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Synthesis and antibacterial activity of 6-*O*-arylpropargyl-9-oxime-11,12-carbamate ketolides

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Abstract—A series of novel 6-*O*-arylpropargyl-9-oxime-ketolides was synthesized and evaluated against various pathogens. These new compounds show promising in vitro antibacterial potency and in vivo efficacy against macrolide resistant strains.

Macrolide antibiotics have been used safely and effectively against respiratory infections since the 1950's.[1] However, the use of erythromycin A is limited due to its poor acid stability and consequent bioavailability and untoward GI side effects.[2] This problem has been addressed by erythromycin derivatives such as clarithromycin in which the 6-OH is methylated and roxithromycin in which the 9-keto group is converted into a more acid stable oxime. These drugs display much better tolerability.[3] However, resistance to known antibiotics is emerging as a major threat to public health.[4] Therefore, it is important to discover new antibiotics that are effective against existing resistant pathogens. Resistance against erythromycin in Gram-positive bacteria occurs via two different mechanisms: efflux by macrolide pumps (*mef*) and ribosome methylation by *erm* methyltransferases. These two mechanisms of resistance have been partially overcome by the discovery of semi-synthetic derivatives of erythromycin such as telithromycin and ABT-773 in which an aryl group is tethered to a ketolide.[5] The aryl group has been appended off of the 11,12-carbamate as in telithromycin or tethered to the C-6 position in ABT-773. The aryl group appears to be crucial for activity against macrolide-lincosamide-streptogramin B (MLS_B) resistant strains (Fig. 1).

We conceived that more potent compounds with improved resistance and pharmacological profiles could be derived by converting the 9-ketone to an oxime and concomitantly varying the 6-*O*-aryl group of the ketolide. This notion was supported by recent publications detailing that certain 9-oxime derivatives of macrolides/ketolides exhibited improved antibacterial activity against resistant streptococci,[6a] staphylococci,[6b] and macrobacteria.[6c] The 2-F ketolides have also been shown to be effective against macrolide resistant strains of bacteria with improved pharmacological profiles.[7] Therefore, a series of 2-F derivatives of our 9-oxime ketolide was also prepared and evaluated. A comparison of the 2-F and the natural 2-H analogues is also presented.

The synthesis of the 9-oxime ketolide started with the 3-hydroxy erythromycin analogue **1**[8] as shown in Scheme 1. The oxime **2** was formed by heating **1** in a sealed tube for 10 days using 2 equiv of boron trifluoride etherate and 15 equiv of methoxylamine hydrochloride.[9] The oxime **2** could also be obtained starting with the 3-cladinose derivative of **1** and using 0.2 equiv of camphorsulfonic acid, which concomitantly formed the 9-oxime and deprotected the 3-cladinose in 73% yield. Triethylamine was also tried as a base but gave partial deprotection of the cladinose as well as the 3′-benzoyl thereby resulting in a mixture of products. When the oxime formation was performed on the 3-keto derivative, bis oxime products were obtained due to a lack of selectivity for the 9-ketone over the 3-ketone on the macrolide. The *E* oxime was obtained exclusively as shown by ^{1}H NMR. The oxime **2** was further elaborated by Corey–Kim oxidation to give ketolide **3** and subsequent 3′-benzyl deprotection to give 2′-alcohol **4**.

The 6-*O*-propargyl group was then derivitized with various aryl bromides using a Sonogashira coupling to give

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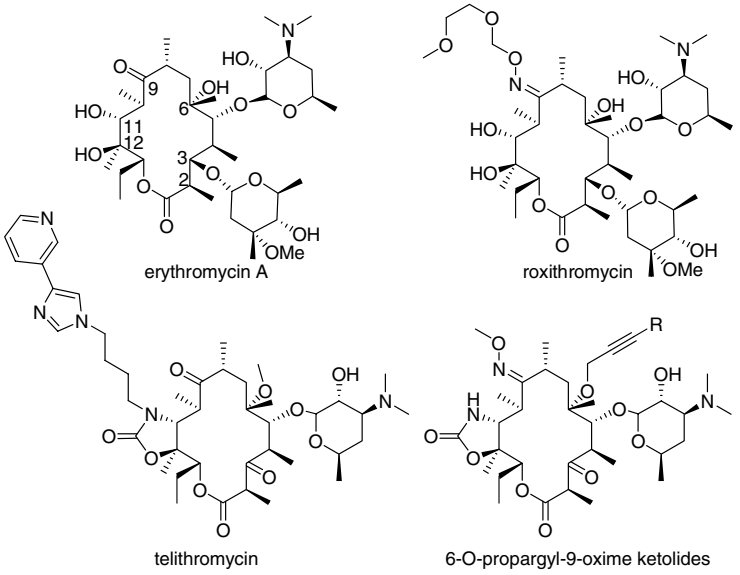
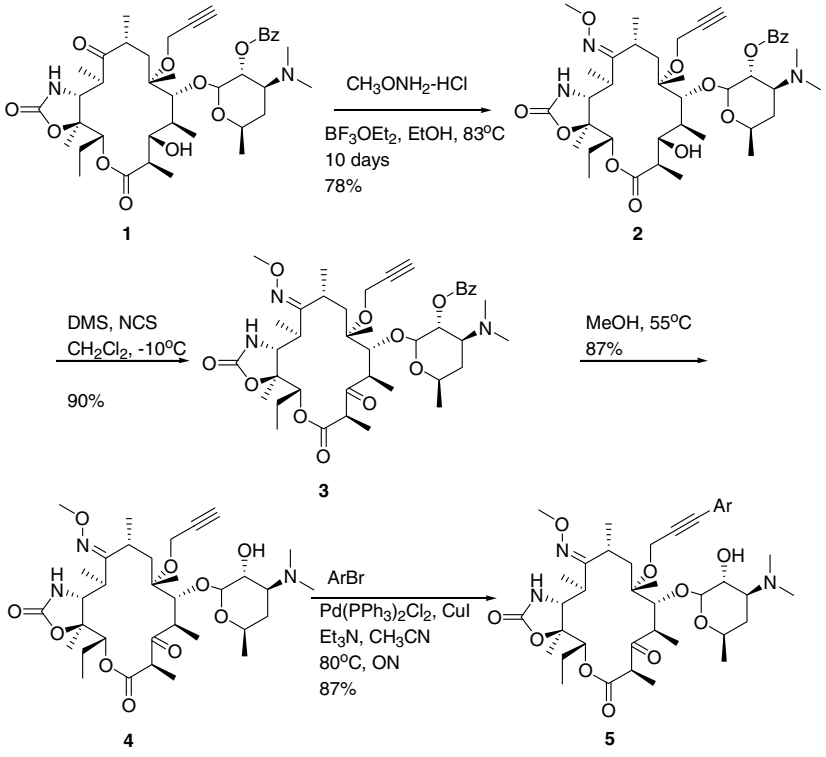


Figure 1. Structures of macrolide and ketolide antibacterials.



Scheme 1. Synthesis of 9-oxime ketolides.

ketolides **5a**–**e** in excellent yields. The aryl groups used for the 6-*O*-propargyl substitutions are shown in Chart 1.

The 2-F series were synthesized as shown in Scheme 2. Starting with the oxime ketolide **3**, the 2-fluorine was introduced using NaH followed by *N*-fluorobenzenesulfonimide to give ketolide **7**. The 6-*O*-propargyl group was arylated as before using a Sonogashira coupling to give derivatives **8b** and **8c**. The ability to perform the Sonogashira coupling as the last step on the free 2′-alcohol allowed a more convergent synthesis when compared to saving the 3′-benzyl deprotection for the end of the synthesis. All new compounds were purified by flash chromatography and were characterized by ^{1}H NMR, ^{13}C NMR, and MS.

The antibacterial activity of each analogue against a panel of representative pathogens selected from the Abbott clinical culture collection is shown in Table 1 and compared to erythromycin A and telithromycin.

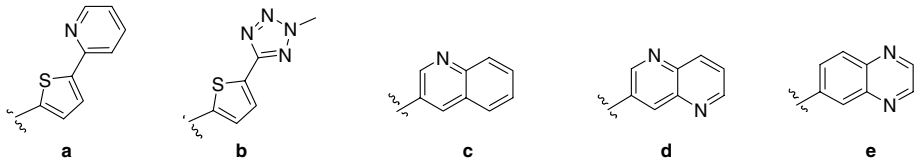
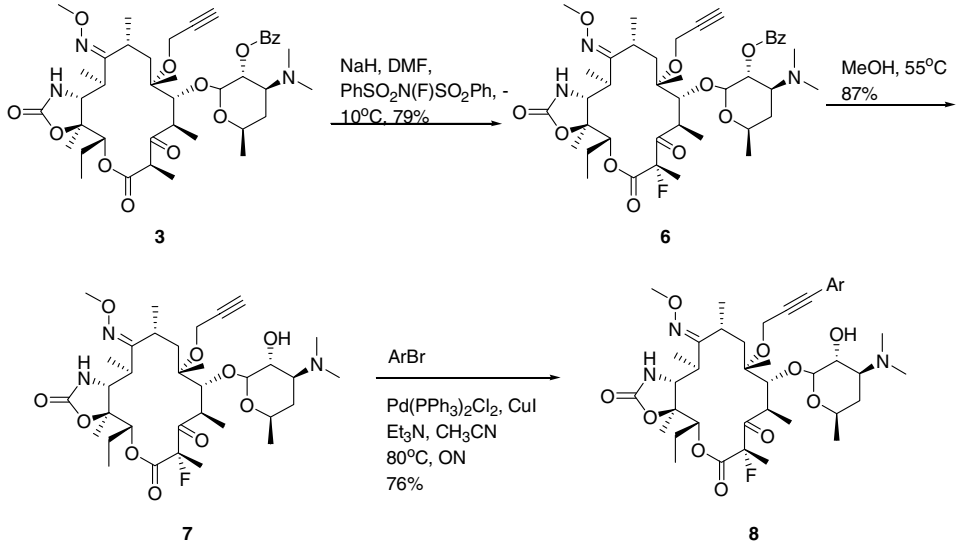


Chart 1. Structures of aryl (Ar) groups.



Scheme 2. Synthesis of 2-fluoro-9-oxime ketolides.

Table 1. In vitro antibacterial activity of 6-*O*-arylpropargyl-9-oxime ketolides against selected bacteria

Organism		MIC (μg/mL)								
		5a	**5b**	**5c**	**5d**	**5e**	**8b**	**8c**	Ery[a]	Teli[b]
S. aureus NCTC 10649M	Ery-S	0.25	0.25	1	1	0.25	1	0.5	0.5	0.1
S. aureus A5177	*erm*A-i	0.008	0.008	0.25	0.008	0.008	0.25	0.25	>128	0.1
S. aureus 1775	*erm*A-c	64	128	128	>128	>128	64	64	>128	>100
S. pyogenes EES61	Ery-S	0.008	0.008	0.008	0.008	0.008	0.008	0.008	0.015	0.004
S. pyogenes 930	*erm*B	16	32	1	0.5	2	8	0.5	>128	8
S. pyogenes PIU 2548	*mef*A	0.03	0.125	0.125	0.06	0.125	0.5	0.25	8	2
S. pneumoniae ATCC 6303	Ery-S	0.008	0.008	0.008	0.008	0.008	0.008	0.008	0.015	0.004
S. pneumoniae 5979	*erm*B	0.008	0.5	0.25	1	4	0.25	0.125	>128	8
S. pneumoniae 5649	*mef*E	0.008	0.06	0.125	0.03	0.06	0.125	0.125	4	0.5
H. flu GYR1435	Amp-R	4	4	4	2	4	8	4	4	2

[a] Ery = erythromycin A.
[b] Teli = telithromycin.

The in vitro antibacterial activities are reported as minimum inhibitory concentrations (MICs) determined using the broth microdilution method as recommended by the National Committee for Clinical Laboratory Standards.[10] Various strains having defined mechanisms of macrolide resistance were included to evaluate each analogue's potential to overcome resistance. The analogues were found to have excellent potency against erythromycin-susceptible strains (Ery-S) *Staphylococcus aureus* NCTC 10649M, *Streptococcus pyogenes* EES61, and *Streptococcus pneumoniae* ATCC 6303. These new analogues also show promising activity against pathogens with either the *erm* or *mef* mechanisms of macrolide resistance when compared to erythromycin A and telithromycin: *S. aureus* A5177 is an inducibly MLS_B resistant strain bearing an *erm*A gene; *S. pyogenes* 930 and *S. pneumoniae* 5979 are MLS_B resistant strains bearing *erm*B genes; *S. pyogenes* PIU 2548 and *S. pneumoniae* 5649 are efflux-resistant strains bearing *mef*A and *mef*E genes, respectively. The new compounds were inactive against *S. aureus* 1775, a methicillin-resistant (MRSA) strain bearing a constitutive *erm*A gene, as is common with ketolides. The analogues show activity comparable to erythromycin against *Haemophilus influenzae* GYR 1435, a fully antibiotic susceptible strain. The 2-F analogues **8b** and **8c** show no improvement in antibacterial activity when compared to the natural 2-H compounds with the exception of the

*erm*B strain *S. pyogenes* 930 for which the MIC of **8b** is 8 μg/mL compared to 32 μg/mL for **5b**. The SAR indicates that compound **5a** exhibits the best overall MIC's against the resistant pathogens.

Compound **5b** was evaluated for in vivo activity against *S. pneumoniae* ATCC 6303 in the rat lung infection model.[11] The doses required to reduce the number of viable bacteria in lungs of treated animals by 3 log 10 in comparison to the number of viable bacteria in untreated control animals was 1.8 mg/kg for **5b** and 4.0 mg/kg for telithromycin. Compound **5b** was also assessed for its potential to cause prolongation of the QT interval, a critical safety issue for new drugs, by measuring the prolongation of the action potential duration in canine Purkinje fibers.[12] At 25 mcg/mL, compound **5b** and telithromycin caused prolongations of the action potential duration of 26.4% and 25.6%, respectively.

In conclusion, we have synthesized a new series of ketolide antibiotics that display excellent in vitro and in vivo efficacy against macrolide resistant pathogens. The 6-*O*-arylpropargyl-9-oxime ketolides have excellent in vitro antibacterial potency against *mef* and *erm* resistant strains. The in vivo efficacy against *S. pneumoniae* shows a slight improvement over telithromycin. In addition to the methoxy oximes, other extended oximes will be reported on in due course. This new series of ketolide antibiotics could be used to effectively combat the growing problem of antibiotic resistance.

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9. Oxime formation: macrolide 1 (1.00 g, 1.35 mmol), methoxylamine hydrochloride (1.69 g, 21.2 mmol), and borontrifluoride etherate (0.34 mL, 2.5 mmol) were combined in 10 mL of ethanol and warmed to 99 °C in a sealed tube for 14 days. The reaction mixture was cooled to rt, and diluted with isopropyl acetate (100 mL), quenched with 5% Na_2CO_3 (25 mL), and diluted with water (100 mL). The organics were removed and the aqueous phase extracted with isopropyl acetate. The combined organics were washed with water, brine, and dried (Na_2SO_4). The solvent was removed in vacuo and the crude material purified by silica gel chromatography using 25% acetone/hexane to give 809 mg (78%) of **2** as a white foam: ^{13}C ($CDCl_3$) δ 174.5, 167.6, 165.3, 158.8, 132.6, 130.6, 129.7, 128.2, 99.6, 84.0, 80.5, 80.4, 79.9, 77.5, 75.5, 73.5, 72.1, 69.0, 63.3, 61.4, 59.9, 51.1, 43.9, 40.8, 36.8, 36.0, 32.5, 31.9, 25.7, 22.4, 21.1, 19.3, 18.8, 16.6, 15.2, 13.2, 10.3, 7.6; MS m/e 772 $(M+H)^+$.
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11. Groups of five Sprague–Dawley rats (200–230 g) were intratracheally inoculated with 3.8 log cfu of *S. pneumoniae*, strains 6303 suspended in 5% hog gastric mucin. Test ketolide compounds were formulated in 2% ethanol, 1 mol equiv HCl, and balance D5W. Oral therapy for infected rats was q.d., 1–3 days, starting 18 h post infection of *S. pneumoniae*. Lungs were removed on day 4. To assess organ burden, lungs were aseptically minced with a Tekmar tissue grinder. The homogenates were serially diluted 100- and 10,000-fold with plating on CNA agar plates. Log cfu per lung was determined from colony counts. Fifty percent effective dosage (ED_{50}) to yield a 2 or 3 log reduction in bacteria count compared to vehicle-treated infected controls was calculated from the group means using linear regression. Percent responder was calculated from animals showing a 3 log reduction in organisms compared to the control mean.
12. Canine cardiac Purkinje fibers were excised, placed in a warmed chamber and superfused (8–10 mL/min) with a Tyrode's solution containing (in mM): NaCl, 131; $NaHCO_3$, 18; NaH_2PO_4, 1.8; $MgCl_2$, 0.5; dextrose, 5.5; KCl, 4; $CaCl_2$, 2 (aerated with 95% O_2/5% CO_2 [pH = 7.2 at rt]). Fibers were stimulated using platinum

electrodes located in the chamber floor and impaled with 3 M KCl-filled microelectrodes, recorded digitally and analyzed using pClamp software. Studies were initiated after a minimum 30-min equilibration period with stimulation. Fibers were sequentially exposed to three ascending drug concentrations. The protocol consisted of pacing in a control (drug-free) solution for 20–25 min at a basic cycle length (BCL) of 2 s, and then consecutively paced at 800 ms BCL (75 bpm) during the transition to a 400 ms BCL for 2–3 min at each stimulation rate. Action potentials recorded at the end of each 25-min equilibration period were used to define drug effects and generate cumulative concentration–response curves.