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Research paper

Design, synthesis and structure-activity relationships of novel 15-membered macrolides: Quinolone/quinoline-containing sidechains tethered to the C-6 position of azithromycin acylides

Bing-Zhi Fan [a], Hiroshi Hiasa [b], Wei Lv [c], Scott Brody [d], Zhao-Yong Yang [e], Courtney Aldrich [d], Mark Cushman [c], Jian-Hua Liang [a, d, *]

[a] School of Chemistry and Chemical Engineering, Beijing Institute of Technology, Beijing, 100081, China
[b] Department of Pharmacology, University of Minnesota Medical School, Minneapolis, MN, 55455, United States
[c] Department of Medicinal Chemistry and Molecular Pharmacology, College of Pharmacy, Purdue University, West Lafayette, 47907, United States
[d] Department of Medicinal Chemistry, College of Pharmacy, University of Minnesota, Minneapolis, MN, 55455, United States
[e] NHC Key Laboratory of Biotechnology of Antibiotics, Institute of Medicinal Biotechnology, Chinese Academy of Medical Sciences, Beijing, 100050, China

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ABSTRACT

In the search for novel hybrid molecules by fusing two biologically active scaffolds into one heteromeric chemotype, we found that hybrids of azithromycin and ciprofloxacin/gatifloxacin **26j** and **26l** can inhibit the supercoiling activity of *E. coli* gyrase by poisoning it in a way similar to fluoroquinolones. This may modestly contribute to their potencies, which are equal to ciprofloxacin against constitutively resistant *Staphylococcus aureus*, whose growth is not inhibited by the presence of macrolides. In contrast, introduction of quinolines (the 3-quinoline **26b** and the 6-quinoline **26o**) with an optimized rigid spacer at the 6-OH of azithromycin acylides did not exert significant potency against constitutively resistant *S. aureus*, despite the fact that the quinoline-containing compounds, exemplified by **26o**, were as active as telithromycin against susceptible, inducibly- and efflux-resistant pathogens. The novel dual modes of action involving protein synthesis inhibition and poisoning DNA replication may pave the way for restoration of antibacterial activities of the current macrolides against constitutively resistant clinical isolates.

1. Introduction

The ribosome is the most important target for antibiotics because it is the target of over half of the antibiotics approved for clinical use. Among them, azithromycin is a long-acting macrolide antimicrobial agent (Fig. 1), which is widely prescribed for curing upper and lower respiratory tract infections. It is characterized by an uncommon aza-15-membered scaffold, which is synthesized by Beckmann rearrangement of erythromycin oxime followed by hydrogenation and methylation [1]. Compared to erythromycin (Fig. 1), azithromycin possesses not only improved pharmacokinetic properties (for example, enhanced acid-stability in the stomach and high tissue distribution in the lung) but also an extended antimicrobial spectrum. However, the use of azithromycin has decreased due to globally emerging antibiotic resistance. A recent international surveillance indicated that azithromycin is only effective against 62.2% of *Streptococcus pneumoniae* and 58.7% of *Staphylococcus aureus* strains [2]. Therefore, the search for an effective chemotype is becoming more urgent.

The underlying resistance mechanism is that azithromycin is an inducer of expression of erm genes (erythromycin ribosomal methylase), which in turn mono- or dimethylates the amino group of A2058Ec (*Escherichia coli* numbering), a vital binding site that is located in bacterial nascent peptide exit tunnel (NPET). The methylation results in dramatically decreased affinity of azithromycin to bacterial ribosomes. Alternative inducible resistance is mediated by mef-encoding efflux proteins, which facilitate the efflux of drug molecules outside the cell and reduce antibiotics' concentration below the effective level when encountering the target [3–5]. The ability to induce resistance should be eradicated, because it is responsible for the recent increases in clinical

* Corresponding author. School of Chemistry and Chemical Engineering, Beijing Institute of Technology, Beijing, 100081, China.
E-mail address: ljhbit@bit.edu.cn (J.-H. Liang).

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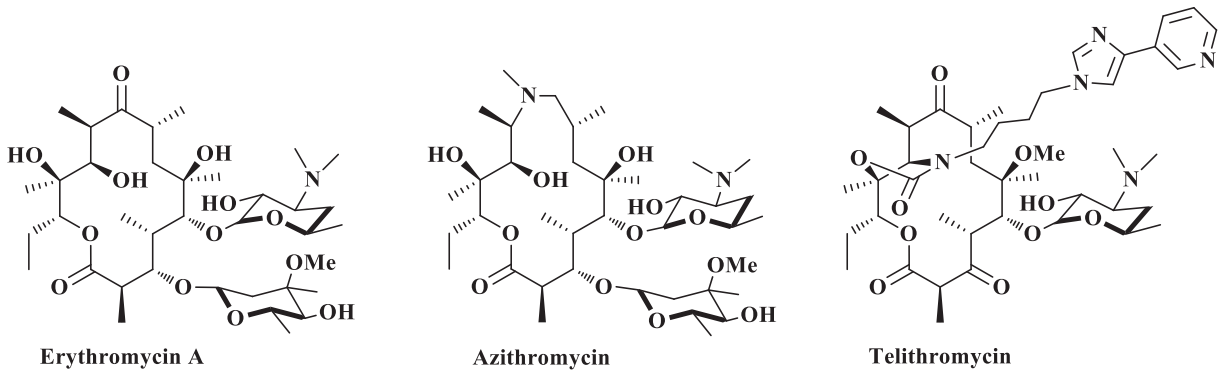


Fig. 1. Structures of the erythromycin, azithromycin, and telithromycin.

resistance and treatment failure. It was found that cladinose, attached to C-3 of the macrolide, plays an important role in induction of the two types of resistance [6,7]. For this reason, the 4"-position of cladinose was structurally modified to circumvent the induction [1,8,9]. However, a more attractive strategy is the replacement of cladinose by means of MIC-guided structural optimization, and the resulting non-inducers are known as ketolides (3-keto) or acylides (3-*O*-acyl) [10–12]. Since the natural 3-keto macrolide picromycin, which was discovered in 1950s, is inactive, the discovery of the synthetic ketolides and acylides disproved the long-held notion that cladinose is necessary to maintain the activities of the macrolides [4,8].

The expression of A2058 methylase can be inducible or constitutive. In the latter case, the binding site A2058 would be constitutively methylated by methylase in absence of the inducers, leading to high-level resistance. Unfortunately, conversion of the cladinose to keto or acyl groups does not restore the activities of the non-inducers against constitutively resistant bacteria [11,12]. Constitutively resistant bacteria, unlike inducible ones, are coresistant to chemically unrelated macrolides, lincosamides, and streptogramin B (MLS_B), which have overlapping binding sites in the bacterial NPET. Thus, non-inducers' inability to restore the activity against constitutively resistant bacteria required the search for an additional high-affinity binding site. This led to telithromycin (Fig. 1), a 14-membered ketolide, which is the first and thus far only marketed erythromycin derivative with the restored activity against erythromycin-resistant pathogens. It has a sidechain at the 11-N carbamate cyclized to the 12-OH, which supplies a key structural element for binding to the base pair A752/U2609 [13,14]. Telithromycin possesses improved activities against constitutively resistant *S. pneumoniae* and *Streptococcus pyogenes*, but not against constitutively resistant *S. aureus*. In addition to telithromycin, at least five 14-membered ketolides are in clinical trials. Although 15-membered azithromycin possesses better PK and safety profiles, none of the azithromycin analogs have entered clinical trials.

To search for novel chemicals with new modes of action, quinolones were tethered to the 4"-position of the cladinose of azithromycin or 6-*O*-methylerythromycin (clarithromycin), and the hybrids were named macrolones [15–23]. 3-Carboxy-4-quinolones are the key pharmacophores that target bacterial DNA gyrase or topoisomerase IV [24,25]. However, extensive studies indicated that the macrolones retained their activities as strong protein synthesis inhibitors and proved to be devoid of DNA gyrase and topoisomerase IV inhibitory activity [22,26]. The lack of the dual mode was corroborated by the full potency of the macrolones against quinolone-resistant *S. aureus* strains [26]. Therefore, the researchers concluded that the macrolones did not exhibit their antibacterial activities through a quinolone mode of action [26].

Despite strenuous efforts on the SARs of macrolides [1,8,9], there are reasons that support continued study. First, constitutive resistance is at a much higher level in comparison with inducible and efflux-mediated resistance. Furthermore, it was reported that mutational frequency in clinical isolates with constitutive resistance is > 57-fold and >3333-fold higher than the inducibly resistant and erythromycin-susceptible strains, respectively [27]. Thus, novel ribosome binding sites or novel modes of action that are different from those of telithromycin need to be identified in order to fight against the constitutively resistant bacteria. Second, none of the macrolides show high activities against constitutively resistant *S. aureus*. An improvement was disclosed by Pavlovic regarding hybrids of quinolones and 8a-azahomoerythromycin ketolides (Fig. 2) [28]. Although these hybrids were 16–64 fold weaker than ciprofloxacin, the results suggest that promising candidates against constitutively resistant *S. aureus* may emerge from 15-membered macrolides. Third, SARs of macrolones and their modes of action are not fully explored yet. Except for some C-6 modified (homo) erythromycin ketolides [28,29], the SAR is unclear regarding azithromycin acylides having quinolones tethered to C-6 position [30,31]. Previously, we found that SARs of acylides are distinct from those of ketolides, probably due to the easily twisted profiles of the flexible macrolides [32,33].

To broaden the SAR of macrolones and their modes of action, we have investigated novel acylide-type macrolones obtained by direct acrylation at the C-6 position of azithromycin followed by facile diversification via Michael addition. As reported herein, the

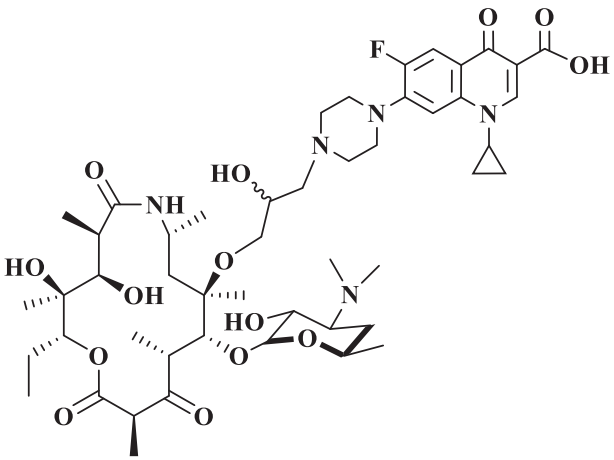


Fig. 2. Structure of the 8a-aza-8a homoerythromycin A ketolide with improved activity against constitutively resistant *S. aureus* (2–8 μg/mL vs 0.125 μg/mL of ciprofloxacin vs > 64 μg/mL of telithromycin).

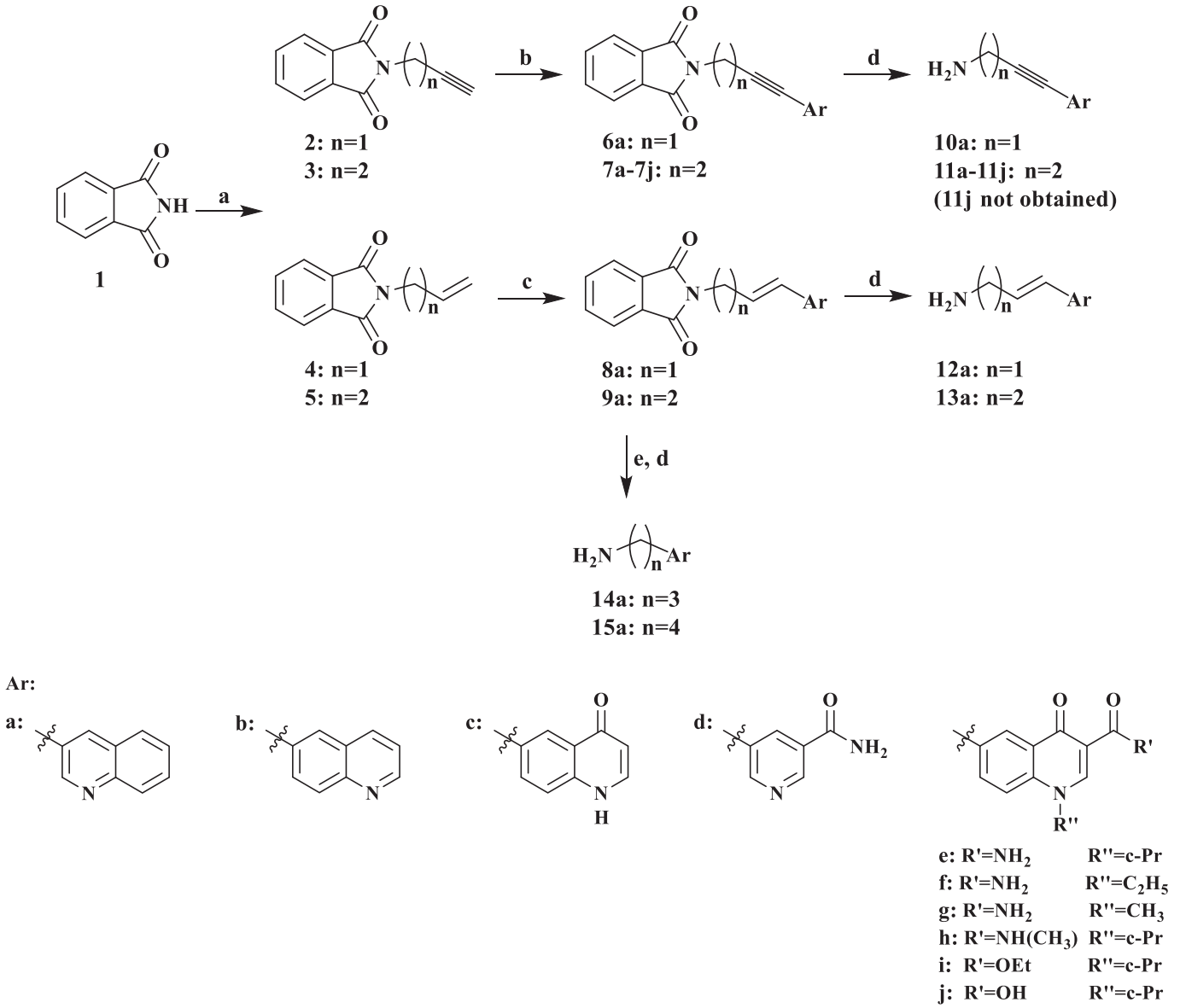
products had enhanced activities against inducibly resistant, efflux-resistant and constitutively resistant pathogens.

2. Results and discussion

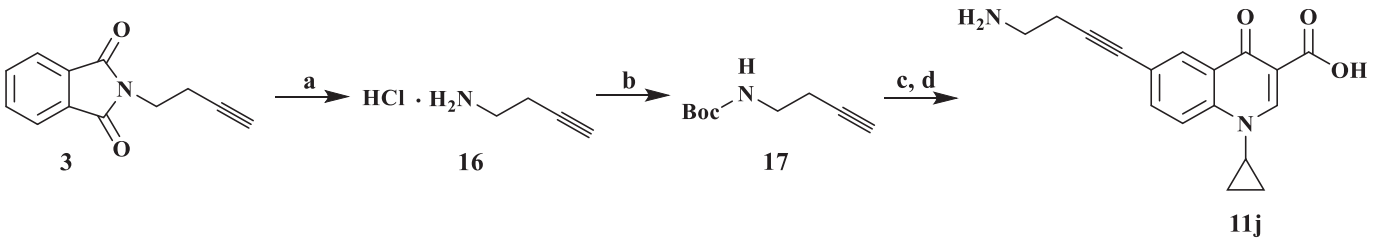
2.1. Chemistry

The synthetic routes leading to the amines **10a**, **11a-11j, 12a**, **13a, 14a**, **15a** and **20** are outlined in Schemes 1–3. Commercially available phthalimide (**1**) was reacted with different reagents (propargyl bromide, but-3-yn-1-ol, allyl bromide or 4-bromobut-1-ene) under different conditions to provide the alkynes and alkenes (**2**, **3**, **4** and **5**). Compounds **6a** and **7a-7j** were synthesized by Sonogashira coupling, followed by treatment with hydrazine hydrate to yield the amines **10a** and **11a-11i**. However, **11j** was not obtained because **7j** did not react in the hydrazinolysis reaction. In a similar way, intermediates **8a** and **9a** were prepared by Heck coupling, followed by treatment with hydrazine hydrate to afford the amines **12a** and **13a**. The amines **14a** and **15a** were obtained by reduction of **8a** and **9a** and subsequent reaction with hydrazine hydrate. Since basic hydrolysis of **11i** resulted in unidentified products, another procedure for **11j** was sought by hydrazinolysis of **3**, Boc-protection, Sonogashira coupling and deprotection (Scheme 2). The ciprofloxacin derivative **20** was prepared in three steps: Boc-protection of ciprofloxacin (**18**), conversion of **19** to the corresponding amide, and subsequent deprotection (Scheme 3).

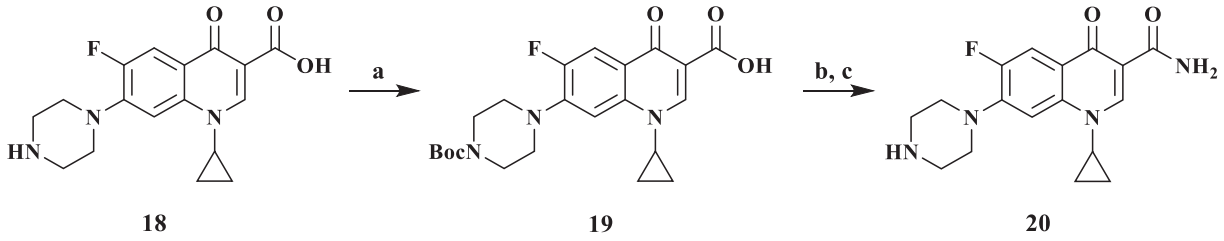
The preparations of the target compounds **26** series, **29** series and **31** series are shown in Schemes 4 and 5 and Chart 1. First, 3-*O*-cladinose was selectively removed from azithromycin (**21**) with 1 M HCl in the presence of EtOH. Next, the 2′-OH was protected by acetylation to give **22** with unprotected hydroxyls at the 3-, 6-, 11-, and 12-positions. It was reported that acrylation occurred easily at



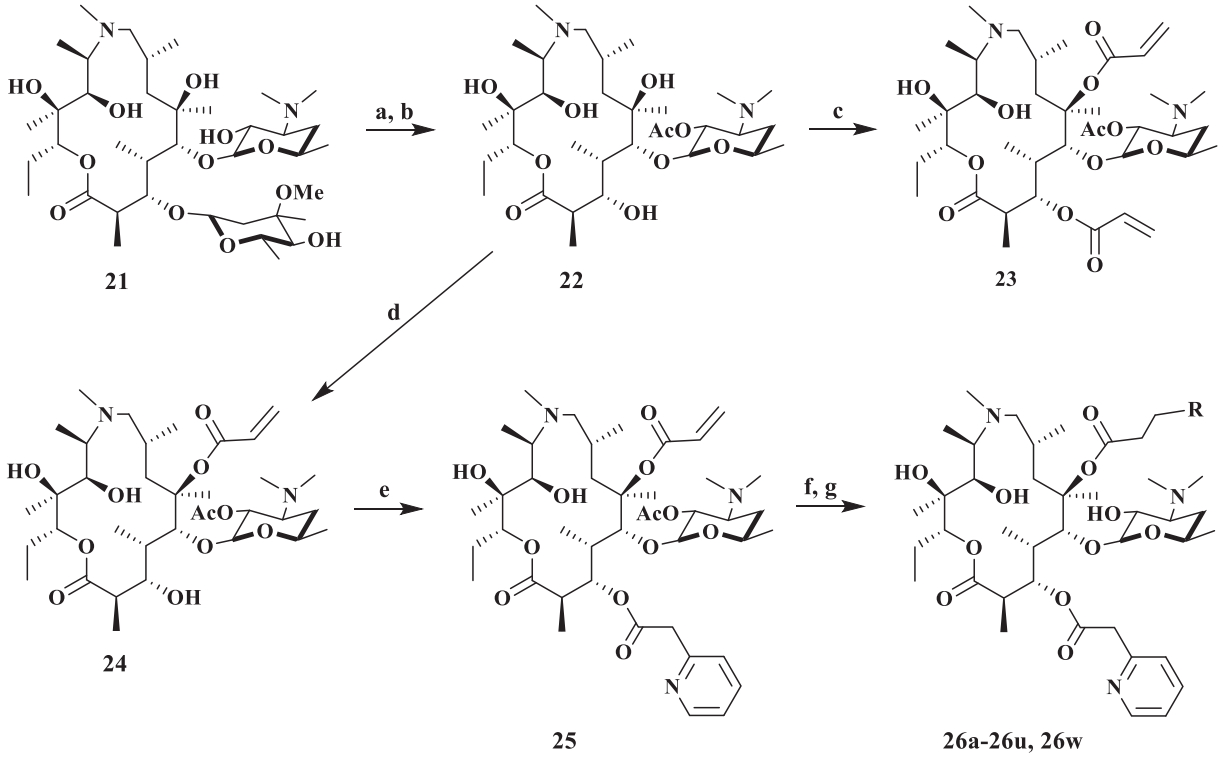
Scheme 1. Synthesis of compounds **10a**, **11a-11i**, **12a**, **13a**, **14a** and **15a**. Reagents and conditions: (a) 3-butyn-1-ol, PPh_3, DIAD, toluene, 0 °C for 10 min, then rt for 1 h; or propargyl bromide or allyl bromide or 4-bromo-1-butene, KOH, EtOH, rt for 2 h, then reflux in DMF for 72 h; (b) ArX, CuI, bis(triphenylphosphine)palladium(II) dichloride, triethylamine, acetonitrile, 80 °C, 4 h; (c) ArX, $Pd(OAc)_2$, tri-*o*-tolylphosphine, triethylamine, acetonitrile, 60 °C for 1 h, then 90 °C for 24 h; (d) 80% hydrazine hydrate, EtOH, reflux; (e) ammonium formate, HCOOH, 10% Pd/C, MeOH, 65 °C, 24 h.



Scheme 2. Synthesis of compound **11j**. Reagents and conditions: (a) 80% hydrazine hydrate, EtOH, reflux; then HCl; (b) di-*tert*-butyl dicarbonate, triethylamine, THF, 0 °C for 10 min, then rt for 24 h; (c) ArX, CuI, bis(triphenylphosphine)palladium(II) dichloride, triethylamine, acetonitrile, 50 °C, 24 h; (d) HCl, EtOAc, 0 °C for 10 min, then rt for 3 h.

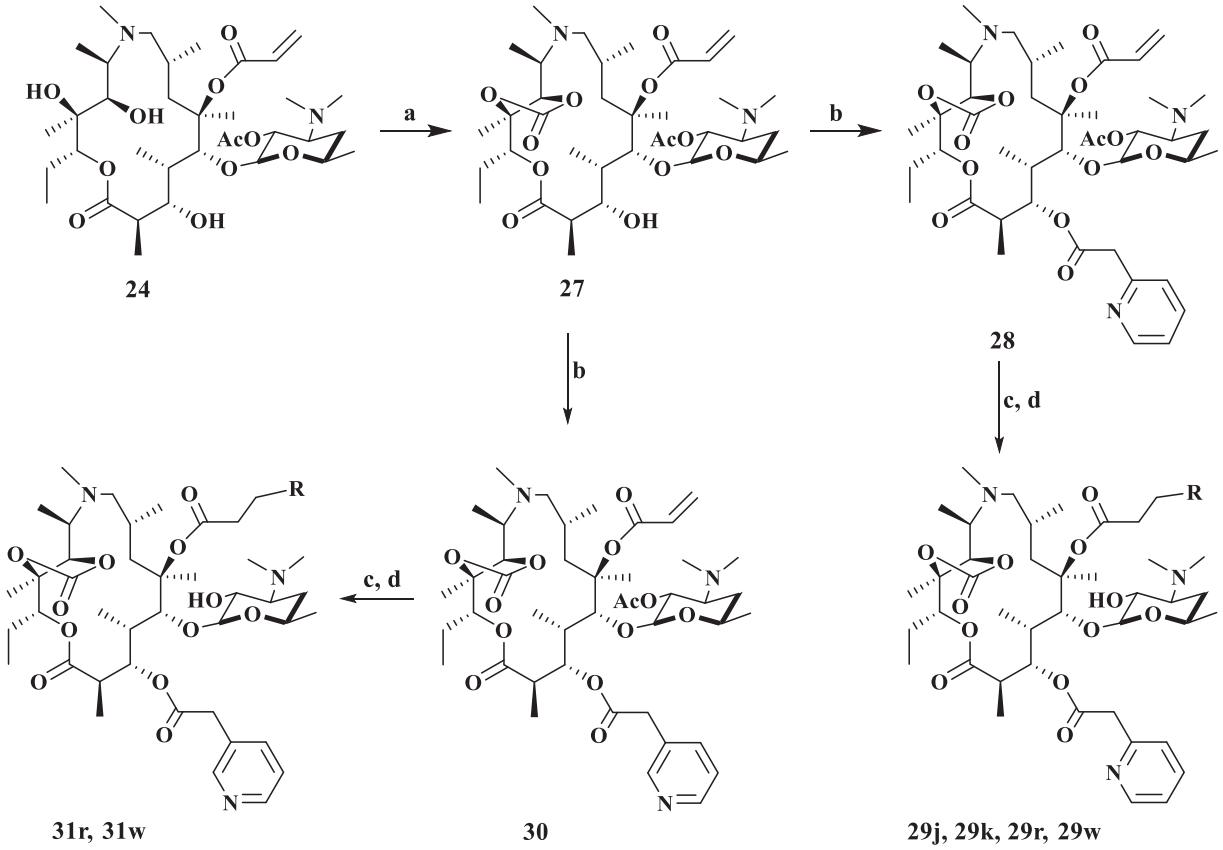


Scheme 3. Synthesis of compound **20**. Reagents and conditions: (a) di-*tert*-butyl dicarbonate, triethylamine, THF, 0 °C for 10 min, then rt for 8 h; (b) CDI, DMF, 65 °C for 2 h, then $NH_3 \cdot H_2O$, rt for 2 h; (c) HCl, EtOAc, 0 °C for 10 min, then rt for 3 h.



Scheme 4. Synthesis of compounds **26a-26u** and **26w**. Reagents and conditions: (a) 1 M HCl, EtOH, 40 °C, 1 h; (b) Ac_2O, CH_2Cl_2, rt, 1 h; (c) 3-chloropropionyl chloride, triethylamine, acetonitrile, 80 °C; (d) 3-chloropropionyl chloride, triethylamine, toluene, rt; (e) 2-pyridylacetic acid hydrochloride, DCC, pyridine, DMAP, CH_2Cl_2, 0 °C; (f) MeOH, reflux, 2 h; (g) amines (**a-u**, **w**; Chart 1), *N*,*N*-diisopropylethylamine, MeOH, 65 °C, 8 h.

the secondary 4"-OH of the cladinose [34]. Although the tertiary 6-OH is highly sterically hindered, treatment **22** with 3-chloropropionyl chloride in toluene at room temperature regioselectively yielded the acrylation derivative at 6-OH (**24**). We found that diacrylation would occur at both 6-OH and 3-OH (**23**) in acetonitrile at elevated temperature. The structures of the acylated products **23** and **24** were determined by analyzing their ^{1}H, COSY, DEPT and ^{13}C NMR spectral data. Then, esterification of the 3-OH produced the acylide **25** by treatment of **24** with 2-pyridineacetic acid hydrochloride in the presence of DCC, pyridine and DMAP. The downshift of H-3 in **24**'s NMR spectrum proved that 3-OH of **24** was successfully acylated to yield **25**. The target compounds **26a-26w** were obtained by Michael addition to 2′-*O*-deacetyl-**25** with a variety of amines in the presence of an excess of *N*,*N*-



Scheme 5. Synthesis of compounds **29j, 29k, 29r**, **29w, 31r** and **31w**. Reagents and conditions: (a) bis(trichloromethyl)carbonate, pyridine, CH_2Cl_2, -10 °C ~ −5 °C for 4 h, then rt for 2 h; (b) 2-pyridylacetic acid hydrochloride or 3-pyridylacetic acid hydrochloride, DCC, pyridine, DMAP, CH_2Cl_2, 0 °C; (c) MeOH, reflux, 2 h; (d) amines (**j**, **k**, **r**, **w**; Chart 1), *N*,*N*-diisopropylethylamine, MeOH, 65 °C, 8 h.

diisopropylethylamine after methanolysis of the 2′-*O*-acetate (Scheme 4). No addition reaction was found to yield **26v** in the presence of **11i**. Cyclic carbonation at 11-OH and 12-OH (**27**) and acylation at the 3-OH (**28** and **30**) further corroborate the structural correctness of **24**. Finally, the target compounds **29** (**29j**, **29k**, **29r** and **29w**) and **31** (**31r** and **31w**) were obtained in a way that is similar to the synthesis of **26** (Scheme 5).

2.2. Structure-activity relationships of azithromycin acylides

2.2.1. Variation of the linker lengths and configuration at the 6-OH of azithromycin

The phenotypes (inducible, constitutive and efflux) and genotypes (mef and erm) of the resistant bacteria were determined and are listed in Table 1. The strains include Gram-positive bacteria such as *S. aureus*, *S. pneumoniae* and *S. pyogenes*, and Gram-negative pathogens such as *H. influenzae*. The constitutive MLS_B phenotypes were differentiated from inducible MLS_B and efflux phenotypes according to a triple-disk test [35].

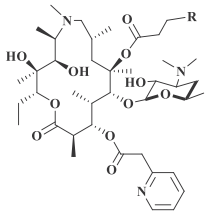
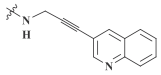
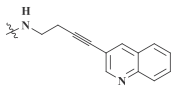
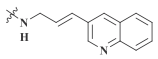
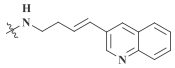
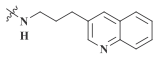
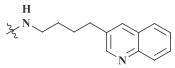
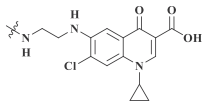
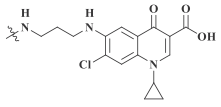
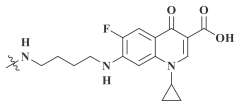
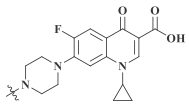
All of the compounds listed in Table 2 showed enhanced activities against resistant strains compared to parent azithromycin. Among the hybrids of macrolides and quinolones bridged by linkers of various length (**26g**, **26h**, **26i** and **26j**), **26h** possessing a linear propanediamine sidechain was the most active. However, **26h** was inactive against *S. aureus* 15B196 containing a constitutive erm gene. Against constitutively resistant *S. pneumoniae* 07P390, **26g** and **26h** (6-quinolones) were 4- to 8-fold more potent than **26i** and **26j** (7-quinolones), which indicated that the attachment positions

Table 1
Selected organisms and their resistant phenotypes and genotypes.

Strain		Phenotype	Genotype
Streptococcus pneumoniae	ATCC 49619	Erythromycin-susceptible	none
Streptococcus pneumoniae	PU 09	Efflux, PSSP[a]	*mef*
Streptococcus pneumoniae	07P390	MLS_B-resistant[b] (constitutive)	*ermB*
Streptococcus pyogenes	12−206	MLS_B-resistant (constitutive)	*ermA*
Staphylococcus aureus	PU 32	MLS_B-resistant (inducible), MRSA[c], Ciprofloxacin-resistant	*ermA*
Staphylococcus aureus	15B196	MLS_B-resistant (constitutive), MRSA	NT[d]
Haemophilus influenzae	ATCC 49247	Azithromycin-susceptible	none

[a] PSSP: penicillin-susceptible *Streptococcus pneumoniae*.
[b] MLS_B: macrolide-lincosamide-streptogramin B.
[c] MRSA: methicillin-resistant *Staphylococcus aureus*.
[d] NT: not tested.

Table 2
Antibacterial activity of **26a**-**26j** against erythromycin-susceptible and -resistant pathogens.

Compound	MIC and MBC (μg/mL)								
	S. pneumoniae ATCC 49619			*S. pneumoniae* PU 09 *efflux*			*S. pneumoniae* 07P390 *constitutive* MLS_B[a]		
	MIC	MBC	MBC/MIC	MIC	MBC	MBC/MIC	MIC	MBC	MBC/MIC
AZM[b]	0.062	0.25	4	8	16	2	256	>256	–
TEL[c]	≤0.008	≤0.008	1	0.25	0.5	2	0.016	>0.125	>8
26 series									
26a		0.125	0.125	1	2	2	1	1	>8	>8
26b		0.062	0.125	2	1	1	1	0.5	>4	>8
26c		0.125	0.25	2	2	2	1	1	>8	>8
26d		0.125	0.125	1	1	1	1	4	>32	>8
26e		0.062	0.062	1	2	4	2	2	16	8
26f		0.25	0.5	2	4	4	1	8	32	4
26g		0.25	0.25	1	4	4	1	1	>8	>8
26h		0.125	0.125	1	1	1	1	1	>8	>8
26i		0.25	0.5	2	4	4	1	8	>64	>8
26j		0.125	0.25	2	2	4	2	4	32	8

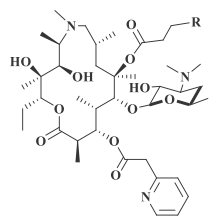
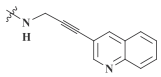
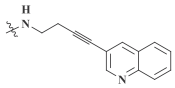
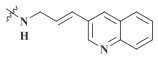
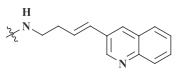
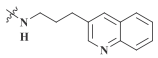
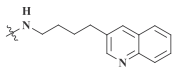
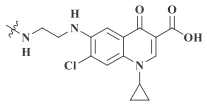
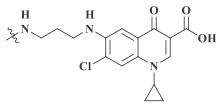
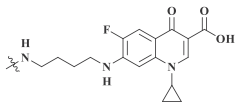
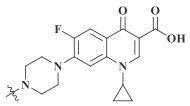
Compound	MIC and MBC (μg/mL)								
	S. aureus PU 32 *MRSA*[d], *inducible* MLS_B			*S. aureus* 15B196 *MRSA*, *constitutive* MLS_B			*H. influenzae* ATCC 49247		
	MIC	MBC	MBC/MIC	MIC	MBC	MBC/MIC	MIC	MBC	MBC/MIC
AZM	32	32	1	>256	>256	–	2	8	4
CFX[e]	64	>256	>4	0.5	0.5	1	0.004	0.008	2
26 series									

Table 2 (*continued*)

Compound		MIC and MBC (μg/mL)								
		S. aureus PU 32 *MRSA*[d], *inducible* MLS_B			*S. aureus* 15B196 *MRSA*, *constitutive* MLS_B			*H. influenzae* ATCC 49247		
		MIC	MBC	MBC/MIC	MIC	MBC	MBC/MIC	MIC	MBC	MBC/MIC
26a		2	8	4	128	>256	–	4	4	1
26b		1	>8	>8	128	>256	–	2	4	2
26c		2	>16	>8	>256	–	–	8	16	2
26d		2	8	4	64	>256	>4	8	16	2
26e		4	32	8	>256	–	–	16	16	1
26f		4	16	4	256	>256	–	16	32	2
26g		8	>64	>8	>256	–	–	16	32	2
26h		8	>64	>8	>256	–	–	16	64	4
26i		16	>128	>8	>256	–	–	4	8	2
26j		16	>128	>8	16	128	8	0.25	0.25	1

[a] MLS_B: macrolide-lincosamide-streptogramin B.
[b] AZM: azithromycin.
[c] TEL: telithromycin.
[d] MRSA: methicillin-resistant *Staphylococcus aureus*.
[e] CFX: ciprofloxacin.

of the linkers at C-6 and C-7 of the quinolones are important. Compound **26j**, with a cyclic piperazine ring that differentiates it from the other macrolones **26g**, **26h** and **26i**, showed improved activity with an MIC of 16 μg/mL against constitutively resistant *S. aureus* 15B196, and **26j** was 8-fold more active than azithromycin against *H. influenzae* ATCC49247.

To understand the impact of sidechain rigidity on activity, we examined the MIC values of **26a** - **26f** (Ar = quinoline) and found **26b**, with a butynyl sidechain, showed better potency in comparison with the corresponding olefin **26d** and the flexible alkane **26f**. Compound **26b** also compared favorably with the one-atom shorter homologs (**26a**, **26c** and **26e**).

2.2.2. Extended antibacterial spectrum test on *26b* and *26h*

Because **26b** and **26h** showed excellent activities in the first round of screening, we assayed their extended antibacterial profiles against more nosocomial isolates, including ESKAPE (*Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa and Enterobacter* spp.). As presented in Table 3, **26b** is much more active than **26h**, regardless of the bacterial phenotypes and genotypes, although both share the same sidechain length. However, the antibacterial spectra of the hybrids are basically similar to those of conventional macrolides.

2.2.3. Variation of aryl groups at the end of the linkers at the 6-OH of azithromycin

Taking advantage of the data in Tables 2 and 3, we fixed the sidechain as in **26b**, and further uncovered the effects of aryl groups (see Chart 1) on activities. Among the analogs of **26b** (the pyridine **26q**, the quinoline **26o** and quinolones **26p**, **26r**, **26s**, **26t**, **26u**, **26w**), **26o** with a 6-quinolyl group was the most active (Table 4). It was as active as telithromycin against susceptible *S. pneumoniae* ATCC49619, efflux-mediated *S. pneumoniae* PU 09, inducibly resistant erm-encoded *S. aureus* PU 32 and *H. influenzae* ATCC49247, but 8-fold less potent than telithromycin against constitutively resistant *S. pyogenes* 12–206 and 128-fold less active against *S. pneumoniae* 07P390. Unfortunately, azithromycin, telithromycin and **26o** are inactive against constitutively resistant *S. aureus*

Table 3
Extended antibacterial spectrum test on **26b** and **26h**.

Strain	Code	Phenotype	Compound, MIC (μg/mL)		
			LEV[a]	26b	26h
Staphylococcus epidermidis	ATCC 12228	MSSE[b]	0.12	0.25	4
Staphylococcus epidermidis	16–5	MRSE[c]	8	>64	>64
Staphylococcus aureus	ATCC 29213	MSSA[d]	0.12	1	8
Staphylococcus aureus	16–30	MRSA[e]	32	>64	>64
Escherichia coli	16–7	ESBLs (−)[f]	0.5	8	64
Escherichia coli	16–1	ESBLs (+)	0.5	8	32
Escherichia coli	ATCC 2469	NDM-1 (+)[g]	16	8	64
Klebsiella Pneumoniae	ATCC BAA-2146	NDM-1 (+)	>128	32	>64
Klebsiella Pneumoniae	16–14	ESBLs (+)	0.5	16	64
Enterococcus faecalis	ATCC 29212	VSE[h]	0.5	1	8
Enterococcus faecium	ATCC 700221	VRE[i]	32	>64	>64
Acinetobacter baumannii	ATCC 19606		0.25	>64	>64
Enterobacter cloacae	ATCC 43560		≤0.03	32	64
Enterobacter aerogenes	ATCC 13048		0.06	32	64
Shigella Castellani	ATCC 12022		≤0.03	8	16
Pseudomonas aeruginosa	ATCC 27853		2	>64	>64

[a] LEV: levofloxacin.
[b] MSSE: methicillin-susceptible *Staphylococcus epidermidis*.
[c] MRSE: methicillin-resistant *Staphylococcus epidermidis*.
[d] MSSA: methicillin-susceptible *Staphylococcus aureus*.
[e] MRSA: methicillin-resistant *Staphylococcus aureus*.
[f] ESBLs: extended spectrum β-lactamases.
[g] NDM-1: new Delhi-metallo-β-lactamase-1.
[h] VSE: vancomycin-susceptible *Enterococcus faecalis*.
[i] VRE: vancomycin-resistant *Enterococcus faecium*.

15B196. Among the hybrids of macrolides and quinolones (**26p**, **26r**, **26s**, **26t**, **26u**, **26w**), the favorable effects of the alkyl groups at N-1 of the quinolones on activities ranked as follows: Et > Me > cyclo-Pr (**26r** vs **26s** vs In addition, we also intended to design the analogs of **26j**, (i.e. **26k**, **26l**, **26m**, **26n** with a piperazine ring) due to its enhanced activities against constitutively resistant *S. aureus* 15B196 and *H. influenzae* ATCC49247 (Table 4). Similar to **26j**, **26l** had an MIC of 8 μg/mL against constitutively resistant *S. aureus* 15B196 and an MIC of 0.5 μg/mL against *H. influenzae* ATCC49247. Significantly, **26l** was just 4-fold less potent than ciprofloxacin, while the previously reported macrolone shown in Fig. 2 had 16 fold weaker activities than ciprofloxacin against constitutively resistant *S. aureus* [28]. But other hybrids of macrolides and quinolones such as **26k**, **26m** and **26n** were not endowed with activities as high as **26l**, which suggested that conversion of the 3-carboxylic acid to an amide or conversion of the cyclopropyl group at N-1 to an ethyl group compromised the potencies against constitutively resistant *S. aureus* and *H. influenzae*.

2.2.4. Variation of substitutions at the 3-, 11-/12- positions of azithromycin

The activities were basically maintained when the 2-pyridylacetyl group at the 3-OH was replaced with 3-pyridyl acetyl group (**29r** vs **31r**; **29w** vs **31w**), as shown in Table 4. Cyclic carbonylation of 11,12-OH (**26k** vs **29k**; **26r** vs **29r**; **26w** vs **29w**) increased MIC values of the resulting compounds by 2 to 16-fold in treatment of susceptible and efflux-mediated *S. pneumoniae* and *H. influenzae*, but it conferred beneficial effects against constitutively resistant *S. pyogenes* 12–206, *S. pneumoniae* 07P390, *S. aureus* 15B196 and *S. aureus* PU 32. Conversion of the 3-carboxylic acid to a 3-carboxamide in the quinolones is unfavorable for the antibacterial activities of the macrolones with uncyclized 11,12-OH (**26r** vs **26w**; **26k** vs **26j**), but the unfavorable effects could be counteracted by cyclic carbonylation at 11,12-OH (**31r** vs **31w**; **29r** vs **29w**).

2.3. Bactericidal activities of azithromycin acylides

The bacteria that were cultured in the presence of 1 to 8-fold MIC of the samples were transported to fresh culture medium without adding antibacterial compounds. After incubation for the required time, the lowest concentrations where the number of colonies was reduced to > $3Log_{10}$ were read as MBCs (minimum bactericidal concentrations). For details see Refs. [36]. The compounds are viewed as bactericidal agents if the ratio of MBC and MIC is less than 4. We found azithromycin acylides, exemplified by highly potent **26j**, **26l**, **26b** and **26o**, were generally bactericidal against *S. pneumoniae* and *H. influenzae*, but were bacteriostatic against *S. aureus* and S. *pyogenes* (Tables 2 and 4).

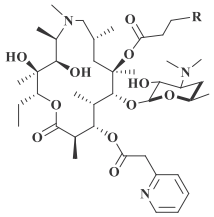
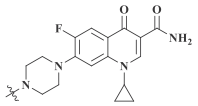
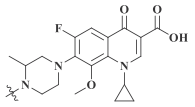
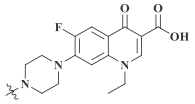
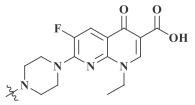
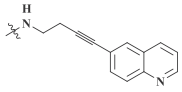
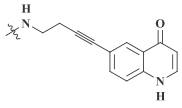
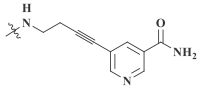
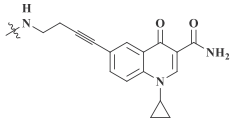
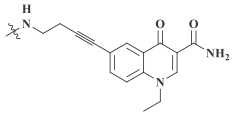
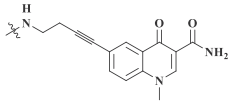
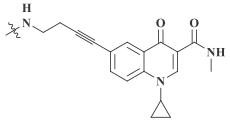
2.4. Mode of action

A molecular docking study was performed to gain insight into the binding mode of azithromycin acylides. The crystal structure of the large (50S) ribosomal subunit of *Haloarcula marismortui* G2099A mutant in complex with azithromycin was retrieved from the PDB ID: 1YHQ [37]. According to the procedure published previously [32], the cladinose was removed and a pyridyl acetyl group was attached to the oxygen at the 3-position, and then the sidechains at the 6-position of **26o** and **26l** were docked in the receptor binding site through a covalent attachment to the oxygen at the 6-position. Only residues within 30 Å of the azithromycin moiety were kept to simplify the calculation. Each sidechain was docked twenty times and the best docking solutions were chosen as the proposed molecular models, which are depicted in Figs. 3 and 4.

Due to constitutive methylation on A2058, the resistant bacteria decrease the binding affinity of macrolides, which are easily flushed away when nascent peptides pass by. To compensate for the decreased binding affinity, additional interactions, such as hydrogen bonding and π-π interactions, are expected to be formed between bacterial ribosomal RNA and drug molecules [38]. It was reported that the cladinose and 3-keto have no interaction with the bases in the NPET [13,38] but the pyridine located at the end of the sidechain at the 3-position interacted with the ribosomal base G2540 (G2505Ec), as shown in Figs. 3 and 4. Similar interaction modes were reported previously in 14-membered acylides [33,39]. In addition to this interaction, the quinoline located at the end of the sidechain at the 6-position of **26o** formed hydrogen bonds to G2540 (G2505Ec), and the quinoline ring also forms π-π stacking interaction with U2645 (U2610Ec). The quinolone located at the end of the sidechain at 6-position of **26l** formed π-π interactions with U2621 (U2586Ec). These proposed additional interactions are expected to increase the affinities of **26o** and **26l** to the ribosomes of resistant bacteria, which in turn should improve their activities against constitutively resistant *S. pneumoniae* and *S. pyogenes* compared to the parent drug azithromycin. However, the MICs of **26o** and **26l** were still fairly high against some of the isolates compared to telithromycin and ciprofloxacin.

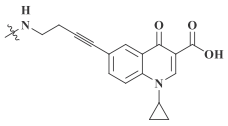
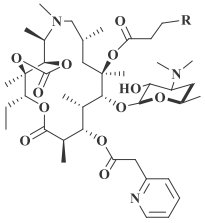
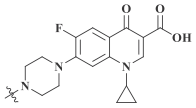
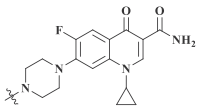
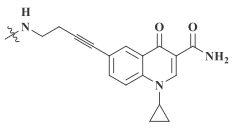
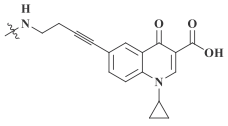
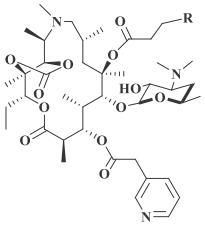
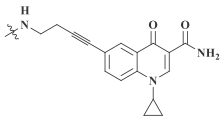
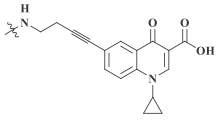
Although both of the tested *S. aureus* strains are MRSA (Table 1), ciprofloxacin is potent against constitutively MLS_B-resistant *S. aureus* 15B196 but inactive against inducibly MLS_B-resistant *S. aureus* PU 32. The resistance of PU 32 to ciprofloxacin is probably due to the presence of mutations of the ciprofloxacin target. Coincidently, quinolone-containing **26l**, contrary to quinoline-containing **26o**, had a significantly lower MIC against the quinolone-susceptible *S. aureus* 15B196 but a higher MIC against the quinolone-resistant *S. aureus* PU 32 (Table 4), which is distinct from the nature of the known macrolones [26]. Moreover, the same trend was observed in quinolone-containing **26j** vs quinoline-containing **26b** (Table 2). To clearly describe this trend, we compared antibacterial activity (in the units of μM) of quinoline-

Table 4
Antibacterial activity of **26k - 26u, 26w, 29j, 29k, 29r, 29w, 31r** and **31w** against erythromycin-susceptible and -resistant pathogens.

Compound		MIC and MBC (μg/mL)								
		S. pneumoniae ATCC 49619			*S. pneumoniae* PU 09 *efflux*			*S. pneumoniae* 07P390 *constitutive* MLS_B[a]		
		MIC	MBC	MBC/MIC	MIC	MBC	MBC/MIC	MIC	MBC	MBC/MIC
CAM[b]		–	–	–	8	–	–	>256	–	–
TEL[c]		0.03	–	–	0.5	–	–	0.25	–	–
CFX[d]		2	–	–	2	–	–	2	–	–
26 series										
26k		0.25	0.5	2	4	4	1	128	128	1
26l		0.5	1	2	2	8	4	8	8	1
26m		0.5	1	2	4	8	2	64	128	2
26n		0.5	1	2	2	4	2	64	128	2
26o		0.03	0.03	1	0.5	1	2	32	64	2
26p		0.25	0.25	1	2	4	2	256	>256	–
26q		0.25	0.25	1	2	4	2	128	>256	–
26r		0.5	0.5	1	4	4	1	256	>256	–
26s		0.12	0.5	4	2	2	1	256	>256	–
26t		0.25	0.5	2	2	4	2	256	>256	–
26u		0.5	1	2	4	8	2	256	256	–

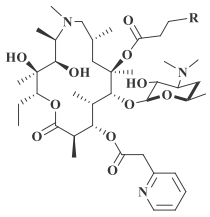
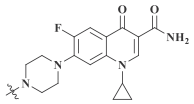
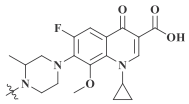
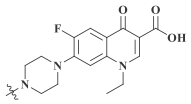
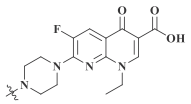
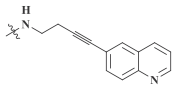
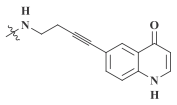
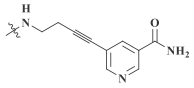
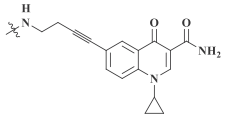
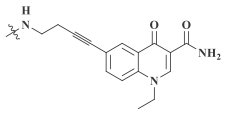
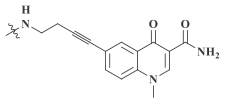
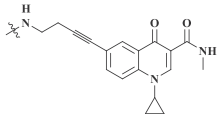
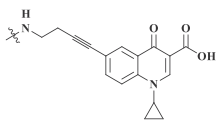
(*continued on next page*)

Table 4 (*continued*)

Compound		MIC and MBC (μg/mL)								
		S. pneumoniae ATCC 49619			*S. pneumoniae* PU 09 *efflux*			*S. pneumoniae* 07P390 *constitutive* MLS_B[a]		
		MIC	MBC	MBC/MIC	MIC	MBC	MBC/MIC	MIC	MBC	MBC/MIC
26w		0.25	0.5	2	2	4	2	64	256	4
29 series										
29j		4	16	4	4	16	4	>256	>256	–
29k		4	8	2	8	16	2	32	32	1
29r		0.5	1	2	4	8	2	64	128	2
29w		1	1	1	4	8	2	64	64	1
31 series										
31r		1	2	2	4	8	2	64	128	2
31w		2	8	4	4	16	4	64	128	2

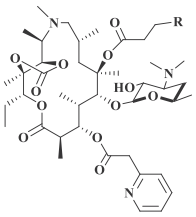
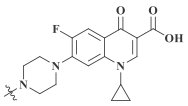
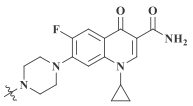
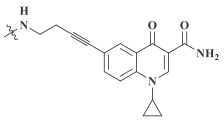
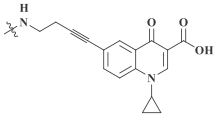
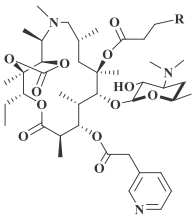
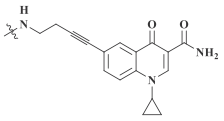
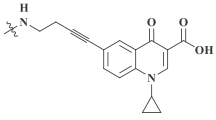
Compound	MIC and MBC (μg/mL)											
	S. aureus PU 32 *MRSA*[e], *inducible* MLS_B			*S. aureus* 15B196 *MRSA*, *constitutive* MLS_B			*H. influenzae* ATCC 49247			*S. pyogenes* 12−206 *constitutive* MLS_B		
	MIC	MBC	MBC/MIC	MIC	MBC	MBC/MIC	MIC	MBC	MBC/MIC	MIC	MBC	MBC/MIC
CAM	64	–	–	>256	–	–	16	–	–	>256	–	–
TEL	2	–	–	>256	–	–	4	–	–	0.25	–	–
CFX	256	–	–	2	–	–	0.004	–	–	0.5	–	–

Table 4 (*continued*)

Compound		MIC and MBC (μg/mL)											
		S. aureus PU 32 *MRSA*[e], *inducible* MLS_B			*S. aureus* 15B196 *MRSA*, *constitutive* MLS_B			*H. influenzae* ATCC 49247			*S. pyogenes* 12—206 *constitutive* MLS_B		
		MIC	MBC	MBC/MIC	MIC	MBC	MBC/MIC	MIC	MBC	MBC/MIC	MIC	MBC	MBC/MIC
26 series													
26k		128	>256	–	>256	>256	–	64	128	2	16	128	8
26l		128	>256	–	8	8	1	0.5	0.5	1	8	>64	>8
26m		128	>256	–	128	128	–	4	4	1	16	128	8
26n		32	>256	>8	256	>256	–	8	8	1	16	>128	>8
26o		4	>32	>8	>256	>256	–	4	8	2	2	>16	>8
26p		16	>128	>8	>256	>256	–	64	128	2	2	>16	>8
26q		16	>128	>8	>256	>256	–	64	128	2	4	>32	>8
26r		128	>256	–	>256	>256	–	64	64	1	32	128	4
26s		32	>256	>8	>256	>256	–	16	16	1	8	>64	>8
26t		16	>128	>8	>256	>256	–	32	64	2	8	>64	>8
26u		>256	>256	–	>256	>256	–	64	128	2	32	128	4
26w		128	>256	–	>256	>256	–	32	64	2	16	128	8

(*continued on next page*)

Table 4 (*continued*)

Compound		MIC and MBC (μg/mL)											
		S. aureus PU 32 *MRSA*[e], *inducible* MLS_B			*S. aureus* 15B196 *MRSA*, *constitutive* MLS_B			*H. influenzae* ATCC 49247			*S. pyogenes* 12−206 *constitutive* MLS_B		
		MIC	MBC	MBC/MIC	MIC	MBC	MBC/MIC	MIC	MBC	MBC/MIC	MIC	MBC	MBC/MIC
29 series													
29j		>256	>256	–	>256	>256	–	8	16	2	64	>256	>8
29k		32	>256	>8	32	>256	>8	>256	>256	–	16	16	1
29r		64	>256	>4	128	>256	–	64	128	2	16	64	4
29w		64	>256	>4	>256	>256	–	64	128	2	32	64	2
31 series													
31r		64	>256	>4	>256	>256	–	64	64	1	32	64	2
31w		128	>256	–	256	>256	–	32	64	2	64	64	1

[a] MLS_B: macrolide-lincosamide-streptogramin B.
[b] CAM: clarithromycin.
[c] TEL: telithromycin.
[d] CFX: ciprofloxacin.
[e] MRSA: methicillin-resistant *Staphylococcus aureus*.

and quinolone-containing compounds vs ciprofloxacin (Table 5). Quinolone-containing azithromycin derivatives (**26j** and **26l**) showed higher activity than quinoline-containing azithromycin derivatives (**26b** and **26o**) against ciprofloxacin-susceptible strains. However, quinolone-containing azithromycin derivatives (**26j** and **26l**) showed weaker activity than quinoline-containing azithromycin derivatives (**26b** and **26o**) against ciprofloxacin-resistant strains. Among them, **26l** showed the minimum variation against the strains of *S. aureus* listed in Table 5. Further SAR studies also revealed that alteration of the fluoroquinolones' pharmacophore moiety, for example, conversion of either the featuring 3-carboxylic acid to an amide (**26j** vs **26k**) or the favorable cyclopropyl group at N-1 to an ethyl group (**26j**, **26l** vs **26m**, **26n**), significantly decreased the potencies against constitutively MLS_B-resistant but quinolone-susceptible *S. aureus*. Therefore, the whole cell-based SAR strongly suggest that the hybrids of quinolone and macrolide reported herein may possess an additional quinolone-associated antibacterial mechanism, which is often referred to as topoisomerase poisoning. In other words, the formation of a topoisomerase-drug-DNA ternary complex would lead to inhibition of DNA replication,

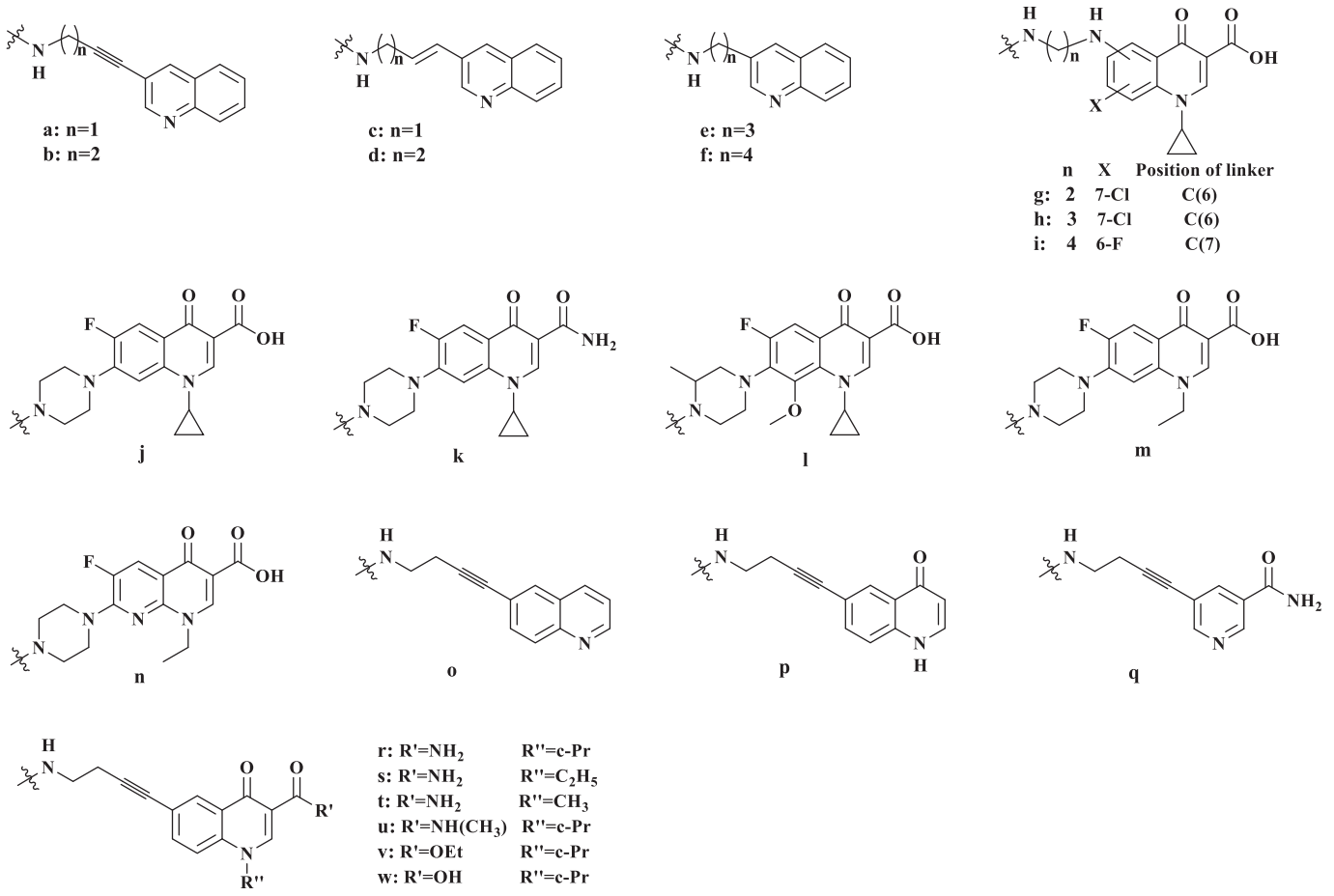


Chart 1. Substructures of the amines for the target compounds **26**, **29** and **31**.

generation of double-strand breaks and subsequent cell death [40].

For this reason, we decided to examine whether **26j** and **26l** are quinolone-like DNA gyrase-targeting agents (Fig. 5). The DNA negative supercoiling assay with *E. coli* gyrase indicated IC_{50} values of macrolones **26j** and **26l**, in contrast to 0.44 μM of ciprofloxacin [41], are 44.1 ± 3.2 μM and 9.6 ± 0.2 μM, respectively (Table 6). Gyrase poisoning involves formation of a stable DNA-gyrase-drug complex containing a double-stranded DNA break. This could be proven by a DNA cleavage gel assay. The assay showed that **26l** generated linear DNA at lower concentration than **26j**, as illustrated in Fig. 5. Thus, **26l** appeared to be more effective in poisoning DNA gyrase than **26j** (Table 6), which is consistent with the order of their bactericidal effects (Tables 2 and 4). In any case, these results demonstrated that both **26j** and **26l** could in fact target and poison DNA gyrase. Since the hybrids are weaker inhibitors than ciprofloxacin, the gyrase poisoning probably contribute only modestly to the overall activity of the hybrids. The MIC data of ciprofloxacin, **26j** and **26l** against *E coli* whole cells (*E coli* BW25113, JW5503-1 and JW5503-KanS) are consistent with IC_{50} values and poisoning effects against *E coli* DNA gyrase (Table 6). Molecules containing typical acid (carboxylic) and good base (pyridine, piperazine, secondary amine of the linker etc.) together within the scaffold of azithromycin can vary in the form of zwitterions in solution. According to the logP and pKa values calculated by using ChemDraw, compounds **26j** and **26l** probably existed in the cation form at pH = 7.4 and would be more hydrophilic because of their lower logD values than parent compounds azithromycin and ciprofloxacin (Table 6).

Despite the fact that a number of hybrids of macrolides and quinolones were documented [9,15–23,34], a systematic study clearly indicated none of the hybrids possess the quinolones' mode of action [22,26]. Therefore, the macrolones presented here are different from previous macrolones and possess a novel mode of action.

3. Conclusion

We designed and synthesized twenty-eight new quinolone/quinoline-containing azithromycin acylides, and most of them showed superior activities over parent azithromycin against clinically isolated pathogens carrying a variety of resistant genotypes and phenotypes.

The whole cell-based SAR studies centered on the 6-position of azithromycin in addition to the 3- and 11-/12-positions. Molecular docking suggested the aryl groups appended on the 3- and 6-positions have interactions with bacterial ribosomal RNA bases. Introduction of quinolines through an optimized rigid linker attached to 6-OH azithromycin acylides resulted in compounds, such as **26o**, that exhibited comparable activities to the marketed drug telithromycin against susceptible *S. pneumoniae*, efflux-mediated *S. pneumoniae*, inducibly resistant erm-encoded *S. aureus* and *H. influenzae*. Meanwhile, **26o** showed reduced MICs over the parent drug azithromycin against constitutively resistant *S. pneumoniae* and *S. pyogenes* but not against constitutively resistant *S. aureus*.

Thus far, hybrids of macrolides and quinolones did not inhibit growth of pathogens in a way similar to fluoroquinolones. However,

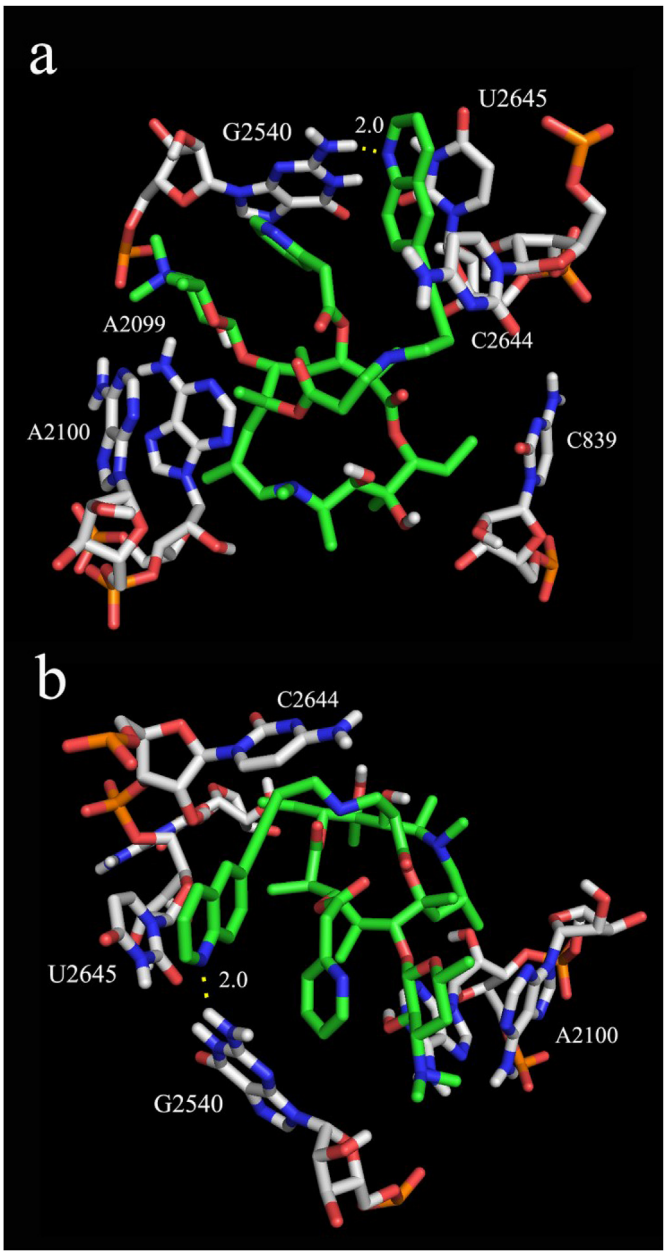


Fig. 3. Molecular model of compound **26o** (green) based on the crystal structure of the complex of azithromycin and G2099A mutant *Haloarcula marismortui* (PDB ID: 1YHQ). a) The pyridine located at the end of the sidechain at the 3-position interacts with G2540 (G2505Ec). b) The quinoline located at the end of the sidechain at the 6-position hydrogen bonds to G2540 (G2505Ec), and the quinoline ring also forms π-π stacking interaction with U2645 (U2610Ec). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

we found that macrolones with a spacer from the 6-position of azithromycin to the 7-position of quinolones could possess a quinolone-associated mode of action, which was corroborated by the observation of their high MIC values against quinolone-resistant strains. The DNA supercoiling assay and the DNA cleavage assay revealed that **26j** and **26l** could poison *E. coli* DNA gyrase, although their IC_{50} values were higher than that of ciprofloxacin. We assume that dual modes of action, namely inhibition of protein synthesis plus poisoning of DNA gyrase and/or topoisomerase IV, may contribute to the enhanced activities of **26j** and **26l** against *S. aureus* that is constitutively resistant to all of the marketed macrolides (MICs of telithromycin, azithromycin and clarithromycin, >256 μg/mL). Considering the fact that the molecular weight of **26l** is 3-fold more than that of ciprofloxacin, the activity of **26l** actually reached that of an equimolar concentration of ciprofloxacin against constitutively resistant *S. aureus* (MICs: 7 μmol/L of **26l** vs 6 μmol/L of ciprofloxacin). This novel mode of action shed light on how to rationally design novel macrolides capable of addressing constitutively resistant *S. aureus* in the future.

4. Experimental section

4.1. DNA supercoiling assay

E. coli DNA gyrase was purchased from New England Biolabs (Ipswich, MA, USA). The activity of DNA gyrase was measured under the reaction conditions described below and one unit was defined as the amount of DNA gyrase required to completely supercoil 0.3 μg of the relaxed DNA at 37 °C in 15 min. The relaxed DNA, used as the substrate in the supercoiling assay, was prepared by incubating negatively supercoiled pBR322 DNA with *E. coli* topoisomerase I.

The supercoiling and DNA cleavage assays were performed as described previously [41,42]. Briefly, supercoiling reaction mixtures (20 μL) containing 50 mM Tris-HCl (pH 8.0 at 23 °C), 10 mM $MgCl_2$, 100 mM potassium glutamate, 10 mM dithiothreitol, 50 μg/mL bovine serum albumin, 1 mM ATP, 0.3 μg of the relaxed DNA, 1 unit of *E. coli* gyrase, and the various concentrations of either **26j** or **26l** were incubated at 37 °C for 15 min. Ciprofloxacin was used as a control gyrase inhibitor. Reactions were terminated by adding EDTA to 25 mM and further incubating at 37 °C for 5 min. The DNA products were analyzed by electrophoresis through vertical 1.2% agarose gels (14 x 10 × 0.3 cm) at 2 V/cm for 15 h in a running buffer of 50 mM Tris-HCl (pH 7.9 at 23 °C), 40 mM sodium acetate, and 1 mM EDTA (TAE buffer). Gels were stained with 0.5 μg/mL ethidium bromide, and then photographed and quantified using a MyECL Imager [Thermo Fisher Scientific (Waltham, MA, USA)].

4.2. DNA cleavage assay

The DNA negative supercoiling and DNA cleavage assays were performed as described previously [41,42]. Briefly, DNA cleavage reaction mixtures (20 μL) containing 50 mM Tris-HCl (pH 8.0 at 23 °C), 10 mM $MgCl_2$, 10 mM dithiothreitol, 50 μg/mL bovine serum albumin, 1 mM ATP, 5 μg/mL tRNA, 0.3 μg of the supercoiled pBR322 DNA, 10 units of *E. coli* gyrase, and the various concentrations of either **26j** or **26l** were incubated at 37 °C for 15 min. SDS was added to a concentration of 1%, and the reaction mixtures were further incubated at 37 °C for 5 min. EDTA and proteinase K were then added to 25 mM and 100 μg/mL, respectively, and incubation was continued for an additional 30 min at 37 °C. The DNA products were purified by extraction with phenol/chloroform/isoamyl alcohol (25:24:1, v/v) and then analyzed by electrophoresis through vertical 1.2% agarose gels at 2 V/cm for 15 h in TAE buffer that contained 0.5 μg/mL ethidium bromide. After destaining in water, gels were photographed and quantified using a MyECL Imager.

4.3. Synthetic procedures

All reagents and solvents were purchased from commercially available sources and used without further purification. The progress of reactions was monitored by TLC (thin-layer chromatography) through pre-coated silica gel HSGF254 plates (0.2 mm). Visualization was performed by iodine staining and UV light. The purification of compounds was carried out by column chromatography with silica gel (100–200 or 200–300 mesh) with various

Table 5
The ratio values of MIC (μmol/L) of quinoline-containing (**26b** and **26o**) and quinolone-containing (**26j** and **26l**) series vs ciprofloxacin, which was normalized to 1.

Compound	The fold numbers of antibacterial activity relative to ciprofloxacin		
	S. aureus PU 32 *MRSA*[a], *inducible* MLS_B[b] Ciprofloxacin-resistant	*S. aureus* 15B196 *MRSA, constitutive* MLS_B Ciprofloxacin-susceptible	*H. influenzae* ATCC 49247 Azithromycin-susceptible Ciprofloxacin-susceptible
Azithromycin	0.22	>226	221
Clarithromycin	0.11	>57	1768
Telithromycin	0.0032	>52	407
26b	0.0054	88	172
26o	0.0054	>44	344
26j	0.076	9.7	19
26l	0.14	1.2	36

[a] MRSA: methicillin-resistant *Staphylococcus aureus*.
[b] MLS_B: macrolide-lincosamide-streptogramin B.

eluent systems. Infrared spectra were recorded on KBr pellets using Nicolet iS5 FT-IR spectrometer. ^{1}H, ^{13}C NMR and COSY spectra were recorded on Bruker Ascend 400 MHz, ARX 500 MHz and Ascend 700 MHz spectrometers using $CDCl_3$, DMSO-d_6 or CD_3OD as solvents, and TMS (tetramethylsilane) as internal standard. The HRMS (high resolution mass spectra) were recorded on Agilent Q-TOF 6520 LC-MS. The melting points of the target compounds were measured with a SGW X-4A melting point apparatus and are uncorrected. The high purities of the target compounds were confirmed by high-performance TLC analysis plus verification by NMR spectrometry (see Supporting Information).

*4.3.1. 2-(Prop-2-yn-1-yl)isoindolin-1,3-dione **(2)***

Compound **1** (1.71 g, 11.6 mmol) was suspended in EtOH (20 mL) and KOH (0.650 g, 11.6 mmol) was added. The mixture was stirred at room temperature for 2 h, and evaporated to remove EtOH. The residue was dissolved in anhydrous DMF (15 mL) and propargyl bromide (1.10 mL, 12.8 mmol) was added. The mixture was stirred at reflux for 72 h. The resulting solution was cooled, diluted with EtOAc and partitioned with saturated $NaHCO_3$. The organic phase was washed with water and brine. The solvent was removed by rotary evaporation. The residue was treated with MeOH, and the suspension was filtered to provide compound **2** (1.25 g, 6.75 mmol, 58.1%) as a white solid.

*4.3.2. 2-(But-3-yn-1-yl)isoindolin-1,3-dione **(3)***

To a suspension of compound **1** (5.00 g, 34.0 mmol) in toluene (60 mL), but-3-yn-1-ol (2.83 mL, 37.4 mmol), PPh_3 (9.81 g, 37.4 mmol), DIAD (8.03 mL, 40.8 mmol) were added. The reaction mixture was stirred at 0 °C for 10 min, and then at room temperature for 1 h. After addition of MeOH (20 mL), the mixture was stirred for another 30 min. The resulting suspension was filtered and washed with MeOH. The filtrate was concentrated via rotary evaporation and the residue was treated with MeOH. The precipitate was collected by filtration to afford compound **3** (5.85 g, 86.4%) as a white solid.

*4.3.3. 2-Allylisoindolin-1,3-dione **(4)***

Compound **4** (1.81 g, 9.67 mmol, 83.7%) was prepared starting from compound **1** (1.70 g, 11.6 mmol) and allyl bromide (1.10 mL, 12.7 mmol) according to the procedure used to prepare compound **2**.

*4.3.4. 2-(But-3-en-1-yl)isoindolin-1,3-dione **(5)***

Compound **5** (1.90 g, 9.44 mmol, 95.8%) was prepared starting from compound **1** (2.20 g, 14.9 mmol) and 4-bromo-1-butene (1.00 mL, 9.85 mmol) according to the procedure used to prepare compound **2**, and purified by silica gel column chromatography (petroleum ether/EtOAc = 12:1). HRMS (ESI) $(M + H)^+$ *m/z* 202.0869, calcd for $C_{12}H_{12}NO_2$ 202.0863. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.83 (d, J = 3.0 Hz, 2 H, H−Ar), 7.71 (d, J = 3.3 Hz, 2 H, H−Ar), 5.85−5.74 (m, 1 H, -C**H**=CH_2), 5.10−4.99 (m, 2 H, -CH=C**H$_2$**), 3.77 (td, J_1 = 7.1 Hz, J_2 = 2.0 Hz, 2 H, -N-C**H$_2$**-), 2.45 (q, J = 7.3 Hz, 2 H, -N-CH_2C**H$_2$**-).

*4.3.5. General procedures for the preparation of intermediates **6a**, **7a**, **7b** and **7d-7i***

To a solution of compound **2** or **3** (1.2 eq) in acetonitrile in a high pressure vessel, CuI (0.1 eq), bis(triphenylphosphine)palladium(II) dichloride (0.05 eq), the corresponding halogenated aryl (1 eq) and triethylamine (1.5 eq) were added. The reaction mixture was stirred at 80 °C for 4 h under Ar. Water was added and the resulting mixture was extracted with EtOAc. The organic layer was washed with water and brine, and concentrated by evaporation. The residue was dissolved with EtOAc and treated with petroleum ether. The product was precipitated and filtered to yield the compounds **6a**, **7a**, **7b** and **7d-7i**.

*4.3.5.1. 2-[3-(Quinolin-3-yl)prop-2-yn-1-yl]isoindolin-1,3-dione **(6a)**.* Compound **6a** (0.660 g, 2.11 mmol, 62.2%) was prepared starting from compound **2** (0.630 g, 3.39 mmol) and 3-bromoquinoline (0.920 mL, 6.78 mmol) according to the general procedure.

*4.3.5.2. 2-[4-(Quinolin-3-yl)but-3-yn-1-yl]isoindolin-1,3-dione **(7a)**.* Compound **7a** (0.500 g, 1.53 mmol, 56.8%) was prepared starting from compound **3** (0.500 g, 2.70 mmol) and 3-bromoquinoline (0.730 mL, 5.40 mmol) according to the general procedure.

*4.3.5.3. 2-[4-(Quinolin-6-yl)but-3-yn-1-yl]isoindolin-1,3-dione **(7b)**.* Compound **7b** (0.570 g, 1.75 mmol, 78.8%) was prepared starting from compound **3** (0.440 g, 2.22 mmol) and 6-bromoquinoline (0.300 mL, 2.22 mmol) according to the general procedure.

*4.3.5.4. 5-(4-(1,3-Dioxoisoindolin-2-yl)but-1-yn-1-yl)nicotinamide **(7d)**.* Compound **7d** (0.481 g, 1.51 mmol, 75.5%) was prepared starting from compound **3** (0.440 g, 2.20 mmol) and 5-bromonicotinamide (0.400 g, 2.00 mmol) according to the general procedure.

*4.3.5.5. 1-Cyclopropyl-6-(4-(1,3-dioxoisoindolin-2-yl)but-1-yn-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxamide **(7e)**.* Compound **7e** (0.540 g, 1.27 mmol, 63.5%) was prepared starting from compound **3** (0.600 g, 3.00 mmol) and 1-cyclopropyl-6-iodo-4-oxo-1,4-dihydroquinoline-3-carboxamide (0.710 g, 2.00 mmol) according to the general procedure.

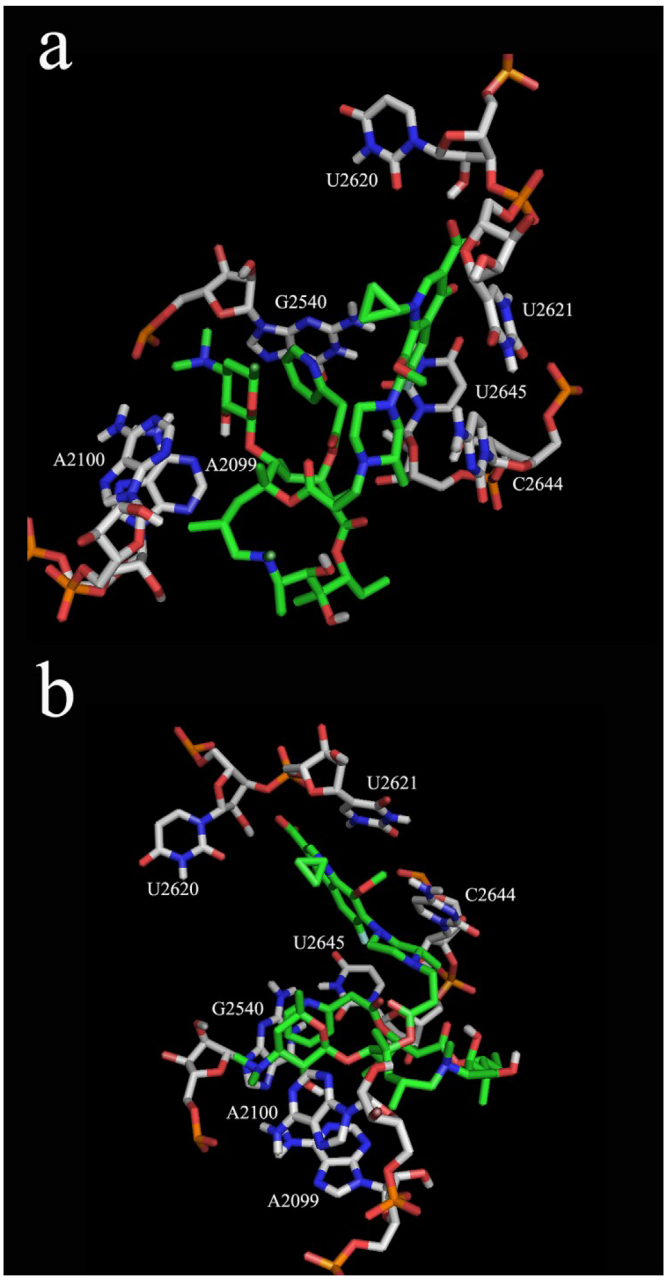


Fig. 4. Molecular model of compound **26l** (green) based on the crystal structure of the complex of azithromycin and G2099A mutant *Haloarcula marismortui* (PDB ID: 1YHQ). a) The pyridine located at the end of the sidechain at the 3-position interacts with G2540 (G2505Ec). b) The quinolone located at the end of the sidechain at the 6-position π-π interacts with U2621 (U2586Ec).

*4.3.5.6. 6-(4-(1,3-Dioxoisoindolin-2-yl)but-1-yn-1-yl)-1-ethyl-4-oxo-1,4-dihydroquinoline-3-carboxamide **(7f)**.* Compound **7f** (0.460 g, 1.11 mmol, 76.0%) was prepared starting from compound **3** (0.350 g, 1.75 mmol) and 1-ethyl-6-iodo-4-oxo-1,4-dihydroquinoline-3-carboxamide (0.500 g, 1.46 mmol) according to the general procedure.

*4.3.5.7. 6-(4-(1,3-Dioxoisoindolin-2-yl)but-1-yn-1-yl)-1-methyl-4-oxo-1,4-dihydroquinoline-3-carboxamide **(7g)**.* Compound **7g** (0.450 g, 1.21 mmol, 79.6%) was prepared starting from compound **3** (0.360 g, 1.82 mmol) and 6-iodo-1-methyl-4-oxo-1,4-dihydroquinoline-3-carboxamide (0.500 g, 1.52 mmol) according to the general procedure.

*4.3.5.8. 1-Cyclopropyl-6-(4-(1,3-dioxoisoindolin-2-yl)but-1-yn-1-yl)-N-methyl-4-oxo-1,4-dihydroquinoline-3-carboxamide **(7h)**.* Compound **7h** (0.370 g, 0.840 mmol, 86.6%) was prepared starting from compound **3** (0.190 g, 0.970 mmol) and 1-cyclopropyl-6-iodo-*N*-methyl-4-oxo-1,4-dihydroquinoline-3-carboxamide (0.360 g, 0.970 mmol) according to the general procedure.

*4.3.5.9. Ethyl 1-cyclopropyl-6-(4-(1,3-dioxoisoindolin-2-yl)but-1-yn-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylate **(7i)**.* Compound **7i** (0.740 g, 1.63 mmol, 81.5%) was prepared starting from compound **3** (0.480 g, 2.40 mmol) and ethyl 1-cyclopropyl-6-iodo-4-oxo-1,4-dihydroquinoline-3-carboxylate (0.770 g, 2.00 mmol) according to the general procedure.

*4.3.6. General procedures for the preparation of intermediates **7c** and **7j***

To a mixture of acetonitrile/trimethylamine (1: 1), CuI (0.1 eq) and corresponding halogenated aryl (1 eq) were added. The mixture was stirred 20 min at room temperature. Compound **3** (1.2 eq) and bis(triphenylphosphine)palladium(II) dichloride (0.05 eq) were added to the reaction mixture. The reaction mixture was stirred at 50 °C for 24 h under Ar. The reaction was quenched with water. The resulting mixture was extracted with EtOAc. The organic layer was washed with water and brine, and evaporated to remove the solvent. The residue was dissolved with EtOAc and treated with petroleum ether. The product was precipitated and filtered to provide compounds **7c** and **7j**.

*4.3.6.1. 2-(4-(4-Oxo-1,4-dihydroquinolin-6-yl)but-3-yn-1-yl)isoindoline-1,3-dione **(7c)**.* Compound **7c** (0.510 g, 1.50 mmol, 90.7%) was prepared starting from compound **3** (0.400 g, 1.99 mmol) and 6-iodoquinolin-4-ol (0.450 g, 1.66 mmol) according to the general procedure.

*4.3.6.2. 1-Cyclopropyl-6-(4-(1,3-dioxoisoindolin-2-yl)but-1-yn-1-yl)-4-oxo-1,4-dihydroquinoline-3-carboxylic acid **(7j)**.* Compound **7j** (0.290 g, 0.680 mmol, 48.2%) was prepared starting from compound **3** (0.340 g, 1.70 mmol) and 1-cyclopropyl-6-iodo-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (0.500 g, 1.41 mmol) according to the general procedure.

*4.3.7. General procedures for the preparation of intermediates **8a** and **9a***

To a mixture of compound **4** or **5** (1 eq) and 3-bromoquinoline (2 eq) in acetonitrile, $Pd(OAc)_2$ (0.3 eq), tri-*o*-tolylphosphine (0.6 eq) and triethylamine (3 eq) were added. The vessel was recharged with Ar. The mixture was stirred at 60 °C for 1 h, and then at 90 °C for another 24 h. Water was added and the mixture was extracted with EtOAc. The organic layer was washed with water and brine, and evaporated to remove the solvent. The resulting mixture was dissolved with EtOAc, treated with petroleum ether, and filtered to collect the products **8a** and **9a**.

*4.3.7.1. 2-(3-(Quinolin-3-yl)allyl)isoindoline-1,3-dione **(8a)**.* Compound **8a** (1.05 g, 3.34 mmol, 58.7%) was prepared starting from compound **4** (1.06 g, 5.69 mmol) according to the general procedure. HRMS (ESI) $(M + H)^+$ *m/z* 315.1123, calcd for $C_{20}H_{15}N_2O_2$ 315.1128.

*4.3.7.2. 2-[4-(Quinolin-3-yl)but-3-en-1-yl]isoindolin-1,3-dione **(9a)**.* Compound **9a** (0.370 g, 1.13 mmol, 45.6%) was prepared starting from compound **5** (0.500 g, 2.48 mmol) according to the general

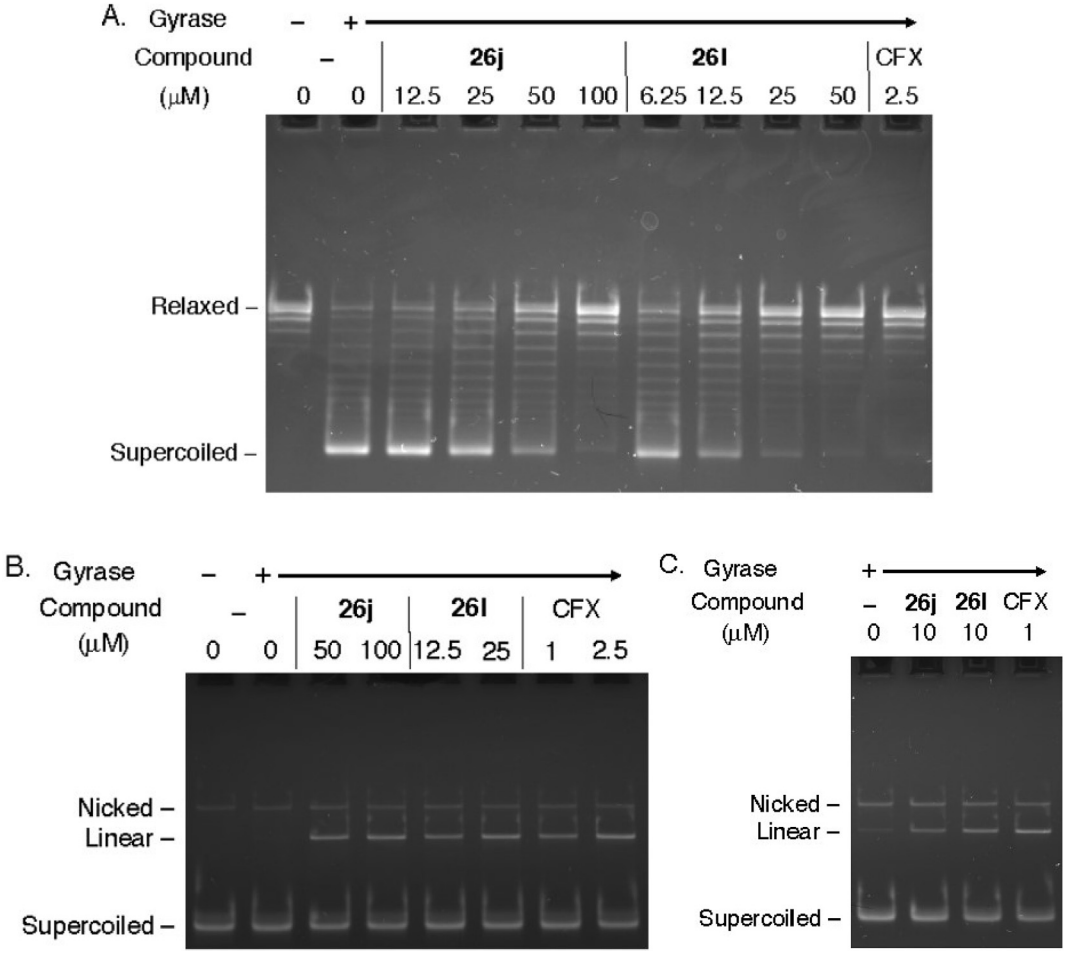


Fig. 5. Activities of **26j** and **26l** against *E. coli* DNA gyrase. Examples of the results of the DNA supercoiling assay (**A**) and DNA cleavage assays (**B** and **C**) are shown here. Ciprofloxacin (CFX) was used as a positive control.

Table 6
Activities of **26j** and **26l** against *E. coli* DNA gyrase and *E. coli* whole cells.

Compound	Catalytic inhibition[a]	Poisoning effect		MIC (μg/mL)			clogD (pH = 7.4)[e]
		Maximum[b]	Relative[c]	*E. coli* BW25113	*E. coli* JW5503-1[d]	*E. coli* JW5503-KanS[d]	
Ciprofloxacin	0.44 ± 0.008[f]	1.2 ± 0.1	100	8	≤0.064	≤0.064	−1.7
26j	44.1 ± 3.2	69.5 ± 6.8	28	4	1	1	−6.3
26l	9.6 ± 0.2	18.2 ± 2.6	71	2	0.5	0.5	−5.8
azithromycin	–	–	–	>16	4	2	1.4

[a] The IC_{50} values (the 50% inhibitory concentration, μM) against DNA gyrase were determined in the supercoiling assay. An example of the result of the supercoiling assay are shown in Fig. 5A.
[b] The concentrations (μM) required to generate the maximum level of linear DNA were determined in the DNA cleavage assay. An example of the result of the DNA cleavage assay are shown in Fig. 5B.
[c] The level of linear DNA generated by 10 μM **26j** or **26l** was normalized to the level of linear DNA generated by 1 μM ciprofloxacin (%). An example of the result of the DNA cleavage assay are shown in Fig. 5C.
[d] *E. coli* BW25113 ΔtolC (depletion of tolC efflux genes).
[e] logD ≈ logP + (pH − pKa), where logP and pKa were calculated by using ChemDraw (Version 16.0.1.4).
[f] Data from references [41].

procedure.

4.3.8. General Gabriel reaction procedures for the preparation of amines *10a*, *11a-11i*, *12a*, *13a*, *14a* and *15a*

Compound **8a** (or **9a**) (1 eq) was dissolved in MeOH. 10% Pd/C, HCOOH (16 eq) and ammonium formate (8 eq) were added. The mixture was stirred at room temperature overnight. The insoluble material was filtered off, and the filtrate was evaporated to remove the solvent. The residue was dissolved with CH_2Cl_2, washed with water and brine, concentrated to afford 2-(3-(quinolin-3-yl)propyl) isoindoline-1,3-dione (or 2-(4-(quinolin-3-yl)butyl)isoindoline-1,3-dione). The hydrogenolysis product was used directly for the Gabriel reaction to yield primary amines without further purification.

The corresponding sidechains capped with phthalimide **6a** (or **7a** - **7j**, **8a**, **9a,** hydrogenation products of **8a** and **9a**) (1 eq) was

dissolved in EtOH and 80% hydrazine hydrate (2 eq) was added. The mixture was stirred at reflux for 2 h. The resulting mixture was cooled to room temperature, and an insoluble solid was observed. The solid was filtered off and washed with EtOH. The filtrate was evaporated to remove the solvent. The residue was dissolved with CH_2Cl_2, and washed with water and brine. The organic layer was dried over anhydrous $MgSO_4$, filtered and concentrated by evaporation to afford compounds **10a**, **11a-11i**, **12a**, **13a**, **14a**, **15a**.

*4.3.8.1. 3-(Quinolin-3-yl)prop-2-yn-1-amine **(10a)**.* Compound **10a** was prepared starting from compound **6a** according to the general procedure, and used directly for the next step without further purification. IR (KBr) ν: 3323, 2870, 2643, 2078, 1492, 1386, 913, 788, 770 cm^{-1}. HRMS (ESI) $(M + H)^+$ *m/z* 183.0904, calcd for $C_{12}H_{11}N_2$ 183.0917. ^1H NMR (DMSO-d_6, 400 MHz) δ: 9.02 (d, J = 2.2 Hz, 1 H, 2′-quinolyl), 8.71 (d, J = 2.2 Hz, 1 H, 4′-quinolyl), 8.18–8.11 (m, 2 H, 5′-quinolyl, 8′-quinolyl), 7.92 (ddd, J_1 = 8.6 Hz, J_2 = 6.9 Hz, J_3 = 1.5 Hz, 1 H, 6′-quinolyl), 7.74–7.72 (m, 1 H, 7′-quinolyl), 6.43 (s, 2 H, -NH_2), 4.09 (q, J = 5.6 Hz, 2 H, -CH_2-). ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 151.0, 145.1, 141.1, 132.2, 128.9, 128.7, 127.8, 127.4, 116.0, 87.1, 82.9, 29.3.

*4.3.8.2. 4-(Quinolin-3-yl)but-3-yn-1-amine **(11a)**.* Compound **11a** was prepared starting from compound **7a** according to the general procedure, and used directly for the next step without further purification. HRMS (ESI) $(M + H)^+$ *m/z* 197.1075, calcd for $C_{13}H_{13}N_2$ 197.1073. ^1H NMR (DMSO-d_6, 700 MHz) δ: 9.19 (d, J = 2.0 Hz, 1 H, 2′-quinolyl), 8.87 (s, 1 H, 4′-quinolyl), 8.67–8.58 (m, 2 H, -NH_2), 8.20 (d, J = 8.5 Hz, 1 H, 5′-quinolyl), 8.11 (d, J = 8.0 Hz, 1 H, 8′-quinolyl), 7.93 (t, J = 7.2 Hz, 1 H, 6′-quinolyl), 7.77 (t, J = 7.5 Hz, 1 H, 7′-quinolyl), 3.06 (q, J = 6.7 Hz, 2 H, -CH_2C≡C-), 2.96 (t, J = 7.0 Hz, 2 H, NH_2–C**H₂**-). ^{13}C NMR (DMSO-d_6, 175 MHz) δ: 150.4, 142.6, 133.1, 132.7, 129.1, 128.8, 127.8, 125.9, 117.4, 91.3, 79.1, 38.0, 18.2.

*4.3.8.3. 4-(Quinolin-6-yl)but-3-yn-1-amine **(11b)**.* Compound **11b** (0.190 g, 1.45 mmol, 82.7%) was prepared starting from compound **7b** (0.570 g, 1.75 mmol) according to the general procedure, and purified by silica gel column chromatography (CH_2Cl_2/EtOH/NH_3·H_2O = 10:0.3:0.05). IR (KBr) ν: 3447, 3025, 2949, 2363, 1654, 1569, 1494, 1023, 842 cm^{-1}. HRMS (ESI) $(M + H)^+$ *m/z* 197.1073, calcd for $C_{13}H_{13}N_2$ 197.1073. ^1H NMR ($CDCl_3$, 400 MHz) δ: 8.91–8.82 (m, 1 H, 2′-quinolyl), 8.11–7.94 (m, 2 H, 5′-quinolyl, 4′-quinolyl), 7.90–7.84 (m, 1 H, 8′-quinolyl), 7.72–6.65 (m, 1 H, 7′-quinolyl), 7.41–7.35 (m, 1 H, 3′-quinolyl), 2.97 (t, J = 6.4 Hz, 2 H, NH_2–C**H₂**-), 2.62 (t, J = 6.4 Hz, 2 H, NH_2–CH_2C**H₂**-). ^{13}C NMR (DMSO-d_6, 175 MHz) δ: 151.4, 146.8, 136.8, 132.7, 131.9, 129.2, 128.1, 122.7, 121.3, 87.9, 82.4, 38.2, 18.2.

*4.3.8.4. 6-(4-Aminobut-1-yn-1-yl)quinolin-4(1H)-one **(11c)**.* Compound **11c** (0.0960 g, 0.450 mmol, 28.1%) was prepared starting from compound **7c** (0.550 g, 1.60 mmol) according to the general procedure, and purified by silica gel column chromatography (CH_2Cl_2/MeOH/NH_3·H_2O = 10:1:0.1). IR (KBr) ν: 3680, 2973, 2866, 2363, 1587, 1483, 1332, 1033, 837 cm^{-1}. HRMS (ESI) $(M + H)^+$ *m/z* 213.1021, calcd for $C_{13}H_{13}N_2O$ 213.1022. ^1H NMR (DMSO-d_6, 400 MHz) δ: 8.07 (d, J = 2.0 Hz, 1 H, 5′-quinolyl), 7.91 (d, J = 7.5 Hz, 1 H, 2′-quinolyl), 7.63 (dd, J_1 = 8.6 Hz, J_2 = 2.0 Hz, 1 H, 7′-quinolyl), 7.51 (d, J = 8.6 Hz, 1 H, 8′-quinolyl), 6.04 (d, J = 7.4 Hz, 1 H, 3′-quinolyl), 2.79 (t, J = 6.8 Hz, 2 H, NH_2–C**H₂**-), 2.55–2.51 (m, 2 H, NH_2–CH_2C**H₂**-). ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 176.6, 140.1, 139.9, 134.6, 128.4, 126.0, 119.3, 118.2, 109.5, 89.4, 81.4, 41.5, 24.0.

*4.3.8.5. 5-(4-Aminobut-1-yn-1-yl)nicotinamide **(11d)**.* Compound **11d** (0.110 g, 0.580 mmol, 26.2%) was prepared starting from compound **7d** (0.700 g, 2.21 mmol) according to the general procedure, and purified by silica gel column chromatography (CH_2Cl_2/MeOH/NH_3·H_2O = 10:1:0.1). IR (KBr) ν: 3440, 2973, 2844, 1593, 1364, 1055, 1033 cm^{-1}. HRMS (ESI) $(M + H)^+$ *m/z* 190.0969, calcd for $C_{10}H_{12}N_3O$ 190.0975. ^1H NMR (DMSO-d_6, 400 MHz) δ: 8.97 (d, J = 2.1 Hz, 1 H, 6′-pyridyl), 8.76 (d, J = 2.0 Hz, 1 H, 2′-pyridyl), 8.30 (d, J = 2.1 Hz, 1 H, 4′-pyridyl), 8.24 (s, 1 H, -CO-N**H₂**), 7.68 (s, 1 H, -CO-N**H₂**), 5.94–4.84 (br, 2 H, -NH_2), 2.93 (t, J = 6.9 Hz, 2 H, NH_2–C**H₂**-), 2.72 (t, J = 6.8 Hz, 2 H, NH_2–CH_2C**H₂**-). ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 166.1, 154.2, 148.0, 138.0, 129.7, 120.0, 92.2, 78.7, 20.9.

*4.3.8.6. 6-(4-Aminobut-1-yn-1-yl)-1-cyclopropyl-4-oxo-1,4-dihydroquinoline-3-carboxamide **(11e)**.* Compound **11e** (0.200 g, 0.680 mmol, 26.2%) was prepared starting from compound **7e** (1.10 g, 2.59 mmol) according to the general procedure, and purified by silica gel column chromatography (CH_2Cl_2/EtOH/NH_3·H_2O = 10:0.8:0.05). IR (KBr) ν: 3452, 2975, 2359, 1651, 1592, 1052 cm^{-1}. HRMS (ESI) $(M + H)^+$ *m/z* 296.1394, calcd for $C_{17}H_{18}N_3O_2$ 296.1394. ^1H NMR (CD_3OD, 400 MHz) δ: 8.80 (s, 1 H, 2′-quinolyl), 8.26 (d, J = 2.0 Hz, 1 H, 5′-quinolyl), 8.10 (d, J = 8.8 Hz, 1 H, 8′-quinolyl), 7.78 (dd, J_1 = 8.8 Hz, J_2 = 2.1 Hz, 1 H, 7′-quinolyl), 3.69 (tt, J_1 = 7.3 Hz, J_2 = 4.0 Hz, 1 H, 1 H-cyclopropyl), 2.92 (s, 2 H, NH_2–C**H₂**-), 2.65 (t, J = 6.6 Hz, 2 H, NH_2–CH_2C**H₂**-), 1.40–1.33 (m, 2 H, 2 H-cyclopropyl), 1.21–1.15 (m, 2 H, 2 H-cyclopropyl). ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 175.6, 165.7, 148.4, 141.2, 128.4, 128.0, 126.9, 126.4, 120.5, 111.9, 93.9, 81.1, 41.4, 35.4, 24.4, 8.0.

*4.3.8.7. 6-(4-Aminobut-1-yn-1-yl)-1-ethyl-4-oxo-1,4-dihydroquinoline-3-carboxamide **(11f)**.* Compound **11f** (0.150 g, 0.530 mmol, 47.8%) was prepared starting from compound **7f** (0.460 g, 1.11 mmol) according to the general procedure, and purified by silica gel column chromatography (CH_2Cl_2/EtOH/NH_3·H_2O = 10:0.75:0.05). IR (KBr) ν: 3410, 2973, 2866, 2359, 1658, 1597, 1490, 1366, 1033 cm^{-1}. HRMS (ESI) $(M + H)^+$ *m/z* 284.1393, calcd for $C_{16}H_{18}N_3O_2$ 284.1394. ^1H NMR (DMSO-d_6, 400 MHz) δ: 9.21 (d, J = 4.6 Hz, 1 H, -CO-N**H₂**), 8.87 (s, 1 H, 2′-quinolyl), 8.30 (d, J = 2.0 Hz, 1 H, 5′-quinolyl), 7.87 (d, J = 8.9 Hz, 1 H, 7′-quinolyl), 7.81 (dd, J_1 = 8.9 Hz, J_2 = 2.1 Hz, 1 H, 8′-quinolyl), 7.52 (d, J = 4.6 Hz, 1 H, -CO-N**H₂**), 4.49 (q, J = 7.1 Hz, 2 H, -C**H₂**CH_3), 2.79 (t, J = 6.7 Hz, 2 H, NH_2–C**H₂**-), 2.56–2.52 (m, 2 H, NH_2–CH_2C**H₂**-), 1.38 (t, J = 7.1 Hz, 3 H, -CH_3). ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 175.2, 165.8, 148.7, 138.5, 135.7, 129.4, 127.8, 120.2, 118.4, 112.3, 91.1, 80.6, 48.8, 41.6, 24.4, 14.9.

*4.3.8.8. 6-(4-Aminobut-1-yn-1-yl)-1-methyl-4-oxo-1,4-dihydroquinoline-3-carboxamide **(11g)**.* Compound **11g** (0.0700 g, 0.260 mmol, 11.4%) was prepared starting from compound **7g** (0.850g, 2.29 mmol) according to the general procedure, and purified by silica gel column chromatography (CH_2Cl_2/EtOH/NH_3·H_2O = 10:0.7:0.05). IR (KBr) ν: 3426, 2973, 2360, 1659, 1597, 1496, 1033, 750 cm^{-1}. HRMS (ESI) $(M + H)^+$ *m/z* 270.1232, calcd for $C_{15}H_{16}N_3O_2$ 270.1237. ^1H NMR (DMSO-d_6, 400 MHz) δ: 9.21 (d, J = 4.6 Hz, 1 H, -CO-N**H₂**), 8.84 (s, 1 H, 2′-quinolyl), 8.30 (d, J = 2.0 Hz, 1 H, 5′-quinolyl), 7.84 (dd, J_1 = 8.8 Hz, J_2 = 2.0 Hz, 1 H, 7′-quinolyl), 7.78 (d, J = 9.0 Hz, 1 H, 8′-quinolyl), 7.50 (d, J = 4.6 Hz, 1 H, -CO-N**H₂**), 4.44 (br, 2 H, -CH_2-N**H₂**), 3.99 (s, 3 H, -CH_3), 2.89 (s, 2 H, NH_2–C**H₂**-), 2.65 (t, J = 6.7 Hz, 2 H, NH_2–CH_2C**H₂**-). ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 175.2, 165.9, 149.8, 139.7, 135.6, 129.1, 127.4, 120.3, 118.6, 111.9, 90.8, 80.8, 41.6, 41.2, 23.8.

*4.3.8.9. 6-(4-Aminobut-1-yn-1-yl)-1-cyclopropyl-N-methyl-4-oxo-1,4-dihydroquinoline-3-carboxamide **(11h)**.* Compound **11h** (0.120 g, 0.390 mmol, 46.4%) was prepared starting from compound **7h** (0.370 g, 0.840 mmol) according to the general procedure, and purified by silica gel column chromatography (CH_2Cl_2/EtOH/NH_3·

H_2O = 10:0.3:0.05). IR (KBr) ν: 3439, 2949, 2831, 2360, 1656, 1599, 1486, 1364, 1033 cm^{-1}. HRMS (ESI) $(M + H)^+$ *m/z* 310.1547, calcd for $C_{18}H_{20}N_3O_2$ 310.1550. ^1H NMR (DMSO-d_6, 400 MHz) δ: 9.63 (q, *J* = 4.8 Hz, 1 H, Ar-CO-NH-), 8.68 (s, 1 H, 2′-quinolyl), 8.27 (d, *J* = 2.0 Hz, 1 H, 5′-quinolyl), 8.12 (d, *J* = 8.8 Hz, 1 H, 7′-quinolyl), 7.87 (dd, J_1 = 8.9 Hz, J_2 = 2.1 Hz, 1 H, 8′-quinolyl), 3.85−3.61 (m, 3 H, 1 H-cyclopropyl, -NH_2), 2.89−2.82 (m, 5 H, NH_2−C**H$_2$**-, -CH_3), 2.61 (t, *J* = 6.8 Hz, 2 H, NH_2−CH_2C**H$_2$**-), 1.33−1.26 (m, 2 H, 2 H-cyclopropyl), 1.13−1.07 (m, 2 H, 2 H-cyclopropyl). ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 175.3, 164.7, 147.7, 140.3, 135.6, 129.0, 127.1, 120.4, 118.7, 111.7, 90.9, 80.7, 55.4, 41.3, 35.5, 26.0, 23.8, 8.0.

*4.3.8.10. Ethyl 6-(4-aminobut-1-yn-1-yl)-1-cyclopropyl-4-oxo-1,4-dihydroquinoline-3-carboxylate **(11i)**.* Compound **11i** (0.250 g, 0.770 mmol, 52.0%) was prepared starting from compound **7i** (0.670 g, 1.48 mmol) according to the general procedure, and purified by silica gel column chromatography (CH_2Cl_2/EtOH/NH_3·H_2O = 10:0.3:0.05). HRMS (ESI) $(M + H)^+$ *m/z* 325.1549, calcd for $C_{19}H_{21}N_2O_3$ 325.1547. ^1H NMR (CD_3OD, 400 MHz) δ: 8.54 (s, 1 H, 2′-quinolyl), 8.31 (d, *J* = 2.0 Hz, 1 H, 5′-quinolyl), 8.18 (dd, J_1 = 8.9 Hz, J_2 = 2.1 Hz, 1 H, 7′-quinolyl), 8.08 (d, *J* = 8.9 Hz, 1 H, 8′-quinolyl), 4.30 (q, *J* = 7.1 Hz, 2 H, -C**H$_2$**CH_3), 4.07−4.01 (m, 2 H, -NH_2), 3.66 (tt, J_1 = 7.3 Hz, J_2 = 4.0 Hz, 1 H, 1 H-cyclopropyl), 3.04−2.97 (m, 2 H, NH_2−C**H$_2$**-), 2.15−2.06 (m, 2 H, NH_2−CH_2C**H$_2$**-), 1.38 (t, *J* = 7.1 Hz, 3 H, -CH_3), 1.36−1.32 (m, 2 H, 2 H-cyclopropyl), 1.24−1.19 (m, 2 H, 2 H-cyclopropyl).

*4.3.8.11. 3-(Quinolin-3-yl)prop-2-en-1-amine **(12a)**.* Compound **12a** was prepared starting from compound **8a** according to the general procedure, and used directly for the next step without further purification. HRMS (ESI) $(M + H)^+$ *m/z* 185.1062, calcd for $C_{12}H_{13}N_2$ 185.1073. ^1H NMR (DMSO-d_6, 700 MHz) δ: 9.25 (s, 1 H, 2′-quinolyl), 8.82 (s, 1 H, 4′-quinolyl), 8.69−8.60 (m, 2 H, -NH_2), 8.23 (d, *J* = 8.5 Hz, 1 H, 5′-quinolyl), 8.20 (d, *J* = 8.3 Hz, 1 H, 8′-quinolyl), 7.93 (t, *J* = 7.7 Hz, 1 H, 6′-quinolyl), 7.78 (t, *J* = 7.6 Hz, 1 H, 7′-quinolyl), 7.02 (d, *J* = 16.1 Hz, 1 H, Ar-C**H**=CH-), 6.74 (dt, J_1 = 16.2 Hz, J_2 = 6.4 Hz, 1 H, Ar-CH=C**H**-), 3.69 (t, *J* = 6.0 Hz, 2 H, -CH_2-).

*4.3.8.12. 4-(Quinolin-3-yl)but-3-en-1-amine **(13a)**.* Compound **13a** was prepared starting from compound **9a** according to the general procedure, and used directly for the next step without further purification.

*4.3.8.13. 3-(Quinolin-3-yl)propan-1-amine **(14a)**.* Compound **14a** was prepared starting from 2-(3-(quinolin-3-yl)propyl)isoindoline-1,3-dione according to the general procedure, and used directly for the next step without further purification. IR (KBr) ν: 3426, 2966, 2736, 2360, 1568, 1389, 1033, 771 cm^{-1}. HRMS (ESI) $(M + H)^+$ *m/z* 187.1220, calcd for $C_{12}H_{15}N_2$ 187.1230. ^1H NMR (DMSO-d_6, 700 MHz) δ: 9.32 (d, *J* = 2.2 Hz, 1 H, 2′-quinolyl), 9.08 (d, *J* = 2.0 Hz, 1 H, 4′-quinolyl), 8.42 (d, *J* = 8.6 Hz, 1 H, 5′-quinolyl), 8.40 (s, 2 H, -NH_2), 8.27 (dd, J_1 = 8.3 Hz, J_2 = 1.2 Hz, 1 H, 8′-quinolyl), 8.07 (ddd, J_1 = 8.5 Hz, J_2 = 6.9 Hz, J_3 = 1.5 Hz, 1 H, 6′-quinolyl), 7.90 (ddd, J_1 = 8.1 Hz, J_2 = 6.8 Hz, J_3 = 1.0 Hz, 1 H, 7′-quinolyl), 3.07 (t, *J* = 7.5 Hz, 2 H, -CH_2-Ar), 2.87−2.80 (m, 2 H, -C**H$_2$**-NH_2), 2.09 (q, *J* = 7.4 Hz, 2 H, -C**H$_2$**CH_2-Ar). ^{13}C NMR (DMSO-d_6, 175 MHz) δ: 146.4, 144.4, 137.5, 135.8, 133.8, 129.9, 129.0, 128.8, 121.5, 38.1, 29.0, 28.2.

*4.3.8.14. 4-(Quinolin-3-yl)butan-1-amine **(15a)**.* Compound **15a** was prepared starting from 2-(4-(quinolin-3-yl)butyl)isoindoline-1,3-dione according to the general procedure, and used directly for the next step without further purification.

*4.3.9. But-3-yn-1-amine hydrochloride **(16)***

Compound **3** (0.500 g, 2.51 mmol) was suspended in 10 mL of EtOH, and 80% hydrazine hydrate (0.300 mL, 5.02 mmol) was added. The mixture was stirred at reflux for 2 h. The resulting mixture was cooled to room temperature. The insoluble solid was filtered off. The filtrate was acidified with 2 M HCl and concentrate to afford compound **16**. The crude product was used directly in the next step without further purification.

*4.3.10. tert-Butyl but-3-yn-1-ylcarbamate **(17)***

Di-*tert*-butyl dicarbonate (0.550 g, 2.51 mmol) was added to a solution of compound **16** (0.260 g, 2.51 mmol) and trimethylamine (1.05 mL, 7.53 mmol) in 8 mL of THF. The mixture was stirred at 0 °C for 10 min, and then at room temperature for 24 h. The THF was removed by means of evaporation at reduced pressure. The residue was dissolved in CH_2Cl_2 and washed with saturated $NaHCO_3$, water and brine. The organic solvent was removed to afford compound **17**. The crude product was used directly for the next step without further purification.

*4.3.11. 6-(4-Aminobut-1-yn-1-yl)-1-cyclopropyl-4-oxo-1,4-dihydroquinoline-3-carboxylic acid **(11j)***

To a mixture of 6 mL of acetonitrile and 6 mL of trimethylamine, CuI (0.0380 g, 0.200 mmol) and 1-cyclopropyl-6-iodo-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (0.710 g, 2.00 mmol) were added. The mixture was stirred for 20 min at room temperature. Compound **17** (0.420 g, 2.51 mmol) and bis(triphenylphosphine) palladium(II) dichloride (0.0700 g, 0.100 mmol) were added to the reaction vessel. The reaction mixture was stirred at 50 °C for 24 h under Ar. The reaction was quenched with water. The resulting mixture was extracted with EtOAc. The organic layer was washed with water and brine, and concentrated to afford intermediate Boc-**11j**. The intermediate was purified by flash column chromatography (CH_2Cl_2/MeOH/NH_3·H_2O = 10:2:0.5) to give pure Boc-**11j** (0.630 g, 1.59 mmol, 63.4% in three step).

Boc-**11j** (0.630 g, 1.59 mmol) was suspended in 32 mL of EtOAc with stirring, and 15.8 mL of concentrated hydrochloric acid was added dropwise at 0 °C. The mixture was stirred for 3 h, and then treated with EtOAc. The precipitate was filtered and washed with EtOAc to provide compound **11j** (0.360 g, 1.21 mmol, 76.1%) as a yellow solid. IR (KBr) ν: 3415, 2969, 2361, 1721, 1606, 1477, 1332, 1238, 952, 811 cm^{-1}. HRMS (ESI) $(M + H)^+$ *m/z* 297.1233, calcd for $C_{17}H_{17}N_2O_3$ 297.1234. ^1H NMR (DMSO-d_6, 400 MHz) δ: 14.87 (s, 1 H, -COOH), 8.75 (s, 1 H, 2′-quinolyl), 8.42 (d, *J* = 2.0 Hz, 1 H, 5′-quinolyl), 8.28 (d, *J* = 8.9 Hz, 1 H, 8′-quinolyl), 8.04 (dd, J_1 = 8.9 Hz, J_2 = 2.0 Hz, 1 H, 7′-quinolyl), 3.85 (tt, J_1 = 7.3 Hz, J_2 = 4.0 Hz, 1 H, 1 H-cyclopropyl), 3.08 (q, *J* = 6.3 Hz, 2 H, NH_2−C**H$_2$**-), 2.88 (t, *J* = 6.9 Hz, 2 H, NH_2−CH_2C**H$_2$**-), 1.36−1.28 (m, 2 H, 2 H-cyclopropyl), 1.23−1.16 (m, 2 H, 2 H-cyclopropyl). ^{13}C NMR (DMSO-d_6, 100 MHz) δ: 177.8, 166.0, 149.4, 141.0, 136.9, 129.0, 125.5, 121.1, 119.6, 108.2, 88.7, 81.4, 38.1, 36.4, 18.2, 8.1.

*4.3.12. 7-(4-(tert-Butoxycarbonyl)piperazin-1-yl)-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydroquinoline-3-carboxylic acid **(19)***

Ciprofloxacin (0.500 g, 1.50 mmol) was suspended in 25 mL of THF, and then trimethylamine (0.210 mL, 1.50 mmol) and di-*tert*-butyl dicarbonate (0.340 g, 1.50 mmol) were added. The mixture was stirred at 0 °C for 10 min, and then at room temperature for 8 h. The resulting mixture was concentrated and treated with petroleum ether. The precipitate was isolated by filtration and dried to yield compound **19** (0.630 g, 1.46 mmol, 97.3%).

*4.3.13. 1-Cyclopropyl-6-fluoro-4-oxo-7-(piperazin-1-yl)-1,4-dihydroquinoline-3-carboxamide **(20)***

Compound **19** (0.630 g, 1.46 mmol) was dissolved in 3 mL of DMF. Then CDI (0.490 g, 3.00 mmol) was added. The mixture was stirred at 65 °C for 2 h, and then 20 mL of NH_3·H_2O was added. The

mixture was stirred at room temperature for another 2 h. The precipitate was filtered off to afford the intermediate Boc-**20** (0.610 g, 1.42 mmol, 97.3%) as a yellow solid.

Boc-**20** (0.610 g, 1.42 mmol) was suspended in 30 mL of EtOAc, and 15 mL of concentrated hydrochloric acid was added dropwise at 0 °C. The mixture was stirred for 3 h. The resulting mixture was evaporated to remove the EtOAc and treated with $NH_3{\cdot}H_2O$. The insoluble solid was filtered off and washed with ether to obtain compound **20** (0.430 g, 1.42 mmol, 97.2%) as a white solid. IR (KBr) ν: 3440, 2981, 2360, 1660, 1597, 1483, 1261, 1033 cm^{-1}. HRMS (ESI) $(M + H)^+$ *m/z* 331.1560, calcd for $C_{17}H_{20}FN_4O_2$ 331.1565. ^1H NMR (DMSO-d_6, 400 MHz) δ: 9.22 (d, J = 4.7 Hz, 1 H, -CO-N**H**$_2$), 8.62 (s, 1 H, 2′-quinolyl), 7.84 (d, J = 13.6 Hz, 1 H, 5′-quinolyl), 7.51–7.44 (m, 2 H, 8′-quinolyl, -CO-N**H**$_2$), 3.77–3.69 (m, 1 H, 1 H-cyclopropyl), 3.26–3.18 (m, 4 H, 4 H-piperazinyl), 2.96 (t, J = 4.7 Hz, 4 H, 4 H-piperazinyl), 1.28 (dd, J_1 = 7.5 Hz, J_2 = 5.5 Hz, 2 H, 2 H-cyclopropyl), 1.13–1.06 (m, 2 H, 2 H-cyclopropyl). ^{13}C NMR (DMSO-d_6, 175 MHz) δ: 174.7, 165.9, 153.8, 152.4, 147.4, 145.0, 139.0, 121.2, 111.8, 111.6, 110.9, 106.4, 50.6, 45.4, 35.4, 8.0.

4.3.14. 2′-O-Acetyl-3-O-descladinosylazithromycin **(22)**

1 M HCl (106 mL) was added dropwise to a stirred mixture of azithromycin (20.0 g, 26.7 mmol) in EtOH (35 mL) at 40 °C for 1 h. The reaction mixture was quenched with $NH_3{\cdot}H_2O$ and extracted twice with CH_2Cl_2 at pH 10. The organic layer was washed with water and brine, and dried to obtain 3-*O*-descladinosylazithromycin as a white solid.

To a stirring solution of 3-*O*-descladinosylazithromycin (16.1 g, 27.2 mmol) in CH_2Cl_2 (150 mL) was added Ac_2O (5.15 mL, 54.5 mmol). The reaction mixture was stirred at room temperature for 1 h and quenched by the addition of saturated $NaHCO_3$. The mixture was washed five times with saturated $NaHCO_3$, water and brine, and concentrated to yield compound **22** (16.4 g, 25.9 mmol, 95.1%) as a white solid.

4.3.15. 2′-O-Acetyl-3-O-descladinosyl-3-O-acryloyl-6-O-acryloylazithromycin **(23)**

To a mixture of compound **22** (0.100 g, 0.160 mmol) and triethylamine (0.220 mL, 1.58 mmol) in acetonitrile (6 mL), a solution of 3-chloropropionyl chloride (0.0430 mL, 0.440 mmol) in acetonitrile (4 mL) was added dropwise at 80 °C. The reaction mixture was quenched with water. Acetonitrile was removed by rotary evaporation. The resulting mixture was diluted with CH_2Cl_2, washed twice with water and brine, and concentrated to yield compound **23**. HRMS (ESI) $(M + H)^+$ *m/z* 741.4542, calcd for $C_{38}H_{65}N_2O_{12}$ 741.4532. ^1H NMR ($CDCl_3$, 700 MHz) δ: 6.56 (d, J = 17.3 Hz, 1 H, -CH=C**H**$_2$), 6.46 (d, J = 16.8 Hz, 1 H, -CH=C**H**$_2$), 6.24 (dd, J_1 = 17.3 Hz, J_2 = 10.4 Hz, 1 H, -C**H**=CH$_2$), 6.18 (dd, J_1 = 17.3 Hz, J_2 = 10.3 Hz, 1 H, -C**H**=CH$_2$), 5.96 (dd, J_1 = 10.4 Hz, J_2 = 1.4 Hz, 1 H, -CH=C**H**$_2$), 5.79 (dd, J_1 = 10.3 Hz, J_2 = 1.7 Hz, 1 H, -CH=C**H**$_2$), 5.11 (d, J = 10.6 Hz, 1 H, H-3), 4.77 (dd, J_1 = 10.5 Hz, J_2 = 7.5 Hz, 1 H, H-2′), 4.67 (d, J = 10.8 Hz, 1 H, H-13), 4.38 (s, 1 H, H-5), 4.20 (d, J = 7.5 Hz, 1 H, H-1′), 3.61 (s, 1 H, H-11), 3.35–3.28 (m, 1 H, H-5′), 3.27–3.21 (m, 1 H, 12-OH), 2.88 (dd, J_1 = 10.8 Hz, J_2 = 6.7 Hz, 1 H, H-2), 2.73 (d, J = 7.2 Hz, 1 H, H-10), 2.66–2.53 (m, 2 H, H-3′, 11-OH), 2.50–2.43 (m, 1 H, H-9a), 2.38 (s, 3 H, -N-CH$_3$), 2.36–2.32 (m, 1 H, H-4), 2.28 (s, 6 H, -N(CH$_3$)$_2$), 2.10 (s, 1 H, 2′-O-CO-CH$_3$), 2.04 (t, J = 11.5 Hz, 1 H, H-9b), 1.97–1.91 (m, 1 H, H-14eq), 1.87–1.77 (m, 2 H, H-7a, H-8), 1.72–1.68 (m, 1 H, H-4′a), 1.62 (s, 3 H, 6-CH$_3$), 1.59–1.53 (m, 1 H, H-14ax), 1.35–1.27 (m, 1 H, H-4′b), 1.24–1.20 (m, 1 H, H-7b), 1.19 (d, J = 6.1 Hz, 3 H, 5′-CH$_3$), 1.11 (d, J = 6.8 Hz, 3 H, 2-CH$_3$), 1.10–1.07 (m, 6 H, 12-CH$_3$, 10-CH$_3$), 1.04 (d, J = 7.4 Hz, 3 H, 4-CH$_3$), 0.93–0.85 (m, 6 H, 8-CH$_3$, 15-CH$_3$). ^{13}C NMR ($CDCl_3$, 175 MHz) δ: 176.2, 169.9, 165.6, 165.0, 131.9, 130.0, 129.9, 128.5, 100.0, 86.9, 78.9, 78.5, 77.7, 74.6, 71.4, 68.9, 63.5, 61.6, 43.4, 40.7, 36.7, 30.6, 29.7, 26.9, 21.5, 21.2, 21.0, 20.8, 16.1, 11.0, 9.0, 8.3.

4.3.16. 2′-O-Acetyl-3-O-descladinosyl-6-acryloylazithromycin **(24)**

To a mixture of compound **22** (5.00 g, 7.90 mmol) and triethylamine (1.65 mL, 11.8 mmol) in toluene (30 mL), a solution of 3-chloropropionyl chloride (1.00 ml, 10.3 mmol) in toluene (20 mL) was slowly added dropwise at room temperature. After completion of the reaction, the mixture was quenched with water. Toluene was removed by rotary evaporation, 40 mL of water was added, and pH was adjusted to 10 using $NH_3{\cdot}H_2O$. The resulting mixture was extracted twice with CH_2Cl_2. The organic layer was washed with water and brine, and concentrated to yield compound **24**. The crude product was used in the next step without further purification. HRMS (ESI) $(M + H)^+$ *m/z* 687.4410, calcd for $C_{35}H_{63}N_2O_{11}$ 687.4426. ^1H NMR ($CDCl_3$, 500 MHz) δ: 6.36 (dd, J_1 = 17.3 Hz, J_2 = 1.6 Hz, 1 H, 6-O-CO-CH=C**H**$_2$), 6.10 (dd, J_1 = 17.3 Hz, J_2 = 10.3 Hz, 1 H, 6-O-CO-C**H**=CH$_2$), 5.77 (dd, J_1 = 10.3 Hz, J_2 = 1.6 Hz, 1 H, 6-O-CO-CH=C**H**$_2$), 4.79 (dd, J_1 = 10.4 Hz, J_2 = 7.6 Hz, 1 H, H-2′), 4.68–4.60 (m, 3 H, H-1′, H-5, H-13), 3.55 (s, 1 H, H-11), 3.52–3.42 (m, 2 H, H-5′, H-3), 3.36–3.24 (m, 1 H, 12-OH), 2.77–2.67 (m, 2 H, H-10, H-3′), 2.63 (dq, J_1 = 10.0, J_2 = 6.8, 1 H, H-2), 2.56–2.46 (m, 2 H, H-9a, 11-OH), 2.38 (s, 3 H, -N-CH$_3$), 2.26 (s, 6 H, -N(CH$_3$)$_2$), 2.14–2.08 (m, 1 H, H-4), 2.07 (s, 3 H, 2′-O-CO-CH$_3$), 2.03–1.95 (m, 1 H, H-9b), 1.95–1.87 (m, 2 H, H-14eq, H-7a), 1.76–1.67 (m, 2 H, H-4′a, H-8), 1.65 (s, 3 H, 6-CH$_3$), 1.59–1.51 (m, 1 H, H-14ax), 1.39–1.29 (m, 2 H, H-7b, H-4′b), 1.27–1.22 (m, 6 H, 5′-CH$_3$, 2-CH$_3$), 1.10–1.05 (m, 6 H, 12-CH$_3$, 10-CH$_3$), 0.93 (d, J = 7.3 Hz, 3 H, 4-CH$_3$), 0.91–0.85 (m, 6 H, 8-CH$_3$, 15-CH$_3$). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 177.5, 170.0, 166.3, 130.1, 129.9, 100.3, 87.6, 78.6, 78.2, 77.2, 75.9, 74.6, 71.6, 68.9, 63.4, 61.3, 44.5, 40.6, 40.2, 38.6, 37.2, 30.8, 21.5, 21.3, 21.1, 21.0, 20.7, 16.2, 11.0, 8.0.

4.3.17. 2′-O-Acetyl-3-O-descladinosyl-3-O-[2-(2-pyridyl)acetyl]-6-O-acryloylazithromycin **(25)**

To a solution of compound **24** (9.61 g, 14.0 mmol) in CH_2Cl_2 (60 mL) under argon, 2-pyridylacetic acid hydrochloride (4.86 g, 28.0 mmol), DCC (5.77 g, 28.0 mmol), pyridine (4.00 mL, 49.0 mmol) and DMAP (0.170 g, 1.40 mmol) were added at 0 °C and the mixture was stirred for 1 h. The *N*,*N*′-dicyclohexylurea was filtered off, and the filtrate was successively washed with saturated NH_4Cl, saturated $NaHCO_3$, water and saturated brine, and concentrated by rotary evaporation. The residue was purified by silica gel column chromatography (CH_2Cl_2/EtOH/$NH_3{\cdot}H_2O$ = 10:0.1:0.05) to afford pure compound **25** (3.98 g, 4.94 mmol, 35.3%) as a yellow solid. HRMS (ESI) $(M + H)^+$ *m/z* 806.4792, calcd for $C_{42}H_{68}N_3O_{12}$ 806.4798. ^1H NMR ($CDCl_3$, 700 MHz) δ: 8.55 (dd, J_1 = 4.9 Hz, J_2 = 0.9 Hz, 1 H, H-pyridyl), 7.70 (td, J_1 = 7.7 Hz, J_2 = 1.8 Hz, 1 H, H-pyridyl), 7.45 (d, J = 7.8 Hz, 1 H, H-pyridyl), 7.23 (ddd, J_1 = 7.5 Hz, J_2 = 4.8 Hz, J_3 = 1.1 Hz, 1 H, H-pyridyl), 6.43 (dd, J_1 = 17.3 Hz, J_2 = 1.7 Hz, 1 H, 6-O-CO-CH=C**H**$_2$), 6.16 (dd, J_1 = 17.3 Hz, J_2 = 10.3 Hz, 1 H, 6-O-CO-C**H**=CH$_2$), 5.77 (dd, J_1 = 10.3 Hz, J_2 = 1.7 Hz, 1 H, 6-O-CO-CH=C**H**$_2$), 5.06 (d, J = 10.5 Hz, 1 H, H-3), 4.80 (dd, J_1 = 10.5 Hz, J_2 = 7.4 Hz, 1 H, H-2′), 4.64 (d, J = 10.5 Hz, 1 H, H-13), 4.41 (s, 1 H, H-5), 4.35 (d, J = 7.4 Hz, 1 H, H-1′), 3.99–3.94 (m, 2 H, 3-O-CO-CH$_2$-), 3.58 (s, 1 H, H-11), 3.45–3.40 (m, 1 H, H-5′), 3.24 (s, 1 H, 12-OH), 2.89–2.79 (m, 2 H, H-3′, H-2), 2.74–2.69 (m, 1 H, H-10), 2.55 (s, 1 H, 11-OH), 2.49–2.43 (m, 1 H, H-9a), 2.37 (s, 3 H, -N-CH$_3$), 2.30 (s, 6 H, -N(CH$_3$)$_2$), 2.29–2.26 (m, 1 H, H-4), 2.10 (s, 3 H, 2′-O-CO-CH$_3$), 2.06–1.98 (m, 1 H, H-9b), 1.97–1.89 (m, 1 H, H-14eq), 1.87–1.82 (m, 1 H, H-7a), 1.81–1.76 (m, 1 H, H-8), 1.74–1.68 (m, 1 H, H-4′a), 1.64 (s, 3 H, 6-CH$_3$), 1.58–1.52 (m, 1 H, H-14ax), 1.36–1.32 (m, 1 H, H-4′b), 1.28–1.24 (m, 1 H, H-7b), 1.21 (d, J = 6.1 Hz, 3 H, 5′-CH$_3$), 1.10–1.06 (m, 6 H, 10-CH$_3$, 12-CH$_3$), 1.04 (d, J = 6.8 Hz, 3 H, 2-CH$_3$), 0.99 (d, J = 7.4, 3 H, 4-CH$_3$), 0.91 (d, J = 6.9 Hz, 3 H, 8-CH$_3$), 0.86 (t, J = 7.3 Hz, 15-CH$_3$). ^{13}C NMR ($CDCl_3$, 175 MHz) δ: 176.1, 169.9, 169.7, 165.7,

153.9, 149.3, 136.6, 130.0, 129.9, 124.1, 122.3, 100.4, 86.9, 78.9, 78.4, 78.1, 77.6, 74.6, 71.5, 69.0, 63.2, 61.5, 49.2, 44.0, 43.5, 40.7, 36.7, 34.0, 30.7, 27.0, 25.6, 25.0, 21.6, 21.3, 21.0, 16.1, 11.0, 9.0, 8.4.

*4.3.18. General procedure for the synthesis of compounds **26a-26u**, and **26w***

Compound **25** was dissolved in MeOH and stirred at reflux for 2 h. MeOH was removed by rotary evaporation to yield the crude product of 3-*O*-descladinosyl-3-*O*-[2-(2-pyridyl)acetyl]-6-*O*-acryloylazithromycin.

To a solution of 3-*O*-descladinosyl-3-*O*-[2-(2-pyridyl)acetyl]-6-*O*-acryloylazithromycin (1 eq) in anhydrous MeOH under argon, amines and *N*,*N*-diisopropylethylamine (5 eq) were added. The reaction mixture was stirred at 65 °C for 8 h, and then cooled to room temperature, diluted with CH_2Cl_2, washed with water and brine, and concentrated by rotary evaporation. The residue was purified by silica gel column chromatography to afford pure products **26a-26u**, **26w**.

*4.3.18.1. 3-O-Descladinosyl-3-O-[2-(2-pyridyl)acetyl]-6-O-(3-{[3-(quinolin-3-yl)prop-2-yn-1-yl]amino}propanoyl)azithromycin **(26a)**.* Compound **26a** (70.1 mg, 0.0740 mmol, 11.2%) was prepared starting from 3-*O*-descladinosyl-3-*O*-[2-(2-pyridyl)acetyl]-6-*O*-acryloylazithromycin (0.510 g, 0.660 mmol) and compound **10a** (0.220 g, 1.19 mmol) according to the general procedure (column chromatography eluents: CH_2Cl_2/EtOH/$NH_3{\cdot}H_2O$ = 10:0.2:0.05). HRMS (ESI) $(M + H)^+$ *m/z* 946.5522, calcd for $C_{52}H_{76}N_5O_{11}$ 946.5536. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 8.89 (d, J = 2.1 Hz, 1 H, H-quinolyl), 8.49 (ddd, J_1 = 5.0 Hz, J_2 = 1.9 Hz, J_3 = 0.9 Hz, 1 H, H-pyridyl), 8.21 (d, J = 1.7 Hz, 1 H, H-quinolyl), 8.07 (d, J = 8.4 Hz, 1 H, H-quinolyl), 7.75 (dd, J_1 = 8.1 Hz, J_2 = 1.4 Hz, 1 H, H-quinolyl), 7.70 (ddd, J_1 = 8.4 Hz, J_2 = 6.9 Hz, J_3 = 1.5 Hz, 1 H, H-quinolyl), 7.63 (td, J_1 = 7.7 Hz, J_2 = 1.8 Hz, 1 H, H-pyridyl), 7.54 (ddd, J_1 = 8.2 Hz, J_2 = 6.9 Hz, J_3 = 1.2 Hz, 1 H, H-quinolyl), 7.39 (d, J = 7.8 Hz, 1 H, H-pyridyl), 7.17 (ddd, J_1 = 7.6 Hz, J_2 = 4.9 Hz, J_3 = 1.2 Hz, 1 H, H-pyridyl), 5.12 (d, J = 11.0 Hz, 1 H, H-3), 4.75 (dd, J_1 = 10.8 Hz, J_2 = 2.0 Hz, 1 H, H-13), 4.34 (d, J = 3.1 Hz, 1 H, H-5), 4.25 (d, J = 7.2 Hz, 1 H, H-1′), 3.93 (dd, J_1 = 18.4 Hz, J_2 = 15.8 Hz, 2 H, 3-O-CO-CH_2-), 3.74 (s, 2 H, -NH-C**H$_2$**C≡C−Ar), 3.60 (s, 1 H, H-11), 3.49−3.40 (m, 2 H, H-5′, 12-OH), 3.24 (dd, J_1 = 10.2 Hz, J_2 = 7.2 Hz, 1 H, H-2′), 3.18−2.97 (m, 2 H, 6-O-CO-CH_2C**H$_2$**−NH-), 2.88−2.80 (m, 1 H, H-2), 2.72−2.56 (m, 5 H, 6-O-CO-C**H$_2$**CH_2−NH-, H-3′, H-10, 11-OH), 2.45−2.40 (m, 1 H, H-9a), 2.38 (s, 3 H, -N-CH_3), 2.32 (s, 6 H, -N(CH_3)$_2$), 2.29−2.25 (m, 1 H, H-4), 2.04−1.97 (m, 1 H, H-9b), 1.94−1.86 (m, 2 H, H-14eq, H-7a), 1.85−1.78 (m, 1 H, H-8), 1.70−1.64 (m, 1 H, H-4′a), 1.62 (s, 3 H, 6-CH_3), 1.58−1.49 (m, 1 H, H-14ax), 1.38−1.31 (m, 1 H, H-7b), 1.26−1.22 (m, 1 H, H-4′b), 1.19 (d, J = 6.1 Hz, 3 H, 5′-CH_3), 1.16 (d, J = 7.4 Hz, 3 H, 4-CH_3), 1.10−1.01 (m, 9 H, 12-CH_3, 10-CH_3, 2-CH_3), 0.87 (d, J = 6.8 Hz, 3 H, 8-CH_3), 0.82 (t, J = 7.3 Hz, 6 H, 15-CH_3). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 176.1, 172.1, 170.0, 154.0, 152.4, 149.2, 146.7, 138.4, 136.6, 129.8, 129.3, 127.6, 127.3, 127.1, 124.2, 122.3, 117.5, 103.2, 91.2, 87.3, 80.7, 78.9, 78.2, 77.8, 77.2, 74.6, 70.8, 69.4, 65.5, 61.7, 44.0, 43.5, 40.4, 39.0, 38.6, 36.9, 35.1, 28.9, 27.4, 21.2, 21.2, 21.0, 16.2, 16.1, 10.9, 9.3, 8.3.

*4.3.18.2. 3-O-Descladinosyl-3-O-[2-(2-pyridyl)acetyl]-6-O-(3-{[4-(quinolin-3-yl)but-3-yn-1-yl]amino}propanoyl)azithromycin **(26b)**.* Compound **26b** (62.1 mg, 0.0650 mmol, 16.7%) was prepared starting from 3-*O*-descladinosyl-3-*O*-[2-(2-pyridyl)acetyl]-6-*O*-acryloylazithromycin (0.300 g, 0.390 mmol) and compound **11a** (0.310 g, 1.56 mmol) according to the general procedure (column chromatography eluents: CH_2Cl_2/EtOH/$NH_3{\cdot}H_2O$ = 10:0.2:0.05). HRMS (ESI) $(M + H)^+$ *m/z* 960.5690, calcd for $C_{53}H_{78}N_5O_{11}$ 960.5692. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 8.89 (d, J = 2.1 Hz, 1 H, H-quinolyl), 8.51 (ddd, J_1 = 4.9 Hz, J_2 = 1.9 Hz, J_3 = 0.9 Hz, 1 H, H-pyridyl), 8.19 (d, J = 2.0 Hz, 1 H, H-quinolyl), 8.06 (d, J = 8.4 Hz, 1 H, H-quinolyl), 7.75 (dd, J_1 = 8.2 Hz, J_2 = 1.4 Hz, 1 H, H-quinolyl), 7.70−7.64 (m, 2 H, H-quinolyl, H-pyridyl), 7.53 (ddd, J_1 = 8.1 Hz, J_2 = 6.9 Hz, J_3 = 1.2 Hz, 1 H, H-quinolyl), 7.41 (dt, J_1 = 7.9 Hz, J_2 = 1.1, 1 H, H-pyridyl), 7.19 (ddd, J_1 = 7.5 Hz, J_2 = 4.9 Hz, J_3 = 1.1 Hz, 1 H, H-pyridyl), 5.11 (dd, J_1 = 10.6 Hz, J_2 = 2.0, 1 H, H-3), 4.76 (dd, J_1 = 10.8 Hz, J_2 = 2.0, 1 H, H-13), 4.32 (d, J = 3.1 Hz, 1 H, H-5), 4.24 (d, J = 7.2 Hz, 1 H, H-1′), 3.94 (dd, J_1 = 17.6 Hz, J_2 = 16.0 Hz, 2 H, 3-O-CO-CH_2-), 3.59 (s, 1 H, H-11), 3.47−3.39 (m, 2 H, H-5′, 12-OH), 3.23 (dd, J_1 = 10.2 Hz, J_2 = 7.2 Hz, 1 H, H-2′), 3.12−2.80 (m, 5 H, 6-O-CO-CH_2C**H$_2$**−NH−C**H$_2$**CH_2C≡C−Ar, H-2), 2.72−2.54 (m, 7 H, 6-O-CO-C**H$_2$**CH_2−NH−CH_2C**H$_2$**C≡C−Ar, H-10, H-3′ 11-OH), 2.46−2.39 (m, 1 H, H-9a), 2.37 (s, 3 H, -N-CH_3), 2.30 (s, 6 H, -N(CH_3)$_2$), 2.28−2.25 (m, 1 H, H-4), 2.04−1.96 (m, 1 H, H-9b), 1.94−1.79 (m, 3 H, H-14eq, H-7a, H-8), 1.66−1.59 (m, 4 H, H-4′a, 6-CH_3), 1.56−1.46 (m, 1 H, H-14ax), 1.34 (dd, J_1 = 14.0 Hz, J_2 = 6.1, 1 H, H-7b), 1.25−1.19 (m, 1 H, H-4′b), 1.18−1.13 (m, 6 H, 5′-CH_3, 4-CH_3), 1.07 (s, 3 H, 12-CH_3), 1.06−1.01 (m, 6 H, 10-CH_3, 2-CH_3), 0.87 (d, J = 6.8 Hz, 3 H, 8-CH_3), 0.78 (t, J = 7.3 Hz, 3 H, 15-CH_3). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 176.0, 172.0, 170.0, 154.0, 152.6, 149.2, 146.6, 138.1, 136.6, 129.7, 129.3, 127.5, 127.3, 127.1, 124.2, 122.3, 118.0, 103.2, 91.8, 87.3, 79.5, 79.0, 78.2, 77.9, 77.3, 74.6, 70.8, 69.5, 65.5, 61.7, 48.1, 44.3, 44.0, 43.5, 40.4, 38.6, 36.8, 35.4, 28.7, 27.5, 21.2, 20.5, 16.3, 16.1, 10.8, 9.3, 8.2.

*4.3.18.3. 3-O-Descladinosyl-3-O-[2-(2-pyridyl)acetyl]-6-O-(3-{[3-(quinolin-3-yl)prop-2-en-1-yl]amino}propanoyl)azithromycin **(26c)**.* Compound **26c** (39.8 mg, 0.0420 mmol, 6.46%) was prepared starting from 3-*O*-descladinosyl-3-*O*-[2-(2-pyridyl)acetyl]-6-*O*-acryloylazithromycin (0.500 g, 0.650 mmol) and compound **12a** (0.350 g, 1.91 mmol) according to the general procedure (column chromatography eluents: CH_2Cl_2/EtOH/$NH_3{\cdot}H_2O$ = 10:0.25:0.05). HRMS (ESI) $(M + H)^+$ *m/z* 948.5684, calcd for $C_{52}H_{78}N_5O_{11}$ 948.5692. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 8.97 (d, J = 2.2 Hz, 1 H, H-quinolyl), 8.48 (dt, J_1 = 4.8 Hz, J_2 = 1.5 Hz, 1 H, H-pyridyl), 8.06 (d, J = 8.4 Hz, 1 H, H-quinolyl), 8.02 (d, J = 2.1 Hz, 1 H, H-quinolyl), 7.76 (d, J = 8.4 Hz, 1 H, H-quinolyl), 7.65 (ddd, J_1 = 8.4 Hz, J_2 = 6.9 Hz, J_3 = 1.5 Hz, 1 H, H-quinolyl), 7.59 (td, J_1 = 7.7 Hz, J_2 = 1.9 Hz, 1 H, H-pyridyl), 7.51 (ddd, J_1 = 8.1 Hz, J_2 = 6.8 Hz, J_3 = 1.2 Hz, 1 H, H-quinolyl), 7.36 (d, J = 8.0 Hz, 1 H, H-pyridyl), 7.15 (ddd, J_1 = 7.6 Hz, J_2 = 4.9 Hz, J_3 = 1.1 Hz, 1 H, H-pyridyl), 6.74 (d, J = 16.0, 1 H, -CH=C**H**-Ar), 6.55 (dt, J_1 = 16.0 Hz, J_2 = 6.2 Hz, 1 H, -C**H**=CH-Ar), 5.11 (d, J = 10.0 Hz, 1 H, H-3), 4.79 (d, J = 9.6 Hz, 1 H, H-13), 4.39 (d, J = 3.0 Hz, 1 H, H-5), 4.25 (d, J = 7.3 Hz, 1 H, H-1′), 3.93 (dd, J_1 = 18.8 Hz, J_2 = 16.0 Hz, 2 H, 3-O-CO-CH_2-), 3.62 (s, 1 H, H-11), 3.57 (d, J = 6.0 Hz, 2 H, -NH-C**H$_2$**CH=CH-), 3.49−3.40 (m, 2 H, H-5′, 12-OH), 3.24 (dd, J_1 = 10.2 Hz, J_2 = 7.2 Hz, 1 H, H-2′), 3.12−2.93 (m, 2 H, 6-O-CO-CH_2C**H$_2$**−NH-), 2.88−2.79 (m, 1 H, H-2), 2.76−2.57 (m, 5 H, 6-O-CO-C**H$_2$**CH_2−NH-, H-10, H-3′, 11-OH), 2.47−2.40 (m, 1 H, H-9a), 2.38 (s, 3 H, -N-CH_3), 2.30 (s, 6 H, -N(CH_3)$_2$), 2.28−2.23 (m, 1 H, H-4), 2.05−1.97 (m, 1 H, H-9b), 1.95−1.85 (m, 2 H, H-14eq, H-7a), 1.84−1.75 (m, 1 H, H-8), 1.68−1.63 (m, 1 H, H-4′a), 1.61 (s, 3 H, 6-CH_3), 1.57−1.48 (m, 1 H, H-14ax), 1.39−1.32 (m, 1 H, H-7b), 1.25−1.20 (m, 1 H, H-4′b), 1.20−1.14 (m, 6 H, 5′-CH_3, 4-CH_3), 1.10−1.04 (m, 6 H, 12-CH_3, 10-CH_3), 1.01 (d, J = 6.8 Hz, 3 H, 2-CH_3), 0.88 (d, J = 6.8 Hz, 3 H, 8-CH_3), 0.80 (t, J = 7.3 Hz, 3 H, 15-CH_3). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 176.1, 172.0, 170.0, 154.0, 149.5, 149.2, 147.4, 136.6, 132.2, 130.0, 129.2, 129.0, 128.8, 128.0, 127.8, 126.8, 124.1, 122.2, 103.2, 87.4, 79.5, 79.1, 78.3, 77.8, 77.3, 74.6, 70.8, 69.4, 65.5, 61.7, 51.3, 44.0, 44.0, 43.4, 40.4, 36.8, 34.8, 28.8, 27.3, 21.2, 21.2, 21.0, 16.4, 16.1, 10.9, 9.3, 8.1.

*4.3.18.4. 3-O-Descladinosyl-3-O-[2-(2-pyridyl)acetyl]-6-O-(3-{[4-(quinolin-3-yl)but-3-en-1-yl]amino}propanoyl)azithromycin **(26d)**.* Compound **26d** (27.1 mg, 0.0280 mmol, 7.37%) was prepared starting from 3-*O*-descladinosyl-3-*O*-[2-(2-pyridyl)acetyl]-6-*O*-

acryloylazithromycin (0.290 g, 0.380 mmol) and compound **13a** (0.360 g, 1.81 mmol) according to the general procedure (column chromatography eluents: CH_2Cl_2/EtOH/$NH_3{\cdot}H_2O$ = 10:0.5:0.03). HRMS (ESI) $(M + H)^+$ *m/z* 962.5852, calcd for $C_{53}H_{80}N_5O_{11}$ 962.5849. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 8.97 (s, 1 H, H-quinolyl), 8.51 (s, 1 H, H-pyridyl), 8.05 (d, *J* = 8.5 Hz, 1 H, H-quinolyl), 8.00 (s, 1 H, H-quinolyl), 7.77 (d, *J* = 8.2 Hz, 1 H, H-quinolyl), 7.69–7.59 (m, 2 H, H-quinolyl, H-pyridyl), 7.54–7.47 (m, 1 H, H-quinolyl), 7.40 (d, *J* = 7.2 Hz, 1 H, H-pyridyl), 7.21–7.15 (m, 1 H, H-pyridyl), 6.60 (d, *J* = 15.9, 1 H, -CH=C**H**-Ar), 6.50–6.40 (m, 1 H, -C**H**=CH-Ar), 5.10 (d, *J* = 10.5 Hz, 1 H, H-3), 4.78 (d, *J* = 10.5 Hz, 1 H, H-13), 4.36 (s, 1 H, H-5), 4.24 (d, *J* = 6.0 Hz, 1 H, H-1′), 4.00–3.89 (m, 2 H, 3-O-CO-CH_2-), 3.60 (s, 1 H, H-11), 3.55–3.31 (m, 2 H, H-5′, 12-OH), 3.27–3.19 (m, 1 H, H-2′), 3.14–2.79 (m, 5 H, 6-O-CO-CH_2C**H$_2$**–NH–C**H$_2$**CH_2CH=CH-Ar, H-2), 2.74–2.50 (m, 7 H, 6-O-CO-C**H$_2$**CH_2–NH–CH_2C**H$_2$**CH=CH-Ar, H-10, H-3′, 11-OH), 2.45–2.38 (m, 1 H, H-9a), 2.36 (s, 3 H, -N-CH_3), 2.30 (s, 6 H, -N$(CH_3)_2$), 2.28–2.22 (m, 1 H, H-4), 2.05–1.95 (m, 1 H, H-9b), 1.94–1.83 (m, 2 H, H-7a, H-14eq), 1.94–1.73 (m, 1 H, H-8), 1.67–1.62 (m, 1 H, H-4′a), 1.60 (s, 3 H, 6-CH_3), 1.57–1.44 (m, 1 H, H-14ax), 1.39–1.30 (m, 1 H, H-7b), 1.30–1.23 (m, 1 H, H-4′b), 1.22–1.12 (m, 6 H, 5′-CH_3, 4-CH_3), 1.11–0.98 (m, 9 H, 12-CH_3, 10-CH_3, 2-CH_3), 0.92–0.83 (m, 3 H, 8-CH_3), 0.83–0.75 (m, 3 H, 15-CH_3). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 176.0, 172.0, 170.1, 154.0, 152.0, 149.4, 149.2, 147.2, 136.6, 134.5, 131.9, 130.5, 130.4, 129.2, 128.9, 128.7, 128.6, 128.1, 127.9, 103.2, 87.5, 79.5, 79.2, 78.2, 77.8, 77.2, 74.6, 70.8, 69.5, 65.5, 61.7, 48.8, 44.4, 44.0, 43.5, 40.4, 40.4, 38.6, 37.7, 36.8, 34.7, 33.0, 28.8, 27.4, 21.2, 21.0, 16.5, 16.1, 10.8, 9.3, 8.0.

4.3.18.5. 3-O-Descladinosyl-3-O-[2-(2-pyridyl)acetyl]-6-O-(3-{[3-(quinolin-3-yl)propyl]amino}propanoyl)azithromycin ***(26e)***. Compound **26e** (40.6 mg, 0.0430 mmol, 6.62%) was prepared starting from 3-*O*-descladinosyl-3-*O*-[2-(2-pyridyl)acetyl]-6-*O*-acryloylazithromycin (0.500 g, 0.650 mmol) and compound **14a** (0.140 g, 0.770 mmol) according to the general procedure (column chromatography eluents: CH_2Cl_2/EtOH/$NH_3{\cdot}H_2O$ = 10:0.25:0.05). HRMS (ESI) $(M + H)^+$ *m/z* 950.5848, calcd for $C_{52}H_{80}N_5O_{11}$ 950.5849. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 8.77 (d, *J* = 2.2 Hz, 1 H, H-quinolyl), 8.47 (ddd, J_1 = 4.9 Hz, J_2 = 1.9 Hz, J_3 = 0.9 Hz, 1 H, H-pyridyl), 8.07 (d, *J* = 8.4 Hz, 1 H, H-quinolyl), 7.94 (d, *J* = 2.2 Hz, 1 H, H-quinolyl), 7.75 (dd, J_1 = 8.2 Hz, J_2 = 1.4 Hz, 1 H, H-quinolyl), 7.65 (ddd, J_1 = 8.4 Hz, J_2 = 6.8 Hz, J_3 = 1.5 Hz, 1 H, H-quinolyl), 7.59 (td, J_1 = 7.7 Hz, J_2 = 1.8 Hz, 1 H, H-pyridyl), 7.51 (ddd, J_1 = 8.1 Hz, J_2 = 6.8 Hz, J_3 = 1.2 Hz, 1 H, H-quinolyl), 7.36 (d, *J* = 8.0 Hz, 1 H, H-pyridyl), 7.14 (ddd, J_1 = 7.5 Hz, J_2 = 4.9 Hz, J_3 = 1.1 Hz, 1 H, H-pyridyl), 5.09 (d, *J* = 10.4 Hz, 1 H, H-3), 4.80 (d, *J* = 9.6 Hz, 1 H, H-13), 4.41 (d, *J* = 2.9 Hz, 1 H, H-5), 4.24 (d, *J* = 7.2 Hz, 1 H, H-1′), 3.92 (dd, J_1 = 18.0 Hz, J_2 = 15.6 Hz, 2 H, 3-O-CO-CH_2-), 3.61 (s, 1 H, H-11), 3.48–3.38 (m, 2 H, H-5′, 12-OH), 3.23 (dd, J_1 = 10.2 Hz, J_2 = 7.2 Hz, 1 H, H-2′), 3.10–2.90 (m, 2 H, 6-O-CO-CH_2C**H$_2$**–NH-), 2.88–2.82 (m, 3 H, H-2, -NH-C**H$_2$**$(CH_2)_2$-Ar), 2.81–2.76 (m, 2 H, -NH-$(CH_2)_2$C**H$_2$**-Ar), 2.74–2.52 (m, 5 H, H-10, 6-O-CO-C**H$_2$**CH_2–NH-, H-3′, 11-OH), 2.44–2.38 (m, 1 H, H-9a), 2.36 (s, 3 H, -N-CH_3), 2.30 (s, 6 H, -N$(CH_3)_2$), 2.28–2.22 (m, 1 H, H-4), 2.04–1.94 (m, 3 H, H-9b, -NH-CH_2C**H$_2$**CH_2-Ar), 1.94–1.85 (m, 2 H, H-14eq, H-7a), 1.81–1.73 (m, 1 H, H-8), 1.67–1.61 (m, 1 H, H-4′a), 1.60 (s, 3 H, 6-CH_3), 1.57–1.48 (m, 1 H, H-14ax), 1.39–1.32 (m, 1 H, H-7b), 1.27–1.20 (m, 1 H, H-4′b), 1.20–1.14 (m, 6 H, 5′-CH_3, 4-CH_3), 1.10–0.99 (m, 9 H, 12-CH_3, 10-CH_3, 2-CH_3), 0.88 (d, *J* = 6.9 Hz, 3 H, 8-CH_3), 0.79 (t, *J* = 7.3 Hz, 3 H, 15-CH_3). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 176.1, 172.0, 170.1, 154.0, 152.0, 149.2, 146.8, 136.6, 134.4, 134.2, 129.1, 128.6, 128.2, 127.4, 126.5, 124.1, 122.2, 103.2, 87.5, 79.4, 79.2, 78.3, 77.7, 77.3, 74.5, 70.8, 69.5, 65.5, 61.7, 48.7, 44.4, 44.0, 43.4, 40.4, 38.6, 36.7, 34.5. 30.7, 30.4, 28.7, 27.2, 21.2, 21.1, 16.5, 16.1, 10.8, 9.3, 8.0.

4.3.18.6. 3-O-Descladinosyl-3-O-[2-(2-pyridyl)acetyl]-6-O-(3-{[4-(quinolin-3-yl)butyl]amino}propanoyl)azithromycin ***(26f)***. Compound **26f** (32.0 mg, 0.0330 mmol, 7.86%) was prepared starting from 3-*O*-descladinosyl-3-*O*-[2-(2-pyridyl)acetyl]-6-*O*-acryloylazithromycin (0.320 g, 0.420 mmol) and compound **15a** (0.260 g, 0.800 mmol) according to the general procedure (column chromatography eluents: CH_2Cl_2/EtOH/$NH_3{\cdot}H_2O$ = 10:0.5:0.05). HRMS (ESI) $(M + H)^+$ *m/z* 964.6009, calcd for $C_{53}H_{82}N_5O_{11}$ 964.6005. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 8.76 (d, *J* = 2.3 Hz, 1 H, H-quinolyl), 8.50 (ddd, J_1 = 5.0 Hz, J_2 = 1.9 Hz, J_3 = 0.9 Hz, 1 H, H-pyridyl), 8.06 (d, *J* = 8.4 Hz, 1 H, H-quinolyl), 7.91 (d, *J* = 2.2 Hz, 1 H, H-quinolyl), 7.76 (dd, J_1 = 8.3 Hz, J_2 = 1.4 Hz, 1 H, H-quinolyl), 7.67–7.61 (m, 2 H, H-quinolyl, H-pyridyl), 7.50 (ddd, J_1 = 8.1 Hz, J_2 = 6.8 Hz, J_3 = 1.2 Hz, 1 H, H-quinolyl), 7.38 (d, *J* = 8.0 Hz, 1 H, H-pyridyl), 7.18 (ddd, J_1 = 7.6 Hz, J_2 = 4.9 Hz, J_3 = 1.2 Hz, 1 H, H-pyridyl), 5.09 (d, *J* = 9.6 Hz, 1 H, H-3), 4.83 (d, *J* = 10.0 Hz, 1 H, H-13), 4.40 (d, *J* = 2.9 Hz, 1 H, H-5), 4.23 (d, *J* = 7.3 Hz, 1 H, H-1′), 3.93 (dd, J_1 = 18.4 Hz, J_2 = 16.0 Hz, 2 H, 3-O-CO-CH_2-), 3.60 (s, 1 H, H-11), 3.47–3.37 (m, 2 H, H-5′, 12-OH), 3.23 (dd, J_1 = 10.2 Hz, J_2 = 7.2 Hz, 1 H, H-2′), 3.12–2.88 (m, 2 H, 6-O-CO-CH_2C**H$_2$**–NH-), 2.88–2.74 (m, 5 H, H-2, -NH-C**H$_2$**$(CH_2)_2$C**H$_2$**-Ar), 2.72–2.53 (m, 5 H, H-10, H-3′, 11-OH, 6-O-CO-C**H$_2$**CH_2-Ar), 2.46–2.38 (m, 1 H, H-9a), 2.36 (s, 3 H, -N-CH_3), 2.29 (s, 6 H, -N$(CH_3)_2$), 2.27–2.19 (m, 1 H, H-4), 2.04–1.94 (m, 1 H, H-9b), 1.94–1.83 (m, 2 H, H-14eq, H-7a), 1.82–1.64 (m, 5 H, H-8, -NH-CH_2C**H$_2$**C**H$_2$**CH_2-Ar), 1.62 (s, 1 H, H-4′a), 1.58 (s, 3 H, 6-CH_3), 1.56–1.45 (m, 1H, H-14ax), 1.39–1.32 (m, 1 H, H-7b), 1.28–1.20 (m, 1 H, H-4′b), 1.20–1.12 (m, 6 H, 5′-CH_3, 4-CH_3), 1.07 (s, 3 H, 12-CH_3), 1.04 (d, *J* = 6.8 Hz, 3 H, 10-CH_3), 1.00 (d, *J* = 6.8 Hz, 3 H, 2-CH_3), 0.87 (d, *J* = 6.8 Hz, 3 H, 8-CH_3), 0.78 (t, *J* = 7.3 Hz, 3 H, 15-CH_3). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 176.0, 171.9, 170.1, 154.0, 152.0, 149.2, 146.8, 136.6, 134.7, 134.2, 128.6, 128.2, 127.4, 126.5, 124.2, 122.3, 103.3, 87.7, 79.6, 79.3, 78.3, 77.8, 77.2, 74.5, 70.7, 69.5, 65.5, 61.7, 49.0, 44.3, 44.0, 43.4, 40.4, 38.6, 36.6, 34.2, 32.9, 32.7, 28.7, 28.5, 28.3, 27.3, 21.3, 21.2, 21.0, 16.6, 16.1, 10.8, 9.3, 7.9.

4.3.18.7. 3-O-Descladinosyl-3-O-[2-(2-pyridyl)acetyl]-6-O-[3-({2-[(3-carboxy-7-chloro-1-cyclopropyl-1,4-dihydro-4-oxoquinolin-6-yl)amino]ethyl}amino)propanoyl]azithromycin ***(26g)***. Compound **26g** (40.0 mg, 0.0370 mmol, 21.8%) was prepared starting from 3-*O*-descladinosyl-3-*O*-[2-(2-pyridyl)acetyl]-6-*O*-acryloylazithromycin (0.130 g, 0.170 mmol) and 6-(2-amino-ethylamino)-7-chloro-1-cyclopropyl-4-oxo-1,4-dihydro-quinoline-3-carboxylic acid (0.110 g, 0.320 mmol) according to the general procedure (column chromatography eluents: CH_2Cl_2/MeOH/$NH_3{\cdot}H_2O$ = 10:0.8:0.5). HRMS (ESI) $(M + H)^+$ *m/z* 1085.5578, calcd for $C_{55}H_{82}ClN_6O_{14}$ 1085.5572. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 8.69 (s, 1 H, H-quinolyl), 8.49 (s, 1 H, H-pyridyl), 8.00 (s, 1 H, H-quinolyl), 7.66 (t, *J* = 7.8 Hz, 1 H, H-pyridyl), 7.48 (s, 1 H, H-quinolyl), 7.39 (d, *J* = 8 Hz, 1 H, H-pyridyl), 7.22–7.15 (m, 1 H, H-pyridyl), 5.42 (s, 1 H, -NH-Ar), 5.07 (d, *J* = 10.7 Hz, 1 H, H-3), 4.70 (d, *J* = 10.9 Hz, 1 H, H-13), 4.38 (s, 1 H, H-5), 4.25 (d, *J* = 5.6 Hz, 1 H, H-1′), 3.98–3.87 (m, 2 H, 3-O-CO-CH_2-), 3.61–3.51 (m, 2 H, H-11, 1 H-cyclopropyl), 3.51–3.31 (m, 4 H, H-5′, 12-OH, -C**H$_2$**-NH-Ar), 3.22 (t, *J* = 8.8 Hz, 1 H, H-2′), 3.08–2.78 (m, 5 H, -C**H$_2$**CH_2–NH-Ar, 6-O-CO-CH_2C**H$_2$**-, H-2), 2.72–2.48 (m, 5 H, H-10, H-3′, 11-OH, 6-O-CO-C**H$_2$**-), 2.44–2.38 (m, 1 H, H-9a), 2.36 (s, 3 H, -N-CH_3), 2.30 (s, 6 H, -N$(CH_3)_2$), 2.27–2.21 (m, 1 H, H-4), 2.05–1.95 (m, 1 H, H-9b), 1.93–1.83 (m, 2 H, H-14eq, H-7a), 1.76 (s, 1 H, H-8), 1.68–1.47 (m, 5 H, H-4′a, 6-CH_3, H-14ax), 1.41–1.30 (m, 3 H, H-7b, 2 H-cyclopropyl), 1.28–1.12 (m, 9 H, H-4′b, 2 H-cyclopropyl, 5′-CH_3, 4-CH_3), 1.09–0.97 (m, 9 H, 10-CH_3, 12-CH_3, 2-CH_3), 0.91–0.78 (m, 6 H, 8-CH_3, 15-CH_3). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 177.5, 176.2, 172.1, 170.0, 167.4, 154.0, 149.2, 145.6, 143.3, 136.6, 132.3, 127.7, 126.2, 124.1, 122.3, 117.9, 107.5, 104.3, 103.2, 87.2, 79.0, 78.3, 77.6, 77.3, 74.6, 70.8, 69.5, 65.5, 61.6, 47.4, 44.2, 44.0, 43.4, 42.7, 40.4, 40.3, 38.6, 36.8, 35.3, 28.7, 27.2, 21.3, 21.2, 21.1, 16.2, 16.1, 10.9, 9.3,

8.2, 8.1.

*4.3.18.8. 3-O-Descladinosyl-3-O-[2-(2-pyridyl)acetyl]-6-O-[3-({2-[(3-carboxy-7-chloro-1-cyclopropyl-1,4-dihydro-4-oxoquinolin-6-yl) amino]propyl}amino)propanoyl]azithromycin **(26h)**.* Compound **26h** (40.4 mg, 0.0370 mmol, 15.4%) was prepared starting from 3-*O*-descladinosyl-3-*O*-[2-(2-pyridyl)acetyl]-6-*O*-acryloylazithromycin (0.180 g, 0.240 mmol) and 6-(3-amino-propylamino)-7-chloro-1-cyclopropyl-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (0.0960 g, 0.300 mmol) according to the general procedure (column chromatography eluents: CH_2Cl_2/MeOH/$NH_3{\cdot}H_2O$ = 10:0.7:0.5). HRMS (ESI) $(M + H)^+$ *m/z* 1099.5727, calcd for $C_{56}H_{84}ClN_6O_{14}$ 1099.5729. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 8.70 (s, 1 H, H-quinolyl), 8.51 (d, J = 4.7 Hz, 1 H, H-pyridyl), 8.01 (s, 1 H, H-quinolyl), 7.67 (td, J_1 = 7.7 Hz, J_2 = 1.8 Hz, 1 H, H-pyridyl), 7.47 (s, 1 H, H-quinolyl), 7.40 (d, J = 8 Hz, 1 H, H-pyridyl), 7.22–7.17 (m, 1 H, H-pyridyl), 5.90 (s, 1 H, -NH-Ar), 5.08 (d, J = 10.4 Hz, 1 H, H-3), 4.72 (d, J = 10.8 Hz, 1 H, H-13), 4.39 (d, J = 3.0 Hz, 1 H, H-5), 4.25 (d, J = 7.2 Hz, 1 H, H-1′), 3.94 (dd, J_1 = 17.6 Hz, J_2 = 16.0 Hz, 2 H, 3-O-CO-CH_2-), 3.60 (s, 1 H, H-11), 3.58–3.51 (m, 1 H, 1 H-cyclopropyl), 3.50–3.35 (m, 4 H, H-5′, 12-OH, -C**H$_2$**-NH-Ar), 3.23 (dd, J_1 = 10.2 Hz, J_2 = 7.2 Hz, 1 H, H-2′), 3.06–2.79 (m, 5 H, 6-O-CO-CH_2C**H$_2$**–NH–C**H$_2$**$(CH_2)_2$–NH-Ar, H-2), 2.73–2.51 (m, 5 H, 6-O-CO-CH_2-, H-3′, H-10, 11-OH), 2.47–2.40 (m, 1 H, H-9a), 2.37 (s, 3 H, -N-CH_3), 2.30 (s, 6 H, -N$(CH_3)_2$), 2.28–2.24 (m, 1 H, H-4), 2.06–1.96 (m, 1 H, H-9b), 1.95–1.85 (m, 4 H, H-14eq, H-7a, -**CH$_2$**CH_2–NH-Ar), 1.78 (s, 1 H, H-8), 1.68–1.63 (m, 1 H, H-4′a), 1.62 (s, 3 H, 6-CH_3), 1.58–1.48 (m, 1 H, H-14ax), 1.41–1.30 (m, 3 H, H-7b, 2 H-cyclopropyl), 1.29–1.23 (m, 1 H, H-4′b), 1.23–1.13 (m, 8 H, 2 H-cyclopropyl, 5′-CH_3, 4-CH_3), 1.10–0.99 (m, 9 H, 2-CH_3, 10-CH_3, 12-CH_3), 0.89 (d, J = 6.9 Hz, 3 H, 8-CH_3), 0.84 (t, J = 7.3 Hz, 3 H, 15-CH_3). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 177.6, 176.3, 172.2, 170.0, 167.5, 154.0, 149.3, 145.5, 143.5, 136.6, 132.1, 127.5, 126.3, 124.1, 122.3, 117.8, 107.5, 103.9, 103.3, 87.2, 79.2, 79.1, 78.3, 77.2, 74.6, 70.8, 69.5, 65.6, 61.6, 47.8, 44.6, 44.0, 43.5, 42.8, 40.4, 36.8, 35.3, 34.8, 28.7, 27.8, 27.2, 21.3, 21.2, 21.1, 16.2, 16.1, 10.9, 9.3, 8.2, 8.1.

*4.3.18.9. 3-O-Descladinosyl-3-O-[2-(2-pyridyl)acetyl]-6-O-[3-({2-[(3-carboxy-6-fluoro1-cyclopropyl-1,4-dihydro-4-oxo-quinolin-7-yl) amino]butyl}amino)propanoyl]azithromycin **(26i)**.* Compound **26i** (38.6 mg, 0.0350 mmol, 20.6%) was prepared starting from 3-*O*-descladinosyl-3-*O*-[2-(2-pyridyl)acetyl]-6-*O*-acryloylazithromycin (0.130 g, 0.170 mmol) and 7-(4-amino-butylamino)-6-fluoro-1-cyclopropyl-4-oxo-1,4-dihydroquinoline-3-carboxylic acid (0.110 g, 0.320 mmol) according to the general procedure (column chromatography eluents: CH_2Cl_2/MeOH/$NH_3{\cdot}H_2O$ = 10:0.7:0.5). HRMS (ESI) $(M + H)^+$ *m/z* 1097.6191, calcd for $C_{57}H_{86}FN_6O_{14}$ 1097.6181. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 8.67 (s, 1 H, H-quinolyl), 8.50 (s, 1 H, H-pyridyl), 7.87 (d, J = 11.5 Hz, 1 H, H-quinolyl), 7.66 (t, J = 8.0 Hz, 1 H, H-pyridyl), 7.37 (d, J = 7.6 Hz, 1 H, H-pyridyl), 7.22–7.16 (m, 1 H, H-pyridyl), 6.95 (d, J = 6.9 Hz, 1 H, H-quinolyl), 5.69 (s, 1 H, -NH-Ar), 5.07 (d, J = 10.6 Hz, 1 H, H-3), 4.74 (d, J = 10.7 Hz, 1 H, H-13), 4.39 (s, 1 H, H-5), 4.25 (d, J = 6.4 Hz, 1 H, H-1′), 3.99–3.89 (m, 2 H, 3-O-CO-CH_2-), 3.60 (s, 1 H, H-11), 3.56–3.37 (m, 3 H, 1 H-cyclopropyl, H-5′, 12-OH), 3.32–3.19 (m, 3 H, -C**H$_2$**-NH-Ar, H-2′), 3.05–2.50 (m, 10 H, H-2, -C**H$_2$**$(CH_2)_3$–NH-Ar, 11-OH, 6-O-CO-C**H$_2$C H$_2$**-, H-10, H-3′), 2.48–2.38 (m, 1 H, H-9a), 2.36 (s, 3 H, -N-CH_3), 2.30 (s, 6 H, -N$(CH_3)_2$), 2.27–2.21 (m, 1 H, H-4), 2.05–1.81 (m, 5 H, H-9b, H-14eq, H-7a, -NH-$(CH_2)_2$C**H$_2$**CH_2–NH-Ar), 1.79–1.69 (m, 3 H, -NH-CH_2C**H$_2$**$(CH_2)_2$–NH-Ar, H-8), 1.65 (d, J = 12.9 Hz, 1 H, H-4′a), 1.60 (s, 3 H, 6-CH_3), 1.58–1.48 (m, 1 H, H-14ax), 1.40–1.30 (m, 3 H, H-7b, 2 H-cyclopropyl), 1.29–1.13 (m, 9 H, H-4′b, 2 H-cyclopropyl, 5′-CH_3, 4-CH_3), 1.09–0.98 (m, 9 H, 10-CH_3, 12-CH_3, 2-CH_3), 0.88 (d, J = 6.7 Hz, 3 H, 8-CH_3), 0.84–0.77 (m, 3 H, 15-CH_3). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 176.7, 176.1, 172.2, 170.1, 167.6, 154.0, 151.5, 149.2, 149.0, 146.6, 142.8, 142.7, 140.4, 136.6, 124.1, 122.3, 115.3, 109.9, 109.7, 107.8, 103.3, 95.6, 87.4, 79.4, 79.2, 78.4, 77.8, 77.2, 74.6, 70.8, 69.5, 65.6, 61.6, 48.7, 44.4, 44.0, 43.5, 43.0, 40.4, 40.4, 38.6, 36.7, 35.3, 34.5, 28.7, 27.2, 26.8, 26.2, 21.2, 21.2, 16.4, 16.1, 10.8, 9.3, 8.2, 8.0.

*4.3.18.10. 3-O-Descladinosyl-3-O-[2-(2-pyridyl)acetyl]-6-O-(3-{2-[(3-carboxy-6-fluoro-1-cyclopropyl-1,4-dihydro-4-oxoquinolin-7-yl) piperazin-1-yl]}propanoyl)azithromycin **(26j)**.* Compound **26j** (48.7 mg, 0.0440 mmol, 25.9%) was prepared starting from 3-*O*-descladinosyl-3-*O*-[2-(2-pyridyl)acetyl]-6-*O*-acryloylazithromycin (0.130 g, 0.170 mmol) and ciprofloxacin (0.280 g, 0.850 mmol) according to the general procedure (column chromatography eluents: CH_2Cl_2/MeOH/$NH_3{\cdot}H_2O$ = 10:0.7:0.5). HRMS (ESI) $(M + H)^+$ *m/z* 1095.6022, calcd for $C_{57}H_{84}FN_6O_{14}$ 1095.6024. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 15.04 (s, 1 H, -COOH), 8.75 (s, 1 H, H-quinolyl), 8.51 (s, 1 H, H-pyridyl), 8.00 (d, J = 13.0 Hz, 1 H, H-quinolyl), 7.68 (t, J = 7.9 Hz, 1 H, H-pyridyl), 7.40 (d, J = 8.0 Hz, 1 H, H-pyridyl), 7.35 (d, J = 7.0 Hz, 1 H, H-quinolyl), 7.24–7.17 (m, 1 H, H-pyridyl), 5.10 (d, J = 10.6 Hz, 1 H, H-3), 4.71 (d, J = 10.6 Hz, 1 H, H-13), 4.34 (s, 1 H, H-5), 4.26 (d, J = 7.2 Hz, 1 H, H-1′), 3.99–3.89 (m, 2 H, 3-O-CO-C**H$_2$**-), 3.61 (s, 1 H, H-11), 3.53 (s, 1 H, 1 H-cyclopropyl), 3.49–3.31 (m, 6 H, H-5′, 12-OH, 4 H-piperazinyl), 3.25 (t, J = 8.9 Hz, 1 H, H-2′), 2.92–2.82 (m, 2 H, 6-O-CO-CH_2C**H$_2$**–N-), 2.82–2.60 (m, 7 H, H-2, H-10, 4 H-piperazinyl, H-3′), 2.60–2.52 (m, 2 H, 6-O-CO-C**H$_2$**CH_2–NH-), 2.51–2.43 (m, 1 H, H-9a), 2.39 (s, 3 H, -N-CH_3), 2.33 (s, 6 H, -N$(CH_3)_2$), 2.30–2.24 (m, 1 H, H-4), 2.08–1.99 (m, 1 H, H-9b), 1.98–1.87 (m, 2 H, H-14eq, H-7a), 1.87–1.78 (m, 1 H, H-8), 1.73–1.66 (m, 1 H, H-4′a), 1.64 (s, 3 H, 6-CH_3), 1.61–1.50 (m, 1 H, H-14ax), 1.44–1.31 (m, 3 H, 2 H-cyclopropyl, H-7b), 1.30–1.13 (m, 9 H, H-4′b, 5′-CH_3, 4-CH_3, 2 H-cyclopropyl), 1.12–1.01 (m, 9 H, 10-CH_3, 2-CH_3, 12-CH_3), 0.95–0.82 (m, 6 H, 8-CH_3, 15-CH_3). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 177.2, 176.3, 171.7, 170.0, 167.1, 154.9, 154.0, 152.4, 149.3, 147.4, 146.0, 145.8, 139.1, 136.6, 124.2, 122.3, 119.7, 112.5, 112.3, 108.1, 104.7, 103.2, 87.2, 79.5, 79.0, 78.2, 77.7, 77.2, 74.7, 70.7, 69.4, 65.6, 61.6, 53.2, 52.5, 49.7, 44.0, 43.5, 40.4, 38.6, 36.9, 35.3, 32.9, 28.9, 27.3, 21.4, 21.2, 16.3, 16.1, 11.0, 9.3, 8.2.

*4.3.18.11. 3-O-Descladinosyl-3-O-[2-(2-pyridyl)acetyl]-6-O-(3-{2-[(3-carbamoyl-6-fluoro-1-cyclopropyl-1,4-dihydro-4-oxoquinolin-7-yl)piperazin-1-yl]}propanoyl)azithromycin **(26k)**.* Compound **26k** (35.2 mg, 0.0320 mmol, 10.7%) was prepared starting from 3-*O*-descladinosyl-3-*O*-[2-(2-pyridyl)acetyl]-6-*O*-acryloylazithromycin (0.300 g, 0.390 mmol) and compound **20** (0.0940 g, 0.300 mmol) according to the general procedure (column chromatography eluents: CH_2Cl_2/EtOH/$NH_3{\cdot}H_2O$ = 10:0.2:0.05). Melting point: 139–141 °C. HRMS (ESI) $(M + H)^+$ *m/z* 1094.6187, calcd for $C_{57}H_{85}FN_7O_{13}$ 1094.6184. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 9.72 (d, J = 5.3 Hz, 1 H, -N**H$_2$**), 8.80 (s, 1 H, H-quinolyl), 8.54–8.49 (m, 1 H, H-pyridyl), 8.01 (d, J = 13.3 Hz, 1 H, H-quinolyl), 7.68 (td, J_1 = 7.7 Hz, J_2 = 1.8 Hz, 1 H, H-pyridyl), 7.40 (d, J = 7.8 Hz, 1 H, H-pyridyl), 7.31 (d, J = 7.1 Hz, 1 H, H-quinolyl), 7.21 (ddd, J_1 = 7.5 Hz, J_2 = 4.8 Hz, J_3 = 1.1 Hz, 1 H, H-pyridyl), 5.77 (d, J = 5.3 Hz, 1 H, -N**H$_2$**), 5.10 (d, J = 10.6 Hz, 1 H, H-3), 4.72 (d, J = 10.4 Hz, 1 H, H-13), 4.35 (s, 1 H, H-5), 4.26 (d, J = 7.2 Hz, 1 H, H-1′), 3.94 (dd, J_1 = 18.4 Hz, J_2 = 16.0 Hz, 2 H, 3-O-CO-C**H$_2$**-), 3.61 (s, 1 H, H-11), 3.51–3.41 (m, 3 H, 1 H-cyclopropyl, H-5′, 12-OH), 3.37–3.23 (m, 5 H, 4 H-piperazinyl, H-2′), 2.91–2.83 (m, 2 H, 6-O-CO-CH_2C**H$_2$**–NH-), 2.82–2.54 (m, 9 H, H-2, H-10, 4 H-piperazinyl, 6-O-CO-C**H$_2$**CH_2–NH-, H-3′), 2.50–2.43 (m, 1 H, H-9a), 2.38 (s, 3 H, -N-CH_3), 2.35 (s, 6 H, -N$(CH_3)_2$), 2.32–2.27 (m, 1 H, H-4), 2.07–1.98 (m, 1 H, H-9b), 1.96–1.88 (m, 2 H, H-14eq, H-7a), 1.86–1.78 (m, 1 H, H-8), 1.75–1.67 (m, 1 H, H-4′a), 1.63 (s, 3 H, 6-CH_3), 1.59–1.51 (m, 1 H, H-14ax), 1.38–1.29 (m, 3 H, 2 H-cyclopropyl, H-7b), 1.29–1.23 (m, 1 H, H-4′b), 1.22–1.13 (m, 8 H, 5′-CH_3, 4-CH_3, 2 H-cyclopropyl), 1.11–1.03 (m, 9 H, 10-CH_3, 2-CH_3, 12-CH_3), 0.91 (d, J = 6.9 Hz, 3 H, 8-CH_3), 0.86 (t, J = 7.3 Hz, 3 H, 15-CH_3). ^{13}C

NMR ($CDCl_3$, 100 MHz) δ: 176.3, 175.4, 171.8, 170.0, 166.8, 154.7, 154.0, 152.2, 149.3, 147.1, 145.0, 144.9, 138.6, 136.6, 124.2, 122.3, 121.8, 121.7, 112.8, 112.6, 111.0, 104.7, 103.1, 87.1, 79.6, 79.0, 78.3, 77.7, 77.2, 74.7, 70.7, 69.4, 65.6, 61.6, 53.2, 52.6, 49.8, 44.0, 43.5, 40.4, 36.9, 34.7, 32.9, 27.3, 21.3, 21.2, 16.1, 11.0, 9.3, 8.2.

*4.3.18.12. 3-O-Descladinosyl-3-O-[2-(2-pyridyl)acetyl]-6-O-(3-{2-[3-(3-carboxy-6-fluoro-8-methoxy-1-cyclopropyl-1,4-dihydro-4-oxoquinolin-7-yl)methylpiperazin-1-yl]}propanoyl)azithromycin **(26l)**.* Compound **26l** (36.7 mg, 0.0320 mmol, 12.3%) was prepared starting from 3-*O*-descladinosyl-3-*O*-[2-(2-pyridyl)acetyl]-6-*O*-acryloylazithromycin (0.200 g, 0.260 mmol) and gatifloxacin (0.120 g, 0.310 mmol) according to the general procedure (column chromatography eluents: CH_2Cl_2/MeOH/$NH_3{\cdot}H_2O$ = 10:0.8:0.5). Melting point: 147–150 °C. HRMS (ESI) $(M + H)^+$ *m/z* 1139.6264, calcd for $C_{59}H_{88}FN_6O_{15}$ 1139.6286. ^{1}H NMR ($CDCl_3$, 700 MHz) δ: 8.80 (s, 1 H, H-quinolyl), 8.52 (d, J = 2.8 Hz, 1 H, H-pyridyl), 7.85 (dd, J_1 = 12.0 Hz, J_2 = 2.7 Hz, 1 H, H-quinolyl), 7.70–7.66 (m, 1 H, H-pyridyl), 7.41 (d, J = 7.9 Hz, 1 H, H-pyridyl), 7.21 (t, J = 6.2 Hz, 1 H, H-pyridyl), 5.10 (t, J = 8.4 Hz, 1 H, H-3), 4.67 (t, J = 9.1 Hz, 1 H, H-13), 4.37 (d, J = 17.4 Hz, 1 H, H-5), 4.27 (d, J = 7.4 Hz, 1 H, H-1′), 4.05–4.00 (m, 1 H, 1 H-cyclopropyl), 3.98–3.90 (m, 2-H, 3-O-CO-CH_2-), 3.74 (d, J = 4.4 Hz, 3 H, Ar-O-CH_3), 3.61 (s, 1 H, H-11), 3.61–3.56 (m, 1 H, 12-OH), 3.50–3.38 (m, 4 H, H-5′, 3 H-piperazinyl), 3.25 (t, J = 8.8 Hz, 1 H, H-2′), 3.21–3.12 (m, 1 H, 1 H-piperazinyl), 3.10–3.04 (m, 1 H, 1 H-piperazinyl), 3.02–2.92 (m, 2 H, 6-O-CO-CH_2C**H$_2$**–NH-), 2.91–2.83 (m, 2 H, H-2, 1 H-piperazinyl), 2.75–2.60 (m, 5 H, H-10, H-3′, 6-O-CO-C**H$_2$**CH_2–NH-, 1 H-piperazinyl), 2.56–2.46 (m, 1 H, H-9a), 2.39 (s, 3 H, -N-CH_3), 2.33 (s, 6 H, -N(CH_3)$_2$), 2.30–2.27 (m, 1 H, H-4), 2.05–1.99 (m, 1 H, H-9b), 1.96–1.88 (m, 2 H, H-14eq, H-7a), 1.85–1.78 (m, 1 H, H-8), 1.71–1.66 (m, 1 H, H-4′a), 1.64 (s, 3 H, 6-CH_3), 1.59–1.53 (m, 1 H, H-14ax), 1.38–1.32 (m, 1 H, H-7b), 1.28–1.19 (m, 6 H, H-4′b, 2 H-cyclopropyl, piperazinyl-C**H$_3$**), 1.18–1.13 (m, 6 H, 5′-CH_3, 4-CH_3), 1.09 (s, 3 H, 12-CH_3), 1.08–1.03 (m, 6 H, 10-CH_3, 2-CH_3), 1.02–0.96 (m, 2 H, 2 H-cyclopropyl), 0.94–0.89 (m, 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: 177.0, 177.0, 176.4, 172.1, 172.0, 170.0, 169.9, 166.8, 156.7, 155.3, 154.0, 149.8, 149.2, 145.1, 139.6, 136.6, 134.0, 124.2, 122.3, 121.5, 108.2, 108.0, 107.7, 103.2, 87.0, 79.3, 79.0, 78.3, 77.6, 74.7, 70.7, 69.4, 65.6, 62.6, 61.5, 57.5, 55.0, 54.9, 51.5, 51.1, 50.9, 48.8, 48.5, 44.0, 43.5, 40.5, 40.4, 38.5, 36.9, 31.6, 31.5, 28.8, 27.2, 21.4, 21.2, 21.1, 21.1, 16.1, 15.8, 15.7, 11.0, 9.6, 9.4, 9.3, 8.2.

*4.3.18.13. 3-O-Descladinosyl-3-O-[2-(2-pyridyl)acetyl]-6-O-(3-{2-[(3-carboxy-6-fluoro-1-ethyl-1,4-dihydro-4-oxoquinolin-7-yl)piperazin-1-yl]}propanoyl)azithromycin **(26m)**.* Compound **26m** (24.1 mg, 0.0220 mmol, 16.9%) was prepared starting from 3-*O*-descladinosyl-3-*O*-[2-(2-pyridyl)acetyl]-6-*O*-acryloylazithromycin (0.100 g, 0.130 mmol) and norfloxacin (0.0500 g, 0.160 mmol) according to the general procedure (column chromatography eluents: CH_2Cl_2/MeOH/$NH_3{\cdot}H_2O$ = 10:1:0.5). Melting point: 144–147 °C. HRMS (ESI) $(M + H)^+$ *m/z* 1083.6035, calcd for $C_{56}H_{84}FN_6O_{14}$ 1083.6024. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 15.14 (s, 1 H, -COOH), 8.67 (s, 1 H, H-quinolyl), 8.51 (d, J = 4.0 Hz, 1 H, H-pyridyl), 8.05 (d, J = 13.1 Hz, 1 H, H-quinolyl), 7.67 (td, J_1 = 7.7 Hz, J_2 = 1.9 Hz, 1 H, H-pyridyl), 7.40 (d, J = 7.8 Hz, 1 H, H-pyridyl), 7.20 (dd, J_1 = 7.6 Hz, J_2 = 4.9 Hz, 1 H, H-pyridyl), 6.82 (d, J = 6.7 Hz, 1 H, H-quinolyl), 5.10 (d, J = 10.4 Hz, 1 H, H-3), 4.70 (d, J = 10.6 Hz, 1 H, H-13), 4.32 (s, 1 H, H-5), 4.37–4.27 (m, 2 H, -C**H$_2$**CH_3), 4.25 (d, J = 7.2 Hz, 1 H, H-1′), 3.98–3.89 (m, 2 H, 3-O-CO-CH_2-), 3.60 (s, 1 H, H-11), 3.50–3.41 (m, 2 H, H-5′, 12-OH), 3.40–3.30 (m, 4 H, 4 H-piperazinyl), 3.24 (dd, J_1 = 10.2 Hz, J_2 = 7.2 Hz, 1 H, H-2′), 2.91–2.60 (m, 10 H, 6-O-CO-C**H$_2$C H$_2$**–NH-, H-2, H-10, 4 H-piperazinyl), 2.58–2.52 (m, 2 H, H-3′, 11-OH), 2.50–2.44 (m, 1 H, H-9a), 2.38 (s, 3 H, -N-CH_3), 2.31 (s, 6 H, -N(CH_3)$_2$), 2.30–2.26 (m, 1 H, H-4), 2.06–1.99 (m, 1 H, H-9b), 1.96–1.88 (m, 2 H, H-14eq, H-7a), 1.86–1.80 (m, 1 H, H-8), 1.70–1.65 (m, 1 H, H-4′a), 1.64 (s, 3 H, 6-CH_3), 1.58 (t, J = 7.1 Hz, 3 H, -CH_2C**H$_3$**), 1.55–1.51 (m, 1 H, H-14ax), 1.35 (dd, J_1 = 14.1 Hz, J_2 = 5.6 Hz, 1 H, H-7b), 1.27–1.21 (m, 1 H, H-4′b), 1.21–1.14 (m, 6 H, 5′-CH_3, 4-CH_3), 1.10–1.02 (m, 9 H, 10-CH_3, 2-CH_3, 12-CH_3), 0.90 (d, J = 6.9 Hz, 3 H, 8-CH_3), 0.86 (t, J = 7.3 Hz, 3 H, 15-CH_3). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 177.0, 176.3, 171.7, 170.0, 167.3, 154.8, 154.0, 149.3, 147.0, 137.1, 136.6, 124.1, 122.3, 112.9, 112.7, 108.4, 103.6, 103.2, 87.2, 79.4, 79.0, 78.3, 77.7, 77.2, 74.7, 70.7, 69.5, 65.6, 61.6, 53.2, 52.5, 49.8, 44.0, 43.5, 40.4, 36.9, 32.9, 27.3, 21.4, 21.2, 21.1, 16.1, 14.5, 11.0, 9.3, 8.3.

*4.3.18.14. 3-O-Descladinosyl-3-O-[2-(2-pyridyl)acetyl]-6-O-(3-{2-[(3-carboxy-6-fluoro-1-ethyl-1,4-dihydro-1,8-naphthyridine-4-oxoquinolin-7-yl)piperazin-1-yl]}propanoyl)azithromycin **(26n)**.* Compound **26n** (34.4 mg, 0.0320 mmol, 24.6%) was prepared starting from 3-*O*-descladinosyl-3-*O*-[2-(2-pyridyl)acetyl]-6-*O*-acryloylazithromycin (0.100 g, 0.130 mmol) and enoxacin (0.0500 g, 0.160 mmol) according to the general procedure (column chromatography eluents: CH_2Cl_2/MeOH/$NH_3{\cdot}H_2O$ = 10:0.8:0.5). Melting point: 137–140 °C. HRMS (ESI) $(M + H)^+$ *m/z* 1084.5987, calcd for $C_{55}H_{83}FN_7O_{14}$ 1084.5977. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 15.09 (s, 1 H, -COOH), 8.68 (s, 1 H, H-quinolyl), 8.52 (dd, J_1 = 5.1 Hz, J_2 = 1.7 Hz, 1 H, H-pyridyl), 8.08 (d, J = 13.4 Hz, 1 H, H-quinolyl), 7.68 (td, J_1 = 7.7 Hz, J_2 = 1.9 Hz, 1 H, H-pyridyl), 7.39 (d, J = 7.8 Hz, 1 H, H-pyridyl), 7.21 (dd, J_1 = 7.6 Hz, J_2 = 4.9 Hz, 1 H, H-pyridyl), 5.10 (d, J = 10.7 Hz, 1 H, H-3), 4.71 (d, J = 10.6 Hz, 1 H, H-13), 4.40 (m, 2 H, -C**H$_2$**CH_3), 4.30 (s, 1 H, H-5), 4.26 (d, J = 7.2 Hz, 1 H, H-1′), 3.99–3.92 (m, 2 H, 3-O-CO-CH_2-), 3.92–3.84 (m, 4 H, 4 H-piperazinyl), 3.82–3.71 (m, 1 H, 2′-OH), 3.60 (s, 1 H, H-11), 3.51–3.41 (m, 2 H, H-5′, 12-OH), 3.25 (dd, J_1 = 10.2 Hz, J_2 = 7.2 Hz, 1 H, H-2′), 2.91–2.79 (m, 3 H, 6-O-CO-CH_2C**H$_2$**–NH-, H-2), 2.77–2.50 (m, 8 H, H-10, 4 H-piperazinyl, H-3′, 6-O-CO-C**H$_2$**CH_2–NH-), 2.48–2.42 (m, 1 H, H-9a), 2.37 (s, 3 H, -N-CH_3), 2.33 (s, 6 H, -N(CH_3)$_2$), 2.30–2.25 (m, 1 H, H-4), 2.07–1.99 (m, 1 H, H-9b), 1.96–1.87 (m, 2 H, H-14eq, H-7a), 1.86–1.80 (m, 1 H, H-8), 1.72–1.66 (m, 1 H, H-4′a), 1.63 (s, 3 H, 6-CH_3), 1.59–1.53 (m, 1 H, H-14ax), 1.49 (t, J = 7.1 Hz, 3 H, -CH_2C**H$_3$**), 1.35 (dd, J_1 = 14.1 Hz, J_2 = 5.7 Hz, 1 H, H-7b), 1.26 (t, J = 11.9 Hz, 1 H, H-4′b), 1.19 (d, J = 6.1 Hz, 3 H, 5′-CH_3), 1.16 (d, J = 7.4 Hz, 3 H, 4-CH_3), 1.11–1.01 (m, 9 H, 10-CH_3, 2-CH_3, 12-CH_3), 0.90 (d, J = 6.9 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: 177.1, 176.3, 171.7, 170.0, 167.1, 154.0, 150.5, 150.4, 149.3, 148.7, 146.3, 146.1, 145.1, 136.6, 124.2, 122.3, 120.2, 120.0, 113.7, 109.3, 103.1, 87.2, 79.5, 79.0, 78.2, 77.7, 77.2, 74.6, 70.7, 69.4, 65.6, 61.6, 53.1, 52.7, 47.8, 47.0, 46.9, 44.0, 43.5, 40.4, 36.9, 32.9, 27.3, 21.4, 21.2, 16.1, 15.0, 11.0, 9.3, 8.3.

*4.3.18.15. 3-O-Descladinosyl-3-O-[2-(2-pyridyl)acetyl]-6-O-(3-{[4-(quinolin-6-yl)but-3-yn-1-yl]amino}propanoyl)azithromycin **(26o)**.* Compound **26o** (20.5 mg, 0.0210 mmol, 4.67%) was prepared starting from 3-*O*-descladinosyl-3-*O*-[2-(2-pyridyl)acetyl]-6-*O*-acryloylazithromycin (0.340 g, 0.450 mmol) and compound **11b** (0.0880 g, 0.450 mmol) according to the general procedure (column chromatography eluents: CH_2Cl_2/EtOH/$NH_3{\cdot}H_2O$ = 10:0.3:0.05). HRMS (ESI) $(M + H)^+$ *m/z* 960.5693, calcd for $C_{53}H_{78}N_5O_{11}$ 960.5692. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 8.88 (dd, J_1 = 4.2 Hz, J_2 = 1.7 Hz, 1 H, H-quinolyl), 8.51 (dd, J_1 = 5.0 Hz, J_2 = 0.9 Hz, 1 H, H-pyridyl), 8.08 (dd, J_1 = 8.3 Hz, J_2 = 1.7 Hz, 1 H, H-quinolyl), 7.99 (d, J = 8.7 Hz, 1 H, H-quinolyl), 7.89 (d, J = 1.7 Hz, 1 H, H-quinolyl), 7.72–7.64 (m, 2 H, H-quinolyl, H-pyridyl), 7.43–7.34 (m, 2 H, H-quinolyl, H-pyridyl), 7.19 (ddd, J_1 = 7.6 Hz, J_2 = 4.9 Hz, J_3 = 1.2 Hz, 1 H, H-pyridyl), 5.12 (dd, J_1 = 10.6 Hz, J_2 = 2.0, 1 H, H-3), 4.78 (dd, J_1 = 10.8 Hz, J_2 = 2.0, 1 H, H-13), 4.32 (d, J = 3.0 Hz, 1 H, H-5), 4.24 (d, J = 7.2 Hz, 1 H, H-1′), 3.94 (dd, J_1 = 16 Hz, J_2 = 15.6 Hz, 2 H, 3-O-CO-CH_2-), 3.60 (s, 1 H, H-11), 3.47–3.40 (m, 2 H, H-5′, 12-OH), 3.24 (dd, J_1 = 10.2 Hz, J_2 = 7.2 Hz, 1 H, H-2′), 3.16–2.80 (m, 5 H, 6-O-CO-

$CH_2C\mathbf{H_2}$—NH—$C\mathbf{H_2}CH_2C{\equiv}C$—Ar, H-2), 2.79—2.59 (m, 7 H, 6-O-CO-$C\mathbf{H_2}CH_2$—NH—$CH_2C\mathbf{H_2}C{\equiv}C$—Ar, H-10, H-3′ 11-OH), 2.44—2.38 (m, 1 H, H-9a), 2.37 (s, 3 H, -N-CH_3), 2.31 (s, 6 H, -N$(CH_3)_2$), 2.29—2.25 (m, 1 H, H-4), 2.00 (t, J = 11.5 Hz, 1 H, H-9b), 1.94—1.80 (m, 3 H, H-14eq, H-7a, H-8), 1.69—1.63 (m, 1 H, H-4′a), 1.62 (s, 3 H, 6-CH_3), 1.58—1.46 (m, 1 H, H-14ax), 1.35 (dd, J_1 = 14.0 Hz, J_2 = 6.2, 1 H, H-7b), 1.25—1.21 (m, 1 H, H-4′b), 1.18—1.14 (m, 6 H, 5′-CH_3, 4-CH_3), 1.07 (s, 3 H, 12-CH_3), 1.06—1.02 (m, 6 H, 10-CH_3, 2-CH_3), 0.86 (d, J = 6.8 Hz, 3 H, 8-CH_3), 0.80 (t, J = 7.3 Hz, 3 H, 15-CH_3). ^{13}C NMR ($CDCl_3$, 175 MHz) δ: 176.0, 171.9, 170.0, 154.0, 150.6, 149.2, 147.4, 136.6, 135.6, 132.5, 130.9, 129.4, 128.0, 124.2, 122.3, 122.1, 121.6, 103.2, 89.3, 87.4, 81.4, 79.6, 79.0, 78.1, 77.9, 74.5, 70.7, 69.4, 65.5, 61.8, 48.0, 44.2, 44.0, 43.4, 40.4, 38.7, 36.8, 35.1, 28.8, 27.4, 21.2, 21.2, 21.0, 20.2, 16.3, 16.1, 10.8, 9.3, 8.2.

*4.3.18.16. 3-O-Descladinosyl-3-O-[2-(2-pyridyl)acetyl]-6-O-(3-{[4-(1,4-dihydro-4-oxo-quinolin-6-yl)but-3-yn-1-yl]amino}propanoyl) azithromycin **(26p)**.* Compound **26p** (38.6 mg, 0.0400 mmol, 12.9%) was prepared starting from 3-*O*-descladinosyl-3-*O*-[2-(2-pyridyl) acetyl]-6-*O*-acryloylazithromycin (0.300 g, 0.390 mmol) and compound **11c** (0.0650 g, 0.310 mmol) according to the general procedure (column chromatography eluents: CH_2Cl_2/MeOH/$NH_3{\cdot}H_2O$ = 10:1.5:0.1). Melting point: 132—135 °C. HRMS (ESI) $(M + H)^+$ *m/z* 976.5631, calcd for $C_{53}H_{78}N_5O_{12}$ 976.5641. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 8.51—8.45 (m, 1 H, H-pyridyl), 8.28 (s, 1 H, H-quinolyl), 7.86—7.75 (m, 1 H, H-quinolyl), 7.66 (td, J_1 = 7.7 Hz, J_2 = 1.9 Hz, 1 H, H-pyridyl), 7.51—7.43 (m, 1 H, H-quinolyl), 7.41—7.34 (m, 2 H, H-quinolyl, H-pyridyl), 7.19 (ddd, J_1 = 7.6 Hz, J_2 = 4.9 Hz, J_3 = 1.1 Hz, 1 H, H-pyridyl), 6.38—6.16 (m, 1 H, H-quinolyl), 5.05 (d, J = 10.7 Hz, 1 H, H-3), 4.84—4.61 (m, 1 H, H-13), 4.41 (d, J = 2.8 Hz, 1 H, H-5), 4.22 (d, J = 7.2 Hz, 1 H, H-1′), 3.93 (dd, J_1 = 18.0 Hz, J_2 = 16.0 Hz, 2 H, 3-O-CO-CH_2-), 3.55 (s, 1 H, H-11), 3.47—3.37 (m, 2 H, H-5′, 12-OH), 3.22 (dd, J_1 = 10.2 Hz, J_2 = 7.1 Hz, 1 H, H-2′), 3.18—2.92 (m, 4 H, 6-O-CO-$CH_2C\mathbf{H_2}$—NH—$C\mathbf{H_2}CH_2C{\equiv}C$—Ar, H-2), 2.86—2.56 (m, 7 H, 6-O-CO-$C\mathbf{H_2}C\mathbf{H_2}$—NH—$CH_2C\mathbf{H_2}C{\equiv}C$—Ar, H-10, H-3′), 2.39—2.34 (m, 1 H, H-9a), 2.28 (s, 9 H, -N-CH_3, -N$(CH_3)_2$), 2.52—2.20 (m, 1 H, H-4), 2.03—1.90 (m, 2 H, H-9b, H-14eq), 1.87—1.78 (m, 1 H, H-7a), 1.76—1.67 (m, 1 H, H-8), 1.66—1.62 (m, 1 H, H-4′a), 1.59 (s, 3 H, 6-CH_3), 1.51—1.38 (m, 1 H, H-14ax), 1.32 (dd, J_1 = 13.7 Hz, J_2 = 5.7 Hz, 1 H, H-7b), 1.27—1.19 (m, 1 H, H-4′b), 1.17—1.10 (m, 6 H, 5′-CH_3, 4-CH_3), 1.06—0.92 (m, 9 H, 2-CH_3, 12-CH_3, 10-CH_3), 0.88—0.80 (m, 6 H, 8-CH_3, 15-CH_3). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 178.2, 175.8, 172.1, 170.2, 153.9, 149.2, 139.4, 139.3, 136.7, 134.6, 129.0, 125.6, 124.2, 122.4, 119.1, 118.5, 109.6, 103.2, 87.4, 81.3, 79.3, 78.9, 78.4, 78.0, 77.2, 74.6, 70.8, 69.5, 65.5, 61.7, 48.1, 44.3, 43.9, 43.4, 40.4, 38.6, 36.8, 35.0, 28.7, 27.2, 21.2, 20.2, 16.5, 16.0, 10.7, 9.3, 8.1.

*4.3.18.17. 3-O-Descladinosyl-3-O-[2-(2-pyridyl)acetyl]-6-O-(3-{[4-(3-carbamoylpyrid-5-yl)but-3-yn-1-yl]amino}propanoyl)azithromycin **(26q)**.* Compound **26q** (48.0 mg, 0.0500 mmol, 12.8%) was prepared starting from 3-*O*-descladinosyl-3-*O*-[2-(2-pyridyl) acetyl]-6-*O*-acryloylazithromycin (0.300 g, 0.390 mmol) and compound **11d** (0.0790 g, 0.420 mmol) according to the general procedure (column chromatography eluents: CH_2Cl_2/EtOH/$NH_3{\cdot}H_2O$ = 10:0.4:0.05). Melting point: 104—107 °C. HRMS (ESI) $(M + H)^+$ *m/z* 953.5584, calcd for $C_{50}H_{77}N_6O_{12}$ 953.5594. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 8.98 (d, J = 2.1 Hz, 1 H, H-carbamoylpyridyl), 8.70 (d, J = 1.9 Hz, 1 H, H-carbamoylpyridyl), 8.59 (dd, J_1 = 5.0 Hz, J_2 = 1.1 Hz, 1 H, H-pyridyl), 8.22 (t, J = 2.1 Hz, 1 H, H-carbamoylpyridyl), 7.67 (td, J_1 = 7.7 Hz, J_2 = 1.9 Hz, 1 H, H-pyridyl), 7.35 (d, J = 7.8 Hz, 1 H, H-pyridyl), 7.21 (ddd, J_1 = 7.6 Hz, J_2 = 4.9 Hz, J_3 = 1.1 Hz, 1 H, H-pyridyl), 7.00 (s, 1 H, -N$\mathbf{H}_2$), 6.45 (s, 1 H, -N$\mathbf{H}_2$), 5.07 (dd, J_1 = 10.6 Hz, J_2 = 1.9 Hz, 1 H, H-3), 4.76 (dd, J_1 = 10.8 Hz, J_2 = 2.0 Hz, 1 H, H-13), 4.37 (d, J = 2.9 Hz, 1 H, H-5), 4.26 (d, J = 7.2 Hz, 1 H, H-1′), 3.95 (q, J = 16.0 Hz, 2 H, 3-O-CO-CH_2-), 3.59 (s, 1 H, H-11), 3.54—3.45 (m, 2 H, H-5′, 12-OH), 3.23 (dd, J_1 = 10.2 Hz, J_2 = 7.2 Hz, 1 H, H-2′), 3.15—2.88 (m, 5 H, 6-O-CO-$CH_2C\mathbf{H_2}$—NH—$C\mathbf{H_2}CH_2C{\equiv}C$—Ar, 11-OH), 2.88—2.80 (m, 1 H, H-2), 2.72—2.54 (m, 6 H, 6-O-CO-$C\mathbf{H_2}CH_2$—NH—$CH_2C\mathbf{H_2}C{\equiv}C$—Ar, H-10, H-3′), 2.47—2.40 (m, 1 H, H-9a), 2.36 (s, 3 H, -N-CH_3), 2.28 (s, 6 H, -N$(CH_3)_2$), 2.27—2.22 (m, 1 H, H-4), 2.01 (dd, J_1 = 12.8 Hz, J_2 = 10.1 Hz, 1 H, H-9b), 1.95—1.85 (m, 2 H, H-14eq, H-7a), 1.80—1.72 (m, 1 H, H-8), 1.68—1.62 (m, 1 H, H-4′a), 1.58 (s, 3 H, 6-CH_3), 1.56—1.50 (m, 1 H, H-14ax), 1.34 (dd, J_1 = 14.1 Hz, J_2 = 6.1 Hz, 1 H, H-7b), 1.25—1.20 (m, 1 H, H-4′b), 1.19—1.14 (m, 6 H, 5′-CH_3, 4-CH_3), 1.09—1.01 (m, 9 H, 2-CH_3, 12-CH_3, 10-CH_3), 0.88 (d, J = 6.9 Hz, 3 H, 8-CH_3), 0.81 (t, J = 7.3 Hz, 3 H, 15-CH_3). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 176.1, 172.5, 170.3, 166.8, 154.2, 153.8, 149.4, 147.4, 137.8, 136.7, 128.5, 124.1, 122.4, 120.6, 103.6, 93.2, 87.4, 79.9, 79.0, 78.3, 78.0, 77.9, 77.3, 74.6, 70.8, 69.5, 65.5, 61.7, 47.7, 44.4, 43.7, 43.5, 40.3, 38.5, 36.9, 35.4, 28.6, 27.3, 21.2, 21.2, 20.5, 16.4, 16.0, 10.9, 9.3, 8.2.

*4.3.18.18. 3-O-Descladinosyl-3-O-[2-(2-pyridyl)acetyl]-6-O-(3-{[4-(3-carbamoyl-1-cyclopropyl-1,4-dihydro-4-oxoquinolin-6-yl)but-3-yn-1-yl]amino}propanoyl)azithromycin **(26r)**.* Compound **26r** (84.3 mg, 0.0800 mmol, 20.5%) was prepared starting from 3-*O*-descladinosyl-3-*O*-[2-(2-pyridyl)acetyl]-6-*O*-acryloylazithromycin (0.300 g, 0.390 mmol) and compound **11e** (0.200 g, 0.680 mmol) according to the general procedure (column chromatography eluents: CH_2Cl_2/EtOH/$NH_3{\cdot}H_2O$ = 10:0.3:0.05). Melting point: 143—147 °C. HRMS (ESI) $(M + H)^+$ *m/z* 1059.6023, calcd for $C_{57}H_{83}N_6O_{13}$ 1059.6013. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 9.61 (d, J = 5.2 Hz, 1 H, -N$\mathbf{H}_2$), 8.83 (s, 1 H, H-quinolyl), 8.52 (d, J = 4.9 Hz, 1 H, H-pyridyl), 8.44 (d, J = 1.9 Hz, 1 H, H-quinolyl), 7.90 (d, J = 8.8 Hz, 1 H, H-quinolyl), 7.76 (dd, J_1 = 8.8 Hz, J_2 = 2.0 Hz, 1 H, H-quinolyl), 7.68 (td, J_1 = 7.7 Hz, J_2 = 1.9 Hz, 1 H, H-pyridyl), 7.40 (d, J = 7.8 Hz, 1 H, H-pyridyl), 7.21 (ddd, J_1 = 7.6 Hz, J_2 = 4.9 Hz, J_3 = 1.1 Hz, 1 H, H-pyridyl), 5.82 (d, J = 5.2 Hz, 1 H, -N$\mathbf{H}_2$), 5.10 (d, J = 10.6 Hz, 1 H, H-3), 4.85 (d, J = 10.7 Hz, 1 H, H-13), 4.40 (s, 1 H, H-5), 4.25 (d, J = 7.2 Hz, 1 H, H-1′), 3.95 (dd, J_1 = 20.8 Hz, J_2 = 16.0 Hz, 2 H, 3-O-CO-CH_2-), 3.62 (s, 1 H, H-11), 3.53—3.41 (m, 3 H, 1 H-cyclopropyl, H-5′, 12-OH), 3.27 (t, J = 8.7 Hz, 1 H, H-2′), 3.22—2.98 (m, 5 H, 6-O-CO-$CH_2C\mathbf{H_2}$—NH—$C\mathbf{H_2}CH_2C{\equiv}C$—Ar, H-2), 2.88—2.61 (m, 7 H, 6-O-CO-$C\mathbf{H_2}CH_2$—NH—$CH_2C\mathbf{H_2}C{\equiv}C$—Ar, H-10, H-3′, 11-OH), 2.42—2.32 (m, 10 H, H-9a, -N-CH_3, -N$(CH_3)_2$), 2.28—2.21 (m, 1 H, H-4), 2.06—1.96 (m, 1 H, H-9b), 1.94—1.83 (m, 2 H, H-14eq, H-7a), 1.82—1.75 (m, 1 H, H-8), 1.74—1.67 (m, 1 H, H-4′a), 1.59 (s, 3 H, 6-CH_3), 1.54—1.48 (m, 1 H, H-14ax), 1.38—1.31 (m, 3 H, H-7b, 2 H-cyclopropyl), 1.28—1.23 (m, 1 H, H-4′b), 1.20—1.13 (m, 8 H, 2 H-cyclopropyl, 5′-CH_3, 4-CH_3), 1.09—1.01 (m, 9 H, 2-CH_3, 12-CH_3, 10-CH_3), 0.87 (d, J = 6.5 Hz, 3 H, 8-CH_3), 0.80 (t, J = 7.3 Hz, 3 H, 15-CH_3). ^{13}C NMR ($CDCl_3$, 175 MHz) δ: 175.9, 175.8, 171.7, 170.1, 166.4, 154.0, 149.2, 147.7, 139.9, 136.7, 135.7, 130.2, 127.2, 124.2, 122.4, 121.0, 116.8, 111.8, 103.1, 87.6, 80.8, 79.6, 79.1, 78.3, 77.8, 74.5, 70.7, 69.3, 65.5, 61.7, 47.8, 44.1, 44.0, 43.4, 40.4, 36.7, 34.8, 29.7, 29.1, 27.3, 21.3, 21.2, 21.1, 19.6, 16.5, 16.1, 10.8, 9.3, 8.3, 8.0.

*4.3.18.19. 3-O-Descladinosyl-3-O-[2-(2-pyridyl)acetyl]-6-O-(3-{[4-(3-carbamoyl-1-ethyl-1,4-dihydro-4-oxoquinolin-6-yl)but-3-yn-1-yl]amino}propanoyl)azithromycin **(26s)**.* Compound **26s** (0.160 g, 0.150 mmol, 48.4%) was prepared starting from 3-*O*-descladinosyl-3-*O*-[2-(2-pyridyl)acetyl]-6-*O*-acryloylazithromycin (0.300 g, 0.390 mmol) and compound **11f** (0.0870 g, 0.310 mmol) according to the general procedure (column chromatography eluents: CH_2Cl_2/EtOH/$NH_3{\cdot}H_2O$ = 10:0.2:0.05). Melting point: 126—129 °C. HRMS (ESI) $(M + H)^+$ *m/z* 1047.6026, calcd for $C_{56}H_{83}N_6O_{13}$ 1047.6013. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 9.68 (d, J = 5.3 Hz, 1 H, -N$\mathbf{H}_2$), 8.75 (s, 1 H, H-quinolyl), 8.53—8.50 (m, 2 H, H-pyridyl, H-quinolyl), 7.73 (dd, J_1 = 8.8 Hz, J_2 = 2.1 Hz, 1 H, H-quinolyl), 7.68 (td, J_1 = 7.7 Hz, J_2 = 1.9 Hz, 1 H, H-pyridyl), 7.45—7.40 (m, 2 H, H-pyridyl, H-

quinolyl), 7.20 (ddd, J_1 = 7.6 Hz, J_2 = 4.9 Hz, J_3 = 1.1 Hz, 1 H, H-pyridyl), 5.89 (d, J = 5.1 Hz, 1 H, -N**H**$_2$), 5.11 (dd, J_1 = 10.6 Hz, J_2 = 2.0, 1 H, H-3), 4.80 (dd, J_1 = 10.8 Hz, J_2 = 2.0, 1 H, H-13), 4.34 (d, J = 3.0 Hz, 1 H, H-5), 4.30 (q, J = 7.3 Hz, 2 H, -C**H**$_2$CH$_3$), 4.25 (d, J = 7.2 Hz, 1 H, H-1′), 3.95 (dd, J_1 = 17.6 Hz, J_2 = 16.0 Hz, 2 H, 3-O-CO-CH$_2$-), 3.60 (s, 1 H, H-11), 3.49–3.39 (m, 2 H, H-5′, 12-OH), 3.23 (dd, J_1 = 10.2 Hz, J_2 = 7.2 Hz, 1 H, H-2′), 3.14–2.80 (m, 5 H, 6-O-CO-CH$_2$C**H**$_2$–NH–C**H**$_2$CH$_2$C≡C–Ar, H-2), 2.71–2.57 (m, 7 H, 6-O-CO-C**H**$_2$CH$_2$–NH–CH$_2$C**H**$_2$C≡C–Ar, H-10, H-3′,11-OH), 2.44–2.39 (m, 1 H, H-9a), 2.37 (s, 3 H, -N-CH$_3$), 2.30 (s, 6 H, -N(CH$_3$)$_2$), 2.28–2.25 (m, 1 H, H-4), 2.05–1.96 (m, 1 H, H-9b), 1.95–1.85 (m, 2 H, H-14eq, H-7a), 1.85–1.77 (m, 1 H, H-8), 1.68–1.63 (m, 1 H, H-4′a), 1.62 (s, 3 H, 6-CH$_3$), 1.53 (t, J = 7.3 Hz, 3 H, -CH$_2$C**H**$_3$), 1.51–1.45 (m, 1 H, H-14ax), 1.35 (dd, J_1 = 14.0 Hz, J_2 = 6.3 Hz, 1 H, H-7b), 1.28–1.20 (m, 1 H, H-4′b), 1.19–1.14 (m, 6 H, 5′-CH$_3$, 4-CH$_3$), 1.07 (s, 3 H, 12-CH$_3$), 1.06–1.01 (m, 6 H, 10-CH$_3$, 2-CH$_3$), 0.87 (d, J = 6.8 Hz, 3 H, 8-CH$_3$), 0.80 (t, J = 7.3 Hz, 3 H, 15-CH$_3$). ^{13}C NMR (CDCl$_3$, 100 MHz) δ: 175.9, 175.7, 171.9, 170.0, 166.6, 154.0, 149.2, 147.5, 137.9, 136.6, 135.8, 130.7, 128.0, 124.2, 122.3, 121.1, 115.8, 112.0, 103.2, 90.0, 87.3, 80.2, 79.5, 78.9, 78.2, 77.8, 77.3, 74.5, 70.8, 69.4, 65.5, 61.7, 49.1, 48.1, 44.2, 44.0, 43.4, 38.6, 36.8, 35.2, 28.8, 27.4, 21.0, 20.3, 16.3, 16.0, 14.6, 10.8, 9.3, 8.2.

4.3.18.20. 3-O-Descladinosyl-3-O-[2-(2-pyridyl)acetyl]-6-O-(3-{[4-(3-carbamoyl-1-methyl-1,4-dihydro-4-oxoquinolin-6-yl)but-3-yn-1-yl]amino}propanoyl)azithromycin ***(26t)***. Compound **26t** (84.0 mg, 0.0810 mmol, 27.9%) was prepared starting from 3-*O*-descladinosyl-3-*O*-[2-(2-pyridyl)acetyl]-6-*O*-acryloylazithromycin (0.270 g, 0.350 mmol) and compound **11g** (0.0790 g, 0.290 mmol) according to the general procedure (column chromatography eluents: CH_2Cl_2/EtOH/$NH_3{\cdot}H_2O$ = 10:0.5:0.05). Melting point: 139–142 °C. HRMS (ESI) $(M + H)^+$ *m/z* 1033.5845, calcd for $C_{55}H_{81}N_6O_{13}$ 1033.5856. ^{1}H NMR (CDCl$_3$, 400 MHz) δ: 9.63 (d, J = 5.0 Hz, 1 H, -N**H**$_2$), 8.69 (s, 1 H, H-quinolyl), 8.51 (d, J = 3.9 Hz, 1 H, H-quinolyl), 8.46 (d, J = 1.9 Hz, 1 H, H-pyridyl), 7.71 (dd, J_1 = 8.9 Hz, J_2 = 2.1 Hz, 1 H, H-quinolyl), 7.70–7.65 (m, 1 H, H-pyridyl), 7.41 (d, J = 7.9 Hz, 1 H, H-quinolyl), 7.37 (d, J = 8.8 Hz, 1 H, H-pyridyl), 7.20 (ddd, J_1 = 7.6 Hz, J_2 = 4.9 Hz, J_3 = 1.1 Hz, 1 H, H-pyridyl), 6.03 (d, J = 5.1 Hz, 1 H, -N**H**$_2$), 5.11 (dd, J_1 = 10.6 Hz, J_2 = 2.0 Hz, 1 H, H-3), 4.81 (dd, J_1 = 10.8 Hz, J_2 = 2.1 Hz, 1 H, H-13), 4.35 (d, J = 3.0 Hz, 1 H, H-5), 4.25 (d, J = 7.2 Hz, 1 H, H-1′), 3.95 (dd, J_1 = 17.2 Hz, J_2 = 16.0 Hz, 2 H, 3-O-CO-CH$_2$-), 3.90 (s, 3 H, Ar-CH$_3$), 3.61 (s, 1 H, H-11), 3.49–3.40 (m, 2 H, H-5′, 12-OH), 3.24 (dd, J_1 = 10.2 Hz, J_2 = 7.2 Hz, 1 H, H-2′), 3.14–2.88 (m, 5 H, 6-O-CO-CH$_2$C**H**$_2$–NH–C**H**$_2$CH$_2$C≡C–Ar, 11-OH), 2.87–2.81 (m, 1 H, H-2), 2.72–2.55 (m, 6 H, 6-O-CO-C**H**$_2$CH$_2$–NH–CH$_2$C**H**$_2$C≡C–Ar, H-10, H-3′), 2.43–2.38 (m, 1 H, H-9a), 2.37 (s, 3 H, -N-CH$_3$), 2.30 (s, 6 H, -N(CH$_3$)$_2$), 2.29–2.27 (m, 1 H, H-4), 2.05–1.97 (m, 1 H, H-9b), 1.93–1.86 (m, 2 H, H-14eq, H-7a), 1.84–1.77 (m, 1 H, H-8), 1.68–1.63 (m, 1 H, H-4′a), 1.62 (s, 3 H, 6-CH$_3$), 1.56–1.47 (m, 1 H, H-14ax), 1.35 (dd, J_1 = 13.9 Hz, J_2 = 6.1 Hz, 1 H, H-7b), 1.25–1.20 (m, 1 H, H-4′b), 1.19–1.14 (m, 6 H, 5′-CH$_3$, 4-CH$_3$), 1.09–1.01 (m, 9 H, 2-CH$_3$, 12-CH$_3$, 10-CH$_3$), 0.87 (d, J = 6.8 Hz, 3 H, 8-CH$_3$), 0.80 (t, J = 7.3 Hz, 3 H, 15-CH$_3$). ^{13}C NMR (CDCl$_3$, 100 MHz) δ: 175.9, 175.7, 171.9, 170.0, 166.6, 154.0, 149.2, 148.7, 138.9, 136.6, 135.8, 130.2, 127.5, 124.2, 122.3. 121.2, 116.0, 111.8, 103.2, 90.0, 87.3, 80.2, 79.5, 78.9, 78.2, 77.9, 77.3, 74.5, 70.7, 69.4, 65.5, 61.7, 48.0, 44.2, 44.0, 43.4, 41.4, 38.6, 36.8, 35.2, 28.7, 27.4, 21.2, 21.2, 21.0, 20.2, 16.4, 16.0, 10.8, 9.3, 8.1.

4.3.18.21. 3-O-Descladinosyl-3-O-[2-(2-pyridyl)acetyl]-6-O-(3-{[4-(3-methylcarbamoyl-1-cyclopropyl-1,4-dihydro-4-oxoquinolin-6-yl) but-3-yn-1-yl]amino}propanoyl)azithromycin ***(26u)***. Compound **26u** (20.6 mg, 0.0190 mmol, 9.05%) was prepared starting from 3-*O*-descladinosyl-3-*O*-[2-(2-pyridyl)acetyl]-6-*O*-acryloylazithromycin (0.190 g, 0.250 mmol) and compound **11h** (0.0640 g, 0.210 mmol) according to the general procedure (column chromatography eluents: CH_2Cl_2/EtOH/$NH_3{\cdot}H_2O$ = 10:0.3:0.05). Melting point: 127–130 °C. HRMS (ESI) $(M + Na)^+$ *m/z* 1095.5991, calcd for $C_{58}H_{84}N_6NaO_{13}$ 1095.5989. ^{1}H NMR (CDCl$_3$, 400 MHz) δ: 9.80 (d, J = 5.1 Hz, 1 H, -CO-N**H**-), 8.87 (s, 1 H, H-quinolyl), 8.51 (d, J = 4.8 Hz, 1 H, H-pyridyl), 8.48 (d, J = 1.9 Hz, 1 H, H-quinolyl), 7.90 (d, J = 8.8 Hz, 1 H, H-quinolyl), 7.74 (dd, J_1 = 8.8 Hz, J_2 = 2.0 Hz, 1 H, H-quinolyl), 7.68 (td, J_1 = 7.7 Hz, J_2 = 1.9 Hz, 1 H, H-pyridyl), 7.41 (d, J = 7.9 Hz, 1 H, H-pyridyl), 7.20 (ddd, J_1 = 7.5 Hz, J_2 = 4.9 Hz, J_3 = 1.1 Hz, 1 H, H-pyridyl), 5.11 (dd, J_1 = 10.6 Hz, J_2 = 1.9 Hz, 1 H, H-3), 4.79 (dd, J_1 = 10.7 Hz, J_2 = 2.0 Hz, 1 H, H-13), 4.34 (d, J = 3.0 Hz, 1 H, H-5), 4.24 (d, J = 7.2 Hz, 1 H, H-1′), 3.94 (dd, J_1 = 18.0 Hz, J_2 = 15.4 Hz, 2 H, 3-O-CO-CH$_2$-), 3.59 (s, 1 H, H-11), 3.53–3.40 (m, 3 H, 1 H-cyclopropyl, H-5′, 12-OH), 3.24 (dd, J_1 = 10.2 Hz, J_2 = 7.2 Hz, 1 H, H-2′), 3.14–3.06 (m, 1 H, 6-O-CO-CH$_2$C**H**$_2$–NH-), 3.00 (d, J = 4.9 Hz, 3 H, Ar-CH$_3$), 2.98–2.89 (m, 3 H, 6-O-CO-CH$_2$C**H**$_2$–NH–C**H**$_2$CH$_2$C≡C–Ar), 2.87–2.80 (m, 1 H, H-2), 2.71–2.56 (m, 7 H, 6-O-CO-C**H**$_2$CH$_2$–NH–CH$_2$C**H**$_2$C≡C–Ar, H-10, H-3′, 11-OH), 2.43–2.39 (m, 1 H, H-9a), 2.37 (s, 3 H, N–CH$_3$), 2.31 (s, 6 H, N(CH$_3$)$_2$), 2.30–2.28 (m, 1 H, H-4), 2.05–1.96 (m, 1 H, H-9b), 1.94–1.85 (m, 2 H, H-14eq, H-7a), 1.85–1.78 (m, 1 H, H-8), 1.69–1.63 (m, 1 H, H-4′a), 1.61 (s, 3 H, 6-CH$_3$), 1.56–1.48 (m, 1 H, H-14ax), 1.38–1.30 (m, 3 H, H-7b, 2 H-cyclopropyl), 1.28–1.20 (m, 1 H, H-4′b), 1.20–1.12 (m, 8 H, 2 H-cyclopropyl, 5′-CH$_3$, 4-CH$_3$), 1.09–1.01 (m, 9 H, 2-CH$_3$, 12-CH$_3$, 10-CH$_3$), 0.87 (d, J = 6.9 Hz, 3 H, 8-CH$_3$), 0.80 (t, J = 7.3 Hz, 3 H, 15-CH$_3$). ^{13}C NMR (CDCl$_3$, 175 MHz) δ: 176.1, 176.0, 172.0, 170.0, 165.4, 154.0, 149.2, 147.0, 139.8, 136.6, 135.6, 130.2, 127.3, 124.2, 122.3, 121.0, 116.7, 112.2, 103.2, 89.7, 87.4, 80.4, 79.5, 79.0, 78.2, 77.9, 74.5, 70.7, 69.4, 65.5, 61.7, 48.1, 44.2, 44.0, 43.4, 40.4, 36.8, 34.7, 28.8, 27.4, 25.9, 21.2, 21.2, 20.2, 16.3, 16.1, 10.8, 9.3, 8.2, 8.2.

4.3.18.22. 3-O-Descladinosyl-3-O-[2-(2-pyridyl)acetyl]-6-O-(3-{[4-(3-carboxy-1-cyclopropyl-1,4-dihydro-4-oxoquinolin-6-yl)but-3-yn-1-yl]amino}propanoyl)azithromycin ***(26w)***. Compound **26w** (25.5 mg, 0.0240 mmol, 9.23%) was prepared starting from 3-*O*-descladinosyl-3-*O*-[2-(2-pyridyl)acetyl]-6-*O*-acryloylazithromycin (0.200 g, 0.260 mmol) and compound **11j** (0.0920 g, 0.310 mmol) according to the general procedure (column chromatography eluents: CH_2Cl_2/MeOH/$NH_3{\cdot}H_2O$ = 10:0.8:0.5). Melting point: 155–159 °C. HRMS (ESI) $(M + H)^+$ *m/z* 1060.5836, calcd for $C_{57}H_{82}N_5O_{14}$ 1060.5853. ^{1}H NMR (CDCl$_3$, 700 MHz) δ: 8.85 (s, 1 H, H-quinolyl), 8.55–8.43 (m, 2 H, H-pyridyl, H-quinolyl), 8.01 (d, J = 8.9 Hz, 1 H, H-quinolyl), 7.85 (dd, J_1 = 8.8 Hz, J_2 = 2.0 Hz, 1 H, H-quinolyl), 7.69 (td, J_1 = 7.7 Hz, J_2 = 1.9 Hz, 1 H, H-pyridyl), 7.42 (d, J = 7.7 Hz, 1 H, H-pyridyl), 7.21 (dd, J_1 = 7.7 Hz, J_2 = 4.9 Hz, 1 H, H-pyridyl), 5.12 (dd, J_1 = 10.6 Hz, J_2 = 2.0 Hz, 1 H, H-3), 4.78 (d, J = 10.9 Hz, 1 H, H-13), 4.32 (s, 1 H, H-5), 4.25 (d, J = 7.3 Hz, 1 H, H-1′), 3.99–3.89 (m, 2 H, 3-O-CO-CH$_2$-), 3.60 (s, 1 H, H-11), 3.49–3.39 (m, 3 H, 1 H-cyclopropyl, H-5′, 12-OH), 3.24 (dd, J_1 = 10.2 Hz, J_2 = 7.1 Hz, 1 H, H-2′), 2.98–2.79 (m, 5 H, 6-O-CO-CH$_2$C**H**$_2$–NH–C**H**$_2$CH$_2$C≡C–Ar, 11-OH), 2.75–2.54 (m, 7 H, 6-O-CO-C**H**$_2$CH$_2$–NH–CH$_2$C**H**$_2$C≡C–Ar, H-2, H-10, H-3′), 2.43–2.38 (m, 1 H, H-9a), 2.38 (s, 3 H, -N-CH$_3$), 2.30 (s, 6 H, -N(CH$_3$)$_2$), 2.27–2.28 (m, 1 H, H-4), 2.04–1.98 (m, 1 H, H-9b), 1.93–1.87 (m, 2 H, H-14eq, H-7a), 1.86–1.80 (m, 1 H, H-8), 1.68–1.64 (m, 1 H, H-4′a), 1.63 (s, 3 H, 6-CH$_3$), 1.56–1.50 (m, 1 H, H-14ax), 1.44–1.39 (m, 2 H, 2 H-cyclopropyl), 1.38–1.34 (m, 1 H, H-7b), 1.28–1.20 (m, 3 H, H-4′b, 2 H-cyclopropyl), 1.20–1.15 (m, 6 H, 5′-CH$_3$, 4-CH$_3$), 1.09–1.02 (m, 9 H, 2-CH$_3$, 12-CH$_3$, 10-CH$_3$), 0.87 (d, J = 6.9 Hz, 3 H, 8-CH$_3$), 0.82 (t, J = 7.4 Hz, 3 H, 15-CH$_3$). ^{13}C NMR (CDCl$_3$, 175 MHz) δ: 178.1, 176.0, 172.0, 170.0, 166.8, 154.0, 149.2, 148.0, 140.0, 136.8, 136.6, 129.9, 125.9, 124.2, 122.6, 122.3, 117.2, 109.0, 103.2, 91.3, 87.3, 79.8, 79.5, 78.9, 78.1, 77.9, 74.5, 70.8, 69.5, 65.5, 61.8, 48.1, 44.2, 44.0, 43.4, 40.4, 36.8, 35.4, 35.4, 28.7, 27.5, 21.2, 20.4, 16.0, 10.9, 9.3, 8.3.

*4.3.19. 2′-O-Acetyl-3-O-decladinosyl-6-acryloylazithromycin-11,12-cyclic carbonate **(27)***

To a solution of compound **24** (0.500 g, 0.730 mmol) in 10 mL of CH_2Cl_2, pyridine (0.700 mL, 8.69 mmol) was added dropwise to the flask, and a solution of bis(trichloromethyl)carbonate (0.430 g, 1.46 mmol) in 8 mL CH_2Cl_2 was slowly added to the reaction mixture. The mixture was stirred at −10 °C ~ −5 °C for 4 h, and then at room temperature for 2 h. The reaction was quenched by the addition of 20 mL of saturated NaCl and stirred slowly for 30 min. The resulting mixture was diluted with CH_2Cl_2, and washed with saturated $NaHCO_3$, water and brine. The organic layer was concentrated and the residue was purified by flash silica gel column chromatography (CH_2Cl_2/EtOH/$NH_3{\cdot}H_2O$ = 10:0.2:0.05) to obtain pure compound **27** (0.390 g, 0.550 mmol, 75.3%). HRMS (ESI) $(M + H)^+$ *m/z* 713.4227, calcd for $C_{36}H_{61}N_2O_{12}$ 713.4219. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 6.34 (dd, J_1 = 17.3 Hz, J_2 = 1.6 Hz, 1 H, 6-O-CO-CH=C**H**$_2$), 6.04 (dd, J_1 = 17.2 Hz, J_2 = 10.3 Hz, 1 H, 6-O-CO-C**H**=CH$_2$), 5.78 (dd, J_1 = 10.3 Hz, J_2 = 1.6 Hz, 1 H, 6-O-CO-CH=C**H**$_2$), 4.92 (dd, J_1 = 9.2 Hz, J_2 = 3.3 Hz, 1 H, H-13), 4.80 (dd, J_1 = 10.4 Hz, J_2 = 7.6 Hz, 1 H, H-2′), 4.70 (d, J = 2.6 Hz, 1 H, H-5), 4.62 (d, J = 7.6 Hz, 1 H, H-1′), 4.51 (d, J = 3.8 Hz, 1 H, H-11), 3.54−3.42 (m, 2 H, H-5′, H-3), 2.84 (dq, J_1 = 6.5 Hz, J_2 = 3.7 Hz, 1 H, H-10), 2.77−2.68 (m, 1 H, H-3′), 2.68−2.61 (m, 1 H, H-2), 2.34−2.29 (m, 1 H, H-9a), 2.27 (s, 6 H, -N(CH$_3$)$_2$), 2.22 (s, 3 H, -N-CH$_3$), 2.07 (s, 3 H, 2′-O-CO-CH$_3$), 2.03−1.97 (m, 1 H, H-9b), 1.97−1.88 (m, 2 H, H-4, H-14eq), 1.88−1.82 (m, 1 H, H-7a), 1.77−1.68 (m, 2 H, H-4′a, H-8), 1.68−1.63 (m, 1 H, H-14ax), 1.61 (s, 3 H, 6-CH$_3$), 1.44 (s, 3 H, 12-CH$_3$), 1.40−1.29 (m, 1 H, H-7b), 1.27−1.23 (m, 6 H, 5′-CH$_3$, 2-CH$_3$), 1.23−1.16 (m, 1 H, H-4′b), 1.06 (d, J = 6.5 Hz, 3 H, 10-CH$_3$), 0.93−0.87 (m, 9 H, 15-CH$_3$, 8-CH$_3$, 4-CH$_3$).

*4.3.20. 2′-O-Acetyl-3-O-descladinosyl-3-O-[2-(2-pyridyl)acetyl]-6-O-acryloylazithromycin-11,12-cyclic carbonate **(28)***

Compound **28** (0.970 g, 1.16 mmol, 84.7%) was prepared starting from compound **27** (0.980 g, 1.37 mmol) and 2-pyridylacetic acid hydrochloride (0.480 g, 2.74 mmol) according to the procedure used to prepare compound **25** (column chromatography eluents: CH_2Cl_2/EtOH/$NH_3{\cdot}H_2O$ = 10:0.1:0.05).

*4.3.21. General procedure for the synthesis of compounds **29j**, **29k**, **29r** and **29w***

The compound **29** series was prepared starting from compound **28** and the corresponding amines according to the procedure used to prepare the compound **26** series.

*4.3.21.1. 3-O-Descladinosyl-3-O-[2-(2-pyridyl)acetyl]-6-O-(3-{2-[(3-carboxy-6-fluoro-1-cyclopropyl-1,4-dihydro-4-oxoquinolin-7-yl) piperazin-1-yl]}propanoyl)azithromycin-11,12-cyclic carbonate **(29j)**.* Compound **29j** (20.0 mg, 0.0180 mmol, 13.8%) was prepared starting from 3-*O*-descladinosyl-3-*O*-[2-(2-pyridyl)acetyl]-6-*O*-acryloylazithromycin-11,12-cyclic carbonate (0.100 g, 0.130 mmol) and ciprofloxacin (0.0560 g, 0.170 mmol) according to the general procedure (column chromatography eluents: CH_2Cl_2/MeOH/$NH_3{\cdot}H_2O$ = 10:0.8:0.5). Melting point: 148−158 °C. HRMS (ESI) $(M + H)^+$ *m/z* 1121.5839, calcd for $C_{58}H_{82}FN_6O_{15}$ 1121.5817. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 15.05 (s, 1 H, -COOH), 8.76 (s, 1 H, H-quinolyl), 8.52−8.49 (m, 1 H, H-pyridyl), 8.00 (d, J = 13.1 Hz, 1 H, H-quinolyl), 7.67 (td, J_1 = 7.7 Hz, J_2 = 1.9 Hz, 1 H, H-pyridyl), 7.38 (d, J = 8.0 Hz, 1 H, H-pyridyl), 7.36 (d, J = 7.3 Hz, 1 H, H-quinolyl), 7.20 (ddd, J_1 = 7.5 Hz, J_2 = 4.9 Hz, J_3 = 1.1 Hz, 1 H, H-pyridyl), 5.10 (d, J = 10.8 Hz, 1 H, H-3), 4.88 (dd, J_1 = 8.3 Hz, J_2 = 3.8 Hz, 1 H, H-13), 4.55 (d, J = 3.1 Hz, 1 H, H-5), 4.46 (d, J = 5.4 Hz, 1 H, H-11), 4.19 (d, J = 7.2 Hz, 1 H, H-1′), 3.93 (dd, J_1 = 19.6 Hz, J_2 = 16.0 Hz, 2 H, 3-O-CO-CH$_2$-), 3.58−3.51 (s, 1 H, 1 H-cyclopropyl), 3.45−3.38 (m, 1 H, H-5′), 3.35 (t, J = 5.0 Hz, 4 H, 4 H-piperazinyl), 3.24 (dd, J_1 = 10.1 Hz, J_2 = 7.2 Hz, 1 H, H-2′), 2.99−2.79 (m, 4 H, 6-O-CO-CH$_2$C**H$_2$**−NH-, H-2, H-10), 2.77−2.66 (m, 7 H, 4 H-piperazinyl, 6-O-CO-C**H$_2$**CH$_2$−NH-, H-3′), 2.37−2.35 (m, 1 H, H-9a), 2.33 (s, 6 H, -N(CH$_3$)$_2$), 2.19 (s, 3 H, -N-CH$_3$), 2.10−2.02 (m, 1 H, H-4), 1.99−1.86 (m, 3 H, H-9b, H-14eq, H-7a), 1.85−1.78 (m, 1 H, H-8), 1.73−1.64 (m, 2 H, H-4′a, H-14ax), 1.60 (s, 3 H, 6-CH$_3$), 1.48 (s, 3 H, 12-CH$_3$), 1.42−1.36 (m, 2 H, 2 H-cyclopropyl), 1.30−1.23 (m, 2 H, H-7b, H-4′b), 1.23−1.16 (m, 5 H, 5′-CH$_3$, 2 H-cyclopropyl), 1.12 (d, J = 7.4 Hz, 3 H, 4-CH$_3$), 1.07 (d, J = 6.6 Hz, 6 H, 10-CH$_3$, 2-CH$_3$), 0.95−0.90 (m, 6 H, 8-CH$_3$, 15-CH$_3$). ^{13}C NMR ($CDCl_3$, 175 MHz) δ: 177.1, 177.1, 174.3, 171.8, 169.9, 167.1, 154.4, 154.0, 153.5, 153.0, 149.2, 147.4, 146.0, 145.9, 139.1, 136.6, 124.3, 122.3, 119.7, 119.6, 112.4, 112.3, 108.1, 104.8, 104.8, 103.4, 86.7, 86.6, 85.2, 80.1, 77.9, 76.6, 70.6, 69.3, 65.7, 60.5, 53.4, 52.5, 49.8, 43.9, 43.4, 40.4, 38.0, 36.9, 35.3, 34.3, 32.8, 29.7, 29.1, 25.8, 21.8, 21.4, 21.2, 21.0, 15.5, 15.2, 10.2, 9.5, 8.2, 7.1.

*4.3.21.2. 3-O-Descladinosyl-3-O-[2-(2-pyridyl)acetyl]-6-O-(3-{2-[(3-carbamoyl-6-fluoro-1-cyclopropyl-1,4-dihydro-4-oxoquinolin-7-yl) piperazin-1-yl]}propanoyl)azithromycin-11,12-cyclic carbonate **(29k)**.* Compound **29k** (56.9 mg, 0.0510 mmol, 20.4%) was prepared starting from 3-*O*-descladinosyl-3-*O*-[2-(2-pyridyl)acetyl]-6-*O*-acryloylazithromycin-11,12-cyclic carbonate (0.200 g, 0.250 mmol) and compound **20** (0.0780 g, 0.250 mmol) according to the general procedure (column chromatography eluents: CH_2Cl_2/EtOH/$NH_3{\cdot}H_2O$ = 10:0.3:0.05). Melting point: 145−148 °C. HRMS (ESI) $(M + H)^+$ *m/z* 1120.5948, calcd for $C_{58}H_{83}FN_7O_{14}$ 1120.5977. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 9.72 (d, J = 5.4 Hz, 1 H, -N**H$_2$**), 8.81 (s, 1 H, H-quinolyl), 8.51 (ddd, J_1 = 4.9 Hz, J_2 = 1.9 Hz, J_3 = 0.9 Hz, 1 H, H-pyridyl), 8.03 (d, J = 13.3 Hz, 1 H, H-quinolyl), 7.67 (td, J_1 = 7.7 Hz, J_2 = 1.8 Hz, 1 H, H-pyridyl), 7.39 (dt, J_1 = 7.8 Hz, J_2 = 1.1 Hz, 1 H, H-pyridyl), 7.32 (d, J = 7.1 Hz, 1 H, H-quinolyl), 7.21 (ddd, J_1 = 7.6 Hz, J_2 = 4.9 Hz, J_3 = 1.1 Hz, 1 H, H-pyridyl), 5.71 (d, J = 5.3 Hz, 1 H, -N**H$_2$**), 5.11 (d, J = 10.4 Hz, 1 H, H-3), 4.88 (dd, J_1 = 8.2 Hz, J_2 = 3.8 Hz, 1 H, H-13), 4.58 (d, J = 3.0 Hz, 1 H, H-5), 4.44 (d, J = 5.7 Hz, 1 H, H-11), 4.17 (d, J = 7.3 Hz, 1 H, H-1′), 3.93 (dd, J_1 = 20.0 Hz, J_2 = 16.0 Hz, 2 H, 3-O-CO-CH$_2$-), 3.50−3.44 (m, 1 H, 1 H-cyclopropyl), 3.43−3.36 (m, 1 H, H-5′), 3.34−3.28 (m, 4 H, 4 H-piperazinyl), 3.28−3.22 (m, 1 H, H-2′), 2.90−2.80 (m, 4 H, 6-O-CO-CH$_2$C**H$_2$**−NH-, H-2, H-10), 2.78−2.66 (m, 6 H, 4 H-piperazinyl, 6-O-CO-C**H$_2$**CH$_2$−NH-), 2.53−2.43 (m, 1 H, H-3′), 2.35 (s, 6 H, -N(CH$_3$)$_2$), 2.34−2.32 (m, 1 H, H-9a), 2.18 (s, 3 H, -N-CH$_3$), 2.10−2.02 (m, 1 H, H-9b), 1.99−1.87 (m, 3 H, H-4, H-14eq, H-7a), 1.86−1.78 (m, 1 H, H-8), 1.74−1.64 (m, 2 H, H-4′a, H-14ax), 1.59 (s, 3 H, 6-CH$_3$), 1.48 (s, 3 H, 12-CH$_3$), 1.37−1.30 (m, 2 H, 2 H-cyclopropyl), 1.30−1.22 (m, 2 H, H-7b, H-4′b), 1.18 (d, J = 6.2 Hz, 3 H, 5′-CH$_3$), 1.17−1.14 (m, 2 H, 2 H-cyclopropyl), 1.11 (d, J = 7.4 Hz, 3 H, 2-CH$_3$), 1.09−1.05 (m, 6 H, 10-CH$_3$, 4-CH$_3$), 0.95−0.90 (m, 6 H, 8-CH$_3$, 15-CH$_3$). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 175.4, 174.2, 171.9, 169.9, 166.8, 154.7, 154.0, 153.5, 152.2, 149.2, 147.2, 145.1, 145.0, 138.6, 136.6, 124.3, 122.3, 121.8, 112.8, 112.6, 111.0, 104.8, 103.4, 86.7, 86.7, 85.0, 80.0, 78.0, 77.2, 76.6, 70.6, 69.4, 65.6, 60.4, 53.4, 52.6, 49.9, 43.9, 43.5, 40.4, 38.0, 37.0, 34.7, 34.4, 32.8, 25.8, 21.8, 21.5, 21.2, 21.0, 15.5, 15.4, 10.1, 9.5, 8.2, 7.2.

*4.3.21.3. 3-O-Descladinosyl-3-O-[2-(2-pyridyl)acetyl]-6-O-(3-{[4-(3-carbamoyl-1-cyclopropyl-1,4-dihydro-4-oxoquinolin-6-yl)but-3-yn-1-yl]amino}propanoyl)azithromycin-11,12-cyclic carbonate **(29r)**.* Compound **29r** (19.8 mg, 0.0180 mmol, 8.18%) was prepared starting from 3-*O*-descladinosyl-3-*O*-[2-(2-pyridyl)acetyl]-6-*O*-acryloylazithromycin-11,12-cyclic carbonate (0.300 g, 0.380 mmol) and compound **11e** (0.0640 g, 0.220 mmol) according to the general procedure (column chromatography eluents: CH_2Cl_2/EtOH/$NH_3{\cdot}H_2O$ = 10:0.3:0.05). Melting point: 143−146 °C. HRMS (ESI) $(M + H)^+$ *m/z* 1085.5815, calcd for $C_{58}H_{81}N_6O_{14}$ 1085.5805. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 9.63 (d, J = 5.2 Hz, 1 H, -N**H$_2$**), 8.86 (s, 1 H, H-quinolyl), 8.51 (ddd, J_1 = 4.9 Hz, J_2 = 1.9 Hz, J_3 = 0.9 Hz, 1 H, H-

quinolyl), 8.47 (d, J = 2.0 Hz, 1 H, H-pyridyl), 7.91 (d, J = 8.8 Hz, 1 H, H-quinolyl), 7.74 (dd, J_1 = 8.8 Hz, J_2 = 2.0 Hz, 1 H, H-quinolyl), 7.68 (td, J_1 = 7.7 Hz, J_2 = 1.8 Hz, 1 H, H-pyridyl), 7.39 (d, J = 8.0 Hz, 1 H, H-pyridyl), 7.20 (ddd, J_1 = 7.6 Hz, J_2 = 4.9 Hz, J_3 = 1.2 Hz, 1 H, H-pyridyl), 5.78 (d, J = 5.1 Hz, 1 H, -N**H**$_2$), 5.07 (d, J = 10.4 Hz, 1 H, H-3), 4.84 (dd, J_1 = 7.5 Hz, J_2 = 4.3 Hz, 1 H, H-13), 4.60 (d, J = 3.2 Hz, 1 H, H-5), 4.40 (d, J = 2.5 Hz, 1 H, H-11), 4.17 (d, J = 7.3 Hz, 1 H, H-1′), 3.93 (dd, J_1 = 20.0 Hz, J_2 = 15.6 Hz, 2 H, 3-O-CO-CH$_2$-), 3.60−3.54 (m, 1 H, 2′-OH), 3.54−3.48 (m, 1 H, 1 H-cyclopropyl), 3.43−3.34 (m, 1 H, H-5′), 3.22 (dd, J_1 = 10.2 Hz, J_2 = 7.2 Hz, 2 H, H-2′), 3.08−2.91 (m, 6 H, 6-O-CO-CH$_2$C**H$_2$**−NH−C**H$_2$**CH$_2$C≡C−Ar, H-2, H-10), 2.79−2.61 (m, 5 H, H-3′, 6-O-CO-C**H$_2$**CH$_2$−NH−CH$_2$C**H$_2$**C≡C−Ar), 2.37−2.33 (m, 1 H, H-9a), 2.32 (s, 6 H, -N(CH$_3$)$_2$), 2.15 (s, 3 H, -N-CH$_3$), 2.06−2.00 (m, 1 H, H-9b), 1.97−1.87 (m, 3 H, H-4, H-14eq, H-7a), 1.83−1.74 (m, 1 H, H-8), 1.72−1.63 (m, 2 H, H-4′a, H-14ax), 1.58 (s, 3 H, 6-CH$_3$), 1.48 (s, 3 H, 12-CH$_3$), 1.38−1.32 (m, 2 H, 2 H-cyclopropyl), 1.27−1.19 (m, 2 H, H-7b, H-4′b), 1.19−1.15 (m, 5 H, 2 H-cyclopropyl, 5′-CH$_3$), 1.10 (d, J = 7.4 Hz, 3 H, 4-CH$_3$), 1.08−1.03 (m, 6 H, 2-CH$_3$, 10-CH$_3$), 0.94−0.85 (m, 6 H, 8-CH$_3$, 15-CH$_3$). ^{13}C NMR (CDCl$_3$, 100 MHz) δ: 175.9, 174.2, 172.4, 170.0, 166.4, 154.0, 153.4, 149.2, 147.7, 139.9, 136.6, 135.7, 130.2, 127.3, 124.3, 122.3, 121.2, 116.8, 111.8, 103.5, 89.7, 86.9, 86.8, 84.2, 80.4, 79.8, 78.0, 77.2, 70.6, 69.4, 66.4, 65.6, 60.2, 48.1, 44.7, 43.9, 43.4, 40.3, 37.7, 36.9, 34.9, 34.8, 34.0, 28.8, 25.4, 21.8, 21.6, 21.2, 21.0, 20.4, 15.8, 15.3, 10.0, 9.5, 8.3, 7.4.

*4.3.21.4. 3-O-Descladinosyl-3-O-[2-(2-pyridyl)acetyl]-6-O-(3-{[4-(3-carboxy-1-cyclopropyl-1,4-dihydro-4-oxoquinolin-6-yl)but-3-yn-1-yl]amino}propanoyl)azithromycin-11,12-cyclic carbonate **(29w)**.* Compound **29w** (22.7 mg, 0.0210 mmol, 8.75%) was prepared starting from 3-*O*-descladinosyl-3-*O*-[2-(2-pyridyl)acetyl]-6-*O*-acryloylazithromycin-11,12-cyclic carbonate (0.190 g, 0.240 mmol) and compound **11j** (0.0960 g, 0.320 mmol) according to the general procedure (column chromatography eluents: CH_2Cl_2/MeOH/$NH_3\cdot H_2O$ = 10:0.8:0.5). Melting point: 167−170 °C. HRMS (ESI) $(M + H)^+$ m/z 1086.5669, calcd for $C_{58}H_{80}N_5O_{15}$ 1086.5645. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 8.85 (s, 1 H, H-quinolyl), 8.53−8.44 (m, 2 H, H-pyridyl, H-quinolyl), 8.01 (d, J = 8.8 Hz, 1 H, H-quinolyl), 7.83 (dd, J_1 = 8.8 Hz, J_2 = 2.0 Hz, 1 H, H-quinolyl), 7.66 (d, J = 8.5 Hz, 1 H, H-pyridyl), 7.39 (d, J = 7.9 Hz, 1 H, H-pyridyl), 7.19 (d, J = 7.0 Hz, 1 H, H-pyridyl), 5.08 (d, J = 10.6 Hz, 1 H, H-3), 4.84 (dd, J_1 = 7.9 Hz, J_2 = 4.3 Hz, 1 H, H-13), 4.58 (d, J = 3.2 Hz, 1 H, H-5), 4.41 (d, J = 6.1 Hz, 1 H, H-11), 4.17 (d, J = 7.3 Hz, 1 H, H-1′), 3.96−3.87 (m, 2 H, 3-O-CO-CH$_2$-), 3.64−3.48 (m, 2 H, 1 H-cyclopropyl, 12-OH), 3.43−3.34 (m, 1 H, H-5′), 3.24−3.16 (m, 1 H, H-2′), 3.02−2.78 (m, 7 H, 6-O-CO-CH$_2$C**H$_2$**−NH−C**H$_2$**CH$_2$C≡C−Ar, H-2, H-10, 11-OH), 2.76−2.41 (m, 5 H, 6-O-CO-C**H$_2$**CH$_2$−NH−CH$_2$C**H$_2$**C≡C−Ar, H-3′), 2.36−2.31 (m, 1 H, H-9a), 2.29 (s, 6 H, -N(CH$_3$)$_2$), 2.15 (s, 3 H, -N-CH$_3$), 2.06−2.00 (m, 1 H, H-4), 1.96−1.85 (m, 3 H, H-9b, H-14eq, H-7a), 1.82−1.74 (m, 1 H, H-8), 1.70−1.61 (m, 1 H, H-4′a), 1.59 (s, 3 H, 6-CH$_3$), 1.57−1.54 (m, 1 H, H-14ax), 1.47 (s, 3 H, 12-CH$_3$), 1.45−1.38 (m, 2 H, 2 H-cyclopropyl), 1.28−1.19 (m, 4 H, H-7b, H-4′b, 2 H-cyclopropyl), 1.17 (d, J = 6.1 Hz, 3 H, 5′-CH$_3$), 1.11 (d, J = 7.3 Hz, 3 H, 4-CH$_3$), 1.09−1.00 (m, 6 H, 2-CH$_3$, 10-CH$_3$), 0.93−0.85 (m, 6 H, 8-CH$_3$, 15-CH$_3$). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 178.1, 174.2, 172.4, 169.9, 166.8, 153.4, 149.2, 148.1, 140.0, 136.8, 136.6, 129.9, 125.9, 124.3, 122.6, 122.2, 117.3, 109.0, 103.6, 86.8, 86.7, 79.8, 78.0, 77.2, 70.6, 69.5, 65.6, 60.3, 48.2, 44.7, 43.9, 43.4, 40.3, 37.8, 36.9, 35.4, 35.2, 34.1, 25.5, 21.7, 21.5, 21.2, 21.0, 20.7, 15.6, 15.4, 10.0, 9.6, 8.3, 7.3.

*4.3.22. 2′-O-Acetyl-3-O-descladinosyl-3-O-[2-(3-pyridyl)acetyl]-6-O-acryloylazithromycin-11,12-cyclic carbonate **(30)***

Compound **30** (0.960 g, 1.15 mmol, 54.8%) was prepared starting from compound **27** (1.50 g, 2.10 mmol) and 3-pyridylacetic acid hydrochloride (0.730 g, 4.20 mmol) according to the procedure used to prepare compound **25** (column chromatography eluents: CH_2Cl_2/EtOH/$NH_3\cdot H_2O$ = 10:0.1:0.05). HRMS (ESI) $(M + H)^+$ m/z 832.4581, calcd for $C_{43}H_{66}N_3O_{13}$ 832.4590. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 8.58 (d, J = 2.3 Hz, 1 H, H-pyridyl), 8.56 (dd, J_1 = 4.8 Hz, J_2 = 1.7 Hz, 1 H, H-pyridyl), 7.77 (td, J_1 = 7.9 Hz, J_2 = 2.0 Hz, 1 H, H-pyridyl), 7.30 (ddd, J_1 = 7.9 Hz, J_2 = 4.8 Hz, J_3 = 0.8 Hz, 1 H, H-pyridyl), 6.39 (dd, J_1 = 17.3 Hz, J_2 = 1.6 Hz, 1 H, 6-O-CO-CH=C**H$_2$**), 6.09 (dd, J_1 = 17.2 Hz, J_2 = 10.3 Hz, 1 H, 6-O-CO-C**H**=CH$_2$), 5.75 (dd, J_1 = 10.3 Hz, J_2 = 1.7 Hz, 1 H, 6-O-CO-CH=C**H$_2$**), 5.06 (dd, J_1 = 10.6 Hz, J_2 = 1.4 Hz, 1 H, H-3), 4.87 (dd, J_1 = 8.1 Hz, J_2 = 4.0 Hz, 1 H, H-13), 4.75 (dd, J_1 = 10.5 Hz, J_2 = 7.4 Hz, 1 H, H-2′), 4.58 (d, J = 3.0 Hz, 1 H, H-5), 4.44 (d, J = 5.4 Hz, 1 H, H-11), 4.08 (d, J = 7.5 Hz,1 H, H-1′), 3.78−3.68 (m, 2 H, 3-O-CO-CH$_2$-), 3.19−3.10 (m, 1 H, H-5′), 2.89−2.87 (m, 2 H, H-3′, H-2), 2.63−2.53 (m, 1 H, H-10), 2.35−2.30 (m, 1 H, H-9a), 2.27 (s, 6 H, -N(CH$_3$)$_2$), 2.17 (s, 3 H, -N-CH$_3$), 2.11 (s, 3 H, 2′-O-CO-CH$_3$), 2.09−2.05 (m, 1 H, H-4), 2.01−1.90 (m, 2 H, H-9b, H-14eq), 1.86−1.76 (m, 2 H, H-7a, H-8), 1.70−1.62 (m, 2 H, H-4′a, H-14ax), 1.58 (s, 3 H, 6-CH$_3$), 1.46 (s, 3 H, 12-CH$_3$), 1.34−1.26 (m, 1 H, H-4′b), 1.26−1.22 (m, 1 H, H-7b), 1.18 (d, J = 6.1 Hz, 3 H, 5′-CH$_3$), 1.07 (d, J = 6.5 Hz, 3 H, 10-CH$_3$), 0.98−0.94 (m, 6 H, 2-CH$_3$, 4-CH$_3$), 0.93−0.87 (m, 6 H, 8-CH$_3$, 15-CH$_3$).

*4.3.23. General procedure for the synthesis of compounds **31r** and **31w***

The compounds **31r** and **31w** were prepared starting from compound **30** and corresponding amines according to the procedure used to prepare the compound **26** series.

*4.3.23.1. 3-O-Descladinosyl-3-O-[2-(3-pyridyl)acetyl]-6-O-(3-{[4-(3-carbamoyl-1-cyclopropyl-1,4-dihydro-4-oxoquinolin-6-yl)but-3-yn-1-yl]amino}propanoyl)azithromycin-11,12-cyclic carbonate **(31r)**.* Compound **31r** (0.110 g, 0.100 mmol, 33.3%) was prepared starting from 3-*O*-descladinosyl-3-*O*-[2-(3-pyridyl)acetyl]-6-*O*-acryloylazithromycin-11,12-cyclic carbonate (0.300 g, 0.380 mmol) and compound **11e** (0.0890 g, 0.300 mmol) according to the general procedure (column chromatography eluents: CH_2Cl_2/EtOH/$NH_3\cdot H_2O$ = 10:0.3:0.05). Melting point: 133−136 °C. HRMS (ESI) $(M + H)^+$ m/z 1085.5814, calcd for $C_{58}H_{81}N_6O_{14}$ 1085.5805. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 9.63 (d, J = 5.2 Hz, 1 H, -N**H$_2$**), 8.87 (s, 1 H, H-quinolyl), 8.55 (d, J = 2.3 Hz, 1 H, H-pyridyl), 8.53 (dd, J_1 = 4.8 Hz, J_2 = 1.7 Hz, H-pyridyl), 8.48 (d, J = 1.9 Hz, 1 H, H-quinolyl), 7.91 (d, J = 8.8 Hz, 1 H, H-quinolyl), 7.78−7.72 (m, 2 H, H-quinolyl, H-pyridyl), 7.31−7.26 (m, 1 H, H-pyridyl), 5.78 (d, J = 5.2 Hz, 1 H, -N**H$_2$**), 5.08 (d, J = 10.8 Hz, 1 H, H-3), 4.84 (dd, J_1 = 7.5 Hz, J_2 = 4.2 Hz, 1 H, H-13), 4.62 (d, J = 3.2 Hz, 1 H, H-5), 4.38 (d, J = 6.7 Hz, 1 H, H-11), 4.03 (d, J = 7.2 Hz, 1 H, H-1′), 3.72 (dd, J_1 = 20.0 Hz, J_2 = 9.6 Hz, 2 H, 3-O-CO-CH$_2$-), 3.54−3.48 (m, 1 H, 1 H-cyclopropyl), 3.43−3.35 (m, 1 H, 2′-OH), 3.25−3.16 (m, 2 H, H-5′, H-2′), 3.05−2.64 (m, 10 H, 6-O-CO-C**H$_2$CH$_2$**−NH−C**H$_2$CH$_2$**C≡C−Ar, H-2, H-10), 2.54−2.45 (m, 1 H, H-3′), 2.37−2.33 (m, 1 H, H-9a), 2.28 (s, 6 H, -N(CH$_3$)$_2$), 2.15 (s, 3 H, -N-CH$_3$), 2.06−2.00 (m, 1 H, H-4), 1.96−1.88 (m, 3 H, H-9b, H-14eq, H-7a), 1.83−1.75 (m, 1 H, H-8), 1.72−1.65 (m, 1 H, H-14ax), 1.62−1.58 (m, 1 H, H-4′a), 1.57 (s, 3 H, 6-CH$_3$), 1.49 (s, 3 H, 12-CH$_3$), 1.38−1.32 (m, 2 H, 2 H-cyclopropyl), 1.24−1.17 (m, 4 H, H-7b, H-4′b, 2 H-cyclopropyl), 1.16 (d, J = 6.1 Hz, 3 H, 5′-CH$_3$), 1.13 (d, J = 7.4 Hz, 3 H, 4-CH$_3$), 1.06 (d, J = 6.4 Hz, 10-CH$_3$), 0.97 (d, J = 6.8 Hz, 2-CH$_3$), 0.91 (d, J = 6.6 Hz, 8-CH$_3$), 0.88 (d, J = 7.4 Hz, 15-CH$_3$). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 175.9, 174.0, 172.5, 169.9, 166.4, 153.4, 150.4, 148.8, 147.7, 139.9, 137.1, 135.6, 130.3, 129.5, 127.3, 123.5, 121.2, 116.8, 111.9, 103.7, 89.9, 87.0, 86.7, 84.1, 80.3, 80.1, 78.3, 77.2, 70.4, 69.6, 66.4, 66.0, 60.2, 48.2, 44.7, 43.5, 40.3, 38.5, 37.7, 36.9, 35.2, 34.8, 34.0, 28.3, 25.5, 21.8, 21.6, 21.2, 21.0, 20.6, 15.8, 15.2, 10.0, 9.6, 8.3, 7.5.

*4.3.23.2. 3-O-Descladinosyl-3-O-[2-(3-pyridyl)acetyl]-6-O-(3-{[4-(3-carboxy-1-cyclopropyl-1,4-dihydro-4-oxoquinolin-6-yl)but-3-yn-1-yl]amino}propanoyl)azithromycin-11,12-cyclic carbonate **(31w)**.*

Compound **31w** (23.9 mg, 0.0220 mmol, 8.80%) was prepared starting from 3-*O*-descladinosyl-3-*O*-[2-(3-pyridyl)acetyl]-6-*O*-acryloylazithromycin-11,12-cyclic carbonate (0.190 g, 0.240 mmol) and compound **11j** (0.0890 g, 0.300 mmol) according to the general procedure (column chromatography eluents: CH_2Cl_2/MeOH/$NH_3 \cdot H_2O$ = 10:0.8:0.5). Melting point: 127–130 °C. HRMS (ESI) $(M + H)^+$ *m/z* 1086.5656, calcd for $C_{58}H_{80}N_5O_{15}$ 1086.5645. ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 8.84 (s, 1 H, H-quinolyl), 8.55 (d, J = 2.3 Hz, 1 H, H-pyridyl), 8.53 (dd, J_1 = 4.8 Hz, J_2 = 1.6 Hz, H-pyridyl), 8.49 (d, J = 1.9 Hz, 1 H, H-quinolyl), 8.01 (d, J = 8.8 Hz, 1 H, H-quinolyl), 7.82 (dd, J_1 = 8.8 Hz, J_2 = 2.0 Hz, 1 H, H-quinolyl), 7.75 (td, J_1 = 7.9 Hz, J_2 = 2.0 Hz, 1 H, H-pyridyl), 7.31–7.26 (m, 1 H, H-pyridyl), 5.08 (d, J = 10.4 Hz, 1 H, H-3), 4.84 (dd, J_1 = 7.7 Hz, J_2 = 4.2 Hz, 1 H, H-13), 4.60 (d, J = 3.2 Hz, 1 H, H-5), 4.39 (d, J = 6.3 Hz, 1 H, H-11), 4.04 (d, J = 7.2 Hz, 1 H, H-1′), 3.72 (dd, J_1 = 20.4 Hz, J_2 = 16.0 Hz, 2 H, 3-O-CO-CH_2-), 3.64–3.57 (m, 1 H, 1 H-cyclopropyl), 3.27–3.15 (m, 2 H, H-5′, H-2′), 3.05–2.63 (m, 10 H, 6-O-CO-C**H$_2$**C**H$_2$**–NH–C**H$_2$**C**H$_2$**C≡C–Ar, H-2, H-10), 2.52–2.43 (m, 1 H, H-3′), 2.37–2.32 (m, 1 H, H-9a), 2.28 (s, 6 H, -N(CH_3)$_2$), 2.15 (s, 3 H, -N-CH_3), 2.08–2.01 (m, 1 H, H-4), 1.97–1.86 (m, 3 H, H-9b, H-14eq, H-7a), 1.83–1.74 (m, 1 H, H-8), 1.71–1.64 (m, 1 H, H-14ax), 1.64–1.60 (m, 1 H, H-4′a), 1.58 (s, 3 H, 6-CH_3), 1.48 (s, 3 H, 12-CH_3), 1.45–1.39 (m, 2 H, 2 H-cyclopropyl), 1.27–1.23 (m, 1 H, H-7b), 1.23–1.19 (m, 3 H, H-4′b, 2 H-cyclopropyl), 1.17 (d, J = 6.2 Hz, 3 H, 5′-CH_3), 1.13 (d, J = 7.4 Hz, 3 H, 4-CH_3), 1.06 (d, J = 6.5 Hz, 10-CH_3), 0.96 (d, J = 6.8 Hz, 2-CH_3), 0.91 (d, J = 6.9 Hz, 8-CH_3), 0.90–0.86 (m, 3 H, 15-CH_3). ^{13}C NMR ($CDCl_3$, 100 MHz) δ: 178.1, 174.0, 172.5, 170.0, 166.7, 153.4, 150.4, 148.8, 148.1, 140.0, 137.1, 136.7, 129.9, 129.5, 125.9, 123.5, 122.5, 117.3, 109.0, 103.8, 91.2, 86.8, 86.7, 84.4, 80.1, 79.8. 78.3, 77.2, 70.4, 69.7, 66.4, 66.0, 60.3, 48.2, 44.7, 43.4, 40.3, 38.5, 37.8, 36.9, 35.4, 35.2, 34.1, 28.3, 25.5, 21.7, 21.6, 21.2, 21.0, 20.6, 15.7, 15.2, 10.0, 9.6, 8.3, 7.4.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

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