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β-Keto-Ester Chemistry and Ketolides. Synthesis and Antibacterial Activity of 2-Halogeno, 2-Methyl and 2,3 Enol-Ether Ketolides

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Abstract—The effect of 2,3 modifications on the antibacterial activity of ketolides was evaluated by introducing substituents in position 2 and converting the C-1, C-2, C-3 β-keto-ester into stable 2,3 enol-ether or 2,3 anhydro derivatives. Introduction of a fluorine in C-2 is beneficial with regard to the overall antibacterial spectrum whereas the enol-ether and 2,3 unsaturated compounds, as well as the bulky *gem* dimethyl or 2-chloro derivatives, are less active particularly against erythromycin resistant strains. A 2-fluoro ketolide derivative demonstrates good antibacterial activity and in vivo efficacy against multi-resistant *Streptococcus pneumoniae*. Compared to azithromycin against *Haemophilus influenzae*, this compound is equivalent in vitro and slightly more active in vivo. These results demonstrate that within the ketolide class, to retain good antibacterial activity, position 2 needs to remain tetrahedral and tolerates only very small substituents such as fluorine.

The ketolides,[1a,b] exemplified by telithromycin,[1b] are a major new class of semisynthetic macrolide derivatives exhibiting antibacterial activity against erythromycin-resistant *Streptococcus pneumoniae* and *Haemophilus influenzae*. The fact that the ketolides do not induce MLS_B resistance[2] bestows therapeutic utility against erythromycin-resistant *S. pneumoniae* and other Gram positive pathogens. The ketolides, are characterized by a 3-keto function that replaces the L-cladinose, and thus define a β-keto-ester at the C-1, C-2, C-3 positions of the macrolactone ring. In addition to the 3-keto function, the most important features for in vitro and in vivo activities of ketolides are: a 11,12-cyclic carbamate moiety and a hetero-aryl side chain, generally linked to the ketolide backbone by the carbamate nitrogen. All these groups are also present in different series synthesized by Abbott[3] (e.g., 6-*O*-substituted ketolides and azaimino tricyclic ketolides). However, apart from the recent report of 2,3 anhydro[4] (Anhydrolides) derivatives, very little is known about the relative importance of position 2 for the overall activity of ketolides (Fig. 1). To address this question, we have exploited the chemical reactivity of the 1,3 β-keto-ester function of ketolides to modify the C-2 and C-3 positions. This approach has allowed us to introduce various electrophiles (halogen atoms or a methyl group) in position 2 and to convert the enolate intermediate into new stable 2,3 enol-ether derivatives. At the same time we have synthesized the 2,3 anhydrolide homologue of telithromycin to compare the overall effect of 2,3 modifications on antibacterial activity of ketolides.

Chemistry

Fluorination at C-2 was achieved in 3 steps from telithromycin (Scheme 1). First quantitative silylation of the 2′ alcohol with $(TMS)_2NH$/imidazole gave a protected intermediate that was reacted with *t*BuOK and *N*-fluorosulfonimide (NSFI) to give stereospecifically, after desilylation with $Bu_4N^+F^-$, the desired fluoro ketolide **I** in 83% yield. The absolute stereochemistry of this compound was later demonstrated by synthesizing **I** from the fluoro-enone **VII.** The starting enone[1a] was

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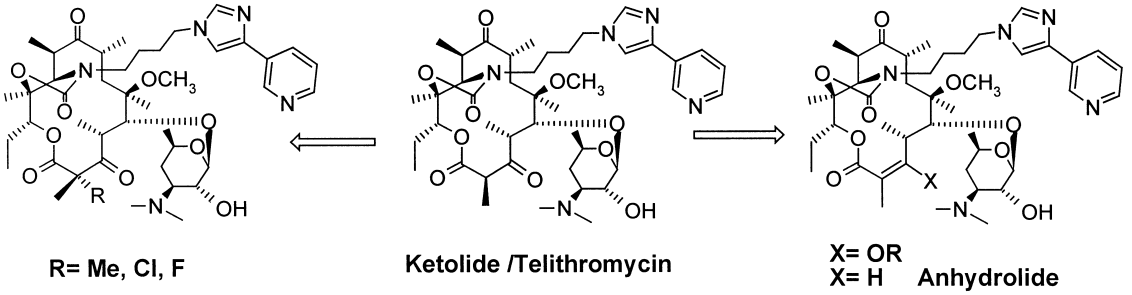
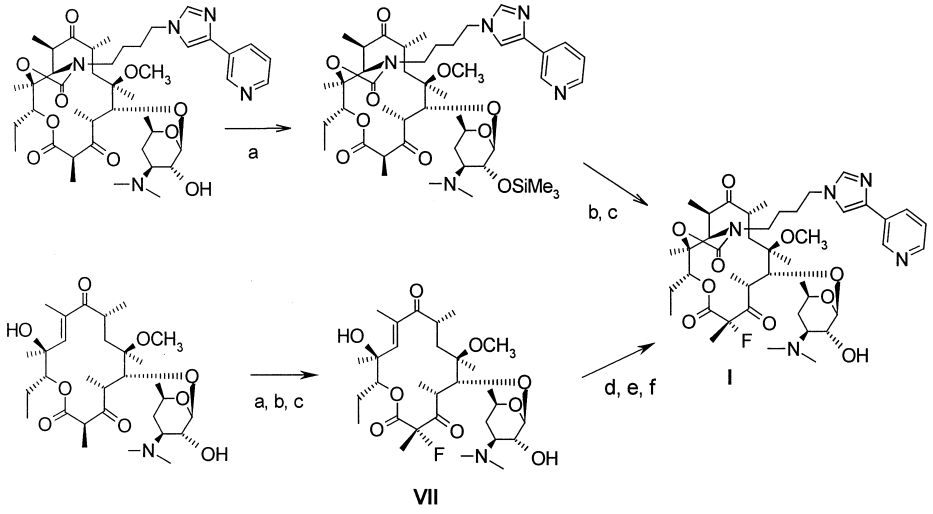


Figure 1.



Scheme 1. (a) $(TMS)_2$ NH/imidazole/THF; (b) *t*BuOK/NSFI/THF/−10°C; (c) $N^+Bu_4F^-$/THF; (d) Ac_2O/ CH_2Cl_2; (e) carbonyldiimidazole /DBU/ RNH_2/THF/rt; (f) MeOH.

first fluorinated similarly to **I** to give **VII** in 69% yield. This compound was then crystallized and the absolute stereochemistry of C-2 determined (Fig. 2). Finally, after acetylation in 2′, **VII** was reacted with carbonyldiimidazole and DBU in THF and the corresponding 4-[4-(3-pyridyl)-imidazolyl]lbutyl amine added to generate **I** in 67% yield. As the two different synthetic pathways yielded the same compound (^{1}H NMR, especially 2-Me δ (ppm) 1.79 (d, $J_{H,F}$ = 21.5 Hz, 3H), and melting point = 118 °C),[5] the absolute *S* configuration was attributed to **I** . It should be mentioned that this fluorination reaction was also carried out stereospecifically in the aza-imino tricyclic series.[6] The chlorine atom was easily introduced by radical chlorination using *N*-chlorosuccinimide with AIBN at 40 °C. **II** was obtained in 38% yield as a single isomer of unknown stereochemistry (Scheme 2). Methylation at C-2 was carried out by treatment of the 2′-OAc protected derivative of telithromycin **VIII** with methyl iodide and sodium hydroxide in the presence of $Bu_4N^+HSO_4^-$. Removing of the 2′-OAc in methanol gave **III** in 13% yield (Scheme 2). The 2,3 enol-ether derivatives were synthesized by *O*-alkylation of **VIII** with known chloromethylethers. **IV** was obtained in 21% yield by treatment with $Br(CH_2)OCH_2Cl$ and NaH in DMF followed by amination using dimethylamine in refluxing ethanol. Similarly **V** was obtained in 40% yield by alkylation with $C_6H_5(CH_2)_3OCH_2Cl$ (Scheme 2). Finally, the 2,3 anhydro compound **VI** was synthesized in 37% yield from the known acyl-imidazole intermediate[4] by reaction with the corresponding amine in acetonitrile and deprotection of the 2′-OAc (Scheme 3); in agreement with the observation made by Elliott[4] for the anhydrolide series, 17% of the corresponding C-10 epimer were also isolated.

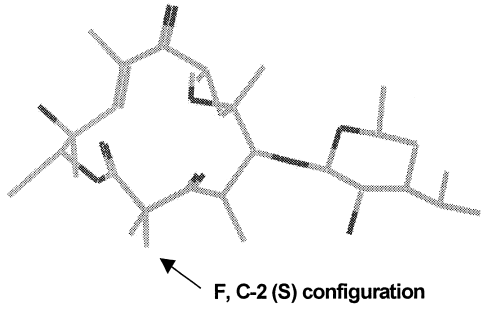
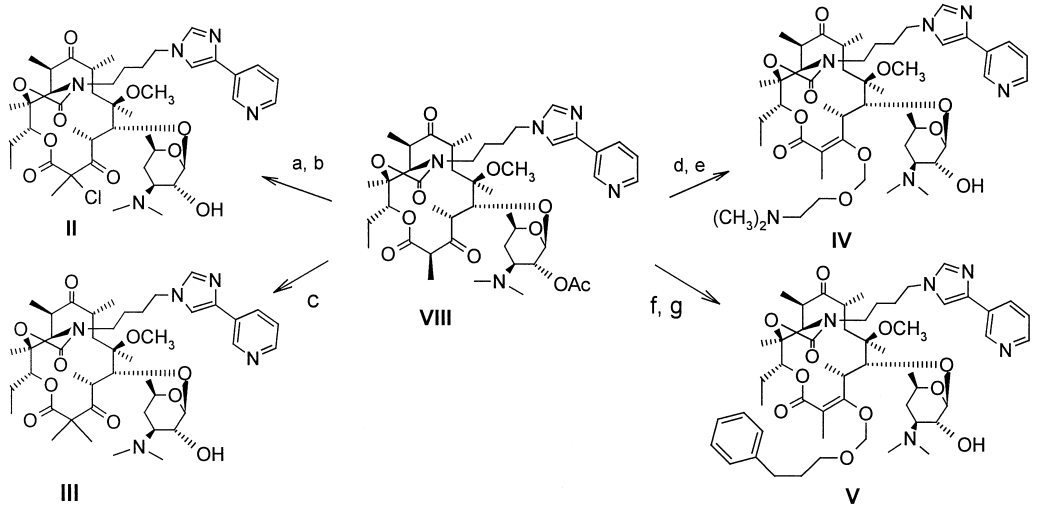


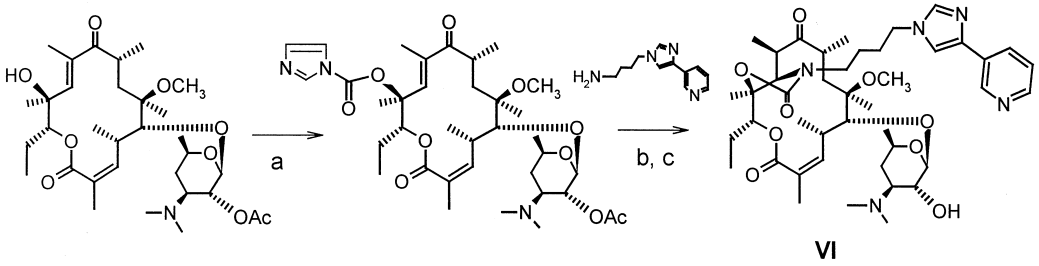
Figure 2. X-ray structure of **VII**.

Results and Discussion

All the ketolides and analogues were tested in vitro by standard agar dilution method against both erythromycin-susceptible and erythromycin-resistant staphylococci, streptococci and pneumococci including constitutive (EryRc) and inducible (EryRi) phenotype. In addition, one strain of *H. influenzae* was also tested. Without



Scheme 2. Synthesis of 2,3-enol-ether, 2-dimethyl and 2-chloro ketolides; (a) MeI/NaOH/ $Bu_4N^+HSO_4^-/CH_2Cl_2$; (b) MeOH; (c) *N*-chlorosuccinimide/AIBN/CCl_4/40 °C; (d) NaH/DMF/ $Br(CH_2)_2OCH_2Cl$; (e) EtOH/$(CH_3)_2NH$/reflux; (f) NaH/DMF/$C_6H_5(CH_2)_3OCH_2Cl$; (g) MeOH.



Scheme 3. Synthesis of 2,3-anhydro telithromycin; (a) NaH/ carbonyldiimidazole/CH_3CN/rt; (b) RNH_2/DMF; (c) MeOH.

exception, the reference macrolides clarithromycin and azithromycin were inactive (MICs >40 μg/mL) against erythromycin resistant strains whatever the phenotype.

All the compounds were inactive against a constitutively erythromycin-resistant strain of *S. aureus* (MIC >40 μg/mL). The replacement of a C-2-hydrogen atom for a fluorine in **I** (HMR3562) gave a compound that demonstrated good activities against strains susceptible to erythromycin (at least one order of magnitude higher than those of clarithromycin). Furthermore **I** was very effective against inducibly resistant *S. aureus* and *S. pneumoniae* as well as constitutively resistant *S. pneumoniae*. **I** was equal to azithromycin and telithromycin against *H. influenzae* (Table 1). The 2-chloro **II** and 2-methyl compounds **III** were both less active than the parent compound; particularly they were almost ineffective against EryRi *S. aureus* and five times less active against EryR *S. pneumoniae*.

The two planar derivatives **IV** and **V** were generally weakly active against most of the strains tested with a

Table 1. In vitro activity of 2,3 modified ketolides[a]

	MIC (μg/mL)								
	S. a.	*S. a.*	*S. a.*	*S. pyo.*	*S. p.*	*S. p.*	*S. p.*	*S. p.*	*H. i.*
	EryS 011UC4	EryRi 011GO25i	EryRc 011CB20	EryS 02A1UC1	EryS 032UC1	EryRc 030PW23c	EryRc 030SJ1	EryRi 030SJ5i	351HT3 (β lactamase +)
AZI	0.3	>40	>40	0.6	0.15	>40	>40	>40	1.2
CLA	0.3	>40	>40	0.08	0.04	>40	>40	>40	5
TEL	0.04	0.08	>40	⩽0.02	⩽0.02	0.04	⩽0.02	⩽0.02	1.2
I	0.02	0.08	>40	⩽0.02	⩽0.02	⩽0.02	⩽0.02	⩽0.02	1.2
II	0.6	>40	>40	0.04	0.04	1.2	5	0.6	2.5
III	0.6	1.2	>40	0.02	0.02	2.5	2.5	0.6	2.5
IV	2.5	20	>40	0.3	0.15	2.5	10	5	20
V	5	>40	>40	0.15	0.8	20	20	20	>40
VI	0.08	0.3	>40	0.02	0.02	0.3	10	2.5	2.5

[a]AZI = azithromycin; CLA: clarithromycin; TEL: telithromycin; EryS = susceptible; EryRc = constitutive MLS resistance; EryRi = inducible MLS resistance. *S. a.* = *Staphylococcus aureus; S. pyo.* = *Streptococcus pyogenes; S. p.* = *Streptococcus pneumoniae; H. i.* = *Haemophilus influenzae*.

Table 2. In vivo efficacy of 2-fluoro ketolide I

	ED_{50} (mg/kg)[a]				
	S. a.[b]	*S. p.*	*S. p.*	*S. p.*	*H. i.*
	EryS 011HT17	EryS 032UC1	EryRc 030MV2	EryRi 030SJ5i	AmpR 351RD7 (β lactamase +)
CLA	12	2	>50	>50	>150
AZI	72	4.5	>50	>50	94
I	9	1.5	2	4.3	56

[a]Effective dosage that protect 50% of mice from lethal infection after oral administration; AmpR = ampicillin resistant.
[b]Abbreviations as footnote in Table 1.

complete loss of activity against the Ery R strains (MICs >2.5–40 μg/mL). In contrast, the anhydrolide **VI** retained activity against susceptible and EryRi *S. aureus* strains. However it was poorly active (4 to 8 times less active than **I**) against erythromycin resistant *S. pneumoniae* whatever the phenotype.

In vivo evaluation

The in vivo efficacies of compound **I** to **VI** and reference compounds clarithromycin and azithromycin were assessed by acute lethal murine infection models caused by susceptible and erythromycin-resistant Gram positive *cocci* and *H. influenzae* (Table 2). Against infections caused by erythromycin susceptible strains, **I** exhibited in vivo efficacies close to clarithromycin and substantially better than azithromycin. Unlike classical macrolides (CLA, AZI) which show complete inactivity with ED_{50} up to 100 mg/kg, **I** demonstrated excellent anti-pneumococcal efficacy in infections caused by EryRi and EryRc *S. pneumoniae*, the corresponding effective doses for **I** ranging between 2 to 4 mg/kg. Against *H. influenzae*, **I** exhibited a 2-fold improvement in efficacy over azithromycin while clarithromycin was inactive under 150 mg/kg.

Conclusions

The β-keto-ester function of ketolides can be chemically exploited to generate new 2-modified ketolides. Introduction of a fluorine at C-2 by electrophilic fluorination results in good antibacterial activities whereas introduction of larger substituents or 2,3 anhydro or enol-ether modifications result in loss of activity and higher MIC. The 2-fluoro ketolide **I** (HMR3562) displays, with the exception of constitutively MLS_B resistant *S. aureus*, good in vitro and in vivo activities against all erythromycin resistant Gram positive cocci, including multi-resistant *S. pneumoniae*. In addition, **I** is active in vitro against *H. influenzae* to a similar extent as azithromycin and demonstrates good in vivo activities against this pathogen. These results demonstrate that within the ketolide class, to retain good antibacterial activity, position 2 needs to remain tetrahedral and tolerates only very small substituents such as fluorine.

Acknowledgements

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5. Spectral data for **I** (HMR 3562): mp: 118 °C; FAB–MS = 830^+ ($M+H^+$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.87 (t, 3H) $\underline{CH_3}CH_2$, 1.01 (d, 3H) 10-CH_3, 1.19 (d, 3H) 8-CH_3, 1.21–1.68 (m, 1H) H_4', 1.24 (d, 3H) 5′-Me, 1.31 (d, 3H) 4-CH_3, 1.34 (s, 3H) 6-CH_3, 1.79 (d, J = 21.5 Hz, 3H) 2-CH_3, 1.50 (s, 3H) 12-CH_3, 1.60–1.83 (m, 1H) H_7, 1.68–1.86 (m, 4H) CH_2–CH_2, 1.50–1.97 (m, 2H) H_{14}, 2.27 (s, 6H) $N(\underline{CH_3})_2$, 2.46 (m, 1H) H_3', 2.59 (m, 1H) H_8, 2.55 (s, 3H) 6-OCH_3, 3.11 (q, 1H) H_{10}, 3.18 (dd, J = 7.5 and 10 Hz, 1H) H_2', 3.53 (m, 2H) H_4 and H_5', 3.42 (s, 1H) H_{11}, 3.63–3.75(m, 2H) $\underline{CH_2}NCO$, 4.01 (t, 2H) $\underline{CH_2}N$, 4.07 (d, J = 10.5 Hz, 1H) H_5, 4.31 (d, 1H) H_1', 4.86 (dd, J = 2 and 10.5 Hz, 1H) H_{13}, [7.55 (d, 1H) H_2, 7.33 (d, 1H) H_5] imidazole, [8.98 (s, 1H) H_2, 8.09 (dt, 1H) H_4, 7.29 (ddd,1H) H_5, 8.46 (dd, 1H) H_6] pyridine. Anal. Calc. (%) for $C_{43}H_{64}N_5O_{10}F$: C 62.33, H 7.77, N 8.44, F 2.29. Found : C 61.9, H 8.1, N 8.7, F 2.2.
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