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Bioorganic & Medicinal Chemistry Letters 15 (2005) 883–887
Bioorganic & Medicinal Chemistry Letters
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Synthesis and antibacterial activity of C2-fluoro, C6-carbamate ketolides, and their C9-oximes

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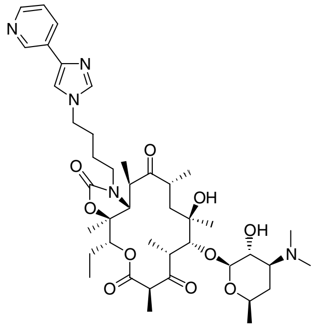
Received 23 November 2004; revised 20 December 2004; accepted 21 December 2004
Available online 21 January 2005

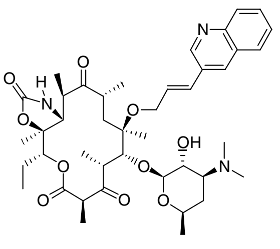
Abstract—Novel C6-carbamate ketolides with C2-fluorination and C9-oximation have been synthesized. The best compounds in this series displayed MIC values of 0.03–0.12 μg/mL against streptococci containing *erm* and *mef* resistance determinants and 2–4 μg/mL against *Haemophilus influenzae*. Several compounds also showed measurable activity against *erm*(B)-containing enterococci with MIC values of 2–8 μg/mL. In vivo activity was adversely affected by fluorination, possibly as a result of increased serum protein binding.

Macrolide antibiotics have been widely used as safe and effective drugs for treatment of upper and lower respiratory tract infections for over 40 years. Due to the increased prevalence of macrolide-resistant bacteria, considerable research effort has been devoted to the identification of next generation macrolide antibiotics with activity against resistant organisms.[1] Ketolides are synthetic derivatives of erythromycin A with a C3-ketone functionality and generally with a C11-, C12-cyclic carbamate. Potent antibacterial activities against both macrolide-susceptible and macrolide-resistant organisms have been achieved by incorporating an arylalkyl side chain into the molecule.[2] Structure–activity relationship (SAR) studies have demonstrated the importance of the heteroaromatic group linked to the erythronolide ring in overcoming macrolide-lincosamide-streptogramin B (MLS_B; *erm*) resistance and the 3-keto group in circumventing efflux (*mef*) resistance.[3] Two of the most advanced ketolides reported to date are telithromycin (Ketek®) from Sanofi-Aventis,[4] which was recently approved for marketing by the FDA and cethromycin (ABT-773) from Abbott Laboratories.[5] Further investigations with ketolides led to the design of analogs with a fluorine atom at the C2 position.[6] The C2-fluoro derivative of cethromycin has shown improved activity against macrolide resistant *Streptococcus pneumoniae* and *Streptococcus pyogenes*[6a] C9-oxime derivatives have also been reported for both nonfluorinated as well as fluorinated ketolides.[7] Some oximes displayed interesting activities against inducibly and constitutively resistant *S. pneumoniae*.[7b,c,g]

We recently identified a series of novel ketolides in which the heteroaryl group is attached to the macrolactone



Telithromycin



Cethromycin

Keywords: Ketolide; Carbamate; Oxime; Fluoro ketolide; Resistant bacteria.

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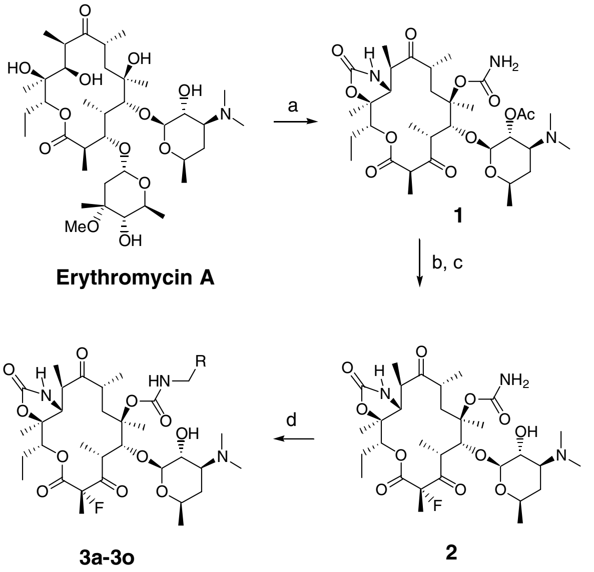
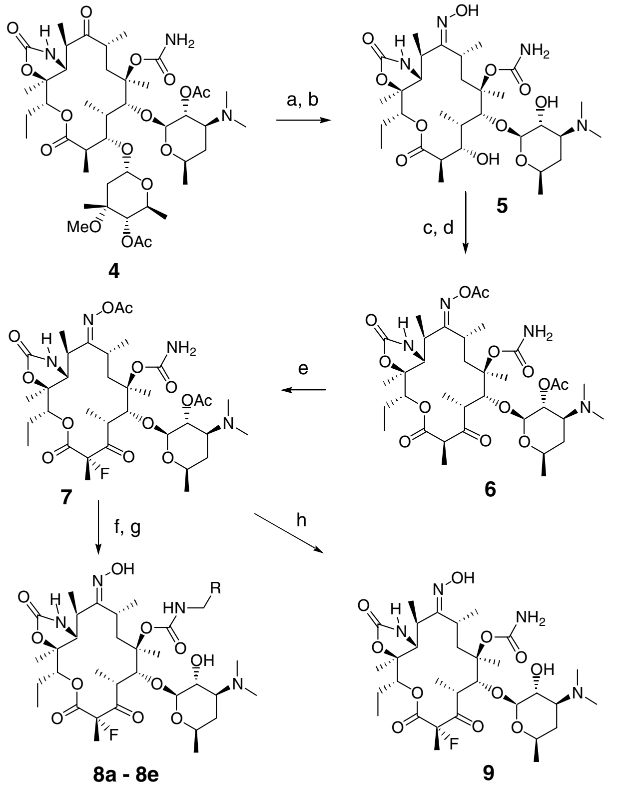
doi:10.1016/j.bmcl.2004.12.067



Scheme 1. Synthesis of C2-fluoro ketolides. Reagents: (a) eight steps, see Ref. 8; (b) NaHMDS, SELECTFLUOR™, THF; (c) MeOH, 40% two steps; (d) RCHO, Et_3SiH, TFA, CH_3CN, 35–80%.



Scheme 2. Synthesis of C2-fluoro, C9-oxime ketolides. Reagents: (a) NH_2OH, AcOH, *i*-PrOH; (b) HCl, EtOH, H_2O, 22% two steps; (c) Ac_2O, Et_3N, CH_2Cl_2; (d) EDCI, pyr·TFA, DMSO, CH_2Cl_2, 55% two steps; (e) NaHMDS, SELECTFLUOR™, THF, 99%; (f) RCHO, Et_3SiH, TFA, CH_3CN; (g) $NaHCO_3$, MeOH, 16–61% two steps; (h) $NaHCO_3$, MeOH, 71%.

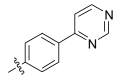
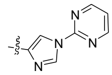
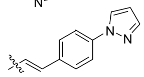
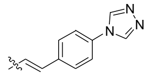
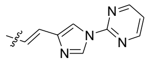
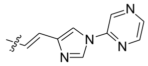
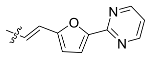
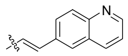
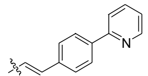
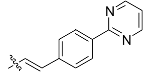
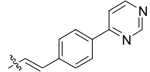
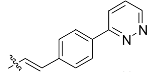
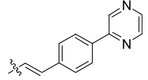
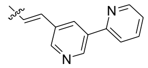
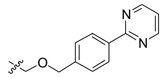
ring via a C6-carbamate linkage.[8] Compounds in this series have displayed antibacterial activity comparable to telithromycin against key respiratory pathogens, including erythromycin-resistant strains. As part of our continuing investigation of this novel ketolide class we have prepared C6-carbamate ketolides with C2-fluorination alone and in combination with C9-oximation. The synthesis, in vitro and in vivo activities of this series of ketolides are presented in this paper.

C2-Fluoro, C6-carbamate ketolides were obtained from commercially available erythromycin A by a sequence of 11 synthetic steps (Scheme 1). Intermediate **1** was prepared in eight steps as reported previously.[8] Treatment of **1** with sodium hexamethyldisilazide followed by SELECTFLUOR™ gave the C2-fluorinated ketolide compound **2** as a single diastereomer.[6] Other bases and fluorinating reagents were also explored without further improvement of yield. Removal of the C2′-acetate and attachment of the side chain by reductive alkylation with aldehydes in the presence of triethylsilane and trifluoroacetic acid[8,9] provided the target compounds **3a**–**o** in 35–80% yield.

Scheme 2 illustrates the synthesis of C6-carbamate ketolides with C2-fluoro and C9-oxime groups. Initial attempts to install the C9-oxime functionality at a late stage in the synthetic sequence (e.g., compounds **1**–**3**) gave very low conversion to the desired compound (<10%) even when heating at elevated temperatures with hydroxylamine for over a week. The reluctance of ketolides that possess a C11-, C12-cyclic carbamate to undergo oximation has been observed by others,[7c] in contrast to the corresponding cyclic carbazates which have been successfully oximated.[7c,10] Instead, the C9-oxime functionality was introduced prior to removal of the cladinose sugar by treatment of **4** with 50% aqueous hydroxylamine in the presence of acetic acid in isopropyl alcohol. The assignment of *E* stereochemistry of the oxime was based on ^1H NMR and literature precedent.[7b,11] Subsequent cleavage of the cladinose sugar gave compound **5** in 22% yield over two steps.[12] Diacetylation of **5** followed by oxidation at C3 yielded the protected ketolide **6**. Introduction of the C2-fluoro group provided **7**, which after installation of the side chain and removal of the protecting groups yielded the target compounds **8a**–**e**. Unsubstituted analog **9** was prepared directly from **7** by hydrolysis of the protecting groups under basic conditions.

The antibacterial activities of the C2-fluoro, C6-carbamate ketolides, and their C9-oximes were measured against both erythromycin-susceptible and erythromycin-resistant bacteria using erythromycin A and telithromycin as comparators. Broth microdilution MIC (lowest concentration of compound inhibiting visible growth) determinations were performed according to procedures established by the National Committee for Clinical Laboratory Standards.[13] Two-fold differences are considered to be within error of the method. Results are presented in Tables 1 and 2 for a set of six strains, including macrolide-susceptible *S. aureus* (Smith); *Enterococcus faecalis* with an *erm*(B)-encoded ribosomal methylase; three strains of *S. pneumoniae*, the first eryth-

Table 1. MICs (µg/mL) for C2-fluoro ketolides[a]

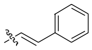
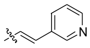
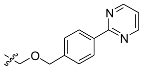
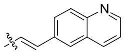
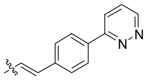
Compound	R	*S. aureus*	*S. aureus* (+ serum)	*E. faecalis erm*(B)	*S. pneum.*	*S. pneum. erm*(B)	*S. pneum. mef*(A)	*H. inf.*
EryA	—	0.5	0.06	>16	0.06	>16	4	8
Telith.	—	0.12	0.5	16	⩽0.015	0.06	0.12	2
2	—	1	0.5	>16	0.12	>16	2	8
3a		0.12	4	16	0.03	0.06	0.12	4
3b		0.25	8	>16	0.03	0.12	0.5	4
3c		0.25	2	8	0.03	0.12	0.5	8
3d		0.5	4	>16	0.06	0.12	0.5	8
3e		0.12	1	8	0.03	0.06	0.25	4
3f		0.25	8	16	0.03	0.06	0.25	8
3g		0.5	4	>16	0.06	0.12	0.5	16
3h		0.25	4	4	0.06	0.06	0.06	4
3i		0.25	2	4	0.06	0.06	0.12	4
3j		0.5	4	8	0.06	0.12	0.12	8
3k		0.25	4	>16	0.03	0.06	0.12	4
3l		0.12	2	4	0.03	0.06	0.12	4
3m		0.25	16	4	0.03	0.06	0.06	4
3n		0.25	4	4	0.03	0.06	0.12	8
3o		0.25	4	>16	0.06	0.12	0.12	8

[a] *S. aureus*: *Staphylococcus aureus* Smith OC4172 (erythromycin-susceptible); *S. aureus* (+ serum): *Staphylococcus aureus* Smith OC4172 in 50% mouse serum; *E. faecalis erm*(B): *E. faecalis* ATCC51299 (constitutively erythromycin-resistant); *S. pneum.*: *Streptococcus pneumoniae* OC9132 (erythromycin-susceptible); *S. pneum. erm*(B): *Streptococcus pneumoniae* OC4051 (ribosomal methylase-mediated resistance); *S. pneum. mef*(A): *Streptococcus pneumoniae* OC4421 (efflux-mediated resistance); *H. inf.*: *Haemophilus influenzae* OC4882.

romycin-susceptible, the second resistant to erythromycin A due to ribosomal methylation [*erm*(B)], and the third erythromycin-resistant due to efflux [*mef*(A)]; and the Gram-negative respiratory pathogen, *H. influenzae*. For *S. aureus* (Smith), MICs were also determined in the presence of 50% mouse serum to assess the effect of serum proteins on in vitro activity.

The unsubstituted parent C2-fluoro ketolide **2** had similar activity to erythromycin A against the organisms in the testing panel. The 16-fold increase in MIC for **2** against the efflux-resistant *S. pneumoniae* strain compared to the erythromycin-susceptible strain was surprising, in view of literature data indicating that ketolides are not good substrates for the Mef efflux pump.[3] Additionally, it stands in contrast to our previous results wherein the nonfluorinated congener of **2** displayed MICs of 2 µg/mL against both the erythromycin-susceptible strain and the *mef* strain, showing that it was intrinsically less active than erythromycin A but that its activity was not diminished by the presence of *mef*[8] Although intriguing, it is not clear whether these differences in MICs are truly reflective of differences in the compounds' susceptibilities to efflux because other

Table 2. MICs (μg/mL) for C2-fluoro, C9-oxime ketolides

Compound	R	*S. aureus*	*S. aureus* (+ serum)	*E. faecalis* *erm*(B)	*S. pneum.*	*S. pneum.* *erm*(B)	*S. pneum.* *mef*(A)	*H. inf.*
EryA	—	0.5	0.06	>16	0.06	>16	4	8
Telith.	—	0.12	0.5	16	⩽0.015	0.06	0.12	2
9	—	1	0.25	>16	0.12	2	2	4
8a		0.25	4	>16	0.03	0.12	0.06	2
8b		0.5	2	>16	0.06	0.12	0.5	2
8c		0.5	2	16	0.06	0.06	0.25	8
8d		0.25	8	2	0.03	0.03	0.12	2
8e		0.5	2	4	0.03	0.06	0.25	2

factors may be operative, especially since the two strains in question are not isogenic.

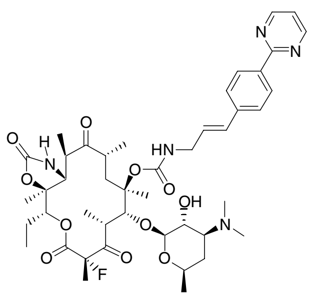
As expected from structure–activity relationship studies of the parent series,[8] attachment of aryl rings to the C6-carbamate functionality, as in compounds **3a**–**o**, significantly improved activity against *erm*(B)- and *mef*(A)-containing *S. pneumoniae* strains. Similarly to the unsubstituted compound **2**, for certain compounds (**3a**–**g**, **k**, **l**, **n**) a significant differential in the MIC values for the *mef* strain versus the erythromycin-susceptible strain was observed, whereas for the rest of the compounds (**3h**–**j**, **m**, **o**) little or no differences were observed between the two strains. Although there appears to be no obvious SAR trend in this regard, it is clear that the C3-ketone alone is not the only determinant of activity against *mef*(A)-containing *S. pneumoniae* strains and that the side chain has an influence as well.

Additional comparison of in vitro data for members of the present series with their nonfluorinated counterpart[8] shows that C2-fluorination generally reduced activities against *S. aureus*, *S. pneumoniae*, and *H. influenzae* strains by 2-fold. Interestingly and in contrast to the nonfluorinated series, some C2-fluoro analogs, especially in the propenyl linker series (e.g., **3h**, **i**, **l**–**n**), showed measurable activity against constitutively-resistant *erm*(B) enterococci, with MIC values as much as 4-fold lower than telithromycin.

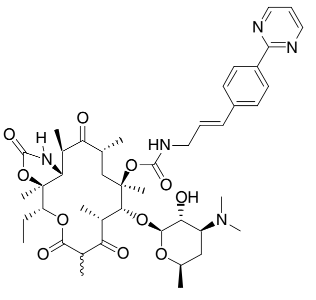
Conversion of the 9-ketone to the corresponding oxime was well-tolerated in this series of ketolides, with the C2-fluoro, C9-oximes having similar antibacterial profiles as the nonoximated analogs (compare **3h** vs **8d**, **3l** vs **8e**, and **3o** vs **8c**). Interestingly, the combination of oximation and fluorination produced measurable activity against the *erm*(B)-containing *S. pneumoniae* strain in the unsubstituted compound **9**. This remarkably good activity against *erm*(B)-containing *S. pneumoniae* for a compound lacking an aryl side chain did not translate into any substantial improvement in the substituted compounds **8a**–**e** relative to the C9-keto compounds. Activity against constitutively-resistant *erm*(B) enterococci was retained in those compounds (**8d** and **e**) whose 9-keto analogs had also shown such activity. In the oxime subseries, ketolides with a single aromatic ring in the side chain (**8a** and **b**) had comparable MIC values against *S. aureus*, *S. pneumoniae*, and *H. influenzae* strains, but meaningful activity against constitutively-resistant *erm*(B) enterococci was lost.

The most profound effect of C2-fluorination on antibacterial activity became evident upon determination of MICs in the presence of 50% mouse serum. Whereas the MIC values of analogs in the parent nonfluorinated series generally increased by 2- to 4-fold in the presence of serum,[8] MICs of the fluorinated congeners increased from 4- to 64-fold, suggesting a high degree of binding or inactivation by serum proteins. Little improvement of activity against *S. aureus* Smith in the presence of mouse serum was observed by adjusting the polarity of the aryl side chain, either by changing the nature of the heterocycle (**3b**, **d**–**g**, **n**) or by incorporating heteroatoms in the linker (**3o**). C9-Oximation also had little effect, with MIC values for ketolides **8a**–**e** ranging from 2 to 8 μg/mL against *S. aureus* Smith in the presence of 50% mouse serum. The lowest MIC value attained in the presence of serum was 1 μg/mL for compound **3e**, a 8-fold increase compared to the MIC determined in broth.

To assess the effect of elevated MIC values against *S. aureus* Smith in the presence of mouse serum on in vivo activity, a selected fluorinated/nonfluorinated ketolide pair was evaluated for efficacy in a *S. aureus* murine systemic lethal infection model.[14] As shown in Table 3, nonfluorinated compound **10** (the ratio of C2 *R* to C2 *S* epimers is approximately 9:1)[8c] displayed excellent in vivo activity with ED_{50} values of 1.0 mg/kg (s.c.) and 10 mg/kg (p.o.). The C2-fluorinated counterpart (**3j**) was 12-fold less active when dosed subcutaneously and was inactive at the highest concentration when dosed orally. One possible explanation is high protein binding as evidenced by the high MIC values in the presence of serum, which significantly reduces the free drug concentration in the systemic circulation.



3j



10

Table 3. Efficacy of **3j** and its nonfluorinated congener **10** in a *S. aureus* murine systemic lethal infection model

Compound	Route	ED_{50} (mg/kg)[a]
10	p.o.	10 (8.1–14)
	s.c.	1.0 (0.5–1.4)
3j	p.o.	>40
	s.c.	12 (6.6–70)

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

In summary, a series of C6-carbamate ketolides with C2-fluorination and C9-oximation was synthesized and shown to have in vitro activity that is superior to erythromycin and comparable to telithromycin. MIC values for the best compounds (**3a**, **h**, **i**, **k**–**m**, and **8d**) were 0.03–0.12 μg/mL against streptococci containing *erm* and *mef* resistance determinants and 2–4 μg/mL against *H. influenzae*. C2-Fluorination had two significant effects on in vitro activity: (1) a decrease in the MIC values against *erm*(B)-containing enterococci and (2) an increase in the MIC values against *S. aureus* in the presence of mouse serum. C9-Oxime analogs of the fluorinated compounds displayed similar in vitro profiles to their nonoximated counterparts. In vivo activity was adversely affected by fluorination, possibly as a result of increased serum protein binding.

Acknowledgements

Contributions to in vitro testing by Ellyn Wira and to in vivo testing by John Melton and Jeff Fernandez are gratefully acknowledged. The authors also wish to thank Dennis Hlasta and Ellen Baum for their scientific insights during the early stages of this investigation.

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