Bioorganic & Medicinal Chemistry 18 (2010) 6569–6577

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

6-Alkylquinolone-3-carboxylic acid tethered to macrolides synthesis and antimicrobial profile

Samra Kapić [a,*], Hana Čipčić Paljetak [a,†], Sulejman Alihodžić [a], Roberto Antolović [a,‡], Vesna Eraković Haber [a], Richard L. Jarvest [b], David J. Holmes [c], John P. Broskey [c], Eric Hunt [d]

[a] GlaxoSmithKline Research Centre Zagreb Ltd, Prilaz baruna Filipovića 29, HR-10000 Zagreb, Croatia
[b] GlaxoSmithKline, Gunnels Wood Road, Stevenage, Herts SG1 2NY, UK
[c] GlaxoSmithKline, 1250 South Collegeville Road, Collegeville, PA 19426, USA
[d] GlaxoSmithKline, New Frontiers Science Park, Harlow, CM19 5AW, UK

ARTICLE INFO

Article history:
Available online 3 July 2010

Keywords:
Tethered macrolides
6-(ω-Carboxy)-alkyl-*N*(1)-ethyl-3-carboxylquinolones
Antimicrobial activity
Structure–activity relationship

ABSTRACT

Two series of clarithromycin and azithromycin derivatives with terminal 6-alkylquinolone-3-carboxylic unit with central ether bond in the linker were prepared and tested for antimicrobial activity. Quinolone-linker intermediates were prepared by Sonogashira-type C(6)-alkynylation of 6-iodo-quinolone precursors. In the last step, 4″ site-selective acylation of 2′-protected macrolides was completed with the EDC reagent, which selectively activated a terminal, aliphatic carboxylic group in dicarboxylic intermediates. Antimicrobial activity of the new series of macrolones is discussed. The most potent compound, 4″-*O*-{6-[3-(3-carboxy-1-ethyl-4-oxo-1,4-dihydroquinolin-6-yl)-propoxy]-hexanoyl}-azithromycin (**10**), is highly active against bacterial respiratory pathogens resistant to macrolide antibiotics and represents a promising lead for further investigation.

1. Introduction

Continuous increase in levels of antibacterial resistance among common pathogens leads to impaired efficacy of available antibiotics and poses a constant need for the development of new, more potent drugs. A number of novel classes of macrolide antibiotics are characterized by the presence of a specific, well defined structural subunit tethered to the macrolide scaffold.[1–3] Terminal units usually comprise an aromatic or heteroaromatic ring, bound by the linkers of various lengths to the selected positions on the macrolide. Some representative structures are given in Figure 1.

Such derivatives of ketolides were for some period regarded as the 'future of macrolides'.[4] In recent years, ketolides become the most frequently reported macrolide derivatives, modified by the C-linked terminal (hetero)aryl units, usually attached to aglycon at C(6)–*O* or C(11)–*N*. Ketolides with C(6)–*O*-allylic chain to heteroaryl group are also reported.[5–7] In addition to marketed antibiotic telithromycin, among the most progressed compounds is cethromycin, which is in phase III clinical research.[8,9] Ketolides with a terminal, C–C linked (hetero)aryl group at C(11)–N usually also contain an annulated cyclic carbamate at C(11), C(12) position.[10–12] Another group of ketolides with a terminal C–C bond to a (hetero) aryl unit contain a carbamate group at C(6)–*O*.[13–15] Linkers with a triple bond are also used to attach (hetero) aryl units.[16,17] A carbon–carbon bond is present at both ends of the linker in ketolides where a (hetero) aryl unit is bound to the C(11)–C atom via α,β, or γ-*S*-alkyl unit and by another C–C bond at the second terminus.[5] Two examples of acylides, C(3)–*O*-acyl derivatives of macrolides with terminal aryl units bound to a short linker, are also reported.[18,19]

Interestingly, tethered derivatives with erythro-, clarithro- or azithro-scaffold are much less explored. We have reported on the synthesis of azithro-congeners, 4″-*O*-acyl derivatives of 8a-aza-8a-homoerythromycin **I** and **II**, Figure 2, and their in vitro activities against different bacteria, sensitive or resistant to macrolide antibiotics.[20,21]

More recently we reported on a series of 4″-*O*-(ω-quinolylamino-alkylamino)propionyl derivatives of selected macrolides and for significant number of derivatives an improved antibacterial profile was observed.[22] In all these compound groups linkers of a varying length, number of heteroatoms, and degree of conformational flexibility are attached to 4″-position of macrolides.[21,22]

Continuing our efforts in obtaining macrolones with improved antimicrobial properties, we have designed synthesis and performed in vitro antimicrobial testing of this new series characterized by the general formula **III** (Fig. 3).

They belong to the class of macrolide derivatives that comprise azithromycin or clarithromycin core, to which quinolone 3-carboxylic unit is bound by two types of linkers. Analysis of data for

* Corresponding author. Tel.: +385 1 605 1242; fax: +385 1 605 1019.
E-mail address: samra.y.kapic@gsk.com (S. Kapić).
† Present address: University of Zagreb, School of Medicine, Center for Translational and Clinical Research, Šalata 2, 10000 Zagreb, Croatia.
‡ Present address: University of Rijeka, Department of Biotechnology, S. Krautzeka bb, 51000 Rijeka, Croatia.

0968-0896/$ - see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2010.06.048

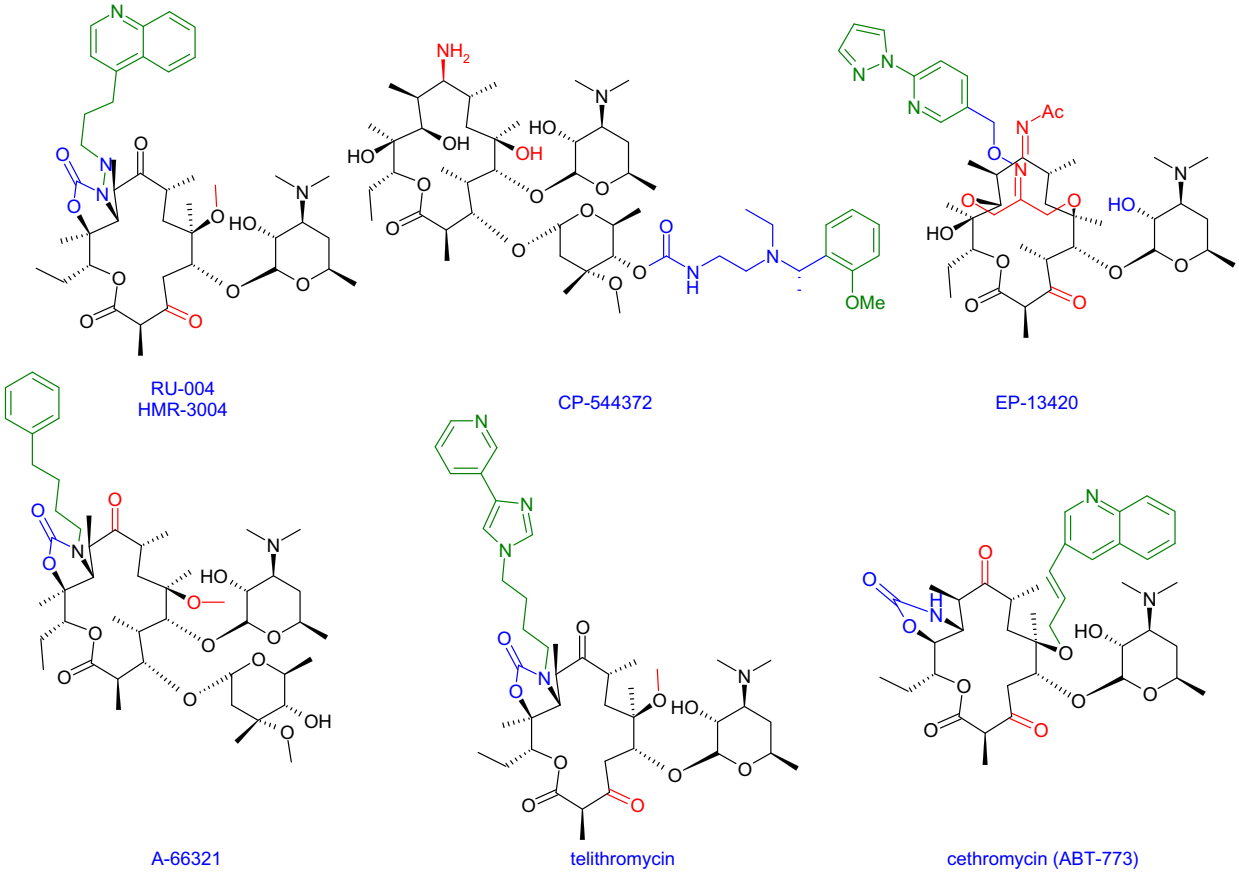


Figure 1. Representative structures of tethered macrolides with a linked (hetero)aromatic group.

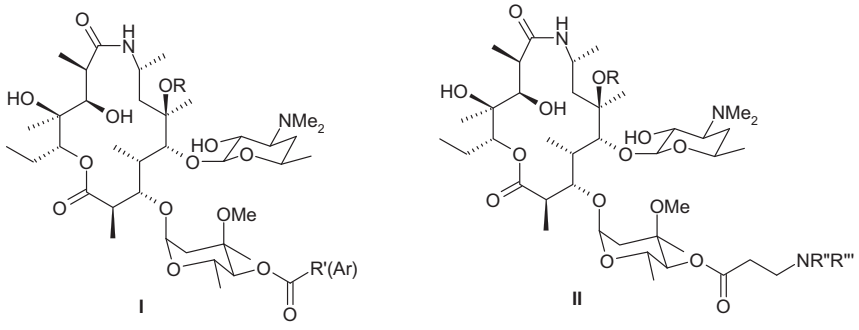


Figure 2. General formula 4″-*O*-acyl derivaties of 8a-aza-8a-homoerythromycin **I** and **II**.

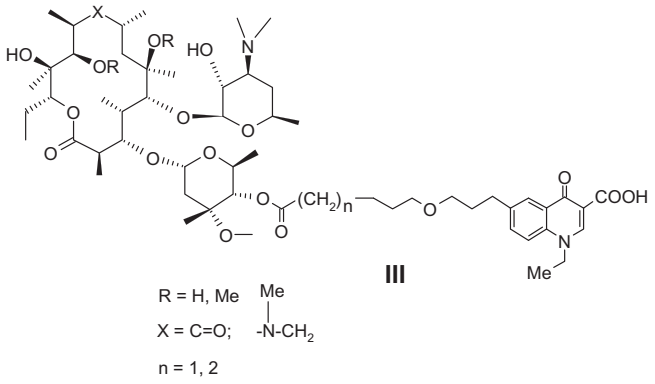


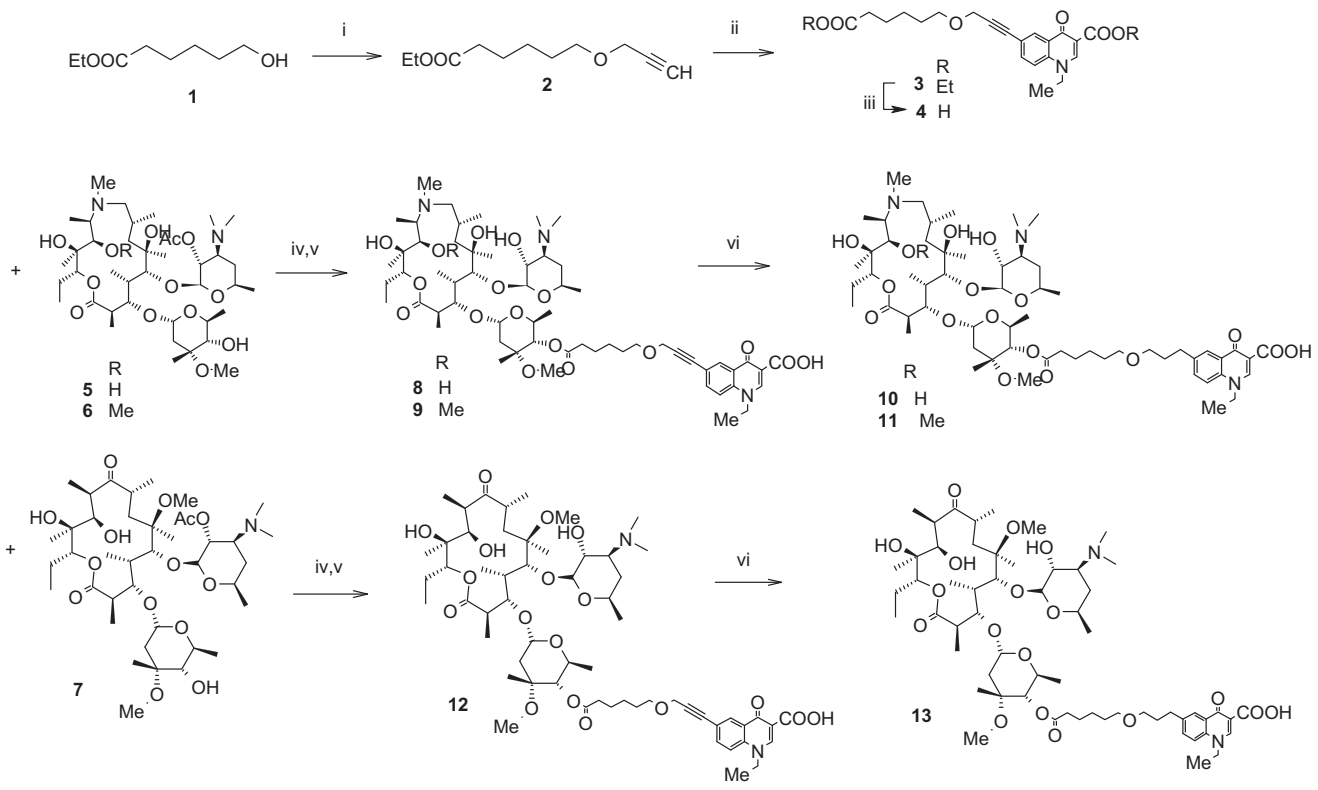
Figure 3. General formula **III**, characterized by *O*-acyl group at C(4″)-*O* of macrolone and C–C bond to C(6) of quinolone unit.

our previous derivatives suggested incorporation of 4″-*O*-acyl unit with the one ether oxygen atom in the polymethylene chain at γ-position to the terminal quinolone unit could be beneficial.

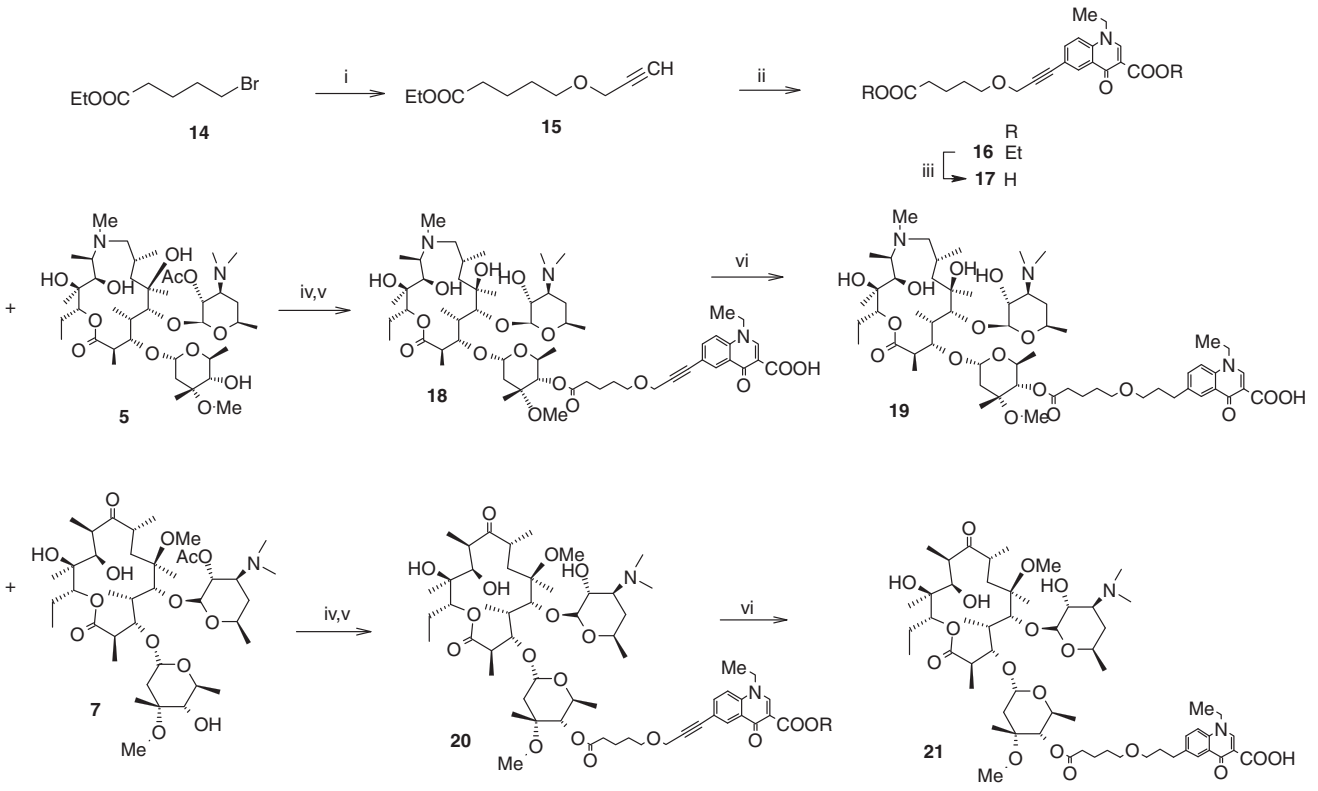
2. Results and discussion

2.1. Chemistry

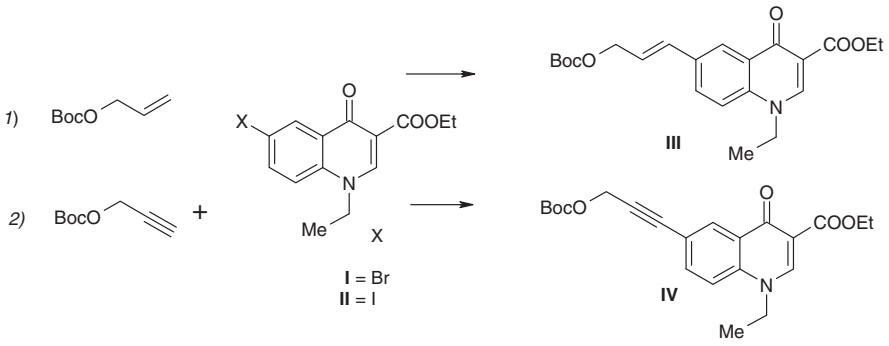
Compounds of the general formula **III** are characterized by the presence of 6-alkyl-*N*(1)-ethyl-3-carboxylquinolone unit bound to the 4″-*O*-atom of cladinose moiety in azithromycin (compounds **8**–**11**; **18**, **19**) or in clarithromycin (compounds **12, 13; 20, 21**). Connection of the linker to 4″-*O* is achieved by an ester bond, and to the 6-position of quinolone via a C–C bond. Within the linker, one oxygen atom has been designed at the γ-position to the



Scheme 1. Synthetic route to the target compounds **8**–**13**. Reagents and conditions: (i) TBAI, NaI, propargyl bromide, KOH, THF, rt, 24 h; (ii) 1-ethyl-6-iodo-4-oxo-1,4-dihydroquinoline-3-carboxylic acid ethyl ester, Cu(I)I, Et_3N, dry MeCN, Pd(II)PPh_3Cl_2, 50 °C, N_2, 2 h, rt, 24 h; (iii) aq soln NaOH, THF, 80 °C, 2 h, rt, 12 h; (iv) **4**, EDC × HCl, DMAP, dry DMF, DCM, 0 °C, 3 h, rt, 24 h; (v) MeOH, 50–55 °C, 12 h; (vi) 10% Pd–C, EtOH, 5 bars, rt, 20 h.



Scheme 2. Synthetic route to the target compounds **18**–**21**. Reagents and conditions: (i) propargyl alcohol, NaH, DMF, 0 °C, N_2, 1 h, 50 °C, overnight; (ii) 1-ethyl-6-iodo-4-oxo-1,4-dihydroquinoline-3-carboxylic acid ethyl ester, Cu(I)I, Et_3N, dry MeCN, Pd(II)PPh_3Cl_2, 50 °C, N_2, 2 h, rt, 24 h; (iii) aq soln NaOH, THF, 80 °C, 2 h, rt, 12 h; (iv) **17**, EDC × HCl, DMAP, dry DMF, DCM, 0 °C, 3 h, rt, 24 h; (v) MeOH, 50–55 °C, 12 h; (vi) 10% Pd–C, EtOH, 5 bars, rt, 20 h.



1)	X	Catalytic system	Solvent; time/h; temp/°C	Yield of **III** (%)
1a)	Br	$Pd(OAc)_2/P(o\text{-}tol.)_3/Et_3N$	DMF/12/100	no reaction
1b)	I	$Pd(OAc)_2/P(o\text{-}tol.)_3/NaHCO_3$	DMF/ 5/ 90	90

2)	X	Catalytic system	Solvent; time/h; temp./ °C	Yield of **IV** (%)
2a)	I	$Pd(PPh_3)_2Cl_2$/9-BBN/$NaHCO_3$	DMF/THF/ 12/ 80	no reaction
2b)	Br	$Pd(OAc)_2$/CuO/PPh_3/n-BuLi/Et_2NH	THF/5 / 45	no reaction
2c)	Br	$Pd(PPh)_3Cl_2$/CuI/Et_3N	MeCN/ 5 / 50	no reaction
2d)	I	$Pd(PPh)_3Cl_2$/CuI/Et_3N	MeCN/ 0,5/ 50	100

Scheme 3. Selection of the catalytic system for C–C(6) bond formation.

quinolone, while variation of the number of carbon atoms of the ω-oxy-carboxylic acid has been investigated.

Synthetic routes to the target compounds are outlined in the Schemes 1 and 2.

Two key intermediates on the route to target compounds, the quinolone carboxylic acid **4** and its congener **17**, were prepared in three steps, differing only in the way the ether bond is formed in terminal alkynes **2** and **15**.

Reactivity of 6-iodo- and 6-bromoquinolone carboxylic acid ethyl ester in alkenylation and alkynylation reactions, and selection of the best catalytic system were studied with Boc-allyl alcohol and Boc-propargyl alcohol. Representative runs are given in the Scheme 3.

Heck reaction *1*) was optimized using conditions *1b*. Relatively long reaction time and high temperature proved impractical, however, for large-scale batches. Suzuki reaction *2a*, with $Pd(PPh_3)_2Cl_2$/9-BBN as the catalyst, also failed. The best results were obtained with Sonogashira catalytic system **2d**, 6-iodo-quinolone **II** as the substrate and Boc-propargyl alcohol.

Having these results at hand, Sonogashira coupling reaction of alkynes **2** and **15** with 6-iodoquinolone derivative[23–25] was completed in deaerated MeCN, in the presence of Et_3N, dichlorobis(triphenylphosphine) palladium (II) and Cu(I) iodide. Base-catalyzed hydrolysis of **3** and **16** afforded dicarboxylic acids **4** and **17**, respectively. To our satisfaction, these dicarboxylic acids were completely site-selectively activated, with the more acidic carboxylic group at the aliphatic terminus forming an activated complex with EDC/DMAP.

Starting 2′-*O*-acetyl macrolides: 2′-*O*-acetyl-azithromycin (**5**), 11-*O*-Me-2′-*O*-acetyl azithromycin (**6**) and 2′-*O*-acetyl clarithromycin (**7**), used as the second components, were prepared by the published route using acetic anhydride/triethylamine.[26]

Selective acylation at 4″-OH of **5**–**7** with **4** and **17**, was completed at 0 °C in a solvent mixture DMF/DCM, and followed by methanolysis of 2′-*O*-acetyl group at slightly elevated temperature. Alkynes **8**, **9**, **12**, **18** and **20** were catalytically hydrogenated at 5 bars and rt, avoiding any over-hydrogenation or hydrolytic split. All alkynes and final compounds were purified by column chromatography and were characterized by ^1H, ^{13}C NMR, HRMS and IR. Proposed structures were confirmed by NMR studies. Downfield shift of 4″-H signal from 3.04 ppm in azithromycin and 3.00 in clarithromycin to 4.70 ppm in new compounds **8**, **9**, **12**, **18** and **20** was observed.[27,28] Also, it was observed that the 4″-H proton in the new derivatives **8**, **9**, **12**, **18** and **20** resonates as a doublet, but as a triplet in the starting compounds, in accordance with acylation of a free hydroxyl group at 4″ positions, hence removal of the 4″-H and 4″-OH coupling.

In addition, the NMR spectra showed the presence of the expected signals arising from the quinolone and linker moiety. The ^1H NMR spectra showed signals characteristic for the quinolone 2H atom at 8.79 ppm resonates as singlet, 1,2,4-trisubstituted ring at 8.60 ppm as doublet, 7.86 ppm as doublet of doublets and 7.61 ppm as doublet, also the *N*-ethyl signals at 4.41 ppm as quartet and 1.60 ppm as triplet.

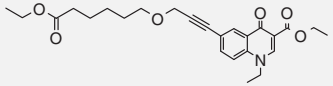
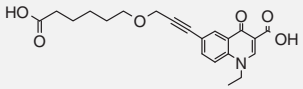
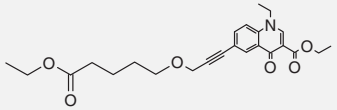
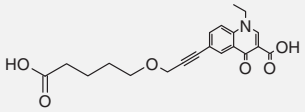
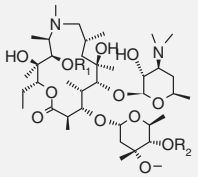
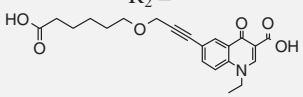
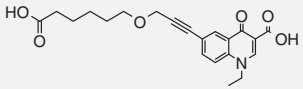
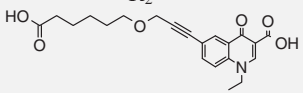
The appearance of signals at 1.95 ppm and 2.89 ppm in ^1H NMR, and at 31 ppm, and disappearance of the signals at 83 ppm in ^{13}C NMR indicated reduction of the triple bond in **8**, **9**, **12**, **18** and **20** to single bond in **10**, **11**, **13**, **19** and **21**.

2.2. Biological results

For all new compounds antibacterial activity was determined against the panel of relevant Gram positive (*Streptococcus pneumoniae*, *Streptococcus pyogenes* and *Staphylococcus aureus*) and Gram negative (*Haemophilus influenzae*) respiratory tract pathogens that were either sensitive or resistant to macrolide antibiotics. Minimum inhibitory concentrations (MICs) were determined by the standard broth microdilution method[29] and are given in units of mg/L, Table 1. Azithromycin, clarithromycin and telithromycin were used as comparators.

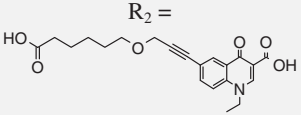
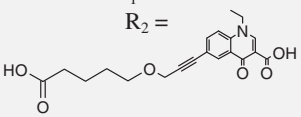
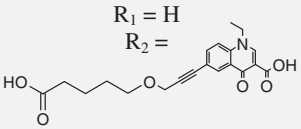
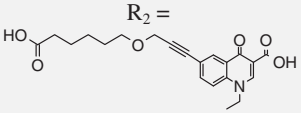
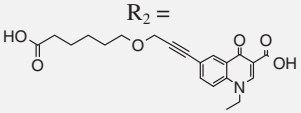
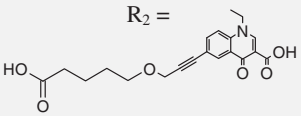
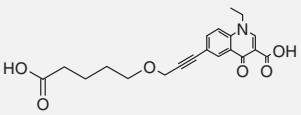
The majority of novel compounds were more potent against selected bacterial strains then azithromycin and some previously reported derivatives of 8a-lactams.[21] In contrast to the final macrolide compounds, it is important to note that non-macrolide molecules **3**, **4** and **16** containing quinolone and linker, proved antibacterialy inactive, while some activity of **17** was observed against streptococci. All macrolide derivatives were highly active against macrolide sensitive isolates of *S. pneumoniae* and *S. pyogenes*, but compared to azithromycin, clarithromycin derivatives

Table 1
Antibacterial activity of reported compounds, given as minimum inhibitory concentration (MIC) in units of mg/L

	Strains	*S. aureus* ATCC13709	*S. pneumoniae* SP 030	*S. pyogenes* 3565	*S. aureus* 90265/97	*S. pneumoniae* 134 GR-M	*S. pyogenes* Finland 11	*S. aureus* PK1	*S. pneumoniae* Ci137	*S. pyogenes* Finland 2	*S. pneumoniae* 58 Spain	*S. pyogenes* 166 GR-Micro	*H. influenzae* ATTC 49247
	Phenotype	eryS	eryS	eryS	iMLS	iMcLS	iMLS	M	M	M	cMLS	cMLS	
	TEL	0.25	<0.125	<0.125	<0.125	0.25	<0.125	0.125	0.5	0.5	0.25	16	1
	AZM	0.5	<0.125	<0.125	>64	>64	16	>64	8	16	>64	>64	1
	CAM	⩽0.125	⩽0.125	⩽0.125	>64	>64	1	32	4	4	>64	>64	8
3		>64	16	8	>64	64	64	>64	16	>64	64	>64	>64
4		>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64
16		>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64	>64
17		>64	1	1	>64	>64	8	>64	4	16	2	>64	>64
	 $R_1 = H$ $R_2 =$												
8		<0.125	<0.125	<0.125	1	<0.125	<0.125	0.25	<0.125	<0.125	<0.125	0.5	0.5
	$R_1 = H$ $R_2 =$												
10		0.25	<0.125	<0.125	0.25	<0.125	<0.125	0.25	<0.125	<0.125	<0.125	<0.125	0.5
	$R_2 =$												
9		0.5	<0.125	<0.125	4	0.5	<0.125	1	<0.125	<0.125	<0.125	2	1

(*continued on next page*)

Table 1 (*continued*)

	Strains	*S. aureus* ATCC13709	*S. pneumoniae* SP 030	*S. pyogenes* 3565	*S. aureus* 90265/97	*S. pneumoniae* 134 GR-M	*S. pyogenes* Finland 11	*S. aureus* PK1	*S. pneumoniae* Ci137	*S. pyogenes* Finland 2	*S. pneumoniae* 58 Spain	*S. pyogenes* 166 GR-Micro	*H. influenzae* ATTC 49247
	Phenotype	eryS	eryS	eryS	iMLS	iMcLS	iMLS	M	M	M	cMLS	cMLS	
	TEL	0.25	<0.125	<0.125	<0.125	0.25	<0.125	0.125	0.5	0.5	0.25	16	1
	AZM	0.5	<0.125	<0.125	>64	>64	16	>64	8	16	>64	>64	1
	CAM	⩽0.125	⩽0.125	⩽0.125	>64	>64	1	32	4	4	>64	>64	8
11	$R_1 = CH_3$, $R_2 =$ 	1	<0.125	<0.125	2	0.5	<0.125	1	<0.125	<0.125	<0.125	0.5	1
18	$R_1 = H$, $R_2 =$ 	0.5	<0.125	<0.125	4	0.5	<0.125	0.5	<0.125	<0.125	<0.125	1	1
19	$R_1 = H$, $R_2 =$ 	0.5	<0.125	<0.125	4	0.25	<0.125	0.5	<0.125	<0.125	<0.125	0.25	2
12	$R_2 =$ 	2	<0.125	<0.125	8	4	<0.125	2	<0.125	<0.125	<0.125	2	16
13	$R_2 =$ 	2	<0.125	<0.125	4	1	<0.125	2	<0.125	<0.125	<0.125	<0.125	8
20	$R_2 =$ 	1	<0.125	<0.125	4	1	<0.125	1	<0.125	<0.125	<0.125	4	4
21		0.5	<0.125	<0.125	2	0.5	<0.125	0.5	<0.125	<0.125	<0.125	<0.125	1

AZM = azithromycin; TEL = telithromycin; CAM = clarithromycin; iMLS = inducible resistance to macrolide, licosamide and streptogramin (MLS) antibiotics; iMcL = inducible resistance to macrolide and constitutive resistance to lincosamide antibiotics; cMLS = constitutive MLS resistance; M = efflux mediated macrolide resistance.

with short linker, compounds **12** and **13**, demonstrated reduced activity against *S. aureus*. Generally, good potency against macrolide-resistant organisms, having either macrolide-lincosamide-streptogramin B (MLS_B) or efflux (M) resistant phenotype, was observed. Compounds with reduced triple bond were more potent compared to their triple bond containing counterparts; **8** versus **10**, **9** versus **11**, **12** versus **13**, **18** versus **19** and **20** versus **21**, and the difference in their potency against constitutively resistant, ribosomal methyltransferase producing cMLS *S. pyogenes* was the most pronounced. These compounds provide full coverage of *S. pyogenes* strains, irrespective of the underlying resistance mechanism, which represents an advantage over telithromycin, that does not cover constitutively resistant isolates.[30,31] However, with the exception of compound **10**, telithromycin still provides better coverage of *S. aureus* strains. In comparison to their azithromycin or 11-*O*-Me counterparts, potency of clarithromycin derivatives against the key Gram negative respiratory pathogen *H. influenzae* is reduced. Elongation of the chain for one methylene group improved antibacterial activity on azithromycin and clarithromycin scaffold (**8** vs **18**, **10** vs **19**, **12** vs **20** and **13** vs **21**).

Regarding overall antimicrobial profile, compound **10** is the most potent one, with the best activity against the *H. influenzae* and clear improvements over both azithromycin and telithromycin (cMLSb *S. pyogenes* MIC $\leqslant$0.125 vs 16 μg/L).

3. Conclusion

In conclusion, two short series of macrolones with terminal *N*-ethyl-3-carboxylquinolone unit, tethered at C(6) by C–C bond and by an ester unit at C(4″)–OH to the macrolide scaffold were prepared. They have improved antibacterial potency against key respiratory pathogens resistant to currently used macrolide antibiotics. The most potent compound **10** has broader spectrum of activity than the marketed ketolide telithromycin, as it provides coverage of cMLSb resistant strains of *S. pyogenes*. As such, it represents a promising lead in our constant efforts to overcome bacterial resistance.

4. Experimental

4.1. Synthetic procedures

4.1.1. 6-Propyn-2-yloxy-hexanoic acid ethyl ester (2)

To the solution of compound **1** (0.50 ml, 3.10 mmol) in THF (5.0 ml) TBAI (57.2 mg, 0.15 mmol), NaI (69.7 mg, 0.46 mmol), propargylbromide (518.0 ml, 4.65 mmol) and solid KOH (173.9 mg, 3.10 mmol) were added. Reaction mixture was stirred for 24 h at rt, solvent was then evaporated and residue was partitioned between EtOAc and water. Organic layer was washed with satd aq NaCl (2 × 10 ml), dried (K_2CO_3) and evaporated at reduced pressure to give 0.347 g of product **2**. MS (*m*/*z*): calcd for MH^+ 199.27; found: 199.27.

4.1.2. 6-[3-(5-Carbethoxy-pentyloxy)-propyn-1-yl]-1-ethyl-4-oxo-1,4-dihydro-quinoline-3-carboxylic acid ethyl ester (3)

1-Ethyl-6-iodo-4-oxo-1,4-dihydro-quinoline-3-carboxylic acid ethyl ester (312.0 mg, 0.84 mmol), Cu(I)I (16.0 mg, 0.08 mmol) and Et_3N (4.1 ml, 29.40 mmol) were dissolved in dry MeCN (10.0 ml). Solution was heated at 50 °C and draught by N_2. After 20 min, $Pd(II)PPh_3Cl_2$ (18.0 mg, 0.03 mmol) and compound **2** (347.0 mg, 1.75 mmol) were added. Reaction mixture was stirred at rt for 24 h, then solvent was evaporated and residue was partitioned between EtOAc and water. Organic layer was washed with satd aq NaCl (2 × 10 ml), dried (K_2CO_3) and evaporated at reduced pressure to give 476.0 mg of product **3**, which is used in the next step without further purification. **HRMS (ES)** calcd for $C_{25}H_{31}NO_6$ $(M+H)^+$ 442.2230; found 442.2224.

4.1.3. 6-[3-(5-Carboxy-pentyloxy)-propyn-1-yl]-1-ethyl-4-oxo-1,4-dihydro-quinoline-3-carboxylic acid (4)

To the solution of compound **3** (476.0 mg, 1.08 mmol) in THF (5.5 ml) solution of NaOH (185.0 mg, 4.62 mmol) in water (5.5 ml) was added and solution was heated at 80 °C for 2 h, than stirred at rt for 12 h. Reaction mixture was partitioned between EtOAc and water. Layers were separated, pH of aqueous adjusted to 5.2 with 2 N HCl, than washed with DCM (3 × 20 ml). Organic extracts were dried (K_2CO_3) and evaporated yielding 184.0 mg of product **4**, which is used in the next step without further purification. **HRMS (ES)** calcd for $C_{21}H_{23}NO_6$ $(M+H)^+$ 386.1604; found 386.1589.

4.1.4. 4″-*O*-{6-[3-(3-Carboxy-1-ethyl-4-oxo-1,4-dihydro-quinoline-6-yl)-propyn-2-yloxy]-hexanoyl}-azithromycin (8)

To the solution of compound **4** (184.0 mg, 0.48 mmol) in dry DMF (6.0 ml), under nitrogen at 0 °C, EDAC × HCl, 131.8 mg, 0.69 mmol) was added and solution of 2′-*O*-acetyl-azithromycine (**5**, 237.3 mg, 0.30 mmol) in dry DCM was added dropwise. Finally, to the reaction mixture DMAP (61.6 mg, 0.50 mmol) was added, resulting suspension stirred at 0 °C for 3 h, than allowed to gradually reach rt over 24 h. Solvent was evaporated and residue was partitioned between EtOAc and water. Organic layer was washed with brine, dried (K_2CO_3) and evaporated to dryness at reduced pressure to give 325.0 mg of crude intermediate. MS spectra confirmed structure of 2′-*O*-acetyl derivative of **8**. MS (*m*/*z*): calcd for MH^+ 1159.45; found: 1158.63.

To complete deacetylation, solution of crude 2′-*O*-acetyl intermediate (325.0 mg, 0.28 mmol) in MeOH (40.0 ml) was heated 55 °C for 12 h. Solvent was evaporated at reduced pressure, and crude **8** was purified by low-pressure column chromatography with solvent mixture DCM/MeOH/NH_3 (90:5:0.5) to give 106.0 mg of pure **8**. **HRMS (ES)** calcd for $C_{59}H_{93}N_3O_{17}$ $(M+H)^+$ 1116.6583; found 1116.6592.

4.1.5. 4″-*O*-{6-[3-(3-Carboxy-1-ethyl-4-oxo-1,4-dihydroquinolin-6-yl)-propoxy]-hexanoyl}-azithromycin (10)

To the solution of **8** (106.0 mg, 0.10 mmol) in EtOH (15.0 ml) was added 10% Pd–C catalyst (14.0 mg) and stirred under hydrogen (5 Barr) for 20 h. The catalyst was removed by filtration, filtrate was concentrated and residual yellow oil (130.0 mg) was diluted with hexane/EtOAc (10:1). The product was collected on filter, washed with hexane/EtOAc (10:1) and dried to give compound **10** (51.0 mg) as yellow crystalline product. **HRMS (ES)** calcd for $C_{59}H_{97}N_3O_{17}$ $(M+H)^+$ 1120.6896; found 1120.6891.

4.1.6. 4″-*O*-{6-[3-(3-Carboxy-1-ethyl-4-oxo-1,4-dihydro-quinoline-6-yl)-propyn-2-yloxy]-hexanoyl}-11-*O*-methyl-azithromycin (9)

Applying the same protocol as for the preparation of **8**, compound **9** was obtained from compound 2′-*O*-Ac-11-*O*-methyl-azithromycin **6**[32] (489.0 mg, 0.61 mmol) and compound **4** (280.0 mg, 0.73 mmol) in the presence of EDAC × HCl (268.0 mg, 1.40 mmol) and DMAP (125.0 mg, 1.03 mmol). Crude product (750.0 mg) was isolated by extraction as yellow oil by the same procedure as described for compound **8**.

To complete deacetylation, solution of oily 2′-*O*-acetyl intermediate (750.0 mg, 0.64 mmol) in MeOH (50.0 ml) was heated 55 °C for 12 h. Solvent was evaporated at reduced pressure, and crude **9** was purified by low-pressure column chromatography with solvent mixture DCM/MeOH/NH_3 (90:9:0.5) to give 66.0 mg of product **9**; **HRMS (ES)** calcd for $C_{60}H_{95}N_3O_{17}$ $(M+H)^+$ 1130.6740; found 1130.6698.

4.1.7. 4″-*O*-{6-[3-(3-Carboxy-1-ethyl-4-oxo-1,4-dihydro-quinoline-6-yl)-propoxy]-hexanoyl}-11-*O*-Me-azithromycin (11)

Applying the same protocol as for the synthesis of compound **10**, compound **11** was obtained from the compound **9** (55.0 mg, 0.05 mmol). Pure product **11** (50.0 mg) was obtained after filtration of catalyst and evaporation of organic solvent. **HRMS (ES)** calcd for $C_{60}H_{99}N_3O_{17}$ $(M+H)^+$ 1134.7053; found 1134.7024.

4.1.8. 4″-*O*-{6-[3-(3-Carboxy-1-ethyl-4-oxo-1,4-dihydro-quinoline-6-yl)-propyn-2-yloxy]-hexanoyl}-clarithromycin (12)

Applying the same protocol as for the synthesis of compound **8**, compound **12** was obtained from 2′-*O*-acetyl clarithromycine (**7**, 233.0 mg, 0.30 mmol) and compound **4** (148.0 mg, 0.38 mmol) in the presence of EDAC × HCl (132.0 mg, 0.69 mmol) and DMAP (62.0 mg, 0.50 mmol). Pure product (302.0 mg) was isolated by extraction as yellow oil by the same procedure as described for compound **8**.

To complete deacetylation, solution of 2′-*O*-acetyl intermediate (302.0 mg, 0.26 mmol) in MeOH (30.0 ml) was heated 50 °C for 12 h. Solvent was evaporated at reduced pressure, and crude product was purified by low-pressure column chromatography with solvent mixture DCM/MeOH/NH_3 (90:9:1.5) to give 78.0 mg of product **12**. **HRMS (ES)** calcd for $C_{59}H_{90}N_2O_{18}$ $(M+H)^+$ 1115.6267; found 1115.6217.

4.1.9. 4″-*O*-{6-[3-(3-Carboxy-1-ethyl-4-oxo-1,4-dihydro-quinoline-6-yl)-propoxy]-hexanoyl}-clarithromycin (13)

To the solution of **12** (44.0 mg, 0.04 mmol) in EtOH (15.0 ml) 10% Pd–C catalyst (20.0 mg) was added, and stirred under hydrogen (5 Barr) for 20 h. The catalyst was removed by filtration and filtrate was concentrated. The residue diluted with mixture of hexane/EtOAc (10:1), product was filtered, washed with the same solvent mixture and dried to give compound **13** (15.0 mg) as yellow crystalline product. **HRMS (ES)** calcd for $C_{59}H_{94}N_2O_{18}$ $(M+H)^+$ 1119.6580; found 1119.6564.

4.1.10. 5-Propyn-2-yloxy-pentanoic acid ethyl ester (15)

To the solution of propargyl alcohol (1.0 ml, 17.2 mmol) in DMF (10.0 ml) at 0 °C under N_2 first was added NaH (0.69 g, 17.2 mmol) then compound **14** (2.72 ml, 17.2 mmol). Reaction mixture was stirred overnight at 50 °C, solvent was evaporated and residue partitioned between EtOAc and water. Organic layer was washed with brine, dried (K_2CO_3) and evaporated to dryness at reduced pressure to give 2.64 g of product **15**. MS (*m*/*z*): calcd MH^+ 185.24; found: 185.26.

4.1.11. 6-[3-(4-Ethoxycarbonyl-butoxy)-propyn-1-yl]-1-ethyl-4-oxo-1,4-dihydro-quinoline-3-carboxylic acid ethyl ester (16)

1-Ethyl 6-iodo-4-oxo-1,4-dihydro.quinoline carboxylic acid ethyl ester (1.0 g, 2.70 mmol), Cu(I)I (51.0 mg, 0.27 mmol) and Et_3N (13.1 ml, 94.5 mmol) were dissolved in dry MeCN (20.0 ml). The solution was stirred at 50 °C under N_2 for 20 min, then $Pd(II)PPh_3Cl_2$ (57.0 mg, 0.08 mmol) and compound **15** (1.2 g, 3.24 mmol) were added. Solvent was evaporated and crude product was partitioned between EtAc and water Organic layer was washed with aq 5% NaCl and satd aq $NaHCO_3$ dried over (K_2CO_3) and evaporated. Crude product was purified by low-pressure column chromatography eluted with solvent mixture DCM/MeOH/NH_3 (90:9:0.5) to give 0.48 g of pure **16**. **HRMS (ES)** calcd for $C_{24}H_{29}NO_6$ $(M+H)^+$ 428.2073; found 428.2068.

4.1.12. 6-[3-(4-Carboxy-butoxy)-propyn-1-yl]-1-ethyl-4-oxo-1,4-dihydro-quinoline-3-carboxylic acid (17)

To the solution of compound **16** (484.0 mg, 1.13 mmol) in THF (5.0 ml) solution of NaOH (250.0 mg, 6.25 mmol) in water (5.0 ml was added) and stirred, first at 80 °C for 2 h than at rt for 12 h. The reaction mixture was partitioned between EtAc (20 ml) and water (20 ml). The pH of aqueous layer was adjusted to 5.1 by 2 N HCl, than washed with DCM (3 × 20 ml). Organic extracts were dried (K_2CO_3) and evaporated yielding 185.0 mg of yellow, oily product **17**, which is used in the next step without further purification. **HRMS (ES)** calcd for $C_{20}H_{21}NO_6$ $(M+H)^+$ 372.1447; found 372.1438.

4.1.13. 4″-*O*-{5-[3-(3-Carboxy-1-ethyl-4-oxo-1,4-dihydro-quinoline-6-yl)-propoxy]-pentanoyloxy}-azithromycin (18)

To the solution of compound **17** (150.0 mg, 0.40 mmol) in dry DMF (6.0 ml), EDAC × HCl (176.0 mg, 0.92 mmol) was added under N_2 at 0 °C, then dropwise solution of 2′-*O*-acetyl-azithromycin (**5**, 380.0 mg, 0.48 mmol) in dry DCM (3.0 ml), and finally DMAP (82.0 mg, 0.67 mmol). Suspension was stirred at 5 °C for 3 h, than gradually heated to rt over 24 h. Solvent was evaporated and residue was partitioned between EtOAc (20 ml) and water (20 ml). Organic layer was washed with brine, dried (K_2CO_3) and evaporated at low pressure to yield 750.0 mg of product **18**. MS (*m*/*z*): calcd for MH^+ 1145.41; found: 1145.20.

Solution of crude 2′-*O*-acetyl-intermediate (750.0 mg, 0.65 mmol) in MeOH (50.0 ml) was stirred at 55 °C over 12 h. Solvent was evaporated at low pressure, crude product was purified by low-pressure column chromatography with the solvent mixture DCM/MeOH/NH_3 (90:9:0.5) to give 33.0 mg of pure **18**. **HRMS (ES)** calcd for $C_{58}H_{91}N_3O_{17}$ $(M+H)^+$ 1102.6427; found 1102.6392.

4.1.14. 4″-*O*-{5-[3-(3-Carboxy-1-ethyl-4-oxo-1,4-dihydro-quinoline-6-yl)-propoxy]-pentanoyloxy}-azithromycin (19)

Hydrogenation of compound **18** (33.0 mg, 0.03 mmol) in EtOH (20.0 ml) over 10% Pd–C (15.0 mg) at hydrogen pressure 5 bars over 20 h afforded, after filtration of catalyst and evaporation of organic solvent, pure **19** (2.08 mg).

HRMS (ES) calcd for $C_{58}H_{95}N_3O_{17}$ $(M+H)^+$ 1106.6740; found 1106.6678.

4.1.15. 4″-*O*-{5-[3-(3-Carboxy-1-ethyl-4-oxo-1, 4-dihydro-quinoline-6-yl)-propyn-2-yloxy]-pentanoyloxy}-clarithromycine (20)

To the solution of compound **17** (185.0 mg, 0.50 mmol) in dry DMF (8.0 ml), EDAC × HCl (220.0 mg, 1.15 mmol) was added under N_2 at 0 °C, then dropwise solution of 2′-*O*-acetyl-clarithromycin (**7**, 475.0 mg, 0.60 mmol) in dry DCM (4.0 ml), and finally DMAP (103.0 mg, 0.84 mmol). Suspension was stirred at 5 °C for 3 h, then gradually heated to rt over 24 h. Solvent was evaporated and residue was partitioned between EtOAc (10 ml) and water 6553333 (10 ml). Organic layer was washed with brine, dried (K_2CO_3) and evaporated at low pressure to give 490.0 mg of 2′-*O*-acetyl-intermediate. MS (*m*/*z*): calcd for MH^+ 1145.41; found: 1145.20.

Deacetylation of crude intermediate (490.0 mg, 0.43 mmol) was completed in MeOH (70.0 ml) at 55 °C under stirring over 12 h. Solvent was evaporated at low pressure, crude product was purified by low-pressure column chromatography with the solvent mixture DCM/MeOH/NH_3 (90:9:0.5) to give 120.0 mg of product **20**.

HRMS (ES) calcd for $C_{58}H_{88}N_2O_{18}$ $(M+H)^+$ 1101.6110; found 1101.6100.

4.1.16. 4″-*O*-{5-[3-(3-Carboxy-1-ethyl-4-oxo-1,4-dihydro-quinoline-6-yl)-propoxy]-pentanoyloxy}-clarithromycine (21)

Hydrogenation of compound **20** (90.0 mg, 0.08 mmol) in EtOH (20.0 ml) over 10% Pd–C (30.0 mg) at hydrogen pressure 5.0 bars for 20 h afforded after filtration of the catalyst and evaporation of organic solvent product **21** (70.0 mg). **HRMS (ES)** calcd for $C_{58}H_{92}N_2O_{18}$ $(M+H)^+$ 1105.6423; found 1105.6331.

Acknowledgements

The authors thank Ana Čikos, Biserka Metelko and Dubravska Gembarovski from the Structure and Analysis Group for recorded data and Mark Vine for constructive advisees. We wish to thank Vitomir Šunjic and Gorjana Lazarevski for their scientific insights during this investigation. We would also like to thank Jadranka Ivetić and Višnja Majzel for excellent technical assistance.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2010.06.048. These data include MOL files and InChiKeys of the most important compounds described in this article.

References and notes

1. Sunitaki, T.; Omura, S.; Iwasaki, S.; Omura, S. Chemical Modification of Macrolides. In *Macrolide Antibiotics, Chemistry, Biology and Practice*; Academic Press, 2002; pp 99–179.
2. Pal, S. *Tetrahedron* **2006**, *62*, 3171.
3. Mutak, S. *J. Antibiot.* **2007**, *60*, 85.
4. Nilius, A. M.; Ma, Z. *Curr. Opin. Pharmacol.* **2002**, *2*, 493.
5. Hunziker, D.; Wyss, P.-C.; Angehrn, P.; Mueller, A.; Marty, H.-P.; Halm, R.; Kellenberger, L.; Bitsch, V.; Biringer, G.; Arnold, W.; Stämpfli, A.; Schmitt-Hoffmann, A.; Cousot, D. *Bioorg. Med. Chem.* **2004**, *12*, 3503.
6. Ma, Z.; Clark, R. F.; Brazzale, A.; Wang, S.; Rupp, M. J.; Li, L.; Griesgraber, G.; Zhang, S.; Yong, H.; Phan, L. T.; Nemoto, P. A.; Chu, D. T. W.; Plattner, J. J.; Zhang, X.; Zhong, P.; Cao, Z.; Nilius, A. M.; Shortridge, V. D.; Flamm, R.; Mitten, M.; Meulbroek, J.; Ewing, P.; Alder, J.; Or, Y. S. *J. Med. Chem.* **2001**, *44*, 4137.
7. Or, Y. S.; Clark, R. F.; Wang, S.; Chu, D. T. W.; Nilius, A. M.; Flamm, R. K.; Mitten, M.; Ewing, P.; Alder, J.; Ma, Z. *J. Med. Chem.* **2000**, *43*, 1045.
8. Keyes, R. F.; Carter, J. J.; Englund, E. E.; Daly, M. M.; Stone, G. G.; Nillius, A. M.; Ma, Z. *J. Med. Chem.* **2003**, *46*, 1795.
9. Plata, D. J.; Leanna, M. R.; Rasmussen, M.; McLaughlin, M. A.; Condon, S. L.; Kerdesky, F. A. J.; King, S. A.; Peterson, M. J.; Stoner, E. J.; Wittenberger, S. J. *Tetrahedron* **2004**, *60*, 10171.
10. Denis, A.; Renou, C. *Tetrahedron Lett.* **2002**, *43*, 4171.
11. Burger, M. T.; Hiebert, C.; Seid, M.; Chu, D. T.; Barker, L.; Langhorne, M.; Shawar, R.; Kidney, J.; Deasi, M. C.; Plattner, J. J. *Bioorg. Med. Chem.* **2006**, *14*, 5592.
12. Kaneko, T.; Romero, K.; Li, B.; Buzon, R. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 5049.
13. Grant, E. B.; Guiadeen, D.; Abbanat, D.; Foleno, B. D.; Bush, K.; Macielag, M. J. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 1929.
14. Xu, X.; Henninger, T.; Abbanat, D.; Bush, K.; Foleno, B.; Hilliard, J.; Macielag, M. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 883.
15. Zhu, B.; Marinelli, B. A.; Abbanat, D.; Foleno, B. D.; Bush, K.; Macielag, M. J. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 3900.
16. Beebe, X.; Yang, F.; Bui, M. H.; Mitten, M. J.; Ma, Z.; Nilius, A. M.; Djuric, S. W. *Bioorg. Med. Chem. Lett.* **2004**, *14*, 2417.
17. Yong, H.; Gu, Y. G.; Clark, R. F.; Marron, T.; Ma, Z.; Soni, N.; Stone, G. G.; Nilius, A. M.; Marsh, K.; Djuric, S. W. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 2653.
18. Tanikawa, T.; Asaka, T.; Kashimura, M.; Misawa, Y.; Suzuki, K.; Sato, M.; Kameo, K.; Morimoto, S.; Nishida, A. *J. Med. Chem.* **2001**, *44*, 4027.
19. Xu, P.; Liu, L.; Jin, Z.; Lei, P. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 3330.
20. Stimac, V.; Alihodzic, S.; Lazarevski, G.; Mutak, S.; Marusic-Istuk, Z.; Fajdetic, A.; Palej, I.; Padovan, J.; Tavcar, B.; Cipcic Paljetak, H.; Erakovic Haber, V. *J. Antibiot.* **2009**, *62*, 133.
21. Hutinec, A.; Đerek, M.; Lazarevski, G.; Šunjić, V.; Čipčić Paljetak, H.; Alihodžić, S.; Eraković Haber, V.; Dumić, M.; Maršić, N.; Mutak, S. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 3244.
22. Fajdetić, A.; Čipčić Paljetak, H.; Lazarevski, G.; Hutinec, A.; Alihodžić, S.; Đerek, M.; Štimac, V.; Andreotti, D.; Šunjić, V.; Berge, J. M.; Mutak, S.; Dumić, M.; Lociuro, S.; Holmes, D. J.; Maršić, N.; Eraković Haber, V.; Spaventi, R. *Bioorg. Med. Chem.* **2010**, *18*, 6559.
23. Ceccheti, V.; Clementi, S.; Cruciani, G.; Fravolini, A.; Pagella, P. G.; Savino, A.; Tabarrini, O. *J. Med. Chem.* **1995**, *38*, 973.
24. Hooper, D. C.; Rubinstein, E. Quinolone Antimicrobial Agents, 3rd ed.; ASM Press, American Society for Microbiology: 1752N Street NW, Washington, DC 20036-2904, 2003; pp 3–19.
25. Ellis, J.; Gellert, E.; Robson, J. *Aust. J. Chem.* **1973**, *26*, 907.
26. Bright, G. M. USP 4,474,768, October 2, 1984, Prior. November 15, 1982.
27. Lazarevski, G.; Vinkovic, M.; Kobrehel, G.; Djokic, S. *Tetrahedron* **1993**, *49*, 721.
28. Steinmetz, W.; Bersch, R.; Towson, J.; Pesiri, D. *J. Med. Chem.* **1992**, *35*, 4842.
29. Clinical Laboratory Standard Institute CLSI *Performance Standards for Antimicrobial Susceptibility Testing: 15th Informational Supplement M100-S15*; Clinical Laboratory Standards Institute: Wayne, Pa, 2005.
30. Drago, L.; Ripa, S.; Zampaloni, C.; De, V. E.; Vitali, L. A.; Petrelli, D.; Prenna, M. *Chemotherapy* **2005**, *51*, 268.
31. Mazzariol, A.; Koncan, R.; Vitali, L. A.; Cornaglia, G. *J. Antimicrob. Chemother.* **2007**, *59*, 1171.
32. Kidemet, D.; Lazarevski, G.; Derek, M.; Leljak, M. PCT WO2004/106354 A1, Dec. 9, 2004.