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Synthesis and Structure–Activity Relationships of α-Amino-γ-lactone Ketolides: A Novel Class of Macrolide Antibiotics

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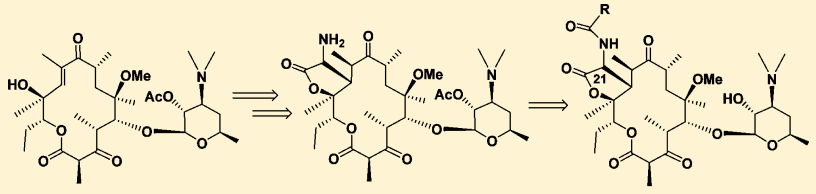
Supporting Information



ABSTRACT: An efficient synthesis of α-amino-γ-lactone ketolide (**3**) was developed, which provided a versatile intermediate for the incorporation of a variety of aryl and heteroaryl groups onto the C-21 position of clarithromycin via HBTU-mediated amidation. The biological data for this important new class of macrolides revealed significantly potent activity against erythromycin-susceptible strains as well as efflux-resistant and erythromycin MLS_B-resistant strains of *S. pneumoniae* and *S. pyogenes*. In addition, ketolide **11o** showed excellent *in vitro* antibacterial activity against *H. influenzae* strain as compared to telithromycin. These results indicate that C-21 substituted γ-lactone ketolides have potential as a next generation macrolide antibiotics.

KEYWORDS: *Macrolide antibiotics, macrolide resistance, ketolides, structure–activity relationships*

Historically, new generations of macrolides have been produced by synthetic modification of existing macrolide core structures.[1−3] Efforts culminated in the late 1980s with the commercialization of clarithromycin[4] and azithromycin,[5] but these compounds remain ineffective against many of the macrolide-resistant pathogenic strains.[6] Discovery of ketolides, exemplified by telithromycin (**1**)[7] and cethromycin (ABT-773; **2**),[8] represented a breakthrough in macrolide structure–activity relationships (Figure 1). Structurally, the ketolides are semisynthetic derivatives of erythromycin A characterized by the presence of a 3-keto group in place of the L-cladinose moiety and an alkyl-aryl extension at the positions 11 and 12 of the cyclic carbamate ring.

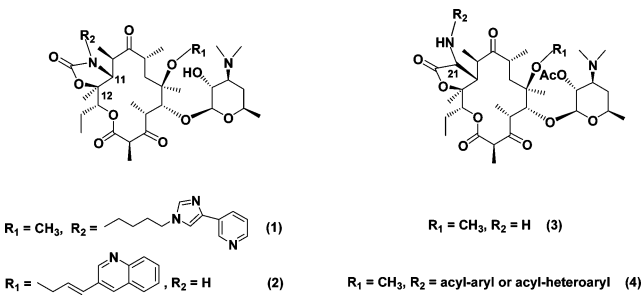


Figure 1. Representative examples of ketolides and novel C-21 substituted γ-lactone ketolides derived from 14-membered macrolides.

Most of the ketolides developed after original disclosure of telithromycin contain a cyclic carbamate fused to C-11 and C-12 of the macrocyclic core.[9] Besides cyclic carbamates, C-11/C-12 cyclic urea,[10] thiocarbamate,[11] and carbazate analogues[12] were also recently synthesized. Our efforts in this area were directed toward the design, synthesis, and functionalization of a new series of ketolides that contain γ-lactone ring fused to C-11 and C-12 position of the clarithromycin scaffold. Although basic unsubstituted[13,14] and arylalkylthio-γ-lactone scaffold[15] were recently synthesized, further efforts directed toward efficient diversification of these important intermediates remain scarce. In addition, researchers at Johnson and Johnson disclosed a novel series of ketolides containing C-6 substituted heteroaryl side chain and C-11/C-12 γ-lactone functionality.[16] In particular, we have developed a new series of γ-lactone ketolides modified at the C-21 position of the lactone ring with an α-amino group, which was further functionalized with an aromatic or heteroaromatic side chain (Figure 1, **3** and **4**). α-Amino lactone derivative of clarithromycin (**3**) was chosen as a

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key intermediate for the introduction of appropriate aryl or heteroaryl side chain because it represents novel proprietary scaffold suitable for straightforward chemical derivatization via N-acylation chemistry.[17] It was hypothesized that the diversification at C-21 carbon atom of the γ-lactone ring with a variety of heterocyclic side-chain appendages similar to those found in our recent paper[18] would result in enhancement of antibacterial activity against resistant pathogens. In this letter, we describe the synthesis and biological properties of C-21-substituted clarithromycin ketolides, as a novel class of macrolide antibiotics, which show good antibacterial activity against Gram-positive pathogens including a macrolide-lincosamide-streptogramin B (MLS_B) and efflux-resistant strains of *S. pneumoniae* and *S. pyogenes*.

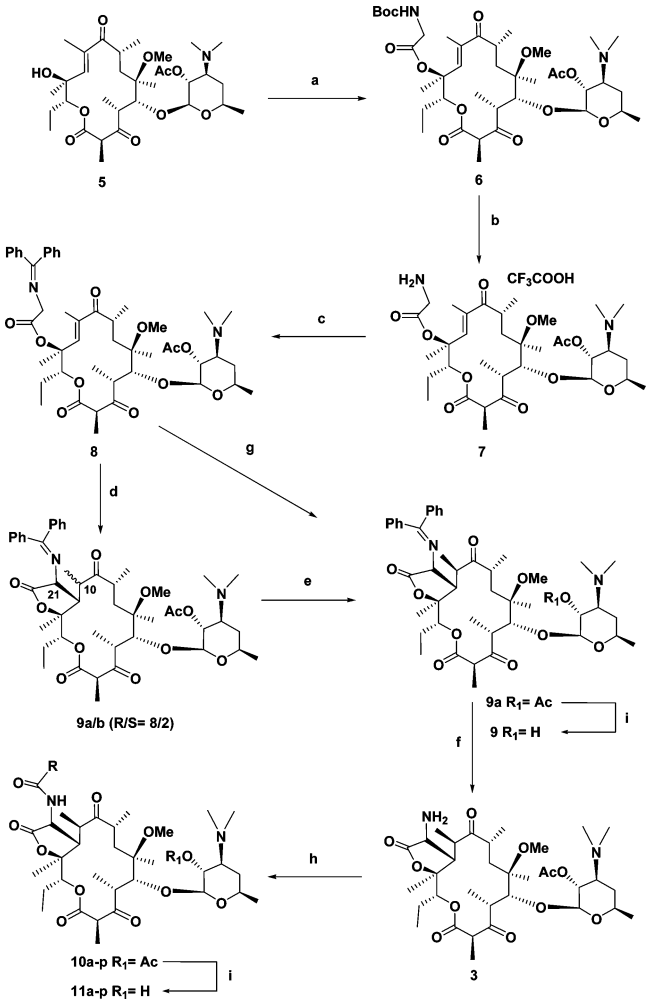
As part of the research program aimed at discovering next generation macrolide antibiotics active against multidrug-resistant respiratory pathogens, we have investigated a broad range of 14- and 15-membered ring macrolides.[19,20] This project was directed toward the development of an efficient synthetic methodology to access α-amino lactone ketolides, a novel class of 14-membered ketolide antibiotic with significant *in vitro* potency against macrolide-resistant strains. The synthetic route for accessing a basic α-amino lactone scaffold (**3**) as a convenient point of attachment for rapid structure–activity relationship (SAR) exploration (Scheme 1) rests on stereoselective incorporation of the α-amino lactone moiety via an intramolecular Michael addition. Central to this strategy is the creation of a suitably oriented C-21 α-amino group attached on the γ-lactone moiety via a stereoselective intramolecular Michael addition. This amino group provides a handle to introduce novel aryl and heteroaryl moieties (Chart 1) directly onto the C-21 position of the macrolide core via HBTU-mediated amidation.

The α,β-unsaturated ketone **5** used as a starting material for the preparation of α-amino lactone (**3**) was prepared by modification of the published procedure.[21] The synthesis of key intermediate **3** began with selective acylation of C-12 hydroxyl group by *tert*-butyloxycarbonyl (Boc) glycine in the presence of 1-[3-(dimethylamino)-propyl]-3-ethylcarbodiimide hydrochloride (EDC·HCl) and the catalytic amount of DMAP (Scheme 1).

The 12-*O*-Boc glycyl derivative **6** thus obtained was subjected to selective deprotection of the Boc group by exposure to trifluoroacetic acid in dichloromethane at room temperature. Acylation of **5** with chloroacetyl chloride or mixed acid anhydride ($ClCH_2CO_2H$, PivCl, Et_3N, DMAP, CH_2Cl_2, −15 °C to room temperature) led to reduced yields (30–50%) and was consequently not explored further.

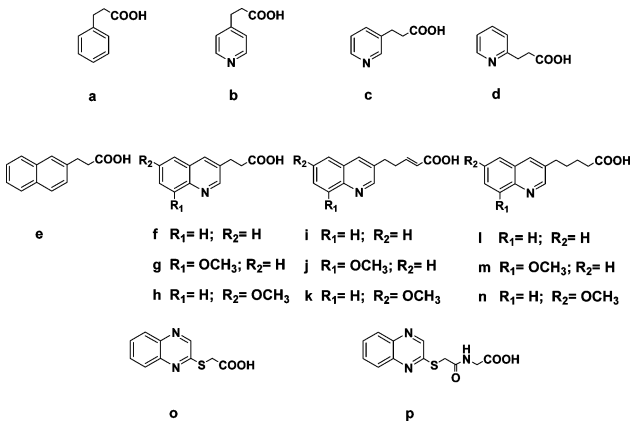
The crystalline ammonium salt **7** was isolated in 90% yield and allowed to react with benzophenone imine to afford the precursor **8** used in the crucial intramolecular Michael addition. Following column chromatography on silica gel benzophenone imine analogue **8** was subjected to DBU-mediated Michael addition in acetonitrile at reflux to afford γ-lactone intermediate **9a/b** as an inseparable mixture of diastereoisomers at C-10 carbon atom (ratio C10-(*R*)/(*S*) = 8/2). Base-catalyzed isomerization of **9a/b** in the presence of lithium hydroxide monohydrate allowed the epimerization at the C-10 position to the desired C10-(*R*) diastereisomer **9a** in 80% yield. Deprotection of benzophenone imine **9a** in aqueous hydrochloric acid provided α-amino-γ-lactone intermediate **3** that was subsequently used in HBTU-mediated coupling reactions.

Scheme 1. Synthesis of 21-Amino-2′-*O*-acetyl-3-*O*-descladinosyl-11,12-dideoxy-6-*O*-methyl-12,11-(oxycarbonylmethylene)-3-oxo-erythromycin A (3) Followed by HBTU Coupling[a]



[a]Reagents and conditions: (a) BocGly, EDC·HCl, DMAP, CH_2Cl_2, r.t., 80%; (b) TFA, CH_2Cl_2, 0 °C to r.t., 90%; (c) Ph_2CNH, Et_3N, CH_3CN, reflux, 90%; (d) DBU, CH_3CN, reflux, 80%; (e) $LiOH·H_2O$, CH_3CN, r.t., 90%; (f) 1 M HCl, CH_3CN, r.t., 80%; (g) $LiOH·H_2O$, CH_3CN, r.t., 80%; (h) RCO_2H, HBTU, DIPEA, DMF, r.t., 50–90%; (i) MeOH, r.t., 95%.

Chart 1. Aryl and Heteroaryl Scaffolds Used as the R Substituents in HBTU Coupling of 3



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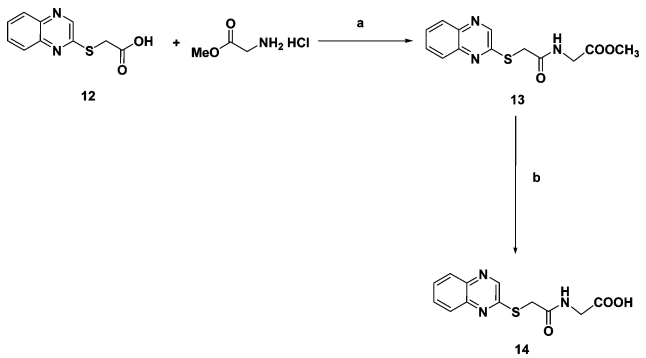
When LiOH was used instead of DBU only one diastereoisomer was observed according to LC/MS analysis of the crude reaction mixture. Thus, upon exposure to LiOH, **8** underwent an intramolecular Michael addition to give exclusively C10-(*R*) diastereoisomer **9a**. The configuration at C-10 was confirmed by coupling constants in ^{1}H NMR spectrum of deprotected analogue **9** (2′-OH, Supporting Information, page S24) suggesting that the 10(*R*)-epimer was indeed formed exclusively.[22] Acid hydrolysis of benzophenone imine **9a** with 1 M HCl in acetonitrile again provided α-amino-γ-lactone ketolide (**3**) with the spectral data that were identical to the sample isolated after base-catalyzed isomerization of the diastereoisomeric mixture **9a**/**b** followed by acid hydrolysis of **9a**.

Coupling of various aryl and heteroaryl carboxylic acids (Chart 1) with α-amino lactone ketolide **3** was carried out in DMF with hydroxybenzotriazole uronium salt (HBTU) in the presence of diisopropylethyl amine (DIPEA) in generally acceptable isolated yields ranging from 50 to 90% (Scheme 1).

Deprotection of the 2′-acetyl group on cladinose was readily accomplished by stirring in methanol at room temperature to give essentially quantitative yield of C-21 functionalized α-amino lactone ketolides (**11a**–**p**). Many of the carboxylic acids used for HBTU coupling reaction were either commercially available or synthesized using well-precedented chemistries.[7]

The synthesis of quinoxaline carboxylic acid **14**, however, had to be developed from more basic building blocks as illustrated in Scheme 2. Therefore, quinoxalin-2-yl thioacetic

Scheme 2. Synthesis of Quinoxalin-2-yl Thioacetyl Glycine 14[a]



[a]Reagents and conditions: (a) HBTU, DIPEA, DMF, r.t., 85%; (b) LiOH·H_2O, THF/H_2O (1:1), r.t.; 2 M aq. HCl, pH = 4, 95%.

acid **12** was treated with methyl ester of glycin hydrochloride in the presence of HBTU as a coupling reagent and DIPEA for 2 h at room temperature (Scheme 2). Subsequent hydrolysis of the glycine ester **13** was carried out with lithium hydroxide in THF/water mixture (1:1) at room temperature. This procedure afforded almost quantitative yield of the corresponding carboxylic acid **14** after acidic workup at pH = 4.

The antibacterial activity of the C-21 substituted α-amino-γ-lactone ketolides was tested against a panel of representative pathogens selected from Pliva Research Institute culture collection. The *in vitro* antibacterial activities are reported as minimum inhibitory concentrations (MICs) that were determined by the agar microdilution method according to NCCLS standards.[23] Table 1 shows the *in vitro* activity of the ketolide analogues and the reference compounds, azithromycin, telithromycin (**1**), and cethromycin (**2**).

The basic, deprotected α-amino-γ-lactone scaffold **3a** exhibited excellent activity against the susceptible strain of *S. pneumoniae*, moderate activity against the efflux resistant strains, but very poor potency against constitutively MLS_B-resistant *S. pneumoniae* and *S. pyogenes*. The antibacterial profile of **3a** is not unexpected knowing the well-established need for an aromatic or heteroaromatic ring attached to the macrolide scaffold as a necessary requirement for potent antibacterial activity against constitutively MLS_B-resistant strains. In general, the ketolides were also inactive against constitutively MLS_B-resistant strain of *Staphylococcus aureus* (MIC > 64 μg/mL). In contrast, most of the C-21 substituted γ-lactone ketolides were active against inducibly resistant *S. aureus* strains. The most interesting feature of these new compounds was their effectiveness against efflux resistant *S. aureus* and *S. pneumoniae* strains as well as constitutively MLS_B-resistant *S. pneumoniae* and *S. pyogenes*. The compounds generally maintained good activity against both, the erythromycin-susceptible and MLS_B constitutively resistant strains of *S. pyogenes* and *S. pneumoniae*.

Attachment of a simple benzyl substituent (**11a**) resulted in dramatic improvement of antibacterial activity against constitutively MLS_B-resistant *S. pneumoniae* and *S. pyogenes*. The activity against efflux- and constitutively MLS_B-resistant strains was further improved with the corresponding pyridylethyl analogues (**11b**, **c**, and **d**). In terms of the site of attachment within the pyridine analogues, positions 2 and 3 appear to be optimal for the *S. pneumoniae* (MLS_B) activity, whereas for *H. influenzae* and *S. pyogenes* (MLS_B) activity position 4 appears to be preferred.

The rest of the analogues presented in Table 1 cover fused bicyclic aryl- and heteroaryl-systems (**11e**–**p**). In reviewing the SAR data of these analogues, it is evident that compounds containing fused bicyclic aryl- and heteroaryl-rings (**11e**–**p**) generally possessed a better overall antibacterial profile than simple monoaryl (**16a**) and monoheteroaryl systems (**11b**–**d**). The quinolyl analogue (**11f**), for example, demonstrated improved activity when compared to its monoaryl and monoheteroaryl counterparts **11a** and **11c**, respectively. In addition, the overall activity spectrum of C-21 substituted γ-lactone ketolides can be improved by the nature and length of the tether connecting heteroaryl ring and the macrolide core. It is a common knowledge that the length of the tether connecting heterocycle and the macrolide is critical for the antibacterial activity, and a four-carbon alkyl chain appeared to be optimal when the tether is attached at the C-11 carbamate nitrogen.[7] In addition to linear alkyl chains, amine-, hydrazine-, amide-, olefin-, and ether-containing linkers have been disclosed.[7,24,25] Most of the linkers used in this work contain four atoms between the aryl- or heteroaryl-unit and C-21 carbon atom of the macrolide core (Chart 1) in analogy with the telithromycin structure. As shown in Table 1 two methylene-unit linkers (**11f**, **11g**, and **11h**) greatly enhance the *in vitro* antibacterial activity compared with four methylene-unit linkers found in **11l**, **11m**, and **11n**, respectively. For example, compounds **11f**–**h** and **11l**–**n** share the same quinoline heterocycle and identical substitution pattern, but compounds **11f**–**h** have significantly improved potency against efflux- and constitutively MLS_B-resistant *S. pneumoniae* and *S. pyogenes* strains as well as *H. influezae* strain.

Introduction of the double bond in the linker additionally improves the activity against constitutively MLS_B-resistant *S. pyogenes* and *H. influenzae* especially in the case of methoxy substituted quinoline analogues (**11j** vs **11m** and **11k** vs **11n**).

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Table 1. *In Vitro* Antibacterial Activity of C-21-α-Amino-γ-lactone Ketolides against Selected Pathogens[a]

	S. aureus			*S. pneumoniae*			*S. pyogenes*			
compd	Ery-S	iMLS	MLS_B	Ery-S	MLS_B	M	Ery-S	MLS_B	M	*H. influenzae*
3a[b]	1	32	>64	≤0.125	>64	8	2	>64	8	>64
11a	4	8	>64	≤0.125	16	4	0.5	16	8	16
11b	4	8	>64	≤0.125	16	8	≤0.125	2	2	2
11c	2	4	>64	≤0.125	4	2	≤0.125	4	2	8
11d	4	4	>64	≤0.125	2	2	≤0.125	32	4	16
11e	1	1	>64	≤0.125	2	2	≤0.125	4	8	2
11f	1	1	>64	≤0.125	1	0.5	≤0.125	1	2	4
11g	0.5	1	>64	≤0.125	0.25	0.25	≤0.125	0.5	0.25	1
11h	2	1	>64	≤0.125	0.5	0.5	≤0.125	0.25	0.5	1
11i	2	2	>64	≤0.125	8	1	≤0.125	8	4	8
11j	1	1	>64	≤0.125	2	2	≤0.125	0.5	2	1
11k	2	2	>64	≤0.125	8	4	≤0.125	0.25	4	1
11l	1	2	>64	≤0.125	8	4	≤0.125	8	16	16
11m	1	1	>64	≤0.125	2	4	≤0.125	4	4	16
11n	1	2	>64	≤0.125	4	4	≤0.125	2	4	8
11o	≤0.125	0.25	>64	≤0.06	≤0.125	0.25	≤0.06	0.25	0.25	0.5
11p	≤0.125	0.25	>64	0.25	0.5	0.5	0.25	1	0.5	2
Azi	1	>64	>64	≤0.125	>64	4	≤0.125	>64	1	1
1	≤0.125	0.5	>64	≤0.06	≤0.125	0.5	≤0.06	4	0.25	2
2	≤0.125	0.25	>64	≤0.06	≤0.125	≤0.06	≤0.06	1	≤0.125	2

[a]Minimum inhibitory concentration (MIC) values are given in μg/mL. Ery-S, erythromycin-susceptible strains; iMLS, inducibly resistant strains; MLS_B, constitutively resistant strains; M, efflux-resistant strains; Azi, azithromycin. [b]Compound **3a** (2′-OH) was synthesized by deprotection of compound **3** (2′-OAc) in MeOH at room temperature.

In the case of unsubstituted quinoline analogues (**11i** vs **11l**), the effect is not as profound but it slightly improves (4-fold) activity against efflux-resistant *S. pneumoniae* and *S. pyogenes*.

To further investigate the SAR we synthesized two representative ketolides in which a quinoxaline ring was appended to the macrolide core. It was found that the activity of the quinoxaline analogue **11o** was in general 2- to 4-fold better in comparison to its glycyl-extended analogue **11p** against most of the strains tested. In addition, comparison of compound **11o** with telithromycin indicates that the former is more active against constitutively MLS_B-resistant *S. pyogenes* and *H. influenzae*.

In summary, a series of clarithromycin γ-lactone ketolides were synthesized and evaluated as a novel class of macrolide antibiotics. By introducing heteroaromatic side-chain instead of α-amino group at the C-21 position of γ-lactone, the antibacterial activity against efflux- and MLS_B-resistant strains of *S. pneumoniae* and *S. pyogenes* could be substantially enhanced. In particular heteroaromatic derivative **11o** exhibited significantly potent antibacterial activity against not only erythromycin-susceptible Gram-positive pathogens but also inducibly MLS_B-resistant *S. aureus*, efflux-resistant *S. pneumoniae*, and MLS_B-constitutively resistant *S. pneumoniae* and *S. pyogenes*. Moreover, compound **11o** is ca. 4-fold more active than telithromycin (**1**) against constitutively MLS_B-resistant *S. pyogenes* and *H. influenzae* strain. It has been demonstrated that γ-lactone ketolides are innovative semisynthetic macrolides that have potential as a next-generation macrolide antibiotic.

■ ASSOCIATED CONTENT

Supporting Information

General experimental methods, experimental procedures, and spectral data for selected new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS

MLS_B, macrolide-lincosamide-streptogramin B; HBTU, *O*-(benzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate; DIPEA, diisopropylethylamine; SAR, structure–activity relationships

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(17) The wide range of carboxylic acid libraries that are commercially available would make this approach particularly attractive and would also ensure that a diverse set of compounds are synthesized. Further extension of N-acylated series of compounds (**4**) has also been made in which the side chain on γ-amino lactone moiety has been replaced with an alkyl heteroaryl group. These results will be published elsewhere.
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