

Abstract

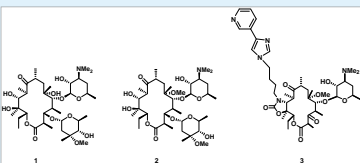
Background: Structural features associated with ketolide antibiotics that may improve activity over their macrolide predecessors typically include: (1) lack of the 3-O-cladinose, (2) presence of 3-keto group, and (3) the presence of a heterocycle tethered to 11,12-carbamate that interacts specifically with domain II of the bacterial rRNA. A novel compound library was designed to optimize domain II binding and antitumor activity by the incorporation of the chemically robust substituted [1,2,3]-triazole group.

Introduction

Macrolide antibiotics such as erythromycin A (1) and clarithromycin (2) are considered safe and effective for the treatment of respiratory tract infections.¹ However, increasing resistance rates to these and other antibiotics are becoming a serious health concern world-wide. It is estimated that nearly 40% of *Streptococcus* strains in the US are resistant to both penicillin and macrolide antibiotics.² Ketolides, such as telithromycin (3), are a newer class of semi-synthetic macrolides that have been developed to help overcome macrolide resistance.³ Telithromycin is effective against penicillin and erythromycin-resistant *S. pneumoniae* and is a non-inducer of Macrolide-Lincosamide-Streptogramin B (MLS_B) resistance. The key structural features of ketolides are: (1) lack of 3-O-cladinose, (2) presence of 3-keto group, and (3) presence of an aromatic functionality to interact with domain II of the bacterial ribosome.

A common mechanism that leads to macrolide resistance involves Erm-mediated methylation of a key bacterial ribosomal nucleotide (A2058EC) found in domain V of the 23S RNA. This nucleotide was shown to play a major role in the ribosomal binding of macrolide antibiotics through interactions with the macrolide desosamine sugar.^{4,5} Methylation of A2058 effectively interferes with the binding of the desosamine sugar thereby reducing the binding affinity of the macrolide to the bacterial ribosome. A possible explanation for the enhanced activity of telithromycin against macrolide-resistant bacteria stems from additional domain II interactions through its four-carbon linked imidazole-pyridyl side chain.⁶ It has been suggested that this additional interaction helps compensate for alterations at the domain V binding site in resistant bacteria. Resistance to all ketolide and macrolide antibiotics due to constitutive expression of the *erm*-gene still remains.^{1,4,7}

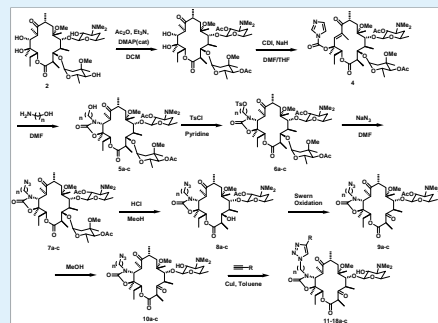
Figure 1. Chemical Structures of Erythromycin A (1), Clarithromycin (2) and Telithromycin (3)



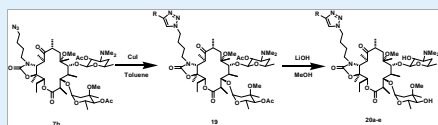
Synthesis

The synthesis of compounds 11-18a-c, 20a-e, and 23a-b are described in Schemes I-III.

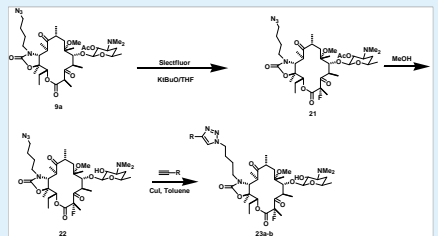
Scheme I.



Scheme II.



Scheme III.



Methods

The macrolides were tested against erythromycin-sensitive (Ery-S) and erythromycin-resistant (Ery-R) strains of *S. aureus* (29213 (Ery-S) and 96:11480 (Ery-R)), *S. pneumoniae* (49619 (Ery-S) and 163 and 303 (Ery-R)) and *H. influenzae* (49247 (Ery-S)). The cladinose analogs **20a-e** were not tested against *H. influenzae*. The micro-dilution method was used to determine the Minimum Inhibitory Concentrations (MICs) against all pathogens as per the Clinical and Laboratory Standards Institute (CLSI).

Results

The chain length of the alkyl side chain had a dramatic effect on activity of ketolides (Table 1). For example, the 3-carbon linked phenyl substituted triazole **11a** was significantly less active against Ery-S and Ery-R *S. aureus* and was completely inactive against Ery-R *S. pneumoniae* 303 (ermB), whereas the corresponding 4- and the 5-carbon linked phenyl substituted triazoles **11b** and **11c** were noticeably more active against these organisms. A similar trend was observed for the 2-pyridyl substituted triazoles **14a-c**, the 2-amino-phenyl substituted triazoles **16a-c**, and the 2,5-dichlorophenoxy substituted triazoles **17a-c**.

Of the 4-carbon linked analogs, the 2-pyridyl substituted triazole **14b** and the 3-amino-phenyl substituted triazole **16b** possessed the highest potency against *S. pneumoniae* 303, both having MIC values (≤ 0.125 $\mu\text{g/mL}$) comparable to telithromycin. Interestingly, the 4-carbon linked 3-pyridyl substituted triazole **15b** was significantly less active against this strain (MIC of 64 $\mu\text{g/mL}$). Surprisingly, within this series antibiatic activity could be recovered by extending the carbon linker to 5 atoms, for example the MIC against *S. pneumoniae* 303 for compound **15c** improved from 64 to 4 $\mu\text{g/mL}$. A similar effect was also observed for the benzo-triazole containing ketolide **18c** against *S. aureus* but it still inactive against *S. pneumoniae* 303. This result illustrates the delicate balance between the length of the linker and nature of the aromatic substitution of the triazole for achieving activity against macrolide resistant *S. pneumoniae* and *S. aureus*. A correlation between linker length and activity was also observed for *H. influenzae* (49247) where the most potent ketolide series had the substituted triazole linked through either 4-carbon (**11b-14b**, **16b**, **17b**) or a 5-carbon (**15c**, **18c**) chain. Interestingly, the most potent aromatic series against *H. influenzae* was the 3-amino-phenyl substitution with a 3-, 4- or 5-carbon linker (**16a**, **16b**, **16c**) having MICs of 16, 2, and 8 $\mu\text{g/mL}$, respectively.

The macrolides containing a cladinose at the 3 position were all highly active against Ery-S *S. pneumoniae* (49619) (Table 2). However, these analogs were less potent than telithromycin against Ery-R strains. The MICs were significantly higher for the cladinose containing analogs with either 2-pyridyl, 2-amino-phenyl or 2,6-dichlorophenyl triazole substituents than for the corresponding ketolides (**20a**, **20c**, and **20d** versus **14b**, **16b**, and **17b**).

Conversely, antibiatic activity was re-established for ketolide analogs **15b** (3-pyridyl) and **18b** (benzo-triazole) by replacing the keto with the cladinose group in analogs **20b** (3-pyridyl) and **20e** (benzo-triazole). The MICs improved from 64 $\mu\text{g/mL}$ for **15b** and **18b** to 1 and 2 $\mu\text{g/mL}$ for **20b** and **20e**, respectively. A similar activity trend was also observed for Ery-R *S. pneumoniae* 163 (MeFa). The ketolides containing a 2-fluoro group retained their potency against resistant strains to the corresponding non-fluorinated analogs (Table 3).

Results (cont.)

Table 1. MICs of Ketolides 11a-18a, 11b-18b, and 11c-18c.

Entry	R	<i>S. aureus</i>				<i>S. pneumoniae</i>				<i>H. influenzae</i>			
		29213 Ery-S	96:11480 Ery-R	49619 Ery-S	303 Ery-R (ermB)	163 Ery-S	303 Ery-R (ermB)	49247 Ery-S	49247 Ery-R	49247 Ery-S	49247 Ery-R	49247 Ery-S	49247 Ery-R
11a	Ph	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125
11b	Ph	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125
11c	Ph	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125
11d	Ph	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125
11e	Ph	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125
11f	Ph	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125
11g	Ph	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125
11h	Ph	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125
11i	Ph	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125
11j	Ph	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125
11k	Ph	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125
11l	Ph	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125
11m	Ph	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125
11n	Ph	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125
11o	Ph	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125
11p	Ph	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125
11q	Ph	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125
11r	Ph	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125
11s	Ph	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125
11t	Ph	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125
11u	Ph	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125
11v	Ph	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125
11w	Ph	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125
11x	Ph	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125
11y	Ph	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125
11z	Ph	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125
11aa	Ph	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125
11ab	Ph	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125
11ac	Ph	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125
11ad	Ph	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125
11ae	Ph	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125
11af	Ph	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125
11ag	Ph	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125
11ah	Ph	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125
11ai	Ph	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125
11aj	Ph	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125
11ak	Ph	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125
11al	Ph	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125
11am	Ph	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125
11an	Ph	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125
11ao	Ph	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125
11ap	Ph	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125
11aq	Ph	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125
11ar	Ph	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125
11as	Ph	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125
11at	Ph	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125
11au	Ph	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125
11av	Ph	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125	>0.125
11aw	Ph	>0.125	&										