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Synthesis and antibacterial activity of C-6 carbamate ketolides, a novel series of orally active ketolide antibiotics ☆

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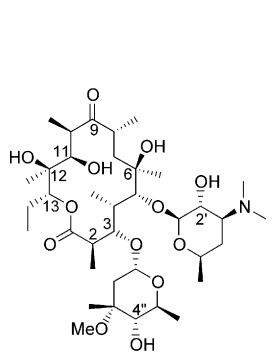
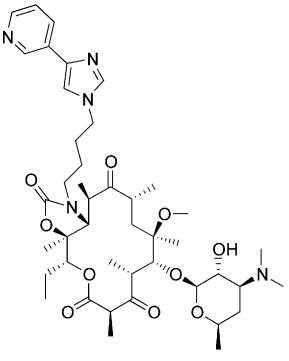
Abstract—A new series of antibacterial ketolides is reported, which features the use of a C-6 carbamate for tethering the arylalkyl sidechain to the macrolide core. The best members of this series display in vitro and in vivo activity comparable to telithromycin. Partial epimerization at C-2, unobserved in previously reported ketolides, was noted for this series.

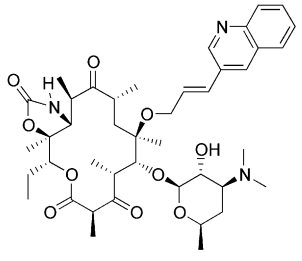
Since its discovery in the 1950s the macrolide antibiotic erythromycin A (**1**) has served as both a clinically useful agent for the treatment of Gram-positive bacterial infections as well as a starting point for the semi-synthesis of derivatives with improved physiochemical and microbiological properties.[1,2] Several of these derivatives, including clarithromycin, azithromycin, and roxithromycin, possess improved acid stability and oral bioavailability and have enjoyed significant commercial success. However, a serious problem that is not adequately addressed by these agents is the growing prevalence of erythromycin-resistant bacteria.[3] The discovery of the ketolides, erythromycin derivatives incorporating a C-3 ketone modification, revealed a class of compounds with excellent activity against some macrolide-resistant bacteria, especially the clinically important respiratory tract pathogen *Streptococcus pneumoniae*.[4,5] The considerable promise shown by ketolides has catalyzed a resurgence in macrolide antibiotic research in the pharmaceutical industry.[6] The first ketolide to reach the clinic, telithromycin (**2**) from Aventis Pharma, was approved for marketing in the EU in 2001 and the US in 2004, and a second ketolide, cethromycin (**3**) from Abbott, reached Phase III clinical trials in the US.



Erythromycin A (**1**)



Telithromycin (**2**)



Cethromycin (**3**)

Macrolides exert their antibacterial effect by binding to the bacterial ribosome and inhibiting protein synthesis. Whereas typical macrolides bind the bacterial ribosome only in domain V, the arylalkyl sidechain of ketolides

Keywords: Ketolide; Carbamate; Antibiotics; Resistant bacteria.
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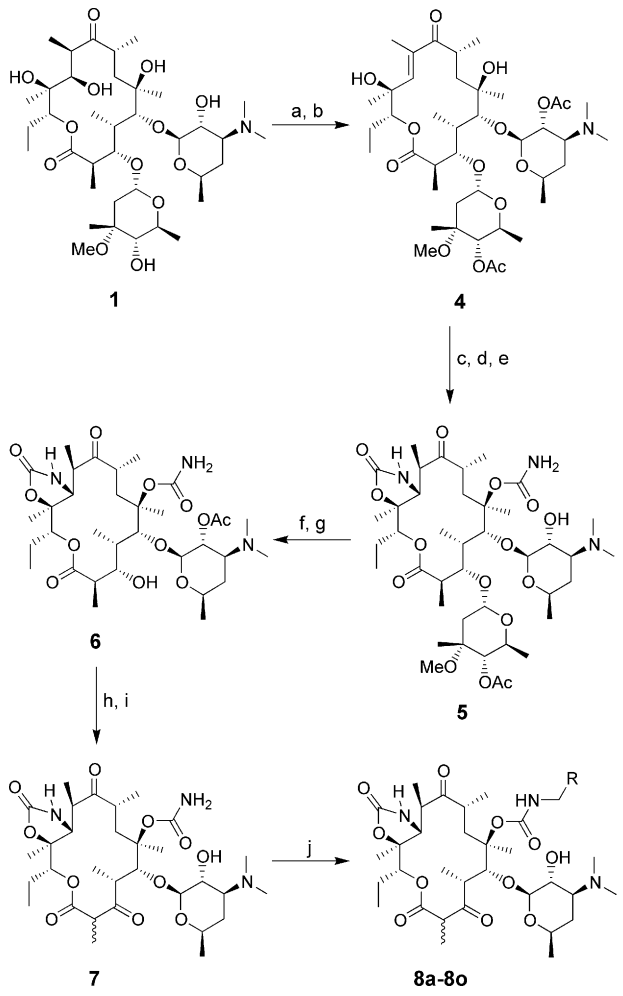
makes an additional binding contact in domain II, which appears to account for the improved activity of ketolides against resistant bacteria with modified ribosomes.[7] Comparison of the telithromycin and cethromycin structures indicates that this sidechain can be attached to the ketolide core through the C-11,C-12 cyclic carbamate or the C-6 oxygen with comparable results. As part of a program aimed at preparing new ketolides, we recognized that the C-6 oxygen offered the possibility of alternative modes of attaching the sidechain other than the ether linkage that had been explored en route to cethromycin. As it had previously been demonstrated that the highly hindered tertiary hydroxyl at C-6 of nonketolide erythromycin derivatives could be carbamoylated with acyl or sulfonyl isocyanates,[8] we reasoned that a series of ketolides having the sidechain attached through a C-6 carbamate linkage should be synthetically feasible. Furthermore, it appeared that incorporation of a carbamate linkage would allow us to combine structural novelty with the potential for a streamlined synthesis if the C-6 carbamate could be installed concurrent with C-11,C-12 cyclic carbamate formation. In this letter, we report the successful implementation of this strategy resulting in a series of C-6 carbamate ketolides with excellent in vitro and in vivo activity.[9–11]

Commercially available erythromycin A was converted into the target compounds by a sequence of ten steps (Scheme 1). Conversion of **1** to its 2′,4″,11-triacetate with acetic anhydride and catalytic DMAP[12] conveniently provided protection of the sugar hydroxyls while activating the C-11 hydroxyl toward β-elimination. This elimination was best achieved in the presence of the strong base sodium hexamethyldisilazide to yield **4**.[13] The C-6 and C-12 hydroxyls were then carbamoylated concurrently by treatment of **4** with trichloroacetylisocyanate.[8] Subsequent hydrolysis of the trichloroacetyl groups resulted in spontaneous cyclization of the liberated C-12 carbamate to generate the C-11,C-12 cyclic carbamate as a ~2:1 mixture of *S* and *R* epimers at C-10 along with the loss of the acetyl protecting group at C-2′. Complete equilibration of this mixture of C-10 epimers to the more stable C-10 *R* epimer **5** was achieved by exposure to potassium *t*-butoxide. The C-2′ acetate was reinstalled and the cladinose sugar was then cleaved by aqueous acid to yield **6**. Oxidation of the C-3 hydroxyl using modified Pfitzner–Moffat conditions[14] was followed by methanolysis of the C-2′ acetate to provide the unsubstituted ketolide **7**. The side chain was then selectively attached to the C-6 carbamate by reductive alkylation of **7** with aldehydes in the presence of triethylsilane and trifluoroacetic acid[15] to provide the target compounds **8a**–**o**. No over-alkylation at either the C-6 carbamate or the C-11,C-12 cyclic carbamate occurred under these conditions and the reaction tolerated a variety of heterocyclic aldehydes.[16]

With compounds **7** and **8a**–**o** in hand, an interesting observation was made regarding the stereochemical configuration of the C-2 methyl group. Despite the potential lability to epimerization of the β-keto ester moiety, telithromycin apparently exists exclusively in the natural C-2 *R* configuration. In compounds **7** and **8a**–**o**, however, we observed the presence of 10–15% of a minor constituent that was not present before C-3 oxidation and which was postulated to be the C-2 *S* epimer. ^{1}H NMR monitoring of solutions of **7** and **8j** in methanol-d_4 over 4 h showed the complete incorporation of deuterium at C-2, which provided evidence of facile proton removal at that position. Although this proton abstraction is a necessary condition for the presence of C-2 epimers, it is evidently not a sufficient condition because we also observed telithromycin to undergo deuteration under the same conditions, and yet it remained as a single isomer. Thus, further proof through isolation and characterization of the two components was deemed necessary, and although the major and minor constituents in **7** and **8a**–**o** were not easily separable by flash chromatography on silica gel, in one case (**8j**) they were separated by semi-preparative reverse phase HPLC. The purified components exhibited sufficient configurational stability in deuteriochloroform to allow for their complete structural assignment by NMR.



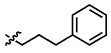
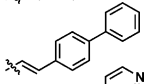
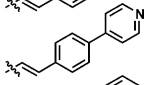
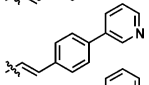
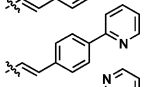
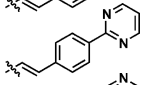
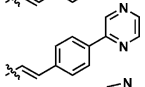
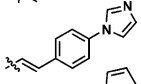
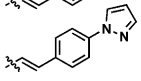
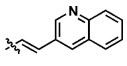
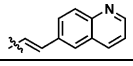
Scheme 1. Synthesis of C-6 carbamate ketolides. Reagents and conditions: (a) Ac_2O, Et_3N, DMAP, CH_2Cl_2, rt, 18 h; (b) NaHMDS, THF, 0 °C, 2 h; (c) $Cl_3CC(O)NCO$, CH_2Cl_2, 0 °C, 3 h; (d) Et_3N, H_2O, CH_3OH, reflux, 2 h; (e) KO*t*-Bu, THF, 0 °C to 15 °C, 6 h; (f) Ac_2O, Et_3N, CH_2Cl_2, rt, 20 h; (g) HCl, EtOH, H_2O, rt, 20 h, 30% overall from **1**; (h) EDCI, pyr·TFA, DMSO, CH_2Cl_2, 0 °C, 2 h; (i) CH_3OH, rt, 24 h, 74% for 2 steps; (j) RCHO, Et_3SiH, TFA, CH_3CN.

A combination of COSY, HMBC, and HMQC experiments confirmed the epimeric nature of the compounds by virtue of their identical connectivities, and NOESY experiments established the identity of the major epimer as C-2 *R* and the minor as C-2 *S*.[17] The isolated minor epimer of **8j** was observed to convert back to an equilibrium mixture of C-2 epimers on standing in methanol solution for 3–4 days. As a result of difficulties in separation of the epimers and their ease of equilibration, compounds **7** and **8a**–**o** were tested in all subsequent assays as epimeric mixtures.

The in vitro antibacterial activity of the C-6 carbamate ketolides was assessed against a panel of bacteria by determining their minimal inhibitory concentrations (MICs) by the broth microdilution method according to NCCLS standards.[18] Data for seven strains are presented in Table 1. *Staphylococcus aureus* (Smith) OC4172 and *S. pneumoniae* OC9132 are erythromycin-susceptible strains. *S. haemolyticus* OC3882 and OC4545 are isogenic strains possessing an *erm*(C)-encoded ribosomal methylase and are, respectively, inducibly or constitutively resistant to erythromycin A. *S. pneumoniae* OC4051 has an *erm*(B)-encoded ribosomal methylase and *S. pneumoniae* OC4421 has a *mef*(A)-encoded efflux pump; both are erythromycin-resistant. *Haemophilus influenzae* OC4882, an important respiratory pathogen with no approved NCCLS susceptibility breakpoint versus erythromycin A,[18] was also included in the testing panel. For *S. aureus* (Smith), the MIC determination was additionally conducted in the presence of 50% mouse serum to gauge the effect of serum proteins on in vitro activity.

In general, the unsubstituted parent compound **7** showed decreased activity relative to erythromycin A. A notable exception to this trend was observed against the *mef*(A)-containing *S. pneumoniae* strain where **7** was

Table 1. In vitro antibacterial activity of C-6 carbamate ketolides[a]

Cmpd	R	MIC (μg/mL)							
		S. aureus	*S. aureus* (+serum)	*S. haemo.* erm_i(C)	*S. haemo.* erm_c(C)	*S. pneum.*	*S. pneum.* *erm*(B)	*S. pneum.* *mef*(A)	*H. inf.*
EryA	—	0.5	0.06	>16	>16	0.06	>16	4	8
Telith	—	0.12	0.5	0.25	>16	⩽0.015	0.06	0.12	2
7	—	4	1	>16	>16	2	>16	2	8
8a		2	1	>16	>16	0.03	8	0.06	8
8b		4	8	>16	>16	1	8	0.25	16
8c		1	2	4	>16	0.25	2	0.12	8
8d		2	8	2	>16	0.06	1	0.12	16
8e		0.25	2	4	>16	0.06	0.5	0.06	4
8f		0.5	2	1	>16	0.06	0.5	0.25	8
8g		0.5	2	2	>16	0.03	0.5	0.12	8
8h		0.25	1	1	>16	0.03	0.12	0.25	4
8i		0.12	0.5	0.12	>16	0.03	0.06	0.06	4
8j		0.12	0.25	0.12	>16	0.03	0.06	0.06	4
8k		0.12	0.25	0.25	>16	0.03	0.06	0.12	4
8l		0.12	0.25	1	>16	⩽0.015	0.12	0.25	4
8m		0.12	0.25	0.12	>16	0.03	0.06	0.06	4
8n		0.25	0.5	0.25	>16	⩽0.015	0.03	0.06	4
8o		0.12	0.5	0.25	>16	⩽0.015	0.03	0.03	2

[a] Strain abbreviations: *S. aureus* OC4172; *S. aureus* OC4172 in the presence of 50% mouse serum; *S. haemolyticus* OC3882; *S. haemolyticus* OC4545; *S. pneumoniae* OC9132; *S. pneumoniae* OC4051; *S. pneumoniae* OC4421; *H. influenzae* OC4882. See text for detailed description.

twofold more potent than erythromycin A. Furthermore, **7** showed no decrease in activity against this strain relative to the macrolide-susceptible strain whereas erythromycin A showed a 64-fold decrease. This insensitivity of **7** (and **8a**–**o**, vide infra) to efflux by the *mef*(A)-encoded pump is in accordance with literature precedence for other ketolides.[4,5] By analogy to other ketolide series, it was anticipated that attaching one or more aryl rings to **7** would lead to an improvement in activity, particularly against the *erm*-containing strains.

Initially, a series of phenyl-substituted compounds **8a**–**e** was prepared to identify the optimum linker between the aryl ring and the C-6 carbamate. It was found that compounds with the longer phenylpropyl (**8c**), phenylbutyl (**8d**), and phenylpropenyl (**8e**) sidechains were superior to those with the shorter phenylmethyl (**8a**) and phenylethyl (**8b**) sidechains. In particular, **8c**–**e** showed good activity against the *erm*(B)-containing strain of *S. pneumoniae* and the inducibly resistant *S. haemolyticus* strain, whereas both **8a** and **8b** were ineffective against the inducible *S. haemolyticus* strain and only marginally active against the *erm*(B)-containing *S. pneumoniae*. All five of the compounds exhibited improved activity against both the erythromycin-susceptible and the *mef*(A)-containing *S. pneumoniae* strains, when compared to the *erm*(B)-containing strain. As with telithromycin, none of the compounds showed measurable activity against the constitutively resistant *S. haemolyticus*. Overall, **8e** appeared to have the best profile, being the most potent of the five against *S. aureus*, *H. influenzae*, and the *erm*(B)-containing *S. pneumoniae*.

Having identified the propenyl linker in compound **8e** as optimal, we prepared a series of compounds in which an additional aromatic ring was appended to that template to further probe the SAR. The biphenyl analog **8f** showed no improvement over **8e**, but more favorable results were anticipated when heteroaryl rings were incorporated, as was generally observed in the 6-O-allyl series.[5] To this end, a series of pyridinylphenyl analogs **8g**–**i** was prepared and the activity was found to depend markedly on the point of attachment of the pyridine ring. Thus, the activity of the (4-pyridinyl)phenyl isomer **8g** was nearly identical to the biphenyl analog **8f**, the activity of the (3-pyridinyl)phenyl isomer **8h** was in general 2-fold better, and that of the (2-pyridinyl)phenyl isomer **8i** was at least an additional 2-fold better against most strains. In fact, compound **8i** has an in vitro profile that compares favorably with telithromycin. The excellent in vitro activity of **8i** was mirrored in the structurally related (2-pyrimidinyl)phenyl analog **8j** and pyrazinylphenyl analog **8k**.

The tolerance of this scaffold for modification of the heterocycle was further explored by incorporating five-membered heterocycles, resulting in the imidazolylphenyl analog **8l** and pyrazolylphenyl analog **8m**. Additionally, the heteroaryl-phenyl motif was replaced with bicyclic aromatic heterocycles to provide the 3-quinolinyl analog **8n** and the 6-quinolinyl analog **8o**. All four of these compounds were comparable to **8i**–**k**, with the exception of the elevated MIC for **8l** against the inducibly resistant *S. haemolyticus* strain. Thus, we observed that variation of the heterocycle had a significant effect on in vitro activity but that it was nonetheless possible to obtain excellent in vitro activity with a variety of different heterocycles as seen in compounds **8i**–**o**. It is interesting to note that the biarylpropenyl sidechains of compounds **8i**–**o**, although resembling the sidechain of cethromycin, are in fact longer because of the presence of the carbamate moiety.

Table 2. Preliminary in vivo assessment of selected compounds against *S. aureus* in a murine lethal infection assay[a]

Cmpd	% Survival	
	s.c. (10 mg/kg)	p.o. (20 mg/kg)
8i	80	20
8j	100	40
8k	100	62
8l	60	0
8m	80	60
8n	75	25
8o	40	0

[a] The number of animals per dose ranged from 5 to 8.

The potential of compounds **8i**–**o** to exhibit antibacterial activity in vivo was initially assessed by screening them in a *S. aureus* murine lethal systemic infection model.[19] The survival rates at a single concentration after subcutaneous or oral administration are presented in Table 2. The most striking finding was that compounds with relatively basic heterocycles such as **8i**, **8l**, **8n**, and **8o** performed poorly (survival rates of 25% or less) when administered orally although all showed some protection when dosed subcutaneously. In contrast, the compounds with less basic heterocycles such **8j**, **8k**, and **8m** showed good protective effects by either route of administration. The incompatibility of basic heterocycles with oral efficacy in this series is surprising in view of the demonstrated oral efficacy of telithromycin and cethromycin, both of which contain basic heterocycles.

The best compounds (**8j**, **8k**, and **8m**) identified by this initial screen were selected for more rigorous evaluation in the *S. aureus* murine model and the ED_{50} values that were generated are presented in Table 3 along with values for telithromycin.[20] All three of the compounds were highly efficacious when dosed subcutaneously, providing ED_{50} values that are essentially equivalent to telithromycin. When dosed orally, however, **8m** displayed a fairly flat dose response (data not shown) and achieved only a 38% survival rate at 30 mg/kg. In contrast, when **8j** (JNJ-17069546) and **8k** (JNJ-17070885)

Table 3. ED_{50} determinations for selected compounds against *S. aureus* in a murine lethal infection assay

Cmpd	*N*	ED_{50} (mg/kg)[a]	
		s.c.	p.o.
8j	8	1.8 (0.5–3.1)	15 (8.9–20)
8k	12	4.8 (2.9–7.7)	17 (11–25)
8m	8	7.1 (4.7–11)	>30
Telith	15	4.4 (3.1–6.0)	15 (9.5–20)

[a] Numbers in parentheses indicate 95% confidence limits.

were dosed orally they were found to be as efficacious as telithromycin with ED_{50} values of 15 and 17 mg/kg, respectively, in comparison to a value of 15 mg/kg for telithromycin. Thus, the (2*E*)-3-[4-(2-pyrimidinyl)-phenyl]-2-propenyl and (2*E*)-3-(4-pyrazinylphenyl)-2-propenyl sidechains were found to provide good oral activity in mice to complement their excellent in vitro profiles.

In conclusion, we have discovered a novel series of ketolide antibiotics that employ a C-6 carbamate for attachment of the heteroaryl sidechain. Optimization of the sidechain led to compounds with in vitro and in vivo activity that is on par with telithromycin. Although structurally similar to 6-O-alkyl ketolides, this series displayed optimal activity with sidechains that are longer and less basic than the cethromycin sidechain. Further investigations into this and related series will be reported in the future.

Supplementary data

^{1}H NMR data and copies of NOESY spectra for both **8j** epimers.

Acknowledgements

Contributions to in vitro testing by Ellyn Wira and to in vivo testing by John Melton and Steven Stryker are gratefully acknowledged. The authors also wish to thank Mary Evangelisto for the HPLC separation and Amy Maden for the NMR structure determination of the C-2 epimers. Preparation of advanced intermediates by Dr. Xun Li is also gratefully acknowledged.

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13. An alternative synthesis of compound **4** has appeared (see Ref. 11b) that involves bis-acetylation of the known 2′,4″-hydroxy derivative, itself prepared in two steps from erythromycin A via elimination of a C11,C12-cyclic carbonate: (a) Hauske, J. R.; Kostek, G. *J. Org. Chem.* **1982**, *47*, 1595; (b) A four-step synthesis of the 4″-formate analog involving elimination of a C11-methanesulfonate is also known: Tadanier, J.; Martin, J. R.; Egan, R. S.; Goldstein, A. W.; Stanaszek, R. S.; Hirner, E.; Fischer, F. *J. Org. Chem.* **1974**, *39*, 2495.
14. Bright, G. M.; Nagel, A. A.; Bordner, J.; Desai, K. A.; Dibrino, J. N.; Nowakowska, J.; Vincent, L.; Watrous, R. M.; Sciavolino, F. C.; English, A. R.; Retsema, J. A.; Anderson, M. R.; Brennan, L. A.; Borovoy, R. J.; Cimochowsky, C. R.; Fiaella, J. A.; Girard, A. E.; Girard, D.; Herbert, C.; Manousos, M.; Mason, R. *J. Antibiot.* **1988**, *41*, 1029.
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16. For syntheses of the various aldehydes, see Ref. 10.
17. The most instructive piece of data was the presence of a NOESY cross-peak between the C-2 and C-5 protons in the minor isomer that was not observed in the major isomer (see Supplementary data). Examination of molecular models suggested reasonable conformations could be generated for the minor isomer, but not for the major isomer, which would place these two protons in sufficient proximity to observe an NOE.
18. National Committee for Clinical Laboratory Standards. *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically*, 5th ed.; Approved standard: NCCLS Document M7-A5, 2000.
19. Female Swiss-Webster mice were infected ip with approximately 6×10^5 CFU/mL of *S. aureus* Smith. Compounds were administered 1 h post-infection in 40 mM sodium citrate buffer, pH 5, and survival was monitored for 3 days.
20. The protocol of Ref. 19 was followed at a range of doses, and the dose allowing survival of 50% of the animals (ED_{50}) was calculated using the Logistic routine of the SAS suite of programs.