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Synthesis and antibacterial activities of a novel alkylide: 3-*O*-(3-aryl-2-propargyl) and 3-*O*-(3-aryl-2-propenyl)clarithromycin derivatives

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ABSTRACT

A series of novel 3-*O*-(3-aryl-*E*-2-propenyl)clarithromycin derivatives **8** and 3-*O*-(3-aryl-2-propargyl)-clarithromycin derivatives **11** were designed, synthesized, and evaluated for their in vitro antibacterial activities. Compared with **8c** and **11c** (Ar was 5-pyrimidyl), 3-*O*-(3-(5′-pyrimidyl)-*Z*-1-propenyl) counterpart **6c** displayed 4- to 64-fold more potent activities against erythromycin-susceptible *Staphylococcus aureus* and *Streptococcus pneumoniae*. Moreover, the activities of **6c**, **8c**, and **11c** against erythromycin-resistant *S. aureus* and *S. pneumoniae* were in general 4-fold higher than those of the reference compound, clarithromycin and azithromycin.

The second-generation erythromycin, exemplified by clarithromycin (6-*O*-methylerythromycin, as shown in Fig. 1), was developed to address the acid instability of erythromycin A, which was associated with an intramolecular ketalization of 6-OH with 9-keto.[1] Clarithromycin was successfully synthesized via regioselective methylation at 6-OH of erythromycin.[2] As a result, clarithromycin exhibits better bioavailability and improved pharmacokinetics.[3]

Recently, the resistance against erythromycin and clarithromycin is increasingly prevalent via two main genotypes: ribosome methylation or dimethylation resistance encoded by *erm* gene and efflux resistance encoded by *mef* gene.[4] Meanwhile, the *erm*-mediated resistance exists in two phenotypes: inducible and constitutive resistance.

To combat the resistance, a series of ketolides, derived from erythromycin by substitution of 3-*O*-cladinose sugar by a 3-keto group, were synthesized and evaluated.[5] Consequently, ketolides are distinguished by telithromycin (HMR3647),[6] cethromycin (ABT-773),[7] and TE-802,[8] as shown in Fig. 2. Telithromycin and cethromycin were shown to effectively address *erm*- and *mef*-containing resistance, and TE-802 proved to effectively overcome *mef* resistance but possessed weak activities against *erm* resistance. However, the discovery of ketolides disproved the long held belief that 3-*O*-cladinose was indispensable moiety for the antibacterial activity.

Encouraged by the important breakthrough, a novel acylide characterized by 3-*O*-acetyl group was prepared after removal of cladinose. Among the candidates, 3-*O*-(3-pyridyl)acetyl acylide (TEA-0929) possessed better activity against *mef* resistant strains[9,10] (Fig. 3). In addition to continuous focus on the synthesis of new ketolides and acylides,[11–13] other macrolides with various substituents at C-3 instead of cladinose have also been designed and investigated over the past decade, such as 3,6-bicyclolide[14] (Fig. 3), 2,3-anhydrolide,[15] 2,3-enol ether,[16] 3-deoxy,[17] 3-*O*-phenyl ether,[18] 3,6-ketal,[19] and 3,6-ether.[20]

The structure–activity relationship study indicated that ketolides, acylides, and 3,6-bicyclolides presented good activities against *mef* resistance. Furthermore, an aryl group tethered to macrolides contributed much to overcome *erm* resistance via additional contacts with domain II of bacterial ribosome.

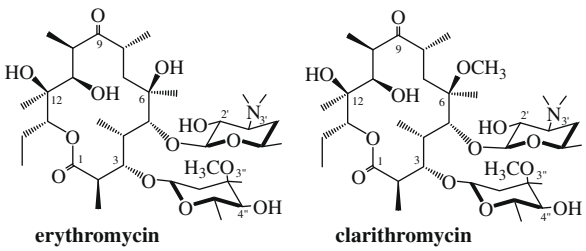

Figure 1. Structure of erythromycin and clarithromycin.

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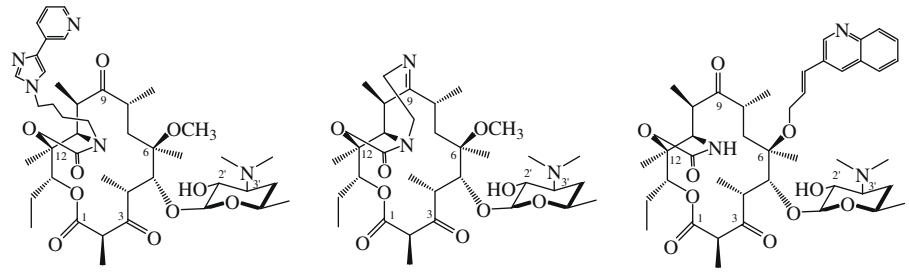


Figure 2. Structure of ketolide.

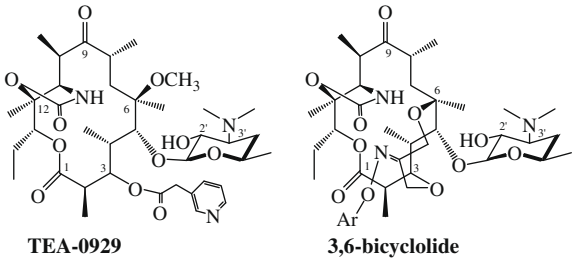
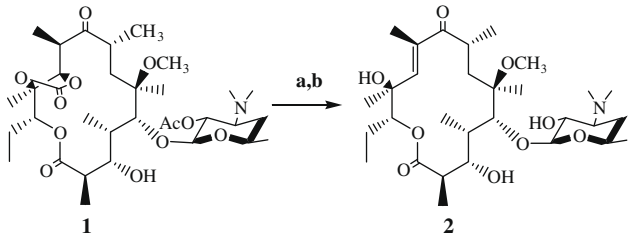


Figure 3. Structure of acylide and 3,6-bicyclolide.

An allyl and a propargyl group have been introduced to 6-OH regioselectively, and the resulting products served as versatile intermediates for a variety of further modification,[7,21–25] such as the introduction of aryl groups via Heck or Sonogashira reaction. Thus, we presumed that 3-*O*-allyl clarithromycin and 3-*O*-propargyl clarithromycin derivatives should be potential lead compounds for further screening. Consequently, we accomplished the regioselective allylation at 3-OH and obtained a series of 3-*O*-(3-aryl-*Z*-1-propenyl)clarithromycin derivatives via Heck reaction, which we named alkylides.[26] To further study of the structure–activity relationships of alkylides, we herein report the synthesis and the antibacterial activities of 3-*O*-(3-aryl-*E*-2-propenyl) and 3-*O*-(3-aryl-2-propargyl)clarithromycin analogues.

A straightforward approach to 3-*O*-allyl alkylide failed via allylation of 3-OH-9-keto **1**,[27] and instead the major product in the resulting mixture was identified as 3-OH-10,11-anhydro **2**, as illustrated in Scheme 1. In contrast, 3-OH-9-*O*-(2-chlorobenzyl)oxime **3** served as the key precursor to produce corresponding 3-*O*-allyl **4** in 95.2% yield,[26] as presented in Scheme 2.

Interestingly, the Heck reaction of 3-*O*-allyl **4**, in the presence of palladium(II) acetate and tri(*o*-tolyl) phosphine as catalysts, easily resulted in the allylic double bond isomerization from 2-position to 1-position as well as the configuration isomerization from *E* to *Z* (**5** and **6**),[26] presumably due to steric reasons in β-hydrogen elimination (Scheme 2). However, further study in our lab disclosed that the catalyst could efficiently give the desirable sidechain without



Scheme 1. Reagents and conditions: (a) allyl bromide, KO*t*Bu, THF/DMSO, 0 °C, 50% (TLC); (b) MeOH, then purified by column chromatography, 11.7% over two steps.

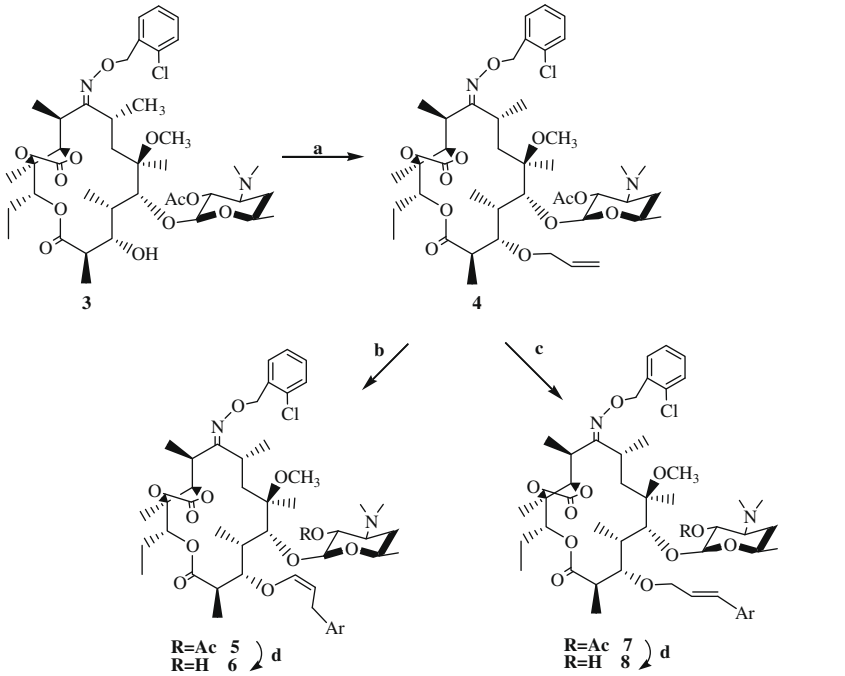
Heck isomerization under the precondition of 2,3-dehydro-3-*O*-allyl analogues, and the results in detail will be published elsewhere. Thus, the Heck reaction of 3-*O*-allyl clarithromycin derivatives may warrant further study.

To prevent the unexpected Heck isomerization of **4**, a new kind of palladacycle catalyst, *trans*-di(μ-acetato)bis[*O*-(di-*O*-tolylphosphino)benzyl]dipalladium(II)[28] was utilized in our synthesis of the alkylides (**7** and **8**), as presented in Scheme 2. The catalyst was extensively used in the synthesis of 3-*O*-aryl-*E*-2-propenyl leucomycin analogues, a 16-membered macrolide.[29] As expected, the application of the palladacycle catalyst successfully furnished the desirable 3-*O*-[3-(3′-pyridyl)-*E*-2-propenyl] (**8b**) and 3-*O*-[3-(5′-pyrimidyl)-*E*-2-propenyl] sidechain (**8c**). Nonetheless, it was noted that the palladacycle catalyst could not afford 3-*O*-[3-(4′-isoquinoly)-*E*-2-propenyl] sidechain (**8e**) efficiently, and the isomerization product (**6e**) still proved to be the majority, as was attributed to the bulky hindrance of 4-isoquinolyl group.

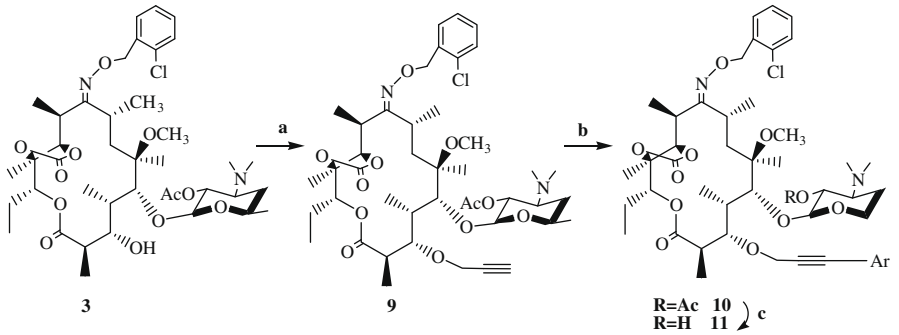
As for the introduction of a propargyl group at 3-OH, it was first reported by Omura group that treatment of 3-OH-6,9-hemiketal derivative, accessible from the acid degradation product of erythromycin, with propargyl bromide and NaH gave 3-*O*-propargyl-6,9-hemiketal counterpart in 72% yield.[30] However, no reaction and activities were reported concerning the Sonogashira coupling products. To further explore the effect of the sidechain's configuration on the activities, the key intermediate 3-*O*-propargyl alkylide **9** was synthesized, in the presence of propargyl bromide and KO*t*Bu, from the precursor 3-OH-9-*O*-(2-chlorobenzyl)oxime **3** in 89.5% yield. Next, treatment of **9** with a variety of aryl halides under the Sonogashira coupling conditions efficiently led to the expected coupling products **10**. The aromatic rings included 4-nitrophenyl (**a**), 3-pyridyl (**b**), 5-pyrimidyl (**c**), 3-quinolyl (**d**), 4-isoquinolyl (**e**), 5-indolyl (**f**) group. Finally, hydrolysis of the 2′-*O*-Ac by heating in methanol provided the desired **11** (Scheme 3).

The antibacterial activities of 3-*O*-(3-aryl-*Z*-1-propenyl) alkylide **6**, 3-*O*-(3-aryl-*E*-2-propenyl) alkylide **8** and 3-*O*-(3-aryl-2-propargyl) alkylide **11** were assessed against a panel of erythromycin-susceptible and erythromycin-resistant bacteria. The selected strains are *Staphylococcus aureus* ATCC 29213 (erythromycin-susceptible strain), *S. aureus* PU-32 (erythromycin-resistant strain bearing inducible *erm* (A) gene), *S. aureus* PU-19 (erythromycin-resistant strain bearing constitutive *erm* (A) gene), *Streptococcus pneumoniae* ATCC 49619 (erythromycin-susceptible strain), *S. pneumoniae* PU-11 (erythromycin-resistant strain bearing both *erm* and *mef* genes), and *S. pneumoniae* PU-13 (erythromycin-resistant strain bearing both *erm* and *mef* genes). Data are listed in Table 1 as the minimal inhibitory concentration (MIC), which is determined by the broth microdilution method as recommended by Clinical and Laboratory Standards Institute (CLSI).[31]

Actually, the activities of the alkylides **6**, **8**, **11** were comparable to those of the corresponding 9-*O*-(2-chlorobenzyl)oxime ketolides[32] and some triazole derivatives started from 3-*O*-propargyl-6,9-hemiketal.[30] The alkylides exhibited improved activities



Scheme 2. Reagents and conditions: (a) allyl bromide, KO*t*Bu, THF/DMSO, 0 °C, 95.2%; (b) $Pd(OAc)_2$, P(*o*-MePh)$_3$, Et_3N, aryl bromide, acetonitrile, 60 °C 1 h then 90 °C 24 h; (c) tetra-*n*-butylammonium bromide, anhydrous NaOAc, *trans*-di(μ-acetato)bis[*O*-(di-*O*-tolylphosphino)benzyl]dipalladium, aryl bromide, DMF, 100 °C 3 days, Ar = 3-pyridyl (**b**), 5-pyrimidyl (**c**), 4-isoquinolyl (**e**); (d) MeOH, 65 °C.



Scheme 3. Reagents and conditions: (a) propargyl bromide, KO*t*Bu, THF/DMSO, 0 °C, 89.5%; (b) $(PPh_3)_2PdCl_2$, CuI, Et_3N, aryl bromide (or aryl iodide), acetonitrile, 80 °C 3 h, Ar = 4-nitrophenyl (**a**), 3-pyridyl (**b**), 5-pyrimidyl (**c**), 3-quinolyl (**d**), 4-isoquinolyl (**e**), 5-indolyl (**f**); (c) MeOH, 65 °C.

Table 1
In vitro antibacterial activities of alkylides against selected respiratory tract pathogens (MIC, μg/mL)

	CLA[g]	AZI[h]	**6c**	**8c**	**11a**	**11b**	**11c**	**11d**	**11e**
ATCC 49619[a]	0.032	0.064	0.25	4	32	16	16	16	16
PU-11[b]	128	128	32	32	32	32	32	16	32
PU-13[c]	128	128	32	32	32	32	32	16	32
ATCC 29213[d]	0.5	0.5	8	64	64	64	32	64	128
PU-19[e]	>256	>256	64	64	128	64	32	128	256
PU-32[f]	128	64	32	64	32	64	32	64	256

[a] *S. pneumoniae* ATCC 49619: erythromycin-susceptible strain.
[b] *S. pneumoniae* PU-11: erythromycin-resistant strain bearing both *erm* and *mef* genes.
[c] *S. pneumoniae* PU-13: erythromycin-resistant strain bearing both *erm* and *mef* genes.
[d] *S. aureus* ATCC 29213: erythromycin-susceptible strain.
[e] *S. aureus* PU-19: erythromycin-resistant strain bearing constitutive *erm* (A) gene
[f] *S. aureus* PU-32: erythromycin-resistant strain bearing inducible *erm* (A) gene.
[g] CLA: clarithromycin.
[h] AZI: azithromycin.

against erythromycin-resistant *S. aureus* and *S. pneumoniae* than those of the reference compound, clarithromycin and azithromycin. Among the series of **11**, **11c** (5-pyrimidyl) was the best candidate with 4- to 16-fold greater activities than clarithomycin against the resistant strains, particularly *S. aureus* bearing constitutive *erm* (A) gene. In comparison, the alkylides with fused biaryl rings **11d** (3-quinolyl) and **11e** (4-isoquinolyl) showed inactive against erythromycin-resistant *S. aureus*, despite a slightly higher activities of **11d** than **11c** against erythromycin-resistant *S. pneumoniae* bearing both erm and *mef* genes. As for the effect of various sidechain's configuration on the activities, **6c**, **8c**, and **11c** shared similar profile against erythromycin-resistant strains. However, **6c** was 4- to 64-fold more potent than **8c** and **11c** against erythromycin-susceptible strains, particularly 64-fold more potent than **11c** against erythromycin-susceptible *S. pneumoniae*.

In conclusion, we designed and synthesized a series of novel alkylides, possessing three-atom length ether linkers instead of cladinose at 3-OH. Among the alkylides (**6**, **8**, and **11**), **8c** was the promising candidate against erythromycin-resistant strains.

Further SAR studies on alkylides are still in progress, varying the length of the linkers included.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.03.038.

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