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Structure-Activity Relationships of Diverse Oxazolidinones for Linezolid-Resistant *Staphylococcus aureus* Strains Possessing the *cfr* Methyltransferase Gene or Ribosomal Mutations[▽]

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Staphylococcal resistance to linezolid (LZD) is mediated through ribosomal mutations (23S rRNA or ribosomal proteins L3 and L4) or through methylation of 23S rRNA by the horizontally transferred Cfr methyltransferase. To investigate the structural basis for oxazolidinone activity against LZD-resistant (LZD^r) strains, we compared structurally diverse, clinically relevant oxazolidinones, including LZD, radezolid (RX-1741), TR-700 (torezolid), and a set of TR-700 analogs (including novel CD-rings and various A-ring C-5 substituents), against a panel of laboratory-derived and clinical LZD^r *Staphylococcus aureus* strains possessing a variety of resistance mechanisms. Potency against all strains was correlated with optimization of C- and D-rings, which interact with more highly conserved regions of the peptidyl transferase center binding site. Activity against *cfr* strains was retained with either hydroxymethyl or 1,2,3-triazole C-5 groups but was reduced by 2- to 8-fold in compounds with acetamide substituents. LZD, which possesses a C-5 acetamide group and lacks a D-ring substituent, demonstrated the lowest potency against all strains tested, particularly against *cfr* strains. 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.

The urgent need for new antibiotics is largely due to the increase in the frequency of bacterial infections with resistant strains, especially Gram-positive organisms, in both the hospital and community settings. Oxazolidinones are a relatively new class of antibiotics that inhibit bacterial protein synthesis by preventing binding of the aminoacyl-tRNA to the A site of the ribosome (30, 40). The potential utility of members of this class include intravenous (i.v.), oral, and stepdown therapy, as well as activity versus multidrug-resistant infections. Linezolid (LZD) (Zyvox) is currently the only FDA-approved and marketed oxazolidinone (1). Since entering the market in 2000, LZD has gained wide use clinically for treating infections caused by Gram-positive pathogens. There has been an ongoing effort to develop second-generation oxazolidinones (71), and currently there are two in clinical development: TR-701 (torezolid phosphate; Trius Therapeutics, Inc.) and radezolid (RX-1741; Rib-X Pharmaceuticals, Inc.).

The structure-activity relationships (SAR) of oxazolidinones have been extensively reported, starting with the original oxazolidinone SAR developed at DuPont (25, 26, 54), followed by SAR studies that led to the discovery of LZD by Pharmacia & Upjohn (5, 9, 10) (see Fig. 1). These studies demonstrated the key role of the 3-(3-fluorophenyl)-oxazolidinone rings and the 5-acetamidomethyl substituent. Later studies demonstrated that substituents in the 4 position of the 3-phenyl-oxazolidinone can enhance antibacterial potency, especially those containing a 3-aryl or heteroaryl ring (6, 31). Some of the most potent compounds are the 3-biphenyloxazolidinones discovered by Rib-X (RX-1741, formerly known as Rx-01_667) (64, 78, 79), the 3-(4-pyrid-3-yl)-phenyloxazolidinones discovered by Dong-A (DA-7867) (77), Kyorin/Merck (AM-7359) (37, 52), and AstraZeneca ("compound 13") (56), and the 3-(azolylphenyl)-oxazolidinones from Pharmacia & Upjohn ("compound 4") (20) (Fig. 1). Continued exploration of the SAR of the oxazolidinone ring C-5 position has led to the discovery of alternatives to acylaminomethyl groups that maintain or enhance antibacterial potency, such as the 1,2,3-triazol-2-yl-methyl (55), pyrid-2-yl-oxymethyl, and isoxazol-3-yl-oxymethyl groups (23). Although the initial 5-hydroxymethyl oxazolidinones examined by DuPont and Pharmacia & Upjohn were significantly weaker in potency than the 5-acylaminomethyl analogs, when combined with a potent ABCD ring system, the Dong-A scientists found that 5-hydroxymethyl-oxazolidinones such as DA-7157 (TR-700) (Fig. 1) had excellent antibacterial activity (29).

The emergence of LZD^r strains soon after the approval of LZD (22, 69) necessitated the requirement of maintenance of good activity against clinically resistant strains for all second-generation oxazolidinones. To date, the most frequently reported type of LZD resistance involves target-based mutations which arise primarily in patients undergoing long-term therapy (11, 17, 22, 69, 74). These mutations map to the domain V region of 23S rRNA genes (67), as well as to the genes encoding the 50S ribosomal proteins L3 and L4 (*rplC* and *rplD*, respectively) (28, 42, 43, 75, 76). Despite documentation of such target-based mutations, they remain infrequent in the clinic (18, 32, 34). Previous studies of TR-700 and radezolid have demonstrated enhanced potency of these molecules

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against LZD-resistant (LZD[r]) ribosomal mutants as a result of optimization of C- and D-ring systems (39, 42, 43, 63, 64), although a thorough SAR analysis has not been reported.

In more recent years, LZD[r] strains possessing the *cfr* gene have emerged around the world (12, 13, 18, 19, 32, 35, 36, 47, 48, 51, 68). Identification of a clinical strain carrying this novel *cfr* gene, previously seen only in veterinary isolates (46), was first reported in an LZD[r] MRSA strain isolated in 2005 in Medellin, Colombia (68). Through carbon-8 methylation of 23S rRNA base A2503 in the peptidyl transferase center (PTC) of the 50S ribosomal subunit, the Cfr methyltransferase greatly diminishes LZD binding, leading to MIC values in excess of the 4-μg/ml breakpoint (21, 36). Cfr methylation also confers resistance to phenicols, lincosamides, pleuromutilins, and streptogramin A antibiotics (46), as well as 16-membered ring macrolides (66). In addition to the potential for selection with multiple classes of 50S targeted antibiotics, Cfr is inherently more worrisome than target-based mutations because it is associated with horizontally transferable elements (2, 36, 46, 48, 49, 68) and has a low fitness cost (38). The influence of Cfr methylation on the antimicrobial activity of members of the oxazolidinone class has not been fully explored. Previous studies have shown that TR-700 maintains its potency against *cfr*-positive strains (MIC_{90}, 0.5 μg/ml) (50). Radezolid has demonstrated activity against a laboratory-generated *Staphylococcus aureus cfr* strain (MIC, 2 μg/ml) (39); however, its activity against clinical *cfr* strains has not been reported.

In contrast to the well-described SAR for oxazolidinones for wild-type *S. aureus* strains, little is known about the SAR of oxazolidinones for LZD[r] strains. Furthermore, when such an SAR data set is reported, it is typically for a single compound and for a limited number of strains. To address this shortfall, we established the SAR of structurally diverse oxazolidinones for a panel of clinical and laboratory-derived *S. aureus* strains possessing ribosomal mutations or the *cfr* methyltransferase gene. We also modeled the binding of these compounds to the PTC using the *Deinococcus radiodurans* and *Haloarcula marismortui* 50S subunit crystal structures to gain insights into the observed SAR trends, with the goal of informing the development of second-generation oxazolidinones that balance potency and activity against clinically relevant resistance determinants.

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MATERIALS AND METHODS

Bacterial strains and culture conditions. *S. aureus* strains were cultured aerobically at 37°C in Mueller-Hinton II cation-adjusted agar (MHA) or in broth (MHB). Strains included 29213 (methicillin-susceptible *S. aureus* [MSSA]; ATCC control strain) and isogenic mutants thereof (43), 33591 (methicillin-resistant *S. aureus* [MRSA]; ATCC control strain) and isogenic mutants thereof (43), NRS127 (MRSA, NRSA collection; ΔSer145 L3 mutation) (42), CM05 (MRSA; chromosomal *cfr*; clinical isolate) (68), CM05Δ (*cfr*-negative strain isogenic to CM05) (43), RN4220(pLI50) (contains the control vector pLI50) (68), RN4220(pLXM1) (pLI50 vector containing the cloned *cfr* gene under the control of a *B. subtilis* promoter, P*spac*) (68), 29213(p42262) (contains the *cfr* plasmid p42262 isolated from 42262 and transformed into ATCC 29213 as previously described [59]), and 42262 (MRSA; Spanish clinical isolate, plasmid-borne *cfr* gene) (50). All 23S rRNA alleles and *rplC* and *rplD* genes in clinical *cfr* isolates were amplified and sequenced as previously described (43) to ensure that no relevant 50S structural mutations were present which may have additionally contributed to reduced susceptibility to oxazolidinones.

Antimicrobial agents. Test solutions of TR-700 (torezolid; Trius Therapeutics, Inc., San Diego, CA), compound 1 (TR-700 B-ring difluoro analog), compound 2 (TR-700 C-5 acetamide analog), compound 3 (TR-700 D-ring 1,3,4-oxadiazole analog), compound 4 (TR-700 C-5 1,2,3-triazole analog) (Dong-A Pharmaceutical Co., Yongin, South Korea) equivalent to "example 1" of AstraZeneca U.S. patent 7,498,350 B2 (24), RX-1741 (radezolid; Medicilon, Chicago, IL), LZD (ChemPacific Corp., Baltimore, MD), and vancomycin (VAN) (Sigma-Aldrich Corp., St. Louis, MO) were freshly prepared in 100% dimethyl sulfoxide (DMSO) prior to use in MIC assays or selective medium (Fig. 1). Quality control of oxazolidinones used in this study was performed via nuclear magnetic resonance (NMR), liquid chromatography-mass spectrometry (LC-MS), and biological activity assays.

MIC testing. MIC assays were performed via broth microdilution in accordance with CLSI guidelines (14), and values were determined visually through detection with alamarBlue (Invitrogen Corp., Carlsbad, CA) as previously described (4). Enumeration of CFU was performed by serially diluting the assay inocula in phosphate-buffered saline (PBS) and plating on MHA. MIC values reported for each strain/drug combination were determined in at least three independent experiments, all yielding identical results.

Modeling analysis of SAR trends. Proposed structural effects for the SAR trends in this study were deduced using the coordinates of the *Deinococcus radiodurans* and *Haloarcula marismortui* LZD-bound 50S crystal structures (PDB accession codes 3DLL and 3CPW) (30, 73). Sequence alignments showed that the regions of the 50S structures discussed in this study are highly conserved, so the structural rationales proposed would be expected to hold for *S. aureus* and other species. Docking studies were conducted using the LigandFit protocol (72) in the Discovery Studio software (Accelrys, Inc.). Both the 3DLL and 3CPW structures were examined, but only the 3CPW structure gave reasonable docking results, likely due to the higher resolution of the 3CPW structure. As a test of the docking protocol, LZD was docked into the 3CPW structure using the flexible ligand mode with minimization of the initial fit using Smart Minimizer. This routine provided a docked LZD pose that had a root mean square (RMS) of 0.46 Å compared to the X-ray structure, indicating the program can retrieve a predictive structure. In addition to LZD, the ABC ring system of TR-700 and compound 4 (triazole) were also docked into the 3CPW structure using the same LigandFit (72) protocol, and images thereof were generated using the PyMOL software program (16). No satisfactory model was obtained with compound 4 (see Fig. 1).

RESULTS

A diverse set of oxazolidinones show similar potency shifts between LZD[s] control strains and isogenic LZD[r] ribosomal mutants. Seven oxazolidinones (Fig. 1) were tested against two sets of isogenic laboratory-derived *S. aureus* strains with mutations in 23S rRNA, ribosomal proteins L3 and L4, and NRS127, an LZD[r] clinical isolate containing an L3 mutation (42, 43). Mutants demonstrated a 4- to 16-fold increase in the LZD MIC, resulting in resistance (MIC > 4 μg/ml) in all but strain 33591-3, where only a 2-fold increase in the MIC was observed (L4 mutation). Compared to TR-700 and the hydroxymethyl analogs (compounds 1 and 3), the acetamide (compound 2) and 1,2,3-triazole C-5 (compound 4) substituents maintained 2- to 4-fold potency advantages against susceptible and isogenic LZD[r] ribosomal mutant strains (Table 1), consistent with findings in previous studies (29).

The most significant shift in MIC was observed with the 3-copy G2447T, G2576T, and G2576T/T2571C mutants (Table 1). Both TR-700 and radezolid had an MIC of 4 μg/ml versus the G2447T mutant; however, the difluoro analog of TR-700 (compound 1) demonstrated an MIC of 8 μg/ml. TR-700, radezolid, and compound 3 had an MIC of 2 μg/ml versus the G2576T mutant, whereas the MIC of compound 1 was again elevated an additional 2-fold to 4 μg/ml (Table 1). Only TR-

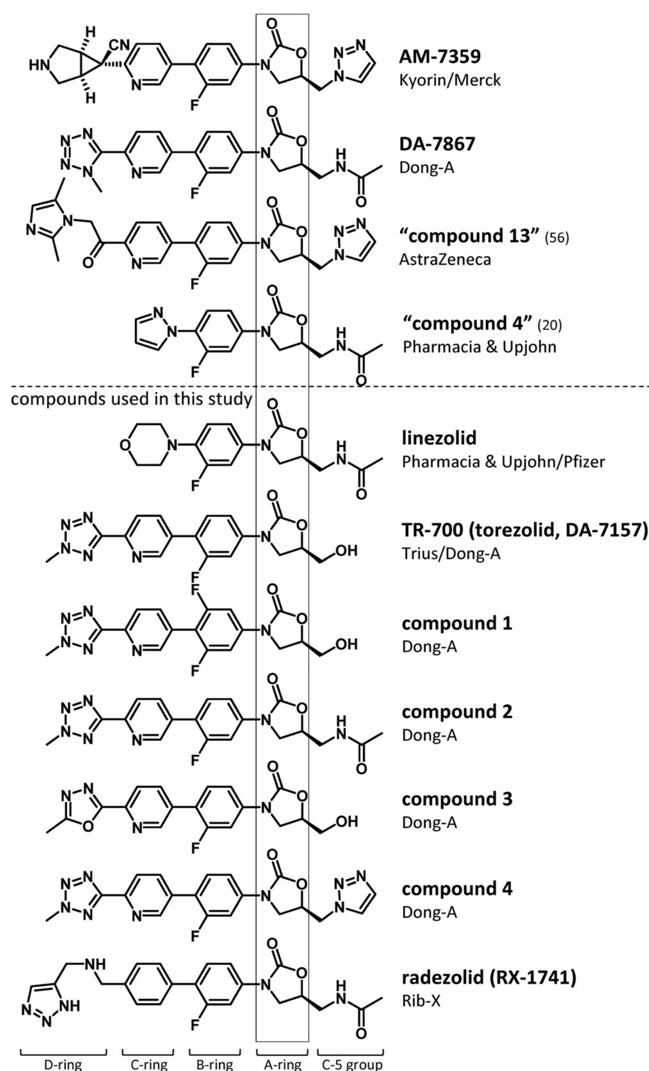

FIG. 1. Chemical structures. Examples of lead oxazolidinones developed to date are given. The oxazolidinones selected for this study vary in the composition of A-ring C-5 substituents and B-, C-, and D-rings.

700 and compounds 1 to 4 had MICs of ≤2 μg/ml against the LZD[r] clinical isolate NRS127 (L3 mutation). Overall, both TR-700 and radezolid performed well against these ribosomal mutants, with TR-700 equal to or 2-fold better than radezolid. TR-700 typically showed ≥4-fold potency advantages over LZD.

Potency of compounds with hydroxymethyl and 1,2,3-triazole C-5 substituents is unaffected by the presence or absence of Cfr methylation, while compounds containing the acetamide substituent demonstrate a 2- to 8-fold loss in activity. Previous studies comparing LZD and TR-700 demonstrated that TR-700 maintains potent activity against *cfr*-positive strains whereas LZD does not (33, 41–43, 50, 63). To probe whether the acetamide substituent accounted for this difference, we compared various C-5 substituents (Fig. 1) against three isogenic *cfr*-carrying or -lacking strain sets (1 laboratory-derived and 2 clinical) (Table 2).

When an isogenic pair of RN4220 strains with or without a cloned *cfr* gene (68) were compared, a 2- to 4-fold increase in the MIC of acetamide-containing compounds was observed, with no change in the MIC of compounds containing a hydroxymethyl or a 1,2,3-triazole substituent (Table 2). The MIC values for radezolid against RN4220(pLI50) and RN4220(pLXM1) are 2-fold lower than previously reported values for this laboratory-derived isogenic set (39).

CM05 was the first documented clinical isolate carrying the *cfr* gene, which was shown to be present in the *S. aureus* chromosome (68). Recent studies have shown that this isolate contains a mixture of strains that possess *cfr* (LZD[r]) and ones in which the *cfr* gene and one copy of the adjacent *ermB* gene are deleted (LZD[s]) (44). The MICs of the isogenic, clonally pure CM05 (*cfr*-positive) and CM05Δ (*cfr*-negative) strains are compared in Table 2. In all cases, compounds with an acetamide (LZD, compound 2, and radezolid) demonstrated a 2- to 4-fold increase in the MIC against CM05 versus CM05Δ. However, compounds with hydroxymethyl (TR-700, compound 1, and compound 3) or 1,2,3-triazole substituents (compound 4) showed no difference in the MIC in the presence or absence of Cfr.

To examine SAR for strains possessing a clinically derived, plasmid-borne *cfr* gene, MICs were determined against 42262

TABLE 1. Oxazolidinone MICs for *S. aureus* ribosomal mutants

Strain[a]	Source or reference	Resistance mechanism[b]	MIC (μg/ml)[c]							
			LZD	TR-700	Compound:				RZD	VAN
					1	2	3	4		
29213	ATCC		2	0.5	0.5	0.25	0.5	0.125	1	1
29213-1	43	23S (G2447T ×3)	32	4	8	2	2	2	4	2
29213-2	43	23S (T2500A ×2)	8	2	2	1	2	1	4	1
29213-3	43	L3 (ΔPhe127-His146)	8	2	2	1	2	0.5	2	2
33591	ATCC		1	0.25	0.25	0.125	0.25	0.125	0.5	1
33591-1	43	23S (G2576T ×3)	16	2	4	1	2	0.5	2	1
33591-2	43	23S (G2576T/T2571C ×3)	16	2	2	1	2	0.5	2	1
33591-3	43	L4 (Lys68Gln)	2	0.5	0.5	0.25	0.5	0.25	1	2
NRS127	NARSA[d]	L3 (ΔSer145)	8	1	2	0.5	1	0.25	4	2

[a] ATCC 29213 and ATCC 33591 isogenic mutant panels were generated through selection in the presence of LZD and/or TR-700. NRS127 is an LZD[r] clinical isolate.
[b] Mutations in 23S rRNA genes (and mutant allele copy number) or in the ribosomal protein L3 or L4 are shown.
[c] MICs (broth microdilution; CLSI) were determined against the oxazolidinone panel (Fig. 1). VAN, vancomycin; RZD, radezolid.
[d] Network of Antimicrobial Resistance in *Staphylococcus aureus*.

TABLE 2. Oxazolidinone MICs for *S. aureus cfr* strains

Strain	Reference	Presence of *cfr*	MIC (μg/ml)[a]							
			LZD	TR-700	Compound:				RZD	VAN
					1	2	3	4		
RN4220(pLI50)	68	−	2	0.5	0.5	0.25	0.5	0.125	0.5	1
RN4220(pLXM1)[b]	68	+	8	0.5	0.5	0.5	0.5	0.125	1	1
CM05Δ[c]	44	−	2	0.5	0.5	0.25	0.5	0.125	1	1
CM05[c]	68	+	8	0.5	0.5	1	0.5	0.125	2	1
29213	ATCC	−	2	0.5	0.5	0.25	0.5	0.25	1	1
29213(p42262)[d]	45	+	16	0.5	0.5	1	0.5	0.25	2	1
42262[e]	51	+	16	0.5	0.5	1	0.5	0.25	4	2

[a] MICs (broth microdilution; CLSI) were performed against the oxazolidinone panel (Fig. 1). VAN, vancomycin; RZD, radezolid.
[b] The pLXM1 *cfr*-containing plasmid is isogenic to the empty pLI50 vector.
[c] CM05Δ is isogenic to the CM05 clinical *cfr*-positive strain but lacks *cfr* and one copy of *ermB*.
[d] 29213(p42262) was generated through transformation of ATCC 29213 with the p42262 *cfr*-containing plasmid isolated from strain 42262.
[e] 42262 is a clinical *cfr*-positive isolate from a 2008 hospital outbreak in Madrid, Spain.

(a representative *cfr*-positive strain from an LZD^r MRSA outbreak in Madrid, Spain [50]) and 29213(p42262) (ATCC 29213 control strain transformed with the *cfr* plasmid isolated from 42262). MIC values for TR-700 and LZD of 0.5 μg/ml versus 16 μg/ml against these two strains (Table 2) are identical to the previously established MIC_{90} for these two compounds against the entire collection of 18 clinical strains (50). Acetamide-containing compounds (radezolid and compound 2) showed a 4-fold higher MIC against the clinical 42262 isolate. MIC values for the 29213(p42262) strain increased 2-fold for radezolid and 4-fold for compound 2 over MIC values of the isogenic wild-type ATCC 29213 background (Table 2). For TR-700 and compounds 1 to 4, there is a small potency advantage for the acetamide (compound 2) compared to the activity of the hydroxymethyl compounds (TR-700, compound 1, and compound 3) on the 29213 control strain; however, for the acetamide compounds, this potency advantage is lost in the presence of the *cfr* gene. Despite the presence of a C-5 acetamide in radezolid, MIC values against *cfr* strains for this compound fall between those of the hydroxymethyl analogs (TR-700 and compounds 1 and 3) and LZD, likely because of additional binding interactions (CD-ring system) with the upper region of the PTC (64). Interestingly, the TR-700 ABCD-ring system containing a triazole (compound 4) had the lowest MIC values against both the *cfr*-negative and *cfr*-positive clinical strains.

DISCUSSION

The discovery and development of new oxazolidinones have remained challenging, due to the need to maintain favorable absorption, distribution, metabolism, and excretion properties and solubility while securing improvements in safety and efficacy. The SAR data herein build on earlier studies and highlight structural areas of interest in the design of oxazolidinones in order to address the evolving variety of resistance mechanisms documented for this anti-infective class.

Prior SAR studies have clearly demonstrated the positive contribution of the acetamidomethyl side chain (65), as seen in LZD, to antimicrobial potency, with N—H acting as a hydrogen bond donor essential for interactions with the ribosome binding site (5, 23, 25, 26, 53, 54). As such, this group has been retained in oxazolidinones, having undergone significant development efforts (eperezolid, RWJ-416457, and radezolid). Although many other heterocyclic substitutions were poorly tolerated (58), certain analogs, such as the 1,2,3-triazoles from AstraZeneca ("compound 2" [57] and "example 1" [24]) and Kyorin/Merck (AM-7359) (37, 52) showed good activity, possibly due to different binding modes in the ribosome (57). Thus, in addition to the 1,2,3-triazole, there is now significant interest in other A-ring C-5 substituents, such as the hydroxymethyl (torezolid) group.

In this study, the activities of compounds with three different A-ring C-5 substituents were evaluated against strains possessing mutations in 50S structural components or carrying the *cfr* methyltransferase gene. Comparing TR-700-series analogs revealed that both acetamide and triazole groups confer small potency advantages (2-fold and 2- to 4-fold, respectively) over hydroxymethyl substituents against all classes of 50S mutation-based resistance determinants characterized to date (23S rRNA, L3, and L4). Evaluation of variable C-5 substituents against *cfr* strains, however, revealed a sensitive SAR, suggesting interactions between these substituents and the Cfr-methylated A2503 base. Docking the ABC ring systems of TR-700 and LZD, which vary in the C-5 position, provided an explanation for the MIC data with the *cfr* strains (Fig. 2). In an unmethylated (wild-type) ribosome, the hydroxymethyl group of TR-700 docked in a fashion wherein the AB-rings replicated the interactions of LZD in the X-ray structure (Fig. 2A and C). The hydroxyl group formed a hydrogen bond to the same phosphate oxygen (G2540 in the 3CPW structure) that LZD binds to (30). Base A2503, highlighted in stick form (Fig. 2), is positioned adjacent to the pocket occupied by the acetamide of LZD or the hydroxymethyl of TR-700 (Fig. 2A and C). To model the Cfr methylation, a methyl group was added to A2503 (3CPW structure) (30). Subsequently, we were unable to retrieve a docked pose for LZD that maintained the binding to the key phosphate oxygen (G2540). Overlay of the methylated model structure with the X-ray coordinates of the LZD bound to the PTC demonstrated the potential for a significant steric clash between the acetamide and the methyl group on the carbon-8 of A2503 (Fig. 2B). In contrast, docking TR-700 demonstrated a similar pose in both the wild-type and the Cfr-methylated ribosome models, without any steric clashes

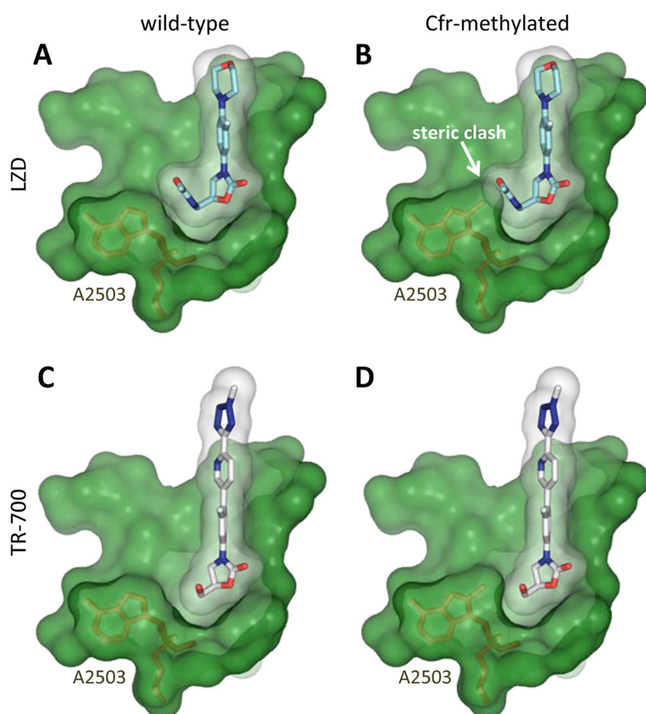

FIG. 2. Structural analysis of oxazolidinone binding in the presence of Cfr methylation. (A) Crystal structure of LZD-bound *H. marismortui* 50S ribosome (30). (B) Model of LZD binding in the Cfr-methylated state. (C and D) Proposed models of TR-700 bound to wild-type (C) or Cfr-methylated (D) ribosome. Substantial steric hindrance between the LZD C-5 acetamide group and the 23S rRNA base A2503 carbon-8 methyl (bonds shown in brown) likely contributes to reduced binding affinity (B). As modeled, the TR-700 hydroxymethyl substituent does not display this steric clash with the A2503 methyl group (D), explaining its retained activity against *cfr* strains. A group of PTC bases were removed from the images to improve clarity. Images were generated with PyMOL (16).

(Fig. 2C and D). The data in this study showed that both the triazole- and hydroxyl-containing compounds maintain full potency against the clinical *cfr* isolates. For the hydroxyl-containing compounds, the excellent potency on *cfr* strains is readily explained by the ability of this smaller substituent to easily fit adjacent to the methylated carbon-8 of A2503 without any steric overlap (Fig. 2D). Alternative docking poses of the triazole analog (compound 4) could not explain the increased potency of this group, suggesting additional conformational adjustments of the bases of the PTC (data not shown).

Although the MICs of acetamide-containing compounds increase approximately 4-fold in strains carrying the Cfr methyltransferase, the steric clash observed in the modeling studies suggests that some conformational movement at the active site is necessary for the acetamide-containing compounds to have any activity at all. One possibility is that A2503 rotates, as seen in many (non-Cfr-methylated) antibiotic-bound *D. radiodurans* structures, such that the 8-methyl is positioned away from the PTC (7, 15, 27, 60, 61). In the methylated state, this "out" conformation would be energetically unfavorable (relative to the unmethylated state) due to subtle but necessary accommodating movements of the RNA backbone. However, it would allow for binding of the acetamide compounds, albeit with greatly diminished potency relative to the hydroxyl-containing oxazolidinones, that would not require this conformational change. The MIC data reported here are consistent with this hypothesis.

Modeling suggests that the binding of the triazole-substituted compound 4 would also require the rotation of the 8-methyl of A2503 away from the PTC, as described above. However, the low MICs observed for this compound suggest that it gains additional interactions that compensate for the somewhat energetically unfavorable "out" rotation of methylated A2503. The known SAR of compound 4 shows that the 3-nitrogen of the triazole group is crucial to its potent MICs and therefore likely functions as a key hydrogen bond acceptor for interaction with the PTC (29). The free hydroxyl groups of A2503 or G2505 represent reasonable candidates for this interaction, and both have previously been found to participate in ribosome-antibiotic hydrogen bond interactions. Specifically, lankacidin and a subset of pleuromutilins hydrogen bond with the hydroxyl of A2503 (3, 15), and clindamycin and dalfopristin have been found to interact with that of G2505 (27, 62, 70).

Previous modeling of oxazolidinones bound in the 50S subunit has revealed that C- and D-ring groups likely gains additional interactions with more highly conserved 23S rRNA bases, such as U2585 (63, 64), supporting their role in the SAR described here. MIC data from both the ribosomal mutant and *cfr* strains revealed that C- and D-rings play a critical role in activity against all strain types. Among the TR-700 analogs, the 1,3,4-oxadiazole (compound 3) and the methyltetrazole (TR-700) were equipotent against all strains tested. The rank order (from least to most potent against both susceptible and resistant strains) of the three acetamide oxazolidinones examined in this study is LZD, which lacks a D-ring substituent, followed by radezolid, followed by compound 2. The 2- to 4-fold potency advantage of compound 2 over radezolid and the potency advantage of TR-700 over radezolid against the majority of strains tested here suggest that the CD-ring system in radezolid does not bind to the target as tightly as does the CD-ring system in the TR-700 analog series. Both TR-700 and radezolid, however, are intrinsically more potent than LZD, supporting the theory that D-ring substituents provide a potency advantage against all strain types.

Additional crystallographic studies demonstrating PTC-bound oxazolidinones would greatly enhance the interpretation of SAR data. A more complete understanding of the SAR behind LZD resistance derived from existing tools, however, is still very useful for informing the development of second-generation oxazolidinones which maintain activity against a variety of resistance mechanisms, including Cfr methylation. This is of high priority now that *cfr* and all three ribosomal mutation-based resistance mechanism classes have been found to co-occur in clinical staphylococci (including *cfr* plus 23S rRNA [13], *cfr* plus L4 [8], and *cfr* plus L3 [45]), resulting in exacerbated reduced susceptibility to oxazolidinones, particularly those containing C-5 acetamide substituents.

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REFERENCES

1. **Ament, P. W., N. Jamshed, and J. P. Horne.** 2002. Linezolid: its role in the treatment of Gram-positive, drug-resistant bacterial infections. Am. Fam. Physician **65:**663–670.
2. **Arias, C. A., M. Vallejo, J. Reyes, D. Panesso, J. Moreno, E. Castaneda, M. V. Villegas, B. E. Murray, and J. P. Quinn.** 2008. Clinical and microbiological aspects of linezolid resistance mediated by the *cfr* gene encoding a 23S rRNA methyltransferase. J. Clin. Microbiol. **46:**892–896.
3. **Auerbach, T., I. Mermershtain, C. Davidovich, A. Bashan, M. Belousoff, I. Wekselman, E. Zimmerman, L. Xiong, D. Klepacki, K. Arakawa, H. Kinashi, A. S. Mankin, and A. Yonath.** 2010. The structure of ribosome-lankacidin complex reveals ribosomal sites for synergistic antibiotics. Proc. Natl. Acad. Sci. U. S. A. **107:**1983–1988.
4. **Baker, C. N., and F. C. Tenover.** 1996. Evaluation of Alamar colorimetric broth microdilution susceptibility testing method for staphylococci and enterococci. J. Clin. Microbiol. **34:**2654–2659.
5. **Barbachyn, M. R., and C. W. Ford.** 2003. Oxazolidinone structure-activity relationships leading to linezolid. Angew. Chem. Int. Ed. Engl. **42:**2010–2023.
6. **Barbachyn, M. R., D. K. Hutchinson, S. J. Brickner, M. H. Cynamon, J. O. Kilburn, S. P. Klemens, S. E. Glickman, K. C. Grega, S. K. Hendges, D. S. Toops, C. W. Ford, and G. E. Zurenko.** 1996. Identification of a novel oxazolidinone (U-100480) with potent antimycobacterial activity. J. Med. Chem. **39:**680–685.
7. **Berisio, R., F. Schluenzen, J. Harms, A. Bashan, T. Auerbach, D. Baram, and A. Yonath.** 2003. Structural insight into the role of the ribosomal tunnel in cellular regulation. Nat. Struct. Biol. **10:**366–370.
8. **Bonilla, H., J. P. Quinn, J. Siedel, H. Schmidt, M. Lescoe, S. P. McCurdy, M. M. Lemmon, M. D. Huband, L. Puzniak, T. Hart, K. Jafri, L. A. Brennan, and A. Tait-Kamradt.** 2009. Abstr. 49th Intersci. Conf. Antimicrob. Agents Chemother., abstr. C2-131.
9. **Brickner, S. J., M. R. Barbachyn, D. K. Hutchinson, and P. R. Manninen.** 2008. Linezolid (ZYVOX), the first member of a completely new class of antibacterial agents for treatment of serious gram-positive infections. J. Med. Chem. **51:**1981–1990.
10. **Brickner, S. J., D. K. Hutchinson, M. R. Barbachyn, P. R. Manninen, D. A. Ulanowicz, S. A. Garmon, K. C. Grega, S. K. Hendges, D. S. Toops, C. W. Ford, and G. E. Zurenko.** 1996. Synthesis and antibacterial activity of U-100592 and U-100766, two oxazolidinone antibacterial agents for the potential treatment of multidrug-resistant gram-positive bacterial infections. J. Med. Chem. **39:**673–679.
11. **Burleson, B. S., D. J. Ritchie, S. T. Micek, and W. M. Dunne.** 2004. *Enterococcus faecalis* resistant to linezolid: case series and review of the literature. Pharmacotherapy **24:**1225–1231.
12. **Campanile, F., D. Bongiorno, S. Borbone, G. Mongelli, M. Baldi, R. Provenzani, and S. Stefani.** 2009. Abstr. 19th Eur. Congr. Clin. Microbiol. Infect. Dis., abstr. P929. European Society of Clinical Microbiology and Infectious Diseases, Munich, Germany.
13. **Cercenado, E., M. Marín, R. Insa, and E. Bouza.** 2010. Abstr. 20th Eur. Congr. Clin. Microbiol. Infect. Dis., abstr. P1104. European Society of Clinical Microbiology and Infectious Diseases, Munich, Germany.
14. **CLSI.** 2006. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Approved standard, CLSI document M7-A7, 7th ed., vol. 26, no. 2. CLSI, Wayne, PA.
15. **Davidovich, C., A. Bashan, T. Auerbach-Nevo, R. D. Yaggie, R. R. Gontarek, and A. Yonath.** 2007. Induced-fit tightens pleuromutilins binding to ribosomes and remote interactions enable their selectivity. Proc. Natl. Acad. Sci. U. S. A. **104:**4291–4296.
16. **DeLano, W. L.** 2002. The PyMOL molecular graphics system. DeLano Scientific, Palo Alto, CA.
17. **Engemann, J. J., M. J. Joyce, L. J. Harrell, S. Evans, L. Reller, and D. Sexton.** 2003. Abstr. 43rd Intersci. Conf. Antimicrob. Agents Chemother., abstr. 1112. American Society for Microbiology, Washington, DC.
18. **Farrell, D. J., R. E. Mendes, J. E. Ross, and R. N. Jones.** 2009. Linezolid surveillance program results for 2008 (LEADER Program for 2008). Diagn. Microbiol. Infect. Dis. **65:**392–403.
19. **Fritsche, T. R., M. Castanheira, R. E. Mendes, R. N. Jones, and L. M. Deshpande.** 2008. Abstr. 108th Gen. Meet. Am. Soc. Microbiol., abstr. A-047. American Society for Microbiology, Washington, DC.
20. **Genin, M. J., D. A. Allwine, D. J. Anderson, M. R. Barbachyn, D. E. Emmert, S. A. Garmon, D. R. Graber, K. C. Grega, J. B. Hester, D. K. Hutchinson, J. Morris, R. J. Reischer, C. W. Ford, G. E. Zurenko, J. C. Hamel, R. D. Schaadt, D. Stapert, and B. H. Yagi.** 2000. Substituent effects on the antibacterial activity of nitrogen-carbon-linked (azolylphenyl)oxazolidinones with expanded activity against the fastidious gram-negative organisms *Haemophilus influenzae* and *Moraxella catarrhalis*. J. Med. Chem. **43:**953–970.
21. **Giessing, A. M., S. S. Jensen, A. Rasmussen, L. H. Hansen, A. Gondela, K. Long, B. Vester, and F. Kirpekar.** 2009. Identification of 8-methyladenosine as the modification catalyzed by the radical SAM methyltransferase Cfr that confers antibiotic resistance in bacteria. RNA **15:**327–336.
22. **Gonzales, R. D., P. C. Schreckenberger, M. B. Graham, S. Kelkar, K. DenBesten, and J. P. Quinn.** 2001. Infections due to vancomycin-resistant *Enterococcus faecium* resistant to linezolid. Lancet **357:**1179.
23. **Gravestock, M. B., D. G. Acton, M. J. Betts, M. Dennis, G. Hatter, A. McGregor, M. L. Swain, R. G. Wilson, L. Woods, and A. Wookey.** 2003. New classes of antibacterial oxazolidinones with C-5, methylene O-linked heterocyclic side chains. Bioorg. Med. Chem. Lett. **13:**4179–4186.
24. **Gravestock, M. B., N. J. Hales, F. Reck, and F. Zhou.** 3 March 2009. Oxazolidinones as antibacterial agents. U.S. patent 7,498,350 B2.
25. **Gregory, W. A., D. R. Brittelli, C. L. Wang, H. S. Kezar III, R. K. Carlson, C. H. Park, P. F. Corless, S. J. Miller, P. Rajagopalan, M. A. Wuonola, et al.** 1990. Antibacterials. Synthesis and structure-activity studies of 3-aryl-2-oxooxazolidines. 2. The "A" group. J. Med. Chem. **33:**2569–2578.
26. **Gregory, W. A., D. R. Brittelli, C. L. Wang, M. A. Wuonola, R. J. McRipley, D. C. Eustice, V. S. Eberly, P. T. Bartholomew, A. M. Slee, and M. Forbes.** 1989. Antibacterials. Synthesis and structure-activity studies of 3-aryl-2-oxooxazolidines. 1. The "B" group. J. Med. Chem. **32:**1673–1681.
27. **Harms, J. M., F. Schlunzen, P. Fucini, H. Bartels, and A. Yonath.** 2004. Alterations at the peptidyl transferase centre of the ribosome induced by the synergistic action of the streptogramins dalfopristin and quinupristin. BMC Biol. **2:**4.
28. **Holzel, C. S., K. S. Harms, K. Schwaiger, and J. Bauer.** 2010. Resistance to linezolid in a porcine *Clostridium perfringens* strain carrying a mutation in the *rplD* gene encoding the ribosomal protein L4. Antimicrob. Agents Chemother. **54:**1351–1353.
29. **Im, W., S. Choi, and J. Rhee.** 2007. Abstr. 47th Intersci. Conf. Antimicrob. Agents Chemother., abstr. F1-1686. American Society for Micriobiology, Washington, DC.
30. **Ippolito, J. A., Z. F. Kanyo, D. Wang, F. J. Franceschi, P. B. Moore, T. A. Steitz, and E. M. Duffy.** 2008. Crystal structure of the oxazolidinone antibiotic linezolid bound to the 50S ribosomal subunit. J. Med. Chem. **51:**3353–3356.
31. **Jo, Y. W., W. B. Im, J. K. Rhee, M. J. Shim, W. B. Kim, and E. C. Choi.** 2004. Synthesis and antibacterial activity of oxazolidinones containing pyridine substituted with heteroaromatic ring. Bioorg. Med. Chem. **12:**5909–5915.
32. **Jones, R. N., S. Kohno, Y. Ono, J. E. Ross, and K. Yanagihara.** 2009. ZAAPS International Surveillance Program (2007) for linezolid resistance: results from 5591 Gram-positive clinical isolates in 23 countries. Diagn. Microbiol. Infect. Dis. **64:**191–201.
33. **Jones, R. N., G. J. Moet, H. S. Sader, R. E. Mendes, and M. Castanheira.** 2009. TR-700 in vitro activity against and resistance mutation frequencies among Gram-positive pathogens. J. Antimicrob. Chemother. **63:**716–720.
34. **Jones, R. N., J. E. Ross, J. M. Bell, U. Utsuki, I. Fumiaki, I. Kobayashi, and J. D. Turnidge.** 2009. Zyvox Annual Appraisal of Potency and Spectrum Program: linezolid surveillance program results for 2008. Diagn. Microbiol. Infect. Dis. **65:**404–413.
35. **Kehrenberg, C., C. Cuny, B. Strommenger, S. Schwarz, and W. Witte.** 2009. Methicillin-resistant and -susceptible *Staphylococcus aureus* strains of clonal lineages ST398 and ST9 from swine carry the multidrug resistance gene *cfr*. Antimicrob. Agents Chemother. **53:**779–781.
36. **Kehrenberg, C., S. Schwarz, L. Jacobsen, L. H. Hansen, and B. Vester.** 2005. A new mechanism for chloramphenicol, florfenicol and clindamycin resistance: methylation of 23S ribosomal RNA at A2503. Mol. Microbiol. **57:** 1064–1073.
37. **Komine, T., A. Kojima, Y. Asahina, T. Saito, H. Takano, T. Shibue, and Y. Fukuda.** 2008. Synthesis and structure-activity relationship studies of highly potent novel oxazolidinone antibacterials. J. Med. Chem. **51:**6558–6562.
38. **LaMarre, J. M., T. Zhu, J. B. Locke, K. J. Shaw, and A. S. Mankin.** 2009. Abstr. 49th Intersci. Conf. Antimicrob. Agents Chemother., abstr. C1-1346. American Society for Microbiology, Washington, DC.
39. **Lawrence, L., P. Danese, J. DeVito, F. Franceschi, and J. Sutcliffe.** 2008. In vitro activities of the Rx-01 oxazolidinones against hospital and community pathogens. Antimicrob. Agents Chemother. **52:**1653–1662.
40. **Leach, K. L., S. M. Swaney, J. R. Colca, W. G. McDonald, J. R. Blinn, L. M. Thomasco, R. C. Gadwood, D. Shinabarger, L. Xiong, and A. S. Mankin.** 2007. The site of action of oxazolidinone antibiotics in living bacteria and in human mitochondria. Mol. Cell **26:**393–402.
41. **Livermore, D. M., S. Mushtaq, M. Warner, and N. Woodford.** 2009. Activity of oxazolidinone TR-700 against linezolid-susceptible and -resistant staphylococci and enterococci. J. Antimicrob. Chemother. **63:**713–715.
42. **Locke, J. B., M. Hilgers, and K. J. Shaw.** 2009. Mutations in ribosomal protein L3 are associated with oxazolidinone resistance in staphylococci of clinical origin. Antimicrob. Agents Chemother. **53:**5275–5278.
43. **Locke, J. B., M. Hilgers, and K. J. Shaw.** 2009. Novel ribosomal mutations in *Staphylococcus aureus* strains identified through selection with the oxazolidi-

nones linezolid and torezolid (TR-700). Antimicrob. Agents Chemother. **53:**5265–5274.

44. **Locke, J. B., A. S. Mankin, J. LaMarre, V. Brown-Driver, and K. J. Shaw.** 2009. Abstr. 49th Intersci. Conf. Antimicrob. Agents Chemother., abstr. C1-1364b. American Society for Microbiology, Washington, DC.
45. **Locke, J. B., G. Morales, M. Hilgers, K. G. C., S. Rahawi, J. J. Picazo, K. J. Shaw, and J. L. Stein.** 2010. Elevated linezolid resistance in clinical *cfr*-positive *Staphylococcus aureus* isolates is associated with co-occurring mutations in ribosomal protein L3. Antimicrob. Agents Chemother. **54:**5352–5355.
46. **Long, K. S., J. Poehlsgaard, C. Kehrenberg, S. Schwarz, and B. Vester.** 2006. The *cfr* rRNA methyltransferase confers resistance to phenicols, lincosamides, oxazolidinones, pleuromutilins, and streptogramin A antibiotics. Antimicrob. Agents Chemother. **50:**2500–2505.
47. **Mendes, R. E.** 2009. Abstr. 19th European Congress of Clinical Microbiology and Infectious Diseases, abstr. O96. European Society of Clinical Microbiology and Infectious Diseases, Munich, Germany.
48. **Mendes, R. E.** 2009. Abstr. 19th European Congress of Clinical Microbiology and Infectious Diseases, abstr. P928. European Society of Clinical Microbiology and Infectious Diseases, Munich, Germany.
49. **Mendes, R. E., L. M. Deshpande, M. Castanheira, J. Dipersio, M. Saubolle, and R. N. Jones.** 2008. First report of *cfr*-mediated resistance to linezolid in human staphylococcal clinical isolates recovered in the United States. Antimicrob. Agents Chemother. **52:**2244–2246.
50. **Morales, G., and J. J. Picazo.** 2009. Abstr. 49th Intersci. Conf. Antimicrob. Agents Chemother., abstr. E-808. American Society for Microbiology, Washington, DC.
51. **Morales, G., J. J. Picazo, E. Baos, F. J. Candel, A. Arribi, B. Pelaez, R. Andrade, M. A. de la Torre, J. Fereres, and M. Sanchez-Garcia.** 2010. Resistance to linezolid is mediated by the *cfr* gene in the first report of an outbreak of linezolid-resistant *Staphylococcus aureus*. Clin. Infect. Dis. **50:**821–825.
52. **Overbye, K. M., and D. Mordekhay.** 2007. AM-7359—a novel oxazolidinone with low resistance potential and potent activity against drug resistant pathogens. J. Chemother. **19:**249–255.
53. **Paget, S. D., B. D. Foleno, C. M. Boggs, R. M. Goldschmidt, D. J. Hlasta, M. A. Weidner-Wells, H. M. Werblood, E. Wira, K. Bush, and M. J. Macielag.** 2003. Synthesis and antibacterial activity of pyrroloaryl-substituted oxazolidinones. Bioorg. Med. Chem. Lett. **13:**4173–4177.
54. **Park, C. H., D. R. Brittelli, C. L. Wang, F. D. Marsh, W. A. Gregory, M. A. Wuonola, R. J. McRipley, V. S. Eberly, A. M. Slee, and M. Forbes.** 1992. Antibacterials. Synthesis and structure-activity studies of 3-aryl-2-oxooxazolidines. 4. Multiply-substituted aryl derivatives. J. Med. Chem. **35:**1156–1165.
55. **Phillips, O. A., E. E. Udo, A. A. Ali, and N. Al-Hassawi.** 2003. Synthesis and antibacterial activity of 5-substituted oxazolidinones. Bioorg. Med. Chem. **11:**35–41.
56. **Reck, F., F. Zhou, C. J. Eyermann, G. Kern, D. Carcanague, G. Ioannidis, R. Illingworth, G. Poon, and M. B. Gravestock.** 2007. Novel substituted (pyridin-3-yl)phenyloxazolidinones: antibacterial agents with reduced activity against monoamine oxidase A and increased solubility. J. Med. Chem. **50:**4868–4881.
57. **Reck, F., F. Zhou, M. Girardot, G. Kern, C. J. Eyermann, N. J. Hales, R. R. Ramsay, and M. B. Gravestock.** 2005. Identification of 4-substituted 1,2,3-triazoles as novel oxazolidinone antibacterial agents with reduced activity against monoamine oxidase A. J. Med. Chem. **48:**499–506.
58. **Renslo, A. R., G. W. Luehr, and M. F. Gordeev.** 2006. Recent developments in the identification of novel oxazolidinone antibacterial agents. Bioorg. Med. Chem. **14:**4227–4240.
59. **Schenk, S., and R. A. Laddaga.** 1992. Improved method for electroporation of *Staphylococcus aureus*. FEMS Microbiol. Lett. **73:**133–138.
60. **Schlünzen, F., J. M. Harms, F. Franceschi, H. A. Hansen, H. Bartels, R. Zarivach, and A. Yonath.** 2003. Structural basis for the antibiotic activity of ketolides and azalides. Structure **11:**329–338.
61. **Schlünzen, F., E. Pyetan, P. Fucini, A. Yonath, and J. M. Harms.** 2004. Inhibition of peptide bond formation by pleuromutilins: the structure of the 50S ribosomal subunit from *Deinococcus radiodurans* in complex with tiamulin. Mol. Microbiol. **54:**1287–1294.
62. **Schlunzen, F., R. Zarivach, J. Harms, A. Bashan, A. Tocilj, R. Albrecht, A. Yonath, and F. Franceschi.** 2001. Structural basis for the interaction of antibiotics with the peptidyl transferase centre in eubacteria. Nature **413:**814–821.
63. **Shaw, K. J., S. Poppe, R. Schaadt, V. Brown-Driver, J. Finn, C. M. Pillar, D. Shinabarger, and G. Zurenko.** 2008. *In vitro* activity of TR-700, the antibacterial moiety of the prodrug TR-701, against linezolid-resistant strains. Antimicrob. Agents Chemother. **52:**4442–4447.
64. **Skripkin, E., T. S. McConnell, J. Devito, L. Lawrence, J. A. Ippolito, E. M. Duffy, J. Sutcliffe, and F. Franceschi.** 2008. Rx-01: a new family of oxazolidinones that overcomes ribosomal-based linezolid resistance. Antimicrob. Agents Chemother. **52:**3550–3557.
65. **Slee, A. M., M. A. Wuonola, R. J. McRipley, I. Zajac, M. J. Zawada, P. T. Bartholomew, W. A. Gregory, and M. Forbes.** 1987. Oxazolidinones, a new class of synthetic antibacterial agents: in vitro and in vivo activities of DuP 105 and DuP 721. Antimicrob. Agents Chemother. **31:**1791–1797.
66. **Smith, L. K., and A. S. Mankin.** 2008. Transcriptional and translational control of the *mlr* operon, which confers resistance to seven classes of protein synthesis inhibitors. Antimicrob. Agents Chemother. **52:**1703–1712.
67. **Swaney, S. M., H. Aoki, M. C. Ganoza, and D. L. Shinabarger.** 1998. The oxazolidinone linezolid inhibits initiation of protein synthesis in bacteria. Antimicrob. Agents Chemother. **42:**3251–3255.
68. **Toh, S. M., L. Xiong, C. A. Arias, M. V. Villegas, K. Lolans, J. Quinn, and A. S. Mankin.** 2007. Acquisition of a natural resistance gene renders a clinical strain of methicillin-resistant *Staphylococcus aureus* resistant to the synthetic antibiotic linezolid. Mol. Microbiol. **64:**1506–1514.
69. **Tsiodras, S., H. S. Gold, G. Sakoulas, G. M. Eliopoulos, C. Wennersten, L. Venkataraman, R. C. Moellering, and M. J. Ferraro.** 2001. Linezolid resistance in a clinical isolate of *Staphylococcus aureus*. Lancet **38:**207–208.
70. **Tu, D., G. Blaha, P. B. Moore, and T. A. Steitz.** 2005. Structures of MLSBK antibiotics bound to mutated large ribosomal subunits provide a structural explanation for resistance. Cell **121:**257–270.
71. **Vara Prasad, J. V.** 2007. New oxazolidinones. Curr. Opin. Microbiol. **10:**454–460.
72. **Venkatachalam, C. M., X. Jiang, T. Oldfield, and M. Waldman.** 2003. LigandFit: a novel method for the shape-directed rapid docking of ligands to protein active sites. J. Mol. Graph. Model. **21:**289–307.
73. **Wilson, D. N., F. Schluenzen, J. M. Harms, A. L. Starosta, S. R. Connell, and P. Fucini.** 2008. The oxazolidinone antibiotics perturb the ribosomal peptidyl-transferase center and effect tRNA positioning. Proc. Natl. Acad. Sci. U. S. A. **105:**13339–13344.
74. **Wilson, P., J. A. Andrews, R. Charlesworth, R. Walesby, M. Singer, D. J. Farrell, and M. Robbins.** 2003. Linezolid resistance in clinical isolates of *Staphylococcus aureus*. J. Antimicrob. Chemother. **51:**186–188.
75. **Wolter, N., A. M. Smith, D. J. Farrell, W. Schaffner, M. Moore, C. G. Whitney, J. H. Jorgensen, and K. P. Klugman.** 2005. Novel mechanism of resistance to oxazolidinones, macrolides, and chloramphenicol in ribosomal protein L4 of the pneumococcus. Antimicrob. Agents Chemother. **49:**3554–3557.
76. **Wong, A., S. P. Reddy, D. S. Smyth, M. E. Aguero-Rosenfeld, G. Sakoulas, and D. A. Robinson.** 2010. Polyphyletic emergence of linezolid-resistant staphylococci in the United States. Antimicrob. Agents Chemother. **54:**742–748.
77. **Yong, D., J. H. Yum, K. Lee, Y. Chong, S. H. Choi, and J. K. Rhee.** 2004. In vitro activities of DA-7867, a novel oxazolidinone, against recent clinical isolates of aerobic and anaerobic bacteria. Antimicrob. Agents Chemother. **48:**352–357.
78. **Zhou, J., A. Bhattacharjee, S. Chen, Y. Chen, E. Duffy, J. Farmer, J. Goldberg, R. Hanselmann, J. A. Ippolito, R. Lou, A. Orbin, A. Oyelere, J. Salvino, D. Springer, J. Tran, D. Wang, Y. Wu, and G. Johnson.** 2008. Design at the atomic level: design of biaryloxazolidinones as potent orally active antibiotics. Bioorg. Med. Chem. Lett. **18:**6175–6178.
79. **Zhou, J., A. Bhattacharjee, S. Chen, Y. Chen, E. Duffy, J. Farmer, J. Goldberg, R. Hanselmann, J. A. Ippolito, R. Lou, A. Orbin, A. Oyelere, J. Salvino, D. Springer, J. Tran, D. Wang, Y. Wu, and G. Johnson.** 2008. Design at the atomic level: generation of novel hybrid biaryloxazolidinones as promising new antibiotics. Bioorg. Med. Chem. Lett. **18:**6179–6183.