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Synthesis and antibacterial activity of novel bifunctional macrolides

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Abstract—We report the discovery of a novel class of macrolide antibiotics that have improved antibacterial activity against Ery-resistant organisms.

Macrolide antibiotics such as erythromycin A (Fig. 1) have been used for many years for the treatment of respiratory tract infections. They exert their action by selectively binding to domains II and V of the bacterial ribosomal RNA and inhibiting protein biosynthesis.[1] Many years of intense research resulted in macrolide derivatives with improved activity and pharmacokinetics, however continuous use of macrolide antibiotics has also resulted in macrolide resistant bacteria. There are two key mechanisms that contribute to macrolide bacterial resistance.[2] One is the dimethylation by *erm* methyltransferases of an adenine group located at the ribosome macrolide binding-site that prevents tight macrolide binding (*erm*-resistance). The other is the action of an efflux pump that recognizes and removes macrolides from the bacterial cell (*mef*-resistance).

Recently, substitution of the 3-cladinose sugar with a keto-group resulted in a new class of drugs, the ketolides as exemplified in Figure 1 by ABT-773[3] and telithromycin.[4] These molecules have addressed macrolide resistance by what is believed to be an alternative, more effective interaction with domain II of the ribosomal RNA via anchoring units attached to various sites on the macrolide molecule instead of the cladinose sugar.[5] Furthermore, several bifunctional macrolides containing rigid anchoring units have also been prepared.[6]

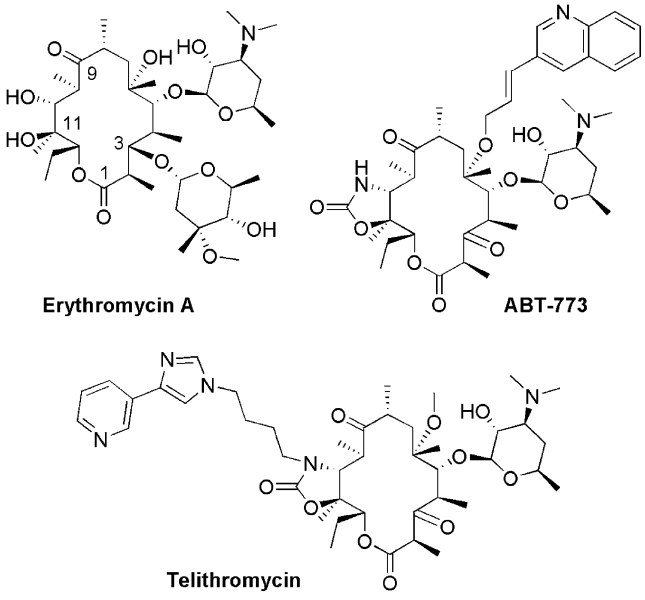

Figure 1. Chemical structures of erythromycin A and ketolides.

Keywords: Bifunctional macrolides; Macrolide resistance; Ketolides.
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In an effort to probe the space around the macrolide molecule and create additional interactions with a secondary site on the ribosome we have prepared two large lead generation libraries of macrolides attached to a variety of tethers and potential secondary binders. We decided to elaborate on two different scaffolds, based on synthetic accessibility. One of the scaffolds allowed us to build on C-3 of the macrolide molecule, the other on C-9.

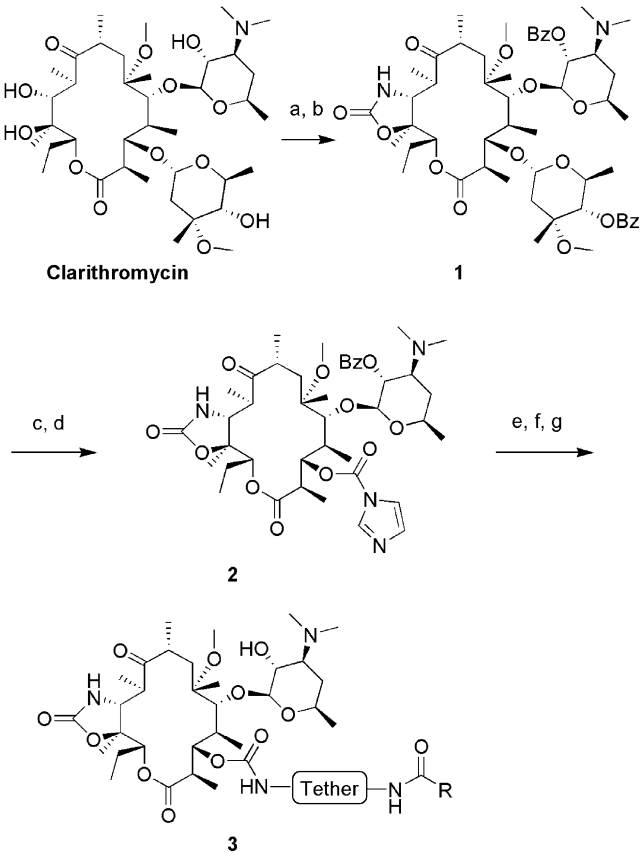
We selected diamines as the bifunctional units that would link the macrolide and the secondary binder because they were readily available in a variety of lengths, rigidity and polarity.

Secondary binders were selected to mimic parts of known antibiotics that bind to domains II and/or V such as chloramphenicol and anisomycin.[7] Additionally, aromatic and heterocyclic substituents were employed as DNA-base surrogates[8] in order to mimic antibiotics containing DNA-bases such as puromycin[9] and blasticidin.[7] A variety of other substituents were also selected based on diversity to probe a large chemical space. All possible combinations of tethers with secondary binders were carried out by a matrix parallel synthesis approach.

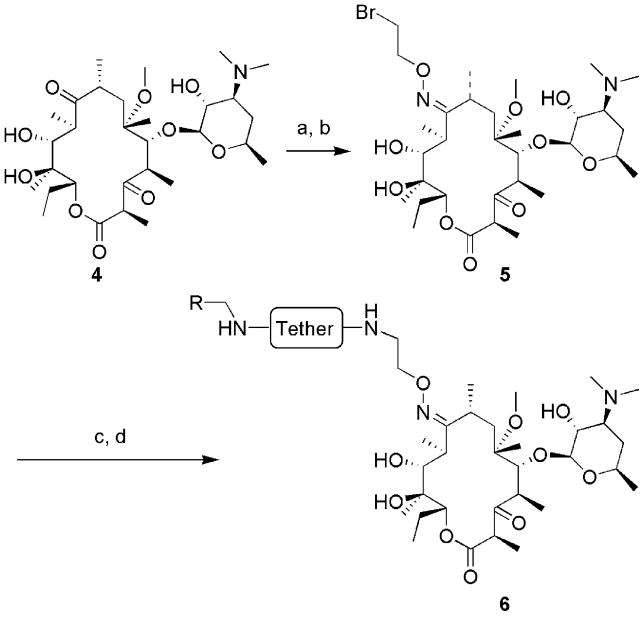
The 3-*O*-carbamate analogs were prepared from clarithromycin in seven steps as shown in Scheme 1. Initially, the sugar hydroxyl groups were protected selectively over the 11, 12 hydroxyls, which were subsequently transformed to the carbamate **1**. Removal of the cladinose sugar with HCl and treatment with 1,1′-carbonyldiimidazole (CDI) provided the desired intermediate **2** in large quantities. This compound was further derivatized with various diamines, which were acylated and deprotected to provide final analogs **3**.[10]

The 9-oxime analogs were assembled as shown in Scheme 2. Starting from ketolide **4**, 9-oxime core **5** was prepared in two steps in large scale and good overall yields. Reactions of 3 equiv of various diamines with bromide **5** in DMF proceeded in good yields to provide the tethered molecules, which were subjected to reductive alkylation using Amberlite–BH_4 as the reducing agent. The presence of base was crucial to the success of the reductive alkylation step, as without it the reaction proceeded in very low yields and starting material was recovered.

All compounds were tested in vitro against *Staphylococcus aureus* NCTC 10649 (an erythromycin-susceptible strain), *S. aureus* 1775 (a methicillin-resistant strain (MRSA) bearing a constitutive *erm* (A) gene), *Streptococcus pneumoniae* ATCC 6303 (an erythromycin-susceptible strain), *S. pneumoniae* 5649 (an efflux-resistant strain bearing a *mef* (E) gene), *S. pneumoniae* 5979 (an MLS_B resistant stain bearing an *erm* (B) gene) and *Haemophilus influenzae* GYR1435 (a fully susceptible strain) by the broth microdilution method according to the reference procedure of the National Committee for Clinical Laboratory Standards.[11]

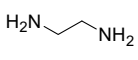
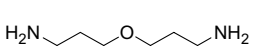
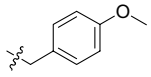
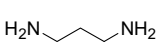
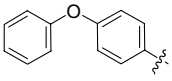
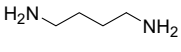
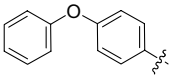
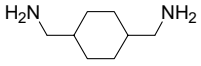
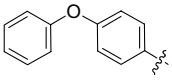
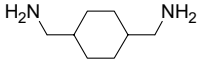
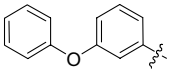
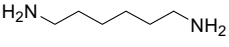
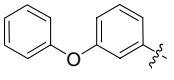
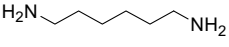
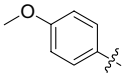
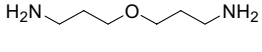
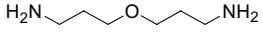
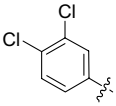
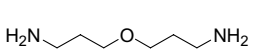
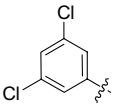
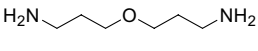
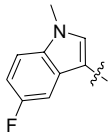
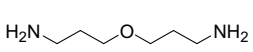
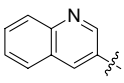
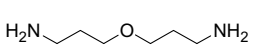
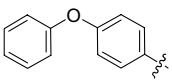
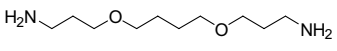
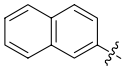


Scheme 1. Reagents and conditions: (a) Bz_2O, DMAP, Et_3N, CH_2Cl_2, 55%; (b) 1. CDI, DBU, THF, DMF, 2. NH_3, *t*-BuOK; (c) 2 M HCl, EtOH, 45 °C, 78%; (d) CDI, THF, dichloroethane; (e) H_2N–tether–NH_2, DMF, 74–85%; (f) RCO_2H, PS-carbodiimide, HOBT, DMF, then PS-trisamine; (g) MeOH, 5–72% in two steps.



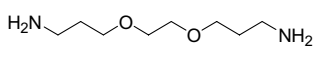
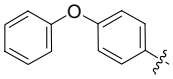
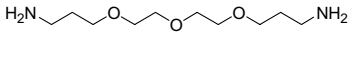
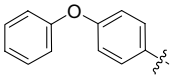
Scheme 2. Reagents and conditions: (a) $NH_2OH{\cdot}HCl$, EtOH, NaOAc, reflux, 99%; (b) dibromoethane, Bu_4NBr, 10% NaOH, CH_2Cl_2, 40 °C, 2 d, 72%; (c) (3 equiv) H_2N–tether–NH_2, K_2CO_3, DMF, 69–85%; (d) RCHO, DIEA, MeOH, Amberlite–BH_4, 11–63%.

Table 1. In vitro antibacterial activity of 3-*O*-carbamate macrolides **3** (Scheme 1) and 9-oxime ketolides **6** (Scheme 2)

Compds	H_2N—Tether—NH_2	R	MIC (μg/mL)[a]					
			S. aureus NCTC 10649	*S. aureus* 1775	*S. pneumo.* ATCC 6303	*S. pneumo.* 5649	*S. pneumo.* 5979	*H. flu* GYR 1435
Ery[b]			0.25	>128	0.03	4	>128	2
Teli[c]			0.06	>128	⩽0.008	0.25	0.06	2
ABT-773			0.12	>128	⩽0.008	⩽0.008	⩽0.008	0.5
4			0.5	>128	0.06	0.12	>128	64
3a			64	>128	0.25	ND[d]	>128	128
3b			8	>128	0.5	0.5	>128	16
6a			32	64	0.12	1	32	13
6b			16	32	0.015	0.25	0.5	16
6c			4	32	0.06	0.12	0.06	8
6d			4	64	⩽0.008	0.12	2	8
6e			2	64	⩽0.008	0.5	0.12	4
6f			1	>128	⩽0.008	0.12	2	4
6g			16	>128	0.12	0.25	1	128
6h			2	>128	0.06	0.5	0.25	16
6i			1	>128	⩽0.008	0.015	4	8
6j			1	64	⩽0.008	0.06	4	8
6k			0.5	>128	0.06	0.5	0.25	64
6l			2	64	⩽0.008	⩽0.008	0.06	2
6m			32	>128	0.12	1	1	32

(*continued on next page*)

Table 1 (*continued*)

Compds	H_2N—Tether—NH_2	R	MIC (μg/mL)[a]					
			S. aureus NCTC 10649	*S. aureus* 1775	*S. pneumo.* ATCC 6303	*S. pneumo.* 5649	*S. pneumo.* 5979	*H. flu* GYR 1435
6n			2	128	0.12	1	2	64
6p			2	128	0.25	1	2	32

[a] Minimum inhibitory concentrations.
[b] Erythromycin A.
[c] Telithromycin.
[d] ND not determined.

Table 1 summarizes the antibacterial activity of the most potent compounds from each series. All 3-*O*-carbamate analogs exhibited a weak antibacterial profile similar to erythromycin, as exemplified by analogs **3a** and **3b**. These compounds were not active against *erm*-resistant strains and were weakly active against susceptible strains.

The 9-oxime series on the other hand provided numerous interesting analogs. Departing from the erythromycin-like activity of the parent compound **4**, analogs in the 9-oxime series exhibited a distinct antibacterial profile. The compounds were not as active in erythromycin-susceptible *S. aureus*, however they showed improved activity against the *S. aureus* strain having the *erm* mechanism of resistance. They were also very active against susceptible and resistant *S. pneumoniae* strains.

A comparison of 9-oxime analogs with identical R substituents such as **6a**, **6b**, **6c**, **6l**, **6n** and **6p**, revealed a bell-shaped curve for antibacterial activity versus tether length reaching an optimal tether length in compound **6l**. This compound exhibited strong antibacterial activity against erythromycin-susceptible and resistant *S. pneumoniae* strains that was better than erythromycin and telithromycin. Antibacterial activity against the *erm*-resistant *S. aureus* strain shifted the curve towards smaller tethers. Compounds **6b** and **6c** exhibited a greater than fourfold improvement against this strain when compared with telithromycin and ABT-773.

Variations of the R substituent attached to the same tether altered the activity and suggested that there were two SARs related with the series. One was based on tether length; the other was based on R substitutions. For example, compound **6i** was more active against *S. pneumoniae* susceptible and *mef*-resistant strains but **6h** was eightfold better against *erm*-resistant *S. pneumoniae*. Similarly, **6f** had stronger activity against *mef*-resistant *S. pneumoniae* but **6e** was more active against *erm*-resistant *S. pneumoniae.*

Interestingly, the majority of the most active analogs had very similar secondary binders that is binders containing phenyl groups suggesting a special interaction between these units and the ribosome.

In conclusion we have identified numerous tether/secondary binder combinations that show comparable antibacterial activity to optimized drugs or drug candidates. The remarkable activity of these unoptimized leads against resistant strains coupled with the fact that the parent compound **4** exhibited only weak antibacterial activity, suggests that additional interactions between the tether/secondary binders and the ribosome take place.

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