

ABSTRACT

Background: TR-701 is the orally-active prodrug of TR-700, a novel oxazolidinone. Linezolid (LZD) inhibits bacterial protein synthesis and mutations in the peptidyl transferase center of 23S rRNA lead to development of resistance in the clinic. The *in vitro* activity of TR-700 was evaluated against clinical isolates of Gram-positive bacteria with defined LZD-resistance mutations.

Methods: *Staphylococcus aureus* isolates included 6 strains harboring either the G2500A or G2576U mutation in the peptidyl transferase center, two laboratory-derived *S. aureus* (G2447U), LZD-resistant *S. aureus* strain CM/05 containing the *cfiR* RNA methyltransferase gene, and plasmid-borne laboratory constructs of this gene. LZD-resistant *Enterococcus* included 4 clinical *E. faecalis*, 1 *E. faecium* and 2 laboratory-derived strains all containing the G2576U mutation. MIC assays were conducted using the CLSI reference broth microdilution method.

Results: TR-700 was 8-16 fold more potent than LZD against the 6 clinical isolates of *S. aureus* (G2500A or G2576U mutants). TR-700 was also more potent than LZD against *S. aureus* containing the G2447U mutation, though the MIC value was 8-16 µg/mL vs. 32-32 µg/mL for LZD. *S. aureus* CM/05 (*cfiR*) was susceptible to 16 µg/mL of LZD vs. 1 µg/mL for TR-700. TR-700 also showed > 8-fold greater potency than LZD in *S. aureus* carrying the cloned *cfiR* gene. TR-700 MIC values for LZD-resistant VRE ranged from 2-8 µg/mL, while LZD was 16 - >32 µg/mL.

Conclusions: TR-701, the orally-active prodrug of TR-700, is a promising new oxazolidinone antibacterial agent. TR-700 has greater *in vitro* potency than LZD against clinical isolates of either *S. aureus* or VRE that are resistant to LZD.

STRUCTURES OF LZD AND TR-700

Fig 1. The model of linezolid in its binding site.
Leach *et al.*, *Molecular Cell* 26, 393-402, 2007

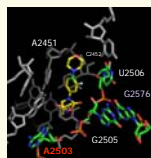
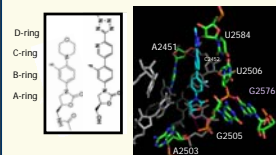


Fig 2. Trius Model of TR-700 in the PTS site



Predictions:

- ◆ Both have H-bond interactions w/G2505 5' O
- ◆ Both have hydrophobic interactions with ring B between A2451 and C2452
- ◆ TR-700 picks up 2 additional H-bonds interactions with rings C & D A2451 & U2584

MATERIALS AND METHODS

◆ MICs were determined using the broth microdilution method as described by the Clinical and Laboratory Standards Institute (1). The CLSI quality control strains *S. aureus* ATCC 29213 and *E. faecalis* ATCC 29212 were tested in each set of assays to ensure the proper performance of the assay.

◆ Mueller Hinton II Broth (MHB II; Becton Dickinson, Sparks, MD) was utilized for MIC testing

◆ The comparator agents were linezolid (ChemPacific Corp), vancomycin (Sigma), oxacillin (Fluka), and penicillin G (Fluka)

◆ Test organisms for the assay included recent clinical isolates from diverse geographical centers as well as laboratory-derived oxazolidinone-resistant mutants

RESULTS

TR-700 shows ≥8-fold better activity than LZD against all MRSA with known PTS mutations as well as the *cfiR* gene

Table 1. *S. aureus* isolates resistant to LZD

Strain	Mutation	Minimal Inhibitory Concentration (µg/mL)				
		TR-700	LZD	VAN	OXA	PEN
1651Beth Israel Deac	G2576U	2	16	0.5	>32	16
1652Beth Israel Deac	U2500A	4	32	1	32	32
1726NRSL127 MRSA	non-23S	1	8	1	32	16
NRS119 MRSA	G2576U	4	64	nd	nd	nd
NRS120 MRSA	G2576U	8	64	nd	nd	nd
NRS121 MRSA	G2576U	4	64	nd	nd	nd
NRS271 MRSA	G2576U	2	32	nd	nd	nd
CM/05 Medinet, Columbia MRSA	<i>cfiR</i> gene acquisition	1	16	nd	nd	nd
1653031 (2003)	nd	2	8	2	>32	nd
1653032 (2005)	nd	1	16	1	>32	nd
1653033 (2005)	nd	1	8	2	>32	nd
1653034 (2006)	nd	2	8	2	>32	nd

lab-derived isolates

Strain	Mutation	Minimal Inhibitory Concentration (µg/mL)				
		TR-700	LZD	VAN	OXA	PEN
190ATCC29213	wildtype	1	4	1	0.12	1
425/LZD-Res dev ¹	G2447U	8	32	2	0.12	1
480/LZD-Res dev ¹	G2447U	16	>32	1	≤0.03	0.25

¹Strains 425 and 480 represent transfers #18 and #19, respectively, from a spiral plating experiment derived from the host ATCC29213 (2)

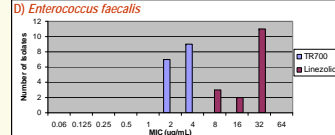
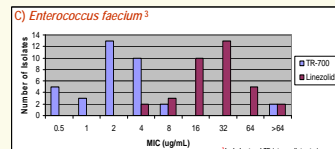
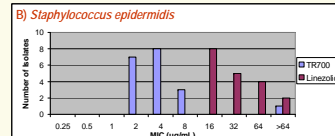
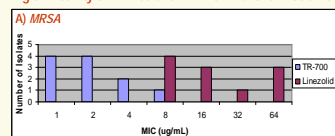
Table 2. *E. faecalis* isolates resistant to LZD

Strain #	Source	Minimal Inhibitory Concentration (µg/mL)				
		TR-700	LZD	VAN	OXA	PEN
411	JMI	2	16	1	>32	4
412	JMI	2	16	1	16	2
413	Mass. Gen.	4	32	1	>32	4
414	Mass. Gen.	4	32	32	16	1
1172	CHP	4	16	> 32	32	4
854	UCLA	2	16	>32	>32	>32
lab-derived isolates						
405	wild type	0.5	2	2	8	2
406	LZD res dev ²	8	>32	2	8	1
407	EZD res dev ²	2	32	>32	>32	>32

²Strain 406 was selected from spiral plating on linezolid, while 407 was selected from spiral plating on eperzolid. In both experiments, the starting strain was 405.

RESULTS

Fig 3. Activity of TR-700 and LZD vs Linezolid-R Strains



# isolates	MIC ₅₀	TR-700		LZD	
		MIC ₅₀	MIC ₉₀	MIC ₅₀	MIC ₉₀
MRSA	11	2	16	4	64
<i>S. epidermidis</i>	19	4	32	8	128
<i>E. faecium</i>	35	2	32	8	64
<i>E. faecalis</i>	16	4	32	8	64

SUMMARY & CONCLUSIONS

- ◆ TR-700 is 8-16 fold more potent than LZD against clinical isolates of *S. aureus* harboring known ribosomal mutations
- ◆ TR-700 is 16-fold more potent against the strain CM/05, which contains the transposon-associated *cfiR* (methyltransferase) gene, whose putative mobile nature is likely to be problematic in the future.
 - Against this strain, the MIC of TR-700 and LZD are 1 µg/mL and 16 µg/mL, respectively
- ◆ TR-700 demonstrates an 8-fold advantage over LZD against other LZD-resistant organisms as well

LITERATURE CITED

- 1) CLSI. *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically: Approved Standard—Seventh Edition*. CLSI Document. M7-A7, 2006.
- 2) Shinabarger D. Mechanism of action of the oxazolidinone antibacterial agents. *Exp. Opin. Invest. Drugs*. 1999; 8:1195-1202.

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