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Synthesis and biological activity of new 5-*O*-sugar modified ketolide and 2-fluoro-ketolide antibiotics

Chang-Hsing Liang, Sulan Yao, Yu-Hung Chiu, Po Yee Leung, Nicole Robert, Jaime Seddon, Pam Sears, Chan-Kou Hwang, Yoshi Ichikawa and Alex Romero*

Department of Chemistry and Department of Biology, Optimer Pharmaceuticals, Inc., 10110 Sorrento Valley Road, Suite C, San Diego, CA 92121, United States

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Abstract—A series of new triazole-containing ketolides and 2-fluoro-ketolides in which the 5-*O*-desosamine was replaced by unnatural sugars were synthesized and evaluated against relevant macrolide-sensitive and macrolide-resistant respiratory pathogens. Excellent in vitro antibacterial activities were demonstrated for ketolide analogues having the 6′-OBz-3′-dimethylamino-glucose and 6′-OBz-4′-deoxy-3′-dimethylamino-glucose substituents.

Ketolide antibiotics, such as telithromycin (**1**) and cethromycin (**2**) (Fig. 1), are a newer generation of macrolide antibiotics that show excellent activity against specific macrolide-resistant organisms, such as inducible macrolide, lincosamide, and streptogramin B phenotype (MLS_B-inducible) and pathogens having efflux (*mef*) resistant mechanisms.[1] This improved activity, over their macrolide counterparts, has been attributed to the 3-keto group, 11,12-cyclic carbamate, and the tethered hetero-aromatic substituent. However, cross-resistance due to constitutive expression of *erm* gene (methylation of the 23S rRNA) still remains an unsolved problem for the efficacy of ketolides and macrolide antibiotics.[2]

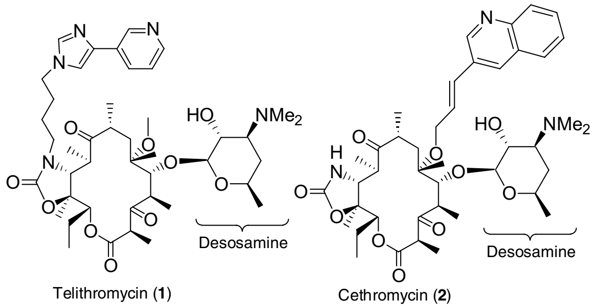

Figure 1.

The 5-*O*-desosamine substituent plays an important role in this ubiquitous *erm*-resistance mechanism and has been considered critical for the overall antibacterial activity of the macrolide and ketolide class of antibiotics.[3] One possible approach to overcome bacterial *erm*-resistance is to replace the resistance causing 5-*O*-desosamine with a optimized sugar scaffold having the potential for increased binding to the resistant bacterial ribosome. Up until our previous report[4] and now with our current disclosure, there have been only few reports describing the removal or modification of this sugar.[1,2,5] The limited SAR investigating the role of the 5-*O*-desoamine substituent is partly due to both the lack of efficient methods for the removal of this sugar and the lack of appropriate and efficient techniques for glycosylating acid sensitive macrolide and ketolide aglycons.

We have recently described an efficient synthetic procedure for preparing ketolide aglycon **4** and its subsequent utilization in glycosylation and triazole-forming reactions.[4] The current communication will highlight the syntheses of other triazole containing 5-*O*-glycosylated ketolide analogues, including novel triazole-containing 5-*O*-glycosylated-2-fluoro-ketolides derived from the

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Keywords: Glycosylated; Ketolide; 2-Fluoro-ketolide; Macrolide resistance.

* Corresponding author. Tel.: +1 858 909 0736; fax: +1 858 909 0737; e-mail: aromero@optimerpharma.com

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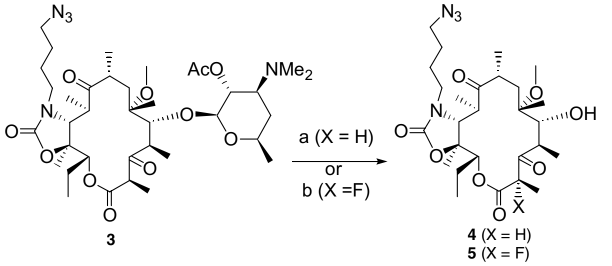
new fluoro-ketolide aglycon **5**, and will present the antimicrobial activities against relevant respiratory pathogens for this novel class of ketolide antibiotics.

Scheme 1 describes the syntheses of the ketolide aglycon **4** (condition a) and 2-fluoro-ketolide aglycon **5** (condition b), while their subsequent glycosylation, deprotection, and triazole-forming reactions are showcased in Scheme 2. Starting with compound **3**, ketolide aglycon **4** was obtained in 54% yield after methanolysis and subsequent one-step Swern oxidation–hydrolysis (Scheme 1, condition a).[4] Similarly, the new 2-fluoro-ketolide aglycon **5** was obtained in 59% overall yield after fluorination using *N*-fluorobenzenesulfonimide followed by one-step Swern oxidation–hydrolysis procedure (condition b).

The *p*-tolyl-thio glycosides that were employed in the glycosylation of the novel ketolide aglycons are shown below (Fig. 2). The *p*-tolyl-thio glycoside of 6-deoxyglucose, **17a**, was chosen in order to investigate the importance of the 3-amino substituent of the naturally occurring desosamine group, while the *p*-tolyl-thio glycosides, **17b–e**, were chosen to investigate the effect on antimicrobial activities resulting from the addition of hydrophobic or hydrophilic groups to 4′- and/or 6′-position of the 5-*O*-desosamine. The *p*-tolyl-thio glycosides having the 3-amino group were protected as the NHFmoc group since the Fmoc group could be easily removed and the amine could be converted to the requisite *N*,*N*-dimethylamino group in the presence of the tethered azide group contained in the ketolide cores.



Scheme 1. Reagents and conditions: (a) (i) MeOH, rt (98%); (ii) $(COCl)_2$, DMSO, Et_3N, CH_2Cl_2; (iii) Silica-gel, MeOH, N_2, 65 °C (55%); (b) (i) NaH, $(PhSO_2)_2NF$, THF (90%); (ii) MeOH, rt (98%); (iii) $(COCl)_2$, DMSO, Et_3N, CH_2Cl_2; (iv) Silica-gel, MeOH, N_2, 65 °C (65%).

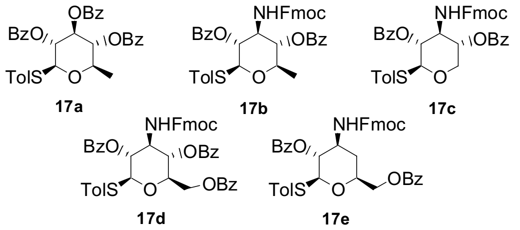
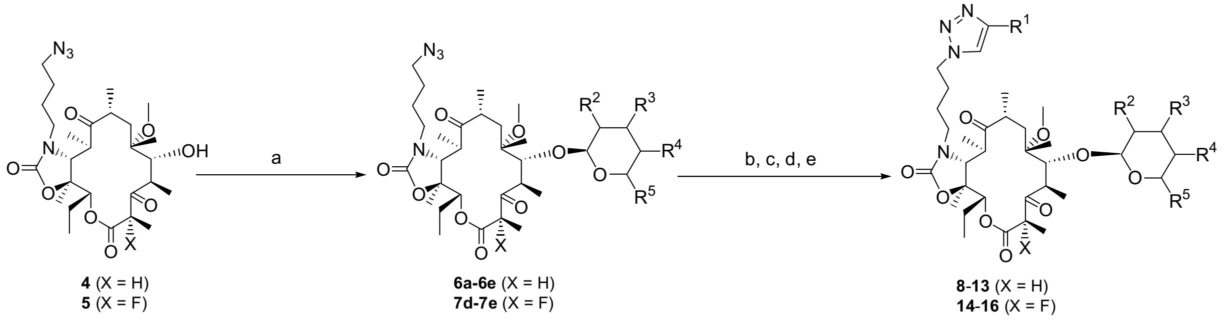


Figure 2. Examples of *p*-tolyl-thio glycosides utilized in the glycosylations of ketolide aglycons.

Ketolide aglycon **4** was successfully glycosylated with appropriately protected *p*-tolyl-thio glycosides **17a–e**[6] (Fig. 2), under mild conditions (NIS/AgOTf/di-*tert*-butyl-pyridine) affording the glycosylated products **6a–e** (X = H) in yields ranging from 60% to 70% (Scheme 2).[4] Similar yields were obtained for ketolides **7d** and **7e** derived from the glycosylation of 2-fluoro-ketolide aglycon **5** with *p*-tolyl-thio glycosides **17d** and **17e**. The desired β-stereoisomer was predominantly obtained (>8:1) from each of the glycosylation reactions due to neighboring group participation of the 2-OBz group of the designed *p*-tolyl-thio glycosides. The deprotection sequences for the newly constructed glycosylated ketolides were as follows (Scheme 2): In cases of where the monosaccharide contained a protected amine (NHFmoc), the Fmoc-protecting group was removed with 10% piperidine in DMF and reductive amination was accomplished with $NaBH(OAc)_3$ and formaldehyde in THF to give the requisite *N*,*N*-dimethylamine substituent. Glycosylated products having 2′- and/or 4′-OBz groups were selectively removed by heating in MeOH. Glycosylated products having additional OBz groups (i.e., 6′-OBz or 3′-OBz) could be deprotected, if desired, by reaction with LiOH in MeOH. For each of the glycosylated-deprotected ketolides, the azide group was converted in excellent yields (>90%) to a 4-substituted-[1,2,3]-triazole as a single regio-isomer by reaction with the corresponding acetylenes to afford ketolides **8–13** (R^1 = 2-pyridyl) and 2-fluoro-ketolides **14–16** (R^1 = 3-NH_2–Ph).[4,7]



Scheme 2. Reagents and conditions: (a) **17a–e**, NIS, AgOTf, −20 °C–rt (60–70%); (b) piperidine, DMF (80–85%); (c) $NaBH(OAc)_3$, CH_2O, THF (80–90%); (d) (i) MeOH, rt (99%) or (ii) LiOH, MeOH (85–90%); (e) 2-pyridyl or 3-NH_2–Ph acteylene, CuI (cat), toluene 80 °C (90–98%).

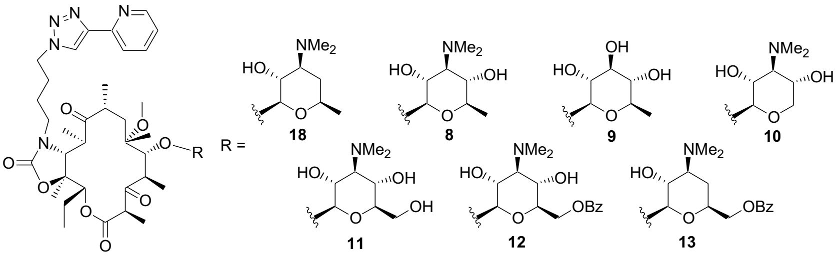


Figure 3. Ketolides **8**–**13**, **18**.

The newly prepared ketolides (Fig. 3) were tested against erythromycin-sensitive (Ery-S) and erythromycin-resistant (Ery-R) strains of *S. aureus*, *S. pyogenes*, *S. pneumoniae* and *H. influenzae* ATCC 49247 (Table 1).[8,9] The ketolide analogue **8**, containing the 5-*O*-mycaminose (6′-deoxy-3′-dimethylamino-glucose) displayed reduced activity against Ery-R *S. pneumoniae* 303 (*erm*) and Ery-S *H. influenzae*, having minimum inhibitory concentrations (MIC) of 16 μg/mL for both strains compared to ⩽0.125 and 4 μg/mL, respectively, for its ketolide counterpart, **18**,[4] having the naturally occurring 5-*O*-desosamine sugar (Table 1). The glycosylated ketolide, **9**, having the 6′-deoxy-glucose substituent, which lacks the 'signature' 3′-*N*,*N*-dimethylamino group, was significantly less active than both compounds **8** and **18** against most of the strains tested. The role of the 5′-methyl substituent was deemed important since the activity of ketolide **10**, having the 3′-dimethlyamino-xylose group, was less active than ketolide **8** against strains of *S. aureus*, Ery-R *S. pneumoniae* 163 (*mef*), and *H. influenzae*. We also prepared and tested ketolides, **11**–**13**, having the 6′-modified groups (OH and OBz) in order to investigate hydrophilic/hydrophobic substituent effects of the 6′-position. Ketolide **11**, having the 3′-dimethylamino-glucose substituent, was significantly less active than ketolides, **12** and **13**, which contained the hydrophobic 6′-OBz-3′-dimethlyamino-glucose and 6′-OBz-4′-deoxy-3′-dimethlyamino-glucose groups, respectively, against strains of *S. aureus*, Ery-R *S. pyogenes* 3029 (*erm*), and Ery-R *S. pneumoniae* 163 (*mef*).

This trend of improved activity with increased hydrophobicity of the 6′-position was also witnessed for the 2-fluoro-ketolides (Fig. 4). The selected 2-fluoro-ketolides, **14**–**16**, having the 4-(3-aniline)-[1,2,3]-triazole group ($R^1 = 3\text{-NH}_2\text{–Ph}$) were tested against a secondary panel of Ery-S and Ery-R strains of *S. pyogenes* and *S. pneumoniae* and *H. influenzae* ATCC 49247 (Table 2).[8,9] The 2-fluoro-ketolide, **14**, was highly active (⩽0.125 μg/mL) against *S. pneumoniae* ATCC 49619 and Ery-R *S. pneumoniae* 163 (*mef*) and 303 (*erm*); however it was completely inactive (>32 μg/mL) against the other

Table 1. In vitro antibacterial activity of triazole-containing 5-*O*-substituted ketolide derivatives **8**–**13**, **18**[a]

Entry	Minimum inhibitory concentration (μg/mL)[9]							
	S. aureus		*S. pyogenes*		*S. pneumoniae*			*H. influenzae*
	ATCC 29213 Ery-S	96:11480 Ery-R (MLS_B-ind)	ATCC 19615 Ery-S	3029 Ery-R (*erm*)	ATCC 49619 Ery-S	163 Ery-R (*mef*)	303 Ery-R (*erm*)	ATCC 49247 Ery-S
Azithromycin	1	>64	⩽0.125	>64	⩽0.125	8	>64	4
Telithromycin	⩽0.125	⩽0.125	⩽0.125	16	⩽0.125	⩽0.125	⩽0.125	4
18	⩽0.125	0.125	⩽0.125	>64	⩽0.125	⩽0.125	⩽0.125	4
8	⩽0.125	0.25	⩽0.125	>64	⩽0.125	0.5	16	16
9	>64	>64	8	nt	4	2	16	>64
10	4	4	⩽0.125	nt	⩽0.125	8	4	>64
11	4	64	⩽0.125	>64	⩽0.125	4	⩽0.125	64
12	⩽0.125	⩽0.125	⩽0.125	2	⩽0.125	⩽0.125	⩽0.125	64
13	nt	nt	nt	0.5	⩽0.125	⩽0.125	⩽0.125	64

[a] nt = not tested.

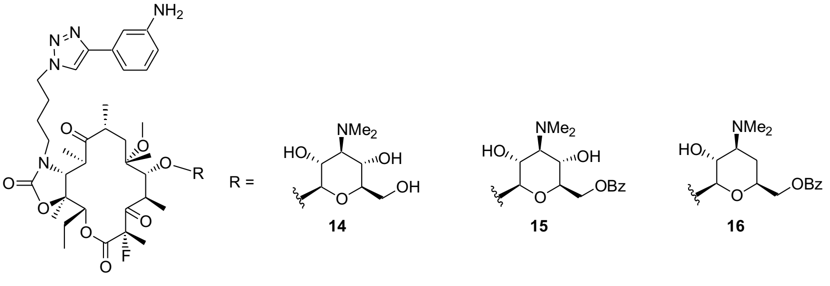


Figure 4. 2-Fluoro-ketolides **14**–**16**.[10]

Table 2. In vitro antibacterial activity of triazole containing 5-*O*-substituted 2-fluoro-ketolide derivatives **14**–**16**[a]

Entry	Minimum inhibitory concentration (μg/mL)[9]										
	S. pyogenes					*S. pneumoniae*					*H. influenzae*
	ATCC 19615 Ery-S	3029 Ery-R (*erm*)	1850 Ery-R (*erm*)	3262 Ery-R (*erm*)	1721 Ery-R (*erm*)	ATCC 49619 Ery-S	163 Ery-R (*mef*)	303 Ery-R (*erm*)	3773 Ery-R (*erm*)	5032 Ery-R (*erm*)	ATCC 49247 Ery-S
Azithromycin	$\leqslant$0.125	>64	>64	>64	>64	$\leqslant$0.125	8	>64	>64	>64	4
Telithromycin	$\leqslant$0.125	16	8	32	64	$\leqslant$0.125	$\leqslant$0.125	$\leqslant$0.125	1	0.5	4
14	$\leqslant$0.125	>32	>32	>32	>32	$\leqslant$0.125	$\leqslant$0.125	$\leqslant$0.125	>32	>32	32
15	$\leqslant$0.125	0.5	0.25	4	8	$\leqslant$0.125	$\leqslant$0.125	$\leqslant$0.125	8	4	4
16	nt	$\leqslant$0.125	0.25	0.5	8	$\leqslant$0.125	$\leqslant$0.125	$\leqslant$0.125	4	0.25	4

[a] nt = not tested.

macrolide-resistant strains, including Ery-R strains of *S. pneumoniae* 3773 and 5032 (*erm*), and all Ery-R strains of *S. pyogenes* 3029, 1850, 3262, and 1721 (*erm*). This compound was also 8-fold less active than azithromycin and telithromycin against Ery-S *H. influenzae*. On the other hand, ketolides **15** and **16**, containing an additional 6′-OBz group such as 6′-OBz-3′-dimethylamino-glucose and 6′-OBz-4′-deoxy-3′-dimethylamino-glucose, respectively, were as active as azithromycin and telithromycin against Ery-S *H. influenzae*. Interestingly, ketolides **15** and **16** were both significantly more active than ketolide **14** against most macrolide-resistant strains of *S. pyogenes* and *S. pneumoniae*. Particularly noteworthy, ketolides **15** and **16** were also significantly more active than telithromycin and azithromycin against Ery-R strains of *S. pyogenes* 3029, 1850, 3262, and 1721 (*erm*).

In summary, series of new triazole containing ketolides and 2-fluoro-ketolides in which the 5-*O*-desosamine was replaced by unnatural sugars were synthesized. Ketolides having the 6′-OBz-3′-dimethylamino-glucose and 6′-OBz-4′-deoxy-3′-dimethylamino-glucose substituents displayed excellent in vitro activity against macrolide-sensitive and macrolide-resistant pathogens. These compounds are promising candidates for further efficacy evaluation. This work should also provide significant opportunity for the discovery of novel 5-*O*-sugar substituted macrolide and ketolide antibiotics having potent activity against bacteria expressing *erm*-mediated resistance.

Acknowledgements

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10. Representative spectroscopic data. Compound **15**: ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.09–8.06 (m, 2H), 7.82 (s, 1H), 7.60–7.51 (m, 1H), 7.49–7.32 (m, 2H), 7.25–7.15 (m, 3H), 6.65–6.60 (m, 1H), 4.95–4.90 (m, 1H), 4.75–4.52 (m, 2H), 4.48–4.32 (m, 3H), 4.13 (d, $J = 8.1$ Hz, 1H), 3.80–3.35 (m, 7H), 3.15–3.05 (m, 1H), 2.80–2.70 (m, 5H), 2.54 (s, 6H), 2.43 (s, 3H), 2.11–1.35 (m, 8H), 1.81 (d, $J = 21.5$ Hz, 3H), 1.51 (s, 3H), 1.23 (s, 3H), 1.21 (d, $J = 6.9$ Hz, 3H), 1.18 (d, $J = 6.9$ Hz, 3H), 1.04 (d, $J = 7.0$ Hz, 3H), 0.90 (t, $J = 7.5$ Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 216.5, 202.5, 167.1, 166.4, 157.2, 147.9, 146.9, 133.5, 131.7, 129.9, 129.5, 128.5, 128.4, 119.7, 116.2, 114.8, 112.4, 103.9, 97.9 (d, $J = 205$ Hz), 82.1, 80.4, 78.6, 78.4, 75.8, 70.3, 70.1, 66.2, 63.9, 60.8, 49.7, 49.1, 44.5, 42.8, 41.7, 40.6, 39.5, 39.2, 27.6, 25.4, 24.3, 22.1, 19.7, 17.9, 15.1, 14.7, 13.6, 10.5. MS: Calcd for $C_{50}H_{70}FN_6O_{13}$ (M+H): 981.5, found: 981.3.