Studies on Macrolide Antibiotics I. Synthesis and Antibacterial Activity of Erythromycin A 9-*O*-Substituted Oxime Ether Derivatives against *Mycobacterium avium* Complex

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A series of erythromycin A 9-*O*-substituted oxime ether derivatives have been synthesized and evaluated for antibacterial activity against *Mycobacterium avium* complex (MAC) and *Staphylococcus aureus.* These compounds possessed stronger *in vitro* activity against MAC including macrolide-resistant strains than clarithromycin (2), although *in vitro* antibacterial activities of these compounds were less than that of 2 against *Staphylococcus aureus.* Our studies found that several factors contribute to the antibacterial activity against MAC. The length and spatial orientation of the substituent at 9-position were found to significantly influenced the anti-MAC activity, especially against macrolide-resistant strains. Of all the compounds prepared, erythromycin A 9-[*O*-(4-phenylbutyl)oxime] (12q) and erythromycin A 9-[*O*-(3-phenoxypropyl)oxime] (12t) possessed 16 times stronger antibacterial activity than 2 against clarithromycin-resistant strains. Surprisingly, the minimum inhibitory concentrations (MICs) of 12q and 12t against the resistant strains were almost same as those against the susceptible strains. These results suggest that the erythromycin A 9-*O*-substituted oxime ether derivatives would be promising macrolide antibiotics.

Key words macrolide; antibiotic; antibacterial activity; *Mycobacterium avium* complex; erythromycin A 9-*O*-substituted oxime ether

Since erythromycin A (**1**)[1] was discovered in 1952, much effort in drug discovery and in searching for a bioactive natural compound has been directed to the development of these exquisite macrolide antibiotics bearing potent activity, broad spectrum, good absorption and lack of serious side effects. New macrolide antibiotics, such as clarithromycin (**2**),[2] roxithromycin (**3**),[3] azithromycin (**4**)[4] and rokitamycin (**5**),[5] were consequently developed in the 1980s. Furthermore, a new class of antibiotics called ketolides, such as telithromycin (**6**)[6] and ABT-773 (**7**),[7] was discovered in the 1990s. These new macrolide agents with improved pharmacokinetic profile and less gastrointestinal side effects, especially the ketolides exhibited excellent activity against not only common strains but also resistant strains induced by the previous macrolide antibiotics. In recent days, therapeutic use of these macrolide agents has expanded into the treatment of respiratory infections by *Mycobacterium avium* complex (MAC). The MAC infection has been a serious worldwide problem and its prevalence has been considered to be fatal for AIDS patients.[8] There have been, however, few effective agents against MAC infection except **2** or **4**.[9]

Although a number of the structure–activity relationships (SAR) of some macrolides against common strains have been found,[10] there have been no systematic studies of

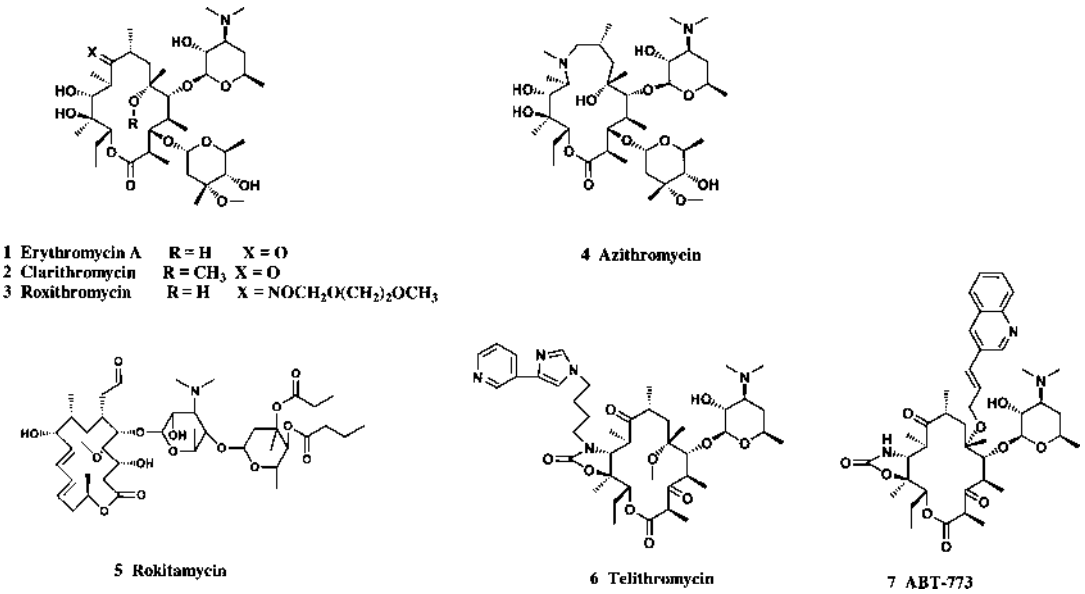

Chart 1

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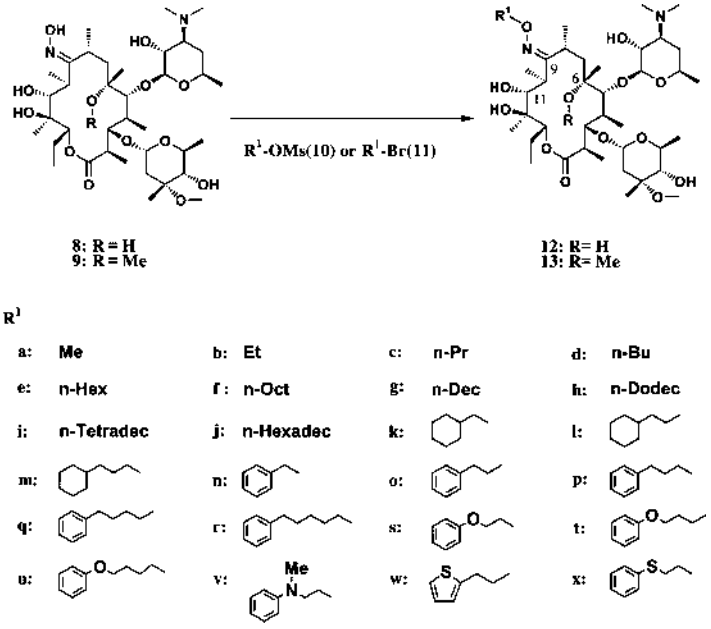


Chart 2

macrolides against MAC. To discover a novel excellent macrolide bearing good activity against macrolide-resistant MAC as well as -susceptible MAC, we started to synthesize various erythromycin A 9-*O*-substituted oxime ether derivatives and evaluate them for anti-MAC activity. In this paper, we describe our SAR studies on erythromycin A 9-*O*-substituted oxime ether derivatives and several our structural insights.

Chemistry The erythromycin A 9-*O*-substituted oxime ether derivatives (**12**, **13**) were basically prepared as shown in Chart 2, according to the literature.[11] In the presence of potassium hydroxide or sodium hydride and tetrabutylammonium iodide, the reaction of erythromycin A 9-oxime (**8**)[12] with various mesylates (**10**) or bromides (**11**) in tetrahydrofuran (THF) afforded the 9-*O*-substituted oxime ether derivatives (**12**). In a similar manner to the preparation of **12**, the 6-*O*-methylerythromycin A analogue (**13t**) was derived from the corresponding 9-oxime (**9**).[13] The physicochemical and spectral data of these compounds (**12d**—**x**, **13t**) are listed in Table 1.

Alteration of the Target Site Clinically isolated MAC (*Mycobacterium intracellulare* 20066, *Mycobacterium avium* 20039, *Mycobacterium avium* 20092, *Mycobacterium avium* 20096) was used in this study. It has been known that resistance to macrolides is caused by mutation at several sites in the peptidyl transferase loop of 23S rRNA from the clinical isolated MAC.[14] To clarify the target mutation of the strains used in this study, we analyzed the base sequence of each 23S rRNA according to the literature.[14] The analytical results are shown in Table 2. The base sequences of *Mycobacterium intracellulare* 20066 and *Mycobacterium avium* 20039 which were considered susceptible to **2** were identified as those of macrolide-susceptible strains reported by Böttger and his co-workers[14a] and Nash and Inderlied[14b] On the other hand, the sequences of *Mycobacterium avium* 20092 and *Mycobacterium avium* 20096 which were resistant to **2** were altered by A→G and A→C transitional mutations at the 2274-position in 23S rRNA, respectively. These alterations were identical to those of macrolide-resistant strains reported by Nash and Inderlied[14b] The same mutation was also reported in the macrolide-resistant *Mycobacterium intracellulare.*[14b] As a result, the strains in this study are known to be resistant to **2** by the mutation in the 2274-position at 23S rRNA.

Results and Discussion

All compounds (**12**, **13**) were evaluated for *in vitro* antibacterial activity against *Mycobacterium intracellulare* 20066, *Mycobacterium avium* 20039, *Mycobacterium avium* 20092, *Mycobacterium avium* 20096, and *Staphylococcus aureus* FDA 209P (gram-positive common strain) and their minimum inhibitory concentrations (MICs, μg/ml) are shown in Table 3. The data for **1** and **2** are included for comparison.

Antibacterial Activity against *Staphylococcus aureus* FDA 209P With regard to the introduction of alkyl groups into the 9-oxime, the lower alkyl oxime ether derivatives (**12a**, **c**, **d**) showed a similar antibacterial activity to that of **2**, but the introduction of more bulky alkyl groups (**12e**—**j**) decreased the potency, especially **12g**—**j** which were quite inactive. The quantitative structure–activity analysis of the 6-*O*-methyl erythromycin A 9-*O*-substituted oxime ether derivatives with the Hansch–Fujita method[15] has been studied by Kawashima *et al.*[10a] They pointed out that the antibacterial activity against *Staphylococcus aureus* FDA 209P was significantly correlated with the hydrophobic parameters such as calculated log P. And they concluded that the activity would be inversely proportional to the calculated log P. A similar tendency was also observed in our studies.

Antibacterial Activity against MAC Although most of the 9-oxime ether derivatives showed good activity against susceptible strains, there were not so many compounds with significant activity against resistant strains. Compounds **12q** and **12t** exhibited the most potent activity against resistant

Table 1. Physicochemical Data for Erythromycin A 9-*O*-Substituted Oxime Ether Derivatives

Compd. No.	Yield (%)	mp (°C) (Solv.)	Formula	HR-MS *m/z* Calcd (Found)
12d	85	109.5—110.5 (iso-Pr_2O)	$C_{41}H_{76}N_2O_{13}$	804.5347 (M^+) (804.5273)
12e	62	Amorphous	$C_{43}H_{80}N_2O_{13}$	674.4479 ($M-C_8H_{16}NO_2$[a])$^+$ (674.4460)
12f	78	112—113 (iso-Pr_2O–hexane)	$C_{45}H_{84}N_2O_{13}$	686.4843 ($M-C_8H_{16}NO_3$[b])$^+$ (686.4842)
12g	42	75.5—76.5 (H_2O–THF)	$C_{47}H_{88}N_2O_{13}$	715.5234 ($M+1-C_8H_{16}NO_3$[b])$^+$ (715.5217)
12h	77	67.5—68.5 (H_2O–THF)	$C_{49}H_{92}N_2O_{13}$	758.5418 ($M-C_8H_{16}NO_2$[a])$^+$ (758.5420)
12i	38	71—72 (H_2O–THF)	$C_{51}H_{96}N_2O_{13}$	770.5782 ($M-C_8H_{16}NO_3$[b])$^+$ (770.5774)
12j	57	77—79.5 (H_2O–THF)	$C_{53}H_{100}N_2O_{13}$	799.6173 ($M+1-C_8H_{16}NO_3$[b])$^+$ (799.6146)
12k	57	115—116.5 (iso-Pr_2O)	$C_{44}H_{80}N_2O_{13}$	844.5660 (M^+) (844.5695)
12l	47	Amorphous	$C_{45}H_{82}N_2O_{13}$	684.4687 ($M-C_8H_{16}NO_3$[b])$^+$ (684.4674)
12m	43	98—100 (iso-Pr_2O)	$C_{46}H_{84}N_2O_{13}$	698.4843 ($M-C_8H_{16}NO_3$[b])$^+$ (698.4859)
12o	93	109—110 (iso-Pr_2O)	$C_{45}H_{76}N_2O_{13}$	678.4217 ($M-C_8H_{16}NO_3$[b])$^+$ (678.4234)
12p	83	99—100 (H_2O–Et_2O)	$C_{46}H_{78}N_2O_{13}$	692.4374 ($M-C_8H_{16}NO_3$[b])$^+$ (692.4384)
12q	56	91—92 (H_2O–Et_2O)	$C_{47}H_{80}N_2O_{13}$	722.4479 ($M-C_8H_{16}NO_2$[a])$^+$ (722.4486)
12r	54	94.5—96 (iso-Pr_2O–AcOEt)	$C_{48}H_{82}N_2O_{13}$	720.4687 ($M-C_8H_{16}NO_3$[b])$^+$ (720.4681)
12s	48	Amorphous	$C_{45}H_{76}N_2O_{14}$	694.4166 ($M-C_8H_{16}NO_3$[b])$^+$ (694.4165)
12t	59	97.5—98 (iso-Pr_2O)	$C_{46}H_{78}N_2O_{14}$	708.4323 ($M-C_8H_{16}NO_3$[b])$^+$ (708.4328)
12u	70	91—92 (H_2O–iso-Pr_2O)	$C_{47}H_{80}N_2O_{14}$	738.4429 ($M-C_8H_{16}NO_2$[a])$^+$ (738.4451)
12v	32	Amorphous	$C_{46}H_{79}N_3O_{13}$	723.4432 ($M-C_8H_{16}NO_2$[a])$^+$ (723.4432)
12w	65	Amorphous	$C_{43}H_{74}N_2O_{13}S$	684.3781 ($M-C_8H_{16}NO_3$[b])$^+$ (684.3788)
12x	24	Amorphous	$C_{45}H_{76}N_2O_{13}S$	726.3887 ($M-C_8H_{16}NO_2$[a])$^+$ (726.3891)
13t	69	105—107 (iso-Pr_2O–heptane)	$C_{47}H_{80}N_2O_{14}$	738.4429 ($M-C_8H_{16}NO_2$[a])$^+$ (738.4446)

a) $C_8H_{16}NO_2$: desosamine. *b*) $C_8H_{16}NO_3$: -*O*-desosamine.

Table 2. The Sequence of Part 23S rRNA Gene of MAC

Strain		Property[a]	Partial 23S rRNA gene sequence
M. intracellulare	20066	Susceptible	GCGCGGCAGGACGAA[b]AAGACCCCGGGACCT
M. avium	20039	Susceptible	A
M. avium	20092	Resistant	G
M. avium	20096	Resistant	C

a) Susceptible or resistant property for **2** determined by agar dilution method are listed. *b*) 2274-position in 23S rRNA of MAC.

strains among all compounds, and their length indices of 9-substituents, which was calculated by Sterimol calculation software,[16] were about 10.3 Å. On the contrary, the compounds bearing shorter length, such as **12k**—**o**, tended to decrease the activity against resistant strains. The inactive compounds against resistant strains actually had somewhat short length of substituents such as lower alkyl (**12a**—**d**), cyclohexylmethyl (**12k**) and benzyl (**12n**) at the 9-position. The substituents at the 9-position of **12i**, **j** were not sufficient to show a good antibacterial activity against both the resistant and susceptible strains. Phenylthioethyl (7.93 Å), phenylpropyl (7.91 Å) or phenoxyethyl (7.80 Å) groups at the 9-position were also insufficient, so the compounds (**12x**, **p**, **s**) did not show good activity accordingly. And one compound (**12w**) showed the same potency as another (**12o**), because a thiophene ring is considered to be an isostere of a benzene ring. Thus, the length of the 9-substituents plays a key role for the antibacterial activity against resistant strains.

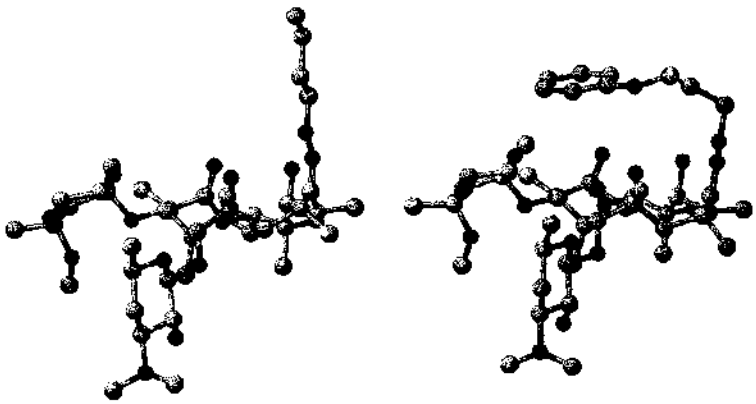
Although the introduction of a 6-methoxy group enhanced activity against common strains,[2] the antibacterial activity against resistant strains of 6-*O*-methoxyerythromycin A 9-*O*-substituted oxime ether derivative (**13t**) was 2—4 times less than that of the parent compound **12t**. So the methoxy group at position 6 of the 9-oxime ether derivative was not appropriate for antibacterial activity.

Molecular Modeling To elucidate factors affecting antibacterial activity in detail, we performed a conformational analysis of the compounds (**12**). The initial conformations were built on the basis of the X-ray structure of **2**[17] using CAChe system.[18] We implemented a systematic conformational analysis around all rotable bonds of the 9-substituents and energy-minimized by -ES force field,[19] which excluded

Table 3. *In Vitro* Antibacterial Activities of Erythromycin A 9-*O*-Substituted Oxime Ether Derivatives

Compd. No.	MIC (μg/ml)[a]				
	S. aureus FDA209P[b]	*M. intracellulare* 20066[b]	*M. avium* 20039[b]	*M. avium* 20092[c]	*M. avium* 20096[c]
12a	0.78	12.5	3.13	>50	>50
12b	3.13	6.25	3.13	>50	>50
12c	0.39	3.13	1.56	>50	>50
12d	0.78	1.56	0.78	>50	>50
12e	6.25	3.13	1.56	25	6.25
12f	3.13	6.25	3.13	25	6.25
12g	>50	6.25	12.5	25	6.25
12h	>50	50	25	25	25
12i	>50	>50	50	>50	>50
12j	>50	>50	>50	>50	>50
12k	1.56	6.25	1.56	>50	>50
12l	12.5	6.25	3.13	>50	50
12m	12.5	6.25	3.13	12.5	6.25
12n	6.25	6.25	3.13	>50	>50
12o	0.78	1.56	1.56	>50	25
12p	0.39	1.56	1.56	12.5	12.5
12q	3.13	1.56	1.56	6.25	3.13
12r	12.5	3.13	3.13	6.25	6.25
12s	0.78	3.13	1.56	12.5	25
12t	0.78	1.56	1.56	3.13	3.13
12u	1.56	3.13	1.56	12.5	6.25
12v	0.78	1.56	1.56	25	25
12w	1.56	3.13	1.56	50	25
12x	0.39	3.13	1.56	25	12.5
13t	1.56	3.13	1.56	12.5	6.25
1	0.78	>50	25	>50	>50
2	0.78	3.13	0.78	>50	>50

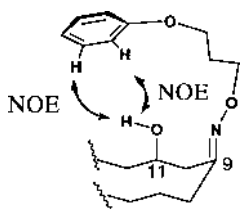
a) Inoculum size, one loopful of 10^6 CFU/ml. *b*) These strains are susceptible to **2**. *c*) These strains are resistant to **2**.



12d (type A) **12t** (type B)

Fig. 1. The Minimized Structure of **12d** and **12t** Calculated by the Molecular Modeling Program CAChe (Version 4.1.1)

the electrostatic energy term and the hydrogen bonding energy term from the total MM2 force field. This analysis consequently suggested that erythromycin A 9-*O*-substituted oxime ether derivatives could be classified into the following two types: 1) the direction of the substituents at 9-position was perpendicular to the plane of the aglycon such as the conformation of **12d** (type A, **12a**—**d**), 2) the direction of the substituents was parallel to the plane of aglycon such as the conformation of **12t** (type B, **12e**—**x**, **13t**). The conformations of **12d** (type A) and **12t** (type B) are shown in Fig. 1. Interestingly, all compounds (**12a**—**d**) classified into type



12t

Fig. 2. NOE Correlations of **12t**

Table 4. Spectral Data for Erythromycin A 9-*O*-Substituted Oxime Ether Derivatives

Compd. No.	^{1}H-NMR ($CDCl_3$) δ (ppm)	IR (KBr) (cm^{-1})	MS *m/z*
12d	0.84 (3H, t, *J*=7.5 Hz), 0.94 (3H, t, *J*=7.5 Hz), 1.02 (3H, d, *J*=6.5 Hz), 1.07—1.80 (35H, m), 1.85—2.07 (2H, m), 2.20 (1H, d, *J*=10.5 Hz), 2.29 (6H, s), 2.36 (1H, d, *J*=15.5 Hz), 2.40—2.50 (1H, m), 2.60—2.70 (1H, m), 2.85—2.95 (1H, m), 3.02 (1H, t, *J*=9.5 Hz), 3.10 (1H, s), 3.22 (1H, dd, *J*=10, 7.5 Hz), 3.32 (3H, s), 3.40 (1H, br s), 3.45—3.55 (1H, m), 3.59 (1H, d, *J*=7.5 Hz), 3.61—3.75 (2H, m), 3.98—4.08 (4H, m), 4.43 (1H, d, *J*=7.5 Hz), 4.45 (1H, s), 4.92 (1H, d, *J*=4.5 Hz), 5.11 (1H, dd, *J*=11, 2.5 Hz)	1738 (C=O)	804 (M^+)
12e	0.84 (3H, t, *J*=7.5 Hz), 0.89 (3H, t, *J*=7 Hz), 1.00—1.80 (42H, m), 1.87—2.05 (2H, m), 2.21 (1H, d, *J*=10.5 Hz), 2.29 (6H, s), 2.36 (1H, d, *J*=15.5 Hz), 2.39—2.48 (1H, m), 2.60—2.70 (1H, m), 2.85—2.96 (1H, m), 3.02 (1H, t, *J*=10 Hz), 3.11 (1H, s), 3.22 (1H, dd, *J*=10.5, 7.5 Hz), 3.32 (3H, s), 3.39 (1H, s), 3.45—3.54 (1H, m), 3.59 (1H, d, *J*=7.5 Hz), 3.64—3.75 (2H, m), 3.95—4.10 (4H, m), 4.43 (1H, d, *J*=6.5 Hz), 4.46 (1H, s), 4.92 (1H, d, *J*=5 Hz), 5.12 (1H, dd, *J*=11.5, 2 Hz)	1738 (C=O)	832 (M^+)
12f	0.84 (3H, t, *J*=7.5 Hz), 0.88 (3H, t, *J*=7 Hz), 0.98—1.80 (46H, m), 1.85—2.06 (2H, m), 2.22 (1H, d, *J*=10.5 Hz), 2.29 (6H, s), 2.36 (1H, d, *J*=15.5 Hz), 2.40—2.50 (1H, m), 2.62—2.70 (1H, m), 2.87—2.97 (1H, m), 3.02 (1H, t, *J*=10 Hz), 3.11 (1H, s), 3.22 (1H, dd, *J*=10.5, 7.5 Hz), 3.32 (3H, s), 3.39 (1H, br s), 3.45—3.57 (1H, m), 3.59 (1H, d, *J*=7.5 Hz), 3.61—3.75 (2H, m), 3.97—4.08 (4H, m), 4.43 (1H, d, *J*=7.5 Hz), 4.46 (1H, s), 4.92 (1H, d, *J*=5 Hz), 5.12 (1H, dd, *J*=11.5, 2 Hz)	1738 (C=O)	861 $(M+1)^+$
12g	0.84 (3H, t, *J*=7.5 Hz), 0.88 (3H, t, *J*=7 Hz), 0.96—1.80 (47H, m), 1.02 (3H, d, *J*=6.5 Hz), 1.87—2.05 (2H, m), 2.20 (1H, d, *J*=10.5 Hz), 2.28 (6H, s), 2.36 (1H, d, *J*=14.5 Hz), 2.38—2.48 (1H, m), 2.60—2.70 (1H, m), 2.81—2.95 (1H, m), 3.02 (1H, t, *J*=10 Hz), 3.11 (1H, s), 3.22 (1H, dd, *J*=10, 7.5 Hz), 3.32 (3H, s), 3.40 (1H, br s), 3.45—3.54 (1H, m), 3.59 (1H, d, *J*=7.5 Hz), 3.65—3.75 (1H, m), 3.68 (1H, s), 3.95—4.09 (4H, m), 4.43 (1H, d, *J*=7.5 Hz), 4.45 (1H, s), 4.92 (1H, d, *J*=5 Hz), 5.11 (1H, dd, *J*=11, 2.5 Hz)	1738 (C=O)	889 $(M+1)^+$
12h	0.84 (3H, t, *J*=7.5 Hz), 0.88 (3H, t, *J*=7 Hz), 1.00—1.70 (54H, m), 1.85—2.10 (2H, m), 2.21 (1H, d, *J*=10 Hz), 2.29 (6H, s), 2.36 (1H, d, *J*=15.5 Hz), 2.39—2.50 (1H, m), 2.60—2.70 (1H, m), 2.85—2.95 (1H, m), 3.02 (1H, t, *J*=10 Hz), 3.10 (1H, s), 3.22 (1H, dd, *J*=10.5, 7.5 Hz), 3.32 (3H, s), 3.38 (1H, br s), 3.45—3.55 (1H, m), 3.59 (1H, d, *J*=7.5 Hz), 3.65—3.75 (2H, m), 3.95—4.08 (4H, m), 4.43 (1H, d, *J*=7.5 Hz), 4.45 (1H, s), 4.92 (1H, d, *J*=5 Hz), 5.08—5.15 (1H, m)	17381 (C=O)	916 (M^+)
12i	0.84 (3H, t, *J*=7.5 Hz), 0.88 (3H, t, *J*=6.5 Hz), 1.02 (3H, d, *J*=7.5 Hz), 1.07—1.80 (55H, m), 1.86—2.07 (2H, m), 2.21 (1H, d, *J*=10.5 Hz), 2.29 (6H, s), 2.36 (1H, d, *J*=15.5 Hz), 2.39—2.48 (1H, m), 2.62—2.70 (1H, m), 2.85—2.95 (1H, m), 3.02 (1H, t, *J*=10 Hz), 3.11 (1H, s), 3.22 (1H, dd, *J*=10.5, 7.5 Hz), 3.32 (3H, s), 3.39 (1H, br s), 3.45—3.55 (1H, m), 3.59 (1H, d, *J*=7.5 Hz), 3.64—3.76 (1H, m), 3.68 (1H, s), 3.94—4.10 (4H, m), 4.43 (1H, d, *J*=7.5 Hz), 4.46 (1H, s), 4.92 (1H, d, *J*=5 Hz), 5.11 (1H, dd, *J*=11, 2.5 Hz)	1738 (C=O)	945 $(M+1)^+$
12j	0.84 (3H, t, *J*=7.5 Hz), 0.88 (3H, t, *J*=7 Hz), 0.95—1.80 (59H, m), 1.02 (3H, d, *J*=6.5 Hz), 1.87—2.04 (2H, m), 2.21 (1H, d, *J*=10.5 Hz), 2.28 (6H, s), 2.36 (1H, d, *J*=15.5 Hz), 2.39—2.46 (1H, m), 2.61—2.68 (1H, m), 2.86—2.93 (1H, m), 3.02 (1H, t, *J*=10 Hz), 3.11 (1H, s), 3.22 (1H, dd, *J*=10, 7.5 Hz), 3.32 (3H, s), 3.40 (1H, br s), 3.42—3.52 (1H, m), 3.59 (1H, d, *J*=7.5 Hz), 3.63—3.72 (1H, m), 3.68 1H, s), 3.95—4.08 (4H, m), 4.43 (1H, d, *J*=7.5 Hz), 4.46 (1H, s), 4.92 (1H, d, *J*=5 Hz), 5.11 (1H, dd, *J*=11, 2 Hz)	1738 (C=O)	973 $(M+1)^+$
12k	0.84 (3H, t, *J*=7.5 Hz), 0.92—1.80 (45H, m), 1.87—2.04 (2H, m), 2.21 (1H, d, *J*=10.5 Hz), 2.29 (6H, s), 2.36 (1H, d, *J*=14.5 Hz), 2.39—2.47 (1H, m), 2.62—2.68 (1H, m), 2.86—2.95 (1H, m), 3.02 (1H, t, *J*=10 Hz), 3.11 (1H, s), 3.22 (1H, dd, *J*=10.5, 7.5 Hz), 3.32 (3H, s), 3.39 (1H, br s), 3.45—3.54 (1H, m), 3.59 (1H, d, *J*=8 Hz), 3.65—3.75 (2H, m), 3.77—3.87 (2H, m), 3.98—4.07 (2H, m), 4.43 (1H, d, *J*=7.5 Hz), 4.46 (1H, s), 4.92 (1H, d, *J*=4.5 Hz), 5.11 (1H, dd, *J*=11, 2 Hz)	1738 (C=O)	844 (M^+)
12l	0.84 (3H, t, *J*=7.5 Hz), 0.88—1.78 (47H, m), 1.86—2.06 (2H, m), 2.20 (1H, d, *J*=10.5 Hz), 2.29 (6H, s), 2.36 (1H, d, *J*=14.5 Hz), 2.38—2.47 (1H, m), 2.62—2.69 (1H, m), 2.86—2.96 (1H, m), 3.02 (1H, t, *J*=10 Hz), 3.10 (1H, s), 3.22 (1H, dd, *J*=10, 7.5 Hz), 3.32 (3H, s), 3.40 (1H, s), 3.44—3.54 (1H, m), 3.59 (1H, d, *J*=7.5 Hz), 3.64—3.74 (2H, m), 3.96—4.09 (4H, m), 4.43 (1H, d, *J*=7.5 Hz), 4.45 (1H, s), 4.91 (1H, d, *J*=5 Hz), 5.12 (1H, dd, *J*=11, 2 Hz)	1738 (C=O)	858 (M^+)
12m	0.80—1.81 (49H, m), 0.84 (3H, t, *J*=7.5 Hz), 1.86—2.06 (2H, m), 2.21 (1H, d, *J*=10.5 Hz), 2.29 (6H, s), 2.36 (1H, d, *J*=15.5 Hz), 2.39—2.48 (1H, m), 2.60—2.70 (1H, m), 2.85—2.96 (1H, m), 3.02 (1H, t, *J*=9.5 Hz), 3.11 (1H, s), 3.22 (1H, dd, *J*=10.5, 7.5 Hz), 3.32 (3H, s), 3.40 (1H, s), 3.44—3.54 (1H, m), 3.59 (1H, d, *J*=7.5 Hz), 3.63—3.75 (2H, m), 3.92—4.09 (4H, m), 4.43 (1H, d, *J*=7.5 Hz), 4.46 (1H, s), 4.92 (1H, d, *J*=4.5 Hz), 5.12 (1H, dd, *J*=11, 2.5 Hz)	1738 (C=O)	872 (M^+)
12o	0.85 (3H, t, *J*=7.5 Hz), 0.98—1.70 (34H, m), 1.88—2.00 (2H, m), 2.20 (1H, d, *J*=10.5 Hz), 2.28 (6H, s), 2.37 (1H, d, *J*=16 Hz), 2.37—2.46 (1H, m), 2.60—2.69 (1H, m), 2.85—2.99 (3H, m), 3.03 (1H, t, *J*=10 Hz), 3.12 (1H, s), 3.20 (1H, dd, *J*=10.5, 7.5 Hz), 3.32 (3H, s), 3.37 (1H, s), 3.42—3.53 (2H, m), 3.54—3.63 (1H, m), 3.71 (1H, s), 3.95—4.05 (2H, m), 4.28 (2H, t, *J*=6.5 Hz), 4.39 (1H, d, *J*=7.5 Hz), 4.46 (1H, s), 4.93 (1H, d, *J*=5 Hz), 5.14 (1H, dd, *J*=11, 2.5 Hz), 7.17—7.34 (5H, m)	1738 (C=O)	679 $(M+1-C_8H_{16}NO_3)^+$
12p	0.85 (3H, t, *J*=7.5 Hz), 1.04—1.70 (33H, m), 1.78 (1H, brs), 1.87—2.06 (4H, m), 2.21 (1H, d, *J*=10.5 Hz), 2.28 (6H, s), 2.36 (1H, d, *J*=15.5 Hz), 2.39—2.48 (1H, m), 2.62—2.73 (3H, m), 2.86—2.95 (1H, m), 3.02 (1H, t, *J*=10 Hz), 3.10 (1H, s), 3.22 (1H, dd, *J*=10.5, 7.5 Hz), 3.32 (3H, s), 3.41 (1H, br s), 3.44—3.53 (1H, m), 3.59 (1H, d, *J*=8 Hz), 3.67—3.78 (2H, m), 3.97—4.09 (4H, m), 4.41 (1H, s), 4.43 (1H, d, *J*=7.5 Hz), 4.92 (1H, d, *J*=5 Hz), 5.11 (1H, dd, *J*=11, 2.5 Hz), 7.13—7.32 (5H, m)	1738 (C=O)	867 $(M+1)^+$
12q	0.84 (3H, t, *J*=7.5 Hz), 0.98—1.76 (38H, m), 1.87—2.04 (2H, m), 2.21 (1H, d, *J*=10.5 Hz), 2.29 (6H, s), 2.36 (1H, d, *J*=15.5 Hz), 2.39—2.46 (1H, m), 2.60—2.68 (3H, m), 2.86—2.95 (1H, m), 3.03 (1H, t, *J*=10 Hz), 3.10 (1H, s), 3.22 (1H, dd, *J*=10, 7 Hz), 3.32 (3H, s), 3.41 (1H, br s), 3.45—3.53 (1H, m), 3.58 (1H, d, *J*=7.5 Hz), 3.63—3.73 (2H, m), 3.97—4.07 (4H, m), 4.42 (1H, s), 4.43 (1H, d, *J*=7.5 Hz), 4.92 (1H, d, *J*=5 Hz), 5.11 (1H, dd, *J*=11, 2 Hz), 7.14—7.20 (3H, m), 7.24—7.30 (2H, m)	1738 (C=O)	880 (M^+)

Table 4. (continued)

Compd. No.	^{1}H-NMR ($CDCl_3$) δ (ppm)	IR (KBr) (cm^{-1})	MS m/z
12r	0.84 (3H, t, J=7.5 Hz), 0.95—1.81 (40H, m), 1.87—2.04 (2H, m), 2.21 (1H, d, J=10 Hz), 2.29 (6H, s), 2.36 (1H, d, J=15.5 Hz), 2.40—2.48 (1H, m), 2.59—2.68 (3H, m), 2.86—2.94 (1H, m), 3.02 (1H, t, J=10 Hz), 3.10 (1H, s), 3.22 (1H, dd, J=10, 7.5 Hz), 3.32 (3H, s), 3.41 (1H, br s), 3.45—3.53 (1H, m), 3.59 (1H, d, J=7.5 Hz), 3.64—3.72 (2H, m), 3.97—4.07 (4H, m), 4.43 (1H, d, J=7.5 Hz), 4.44 (1H, s), 4.91 (1H, d, J=5 Hz), 5.11 (1H, dd, J=11, 2.5 Hz), 7.13—7.20 (3H, m), 7.23—7.30 (2H, m)	1738 (C=O)	720 $(M-C_8H_{16}NO_3)^+$
12s	0.84 (3H, t, J=7.5 Hz), 0.97—1.75 (33H, m), 1.86—2.03 (3H, m), 2.22 (1H, d, J=10.5 Hz), 2.28 (6H, s), 2.34 (1H, d, J=14.5 Hz), 2.38—2.47 (1H, m), 2.64—2.74 (1H, m), 2.83—2.93 (1H, m), 3.00 (1H, t, J=10 Hz), 3.09 (1H, s), 3.21 (1H, dd, J=10, 7.5 Hz), 3.30 (3H, s), 3.37 (1H, br s), 3.42—3.57 (2H, m), 3.66—3.80 (2H, m), 3.87—4.04 (2H, m), 4.08—4.24 (2H, m), 4.33—4.44 (4H, m), 4.77 (1H, d, J=4.5 Hz), 5.10 (1H, dd, J=11, 2.5 Hz), 6.84—6.99 (3H, m), 7.22—7.33 (2H, m)	1738 (C=O)	694 $(M-C_8H_{16}NO_3)^+$
12t	0.85 (3H, t, J=7.5 Hz), 1.02 (3H, d, J=6.5 Hz), 1.08—1.80 (31H, m), 1.87—2.05 (2H, m), 2.08—2.18 (2H, m), 2.21 (1H, d, J=10.5 Hz), 2.29 (6H, s), 2.33—2.48 (1H, m), 2.36 (1H, d, J=15.5 Hz), 2.63—2.72 (1H, m), 2.85—2.96 (1H, m), 3.02 (1H, t, J=10 Hz), 3.10 (1H, s), 3.22 (1H, dd, J=10, 7.5 Hz), 3.32 (3H, s), 3.41 (1H, s), 3.44—3.53 (1H, m), 3.57 (1H, d, J=7.5 Hz), 3.64—3.76 (2H, m), 3.96—4.10 (4H, m), 4.16—4.27 (2H, m), 4.38 (1H, s), 4.42 (1H, d, J=7.5 Hz), 4.92 (1H, d, J=5 Hz), 5.12 (1H, dd, J=11, 2 Hz), 6.84—6.96 (3H, m), 7.21—7.32 (2H, m)	1738 (C=O)	708 $(M-C_8H_{16}NO_3)^+$
12u	0.85 (3H, t, J=7.5 Hz), 0.96—2.05 (40H, m), 2.21 (1H, d, J=10.5 Hz), 2.25—2.40 (1H, m), 2.32 (6H, s), 2.40—2.55 (1H, m), 2.60—2.70 (1H, m), 2.85—2.95 (1H, m), 3.02 (1H, t, J=9.5 Hz), 3.10 (1H, s), 3.20—3.27 (1H, m), 3.32 (3H, s), 3.40 (1H, s), 3.45—3.55 (1H, m), 3.59 (1H, d, J=7.5 Hz), 3.65—3.75 (2H, m), 3.95—4.15 (6H, m), 4.41 (1H, s), 4.43 (1H, d, J=7.5 Hz), 4.90 (1H, d, J=5 Hz), 5.11 (1H, dd, J=11, 2 Hz), 6.90—7.00 (3H, m), 7.20—7.30 (2H, m)	1738 (C=O)	896 (M^+)
12v	0.85 (3H, t, J=7.5 Hz), 0.92—1.70 (33H, m), 1.80—2.01 (3H, m), 2.21 (1H, d, J=10 Hz), 2.28 (6H, s), 2.36 (1H, d, J=14.5 Hz), 2.37—2.45 (1H, m), 2.60—2.70 (1H, m), 2.85—2.98 (1H, m), 2.95 (3H, s), 3.02 (1H, t, J=10 Hz), 3.09 (1H, s), 3.21 (1H, dd, J=10, 7.5 Hz), 3.32 (3H, s), 3.39 (1H, br s), 3.43—3.58 (4H, m), 3.60—3.75 (2H, m), 3.95—4.05 (2H, m), 4.17—4.23 (2H, m), 4.35 (1H, s), 4.41 (1H, d, J=6.5 Hz), 4.90 (1H, d, J=4.5 Hz), 5.11 (1H, dd, J=11, 2 Hz), 6.68—6.77 (3H, m), 7.20—7.30 (2H, m)	1738 (C=O)	723 $(M-C_8H_{16}NO_2)^+$
12w	0.85 (3H, t, J=7.5 Hz), 0.97—1.70 (34H, m), 1.87—2.01 (2H, m), 2.20 (1H, d, J=10.5 Hz), 2.28 (6H, s), 2.37 (1H, d, J=15.5 Hz), 2.37—2.46 (1H, m), 2.61—2.70 (1H, m), 2.86—2.96 (1H, m), 3.03 (1H, t, J=10 Hz), 3.08—3.25 (4H, m), 3.32 (3H, s), 3.40 (1H, s), 3.43—3.54 (2H, m), 3.61—3.70 (1H, m), 3.72 (1H, s), 3.96—4.06 (2H, m), 4.28 (2H, t, J=6.5 Hz), 4.36—4.42 (2H, m), 4.93 (1H, d, J=5 Hz), 5.13 (1H, dd, J=11, 2 Hz), 6.85 (1H, d, J=2.5 Hz), 6.94 (1H, dd, J=5, 3.5 Hz), 7.15 (1H, dd, J=5, 1 Hz)	1738 (C=O)	858 (M^+)
12x	0.85 (3H, t, J=7.5 Hz), 0.98—1.70 (33H, m), 1.85—2.05 (3H, m), 2.20 (1H, d, J=10.5 Hz), 2.29 (6H, s), 2.35 (1H, d, J=15.5 Hz), 2.40—2.48 (1H, m), 2.65—2.72 (1H, m), 2.85—2.93 (1H, m), 3.01 (1H, t, J=10 Hz), 3.08 (1H, s), 3.09—3.18 (2H, m), 3.22 (1H, dd, J=10.5, 7.5 Hz), 3.32 (3H, s), 3.39 (1H, brs), 3.45—3.53 (1H, m), 3.59 (1H, d, J=3.5 Hz), 3.65—3.75 (2H, m), 3.98—4.07 (2H, m), 4.14—4.25 (3H, m), 4.42 (1H, d, J=6.5 Hz), 4.89 (1H, d, J=5 Hz), 5.11 (1H, dd, J=11, 2.5 Hz), 7.18—7.42 (5H, m)	1738 (C=O)	884 (M^+)
13t	0.84 (3H, t, J=7.5 Hz), 0.97 (3H, d, J=7.5 Hz), 1.02—1.75 (30H, m), 1.87—2.00 (2H, m), 2.07—2.25 (3H, m), 2.31 (6H, s), 2.36 (1H, d, J=15.5 Hz), 2.39—2.49 (1H, m), 2.51—2.59 (1H, m), 2.82—2.92 (1H, m), 3.02 (1H, t, J=9.5 Hz), 3.05 (3H, s), 3.15—3.25 (2H, m), 3.32 (3H, s), 3.37 (1H, br s), 3.43—3.53 (1H, m), 3.59—3.80 (4H, m), 3.95—4.24 (5H, m), 4.43 (1H, d, J=7.5 Hz), 4.59 (1H, s), 4.93 (1H, d, J=4.5 Hz), 5.11 (1H, dd, J=11.5, 2 Hz), 6.86—6.94 (3H, m), 7.22—7.29 (2H, m)	1736 (C=O)	897 $(M+1)^+$

A showed no activity against the resistant strains. On the other hand, some compounds (**12e**—**g**, **m**, **p**—**u**, **x**, **13t**) in type B showed good activity against both the resistant and susceptible strains. Our modeling study suggested that the difference in the spatial orientation of the 9-substituents might be an important factor for the antibacterial activity against resistant strains. Six compounds (**12i**—**l**, **n**, **o**) in type B, however, showed no activity against the resistant strains. The insufficient activity of these compounds would stem from the inappropriate length at the 9-position, as mentioned above. To demonstrate the spatial difference of the orientation of the 9-substituents, nuclear Overhauser effect (NOE) technique was considered to be useful, so we measured the NOE in the representative type A (**12d**) and type B (**12t**) compounds. In the actual experiments, the NOE between the 11-hydroxy group H atom and aromatic H atoms on the 9-phenyl group was observed in **12t** (Fig. 2), while it was not observed in **12d**.

In conclusion, our SAR studies of a series of erythromycin A 9-oxime ether derivatives allowed us to find that erythromycin A 9-[*O*-(4-phenylbutyl)oxime] (**12q**) and erythromycin A 9-[*O*-(3-phenoxypropyl)oxime] (**12t**) exhibited potent antibacterial activities against both the resistant and susceptible strains. We also found that a couple of factors influenced the antibacterial activity against macrolide-resistant strains. Our results suggest that the orientation of the substituent of the 9-oxime ether group to the aglycon could be an important factor for improved anti-MAC activity as well as the length of the 9-substituents. Further evaluations of the potent macrolides (**12q**, **12t**), bearing antibacterial activity against macrolide-resistant strains, are underway.

Experimental

Melting points were measured with a Yanagimoto melting point apparatus and are uncorrected. Infrared (IR) spectra were recorded on a Hitachi 270-30 spectrometer. Proton nuclear magnetic resonance (^{1}H-NMR) spectra were recorded on a JEOL JNM-GX500 spectrometer. Chemical shifts are reported in δ downfield from internal tetramethylsilane. The difference ^{1}H-NOE experiments were carried out on the same spectrometer. Mass spectra (MS) and high-resolution mass spectra (HR-MS) were taken on a Waters ZMD 2690 mass spectrometer and JEOL JMS-DX 300 mass spectrometer. HPLC analyses were performed with Shimadzu LC-3A pumping system and Shimadzu ultraviolet–visible spectrophotomeric detector (210 nm). Column chromatography was carried out with silica gel [Kieselgel 60 (Merck)]. TLC

was run on 0.25 mm pre-coated silica gel plates (60 F_{254}, Merck). All extracted solvents were dried over anhydrous Na_2SO_4 and removed on a rotary evaporator under reduced pressure. The known compounds (**12a**—**c**, **12n**) were prepared according to the literature.[11)]

4-Phenoxylbutyl Methanesulfonate (10u) To the mixture of 4-phenoxybutyl alcohol (10.3 g, 62.0 mmol) and NEt_3 (10.3 ml, 73.9 mmol) in Et_2O (100 ml), methanesulfonyl chloride (5.26 ml, 68.0 mmol) was added dropwise under ice-cooling and the mixture was stirred at the same temperature for 30 min. To the reaction mixture was added saturated $NaHCO_3$, and it was washed with water and brine successively, dried and concentrated to give **10u** (14.5 g, 96%) as a colorless oil. IR (liq.): 1355 (SO_2), 1175 (SO_2) cm^{-1}. MS *m/z*: 245 $(M+1)^+$. ^{1}H-NMR ($CDCl_3$) δ: 1.87—2.00 (4H, m), 3.01 (3H, s), 3.97—4.03 (2H, m), 4.27—4.37 (2H, m), 6.84—7.00 (3H, m), 7.22—7.35 (2H, m).

2-(*N*-Methylanilino)ethyl Methanesulfonate (10v) In a similar manner to that described above **10v** (87 %) was obtained as a colorless oil. IR (liq.): 1350 (SO_2), 1175 (SO_2) cm^{-1}. MS *m/z*: 230 $(M+1)^+$. ^{1}H-NMR ($CDCl_3$) δ: 2.92 (3H, s), 3.01 (3H, s), 3.67—3.73 (2H, m), 4.35—4.40 (2H, m), 6.67—6.79 (3H, m), 7.21—7.28 (2H, m).

Erythromycin A 9-[*O*-(3-Phenoxypropyl)oxime] (12t) Method A: A suspension of **8** (4.00 g, 5.34 mmol), 3-phenoxypropyl bromide (1.30 ml, 8.25 mmol), tetrabutylammonium iodide (0.10 g, 0.271 mmol), NaI (0.12 g, 0.801 mmol) and KOH (0.43 g, 6.51 mmol) in THF (40 ml) was stirred at room temperature for 16.5 h. The reaction mixture was concentrated under reduced pressure and the residue was made alkaline with saturated $NaHCO_3$, and extracted with AcOEt. The organic layer was washed with brine, dried and concentrated. The residue was crystallized from Et_2O and the precipitate was filtered and washed with iso-Pr_2O to give **12t** as a colorless solid (2.80 g, 59%). Recrystallization from iso-Pr_2O gave **12t** as colorless needles. Geometrical ratio: $E:Z=99.7:0.3$; HPLC using a TSK gel CN [4.6 mm×100 mm, solvent; $KH_2PO_4:CH_3CN$ (7:3), flow rate 1.0 ml/min]. The physicochemical and spectral data are listed in Tables 1 and 4, respectively. A NOE was observed between the 11-hydroxy group H atom (δ 4.38) and the aromatic H atoms on the 9-phenyl group (δ 6.84—6.96, 7.21—7.32).

Compounds **12d**—**k**, **o**, **p**, **s**, **x**, **13t** were prepared in a similar manner, and their physicochemical and spectral data are listed in Tables 1 and 4, respectively.

Method B: To a solution of **8** (0.50 g, 0.668 mmol) in THF (5.0 ml), NaH (0.032 g, 0.800 mmol, 60% mineral oil dispersion) was added portionwise under ice-cooling and the mixture was stirred at the same temperature for 5 min. Tetrabutylammonium iodide (0.012 g, 0.025 mmol) and 3-phenoxypropyl bromide (0.16 ml, 1.02 mmol) were successively added to the reaction mixture and the mixture was stirred at room temperature for 27.5 h. To the reaction mixture was added water, it was made alkaline with saturated $NaHCO_3$, and extracted with AcOEt. The organic layer was washed with brine, dried and concentrated. The residue was recrystallized from iso-Pr_2O to give **12t** as colorless needles (0.28 g, 47%), which were identical with the compound (**12t**) synthesized by method A.

Erythromycin A 9-[*O*-(3-(Cyclohexyl)propyl)oxime] (12m) To a solution of **8** (15.0 g, 20.0 mmol) in THF (150 ml), NaH (0.96 g, 24.0 mmol, 60% mineral oil dispersion) was added portionwise under ice-cooling and the mixture was stirred at the same temperature for 5 min. Tetrabutylammonium iodide (0.37 g, 1.00 mmol) and 3-(cyclohexyl)propyl methanesulfonate (6.60 g, 30.0 mmol) were successively added to the reaction mixture and the mixture was stirred at room temperature for 24.5 h. The reaction mixture was concentrated under reduced pressure and to the residue was added water, and the mixture was crystallized from iso-Pr_2O. The precipitate was dissolved in AcOEt to remove impurities and filtered. The filtrate was concentrated to give a pale brown amorphous solid (7.50 g, 43%). Recrystallization from iso-Pr_2O gave **12m** as colorless crystals. Geometrical ratio: $E:Z=99.3:0.7$; HPLC using a TSK gel CN [4.6 mm×100 mm, solvent; $KH_2PO_4:CH_3CN$ (7:3), flow rate 1.0 ml/min]. The physicochemical and spectral data are listed in Tables 1 and 4, respectively.

Compounds **12l**, **q**, **r**, **u**—**w** were prepared in a similar manner, and the physicochemical and spectral data are listed in Tables 1 and 4, respectively.

***In Vitro* Antibacterial Test** The MICs of the test drugs were determined by the agar dilution method. *Mycobacterium avium* and *Mycobacterium intracellulare* were tested in Middlebrook 7H11 agar (Becton Dickinson and Company, Sparks, MD, U.S.A.). *Staphylococcus aureus* was tested in Mueller Hinton Agar (Becton Dickinson and Company). One loopful (5 μl) of an inoculum corresponding to 10^6 CFU/ml was inoculated on drug-containing agar plates, and the plates were incubated for 7 d (Mycobacteria) or 18 h (*Staphylococcus aureus*) at 37 °C. The MIC was defined as the lowest drug concentration which prevented visible growth of bacteria.

Nucleotide Sequencing Test *Mycobacterium avium* strains were suspended in tris(hydromethyl)aminomethane–ethylenediaminetetraacetic acid (TE) buffer (12.0 ml) and mixed with phenol/$CHCl_3$/isopentyl alcohol (12.0 ml), (Life Technologies, Inc., Grand Island, NY, U.S.A.) and 0.1 mm diameter glass beads (10.0 ml). The mixture was sonicated (Sonifier 250, Branson, Danbury, CT, U.S.A.) on ice for two 3 min periods, with a cooling period in between. After centrifugation at 8000×***g*** at room temperature for 15 min, the aliquot phase was removed to a new tube, and the DNA was precipitated with 1/10 volume of NaOAc (pH 5.2) and 2 volumes of EtOH. The DNA was collected by centrifugation at 15000×***g*** at 4 °C for 20 min, and the pellet was dissolved in 100 μl of TE buffer. The mycobacterial DNA was amplified by a PCR designed in domain V of 23S rRNA gene using primers MA1 (5′-AACGGCGGTGGTAACTATAAC-3′) and 23.3 (5′-CCGACTTT-CGTCCCTGCTTGA-3′). These primers were designed with reference to the 23S rRNA gene sequence of *Mycobacterium avium*.[14b,c)] DNA fragments were amplified with TakaRa Ex Taq polymerase (Takara, Osaka, Japan) by using the following temperature profile in Thermal cycler 480 (Perkin-Elmer Cetus, Norwalk, CN, U.S.A.): One cycle of 4 °C for 10 min; 30 cycles of 94 °C for 1 min and 65 °C for 2 min and 72 °C for 1.5 min. The PCR products were purified with Microcon-100 column (Millipore, Bedford, MA, U.S.A.). The DNA sequencing of the region was determined directly using primer MA1 with Sequenase 7-deaza-dGTP sequencing kit (Amersham, Cleveland, OH, U.S.A.) in accordance with standard protocol,[14d)] or with Thermo sequence DNA polymerase (Amersham Pharmacia Biotech, Inc. Piscataway, NJ, U.S.A.) on auto sequencer (DSQ-2000L, Shimadzu, Kyoto, Japan) in according with the manufacturer's instructions.

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