Supplementary Material

Crystal Structure of the Oxazolidinone Antibiotic Linezolid Bound to the 50S Ribosomal Subunit

Joseph A. Ippolito[a], Zoltan F. Kanyo[a], Deping Wang[†], Francois J. Franceschi[a], Peter B. Moore[b], Thomas A. Steitz[b] and Erin M. Duffy[a]*

Department of Structure-Based Drug Design, Rib-X Pharmaceuticals Inc, 300 George Street, Suite 301, New Haven, CT 06511

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METHODS 50S ribosomal subunits from *H. marismortui* were purified and crystallized as reported previously[S1]. CCA-Phe was purchased from Dharmacon. Crystals of *H. marismortui* 50S were soaked for 3 hours in a stabilizing solution containing 5 mM linezolid plus 1 mM CCA-Phe. Crystals were frozen in propane and diffraction data were collected at beamline X25 at the National Synchrotron Light Source, Brookhaven National Laboratory. The data were integrated with DENZO and scaled with SCALEPACK[S2]. Initial electron density maps were calculated using CNX[S3, S4] following rigid body refinement of the native 50S structure (PDB ID code 1JJ2) against the x-ray data and revealed the binding positions of both linezolid and CCA-Phe. The ligands were modeled using the graphics program O[S5]. The structures were refined using Powell minimization and simulated annealing algorithms contained in CNX. The atomic coordinates and corresponding structure factors for the HMA 50S-linezolid-CCA-Phe complex have been deposited into the RCSB Protein Data Bank with accession code 3CPW. All 3-D graphical figures were prepared with VMD[S6] and Raster3D[S7].

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TABLE S1. Data Collection and Refinement Statistics for the Crystal Structure of Linezolid and CCA-Phe Bound to H50S

Data Collection	
Unit Cell	a=211.73, b=298.58, c=575.29, α=90.0, β=90.0, γ=90.0
Space Group	$C222_1$
Resolution, Å	40.0 - 2.70
Total reflections measured	1,080,057
Unique reflections measured[a]	470,299 (41,142)
Rmerge [a,b]	0.054 (0.412)
I/•(I)[a]	15.9 (2.3)
Completeness (%)[a]	95.6 (84.2)
Multiplicity[a]	2.3 (2.0)
Refinement	
Reflections used in refinement/test set	446,872/4,438
R_{cryst} [a,c]	0.191 (0.283)
R_{free} [a,d]	0.230 (0.286)
R.m.s. deviations Bond lengths, Å	0.005
R.m.s. deviations Bond angles, °	1.07

[a]Number in parentheses refer to the outermost 2.8-2.7 Å shell of data.

[b]$R_{merge} = \{\bullet_{hkl} \bullet_i | \ I_i(hkl) - \langle I(hkl)\rangle |\} / \{\bullet_{hkl} \bullet_i [I_i(hkl]\}$, where $\langle I(hkl)\rangle$ is the average intensity of reflection (*hkl*), and $I_i(hkl)$ is the i^{th} observation.

[c]$R_{cryst} = \{\bullet hkl \ | \ F_c(hkl) - F_o(hkl) |\} / \{\bullet hkl \ [F_o hkl]\}$, for data used for refinement, where $F_c(hkl)$ is the calculated model based amplitude for reflection *hkl* and F_o is the measured amplitude.

[d]R_{free} is calculated in the same manner as R_{cryst} for a test reflection set excluded from all refinement

TABLE S2. Corresponding Haloarcula marismortui (HMA) 23S rRNA Residues in Escherichia coli (EC)

HMA Residue	EC Residue
A2486	A2451
C2487	C2452
A2488	A2453
G2489	G2454
U2539	U2504
G2540	G2505
U2541	U2506
U2607	A2572
G2611	G2576
U2620	U2585

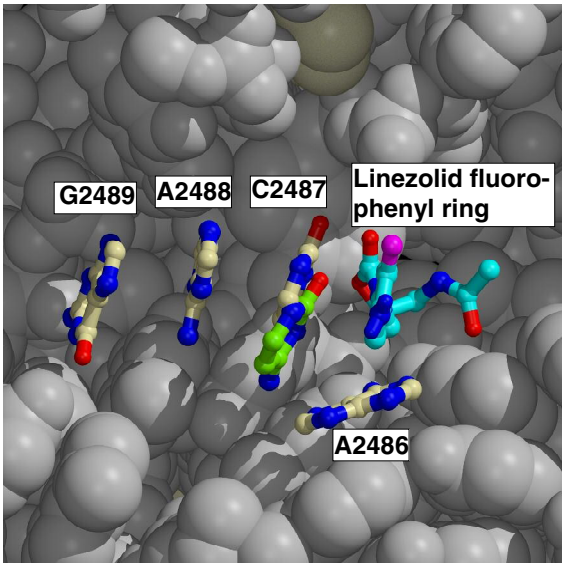

Figure S1. Pi-stacking interactions between the phenyl B-ring of linezolid (cyan) and 23S rRNA bases in HMA 50S are indicated. C2487 (EC 2452) is depicted in both its native (green) and linezolid-bound (yellow) conformations.

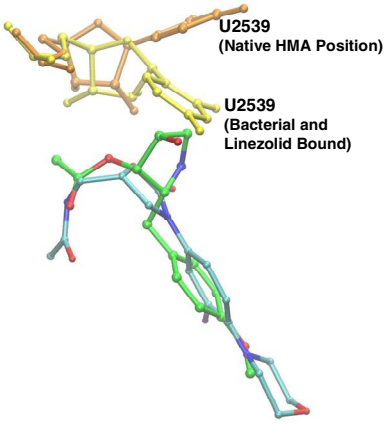

Figure S2. Superposition of linezolid (cyan) and anisomycin (green) (PDB 1K73) bound to the H50S A-site. The eukaryotic-specific inhibitor anisomycin cannot bind to the A site in bacterial 50S due to steric clash with the "down" conformation of U2539 (EC 2504) (yellow). The larger A-site pocket resulting from the "up" U2539 (EC 2504) conformer in native H50S (orange) accommodates anisomycin binding.