Bioorganic & Medicinal Chemistry 18 (2010) 6559–6568

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

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

4″-*O*-(ω-Quinolylamino-alkylamino)propionyl derivatives of selected macrolides with the activity against the key erythromycin resistant respiratory pathogens

A. Fajdetić [a,*], H. Čipčić Paljetak [a,†], G. Lazarevski [a], A. Hutinec [a], S. Alihodžić [a], M. Đerek [b,‡], V. Štimac [a], D. Andreotti [c], V. Šunjić [a], J. M. Berge [d], S. Mutak [b,§], M. Dumić [e,¶], S. Lociuro [c], D. J. Holmes [f], N. Maršić [b,‖], V. Eraković Haber [a], R. Spaventi [a]

[a] *GlaxoSmithKline Research Centre Zagreb Ltd, Prilaz baruna Filipovića 29, HR-10000 Zagreb, Croatia*
[b] *PLIVA Research Institute Ltd, Prilaz baruna Filipovića 29, HR-10000 Zagreb, Croatia*
[c] *GlaxoSmithKline Medicine Research Centre, Via A. Fleming 4, I-37135 Verona, Italy*
[d] *GlaxoSmithKline, New Frontiers Science Park South Third Avenue, Harlow Essex CM19 5AW, UK*
[e] *PLIVA Research and Development, Prilaz baruna Filipovića 29, HR-10000 Zagreb, Croatia*
[f] *GlaxoSmithKline, 1250 South Collegeville Road, Collegeville, PA 19426, USA*

ARTICLE INFO

Article history:
Available online 22 June 2010

Keywords:
Macrolide
Quinolone
Antibacterial activity
Resistance

ABSTRACT

Four macrolides—6-*O*-methyl-8a-aza-8a-homoerythromycin, clarithromycin, azithromycin and azithromycin 11,12-cyclic carbonate, have been selected for the construction of a series of new quinolone derivatives. The quinolone moiety is connected to the macrolide scaffold via a diaminoaklyl 4″-*O*-propionyl ester chain of varying length. At the terminus the linker is attached via one of the nitrogen atoms in the linker at C(6) or C(7) of the quinolone. Many of compounds described, particularly clarithromycin derivative **37**, and azithromycin derivatives **48** and **55**, exhibited excellent antibacterial activity against a wide range of clinically relevant macrolide-resistant organisms, with profiles superior to that of telithromycin, an enhanced spectrum ketolide.

1. Introduction

The successful clinical use of macrolide antibiotics started in 1952 with the introduction of erythromycin A for the treatment of infections caused by Gram-positive cocci. Despite its numerous limitations (i.e., poor oral bioavailability, short half-life, acidic instability, gastrointestinal side effects) erythromycin is still utilized in general practice.[1] Second generation macrolide antibiotics, such as azithromycin and clarithromycin, overcome these issues and because of their superior antibacterial spectrum, pharmacokinetic properties and fewer side effects became widely prescribed, mainly for the treatment of upper and lower respiratory tract infections.[1]

However, as with all antibacterial agents continuous use of macrolides has greatly increased the number of infections caused by macrolide-resistant bacteria.[2,3] Such impaired efficacy of available antibiotics requires a constant effort to discover and develop new agents with an improved spectrum of activity.

It is a well established concept[4–6] that chemical modifications of the macrolide scaffold can lead to improved antibacterial activity against macrolide-resistant organisms. Erythromycin, clarithromycin and azithromycin are the most extensively explored macrolides in the search for new classes of broad spectrum antibacterial agents.[7,8] In the past we have performed chemical modification of macrolides first by varying substituents at 9a-N-position of the azithromycin scaffold,[9] and then by modifying 8a-lactam at C(3)–OH[10] and C(4″)–OH positions.[11] Our recent communication reported on synthesis and antibacterial activities of the first macrolide compounds with 4″-*O*-(3-amino)propionyl unit as a part of the linker tethering the second structural unit.[12] This position was lately explored by other groups as well and they have shown that introducing carbamate functionality on position 4″ of azithromycin lead to improved in vitro antibacterial activity against resistant *Streptococcus pneumoniae*.[13,14]

Initially, we have observed that introduction of specific (hetero)aromatic units at the terminal position of the linker extends the antibacterial spectrum,[12] and in this paper we report on

* Corresponding author. Tel.: +385 16051234; fax: +385 16051019.
E-mail address: andrea.z.fajdetic@gsk.com (A. Fajdetić).
† University of Zagreb, School of Medicine, Centre for Translational and Clinical Research, Šalata 2, 10000 Zagreb, Croatia.
‡ Private address: Grada Gualdo Tadino 28, HR-10000 Zagreb, Croatia.
§ Private address: Hercegovačka 99, HR-10000 Zagreb, Croatia.
¶ Present address: Department of Biotechnology, University of Rijeka, S. Krautzeka bb, HR-51000 Rijeka, Croatia.
‖ Private address: Prvi Vranovinski ogranak 2, HR-10000 Zagreb, Croatia.

0968-0896/$ - see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2010.06.049

exploration of novel 6-*O*-methyl-8a-aza-8a-homoerythromycin, clarithromycin, azithromycin and azithromycin 11,12-carbonate derivatives with quinolone-3-carboxylic acid moiety. A linker containing two nitrogen atoms was selected, the central one at β-position to the 4″-ester group, and the terminal one binding quinolone-3-carboxylic acid moiety at C(6) or C(7) position. Linkers of various lengths were selected, most of them with 2 carbon atoms between central and terminal nitrogen but also longer linkers containing 3 and 4 carbon atoms were explored.

2. Results and discussion

2.1. Chemistry

The synthesis of novel macrolide-quinolone derivatives is outlined in Scheme 1.

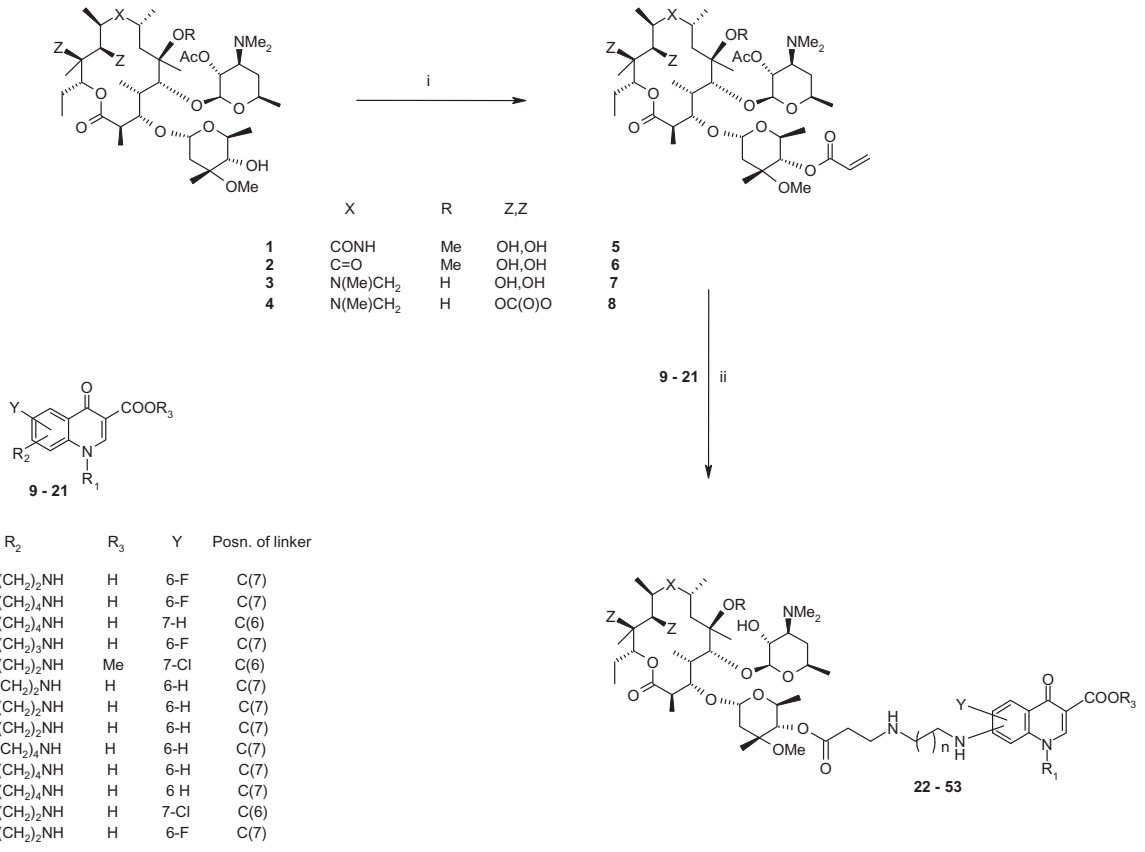
Clarithromycin and azithromycin, used for the preparation of starting molecules **2** and **3** in Scheme 1, are commercially available, whereas 8a-lactam **1**[15] and 11,12-carbonate of azithromycin **4**[16] were prepared by modified reported procedures. Preparation of the 4″-*O*-propenoyl esters **5–8** could be achieved using a previously described procedure.[17] Subsequent Michael reaction and removal of the 2′-*O*-acetyl protecting group was the route used to prepare compounds **30–35**.[18] Alternatively the 2′-*O*-acetyl protection can be removed prior to Michael addition and this route afforded target compounds **22–29** and **36–53**. Synthesis of the quinolone units **9–13**, and **20**, present in clarithromycin derivatives **30–35**, was achieved by heating of the appropriately substituted mono or dihalo-heterocycle with excess of diamine or the mono *N*-*t*-butoxycarbonyl diamine, followed by a separation of the formed regioi somers. Other quinolone-3-carboxylic derivatives with a linker at C(6)- or C(7)-position, were prepared either from commercially available quinolone compounds, or from 2,4-dichloroacetophenone according to the reported method.[19,20]

Azithromycin scaffold was linked to unsubstituted and C(7)-chloro and C(6)-fluoro substituted quinolones via proper propyl alkylamino chain. The nitrogen atom in the linker of the compounds **48**, **49**, **50** and **54** was methylated under the Eschweiler–Clark conditions to give compounds **55–58** with complete site-selectivity regarding two nitrogen atoms in the linker, Scheme 2. The same methylation method was used to prepare 11,12-carbonate derivatives **60–62**. Dehalogenation of compounds **48** and **51–54** and **59**, respectively, was achieved by the catalytic hydrogenolysis, Scheme 2.

In addition to methylation of the linker nitrogen, analogues **60–62** described above, the formation of a C(11)–C(12)-cyclic carbonate unit[21] on azithromycin, derivatives **51–59**, was used to offset the hydrophilicity of this more polar scaffold.

Crude products **22–53** were purified either by column chromatography eluting with dichloromethane containing an increasing concentration of methanol/ammonium hydroxide mixture (9:0.5), or by preparative LC using molecular mass detection. Desired structures of final compounds were confirmed by high resolution mass spectroscopy (HRMS) and ^{1}H and ^{13}C NMR spectroscopy.

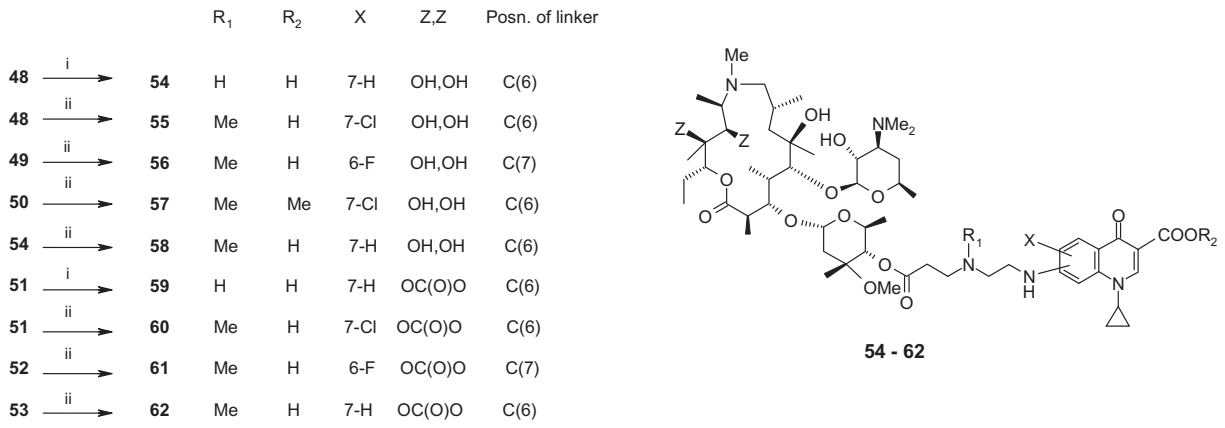
In the ^{13}C NMR spectra of compounds **22–52** two methylene signals at around 34 and 44 ppm appeared, while two signals at around 128 and 131 ppm, corresponding to the precursors **5–8** olefinic carbon atoms disappeared. These signals correlated with the proton signals at around 2.7, 2.6 and 3.0 ppm in the ^{1}H–^{13}C COSY spectra. The hydrogenolytic cleavage of carbon–chlorine was indicated by the presence of a new signal in the aromatic region (around 7.3 ppm) of ^{1}H NMR spectra for molecules **54** and **59** as



	X	R	Z,Z	
1	CONH	Me	OH,OH	**5**
2	C=O	Me	OH,OH	**6**
3	N(Me)CH_2	H	OH,OH	**7**
4	N(Me)CH_2	H	OC(O)O	**8**

	R_1	R_2	R_3	Y	Posn. of linker
9	cPr	$NH_2(CH_2)_2NH$	H	6-F	C(7)
10	cPr	$NH_2(CH_2)_4NH$	H	6-F	C(7)
11	cPr	$NH_2(CH_2)_4NH$	H	7-H	C(6)
12	cPr	$NH_2(CH_2)_3NH$	H	6-F	C(7)
13	cPr	$NH_2(CH_2)_2NH$	Me	7-Cl	C(6)
14	Me	$NH_2(CH_2)_2NH$	H	6-H	C(7)
15	Et	$NH_2(CH_2)_2NH$	H	6-H	C(7)
16	cPr	$NH_2(CH_2)_2NH$	H	6-H	C(7)
17	Me	$NH_2(CH_2)_4NH$	H	6-H	C(7)
18	Et	$NH_2(CH_2)_4NH$	H	6-H	C(7)
19	cPr	$NH_2(CH_2)_4NH$	H	6 H	C(7)
20	cPr	$NH_2(CH_2)_2NH$	H	7-Cl	C(6)
21	cPr	$NH_2(CH_2)_2NH$	H	6-F	C(7)

Scheme 1. Reagents and conditions: (i) 3-chloropropanoyl chloride, TEA, DCM, rt, 20 h; (ii) MeOH, 50 °C, 20 h; quinolone **14–21**, 2-PrOH, 75 °C, 24–28 h (for compounds **22–29** and **36–53**) or quinolone **9–13** and **20**, DMSO, 100 °C, 8–24 h; MeOH, 50 °C, 20 h (for compounds **30–35**).



Scheme 2. Reagents and conditions: (i) Pd/C, H_2, 2 barr, 16 h; (ii) 36% CH_2O/HCOOH, 65 °C, 48 h.

also C(7)-dehalogenated compounds **58** and **62**, N-methylated in the linker. New signals in compounds **55**–**58** and **60**–**62** at around 2.3 ppm in ^{1}H NMR spectra and around 43 ppm in ^{13}C NMR spectra provided evidence that the central nitrogen atom in the linker is methylated.

Although C-6, C-7, and N-1 positions in the quinolone part of target molecules should correctly be C-6‴, C-7‴, and N-1‴, the designations used in this article are C(6), C(7), and N(1) because of the clearness.

2.2. Antibacterial activity

Antibacterial activity of all novel compounds was determined by a standard broth microdilution method[21] and the ensuing data is expressed as minimum inhibitory concentrations (MICs) in units of μg/mL. The organisms studied represent relevant Gram-positive (*S. pneumoniae*, *Streptococcus pyogenes* and *Staphylococcus aureus*) and Gram-negative (*Haemophilus influenzae*) respiratory tract pathogens, and are either sensitive or resistant to macrolide antibiotics. Underlying resistant phenotypes are two major mechanisms:production of efflux pumps (M), or ribosome modification by methylation, where methylase expression is inducible (iMLS) or constitutive (cMLS).

As expected all compounds showed MIC ⩽0.125 μg/mL against erythromycin sensitive *S. pneumoniae* and *S. pyogenes*.

In our previous communication[12] we reported on excellent antibacterial activity of 4″-*O*-quinolone derivative of 6-*O*-methyl-8a-aza-8a-homoerythromycin, **22**. Compound **23**, 6-linked analogue with chlorine atom at C(7) position, had similar potency. In order to further investigate the importance of linker length and nature of quinolone moiety for antibacterial activity, we have synthesised a series of 8a-aza-8a-homoerythromycin derivatives without a halogen atom, bearing a methyl, ethyl or cyclopropyl groups at the position N(1) of quinolone unit, with linker lengths of 7 or 9 atoms (Table 1). Linker elongation proved to be detrimental for overall antibacterial potency. In compounds with shorter linkers, defluoro derivative **25** with ethyl group attached to the N(1) position retains acceptable, though somewhat inferior potency on efflux resistant Gram-positive organisms compared to **22**, while a decrease in activity is more pronounced for methyl compound **24**. Conversely, absence of fluorine atom in N(1)-cyclopropyl compound **26** is detrimental for the potency, especially against organisms with macrolide resistance due to ribosome methylation.

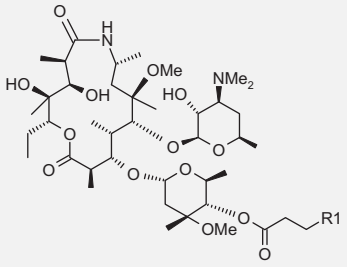
Additionally, we have explored a 14-membered macrolide scaffold, clarithromycin. Data in Table 2 shows that clarithromycin quinolone derivatives generally possess enhanced antibacterial potency compared to clarithromycin. Within this series a number of SAR trends were identified. Of particular relevance are the nature of both the linker and the quinolone moiety. The position of attachment of the linker to the quinolone unit also has an influence on potency such that C(6)-substituted compound **32** has improved activity against *H. influenzae* compared with C(7)-substituted compound **30** (MIC of 2 and 8 μg/mL, respectively). A discernible trend in the linker length is also observed such that analogues **34**, **31** and **41**, containing 8 and 9 atoms in the linker, have lower *S. aureus* and *H. influenzae* potency than 7-atom derivative **30**. There is also a particularly marked drop in potency against the constitutively resistant *S. pneumoniae* and *S. pyogenes* for the compounds containing longer linkers (i.e., cMLS *S. pyogenes* MIC drops from 0.5 μg/mL for 7-atom linker derivative **30** and 2 μg/mL for 8-atom linker derivative **34** to 8 μg/mL for 9-atom linker compound **31**).

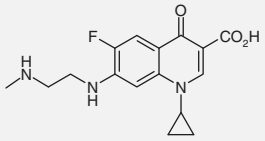
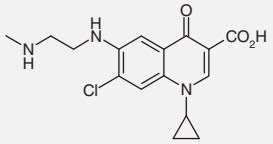
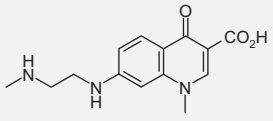
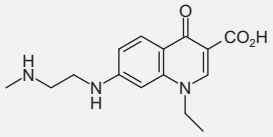
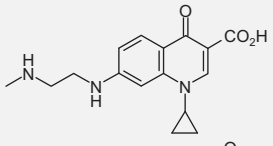
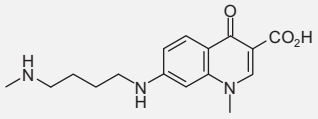
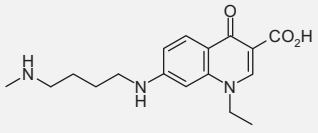
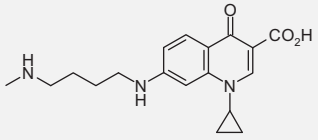
Slight difference in the potency of clarithromycin derivatives with minor changes to the quinolone N(1)-substituent is present (**36**–**38**), ethyl analogue **37** being the most active. The same analogy was also noticed on 8a-lactam derivatives.

Conversion of the acid **32** to the methyl ester **35** appears to retain good potency against all organisms except for M and iMLS *S. aureus* strains.

The next two series of targeted compounds comprise the 15-membered azithromycin and azithromycin 11,12-carbonate scaffold. Regarding azithromycin (Table 3), dehalogenation of the quinolone unit was not as detrimental to potency as in 8a-aza-8a-homoerythromycin series, and antibacterial activity of compounds with ethylene linker **42**–**44** was excellent. In contrast to 8a-lactam derivative **26**, the cyclopropyl compound **44** was very active against bacterial isolates exhibiting resistance due to ribosomal methylation. Analogues having a halogen atom, linked at positions either C(6) and C(7) are both antibacterially active, but the C(7)-chloro compound, **48**, was more potent against bacteria with macrolide resistance due to ribosome methylation. On azithromycin scaffold, methylation of the central nitrogen atom in the linker did not significantly impair potency of both C(6)-linked derivatives (**57** vs **50**, **55** vs **48** and **58** vs **54**), and C(7)-linked compounds (**56** vs **49**). In contrast, methylation on azithromycin 11,12-carbonate scaffold reduced potency regardless of quinolone attachment position (**60**–**62**, Table 4). Esterification of quinolone acid significantly decreased potency of compounds against both M and iMLS *S. aureus*, and *H. influenzae* as well (esters **50** and **57** vs acids **48** and **55**). In contrast to C(7)-fluorine derivative on azithromycin scaffold **49**, analogue on azithromycin 11,12-carbonate **52** had high overall potency, most significant difference was observed against cMLS *S. pyogenes* (MIC of 2 and <0.125 μg/mL, respectively).

Table 1
Antibacterial activity of 8a-aza-8a-homoerythromycin derivatives, given as minimum inhibitory concentration (MIC) in units of μg/mL



		Organism and strain								
		S. aureus PK1	*S. pneumoniae* Ci137	*S. pyogenes* 2 Finland	*S. aureus* 90256	*S. pneumoniae* 134 GR M	*S. pyogenes* Finland 11	*S. pneumoniae* 58 Spain	*S. pyogenes* 166 GR-Micro	*H. influenzae* ATCC 49247
	Phenotype	M	M	M	iMLS	iMcLS	iMLS	cMLS	cMLS	
No.	R1									
AZM	—	>64	8	8	>64	>64	16	>64	>64	1
TEL	—	⩽0.125	0.25	0.5	⩽0.125	0.25	0.06	0.25	16	2
22[12]		⩽0.125	⩽0.125	0.5	1	⩽0.125	⩽0.125	⩽0.125	⩽0.125	4
23		0.5	⩽0.125	⩽0.125	1	⩽0.125	⩽0.125	⩽0.125	⩽0.125	2
24		4	1	2	32	1	⩽0.125	1	0.5	16
25		2	0.25	0.5	8	⩽0.125	⩽0.125	⩽0.125	⩽0.125	16
26		64	⩽0.125	⩽0.125	64	64	1	32	32	8
27		16	4	8	>32	16	8	32	32	>32
28		8	2	8	>32	8	2	16	32	>32
29		4	0.25	⩽0.125	>64	8	0.25	8	16	16

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

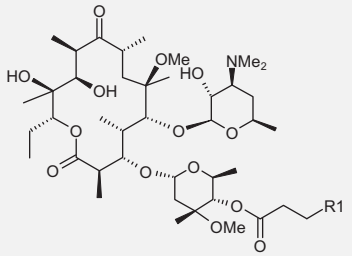
3. Conclusion

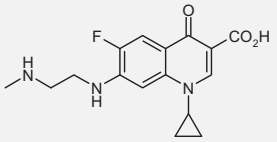
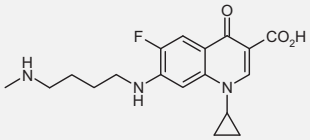
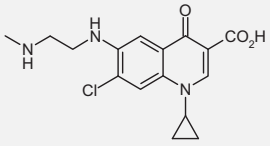
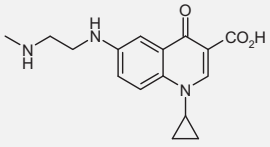
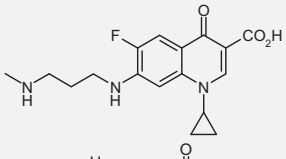
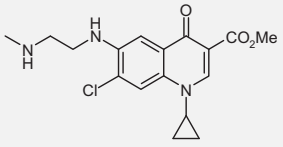
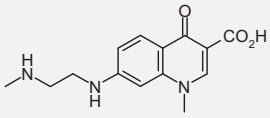
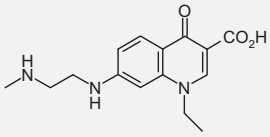
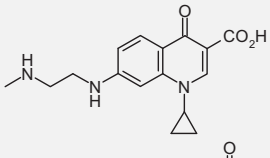
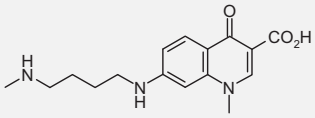
A series of 4″-*O*-(ω-quinolylamino-alkylamino)propionyl derivatives of macrolides have been prepared by combining several 14- and 15-membered macrolide scaffolds, quinolone unit and linker binding quionolone part via ester bond to the 4″-position of selected macrolides.

Optimal linker length in macrolide-quinolone derivatives was 2 carbon atoms between central and terminal nitrogen atoms, as elongation (i.e., 3 and 4 C-atoms) was detrimental to the antibacterial potency across all explored scaffolds.

The halogenated analogues showed enhanced overall potency in comparison to corresponding des-halogenated derivatives, regardless of the scaffold.

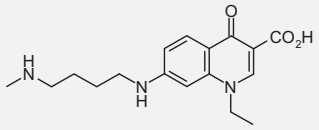
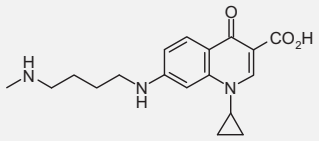
Table 2
Antibacterial activity of clarithromycin derivatives, given as minimum inhibitory concentration (MIC) in units of μg/mL



		Organism and strain								
		S. aureus PK1	*S. pneumoniae* Ci137	*S. pyogenes* 2 Finland	*S. aureus* 90256	*S. pneumoniae* 134 GR M	*S. pyogenes* Finland 11	*S. pneumoniae* 58 Spain	*S. pyogenes* 166 GR-Micro	*H. influenzae* ATCC 49247
	Phenotype	M	M	M	iMLS	iMcLS	iMLS	cMLS	cMLS	
No.	R1									
CAM	—	>64	4	4	>64	>64	1	>16	>16	8
TEL	—	⩽0.125	0.25	0.5	⩽0.125	0.25	0.06	0.25	16	2
30		0.5	⩽0.125	⩽0.125	4	0.25	⩽0.125	⩽0.125	0.5	8
31		2	⩽0.125	⩽0.125	>64	2	⩽0.125	8	8	2
32		0.5	⩽0.125	⩽0.125	4	⩽0.125	⩽0.125	⩽0.125	⩽0.125	2
33		8	⩽0.125	⩽0.125	4	⩽0.125	⩽0.125	⩽0.125	⩽0.125	1
34		1	⩽0.125	⩽0.125	64	2	⩽0.125	0.25	2	8
35		16	⩽0.125	⩽0.125	>64	⩽0.125	⩽0.125	⩽0.125	0.125	2
36		1	⩽0.125	1	4	⩽0.125	⩽0.125	⩽0.125	⩽0.125	8
37		0.5	⩽0.125	⩽0.125	1	⩽0.125	⩽0.125	⩽0.125	⩽0.125	2
38		8	⩽0.125	⩽0.125	8	⩽0.125	⩽0.125	⩽0.125	⩽0.125	2
39		4	4	0.25	>32	2	1	4	16	>32

(*continued on next page*)

Table 2 (*continued*)

		Organism and strain								
		S. aureus PK1	*S. pneumoniae* Ci137	*S. pyogenes* 2 Finland	*S. aureus* 90256	*S. pneumoniae* 134 GR M	*S. pyogenes* Finland 11	*S. pneumoniae* 58 Spain	*S. pyogenes* 166 GR-Micro	*H. influenzae* ATCC 49247
	Phenotype	M	M	M	iMLS	iMcLS	iMLS	cMLS	cMLS	
No.	R1									
40		2	1	⩽0.125	32	2	0.25	4	16	>32
41		2	⩽0.125	⩽0.125	64	1	⩽0.125	2	4	4

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

Analogues derived from the azithromycin scaffold resulted with the most favourable antibacterial spectrum, of which compounds **48** (C(6)-linked C(7)-chloro derivative) and **55** (C(6)-linked C(7)-chloro compound with methylated central nitrogen in the linker) were the most potent, giving full coverage of resistant organisms regardless of underlying resistance mechanisms as well as good potency against *H. influenzae* (MIC of 1 μg/mL). This provides a clear advantage over currently used macrolide antibiotics (i.e., azithromycin and clarithromycin) as well as the ketolide telithromycin that is not active against constitutively resistant *S. pyogenes*. However, compared to our compounds, telithromycin showed higher potency against *S. aureus* isolates. Enhancement of lipophilicity by methylation of the central nitrogen atom in the linker and by introduction of cyclic carbonate unit at C(11)–C(12) positions of azithromycin did not diminish antimicrobial potency of the compounds. As lipophilicity affects pharmacokinetic properties of drugs, this may allow optimisation of the potential drug candidates.

4. Experimental

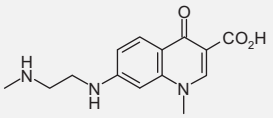
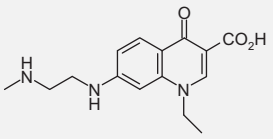
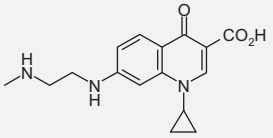
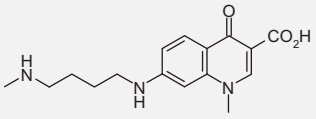
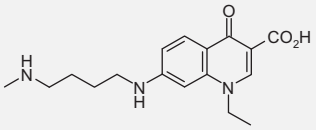
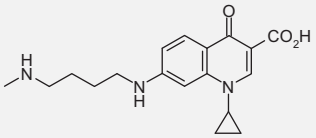
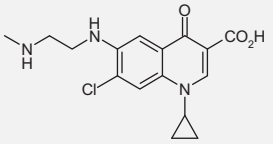
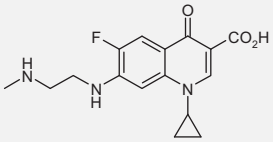
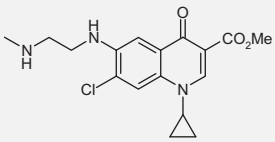
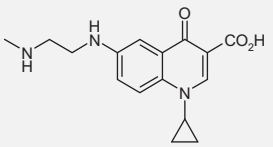
4.1. Chemistry

All commercial reagents (Merck, Sigma–Aldrich) were used as provided unless otherwise indicated, and all solvents are of the highes purity unless otherwise noted. NMR spectra were recorded on a Bruker Avance DRX500 or Bruker Avance DPX300 spectrometer in $CDCl_3$ or DMSO and chemical shifts are reported in ppm using TMS as an internal standard. Mass spectra were obtained on a Waters Micromass ZQmass spectrometer for ES^+-MS. Electrospray positive ion mass spectra were acquired using a Micromass Q-Tof2 hybrid quadrupole time-of-flight mass spectrometer, equipped with a Z-spray interface, over a mass range of 100–2000 Da, with a scan time of 1.5 s and an interscan delay of 0.1 s in a continuum mode. Reserpine was used as the external mass calibrant lock mass ($[M+H]^+$ = 609.2812 Da). The elemental composition was calculated using a MassLynx v4.1 for the $[M+H]^+$ and the mass error quoted within ±5 ppm range. In synthetic procedures, column chromatography was carried out over Merck Kieselgel 60 (230–400 mesh) or on SPE cartridge with average size silica 50 μm. Thin layer chromatography was performed on 0.24 mm silica gel plates Merck TLC 60F254. The eluent used was indicated and solvent ratios refer to volume. In general, organic solutions were dried with anhydrous Na_2SO_4 or K_2CO_3, evaporation and concentration were carried out under reduced pressure below 40 °C, unless otherwise noted. All compounds were isolated as amorphous solid. The acronym DCM is an abbreviation for dichloromethane.

4.1.1. General method for compounds 22–29 and 36–53

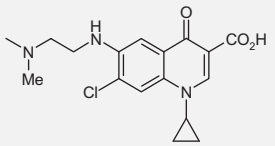
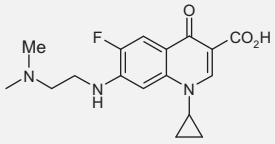
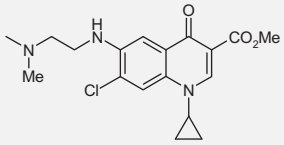
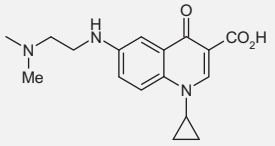
4.1.1.1. 4″-*O*-[3-({2-[(3-Carboxy-7-chloro-1-cyclopropyl-1,4-dihydro-4-oxoquinolin-6-yl)amino]ethyl}amino)propanoyl]-6-*O*-methyl-8a-aza-8a-homoerythromycin A (23). To a solution of 2′-*O*-acetyl-6-*O*-methyl-4″-*O*-propenoyl-8a-aza-8a-homoerythromycin **5** (0.10 g, 0.12 mmol) in 2-PrOH (3 mL) 6-[(2-aminoethyl)amino]-7-chloro-1-cyclopropyl-1,4-dihydro-4-oxo-3-quinolinecarboxylic acid **20** (0.078 g, 0.24 mmol) was added and stirred at 75 °C for 72 h. The reaction mixture was filtered from remaining quinolone. The mother liquor was evaporated to dryness and the residue dissolved in EtOAc (2 mL) followed by addition of *n*-hexane (10 mL). The resulting suspension was filtered and the crude product was purified by column chromatography on SPE cartridge [1 g flash silica] using a gradient method starting with 100% DCM and finishing with a system DCM/MeOH/aq NH_4OH = 90:9:0.5. The appropriate fractions were combined and evaporated to dryness yielding 0.053 g (yield 39%) of compound **23** as white solid; IR (KBr, cm^{-1}): 3408, 3093, 2975, 2937, 2833, 1729, 1640, 1609, 1547, 1509, 1462, 1379, 1354, 1275, 1172, 1111, 1090, 1049, 1015, 962, 901, 871, 836, 806, 639; ^1H NMR (500 MHz, $CDCl_3$) δ: 8.72 (s, 1H), 8.04 (s, 1H), 7.52 (s, 1H), 5.69 (s, CONH), 5.28 (t, NH), 5.08 (d, 1H), 4.94 (dd, 1H), 4.70 (d, 1H), 4.50 (d, 1H), 4.37 (m, 1H), 4.18 (k, 1H), 3.98 (d, 1H), 3.71 (m, 1H), 3.67 (d, 1H), 3.57 (m, 1H), 3.51 (s, 1H), 3.38 (k, 2H), 3.31 (s, 3H), 3.18 (m, 1H), 3.16 (s, 3H), 3.02 (t, 2H), 2.98 (k, 2H), 2.78 (m, 1H), 2.67 (t, 1H), 2.65 (t, 1H), 2.56 (m, 1H), 2.40 (d, 1H), 2.32 (s, 6H), 2.29 (m, 1H), 1.93 (m, 1H), 1.94 (m.1H), 1.70 (d, 1H).1.63 (dd, 1H), 1.57 (d, 1H), 1.46 (m, 1H), 1.40 (m, 2H), 1.36 (s, 3H), 1.24 (m, 1H), 1.21 (d, 3H), 1.19 (d, 3H), 1.18 (d, 3H), 1.14 (d, 3H), 1.13 (s, 2 × 3H),

Table 3
Antibacterial activity azithromycin derivatives, given as minimum inhibitory concentration (MIC) in units of μg/mL

		Organism and strain								
		S. aureus PK1	*S. pneumoniae* Ci137	*S. pyogenes* 2 Finland	*S. aureus* 90256	*S. pneumoniae* 134 GR M	*S. pyogenes* Finland 11	*S. pneumoniae* 58 Spain	*S. pyogenes* 166 GR-Micro	*H. influenzae* ATCC 49247
	Phenotype	M	M	M	iMLS	iMcLS	iMLS	cMLS	cMLS	
No.	R1									
AZM	—	>64	8	8	>64	>64	16	>64	>64	1
TEL	—	⩽0.125	0.25	0.5	⩽0.125	0.25	0.06	0.25	16	2
42		2	⩽0.125	⩽0.125	4	⩽0.125	⩽0.125	⩽0.125	⩽0.125	8
43		2	⩽0.125	⩽0.125	4	⩽0.125	⩽0.125	⩽0.125	⩽0.125	4
44		1	⩽0.125	⩽0.125	2	⩽0.125	⩽0.125	⩽0.125	⩽0.125	4
45		8	2	4	>64	32	0.5	32	8	16
46		4	1	8	>32	8	0.5	2	8	>32
47		2	0.5	1	8	8	⩽0.125	4	4	8
48		0.25	⩽0.125	⩽0.125	0.5	⩽0.125	⩽0.125	⩽0.125	⩽0.125	1
49		0.5	0.25	1	0.5	1	⩽0.125	0.25	2	2
50		8	⩽0.125	⩽0.125	>64	⩽0.125	⩽0.125	⩽0.125	⩽0.125	8
54		1	⩽0.125	⩽0.125	1	⩽0.125	⩽0.125	⩽0.125	⩽0.125	4

(*continued on next page*)

Table 3 (*continued*)

		Organism and strain								
		S. aureus PK1	*S. pneumoniae* Ci137	*S. pyogenes* 2 Finland	*S. aureus* 90256	*S. pneumoniae* 134 GR M	*S. pyogenes* Finland 11	*S. pneumoniae* 58 Spain	*S. pyogenes* 166 GR-Micro	*H. influenzae* ATCC 49247
	Phenotype	M	M	M	iMLS	iMcLS	iMLS	cMLS	cMLS	
No.	R1									
55		0.5	⩽0.125	⩽0.125	0.5	⩽0.125	⩽0.125	⩽0.125	⩽0.125	1
56		1	⩽0.125	⩽0.125	2	0.25	⩽0.125	0.25	2	4
57		16	⩽0.125	⩽0.125	>64	0.5	⩽0.125	0.5	1	8
58		1	⩽0.125	0.5	1	0.25	⩽0.125	⩽0.125	⩽0.125	4

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

1.12 (d, 3H), 1.07 (d, 3H), 0.89 (t, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) δ: 177.43, 177.21, 174.21, 172.18, 167.27, 145.74, 143.02, 132.43, 127.51, 126.19, 117.92, 107.46, 104.39, 102.50, 95.23, 79.95, 78.82, 78.73, 77.26, 77.01, 74.16, 72.84, 70.76 70.30, 68.03, 65.30, 62.76, 51.75, 49.41, 47.45, 45.42, 44.37, 42.89, 42.76, 42.23, 40.86, 40.26, 35.28, 34.98, 34.61, 28.98, 23.78, 21.64, 21.54, 21.21, 21.11, 17.88, 16.05, 14.91, 11.08, 9.51, 9.26, 8.02; MS (ESI) *m/z* calcd for $C_{58}H_{88}ClN_5O_{17}$ $[M+H]^+$ 1138.5942; found 1138.5927.

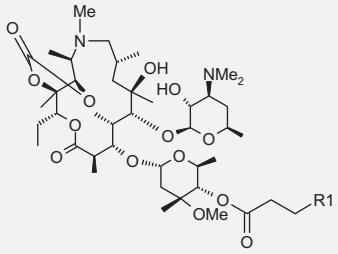
4.1.2. General method for compounds 30–35

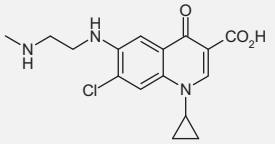
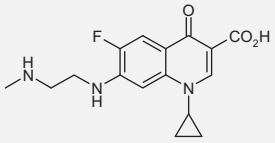
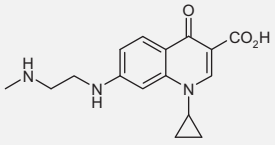
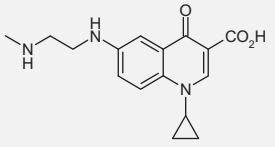
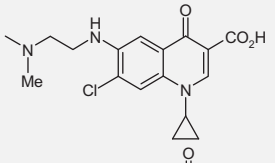
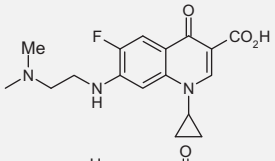
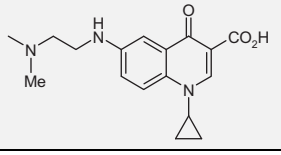
4.1.2.1. 4″-*O*-[3-({2-[(3-Carboxy-7-chloro-1-cyclopropyl-1,4-dihydro-4-oxoquinolin-6-yl)amino]ethyl}amino)propanoyl]-6-*O*-methylerythromycin A (32). To a solution of hydrochloride salt of 6-[(2-aminoethyl)amino]-7-chloro-1-cyclopropyl-4-oxo-1,4-dihydro-3-quinolinecarboxylic acid **20** (0.05 g) in dry DMSO (1 mL) di-*i*-isopropylethylamine (0.02 mL) was added and the mixture was stirred at room temperature for 1 h. The resulting mixture was added to a solution of 2′-*O*-acetyl-6-*O*-methyl-4″-propenyl-erythromycin A **6** (0.05 g) in dry DMSO (1 mL) and heated at 80 °C for 12 h. The reaction mixture was cooled to room temperature, diluted with DCM (10 mL), washed with water (3 × 10 mL), dried and the solvent was evaporated to dryness. The crude product was dissolved in MeOH (0.5 mL) and the solution was stirred at 40 °C for 12 h. The solvent was evaporated, the residue dissolved in DCM (0.1 mL) and treated with diethyl ether (0.5 mL), The resulting suspension was filtered yielding 0.023 mg (yield 60%) of compound **32** as white solid; IR (KBr, cm^{-1}): 3435, 2974, 2939, 2882, 2833, 1734, 1689, 1610, 1546, 1500, 1462, 1380, 1354, 1299, 1171, 1111, 1074, 1050, 1014, 935, 903, 824, 789, 734, 694, 636; ^{1}H NMR (500 MHz, $CDCl_3$) δ: 8.73 (s, 1H), 8.03 (s, 1H), 7.54 (s, 1H), 5.05 (dd, 1H), 4.97 (d, 1H), 4.67 (d, 1H), 4.55 (d, 1H), 4.28 (m, 1H), 3.78 (d, 1H), 3.75 (s.1H), 3.71 (m, 1H), 3.64 (m, 1H + 1H), 3.30 (s, 3H + 2H), 3.18 (m, 1H), 3.03 (s, 3H), 3.00 (m, 1H), 2.91 (m, 2H + 2H), 2.58 (m, 2H), 2.54 (m, 1H), 2.52 (m, 1H), 2.41 (d, 1H), 2.28 (s, 6H), 1.94 (m, 1H), 1.92 (m, 1H), 1.84 (d, 1H), 1.70 (d, 1H), 1.66 (d, 1H), 1.64 (dd, 1H), 1.47 (m, 1H), 1.37 (s, 3H), 1.20 (d, 3H), 1.18 (d, 3H), 1.14–1.12 (m, 6 × 3H), 0.84 (t, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 221.01, 177.65, 175.76, 172.29, 145.99, 141.09, 132.03, 127.46, 124.47, 117.20, 107.43, 104.56, 102.14, 95.96, 80.29, 78.73, 78.28, 78.07, 77.19, 74.23, 72.63, 71.06, 69.09, 68.31, 65.16, 62.94, 50.58, 49.49, 47.79, 45.31, 44.84, 44.53, 42.79, 40.25, 39.18, 38.85, 37.18, 35.17, 34.72, 38.82, 28.80, 21.86, 21.14, 21.00, 19.67, 18.37, 17.98, 15.90, 12.31, 10.57, 9.04, 7.78; MS (ESI) *m/z* calcd for $C_{56}H_{87}ClN_4O_{17}$ $[M+H]^+$ 1123.5833; found 1123.5854.

4.1.3. General method for Eschweiler–Clark methylation

4.1.3.1. 4″-*O*-[3-({2-[(3-Carboxy-7-chloro-1-cyclopropyl-1,4-dihydro-4-oxoquinolin-6-yl)amino]ethyl}methylamino)propanoyl]-azithromycin (55). To a solution of compound **48** (0.15 g, 0.13 mmol) in $CHCl_3$ (5 mL) 36% aqueous solution of HCHO (0.021 mL, 0.27 mmol) and HCOOH (0.018 mL, 0.47 mmol) were added and the reaction mixture was stirred at 65 °C for 2 days. To the reaction mixture water (10 mL) was added and the reaction mixture was extracted at pH 9 with DCM (2 × 10 mL). The combined organic extracts were concentrated to

Table 4
Antibacterial activity of azithromycin 11,12-carbonate derivatives, given as minimum inhibitory concentration (MIC) in units of μg/mL



		Organism and strain								
		S. aureus PK1	*S. pneumoniae* Ci137	*S. pyogenes* 2 Finland	*S. aureus* 90256	*S. pneumoniae* 134 GR M	*S. pyogenes* Finland 11	*S. pneumoniae* 58 Spain	*S. pyogenes* 166 GR-Micro	*H. influenzae* ATCC 49247
	Phenotype	M	M	M	iMLS	iMcLS	iMLS	cMLS	cMLS	
No.	R1									
AZM	—	>64	8	8	>64	>64	16	>64	>64	1
TEL	—	⩽0.125	0.25	0.5	⩽0.125	0.25	0.06	0.25	16	2
51		0.5	⩽0.125	⩽0.125	1	⩽0.125	⩽0.125	⩽0.125	⩽0.125	2
52		0.25	⩽0.125	⩽0.125	1	⩽0.125	⩽0.125	⩽0.125	⩽0.125	2
53		2	⩽0.125	⩽0.125	16	0.25	⩽0.125	⩽0.125	0.25	8
59		1	⩽0.125	0.25	2	0.25	⩽0.125	0.25	⩽0.125	2
60		1	⩽0.125	⩽0.125	2	2	⩽0.125	1	1	4
61		2	⩽0.125	⩽0.125	4	2	⩽0.125	1	16	8
62		1	⩽0.125	⩽0.125	8	1	⩽0.125	1	0.5	4

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

dryness. The residue was dissolved in EtOAc (3 mL) and treated with *n*-hexane (15 mL). The resulting suspension was filtered yielding 110 mg (yield 73%) of compound **55** as white solid; IR (KBr, cm^{-1}): 3404, 2974, 2937, 1724, 1609, 1545, 1477, 1379, 1347, 1174, 1110, 1043, 1015, 960, 900, 819, 643; ^1H NMR (500 MHz, $CDCl_3$) δ: 8.70 (s, 1H), 8.03 (s, 1H), 7.59 (s, 1H), 5.26 (t, NH), 5.25 (d, 1H), 4.74 (d, 1H + 1H), 4.54 (d, 1H), 4.45 (m, 1H), 4.23 (s, 1H), 3.77 (m, 1H), 3.60 (s, 1H), 3.58 (d, 1H), 3.56 (m, 1H), 3.35 (t, 2H), 3.32 (s, 3H), 3.29 (m, 1H), 2.81 (m, 2H), 2.76 (m, 2H), 2.71 (m, 1H), 2.63 (m, 1H), 2.61 (m, 1H), 2.58 (m, 1H), 2.51 (m, 2H), 2.43 (d, 1H), 2.33 (s, 6H), 2.32 (s, 3H), 2.06 (t, 1H), 2.03 (m, 1H), 1.94 (m, 1H), 1.92 (m, 1H), 1.74 (d, 1H), 1.68 (d, 1H), 1.63 (dd, 1H), 1.46 (m, 1H), 1.43 (m, 2H), 1.28 (s, 3H), 1.27 (m, 1H), 1.20 (m, 2H), 1.19 (d, 3H), 1.17 (d, 3H), 1.16 (d, 3H), 1.14 (s, 3H), 1.12 (s, 3H), 1.08 (d, 3H), 1.05 (d, 3H), 0.93 (d, 3H), 0.89 (t, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 178.96, 177.51, 172.36, 167.30, 145.81, 143.11, 132.54, 127.66, 126.36, 118.02, 107.63, 104.49, 102.03, 94.58, 83.09, 78.92, 77.66, 74.27, 73.66, 73.44, 72.91, 70.91, 70.08, 67.74, 65.54, 62.97, 62.60, 55.43, 52.61, 49.50, 45.26, 42.35, 42.11, 40.63, 40.24,

36.13, 35.30, 34.95, 32.83, 29.22, 27.55, 26.74, 21.95, 21.82, 21.38, 21.35, 17.78, 16.27, 14.49, 11.21, 8.94, 8.09, 7.26; MS (ESI) *m/z* calcd for $C_{57}H_{92}ClN_5O_{16}$ $[M+H]^+$ 1139.0112; found 1139.0124.

4.1.4. General method for catalytic dehalogenation

4.1.4.1. 4″-*O*-[3-({2-[(3-Carboxy-1-cyclopropyl-1,4-dihydro-4-oxo-quinolin-6-yl)amino]ethyl}amino)propanoyl]-azithromycin (54). To a solution of compound **48** (0.30 g, 0.27 mmol) in MeOH (30 mL) 10% Pd/C (0.01 g) was added and the reaction mixture was hydrogenated at 2 bars pressure for 16 h at rt. The catalyst was filtered off, washed with MeOH and the mother liquor concentrated to dryness. To the reaction mixture water (10 mL) was added and the reaction mixture was extracted at pH 9 with DCM (2 × 10 mL). The combined organic extracts were concentrated to dryness. The residue was dissolved in EtOAc (5 mL) and treated with *n*-hexane (20 mL). The resulting suspension was filtered yielding 240 mg (Y = 82%) compound **54** as white solid; IR (KBr, cm^{-1}): 3425, 2972, 2937, 1723, 1610, 1546, 1479, 1462, 1380, 1345, 1255, 1175, 1110, 1076, 1042, 1016, 959, 901, 822; ^1H NMR (500 MHz, $CDCl_3$) δ: 8.70 (s, 1H), 7.91 (d, 1H), 7.35 (d, 1H), 7.27 (d, 1H), 5.17 (dd, H), 4.74 (d, 1H), 4.70 (d, 1H), 4.60 (m, 1H), 4.37 (m, 1H), 4.24 (d, 1H), 3.84 (m, 1H), 3.69 (s, 1H), 3.65 (m, 1H), 3.60 (m, 1H), 3.48 (m, 2H), 3.40 (m, 1H), 3.30 (s, 3H), 3.12 (m, 2H), 3.10 (m, 2H), 2.98 (m, 1H), 2.75 (m, 1H + 1H), 2.63 (m, 3H + 1H), 2.60 (m, 2H), 2.41 (d, 1H), 2.35 (s, 3H), 2.13 (m, 1H), 2.04 (m, 1H), 2.00 (m, 1H), 1.98 (m, 1H), 1.91 (m, 1H), 1.72 (d, 1H), 1.64 (dd, 1H), 1.46 (m, 1H), 1.37 (m, 2H), 1.35 (m, 1H), 1.30 (s, 3H + 1H), 1.20 (d, 3H), 1.19 (d, 3H), 1.15 (d, 3H), 1.14 (d, 3H), 1.13 (s, 3H), 1.10 (s, 3H), 1.03 (d, 3H), 0.92 (d, 3H), 0.89 (t, 3H); ^{13}C NMR (75 MHz, $CDCl_3$) δ: 178.69, 177.90, 172.01, 167.78, 147.05, 145.09, 133.30, 127.59, 122.40, 118.42, 107.38, 104.10, 102.11, 94.82, 83.61, 78.99, 78.00, 74.32, 73.69, 73.63, 73.09, 71.05, 70.04, 67.65, 65.24, 63.19, 62.81, 49.49, 47.63, 45.25, 44.22, 42.57, 42.29, 42.02, 40.56, 36.36, 35.42, 35.07, 34.12, 29.20, 27.47, 26.77, 22.01, 21.66, 21.45, 21.35, 17.87, 16.29, 14.66, 11.26, 9.28, 8.09, 7.51; MS (ESI) *m/z* calcd for $C_{56}H_{91}N_5O_{16}$ $[M+H]^+$ 1090.6533; found 1090.6518.

4.2. Biological evaluation (or In vitro antibacterial activity assays)

Minimum inhibitory concentrations (MICs) were determined by the broth microdilution method according to guidelines of the Clinical Laboratory Standards Institute,[22] except that for *Streptococcus* medium, lysed blood was substituted with 5% horse serum. Double dilutions of tested compounds in 96-well microtitre plates were prepared using TECAN Genesis 150. Bacteria were grown on appropriate agar plates (by Becton Dickinson, USA)—Columbia agar with 5% sheep blood for Streptococci, Mueller-Hinton chocolate agar for *H. influenzae* and Mueller-Hinton agar for Staphylococci.

Acknowledgments

The authors thank Ana Čikoš, Biserka Metelko and Dubravka Gembarovski from Structure and Analysis Department for their supports and services. We greatfully acknowledge Dragica Muhvić, Božica Škrinjar, Jadranka Ivetić and Višnja Majzel for their excellent technical assistance.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2010.06.049.

References and notes

1. Blondeau, J. M.; DeCarolis, E.; Metzler, K. L.; Hansen, G. T. *Expert Opin. Invest. Drugs* **2002**, *11*, 189.
2. Beekmann, S. E.; Heilmann, K. P.; Richter, S. S.; Garcia-de-Lomas, J.; Doern, G. V. *Int. J. Antimicrob. Agents* **2005**, *25*, 148.
3. Spellberg, B.; Guidos, R.; Gilbert, D.; Bradley, J.; Boucher, H. W.; Scheld, W. M.; Bartlett, J. G.; Edwards, J., Jr. *J. Clin. Infect. Dis.* **2008**, *46*, 155.
4. Pal, S. *Tetrahedron* **2006**, *62*, 3171.
5. Mutak, S. *J. Antibiot.* **2007**, *60*, 85.
6. Mutak, S.; Maršić, N.; Dominis Kramarić, M.; Pavlović, D. *J. Med. Chem.* **2004**, *4*, 411.
7. Bryskier, A.; Denis, A. Ketolides: Novel Antibacterial Agents Designed to Overcome Resistance of Erythromycin A within Gram-positive Cocci. In *Macrolide Antibiotics*; Schonfeld, W., Kirst, H. A., Eds.; Birkhauser Verlag, 2002; pp 97–140.
8. Nomura, T.; Iwaki, T.; Yasukata, T.; Nishi, K.; Narukawa, Y.; Uotani, K.; Hori, T.; Miwa, H. *Bioorg. Med. Chem.* **2005**, *13*, 6615.
9. (a) Kujundžić, N.; Kobrehel, G.; Banić, Z.; Kelnerić, Ž.; Kojić-Prodić, B. *Eur. J. Med. Chem.* **1995**, *30*, 455; (b) Marušić Ištuk, Z.; Mutak, S.; Kujundžić, N.; Kragol, G. *Bioorg. Med. Chem.* **2007**, *15*, 4498; (c) Bukvić Krajačić, M.; Kujundžić, N.; Dumić, M.; Cindrić, M.; Brajša, K.; Metelko, B.; Novak, P. *J. Antibiot.* **2005**, *58*, 380; (d) Bukvić Krajačić, M.; Novak, P.; Cindrić, M.; Brajša, K.; Dumić, M.; Kujundžić, N. *Eur. J. Med. Chem.* **2007**, *42*, 138; (e) Bukvić Krajačić, M.; Novak, P.; Dumić, M.; Cindrić, M.; Čipčić Paljetak, H.; Kujundžić, N. *Eur. J. Med. Chem.* **2009**, *44*, 3459.
10. Alihodžić, S.; Fajdetić, A.; Kobrehel, G.; Lazarevski, G.; Mutak, S.; Pavlović, D.; Štimac, V.; Čipčić, H.; Dominis Kramarić, M.; Eraković, V.; Hasenhorl, A.; Maršić, N.; Schonfeld, W. *J. Antibiot.* **2006**, *59*, 753.
11. Štimac, V.; Alihodžić, S.; Lazarevski, G.; Mutak, S.; Marušić Ištuk, Z.; Fajdetić, A.; Palej, I.; Čipćić Paljetak, H.; Padovan, J.; Tavčar, B.; Eraković Haber, V. *J. Antibiot.* **2009**, *62*, 133.
12. Hutinec, A.; Đerek, M.; Lazarevski, G.; Šunjić, V.; Čipčić Paljetak, H.; Alihodžić, S.; Eraković Haber, V.; Dumić, M.; Mutak, S. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 3244.
13. Ma, S.; Jiao, B.; Liu, Z.; Wang, H.; Xian, R.; Zheng, M.; Lou, H. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 1698.
14. Ma, S.; Ma, R.; Liu, Z.; Ma, C.; Shen, X. *Eur. J. Med. Chem.* **2009**, *44*, 4010.
15. (a) Waddel, S. T.; Santorelli, G. M.; Blizzard, T. A.; Graham, A.; Occi, J. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 1321; (b) Denis, A.; Augoridas, C. *Bioorg. Med. Chem. Lett.* **1998**, *8*, 2427.
16. Đokić, S.; Kobrehel, G.; Lopotar, N.; Kamenar, B.; Nagl, A.; Mrvoš, D. *J. Chem. Res. (S)* **1988**, 152.
17. Alihodžić, S.; Andreotti, D.; Berdik, A.; Bientinesi, I.; Biondi, S.; Ciraco, M.; Damiani, F.; Đerek, M.; Dumić, M.; Eraković, V.; Hutinec, A.; Lazarevski, G.; Lociuro, S.; Maršić, N.; Marušić Ištuk, Z.; Mutak, S.; Paio, A.; Pavlović, D.; Quaglia, A.; Schoenfeld, W.; Štimac, V.; Tibasco, J. WO 03042228, 2003.
18. Berge, J.; Forrest, A.; Jarvest, R. WO 04101589, 2004.
19. Chu, D. T. W.; Fernandes, P. B.; Claiborne, A. K.; Pihuleac, E.; Nordeen, C. W.; Maleczka, R. M.; Pernet, A. G. *J. Med. Chem.* **1985**, *28*, 1558.
20. Wentland, M. P.; Perni, R. B.; Dorff, P. H.; Brundage, R. P.; Castaldi, M. J.; Bailey, T. R.; Carabataes, P. M.; Bacon, E. R.; Young, D. C.; Woods, M. G.; Rossi, D.; Drod, M. L.; Kullnig, R. K.; Dutko, F. J. *J. Med. Chem.* **1993**, *36*, 1580.
21. Kowals, J. *Drugs Today* **1981**, 26.
22. Clinical Laboratory Standard Institute CLSI, Performance Standards for Antimicrobial Susceptibility Testing: 15th Informational Supplement M100-S15, Clinical Laboratory Standards Institute, Wayne, Pa, 2005.