

C1-1432 STRUCTURE-ACTIVITY RELATIONSHIPS OF DIVERSE OXAZOLIDINONES FOR LINEZOLID-RESISTANT *S. aureus* STRAINS POSSESSING THE *cfr* METHYLTRANSFERASE GENE OR RIBOSOMAL MUTATIONS

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ABSTRACT

Background: Resistance to linezolid (LZD) is mediated through ribosomal gene mutations (23S rRNA or ribosomal proteins L3 and L4) or through methylation of 23S rRNA by the horizontally-transferred Cfr methyltransferase. Here we investigated the structure-activity relationships behind oxazolidinone potency against LZD^r *Staphylococcus aureus*.

Methods: MICs of compounds including LZD, TR-700 (torezolid) and a set of 5 additional oxazolidinone analogs (including novel CD rings and variable A-ring C-5 substituents) were determined via broth microdilution (CLSI). LZD^r *S. aureus* representatives included a variety of laboratory and clinically-derived strains possessing ribosomal mutations (23S rRNA, L3 or L4) or the *cfr* gene.

Results: Potency against all strains correlated with optimization of C- and D-rings which interact with more highly conserved regions of the peptidyl transferase center binding site. In the TR-700 analog series, C-5 acetamide and 1,2,3-triazole substituted compounds maintained a ≥2-fold potency advantage over hydroxymethyl groups vs. ribosomal mutants and LZD^r strains. Activity against *cfr* strains, however, was unaffected with either hydroxymethyl or triazole C-5 groups, but was reduced by 2- to 8-fold in compounds with acetamide substituents. Compound 4 (TR-700 C-5 triazole analog) had the greatest potency against all strain classes.

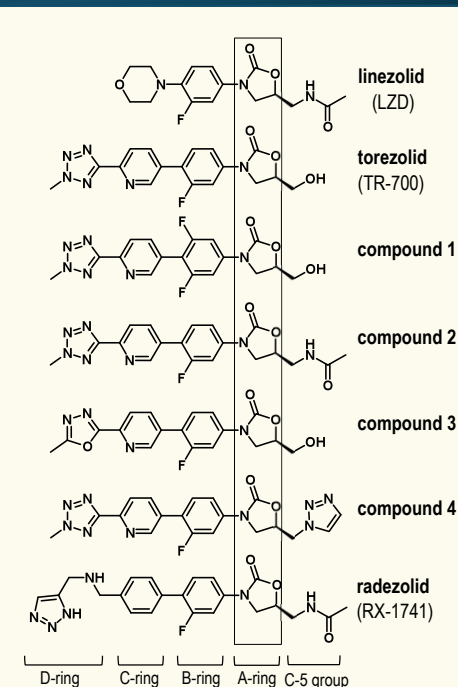
Conclusions: These data reveal key features contributing to oxazolidinone activity and highlight structural tradeoffs between potency against susceptible strains and potency against strains with various resistance mechanisms. TR-700 maintains ≥4-fold potency advantage over LZD against all *S. aureus* classes and was unaffected by Cfr methylation.

INTRODUCTION

❖ Oxazolidinone resistance is linked to mutations in 23S rRNA domain V (especially G2576T), ribosomal proteins L3 and L4, or through methylation by the Cfr methyltransferase (modifies 23S rRNA base A2503)

❖ To better understand how structural features correlate with activity against various resistance mechanisms, we tested a panel of structurally-diverse and clinically-relevant oxazolidinones against representative *S. aureus* strains for each of the major classes of linezolid resistance determinants described to date

COMPOUNDS



METHODS

- ❖ All *S. aureus* strains were cultured at 37°C on Mueller-Hinton II agar (MHA) or in liquid broth (MHB)
- ❖ Compounds included: TR-700 (torezolid, Trius Therapeutics, Inc.), linezolid (LZD, ChemPacific), Compounds 1, 2, 3, and 4 (Dong-A Pharmaceutical Co.), RX-1741 (radezolid, Medicilon), and VAN (vancomycin, Sigma-Aldrich)
- ❖ MIC assays (broth microdilution, CLSI) were repeated at least 3 times independently per compound/strain pairing
- ❖ Modeling of ribosomal mutations is based on the crystallographic structure of the *Haloarcula marismortui* LZD-bound 50S subunit (Ippolito et al., 2008, J. Med. Chem., 51:3353-56; PDB accession code 3CPW)

RESULTS

I.a. Oxazolidinone MICs for *S. aureus* ribosomal mutants

Strain	Resistance mechanism	MIC (µg/ml)								Reference/Source
		LZD	TR-700	1	2	3	4	RX-1741	VAN	
29213 ^a	-	2	0.5	0.5	0.25	0.5	0.125	1	1	ATCC
29213-1 ^a	23S (G2447T x3)	32	4	8	2	2	2	4	2	Locke et al., 2009, AAC, 53(12): 5265-5274
29213-2 ^a	23S (T2500A x2)	8	2	2	1	2	1	4	1	Locke et al., 2009, AAC, 53(12): 5265-5274
29213-3 ^a	L3 (ΔPhe127-His146)	8	2	2	1	2	0.5	2	2	Locke et al., 2009, AAC, 53(12): 5265-5274
33591 ^b	-	1	0.25	0.25	0.125	0.25	0.125	0.5	1	ATCC
33591-1 ^b	23S (G2576T x3)	16	2	4	1	2	0.5	2	1	Locke et al., 2009, AAC, 53(12): 5265-5274
33591-2 ^b	23S (G2576T/T2571C x3)	16	2	2	1	2	0.5	2	1	Locke et al., 2009, AAC, 53(12): 5265-5274
33591-3 ^b	L4 (Lys68Gln)	2	0.5	0.5	0.25	0.5	0.25	1	2	Locke et al., 2009, AAC, 53(12): 5265-5274
NRS127 ^c	L3 (ΔSer145)	8	1	2	0.5	1	0.25	4	2	NARSA

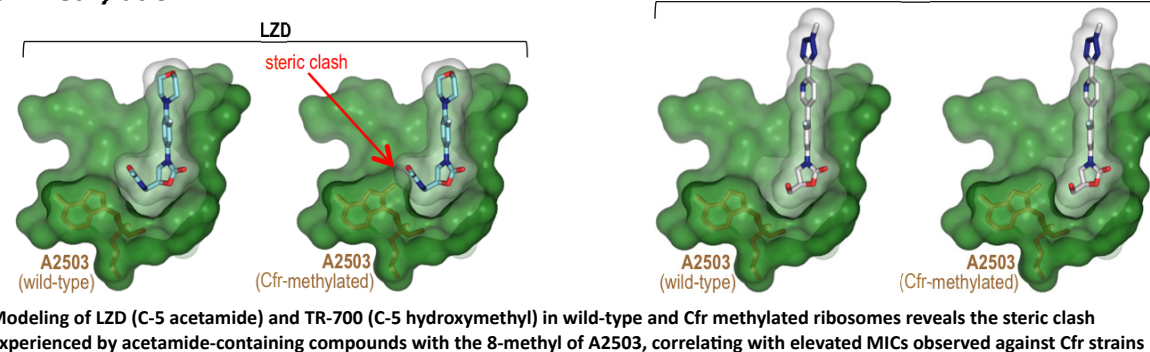
^aATCC 29213 and ^b33591 isogenic strain panels were generated through in vitro selection with LZD and/or TR-700 in a previous study; ^cNRS127 is a clinical LZD^r isolate from the NARSA collection

I.b. Oxazolidinone MICs for *S. aureus* *cfr* strains

Strain	<i>cfr</i> gene	MIC (µg/ml)								Reference/Source
		LZD	TR-700	1	2	3	4	RX-1741	VAN	
RN4220 + pLI50 ^a	-	2	0.5	0.5	0.25	0.5	0.125	0.5	1	Toh et al., 2007, Mol. Microbiol., 64(6):1506-1514
RN4220 + pLXM1 ^a	+	8	0.5	0.5	0.5	0.5	0.125	1	1	Toh et al., 2007, Mol. Microbiol., 64(6):1506-1514
CM05Δ ^b	-	2	0.5	0.5	0.25	0.5	0.125	1	1	Locke et al., ICAAC 2009, abstr. C1-1364b
CM05 ^b	+	8	0.5	0.5	1	0.5	0.125	2	1	Toh et al., 2007, Mol. Microbiol., 64(6):1506-1514
29213 ^c	-	2	0.5	0.5	0.25	0.5	0.25	1	1	ATCC
29213 + p42262 ^c	+	16	0.5	0.5	1	0.5	0.25	2	1	Locke et al., ICAAC 2010, abstr. C1-1431
42262 ^c	+	16	0.5	0.5	1	0.5	0.25	4	2	Morales et al., 2010, Clin. Infect. Dis., 50:821-825

^apLI50 is a non-*cfr* control construct of pLXM1; ^bCM05Δ is a *cfr*-negative isogenic derivative of the wild-type clinical CM05 isolate (chromosomal *cfr* gene); ^cATCC 29213 was transformed with the p42262 *cfr* plasmid from clinical isolate 42262

II. Structural analysis of acetamide vs. hydroxymethyl C-5 groups binding in the presence of Cfr methylation



RESULTS

- ❖ The addition of D-ring substituents increases the potency of oxazolidinones against all strain classes as compared to LZD
- ❖ From least potent to most potent activity against control and ribosomal mutant strains C-5 side groups rank order: hydroxymethyl, acetamide, 1,2,3-triazole
- ❖ All compounds possessing acetamide C-5 groups (LZD, RX-1741, 2) demonstrate losses in potency against *cfr* strains, while hydroxymethyl and 1,2,3-triazole C-5 compounds do not (TR-700, compounds 1, 3, and 4)
- ❖ Modeling of A2503 carbon 8 methylation by Cfr reveals steric hindrance with the C-5 acetamide group of LZD but not with the smaller hydroxymethyl group of TR-700

CONCLUSIONS

- ❖ TR-700 maintains a balanced and potent profile against all strain classes (hydroxymethyl group is unaffected by Cfr methylation and the methyltetrazole D-ring has high potency against all strain classes)
- ❖ TR-700 demonstrates significant potency advantages over LZD and RX-1741 against a variety of oxazolidinone resistance mechanisms, especially Cfr methylation
- ❖ The activity of triazole C-5 compounds against *cfr* strains is not readily explained, but may occur through flipping of A2503 into an alternative conformation that facilitates an additional H-bond between the inhibitor and 23S rRNA

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