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Letters

Synthesis and Antibacterial Activity of Acylides (3-*O*-Acyl-erythromycin Derivatives): A Novel Class of Macrolide Antibiotics

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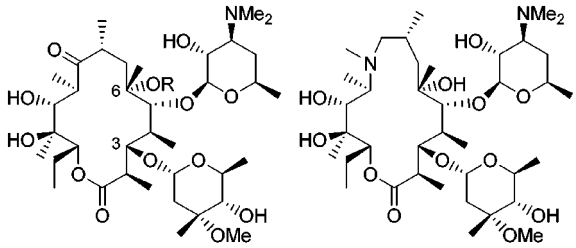
Abstract: Introduction of an acyl group to the 3-*O*-position of erythromycin A derivatives instead of L-cladinose led to a novel class of macrolide antibiotics that we named "acylides". The 3-*O*-nitrophenylacetyl derivative TEA0777 showed significantly potent activity against not only erythromycin-susceptible Gram-positive pathogens but also inducibly macrolides-lincosamides-streptogramin B (MLS_B)-resistant *Staphylococcus aureus* and efflux-resistant *Streptococcus pneumoniae*. These results indicated that acylides have potential as next-generation macrolide antibiotics.

Introduction. Macrolide antibiotics such as erythromycin A have been clinically used for more than 45 years and are considered preferable for the treatment of upper and lower respiratory tract infections. However, the first-generation macrolide erythromycin A easily loses its antibacterial activity under acidic conditions by degradation, and the degraded products are known to be responsible for undesirable gastrointestinal side effects.[1] Numerous chemical modifications of erythromycin A, to overcome this acid-instability problem, have been investigated by many groups. As a result, several second-generation macrolide antibiotics have been launched for clinical use.



Clarithromycin[2] (CAM, 6-*O*-methylerythromycin A) and azithromycin[3] (15-membered aza-macrolide) are widely prescribed due to their efficacy and safety, but this has led to rapid increases in the rates of resistance in bacteria isolated clinically.[4] Therefore, we have sought to identify a next-generation macrolide that exhibits greater efficacy and safety, has a broader spectrum of activities, and is particularly effective against resistant pathogens.

In our search for next-generation macrolide antibiotics, we initially focused on the L-cladinosyl moiety at the 3-*O*-position of the macrolactone skeleton as a target: modification of this moiety has not yet been systemically investigated since it has been considered to be essential for activity.[5] By substituting L-cladinose with various functional groups, we have obtained some new classes of macrolide antibiotics, such as 3-oxo derivatives,[6] so-called "ketolides", and 3-alkoxy,[7] 3-carbamoyloxy,[8] 3-alkoxycarbonyloxy,[9a] and acyloxy[9] derivatives. In this paper, we describe the synthesis and biological properties of 3-*O*-acyl-erythromycin A derivatives, which we named "acylides", as a novel class of macrolide antibiotics which show good antibacterial activities against Gram-positive pathogens including a macrolides-lincosamides-streptogramin B (MLS_B)-resistant strain and an efflux-resistant strain.

Chemistry. 3-*O*-Substituted-6-*O*-methylerythromycin A derivatives were synthesized as follows. Treat-

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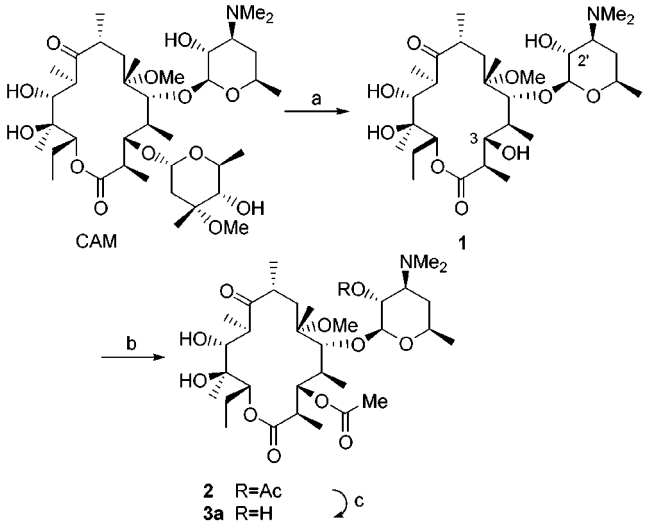
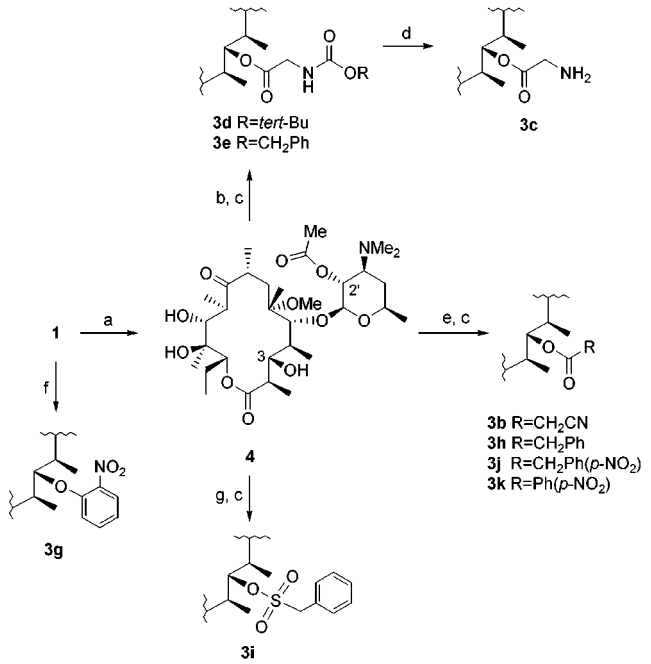
Scheme 1[a]



[a] (a) 2 M HCl, rt, 53%; (b) AcCl, DMAP, pyridine, THF, rt, 68%; (c) MeOH, rt, 98%.

Scheme 2[a]



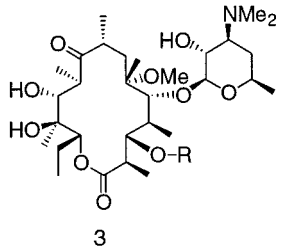
[a] (a) Ac_2O, Me_2CO, rt, 96%; (b) Boc-glycine or Cbz-glycine, EDC−HCl, DMAP, CH_2Cl_2, rt, 96−97%; (c) MeOH; (d) HCO_2NH_4, 10% Pd−C, MeOH, 50%; (e) RCO_2H, EDC−HCl, DMAP, CH_2Cl_2, rt, 80−98% or $PhCH_2COCl$, DMAP, pyridine, rt, 49%; (f) 1-fluoro-2-nitrobenzene, NaH, THF, rt, 39%; (g) $PhCH_2SO_2Cl$, pyridine, CH_2Cl_2, rt, 87%.

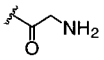
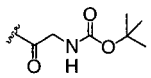
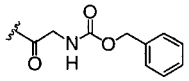
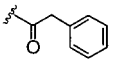
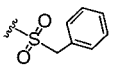
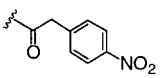
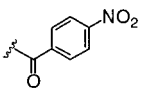
ment of clarithromycin with 2 M aqueous hydrochloric acid at room temperature afforded selective cleavage of the 3-*O*-sugar moiety to give 3-OH derivative **1** (Scheme 1).

Treatment of the alcohol **1** with excess acetyl chloride in the presence of 4-(dimethylamino)pyridine (DMAP) in tetrahydrofuran (THF)/pyridine gave 2′,3-diacetate **2**. 3-Acetate **3a** was easily obtained by selective 2′-*O*-deacetylation of **2** in methanol (MeOH) at room temperature. The 2′-acetate **4**[10] was used as a common intermediate to introduce various functional groups at the 3-*O*-position (Scheme 2). Treatment of the alcohol **1** with acetic anhydride in acetone at room temperature selectively gave 2′-acetate **4** as a sole product.

3-*O*-Acyl derivatives **3b**,**d**,**e**,**h**,**j**,**k** were prepared in good yields (65−97%) by acylation of **4** with the corresponding carboxylic acids and 1-[3-(dimethylamino)-propyl]-3-ethylcarbodiimide hydrochloride (EDC−HCl) in the presence of DMAP in dichloromethane (CH_2Cl_2), followed by selective removal of the 2′-*O*-acetyl group

Table 1. In Vitro Evaluation of 3-*O*-Substituted-6-*O*-methyl-erythromycin A Derivatives **3**



Entry	R	MIC (μg/mL) *S. aureus* 209P-JC[a]	*S. aureus* B1[b]	*S. aureus* SR138[c]
CAM	L-cladinose	0.10	>100	>100
1	H	>100	>100	>100
3a		50	>100	>100
3b		25	>100	>100
3c		12.5	>100	>100
3d		12.5	>100	>100
3e		3.13	>100	>100
3f		12.5	>100	>100
3g		3.13	>100	>100
3h		0.78	>100	>100
3i		100	>100	>100
3j (TEA0777)		0.20	0.39	>100
3k		50	>100	>100

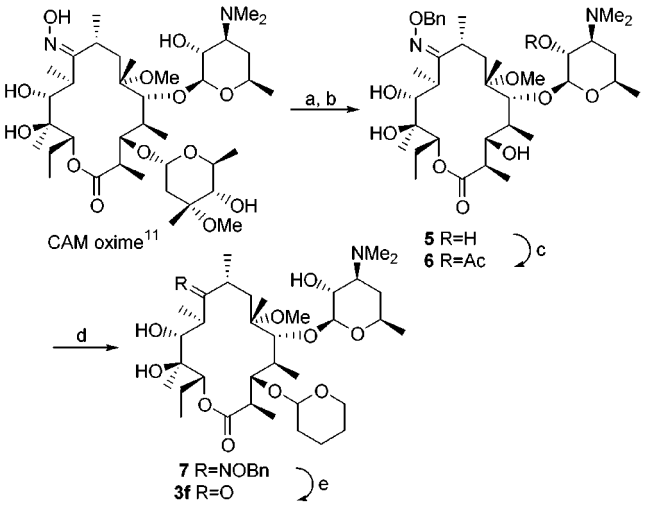
[a] *S. aureus* 209P-JC: erythromycin-susceptible strain. [b] *S. aureus* B1: inducibly MLS_B-resistant strain. [c] *S. aureus* SR138: constitutively MLS_B-resistant strain encoded by an *ermA* gene.

Table 2. Antibacterial Effects of TEA0777 against Representative Pathogens

	MIC (μg/mL)					
	S. pneumoniae			*E. faecalis*	*E. faecium*	*H. influenzae*
compd	IID553[a]	210[b]	221[c]	ATCC29212	ATCC19434	ATCC43095
TEA0777	0.20	0.20	>100	0.20	0.05	25
erythromycin A	0.10	1.56	>100	1.56	3.13	6.25
clarithromycin	0.05	1.56	>100	1.56	1.56	6.25

[a] *S. pneumoniae* IID553: erythromycin-susceptible strain. [b] *S. pneumoniae* 210: efflux-resistant strain. [c] *S. pneumoniae* 221: MLS_B-resistant strain encoded by an *erm B* gene.

Scheme 3[a]



[a] (a) 2 M HCl, rt, 45%; (b) BnCl, *n*-Bu_4NI, KOH, THF, 62%; (c) Ac_2O, Me_2CO, rt, (d) (i) 3,4-dihydro-2*H*-pyran, *p*-TsOH-H_2O, MS-4A, CH_2Cl_2, (ii) MeOH, 56% in three steps; (e) (i) 10% Pd−C, HCO_2H, HCO_2NH_4, MeOH, 50 °C, (ii) $NaHSO_3$, HCO_2H, EtOH, H_2O, reflux, 51% in two steps.

by heating with methanol for 2 h. Acylation of **4** with the corresponding acyl chlorides or mixed acid anhydrides led to reduced yields (36−74%). Glycinate **3c** was prepared by deprotection of the benzyloxycarbonyl group of **3e** by catalytic transfer hydrogenation (CTH). Sulfonate **3i** was obtained in 40% yield by treatment of the alcohol **4** with benzylsulfonyl chloride in the presence of pyridine in CH_2Cl_2 followed by methanolysis.

o-Nitrophenyl ether **3g** was straightforwardly synthesized by the 3-*O*-substitution reaction of the alcohol **1** using 2-fluoronitrobenzene and sodium hydride in THF without protection of the 2′-hydroxy group (39% yield). Tetrahydropyranyl (THP) etherification of the alcohol **4** was achieved by protection of 9-keto with a benzyloxime group (Scheme 3), since treatment of **4** under acidic conditions caused degradation to form the 9,12-ketal compound. 3-*O*-Acetalization of **6** with 3,4-dihydro-2*H*-pyran in the presence of *p*-toluenesulfonic acid and 4 Å molecular sieves in CH_2Cl_2 afforded the desired 3-*O*-THP. Deprotection of the benzyl group of the 3-*O*-THP ether with CTH followed by conversion of the oxime to a ketone at the 9-position using sodium hydrogen sulfite and formic acid in aqueous ethanol[12] gave the desired ether **3f**.

Results and Discussion. The 3-*O*-substituted-6-*O*-methylerythromycin A derivatives and clarithromycin as a reference were tested for in vitro antibacterial activity against three strains of *Staphylococcus aureus*. The activities are reported in Table 1 as minimum inhibitory concentrations (MICs) determined according to the Japan Society of Chemotherapy.[13] *S. aureus* 209P-JC is an erythromycin-susceptible strain, *S. aureus* B1 is an inducibly MLS_B-resistant strain, and *S. aureus* SR138 is a constitutively MLS_B-resistant strain that is also encoded by an *ermA* gene.

Removal of the L-cladinosyl moiety of clarithromycin resulted in a complete loss of antibacterial activity as defined (MICs > 100 μg/mL). Simple introduction of an acetyl group at the 3-*O*-position did not completely restore the antibacterial activity, although it did seem to be slightly effective against the erythromycin-susceptible strain.

Accordingly, cyanoacetate **3b** and aminoacetates **3c**−**e** were synthesized to investigate the structure−activity relationship at the acetyl position. Both the electron-withdrawing group in **3b** and the electron-releasing group in **3c** appeared to be effective at increasing the activity against the erythromycin-susceptible strain. Carbamates **3d**,**e** showed greater activities than the parent acetate **3a**; benzyloxycarbamate **3e** was 16-fold more potent than **3a**. In contrast to the weak activity of THP ether **3f**, *o*-nitrophenyl ether **3g** showed potent antibacterial activity. The results described above suggested that a phenyl group may increase the activity against the erythromycin-susceptible strain. Therefore, we tried to introduce a phenyl group at the 3-*O*-acetyl group. Phenylacetate **3h** showed considerably potent antibacterial activity against the erythromycin-susceptible strain, as anticipated (MIC 0.78 μg/mL). In marked contrast, the corresponding sulfonate **3i** did not show any antibacterial activity. Therefore, a carbonyl function in the 3-*O*-linkage appeared to have a profound effect on this activity.

After further investigation, we identified 3-*O*-(4-nitrophenyl)acetyl-5-*O*-desosaminyl-6-*O*-methylerythronolide **3j** (TEA0777), which showed 250-fold greater activity against the erythromycin-susceptible strain than that of the parent acetate **3a**. In addition, this acylide showed significantly potent activity against the inducibly MLS_B-resistant strain (0.39 μg/mL). Conversion of the phenylacetyl group (**3j**) to a corresponding benzoyl group (**3k**) resulted in a drastic decrease in antibacterial activity. The poor activity is consistent with the results with 3-*O*-benzoyl-erythromycin A oxime derivatives reported by LeMahieu.[5] The phenylacetyl group was a promising mimic for L-cladinose at the 3-*O*-position.

The antibacterial activities of TEA0777 against representative pathogens are summarized in Table 2. This acylide demonstrated potent activity against the erythromycin-susceptible strain of *Streptococcus pneumoniae*, like other macrolides. Furthermore, it was also highly effective against *Enterococcus* strains and the efflux-resistant strain of *S. pneumoniae*.

Table 3. In Vivo Efficacy of Acylide TEA0777 in Mouse Protection Tests (ED_{50}, mg/kg)

compd	*S. aureus* Smith[a]	
	MIC (μg/mL)	ED_{50} (95% CL[b])
TEA0777	0.39	15.4 (11.2−21.1)
erythromycin A	0.20	56.6 (40.4−79.2)
clarithromycin	0.20	7.6 (4.9−12.0)

[a] *S. aureus* Smith: erythromycin-susceptible strain. [b] CL: confidence limits.

In Vivo Evaluation. The in vivo efficacies of acylide TEA0777, erythromycin A, and clarithromycin were assessed by mouse protection tests, using the erythromycin-susceptible strain of *S. aureus* Smith. The mice were inoculated with 5.80×10^7 CFU/mouse intraperitoneally, and the macrolides were then administered orally 1 h after inoculation. The efficacy of each macrolide was reported as the effective drug dosage (ED_{50}) which gave a survival rate of 50% following lethal infection over the duration of the trial (Table 3).

Acylide TEA0777 was significantly more active than erythromycin A and comparable to clarithromycin.

Conclusion. In summary, a series of acylides (3-*O*-acyl-erythromycin A derivatives) were synthesized and evaluated as a novel class of macrolide antibiotics. By introducing a phenylacetyl group instead of L-cladinose at the 3-*O*-position, the abolished antibacterial activity could be restored. In particular, the 3-*O*-(4-nitrophenyl)-acetyl erythromycin A derivative TEA0777 exhibited significantly potent antibacterial activity against not only erythromycin-susceptible Gram-positive pathogens but also inducibly MLS_B-resistant *S. aureus* and efflux-resistant *S. pneumoniae*. It has been demonstrated that acylides are innovative semisynthetic macrolides that have potential as next-generation macrolide antibiotics.

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Supporting Information Available: Experimental procedures, and spectral and analytical data for all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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