 (m, 3H, H-14a, H-8, H-4), 1.76 (d, 1H, H-7a), 1.68 (m, 1H, H-4’a), 1.57 (dd, 1H, H-2”b), 1.50 (m, 2H), 1.43-1.35 (m, 3H, H-14b, cyclopropyl $CH_2$), 1.31 (s, 3H, 6-Me), 1.26-1.24 (m, 2H, H-7b, H-4’b), 1.21-1.18 (m, 5H, 2-Me, cyclopropyl $CH_2$), 1.17-1.14 (m, 6H, 5”-Me, 5’-Me), 1.10 (s, 3H, 3”-Me), 1.08 (s, 3H, 12-Me), 1.06 (d, 3H, 10-Me), 1.01 (d, 3H, 4-Me), 0.95 (d, 3H, 8-Me), 0.87 (t, 3H, 15-Me). $^{13}$C NMR (125 MHz, $CDCl_3$) δ: 177.5 (4”’-CO), 177.5 (C-1), 172.7 (4”-O$\underline{CO}CH_2NH_2$), 167.0 (COOH), 149.7, 138.4, 135.1, 129.4, 128.3, 119.3, 118.1, 110.9 (C-Ar), 102.2 (C-1’), 94.6 (C-1”), 83.1 (C-5), 79.6 (C-4”), 77.2 (C-3), 77.0 (C-13), 74.8 (C-6), 74.0 (C-12), 72.9 (C-11), 71.7 (C-3”), 70.4 (C-2’), 69.5 (C-9), 68.1 (C-5’), 66.9 (C-5”), 65.7 (C-3’), 62.5 (C-10), 49.4 (3”OMe), 48.0, 46.8, 46.2, 45.3, 44.9 (C-2), 41.9 (C-4), 41.3 (C-7), 40.2 (3’-$NMe_2$), 37.7, 35.8, 35.0 (9N-Me), 34.6 (C-2”), 29.2, 28.3 (C-4’), 27.0 (6-Me), 25.4 (C-8), 21.5 (8-Me), 21.1 (3”-Me), 20.7 (5’-Me), 20.2 (C-14),

18.5 (5”-Me), 16.3 (12-Me), 14.9 (2-Me), 11.0 (15-Me), 9.6, 9.5, 8.6 (4-Me), 7.2 (10-Me). Anal. Calcd. For $C_{58}H_{95}ClN_6O_{16}$: C, 59.65; H, 8.20; N, 7.20. Found: C, 59.74; H, 8.34; N, 6.99.

*4.3.7.2. Spectral data for compounds* **22b**-**22p**

The following compounds were also prepared by using the same general procedure for LiOH hydrolysis:

**22b**: Yield= 65% (for 2 step sequence starting from **7**). HRMS (ES) calcd for $C_{50}H_{83}N_5O_{13}S$ $(M+H)^+$ 994.5708, found 994.5716. $^1$H NMR (500 MHz, $CDCl_3$) δ: 8.68 (m, 1H), 8.18 (m, 1H), 8.08 (m, 1H), 7.76 (m, 1H), 7.60 (m, 1H), 5.31 (dd, 1H), 4.74-4.62 (m, 2H), 4.51 (d, 1H), 4.34-3.72 (m, 2H), 3.68 (s, 1H), 3.63 (m, 1H), 3.58 (d, 1H), 3.54 (m, 2H), 3.31 (s, 3H), 3.24 (dd, 1H), 3.05 (m, 2H), 2.95 (m, 2H), 2.74-2.48 (m, 4H), 2.36 (d, 1H), 2.31 (s, 3H, 9-NMe), 2.29 (s, 6H, 3’-$NMe_2$), 2.15-1.89 (m, 4H), 1.75 (d, 1H), 1.63 (m, 1H), 1.54 (dd, 1H), 1.42 (m, 1H), 1.34 (s, 3H, 6-Me), 1.31-1.22 (m, 2H), 1.19 (d, 3H, 2-Me), 1.17-1.15 (m, 6H), 1.14 (s, 3H, 3”-Me), 1.10 (s, 3H, 12-Me), 1.07 (d, 3H, 10-Me), 1.02 (d, 3H, 4-Me), 0.94 (d, 3H, 8-Me), 0.89 (t, 3H, 15-Me). $^{13}$C NMR (125 MHz, $CDCl_3$) δ: 179.1, 172.6, 153.9, 143.8, 142.5, 140.6, 130.7, 129.0, 128.2, 127.2, 101.5, 95.7, 82.3, 78.4, 77.9, 77.1, 74.6, 74.4, 73.0, 72.3, 71.1, 70.0, 69.0, 67.3, 65.5, 63.1, 51.5, 49.9, 44.8, 43.5, 42.3, 41.3, 40.5, 39.8, 37.1, 35.1, 29.6, 27.8, 26.2, 23.1, 22.5, 21.9, 20.7, 18.8, 17.1, 15.2, 11.9, 9.7, 8.1. Anal. Calcd. For $C_{50}H_{83}N_5O_{13}S$: C, 60.40; H, 8.41; N, 7.04. Found: C, 60.50; H, 8.50; N, 6.88.

**22d**: Yield= 74% (for 2 step sequence starting from **7**). HRMS (ES) calcd for $C_{52}H_{86}N_6O_{14}S$ $(M+H)^+$ 1051.5923, found 1051.5927. $^1H$ NMR (500 MHz, $CDCl_3$) δ: 8.69 (m, 1H), 8.24 (m, 1H), 8.08 (m, 1H), 7.68 (m, 1H), 7.59 (m, 1H), 6.54 (bs, 1H, CONH), 5.40 (dd, 1H), 4.73-4.30(m, 4H), 3.82-3.59 (m, 4H), 3.58 (d, 1H), 3.50 (m, 2H), 3.32 (s, 3H), 3.30 (m, 2H), 3.21 (dd, 1H), 2.82-2.49 (m, 6H), 2.34 (d, 1H), 2.30 (s, 3H, 9-NMe), 2.28 (s, 6H, 3'-$NMe_2$), 2.15-1.93 (m, 4H), 1.76 (d, 1H), 1.64 (m, 1H), 1.51 (dd, 1H), 1.40 (m, 1H), 1.34 (s, 3H, 6-Me), 1.30-1.22 (m, 2H), 1.19 (d, 3H, 2-Me), 1.17-1.14 (m, 6H), 1.12 (s, 3H, 3"-Me), 1.10 (s, 3H, 12-Me), 1.06 (d, 3H, 10-Me), 1.01 (d, 3H, 4-Me), 0.99 (d, 3H, 8-Me), 0.87 (t, 3H, 15-Me). $^{13}C$ NMR (125 MHz, $CDCl_3$) δ: 178.1, 173.0, 172.1, 154.1, 143.4, 142.0, 140.2, 130.6, 129.4, 128.5, 127.2, 101.8, 95.5, 83.1, 78.6, 78.0, 76.9, 75.5, 73.9, 73.1, 72.3, 71.7, 71.0, 70.0, 67.3, 65.3, 63.7, 55.5, 52.0, 49.9, 45.1, 43.5, 42.7, 41.9, 41.1, 40.0, 35.3, 34.3, 30.5, 29.3, 27.8, 23.4, 22.0, 21.3, 20.7, 18.5, 17.3, 14.9, 11.2, 10.1, 8.1. Anal. Calcd. For $C_{52}H_{86}N_6O_{14}S$: C, 59.41; H, 8.25; N, 7.99. Found: C, 59.69; H, 8.40; N, 7.76.

**22h**: Yield= 80% (for 2 step sequence starting from **7**). HRMS (ES) calcd for $C_{58}H_{95}FN_6O_{16}$ $(M+H)^+$ 1151.6789, found 1151.6780. $^1H$ NMR (500 MHz, $CDCl_3$) δ: 8.60 (s, 1H), 8.15 (s, 1H), 7.68 (s, 1H), 5.35 (dd, 1H), 4.76-4.63(m, 2H), 4.53 (d, 1H), 4.36 (m, 1H), 4.18-3.82 (m, 3H), 3.75 (s, 1H), 3.65-3.52 (m, 3H), 3.38 (s, 3H), 3.29 (t, 2H), 3.20 (dd, 1H), 3.10 (t, 2H), 2.81 (t, 2H), 2.77-2.53 (m, 4H), 2.44 (d, 1H), 2.36 (t, 2H), 2.33 (s, 3H, 9-NMe), 2.30 (s, 6H, 3'-$NMe_2$), 2.15-1.95 (m, 4H), 1.76 (d, 1H), 1.68-1.46 (m, 3H), 1.40 (m, 2H, cyclopropyl

$CH_2$), 1.35 (s, 3H, 6-Me), 1.30-1.18 (m, 7H), 1.16-1.12 (m, 6H), 1.10 (s, 3H, 3”-Me), 1.08 (s, 3H, 12-Me), 1.04 (d, 3H, 10-Me), 1.02 (d, 3H, 4-Me), 0.99 (d, 3H, 8-Me), 0.88 (t, 3H, 15-Me). $^{13}$C NMR (125 MHz, $CDCl_3$) δ: 177.8, 177.2, 172.4, 167.4, 148.4, 140.7, 143.7, 137.2, 117.4, 116.5, 109.4, 107.9, 101.6, 95.1, 83.5, 79.4, 77.8, 77.1, 75.1, 74.5, 72.0, 71.2, 70.9, 69.6, 68.0, 67.2, 66.0, 62.1, 49.6, 48.4, 46.4, 46.0, 45.1, 44.1, 42.5, 41.8, 40.1, 38.2, 36.4, 35.1, 34.2, 29.8, 28.6, 27.0, 25.8, 22.2, 21.0, 20.3, 19.2, 18.8, 17.2, 15.3, 11.6, 9.9, 9.4, 8.9, 7.8. Anal. Calcd. For $C_{58}H_{95}FN_6O_{16}$: C, 60.50; H, 8.32; N, 7.30. Found: C, 60.68; H, 8.41; N, 7.21.

**22j**: Yield= 77% (for 2 step sequence starting from **7**). HRMS (ES) calcd for $C_{60}H_{99}ClN_6O_{17}$ $(M+H)^+$ 1211.6755, found 1211.6774. $^1$H NMR (500 MHz, $CDCl_3$) δ: 8.68 (s, 1H), 8.15 (s, 1H), 7.69 (s, 1H), 5.38 (dd, 1H), 4.77-4.62 (m, 2H), 4.52 (d, 1H), 4.36-3.73 (m, 2H), 3.70 (s, 1H), 3.65-3.62 (m, 3H), 3.59 (d, 1H), 3.56-3.36 (m, 5H), 3.30 (s, 3H), 3.25 (dd, 1H), 3.24 (m, 2H), 2.74-2.70 (m, 6H), 2.57-2.45 (m, 6H), 2.42 (d, 1H), 2.31 (s, 3H, 9-NMe), 2.29 (s, 6H, 3’-$NMe_2$), 2.16-1.92 (m, 4H), 1.79 (d, 1H), 1.65 (m, 1H), 1.62 (dd, 1H), 1.55-1.40 (m, 5H), 1.36 (s, 3H, 6-Me), 1.34-1.19 (m, 5H), 1.17-1.12 (m, 8H), 1.15-1.12 (m, 6H), 1.10 (s, 3H, 3”-Me), 1.07 (s, 3H, 12-Me), 1.05 (d, 3H, 10-Me), 1.00 (d, 3H, 4-Me), 0.97 (d, 3H, 8-Me), 0.88 (t, 3H, 15-Me). $^{13}$C NMR (125 MHz, $CDCl_3$) δ: 178.1, 176.1, 172.6, 167.4, 149.5, 138.6, 135.4, 130.1, 128.8, 119.0, 118.3, 110.8, 101.2, 94.4, 84.4, 79.4, 77.5, 76.1, 75.0, 74.7, 72.4, 71.3, 70.8, 70.4, 70.0, 69.3, 68.3, 67.3, 65.9, 62.6, 49.0, 47.4, 46.9, 46.0, 45.5, 43.9, 42.1,

40.9, 40.0, 38.7, 36.4, 35.3, 34.2, 29.5, 28.3, 27.2, 26.9, 22.3, 21.1, 20.6, 20.0, 19.5, 15.9, 14.6, 11.2, 10.1, 9.6, 8.7, 7.4. Anal. Calcd. For $C_{60}H_{99}ClN_6O_{17}$: C, 59.46; H, 8.23; N, 6.93. Found: C, 59.58; H, 8.28; N, 6.86.

**22l**: Yield= 68% (for 2 step sequence starting from **7**). HRMS (ES) calcd for $C_{60}H_{99}FN_6O_{17}$ $(M+H)^+$ 1195.7051, found 1195.7063. $^1$H NMR (300 MHz, $CDCl_3$) δ: 8.72 (s, 1H), 8.21 (s, 1H), 7.68 (s, 1H), 5.41 (dd, 1H), 4.76-4.33 (m, 4H), 3.75 (s, 1H), 3.72-3.35 (m, 14H), 3.26 (s, 3H), 3.24 (dd, 1H), 3.20-2.70 (m, 10H), 2.62-2.52 (m, 6H), 2.39 (d, 1H), 2.31 (s, 3H, 9-NMe), 2.29 (s, 6H, 3'-$NMe_2$), 2.15-1.91 (m, 4H), 1.81 (d, 1H), 1.67 (m, 1H), 1.60 (dd, 1H), 1.52-1.41 (m, 3H), 1.35 (s, 3H, 6-Me), 1.33-1.12 (m, 13H), 1.09 (s, 3H, 3''-Me), 1.07 (s, 3H, 12-Me), 1.05 (d, 3H, 10-Me), 1.02 (d, 3H, 4-Me), 0.96 (d, 3H, 8-Me), 0.89 (t, 3H, 15-Me). $^{13}$C NMR (125 MHz, $CDCl_3$) δ: 177.6, 176.6, 172.8, 167.0, 149.3, 143.6, 141.9, 137.6, 117.6, 116.9, 110.4, 108.1, 101.2, 95.2, 85.1, 79.9, 77.3, 76.6, 75.6, 74.3, 72.8, 71.6, 71.0, 70.7, 70.1, 69.5, 69.0, 68.1, 66.1, 63.3, 49.4, 47.7, 47.0, 46.0, 45.1, 44.0, 42.4, 41.0, 39.8, 38.6, 36.6, 35.6, 35.0, 30.5, 29.2, 27.7, 26.8, 22.1, 21.1, 20.7, 20.2, 19.0, 16.3, 14.7, 11.1, 10.0, 9.5, 8.5, 7.6. Anal. Calcd. For $C_{60}H_{99}FN_6O_{17}$: C, 60.28; H, 8.35; N, 7.03. Found: C, 60.34; H, 8.39; N, 7.00.

**22n**: Yield= 55% (for 2 step sequence starting from **7**). HRMS (ES) calcd for $C_{62}H_{103}ClN_6O_{18}$ $(M+H)^+$ 1255.7017, found 1255.7023. $^1$H NMR (300 MHz, $CDCl_3$) δ: 8.68 (s, 1H), 8.35 (s, 1H), 7.44 (s, 1H), 5.42 (dd, 1H), 4.74-4.34 (m, 4H), 3.76-3.65 (m, 3H), 3.60-3.51 (m, 8H),

3.48-3.23 (m, 5H), 3.30 (s, 3H), 3.25 (dd, 1H), 2.72-2.46 (m, 8H), 2.40 (d, 1H), 2.31 (s, 3H, 9-NMe), 2.29 (s, 6H, 3'-$NMe_2$), 2.15-1.95 (m, 4H), 1.79 (d, 1H), 1.68-1.53 (m, 4H), 1.46-1.39 (m, 3H), 1.34 (s, 3H, 6-Me), 1.31-1.15 (m, 7H), 1.14-1.12 (m, 6H), 1.11 (s, 3H, 3''-Me), 1.07 (s, 3H, 12-Me), 1.05 (d, 3H, 10-Me), 1.02 (d, 3H, 4-Me), 0.97 (d, 3H, 8-Me), 0.85 (t, 3H, 15-Me). $^{13}$C NMR (125 MHz, $CDCl_3$) δ: 177.6, 176.5, 171.8, 166.5, 149.8, 139.1, 136.5, 131.0, 128.5, 119.5, 118.1, 109.6, 101.7, 95.4, 85.0, 80.1, 77.9, 76.5, 75.3, 74.5, 72.3, 71.8, 70.9, 70.6, 70.2, 70.0, 69.0, 68.1, 67.2, 66.2, 62.8, 50.1, 49.6, 47.5, 46.4, 46.0, 45.5, 44.1, 42.0, 41.2, 40.3, 38.9, 36.6, 35.5, 34.5, 30.0, 29.4, 28.2, 27.1, 23.0, 21.5, 20.5, 19.8, 18.8, 16.1, 15.6, 11.3, 10.5, 9.5, 8.8, 7.6. Anal. Calcd. For $C_{62}H_{103}ClN_6O_{18}$: C, 59.29; H, 8.27; Cl, N, 6.69. Found: C, 59.43; H, 8.35; N, 6.58.

**22p**: Yield= 60% (for 2 step sequence starting from **7**). HRMS (ES) calcd for $C_{62}H_{103}FN_6O_{18}$ $(M+H)^+$ 1239.7313, found 1239.7340. $^1$H NMR (300 MHz, $CDCl_3$) δ: 8.63 (s, 1H), 8.29 (s, 1H), 7.48 (s, 1H), 5.38 (dd, 1H), 4.77-4.30(m, 4H), 3.76-3.64 (m, 3H), 3.60-3.51 (m, 9H), 3.46 (m, 2H), 3.31 (s, 3H), 3.28-3.20 (m, 3H), 2.70-2.42 (m, 4H), 2.54-2.34 (m, 5H), 2.31 (s, 3H, 9-NMe), 2.28 (s, 6H, 3'-$NMe_2$), 2.16-1.68 (m, 6H), 1.60 (dd, 1H), 1.53-1.39 (m, 3H), 1.38 (s, 3H, 6-Me), 1.17-1.11 (m, 6H), 1.07 (s, 3H, 3''-Me), 1.05 (s, 3H, 12-Me), 1.01 (d, 3H, 10-Me), 1.00 (d, 3H, 4-Me), 0.99 (d, 3H, 8-Me), 0.87 (t, 3H, 15-Me). $^{13}$C NMR (125 MHz, $CDCl_3$) δ: 177.9, 176.3, 172.1, 167.0, 149.8, 143.0, 141.4, 137.9, 117.0, 116.3, 111.4, 109.2, 101.3, 95.8, 85.5, 81.2, 78.7, 76.7, 75.9, 74.7, 72.7, 71.2, 71.5, 70.9, 70.4, 70.0, 69.1, 68.0,

67.0, 65.7, 63.7, 50.0, 49.2, 47.3, 46.9, 46.2, 45.2, 44.2, 42.3, 41.4, 40.5, 39.5, 37.1, 35.6, 34.2, 30.3, 29.2, 28.1, 27.1, 23.4, 22.5, 21.5, 20.8, 18.9, 17.5, 15.8, 12.6, 11.5, 9.4, 8.1, 7.3. Anal. Calcd. For $C_{62}H_{103}FN_6O_{18}$: C, 60.08; H, 8.38; N, 6.78. Found: C, 60.31; H, 8.58; N, 6.59.

*4.3.8. General procedure for the synthesis of quinoxaline aldehydes (***b** *and* **d***). DIBAL-H reduction of quinoxalin-2-yl thioacetyl glycine methyl ester.*

To a solution of quinoxalin-2-yl thioacetyl glycine methyl ester[13] (320.4 mg, 1.1 mmol) in dry THF (10 mL) at -78°C was added dropwise via syringe a 1M solution (1.32 mL, 1.32 mmol) of DIBAL-H in toluene. The reaction mixture was stirred for 45 min at -78 °C, quenched with ethyl acetate (1.3 mL), followed by addition of ethyl acetate (20 mL) and saturated aqueous sodium tartrate (5 mL). The reaction mixture was allowed to warm up to RT and stirring was continued until phase separation. The organic phase was separated, the aqueous phase was further extracted with ethyl acetate (2 x 40 mL), the combined organic phase was concentrated under reduced pressure and the residue filtered through a short plug of celite. Evaporation under reduced pressure afforded crude aldehyde **d** (206.9 mg, 72%) which was used in the next step without further purification. HRMS (ES) calcd for $C_{12}H_{11}N_3O_2S$ $[M+H]^+$ 262.0572, found 262.0598. $^1$H NMR (500 MHz, $CDCl_3$) δ 9.72 (s, 1H, CHO), 8.68 (s, 1H, Ar-H), 8.45 (bs, 1H, CO*NH*), 8.10 (m, 2H, Ar-H), 7.65 (m, 2H, Ar-H), 4.31 (d, 2H, NH*CH*$_2$CHO), 3.82 (s, 2H, S*CH*$_2$CONH). $^{13}$C NMR (125 MHz, $CDCl_3$) δ 198.0

(CHO), 172.5 (CONH), 148.1 (1C, Ar-C), 145.5 (1C, Ar-C), 142.8 (2C, Ar-C), 129.8 (2C, Ar-C), 129.0 (2C, Ar-C), 56.0 (NH$\underline{CH_2}$CHO), 38.9 (S$\underline{CH_2}$CONH).

The same general procedure for DIBAL-H reduction was used to prepare aldehyde **b**. Yield= 80%. HRMS (ES) calcd for $C_{10}H_8N_2OS$ $[M+H]^+$ 205.0357; found 205.0388. $^1$H NMR (500 MHz, $CDCl_3$) δ 9.74 (s, 1H, CHO), 8.79 (s, 1H, Ar-H), 8.10 (m, 2H, Ar-H), 7.73 (m, 2H, Ar-H), 3.98 (d, 2H, S$\underline{CH_2}$CHO). $^{13}$C NMR (125 MHz, $CDCl_3$) δ 199.8 (CHO), 148.5 (1C, Ar-C), 144.5 (1C, Ar-C), 141.9 (2C, Ar-C), 129.3 (2C, Ar-C), 128.8 (2C, Ar-C), 40.9 (S$\underline{CH_2}$CHO).

*4.3.9. Synthesis of 7-(2-(2-formylethylamino)ethylamino)-6-fluoro-1-cyclopropyl-1,4-dihydro-4-oxoquinoline-3-carboxylic acid* (**h**). *General Procedure for Michael addition and DIBAL-H reduction.*

To a solution of 3-acryloyl oxazolidin-2-one **17**[39] (705.6 mg, 5.0 mmol, 5 mol. equiv., for NMR spectra of **17** see the Supporting Information, pages S11-S15) was added 7-[(2-aminoethyl)amino]-6-fluoro-1-cyclopropyl-1,4-dihydro-4-oxo-3-quinoline-carboxylic acid **11b** (305.3 mg, 1.0 mmol) in i-PrOH (15 mL) and stirred under reflux (75°C) for 16 h. The reaction mixture was cooled down to room temperature and the remaining quinolone was removed via filtration. The filtrate was evaporated to dryness and the residue dissolved in $CH_2Cl_2$ (50 mL). The resulting solution was washed with saturated $Na_2CO_3$ (50 mL) and brine (50 mL), dried over $K_2CO_3$ and evaporated to dryness yielding 312.5 mg (70 % yield)

of compound **18b** (for NMR spectra of **18b** see the Supporting Information, pages S16-S20) as white solid. The crude product was used immediately in the next step without further purification. To a cooled (-78 °C) solution of the total sample of unpurified oxazolidinone **18b** (312.5 mg, 0.7 mmol) in 10 mL of anhydrous THF was added 0.71 mL (0.71 mmol) of DIBAL-H (1.0 M in toluene) over 1 h, and the resulting solution was stirred for an additional 15 min. The excess DIBAL-H was quenched by the addition of 0.7 mL of acetone followed by transfer of the solution via cannula into a vigorously stirred mixture of 10 mL of 1 M aqueous tartaric acid and 10 mL of hexane. After 1 h, 100 mL of ethyl acetate was added, the layers were separated, and the aqueous layer was extracted with two 10 mL portions of ethyl acetate. The combined organic extracts were washed with brine, dried over anhydrous $Na_2SO_4$, and concentrated *in vacuo***.** The residue was dissolved in $CH_2Cl_2$ (0.5 mL) and treated with diethyl ether (5 mL). The resulting suspension was filtered yielding 172.0 mg (68% yield) of aldehyde **h** as a colorless solid; $^1$H NMR($CDCl_3$, 500 MHz) δ 9.76 (s, 1H), 8.59 (s, 1H), 7.89 (d, 1H), 7.32 (d, 1H), 3.82 (m, 1H), 3.18 (m, 2H), 2.88-2.78 (m, 4H), 2.52 (m, 2H), 1.38 (m, 2H), 1.21 (m, 2H). MS (ESI) m/z calcd. for $C_{18}H_{20}FN_3O_4$ $[M+H]^+$ 362.1438, found 362.1473.

*4.3.9.1. Spectral data for compounds **f, j, l, n,** and **p***

The following compounds were also prepared by using the same general procedure for Michael addition and DIBAL-H reduction:

**f**: Yield= 52% (for 2 step sequence starting from **11a**). HRMS (ES) calcd for $C_{18}H_{20}ClN_3O_4$ $[M+H]^+$ 378.1142; found 378.1163. $^1H$ NMR ($CDCl_3$, 500 MHz) δ 9.78 (s, 1H), 8.73 (s, 1H), 8.06 (s, 1H), 7.57 (s, 1H), 3.69 (m, 1H), 3.25 (m, 2H), 2.90 (m, 2H), 2.65 (m, 2H), 2.36 (m, 2H), 1.35 (m, 2H), 1.20 (m, 2H).

**j**: Yield= 64% (for 2 step sequence starting from **12a**). HRMS (ES) calcd for $C_{20}H_{24}ClN_3O_5$ $[M+H]^+$ 422.1404, found 422.1419. $^1H$ NMR ($d_6$-DMSO, 500 MHz) δ 9.85 (m, 1H), 8.78 (s, 1H), 7.99 (s, 1H), 7.40 (s, 1H), 3.75 (m, 1H), 3.60-3.49 (m, 4H), 3.23 (m, 2H), 2.88-2.72 (m, 4H), 2.52 (m, 2H), 1.29 (m, 2H), 1.18 (m, 2H).

**l**: Yield= 68% (for 2 step sequence starting from **12b**). HRMS (ES) calcd for $C_{20}H_{24}FN_3O_5$ $[M+H]^+$ 406.1700, found 406.1711. $^1H$ NMR ($d_6$-DMSO, 500 MHz) δ 9.69 (m, 1H), 8.55 (s, 1H), 7.95 (d, 1H), 7.43 (d, 1H), 3.79 (m, 1H), 3.65-3.20 (m, 6H), 2.79-2.49 (m, 6H), 1.32 (m, 2H), 1.16 (m, 2H).

**n**: Yield= 65% (for 2 step sequence starting from **13a**). HRMS (ES) calcd for $C_{22}H_{28}ClN_3O_6$ $[M+H]^+$ 466.1667, found 466.1690. $^1H$ NMR ($d_6$-DMSO, 500 MHz) δ 9.81 (m, 1H), 8.72 (s, 1H), 8.15 (s, 1H), 7.44 (s, 1H), 3.72 (m, 1H), 3.60-3.54 (m, 6H), 3.49-3.23 (m, 4H), 2.88-2.72 (m, 4H), 2.52 (m, 2H), 1.26 (m, 2H), 1.17 (m, 2H).

**p**: Yield= 60% (for 2 step sequence starting from **13b**). HRMS (ES) calcd for $C_{22}H_{28}FN_3O_6$ $[M+H]^+$ 450.1962, found 450.1964. $^1H$ NMR ($d_6$-DMSO, 500 MHz) δ 9.75 (m, 1H), 8.58 (s, 1H), 8.05 (d, 1H), 7.48 (d, 1H), 3.80 (m, 1H), 3.62-3.28 (m, 10H), 2.85-2.70 (m, 4H), 2.50 (m,

2H), 1.31 (m, 2H), 1.20 (m, 2H).

**Supporting Information**

Supplementary data associated with this article can be found in the online version.

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**References and notes**

(1) Doern, G. V.; Heilmann, K. P.; Huynh, H. K.; Rhomberg P. R.; Coffman, S. L.; Brueggemann, A. B. Antimicrobial Resistance Among Clinical Isolates of *Streptococcus pneumoniae* in the United States During 1999-2000, Including a Comparison of Resistance Rates Since 1994-1995. *Antimicrob. Agents Chemother.* **2001**, *45*, 1721-1729.

(2) Ma, Z.; Nemoto, P. A. Discovery and Development of Ketolides as a New Generation of Macrolide Antimicrobial Agents. *Curr. Med. Chem.-Anti-Infect. Agents* **2002**, *1*, 15-34.

(3) Agouridas, C.; Denis, A.; Auger, J.-M.; Benedetti, Y.; Bonnefoy, A.; Bretin, F.; Chantot,

J.-F.; Dussarat, A.; Fromentin, C.; D'Ambrieres, S. G.; Lachaud, S.; Laurin, P.; Le Martret, O.; Loyau, V.; Tessot, N. Synthesis and Antibacterial Activity of Ketolides (6-O-Methyl-3-oxoerythromycin Derivatives): A New Class of Antibacterials Highly Potent Against Macrolide-Resistant and Susceptible Respiratory Pathogens. *J. Med. Chem.* **1998**, *41*, 4080-4100.

(4) Ross, D. B. The FDA and the Case of Ketek. *N. Engl. J. Med.* **2007**, *356*, 1601-1604.

(5) Revill, P.; Bolo´s, J.; Serradell, N. EDP-420: Ketolide Antibiotic. *Drugs Future* **2006**, *31*, 479-483.

(6) Fernandes, P.; Pereira, D.; Jamieson, B.; Keedy, K. Solithromycin. Macrolide Antibiotic. *Drugs Future* **2011**, *36*, 751-758.

(7) Georgopapadakou, N. H. The Wobbly Status of Ketolides: Where do we Stand?. *Expert Opin. Investig. Drugs* **2014**, *23*, 1-7.

(8) Brinker, A. D.; Wassel, R. T.; Lyndly, J.; Serrano, J.; Avigan, M.; Lee, W.M.; Seeff, L.B. Telithromycin-associated Hepatoxicity: Clinical Spectrum and Causality Assessment of 42 cases. *Hepatology*, **2009**, *49*, 250-257.

(9) Como, J. A.; Dismukes, W. E. Oral Azole Drugs as Systemic Antifungal Therapy. *N. Engl. J. Med.* **1994**, *330*, 263-272.

(10) Echols, R. M. Understanding the Regulatory Hurdles for Antibacterial Drug Development in the post-Ketek World. *Ann. N. Y. Acad. Sci.* **2011**, *1241*, 153-161.

(11) Ma, Z.; Clark, R. F.; Brazzale, A.; Wang, S.; Rupp, M. J.; Li, L.; Griesgraber, G.; Zhang, S.; Yong, H.; Phan, L. T.; Nemoto, P. A.; Chu, D. T. W.; Plattner, J. J.; Zhang, X.; Zhong, P.; Cao, Z.; Nilius, A. M.; Shortridge, V. D.; Flamm, R.; Mitten, M.; Meulbroek, J.; Ewing, P.; Alder, J.; Or, Y. S. Novel Erythromycin Derivatives with Aryl Groups Tethered to the C-6 Position are Potent Protein Synthesis Inhibitors and Active against Multidrug-Resistant Respiratory Pathogens. *J. Med. Chem.* **2001**, *44*, 4137-4156.

(12) Andreotti, D.; Bientinesi, I.; Biondi, S.; Donati, D.; Erbetti, I.; Lociuro, S.; Marchioro, C.; Pozzan, A.; Ratti, E.; Terreni, S. A Novel Ketolide Class: Synthesis and Antibacterial Activity of a Lead Compound. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 5265-5269.

(13) Pavlović, D.; Mutak, S.; Andreotti, D.; Biondi, S.; Cardullo, F.; Paio, A.; Piga, E.; Donati, D.; Lociuro, S. Synthesis and Structure–Activity Relationships of α-Amino-γ-lactone Ketolides: A Novel Class of Macrolide Antibiotics. *ACS Med. Chem. Lett.*, **2014**, *5*, 1133-1137.

(14) Wang, J.; De Clercq, P. J. Estramycins: A New Diyl Precursor Family Derived from Estradiol. *Angew. Chem., Int. Ed. Engl.* **1995**, *34*, 1749-1752.

(15) Depew, K. M.; Zeman, M.; Boyer, S. H.; Denhart, D. J.; Ikemoto, N.; Crothers, D. M.; Danishefsky, S. J. Synthesis and a Preliminary DNA Binding Study of Hybrids of the Carbohydrate Domain of Calicheamicin γmath image and the Aglycone of Daunorubicin: Calichearubicins A and B. *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 2797-2801.

(16) Tietze, L. F.; Schneider, G.; Wölfling, J.; Nöbel, T.; Wulff, C.; Schubert, I.; Rübeling, A. Efficient Synthesis of a Novel Estrone-Talaromycin Hybrid Natural Product. *Angew. Chem., Int. Ed. Engl.* **1998**, *37*, 2469-2470.

(17) Dräger, G.; Garming, A.; Maul, C.; Noltemeyer, M.; Thiericke, R.; Zerlin, M.; Kirschning, A. First Glycosylation of Decarestrictine B and D: A Route to Hybrid Antibiotics. *Chem. Eur. J.* **1998**, *4*, 1324-1333.

(18) Cornia, M.; Binacchi, S.; Del Soldato, T.; Zanardi, F.; Casiraghi, G. Synthesis of Novel Porphyrin-Uridine Carbon-Carbon Conjugates. *J. Org. Chem.* **1995**, *60*, 4964-4965.

(19) Jiang, X.; Pandey, R. K.; Smith, K. M. Synthesis of Nucleoside Adducts of Porphyrins and Chlorophyll Derivatives. *Tetrahedron Lett.* **1995**, *36*, 365-368.

(20) Dömling, A.; Starnecker, M.; Ugi, I. The β-Lactam-Nucleoside Chimera. *Angew. Chem., Int. Ed. Engl.* **1995**, *34*, 2238-2239.

(21) Manoharan, M.; Tivel, K. L.; Cook, P. D. Lipidic Nucleic Acids. *Tetrahedron Lett.* **1995**, *36*, 3651-3654.

(22) Uhlmann, E.; Peyman, A.; Breipohl, G.; Will, D. W. PNA: Synthetic Polyamide Nucleic Acids with Unusual Binding Properties. *Angew. Chem., Int. Ed. Engl.* **1998**, *37*, 2796-2823.

(23) Nielsen, P. E.; Haaima, G. Peptide Nucleic Acid (PNA). A DNA Mimic with a Pseudopeptide Backbone. *Chem. Soc. Rev.* **1997**, *26*, 73-78.

(24) Albrecht, H. A.; Beskid, G.; Chan, K. K.; Christenson, J. G.; Cleeland, R.; Deitcher, K.

H.; Georgopapadakou, N. H.; Keith, D. D.; Pruess, D. L.; Sepinwall, J.; Specian, Jr. A. C.; Then, R. L.; Weigele, M.; West, K. F.; Yang, R. Cephalosporin 3'-Quinolone Esters with a Dual Mode of Action. *J. Med. Chem.* **1990**, *33*, 77-86.

(25) Demuth, T. P., Jr.; White, R. E.; Tietjen, R. A.; Storrin, R. J.; Skuster, J. R.; Andersen, J. A.; McOsker, C. C.; Freedman, R.; Rourke, F. J. Synthesis and Antibacterial Activity of New C-10 Quinolonyl-Cephem Esters. *J. Antibiot.* **1991**, *44*, 200-209.

(26) Jones, R. N.; Barry, A. L.; Thornsberry, C. Antimicrobial Activity of Ro-23-9424, A Novel Ester-linked Codrug of Fleroxacin and Desacetylcefotaxime. *Antimicrob. Agents Chemother.* **1989**, *33*, 944-950.

(27) Hubschwerlen, C.; Specklin, J.-L.; Sigwalt, C.; Schroeder, S.; Locher, H. H. Design, Synthesis and Biological Evaluation of Oxazolidinone–Quinolone Hybrids *Bioorg. Med. Chem.* **2003**, *11*, 2313-2319.

(28) Alovero, F.; Nieto, M.; Mazzieri, M. R.; Then, R.; Manzo, R. H. Mode of Action of Sulfanilyl Fluoroquinolones. *Antimicrob. Agents Chemother.* **1998**, *42*, 1495-1498.

(29) Gordeev, M. F.; Hackbarth, C.; Barbachyn, M. R.; Banitt, L. S.; Gage, J. R.; Luehr, G. W.; Gomez, M.; Trias, J.; Morin, S. E.; Zurenko, G. E.; Parker, C. N.; Evans, J. M.; White, R. J.; Patel, D. V. Novel Oxazolidinone-Quinolone Hybrid Antimicrobials. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 4213-4216.

(30) Đokić, S.; Kobrehel, G.; Lazarevski, G.; Lopotar, N.; Tamburašev, Z. Erythromycin

Series. Part 11. Ring Expansion of Erythromycin A Oxime by the Beckmann Rearrangement. *J. Chem. Soc., Perkin Trans.* 1 **1986**, 1881-1890.

(31) Alihodžić, S.; Pavlović, D.; Hunt, E.; Forrest, A. K.; Palej, I.; Kapić, S.; Štimac, V. Ether Linked Macrolides Useful for the Treatment of Microbial Infections. US Patent 7,547,679 B2, **2009**.

(32) Pavlović, D.; Mutak, S. Discovery of 4"-Ether Linked Azithromycin-Quinolone Hybrid Series: Influence of the Central Linker on the Antibacterial Activity. *ACS Med. Chem. Lett.* **2011**, *2*, 331-336.

(33) Plata, D. J.; Leanna, M. R.; Rasmussen, M.; McLaughlin, M. A.; Condon, S. L.; Kerdesky, F. A. J.; King, S. A.; Peterson, M. J.; Stoner, E. J.; Wittenberger, S. J. The Synthesis of Ketolide Antibiotic ABT-773 (Cethromycin). *Tetrahedron* **2004**, *60*, 10171-10180.

(34) Magee, T. V.; Ripp, S. L.; Li, B.; Buzon, R. A.; Chupak, L.; Dougherty, T. J.; Finegan, S. M.; Girard, D.; Hagen, A. E.; Falcone, M. J.; Farley, K. A.; Granskog, K.; Hardink, J. R.; Huband, M. D.; Kamicker, B. J.; Kaneko, T.; Knickerbocker, M. J.; Liras, J. L.; Marra, A.; Medina, I.; Nguyen,T.-T.; Noe, M. C.; Obach, R. S.; O'Donnell, J. P.; Penzien, J. B.; Reilly, U. D.; Schafer, J. R.; Shen, Y.; Stone, G. G.; Strelevitz, T. J.; Sun, J.; Tait-Kamradt, A.; Vaz, A. D. N.; Whipple, D. A.; Widlicka, D. W.; Wishka, D. G.; Wolkowski, J. P.; Flanagan M. E. Discovery of Azetidinyl Ketolides for the Treatment of Susceptible and Multidrug Resistant

Community-Acquired Respiratory Tract Infections. *J. Med. Chem.* **2009**, *52*, 7446-7457.

(35) Woosley, L. N.; Castanheira, M.; Jones, R. N. CEM-101 Activity against Gram-positive Organisms. *Antimicrob. Agents Chemother.* **2010**, *54*, 2182-2187.

(36) Đokić, S.; Kobrehel, G.; Lopotar, N.; Kamenar, B.; Nagl, A.; Mrvoš, D. Erythromycin Series. Part 13. Synthesis and Structure Elucidation of 10-Dihydro-10-deoxo-11-methyl-11-azaerythromycin A. *J. Chem. Research (S)* **1988**, 152-153.

(37) Separation of the pure regioisomeric quinolones was achieved by adjustment of the pH of the crude reaction mixture to 6 followed by recrystallization from water at RT. This procedure afforded isolation of pure C(7)-chloro and C(6)-fluoro substituted quinolones **11a-13a** and **11b-13b** in 25-35% yield starting from the commercially available quinolone carboxylic acid **10**.

(38) Fajdetić, A.; Vinter, A.; Čipčić Paljetak, H.; Padovan, J.; Palej Jakopović, I.; Kapić, S.; Alihodžić, S.; Filić, D.; Modrić, M.; Košutić-Hulita, N.; Antolović, R.; Ivezić Schoenfeld, Z.; Mutak, S.; Eraković Haber, V.; Spaventi, R. Synthesis, Activity and Pharmacokinetics of Novel Antibacterial 15-Membered Ring Macrolones. *Eur. J. Med. Chem.* **2011**, *46*, 3388-3397.

(39) Okazaki, K.; Seki, R.; Nakatsuka, S. Organic polymers and novel polymerizable compounds. US Patent 6,797,805 B2, **2004**.

(40) Debono, M.; Willard, K. E.; Kirst, H. A.; Wind, J. A.; Crouse, G. D.; Tao, E. V.;

Vincenzi, J. T.; Counter, F. T.; Ott, J. L.; Ose, E. E.; Omura, S. Synthesis and Antimicrobial Evaluation of 20-Deoxo-20-(3,5-dimethylpiperidin-1-yl)desmycosin (Tilmicosin, EL-870) and Related Cyclic Amino Derivatives. *J. Antibiot.* **1989**, *42*, 1253-1267.

(41) National Committee for Clinical Laboratory Standards. *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically*, 5th ed. Approved standard: NCCLS Document M7-A5, 200.

## Graphical Abstract