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Synthesis and antibacterial activity of 6-*O*-heteroarylcarbamoyl-11,12-lactoketolides

Eugene B. Grant,* Deodialsingh Guiadeen, Darren Abbanat, Barbara D. Foleno, Karen Bush and Mark J. Macielag

Johnson & Johnson Pharmaceutical Research & Development, L.L.C., 1000 Route 202, PO Box 300, Raritan, NJ 08869, USA

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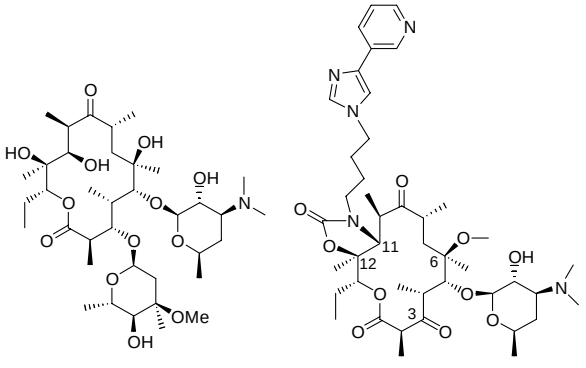
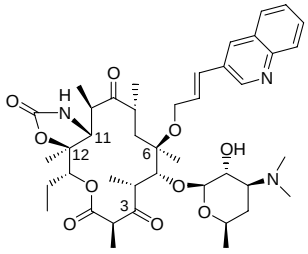
Abstract—A new series of erythromycin A derivatives, the 6-*O*-heteroarylcarbamoyl-11,12-lactoketolides, with activity against macrolide-resistant streptococci, are described. Structurally, these macrolide antibiotics are characterized by a heteroaryl side chain attached to the macrolactone core through a carbamate linkage at the C6 position, as well as 11,12-γ-lactone and 3-keto functionalities. The synthesis and antibacterial activity of this new series of ketolides are discussed.

The macrolide antibiotics, erythromycin A (**1**), clarithromycin, and azithromycin, have been widely prescribed to treat respiratory tract infections.[1] Due to their widespread use, resistance in the important respiratory pathogen, *Streptococcus pneumoniae*, has emerged at an alarming rate.[2] The primary mechanisms of resistance in *S. pneumoniae* are ribosomal methylation (catalyzed by the *erm* methylase) and intracellular removal of the macrolide by efflux (mediated by the *mef*-gene product).[3] As the prevalence of erythromycin resistance has increased, the interest in discovery of new, more effective macrolide antibiotics with activity against resistant respiratory pathogens has intensified.

The ketolides, which include telithromycin (**2**)[4] and cethromycin (**3**),[5] are the latest generation of semisynthetic macrolide antibiotics, with improved activity against erythromycin- and penicillin-resistant isolates of *S. pneumoniae*. Telithromycin (**2**) has demonstrated clinical efficacy against most erythromycin-susceptible and -resistant strains of *S. pneumoniae*, *Streptococcus pyogenes*, and *Haemophilus influenzae*, and is marketed for the treatment of community-acquired upper and lower respiratory tract infections.[6] Cethromycin has progressed to late stage clinical development.[5]

Telithromycin (**2**) and cethromycin (**3**) share certain structural features, including an 11,12-cyclic carbamate functionality and a 3-keto group. The cyclic carbamate appears to play a conformational role in ribosomal





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* Corresponding author. Tel.: +1 908 218 6589; e-mail: egrant@prdus.jnj.com

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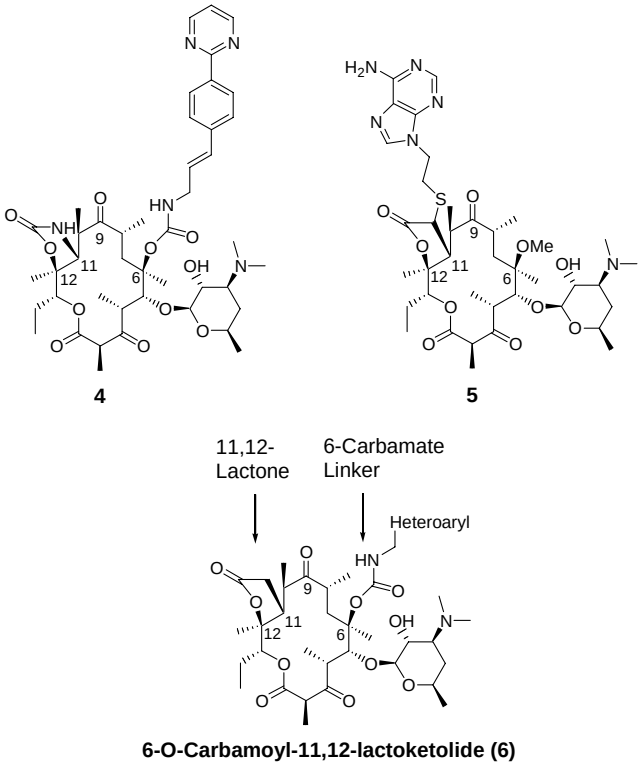
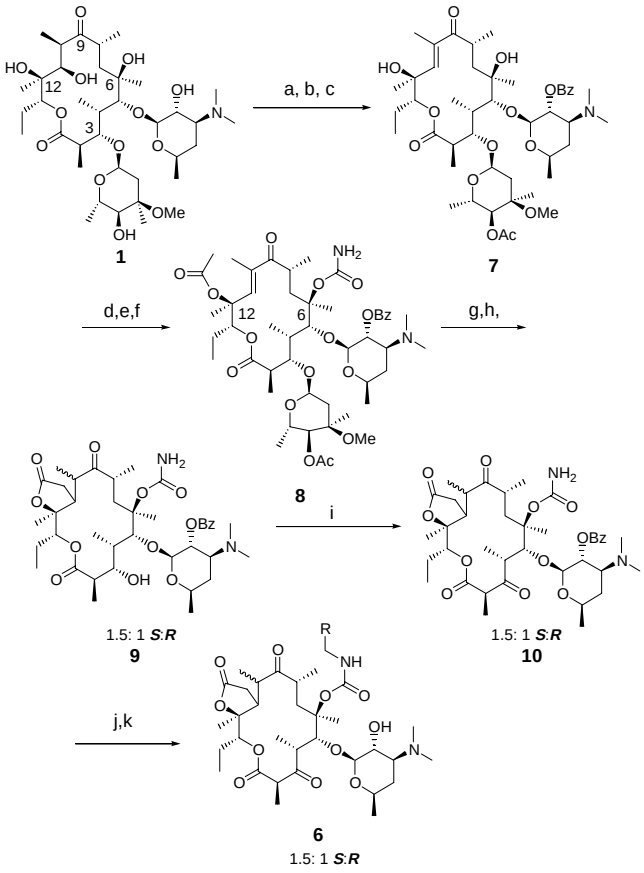
binding, whereas the ketone is important in circumventing efflux resistance. Both **2** and **3** contain heteroarylalkyl side chains that are critical in overcoming resistance due to ribosomal methylation. Although the side chains are tethered to different positions of the macrolactone nucleus (i.e., at the carbamate nitrogen and O6, respectively), structural studies have shown that they bind to similar sites within domain II of the bacterial ribosome.[7]

Recent structure–activity relationship (SAR) studies have shown that a variety of synthetic modifications of the basic ketolide core structure can provide analogs with similar activity as **2** and **3** against erythromycin-susceptible and -resistant respiratory pathogens. In particular, researchers at Johnson & Johnson Pharmaceutical Research & Development have reported on a novel series of ketolides, represented by **4**, in which the heteroarylalkyl group is attached to the macrolactone ring by a C6-carbamate linkage.[8] In addition, scientists at Roche and Basilea have described a new series of ketolides with a fused five-membered lactone ring in place of the cyclic carbamate.[9] Compound **5** has an antimicrobial spectrum similar to that of **2**. We were interested in the effect on antibacterial activity of combining these two structural modifications in a single molecule. Herein, we report the synthesis and antibacterial activity of a new series of macrolide antibiotics, the 6-*O*-carbamoyl-11,12-lactoketolides represented by structure **6**.

The synthesis of **6** started with commercially available erythromycin A (**1**) (Scheme 1). Selective acylation of the 2′ hydroxyl group of **1** with benzoic anhydride was followed by exhaustive acetylation to provide the 4″,11-diacetate. Elimination of the C11-acetate under basic conditions afforded the 10,11-anhydroerythromycin A derivative **7**. Selective acetylation of the 12-hydroxyl group was readily accomplished upon treatment with acetic anhydride in pyridine containing a catalytic amount of dimethylaminopyridine. Carbamoylation of the C-6 hydroxy with trichloroacetylisocyanate, followed by treatment with 10% aqueous sodium hydroxide, afforded primary carbamate **8** in good yield.[8] Ester **8** was treated with 5 equivalents of lithium diisopropylamide in THF at −78 °C, and the resulting heterogeneous mixture was allowed to warm to 0 °C over 5 h. Following acidic workup that effected the removal of the cladinose sugar, and careful chromatography, alcohol **9** was isolated as a 1.5:1 inseparable mixture of C10-epimers in 30% yield. With the core structure prepared, alcohol **9** was converted to ketolide **10** by Pfitzner–Moffatt oxidation. Attempts to epimerize the C10-methyl group of **9** by treatment with the equilibrating base potassium *tert*-butoxide, the strong base lithium diisopropylamide, or simply by stirring in methanol were unsuccessful,[9] with no change in the ratio of diastereomers. At this point, the stereochemical outcome of the intramolecular conjugate enolate addition was studied further.[10] While the selective attack of the C12-ester enolate of **8** to the C11-position must

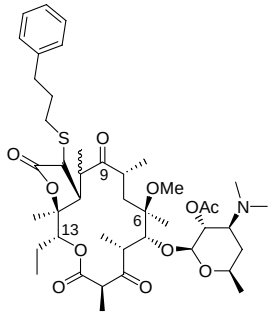




Scheme 1. Synthesis of 6-*O*-carbamoyl-11,12-lactoketolides. Reagents and conditions: (a) Bz_2O, Et_3N, CH_2Cl_2, rt, 18 h; (b) Ac_2O, Et_3N, DMAP, CH_2Cl_2, rt, 18 h; (c) NaHMDS, THF, 0 °C, 2 h; (d) Ac_2O, Et_3N, DMAP, pyridine, rt, 18 h; (e) $Cl_3CC(O)NCO$, CH_2Cl_2, 0 °C, 3 h; (f) 10% aq NaOH, rt, 2 h; (g) LDA, THF, −78 to 0 °C, 5 h; (h) HCl, EtOH, H_2O, rt, 20 h; (i) EDCI, pyr.TFA, DMSO, CH_2Cl_2, 0 °C, 3 h; 12% overall yield from **1**; (j) RCHO, Et_3SiH, TFA, CH_3CN; (k) MeOH, reflux, 12 h 14–25%.

occur from the *Si*-face of the double bond leading to the 11-(*S*) absolute configuration,[11] the stereochemistry at C10 could not be predicted a priori. A small amount of the major diastereomer of **9** could be isolated by RP-HPLC and the absolute configuration at C10 was investigated by NMR. After assignment of the resonances by COSY, HETCOR, and HMBC, NOESY experiments revealed the unnatural (*S*) stereochemistry at C10, with C2, C11, and C12 retaining the expected *R*, *S*, and *R* absolute configurations, respectively.[12] Additional support for this structural assignment was provided by the NMR data for the C10-diastereomers of compound **11**,[9] an intermediate in the synthesis of a ketolide lactone analog from Roche/Basilea. In **11**, the chemical shift of the proton at C13 appeared at 5.01 ppm in the unnatural C10-(*S*) diastereomer and at 5.53 ppm in the natural C10-(*R*) diastereomer. In the case of **9**, the chemical shift of the C13-proton appeared at 5.10 ppm, again suggesting the (*S*)-configuration at C10. The heteroaryl side chain was appended to the primary carbamate of **9** (used as a 1.5:1 mixture of *S*:*R* diastereomers) by treatment with an appropriately substituted aldehyde in the presence of trifluoroacetic acid and triethylsilane in acetonitrile at 80 °C for 12 h. This was followed by methanolysis of the 2′-benzoyl protecting group to afford a 10–25% yield of heteroaryl-substituted **6** as a mixture of epimers.

As expected, the diastereomeric ratio observed for **9** remained constant after alkylation and deacetylation to afford **6a**–**l**. Generally, Attempts to separate the diastereomers of **6a**–**l** by chromatography were unsuccessful; thus, these analogs were tested as a 1.5:1 mixture of *S*:*R* isomers at C10.



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In vitro antibacterial activities of ketolides **6a**–**l** were established against a five-membered panel of bacteria, including erythromycin-susceptible and -resistant strains. Minimum inhibitory concentrations (MIC) were determined by the NCCLS-approved broth microdilution method and are reported in Table 1.[13] Twofold differences are considered to be within the error of the method. Among the strains tested, *Staphylococcus aureus* (Smith) OC4172 and *S. pneumoniae* ATCC6301 are erythromycin-susceptible, *S. pneumoniae* OC4051 is erythromycin-resistant due to an *erm*(B)-encoded ribosomal methylase, and *S. pneumoniae* OC4421 is erythromycin-resistant due to a *mef*(A)-encoded efflux-pump. A representative strain (OC4882) of the important Gram-negative respiratory pathogen *H. influenzae* was also included in the testing panel. For *S. aureus* (Smith), MIC values were determined in the absence and presence of 50% mouse serum to assess the effect of serum proteins on antibacterial activity in the ketolides.

Activities of the ketolides against the susceptible *S. aureus* and *S. pneumoniae* strains were essentially independent of the composition of the heteroaryl side chain, with MIC values ranging from 0.25 to 1 μg/ml and $\leqslant$0.015 to 0.06 μg/ml, respectively, comparable to those of erythromycin and telithromycin. MIC values of the ketolides against the *mef*(A)-containing *S. pneumoniae* strain were 8- to 16-fold lower than for erythromycin, but once again the structure of the heteroaryl side chain had little impact on the level of activity.

In contrast, the side-chain substituent had a more dramatic effect on activity against the *erm*(B)-containing *S. pneumoniae* strain. Generally, lower MIC values were observed for ketolides containing a biaryl- or a fused heteroarylpropenyl substituent than for analogs with a single aromatic ring in the side chain (**6i**) or with a benzylic linker (**6a** and **b**). Quinoline analogs **6c**, **h**, and **j** had comparable activity to telithromycin (**2**) against this strain.

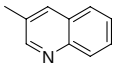
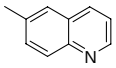
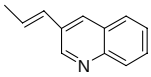
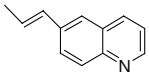
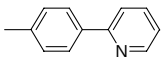
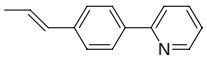
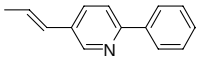
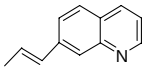
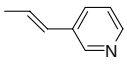
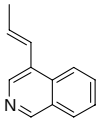
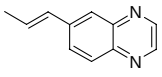
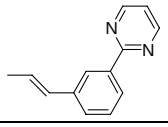
Activity against the Gram-negative respiratory pathogen, *H. influenzae*, was somewhat more capricious, with MIC values ranging from 2 μg/ml for the 6- and 7-quinolinyl analogs **6d** and **6h** to 16 μg/ml for pyridylphenyl analog **6e**. The ketolides with the best activity against *H. influenzae* (**6d**, **h**, **i**, **j**, **k**, and **l**) had similar MIC values as telithromycin (**2**).

An unanticipated property of this novel series of ketolides was the significant reduction of antibacterial activity (4- to 16-fold increase in MIC) for nearly all analogs in the presence of mouse serum, presumably due to binding to serum proteins. It was possible to reduce protein binding by decreasing the number of aryl rings in the side chain (**6i**), but as mentioned above this came at a cost of activity against the *erm*-containing *S. pneumoniae* strain.

In the case of the pyrimidinylphenyl analog **6m**, separation of the C-10 epimers was accomplished by HPLC in order to determine the effect of C-10 stereochemistry on antibacterial activity (Table 2). As expected from previous literature reports,[10] the MIC values of the lactoketolides were significantly influenced by C-10-stereochemistry, with MIC values for the (*S*)-isomer 2- to 32-fold higher than those for the natural (*R*)-isomer (compare (10*R*)-**6m** to (10*S*)-**6m**). It is noteworthy that the antibacterial activity of (10*R*)-**6m** was comparable to that of the corresponding analog from the ketolide series with the 11,12-cyclic carbamate (**4**),[8] but with a slight decrease in activity against the *mef*(A)-containing *S. pneumoniae* strain.

In conclusion, the SAR study of the heteroaryl-substituted lactoketolides (**6**) showed the importance of tether length, the nature of the heteroaromatic substituent, and C10-stereochemistry. As a class, the lactoketolides

Table 1. In vitro antibacterial activity of 6-*O*-heteroarylcarbamoyl-11,12-lactoketolides[a]

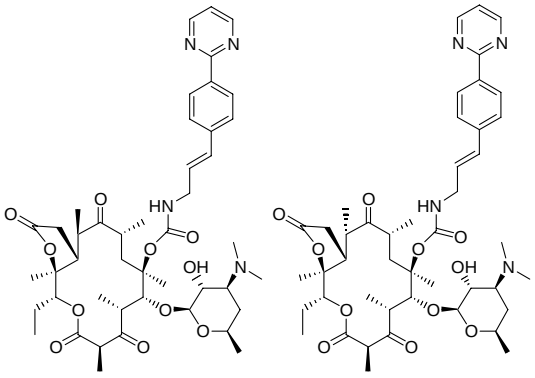
Compound	Heteroaryl	MIC (μg/mL)					
		S. aureus	*S. aureus* (+serum)	*S. pneum.*	*S. pneum. erm*(B)	*S. pneum. mef*(A)	*H. inf.*
1	—	0.5	0.12	0.06	>16	4	8
2	—	0.12	0.5	0.015	0.06	0.12	2
6a		0.5	4	0.06	0.5	0.5	8
6b		1	4	0.06	2	0.5	8
6c		0.5	2	⩽0.015	0.12	0.25	8
6d		0.5	2	0.03	0.12	0.25	2
6e		1	8	0.03	0.25	0.5	16
6f		0.5	2	0.06	0.25	0.5	8
6g		0.5	2	ND[b]	0.5	0.5	8
6h		0.25	4	0.03	0.12	0.25	2
6i		1	1	0.03	2	0.5	4
6j		0.5	4	⩽0.015	0.25	0.25	4
6k		0.5	2	0.03	0.25	0.5	4
6l		0.5	2	0.06	0.25	0.5	4

[a] *S. aureus*: *Staphylococcus aureus* (Smith) OC4172; *S. aureus* (+serum): *Staphylococcus aureus* (Smith) OC4172 in the presence of 50% mouse serum; *S.pneum.*: *Streptococcus pneumoniae* ATCC6301; *S. pneum. erm*(B): *Streptococcus pneumoniae* OC4051; *S. pneum. mef*(A): *Streptococcus pneumoniae* OC4421; *H. inf.*: *Haemophilus influenzae* OC4882. See text for detailed description.
[b] Not determined.

Table 2. In vitro antibacterial activity

Compound	MIC (μg/mL)					
	S. aureus	*S. aureus.* (+serum)	*S. pneum.*	*S. pneum. erm*(B)	*S. pneum. mef*(A)	*H. inf.*
1	0.5	0.12	0.06	>16	4	8
2	0.12	0.5	0.015	0.06	0.12	2
4	0.12	0.25	0.03	0.06	0.06	4
(10***R***)-**6m**	0.12	0.5	0.03	0.06	0.25	8
(10***S***)-**6m**	2	16	0.12	2	1	8

See Table 1 for detailed description of strains.



(10*R*)-6m (10*S*)-6m

appeared to be more highly protein bound than corresponding analogs bearing an 11,12-cyclic carbamate.[8] The best compounds in this series, however, possess in vitro antibacterial activity comparable to telithromycin (**2**). In preparing this new series of ketolides, the dual synthetic challenges of intramolecular 1,4-addition of an unsubstituted acetate in the presence of a base labile carbamate and selective acetylation of the C12-tertiary alcohol were overcome.

Acknowledgments

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