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Exploiting translational stalling peptides in an effort to extend azithromycin interaction within the prokaryotic ribosome nascent peptide exit tunnel

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ABSTRACT

The ribosome is the primary protein synthesis machine in the cell and is a target for treatment of a variety of diseases including bacterial infection and cancer. The ribosomal peptide exit tunnel, the route of egress for the nascent peptide, is an inviting site for drug design. Toward a rational engagement of the nascent peptide components for the design of small molecule inhibitors of ribosome function, we designed and disclosed herein a set of N-10 indole functionalized azithromycin analogs. The indole moiety of these compounds is designed to mimic the translation stalling interaction of SecM W155 side-chain with the prokaryotic (*Escherichia coli*) ribosome A751 residue. Many of these N-10 functionalized compounds have enhanced translation inhibition activities against *E. coli* ribosome relative to azithromycin while a subset inhibited the growth of representative susceptible bacteria strains to about the same extent as azithromycin. Moreover, the inclusion of bovine serum in the bacterial growth media enhanced the anti-bacterial potency of the N-10 functionalized azithromycin analogs by as high as 10-fold.

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1. Introduction

The ribosome is the primary protein synthesis machine in the cell, and is among the most important and best studied systems in biology. The details of its function are central to our understanding of biology and treatment of a variety of diseases including bacterial infection and cancer.[1,2] Translation, ribosome-mediated peptide synthesis, proceeds through a series of highly ordered steps in which messenger RNA (mRNA) is matched with transfer RNA (tRNA) through codon/anticodon pairing. These tRNAs carry with them the matched amino acid on the charged end opposite that of the pairing. Depending on their position in the sequence of events in this assembly-line-like system, tRNAs occupy three distinct locations within the ribosome named the aminoacyl- (A-), peptidyl- (P-), and exit- (E-) sites. The proximity of their charged ends (ester bonds) at the P- and A-sites allows for peptide bond formation. This catalytic step where the nascent peptide is transferred to the A-site bound tRNA occurs within the peptidyl transferase center (PTC). As the protein grows, it extends through the ribosomal nascent peptide exit tunnel, an 80 Å × 20 Å pathway once thought to be passive route of egress for the nascent peptide. However, increasingly more evidence suggests that the exit tunnel may play an active role in translation including preliminary folding and outright translational stalling.[2–8]

Efforts aimed at elucidating the nascent peptide-tunnel interaction have been hampered by a dearth of customizable molecular probes. Recently, we reported a class of oligopeptide-linked ketolide (peptolides) probes which furnished atomic level information about specific interactions between the ribosomal exit tunnel and models of nascent peptides.[9] Earlier studies with translation stalling peptide sequences, including SecM, ErmBL and TnaC, have also provided evidence of direct interaction of the nascent peptide with the components of the exit tunnel.[10–12] Inspired by these observations, we sought to rationally target the components of the exit tunnel to enhance the binding affinity of azithromycin, a class of macrolide antibiotics (Fig. 1), for the prokaryotic ribosomes. We showed that derivatization of the N-10 endocyclic amine of azithromycin with moieties which mimicked the SecM W155 side-chain resulted in a sub-set of analogs with enhanced translation inhibition activities against *Escherichia coli* ribosome. Many of

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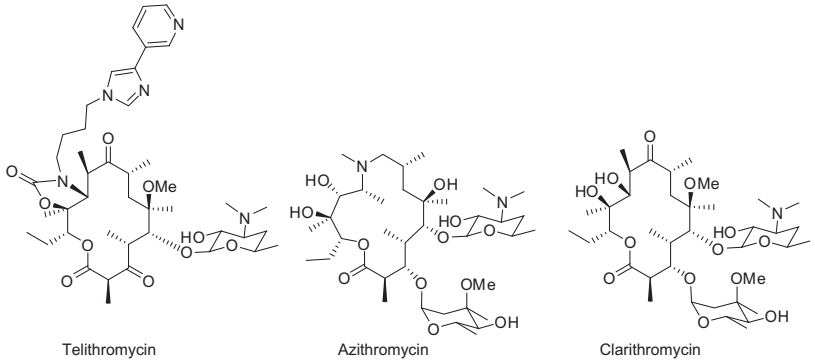


Figure 1. Structures of representative examples of clinically useful macrolides.

these functionalized azithromycin inhibited the growth of representative susceptible bacteria strains to about the same extent as azithromycin. Moreover, the inclusion of bovine serum in the bacterial growth media enhanced the anti-bacterial potency of the N-10 functionalized azithromycin analogs by as high as 10-fold while only 6-fold enhancement was observed for azithromycin.

1.1. Design and chemistry

Macrolides (Fig. 1), a class of clinically useful antibiotics, inhibit prokaryotic translation by partially blocking the exit tunnel just before the constriction point where ribosomal large subunit proteins L4 and L22 narrow the tunnel to about 10 Å.[13] However the efficacy of macrolides is being hampered by the increase in the prevalence of resistant bacteria.[14–16] Previous optimization of the macrolides has furnished ketolides, such as telithromycin, with enhanced potency against some macrolide-resistant bacteria.[17,18]

Toward an alternative structure-guided optimization of macrolides, we have analyzed the X-ray structures of azithromycin bound to the ribosomes from various prokaryotes[19,20] and the simulated structure of SecM bound to *E. coli* ribosome.[10] SecM is a translation stalling peptide. The minimum sequence of SecM required for ribosomal stalling has been identified as

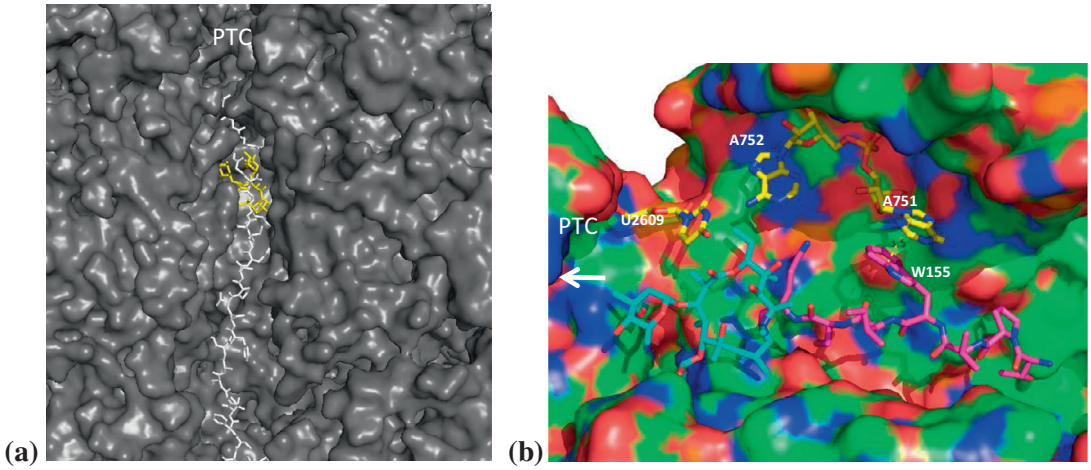
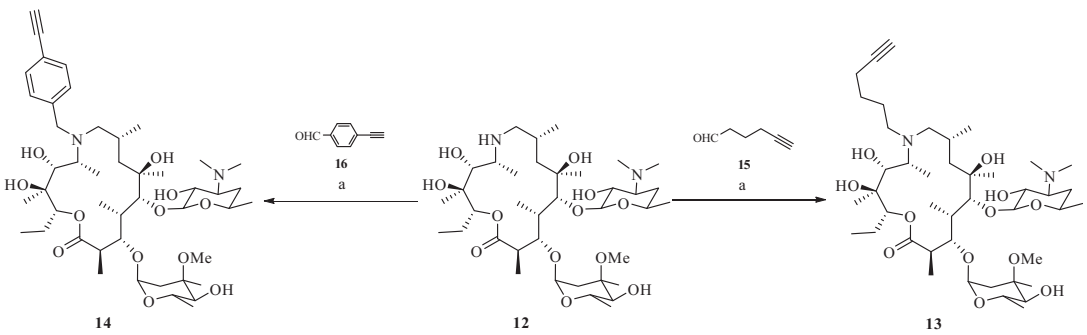


Figure 2. PDB 3OI1[19] showing the overlay of ribosome-bound azithromycin and modeled SecM. (a) Cross section of the exit tunnel. 50S is shown in grey, SecM model in white, azithromycin in yellow. PTC is at the top of the image. (b) View atop the exit tunnel where W155 and A751 are shown with 3.5 Å separation. Images generated using PyMOL.[23]



Scheme 1. Reagents and conditions: (a) $NaBH_3CN$, AcOH, DMF, 70 °C, 7 h.