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Novel 8a-aza-8a-homoerythromycin—4″-(3-substituted-amino)propionates with broad spectrum antibacterial activity

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ABSTRACT

Fifteen-membered 8a-aza-8a-homoerythromycins derived from either erythromycin or clarithromycin have been acylated to form 4″-*O*-propenoyl derivative. These functionalized analogues underwent Michael reaction with primary or secondary amines to afford novel 8a-aza-8a-homoerythromycin—4″-(3-substituted-amino)propionates. This preparative sequence was adapted so that analogues could be made by parallel synthesis. Among them, 4-quinolone derivatives show particularly good antibacterial potency against macrolide resistant bacteria, comparable or better than azithromycin and telithromycin.

The search for new classes of broad spectrum antibacterial agent remains a challenging goal for medicinal chemistry particularly in view of the relentless advance of drug resistant bacteria.[1] A significant effort has also been made to modifying existing antibacterial agents in order to overcome bacterial resistance mechanisms. The macrolide class of antibacterial agents, such as erythromycin, clarithromycin and azithromycin, are particularly suited to the latter approach as selected modification of the macrolactone scaffold or either of the sugar substituents[2,3] can lead to analogues with improved antibacterial potency; the ketolides telithromycin[4] and cethromycin[5] are typical of this approach. Figure 1.

Despite these advances there is still a genuine need for additional novel macrolide analogues which display improved levels of antibacterial activity against resistant organisms and have an acceptable safety profile. Owing to the azithromycin good stability, pharmacokinetics and safety profile, design and discovery of the novel azalides represent the main stream.[6,7]

In a previous communication[8] we reported that the 4″-hydroxyl group of 2′-*O*-acetyl 8a-aza-8a-homoerythromycin analogues **1a** or **2a** could be selectively acylated with a variety of arylalkyl carboxylic acids to form the corresponding 4″-*O*-acyl derivatives. Subsequent 2′-deacetylation yielded 2′-OH derivatives with antibacterial activity. The scope of this chemistry has now been extended to design of the novel 8a-aza-8a-homoerythromycin—4″-(3-substituted-amino)propionates with broad spectrum antibacterial activity.

Strategies which involved macrolide conjugates incorporating heteroaromatic rings,[9] as well as macrolide–nucleoside and macrolide–nucleobase conjugates[10] have already been introduced and these compounds showed an increased affinity for the ribosome. Similarly, we expect that by introducing novel interactive groups into the 8a-aza-8a-homoerythromycin backbone via suitable linker at 4″-O-position further improvements in activity might be achieved.

The suitable (alkylamino)propionyl linker at 4″-O-position was prepared by acylation of **1a** and **2a**[11] with 3-chloropropionyl chloride/triethylamine in toluene, and the subsequent methanolysis of 2′-acetyl 4″-*O*-propenoyl derivatives **1b** and **2b** to the corresponding 4″-*O*-propenoyl derivatives **3** and **4**.[12] Compounds **3** and **4** readily undergo Michael addition with primary or secondary amines in methanol at reflux to yield a series of novel hybrid compounds, 4″-*O*-[3-(aryl/heteroaryl-alkylamino)propionyl derivatives **5–28**, that is, the 8a-aza-8a-homoerythromycin—arene/heteroarene conjugates, Scheme 1.

Unlike the direct 4″-acylation reported previously the route described above is amenable to parallel synthesis. To make this

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more practical the excess amine, used to drive the Michael addition to completion and minimize the propensity for retro-Michael reaction, was removed with aid of scavenger resins. Removal of primary amines was achieved with polymer-bounded benzaldehyde while secondary amines were scavenged with polymer-bounded isocyanate. Employing these modifications parallel synthesis afforded the target compounds **5–23** in 50–70% yield (Table 1) and ca. 85% purity.[13,14] The most facile Michael additions occurred with sterically unhindered amines. Attempts to use aromatic amines as Michael donors failed using these experimental conditions.

Parallel synthesis was less successful for the preparation of 8a-aza-8a-homoerythromycin—4-quinolone conjugates **24–28** (35–50%), which were obtained reacting of 4″-*O*-propenoyl **4** with corresponding aminoquinolones in MeOH as solvent in the presence of DIPEA (Table 2).[12,13]

The organisms studied are either macrolide-sensitive (S) or resistant, via two different mechanisms—efflux pumps (M), or

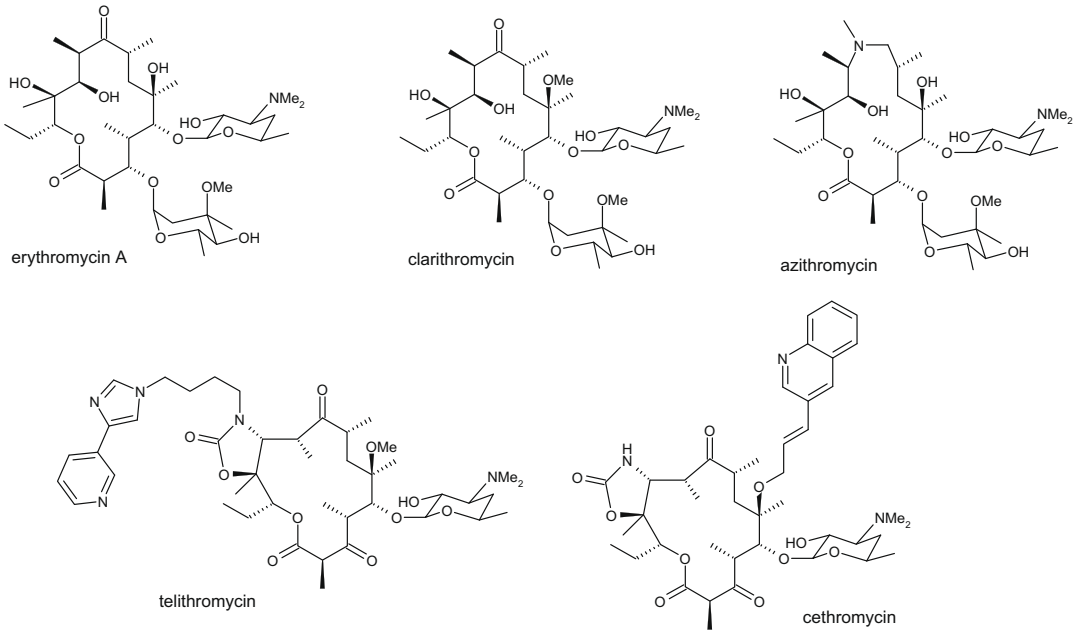
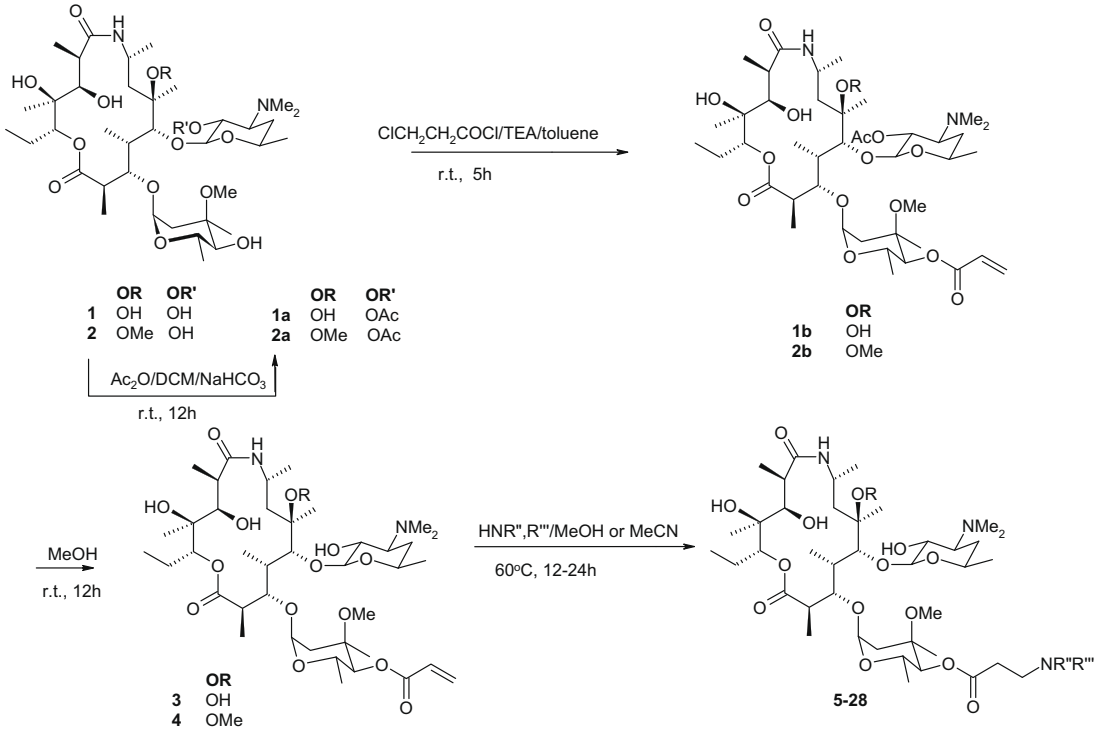
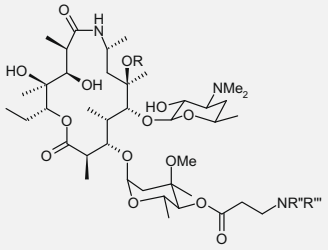


Figure 1. Antibacterial active macrolides.



Scheme 1. Synthesis of 4″-propionates.

Table 1
Yield (%) and antibacterial activity (MICs/μg/mL) of 8a-aza-8a-homoerythromycin—4″-(3-substituted-amino)propionates **5–23**



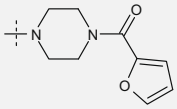
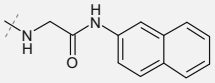
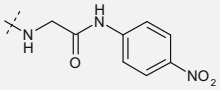
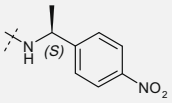
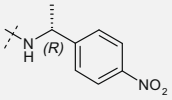
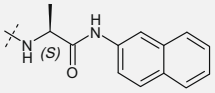
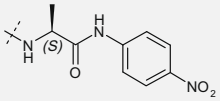
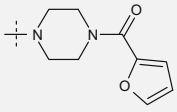
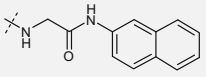
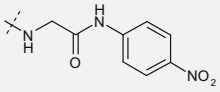
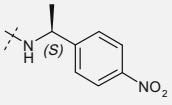
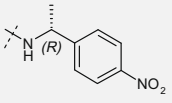
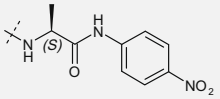
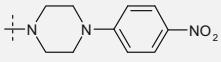
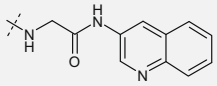
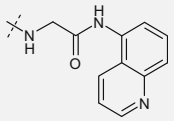
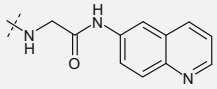
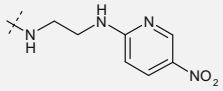
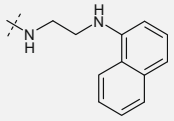
No.	R	NR″R‴	Yield (%)	*S. aureus* ATCC 13709	*S. pneumoniae* SPO 30	*S. pyogenes* 3665	*S. pneumoniae* Ci137	*S. pyogenes* Finland 11	*S. pneumoniae* 4636	*H. influenzae* ATCC 49247
				Phenotype						
				S	S	S	M	iMLS	iMcL	
azi		—		0.5	<0.13	<0.13	8	16	>64	1
1		—		1	<0.13	<0.13	32	16	>64	2
2		—		0.5	<0.13	<0.13	16	8	>64	4
5	H		68	4	<0.13	<0.13	2	8	>64	16
6	H		69	4	<0.13	<0.13	2	8	32	4
7	H		61	2	<0.13	<0.13	4	4	32	8
8	H		62	4	<0.13	<0.13	4	4	16	8
9	H		61	4	<0.13	<0.13	4	8	32	4
10	H		58	2	<0.13	<0.13	0.5	8	32	2
11	H		60	4	<0.13	<0.13	2	4	32	4
12	Me		68	2	<0.13	<0.13	2	16	32	4
13	Me		62	1	<0.13	<0.13	2	16	16	2
14	Me		51	1	<0.13	<0.13	2	4	16	2
15	Me		58	2	<0.13	<0.13	2	4	>32	32
16	Me		59	1	<0.13	<0.13	2	4	>32	32
17	Me		56	0.5	<0.13	<0.13	0.5	1	16	2
18	Me		53	2	<0.13	<0.13	1	8	8	2

Table 1 (*continued*)

				S. aureus ATCC 13709	*S. pneumoniae* SPO 30	*S. pyogenes* 3665	*S. pneumoniae* Ci137	*S. pyogenes* Finland 11	*S. pneumoniae* 4636	*H. influenzae* ATCC 49247
				Phenotype						
19	Me		66	2	<0.13	<0.13	2	16	>32	>32
20	Me		59	8	<0.13	0.25	16	32	>32	>32
21	Me		58	2	<0.13	<0.13	4	16	>32	>32
22	Me		65	0.25	<0.13	<0.13	0.5	0.25	4	4
23	Me		51	1	<0.13	<0.13	2	4	>32	nd

azi = azithromycin; S = macrolide-sensitive strain; iMLS = inducible resistance to macrolide, lincosamide and streptogramin (MLS) antibiotics; iMcL = inducible resistance to macrolides and constitutive to lincosamides and streptogramines; cMLS = constitutive MLS resistance; M = efflux mediated macrolide resistance, nd = not determined.

ribosome modification by methylation; methylase expression is inducible (iMLS, iMcL) or constitutive (cMLS).

Data in Table 1 shows that all tested 8a-aza-8a-homoerythromycin derivatives maintained high antibacterial potency against macrolide-sensitive *Streptococcus pneumoniae* and *Streptococcus pyogenes* strains. The potency toward macrolide-sensitive *Staphylococcus aureus*, however, is more variable, the most encouraging compound in this respect is 3-nitropyridine conjugate **22** which displays a similar profile (MIC = 0.25 μg/mL) to azithromycin (MIC = 0.5 μg/mL). A marked improvement in profile was also observed for most analogues against the efflux mutant *S. pneumoniae*. On the whole only a moderate improvement in potency was observed against iMcL *S. pneumoniae,* except conjugate **22** which shows significantly improved potency (MIC = 4 μg/mL) in comparison to azithromycin (MIC = >64 μg/mL).

Antimicrobial activity of arene/heteroarene conjugates against iMLS *S. pyogenes* is more encouraging, particularly for the nitro analogues **17** (MIC = 1 μg/mL) and **22** (MIC = 0.25 μg/mL) in comparison to azithromycin (MIC = 16 μg/mL).

Generally potency against the Gram negative organism *Haemophillus influenzae* is decreased compared with azithromycin, apart from the naphthalene analogues **10** and **13** and the nitro compounds **14, 17** and **18** all of which show a similar potency(all MICs = 2 μg/mL) to that of azithromycin (MIC = 1 μg/mL).

Regarding the nature of the scaffold, there is a trend for higher antibacterial potency with the 6-*O*-methyl analogues compared to the 6-hydroxy congeners. A distinct SAR trend for the linker is harder to identify, although the 1,2-diaminoethane appears to be favoured when combined with the 3-nitropyridyl substituent, **22**. Finally, compounds possessing a 7-atom linker tend to have higher potency than conjugates with a 5-atom linker.

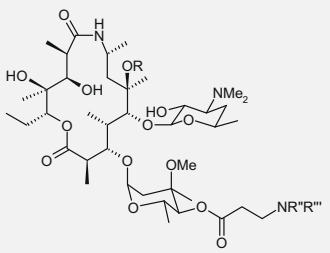
The introduction of nitrogen atom in naphthalene ring decreased antimicrobial activity of quinoline conjugates **19**–**21** against *H. influenzae* (all MICs = 32 μg/mL) compared with the naphthalene derivative **13** (MIC = 2 μg/mL) (Table 1). However, the 4-quinolone conjugates **24**–**28** show a much more encouraging profile with MICs 1–8 μg/mL (Table 2).

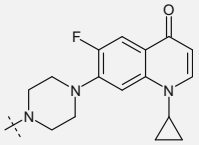
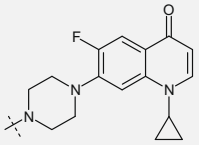
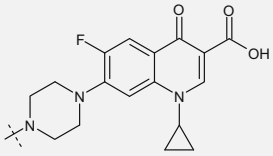
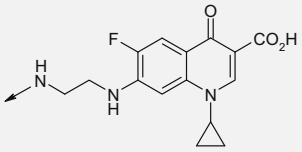
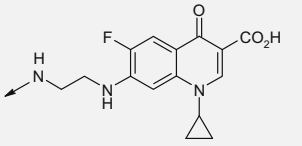
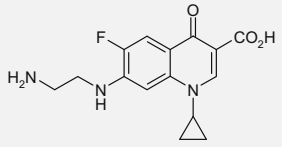
Generally, 4-quinolone derivatives **24**–**28** show a clear improvement in potency against macrolide resistant, both, iMLS and cMLS *S. pneumoniae*, iMLS *S. pyogenes* and *H. influenzae* in comparison to azithromycin. The inclusion of a carboxylic group at the 3-position of the 4-quinolone gave a very marked increase in antibacterial potency across the entire screen (Table 2).

A further significant improvement in potency was then achieved by switching from the piperazine linker to the 1,2-diaminoethane linker, analogues **27** and **28**. These conjugates exhibit excellent antibacterial potency against the resistant organisms, comparable or better than the potency shown by azithromycin and telithromycin. The data contrast strongly with the MICs displayed by intermediate, 7-[(2-aminoethyl)amino]-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydro-3-quinolinecarboxylic acid **29**, which is devoid of any significant antibacterial activity (MIC = >64 μg/mL) (Table 2).

In conclusion, an efficient and flexible parallel synthesis was developed for the rapid preparation of 8a-aza-8a-homoerythromycin derivatives via Michael addition. Among them, the 4-quinolone conjugates are highly potent antibacterial agents and have activity against clinically relevant macrolide resistant strains.

Table 2
Yield (%) and antibacterial activity (MICs/μg/mL) of 8a-aza-8a—4″-(3-substituted-amino)propionates **24–28**



No.	R	NR″R‴	Yield (%)	*S. aureus* ATCC 13709	*S. aureus* PK1	*S. pneumoniae* Ci137	*S. pyogenes* 3 Finland	*S. pneumoniae* 134 GR M	*S. pyogenes* Finland 11	*S. pneumoniae* 58 Spain	*S. pyogenes* 166 GR-Micro	*H. influenzae* ATCC 49247
				Phenotype								
azi		—		0.5	>64	8	8	>64	16	>64	>64	1
teli		—		<0.06	0.125	0.25	0.5	0.25	0.06	0.25	16	2
1		—		1	>64	32	32	>64	16	>64	>64	2
2		—		0.5	>64	16	16	>64	8	>64	>64	4
24	H		45	1	nd	<0.13	nd	>32	0.5	>32	nd	8
25	Me		49	1	8	<0.13	<0.13	>64	1	64	>64	8
26	Me		44	1	2	<0.13	0.5	8	0.5	16	8	1
27	H		36	0.25	0.5	<0.13	0.25	<0.13	<0.13	<0.13	0.25	nd
28	Me		35	<0.13	<0.13	<0.13	0.5	<0.13	<0.13	<0.13	0.25	4
29				>64	>64	>64	>64	>64	>64	>64	>64	>64

azi = azithromycin; teli = telitromycin; S = macrolide-sensitive strain; iMLS = inducible resistance to macrolide, licosamide and streptogramin (MLS) antibiotics; cMLS = constitutive MLS resistance; M = efflux mediated macrolide resistance, nd = not determined.

References and notes

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11. *Typical experimental procedures*: *2′-O-Acetyl-6-O-methyl-4″-O-propenoyl-8a-aza-8a-homoeythromycin A*, **2b**: To a stirred solution of **2a**[15] (1.8 g) in dry toluene (30 mL), Et_3N (2.0 mL) and 3-chloropropionyl chloride (0.63 mL) were sequentially added at room temperature. After 2 h additional amounts of Et_3N (2 equiv) and 3-chloropropionyl chloride (1 equiv) were added and stirred for

further 2 h. The reaction was quenched with saturated $NaHCO_3$ (60 mL), the layers separated and the aqueous extracted with toluene (3 × 30 mL). The combined organic extracts washed with brine (20 mL), dried and the solvent evaporated to give the title compound (1.8 g, 95.2%), MS: *m*/*z* (ES) = 859 $(MH)^+$.

12. *Typical experimental procedures*: *6-O-Methyl-4″-O-propenoyl-8a-aza-8a-homoerythromycin A*, **4**: Compound **2b** (1.8 g) was dissolved in MeOH (100 mL) and stirred at room temperature for 24 h then at 60 °C for a further 2 h. The solvent was evaporated and the residue purified by flash chromatography over silica gel eluenting with DCM/MeOH/NH_4OH 90:9:1 to yield the title compound (1.5 g, 87.4%), MS: *m*/*z* (ES) = 817.5 $(MH)^+$.
13. *General Procedure for the Michael addition* (array synthesis): To a solution of **4** (5.0 mg, 0.006 mmol) in MeOH (200 μL) was added the primary or secondary amine (5 equiv). If the amine contained an acid functionality or a salt DIPEA (5 equiv) was then added. The resultant mixture was heated at 60 °C. After 12–24 h the reaction cooled, diluted with DCM (600 μL) and the appropriate scavenger resin (30 mg) added (isocyanate-resin for secondary amines or 4-benzyloxybenzaldehyde-resin for primary amines). After further 12 h the resin was filtered off, washed sequentially with MeOH (300 μL), DCM (300 μL) and MeOH (300 μL). The combined solvents were evaporated to yield the corresponding product, characterized by hyphenated MS techniques.
14. *Typical procedure for the synthesis of acylamino-quinolyl intermediates*: To a solution of Boc-Gly-OH or Boc-Ala-OH (2.85 mmol) in dry DMF (5.0 mL) was added HBTU (1.03 g, 2.7 mmol) and heated at 50 °C for 10 min. To this mixture, 3-aminoquinoline (289 mg, 2 mmol) were added and the resulting mixture stirred at this temperature for 48 h. The cooled reaction mixture was diluted with water (50 mL) and extracted with EtOAc (3 × 30 mL). The organic layer was washed with satd $NaHCO_3$ (3 × 30 mL) and brine (3 × 30 mL), dried over anhydrous Na_2SO_4 and evaporated to yield 459 mg of the desired Boc-protected raw product. The product was dissolved in TFA (2.0 mL) and stirred at room temperature for 24 hours. Diethylether (50 mL) and water (20 mL) were added and the organic layer was washed with water (3 × 30 mL). The combined aqueous layers were washed with DCM (3 × 30 mL). The pH of the aqueous layer was adjusted to pH 10 and extracted with DCM (4 × 30 mL). This organic layer (at pH 10) was dried over anhydrous Na_2SO_4 and evaporated to yield 30–40% of the desired crystalline product.
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