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Synthesis and antibacterial activity of 6-*O*-(heteroaryl-isoxazolyl)propynyl 2-fluoro ketolides

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ABSTRACT

Macrolide antibiotics are widely prescribed for the treatment of respiratory tract infections; however, the increasing prevalence of macrolide-resistant pathogens is a public health concern. Therefore, the development of new macrolide derivatives with activities against resistant pathogens is urgently needed. A series of novel 6-*O*-(heteroaryl-isoxazolyl)propynyl 2-fluoro ketolides has been synthesized from erythromycin A. These compounds have shown very promising in vitro and in vivo antibacterial activities against key respiratory pathogens including erythromycin-susceptible/resistant strains.

Macrolide antibiotics[1–4] (see Fig. 1 for some structures) are a safe and effective drug class for the treatment of bacterial infections in the respiratory tract. The first macrolide antibiotic, erythromycin A (EM-A) **1**, was commercialized in 1952. Since EM-A decomposes to antibacterially inactive spiroketal products[5] under acidic conditions in the stomach, its bioavailability is relatively low and varies interindividually.[6] To improve the pharmacokinetic profile of EM-A caused by its acid instability, an enteric coating is applied to EM-A tablets and further chemical modifications of EM-A have been performed.[1–4] Second-generation macrolides, such as clarithromycin[7] (CAM) **2** and azithromycin[8] (AZM) **3**, were investigated in the 1980s and were eventually launched in the 1990s as a result of these chemical modification efforts. These macrolide antibiotics have been widely prescribed for more than five decades. Because of their widespread use, the increasing prevalence of macrolide-resistant pathogens among clinical isolates has become a public health concern.[9–12] The major mechanisms of resistance against Gram-positive pathogens are ribosome methylation by *erm* methyltransferase and efflux by macrolide efflux pumps (mediated by the *mef*-gene product).[13–15]

Ketolides are a chemical class of semi-synthetic erythromycin derivatives, in which the natural C3-cladinose sugar is replaced by a keto group. The most advanced ketolides are telithromycin **4** and cethromycin **5**. These agents are known to be effective against erythromycin-susceptible and -resistant strains of *Streptococcus pneumoniae*, *Streptococcus pyogenes*, and *Haemophilus influenzae*.[16,17] These two ketolides have similar structural features, such as a 3-keto group and a heteroaryl-alkyl side chain. The 3-keto group is important for preventing efflux resistance. The heteroaryl-alkyl side chain is believed to play a key role in overcoming resistance caused by ribosome methylation.[18–22] The aryl-alkyl side chains are attached to different positions of the macrolactone skeleton (11, 12-cabamate nitrogen for telithromycin and C-6 position for cethromycin). Despite this difference in the attached positions, the side chains interact with similar sites in domain II

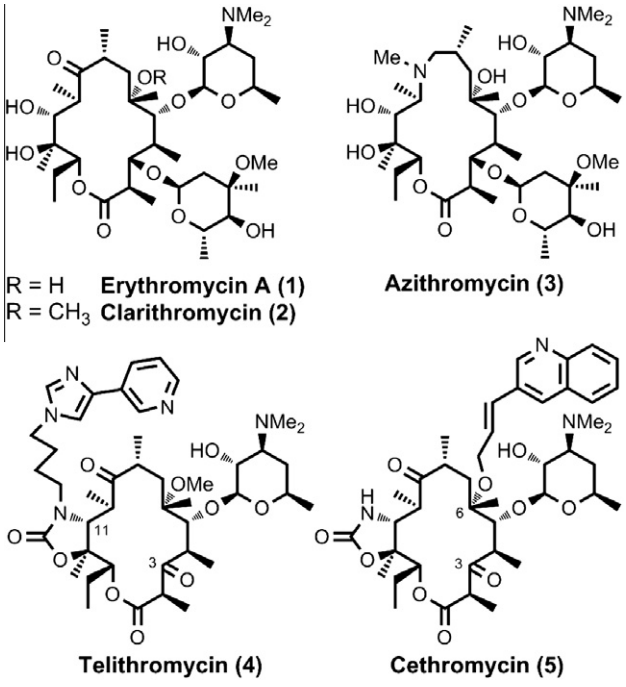

Figure 1. Structures of macrolide antibiotics.

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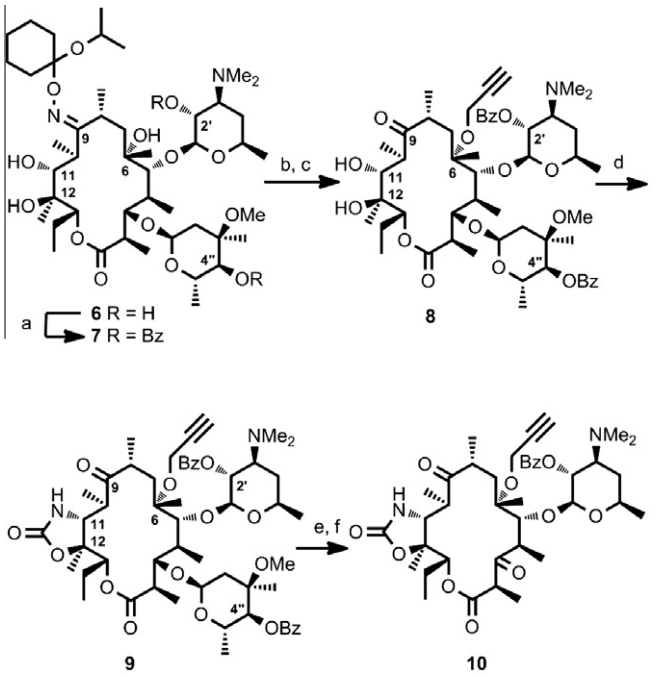
of 23S rRNA.[23] 2-Fluoro ketolides are one of the most successful modification of ketolides. Introduction of a fluorine atom to the C-2 position tends to enhance the antibacterial activity of the corresponding ketolides both in vitro and in vivo.[19,24]

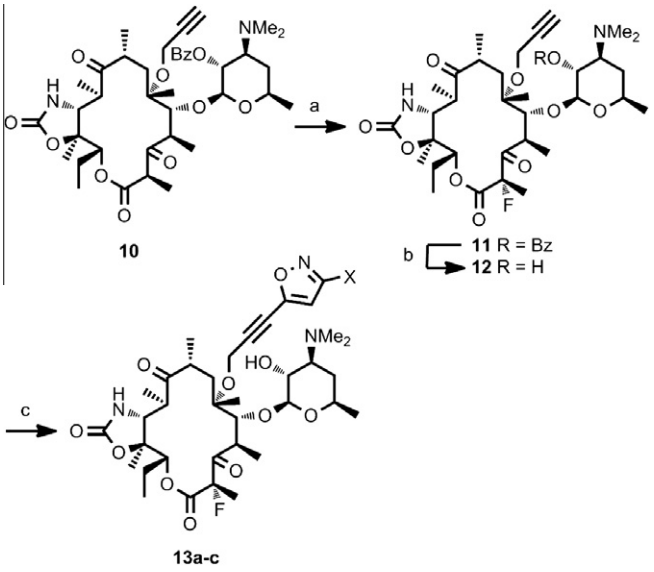
Modifications at the C-6 position, such as in cethromycin, are thought to be a promising approach for improving the antibacterial activity and pharmacokinetic profile. Most of these studies involving the modification of side chain linkage produced good activities against erythromycin-resistant *S. pneumoniae*.[18–22] These results encouraged us to develop novel 2-fluoro ketolides with in vitro and in vivo potency against resistant strains. As a result of our continuing medicinal chemistry efforts, we have identified a novel series of 2-fluoro ketolides in which the heteroaryl-isoxazolyl group is attached to the 6-*O*-propargyl side chain.

The synthesis of novel 6-*O*-(heteroaryl-isoxazolyl)propynyl 2-fluoro ketolides is shown in Schemes 1–3. Compound **6** was prepared from EM-A in 2 steps.[25] EM-A was treated with hydroxylamine to produce 9-oxime erythromycin. Subsequent transketalization with O-isopropyl cyclohexylketal led to cyclohexyl ketal **6**. Intermediate **10** was prepared from ketal **6** using a previously reported method.[26] Briefly, the selective protection of 2′,4″ hydroxyl groups was achieved using benzoyl anhydride (Bz_2O) in the presence of triethyl amine and 4-dimethylaminopyridine, and the subsequent selective 6-*O*-propargylation was achieved by treatment with propargyl bromide in the presence of potassium tert-butoxide as the base. Deoximation with sodium nitrate produced compound **8**. The formation of the carbamate functionality was performed using a three-step, one-pot sequence to yield compound **9**. The cladinose sugar of **9** was removed under acidic conditions, and the subsequent Corey-Kim oxidation of the 3-hydroxyl group produced the 3-ketolide derivative **10** (Scheme 1).

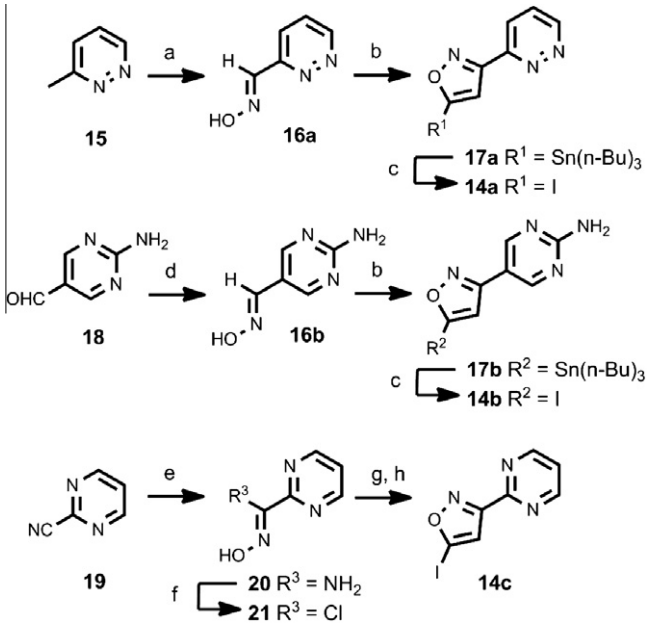
Compound **10** was treated with NaH followed by N-fluorobenzene sulfonamide, and the 2′-*O*-benzoyl group was then deprotected with methanol to yield intermediate **12**. Sonogashira coupling of **12** with iodide reagents **14a–c** was achieved using bis(triphenylphosphino)palladium dichloride in the solvent as triethylamine and acetonitrile to give 6-*O*-(heteroaryl-isoxazolyl) propynyl 2-fluoro ketolides **13a–c**(Scheme 2). The stereochemistry of the fluorine was determined from an X-ray crystal structure of **12** (Fig. 2).[27]



Scheme 1. Reagents and conditions: (a) Bz_2O, DMAP, Et_3N, AcOEt, 73%; (b) propargylbromide, *t*-BuOK, THF-DMSO, 61%; (c) $NaNO_2$, HCl, 60%; (d) DBU, CDI, NH_3, *t*-BuOK, THF, 63%; (e) 2 M HCl, EtOH; (f) dimethylsulfide, NCS, Et_3N, THF, 93% for two steps.



Scheme 2. Reagents and conditions: (a) $(PhSO_2)_2NF$, NaH, DMF; (b) MeOH, reflux, 54% for two steps; (c) iodide reagents (**14a–c**), $PdCl_2(PPh_3)_2$, Et_3N, CH_3CN, 60–83%.



Scheme 3. Reagents and conditions: (a) *t*-BuOK, *t*-$BuNO_2$, THF, 71%; (b) tributylethynylstannane, NCS, sat. $NaHCO_3$–AcOEt; (c) I_2, THF, 17–35%; (d) $HONH_2$–HCl, pyridine, EtOH; (e) $HONH_2$–HCl, NaOMe, MeOH, 78%; (f) $NaNO_2$, HCl, 68%; (g) tributylethynylstannane, Et_3N, THF; (h) I_2, THF, 62% for two steps.

The preparation of 3-heteroaryl-5-iodoisoxazole reagents **14a–c** is shown in Scheme 3. Generally, isoxazoles are constructed by the [2+3] cycloaddition of a nitrile oxide to an alkyne. In particular, 5-iodoisoxazoles are synthesized by the electrophilic halogenation of 5-tributylstannylisoxazole,[28–30] prepared by the 1,3-dipolar cycloaddition of tributylethynylstannane with nitrile oxides. We applied this methodology to the preparation of 3-heteroaromatic-substituted 5-iodoisoxazoles. Isoxazole **14a** and **14b** were prepared as follows. The oximation of 3-methyl pyridazine **15** with

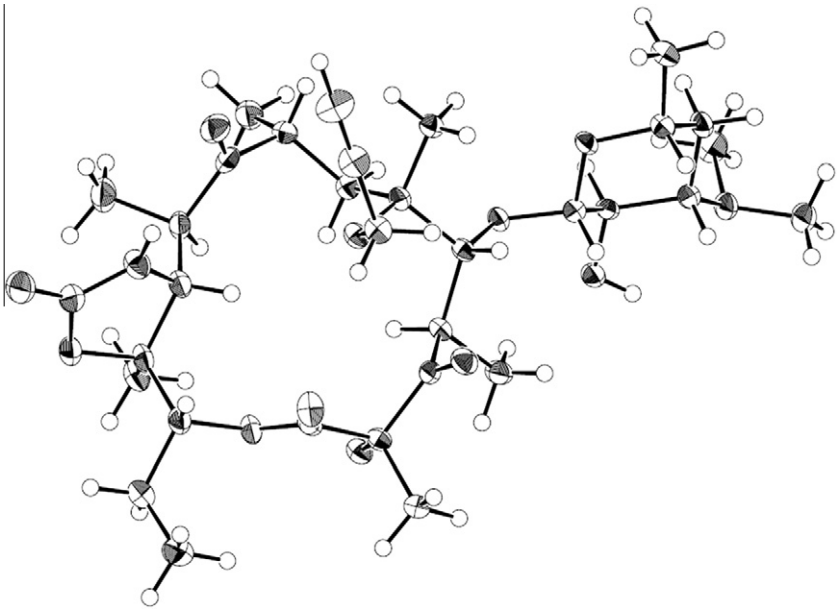


Figure 2. X-ray crystal structure of **12**.

tert-butylnitrate produced oxime **16a**. The oximation of aldehyde **18** produced oxime **16b**. The formation of isoxazole **17a/b** was smoothly advanced by the addition of NCS to the ethyl acetate solution of oxime **16a/b**, tributylethynylstannane, and aq. $NaHCO_3$ as a base. In this reaction, nitrile oxide was generated in situ from hydroximoyl chloride, formed by the chlorination of oxime **16a/b** with NCS, and the subsequent [2+3] cycloaddition of the nitrile oxide with tributylethynylstannane was performed in one-pot to yield isoxazole **17a/b**. The iodination of **17a/b** was smoothly reacted with iodine in THF to give iodoisoxazole **14a**/**b**.[31] **14c** was prepared as follows. Aminooxime **20** was prepared from cyanide **19**, and the subsequent chlorination of **20** with sodium nitrate in hydrogen chloride yielded hydroximoyl chloride **21**. The 1,3-dipolar cycloaddition of tributylethynylstannane with nitrile oxides generated from **21**, and the subsequent iodination yielded isoxazole **14c**.

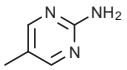
The in vitro antibacterial activity of 6-*O*-(heteroaryl-isoxazolyl)propynyl 2-fluoro ketolides **13a–c** was evaluated, as shown in Table 1.

The minimal inhibitory concentrations (MICs) were determined against selected respiratory pathogens including erythromycin-susceptible *S. pneumoniae* ATCC49619, erythromycin-resistant *S. pneumoniae* 210 with the *mef*(A) efflux pump gene coded, erythromycin-resistant 205 and 1104 with the *erm*(B) ribosomal methylase gene coded, erythromycin-resistant *S. pyogenes* with the *erm*(B) gene coded, and *Haemophilus influenzae* ATCC43095.[32]

All three ketolides **13a–c** were at least four-fold more active (lower MIC values) than CAM and AZM against erythromycin-susceptible *S. pneumoniae*. Furthermore, **13a–c** had excellent activities against *mef*(A) and *erm*(B) gene coded erythromycin-resistant *S. pneumoniae*. Especially, the MIC values of **13a–c** against *erm*(B) gene coded erythromycin-resistant *S. pneumoniae* were dramatically improved, compared with those of second-generation macrolides (CAM and AZM). The activities of **13a–c** against *S. pyogenes* were sufficient but were slightly weaker than the case in *S. pneumoniae*. Compounds **13a–c** were two-fold more active than CAM against *H. influenzae*, but four-fold less active than AZM.

The in vivo efficacy of 2-fluoro ketolides **13a–c** was evaluated in murine pulmonary infection models,[33,34] as shown in Figures 3 and 4. In an erythromycin-resistant *S. pneumoniae* infection model, **13a–c** decreased the lung bacterial count in a dose-dependent manner. In a *H. influenzae* infection model, **13a–c** decreased the lung bacterial count in a dose-dependent manner. Compound **13a** had the most potent effect at a dose of 50 mg/kg. Although, **13a** was four-fold less active in vitro than AZM, its in vivo efficacy was comparable to that of AZM.

Table 1
In vitro antibacterial activities of 6-*O*-(heteroaryl-isoxazolyl)propynyl 2-fluoro ketolides

Compound	X	MIC (μg/mL)				
		S. pneumoniae			*S. pyogenes*	*H. influenzae*
		ATCC49619 Ery-S	210 *mef* (A)	205 *erm* (B)	1104 *erm* (B)	ATCC43095 Amp-S
CAM (**2**)		0.03	2	>128	>128	4
AZM (**3**)		0.06	2	>128	>128	0.5
13a		0.008	0.03	0.008	1	2
13b		0.008	0.03	0.008	0.06	2
13c		0.008	0.03	0.008	2	2

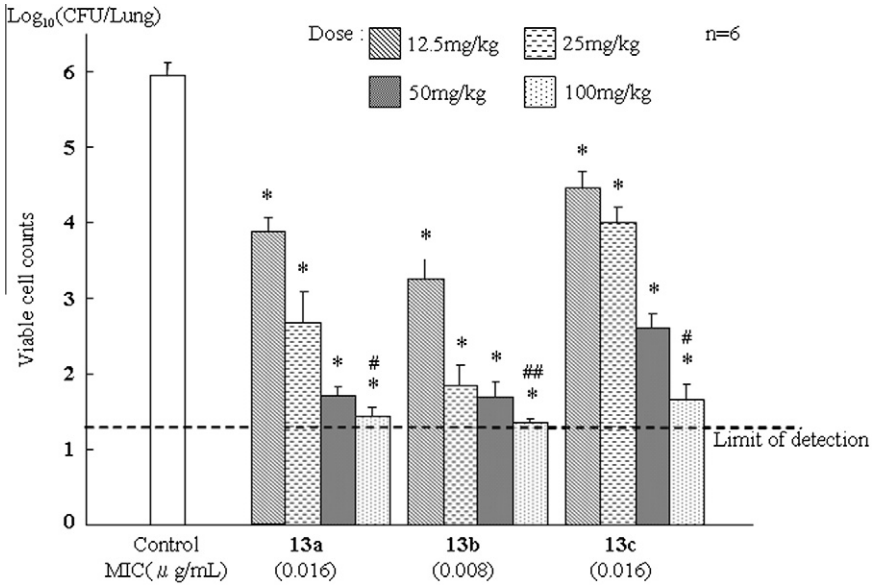


Figure 3. In vivo efficacy of **13a–c** against murine pulmonary infection caused by *S. pneumoniae* 1101 (*erm*(B) gene coded) in mice. Values are mean ± S.E. Statistical analysis was performed using Steel's test. $^{*}p$ <0.05 versus control.

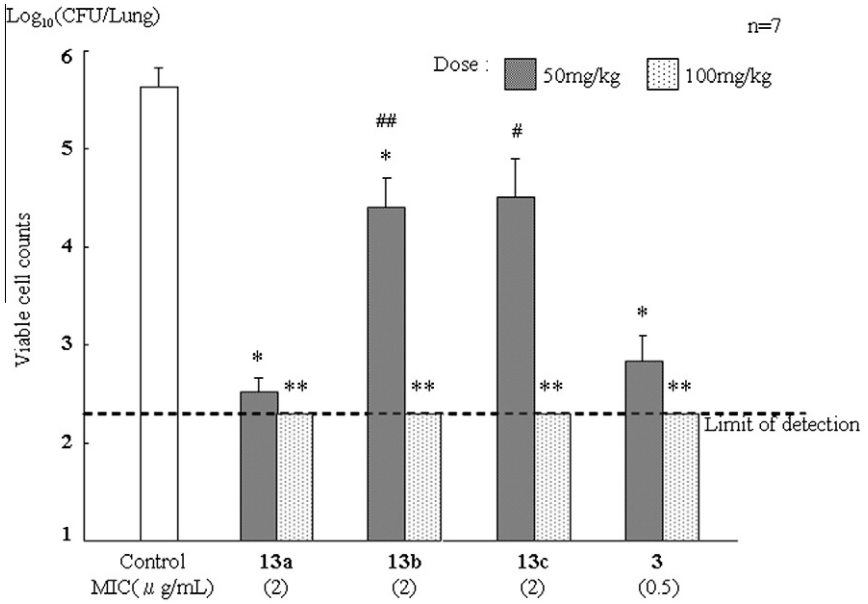


Figure 4. In vivo efficacy of **13a–c** against murine pulmonary infection caused by *H. influenzae* ATCC43095 in mice. Values are mean ± S.E. Statistical analysis was performed using Steel's test. $^{*}p$ <0.05, $^{**}p$ <0.01 versus control, $^{\#}p$ <0.05, $^{\#\#}p$ <0.01 versus **13a** (comparison at 50 mg/kg).

In conclusion, a novel 6-*O*-(heteroaryl-isoxazolyl)propynyl 2-fluoro ketolides has been synthesized. These 2-fluoro ketolides showed very promising in vitro antibacterial activity against key respiratory pathogens including erythromycin-susceptible/resistant *S. pneumoniae*, erythromycin-resistant *S. pyogenes* and *H. influenzae*. These ketolides exhibited good in vivo efficacy against *erm*-containing *S. pnuemoniae*. Especially, piridazinyl derivative **13a** showed most potent efficacy comparable to AZM. Further exploration of these heteroaryl-isoxazolyl ketolide will be reported in the future.

Acknowledgments

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bmcl.2012.06.092.

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32. The minimal inhibitory concentration (MIC) was determined using the broth microdilution method according to the Clinical and Laboratory Standards Institute's guidelines for *S. pnuemoniae*, *S. pyogenes* and *H. influenzae*.
33. Five-week-old male CBA/JN mice were intranasally inoculated with *S. pneumoniae* 1101 (*erm*(B) gene coded, Challenge dose: 5.5×10^4 CFU/mouse). Oral administration was commenced on the day after inoculation and was continued for 2 days, with the drugs being administered once a day. The bacterial count in the lung was examined on the day after the last dosing.
34. Four-week-old male ICR mice were intratracheally inoculated with *H. influenzae* ATCC43095 (Challenge dose: 5.2×10^6 CFU/mouse). Oral administration was commenced on the day after inoculation and was continued for 2 days, with the drugs being administered once a day. The bacterial count in the lung was examined on the day after the last dosing.