

# In Vitro and In Vivo Antibacterial Activity of TR-701 (DA-7218) against Penicillin-Resistant *Streptococcus pneumoniae*

F1-1689

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## Abstract

### Background:

With the approval of linezolid as the first therapeutically acceptable oxazolidinone, efforts have been made to identify new oxazolidinones with improved antibacterial properties. TR-701 (DA-7218) is a prodrug of TR-700 (DA-7157). This study was performed to establish the *in vitro* antibacterial activity of TR-700 and the *in vivo* efficacy of TR-701 in a mouse *Streptococcus pneumoniae* systemic infection model compared to linezolid.

### Methods:

MICs were determined against *S. pneumoniae* using the agar dilution method. Time-kill studies were evaluated at 1, 2, 4 and 8X MIC of TR-700 and linezolid at time intervals of 0, 1, 3, 5 and 7 h. *In vivo* protection assays of TR-701 were carried out in a mouse systemic infection model. Drugs were administered intravenously (i.v.) or orally (p.o.).

### Results:

The MIC range for TR-700 was 0.125 to 0.25 µg/mL against penicillin-resistant *S. pneumoniae*. The MIC<sub>90</sub> of TR-700 and linezolid for these strains were 0.25 µg/mL and 1 µg/mL, respectively. TR-700 and linezolid showed bacteriostatic activity against penicillin-resistant *S. pneumoniae*. In a pneumococcal systemic infection model, the ED<sub>50</sub> of TR-701 was 3-8 fold lower than that of linezolid after i.v. administration and was 2 fold lower after p.o. administration.

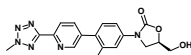
### Conclusions:

TR-700 showed 4 fold better antibacterial activity than linezolid against penicillin-resistant *S. pneumoniae* and TR-701, a prodrug of TR-700, had excellent *in vivo* efficacy against pneumococcal systemic infections.

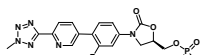
## Introduction

Oxazolidinones are a novel class of totally synthetic antibacterial agents that are active against Gram-positive organisms. The antibacterial activity of oxazolidinones was originally discovered by E. I. Du Pont de Nemours & Co. scientists in the late 70's- early 80's. Linezolid (Zyvox®), the first commercial drug of this class, has been approved by the U.S.FDA for the treatment of infections caused by Gram-positive bacteria. The unique mechanism of oxazolidinones involves inhibition of an early stage of protein biosynthesis and results in a lack of cross-resistance with existing antimicrobials. Thus, linezolid is an important agent for the treatment of multidrug-resistant (MDR) bacterial infections. *S. pneumoniae* is an important pathogen in bacterial pneumonia, meningitis, otitis media, sinusitis. Moreover, MDR strains of penicillin-intermediate and penicillin-resistant *S. pneumoniae* is of great concern.

In our program, we have discovered a second generation oxazolidinones with improved potency and extended antimicrobial spectrum, TR-701 (DA-7218). As shown in this poster, TR-701 (DA-7218) has improved *in vitro* and *in vivo* antibacterial activity compared to linezolid against penicillin-resistant *S. pneumoniae*.



TR-700 (DA-7157)



TR-701 (DA-7218)

## Methods

### Susceptibility test

Recent clinical isolates of Penicillin-resistant *S. pneumoniae* were selected from our culture collection. The Minimum Inhibitory Concentration (MIC) was determined by the agar dilution method according to NCCLS (CLSI) recommendations.

### Time-kill study

Two recent clinical isolates of Penicillin-resistant *S. pneumoniae* were studied. Time-kill studies were determined at 1, 2, 4 and 8X MIC of TR-700 (DA-7157) and linezolid, at time intervals of 0, 1, 3, 5 and 7 h. Because bacteriostatic activity is defined as a 3 log<sub>10</sub> decrease in CFU/ml (99.9% kill), we defined bacteriostatic activity as < 99.9% kill.

### Murine systemic infection

Male ICR mice (18-20g) were given an intraperitoneal injection of penicillin-resistant *S. pneumoniae* suspended in 10% mucin, which contained sufficient bacteria to kill 100% of the untreated controls. TR-701 (DA-7218) and linezolid were administered intravenously or orally (8 mice per dose) at 1 hour post infection. Mice were observed for mortality over 7 days. The total number of survivors at each dose was used to calculate the effective dose that protected 50% of the infected mice from death (ED<sub>50</sub>). ED<sub>50</sub> and 95% confidence limits determinations were performed by Probit analysis.

## Results

- In vitro* antibacterial activities of TR-700 (DA-7157) and linezolid against clinical isolates are shown in Table 1. MIC<sub>90</sub> value of TR-700 (DA-7157) was 0.25 µg/mL for penicillin-resistant *S. pneumoniae*, compared to linezolid MIC<sub>90</sub> of 1 µg/mL for these strains. All clinical isolates of penicillin-resistant *S. pneumoniae* tested in this study were inhibited by TR-700 (DA-7157) at 0.25 µg/mL.
- Table 2 summarizes the logarithmic changes in colony forming units observed in time kill studies. Seven hour results for penicillin-resistant *S. pneumoniae* show that both TR-700 (DA-7157) and linezolid exhibited bacteriostatic activity at 1-8X MIC.
- Table 3 shows *in vivo* efficacy of TR-701 (DA-7218) against a streptococcal systemic infection in mice. The ED<sub>50</sub> of TR-701 (DA-7218) was 3-8 fold lower than that of linezolid after i.v. administration and was 2 fold lower after p.o. administration.

Table 1. *In vitro* antibacterial activity of TR-700 (DA-7157) and Linezolid against penicillin-resistant *S. pneumoniae*.

Organism (No)	Drug	MIC (µg/mL)		
		MIC <sub>range</sub>	MIC <sub>50</sub>	MIC <sub>90</sub>
Penicillin-resistant <i>Streptococcus pneumoniae</i> (28)	TR-700 (DA-7157)	0.125 - 0.25	0.25	0.25
	Linezolid	0.125 - 1	0.5	1

Table 2. Time-kill study of TR-700 (DA-7157) and linezolid against penicillin-resistant *S. pneumoniae*.

Organism	Drug	MIC (µg/mL)	Log CFU/ml Change				
			1 X MIC	2 X MIC	4 X MIC	8 X MIC	Control
PRSP 98-3-3588	TR-700 (DA-7157)	0.125	-0.2	-0.7	-0.9	-1.3	0.7
	Linezolid	0.5	-0.6	-0.7	-0.9	-1.6	
00-4-R459	TR-700 (DA-7157)	0.125	0.7	-0.1	-0.7	-1.7	0.9
	Linezolid	0.5	0.7	0.3	-0.9	-1.5	

Table 3. *In vivo* efficacy of TR-701 (DA-7218) and Linezolid in a murine systemic infection model.

Organism	Drug	MIC* (µg/mL)	Route	ED <sub>50</sub> (mg/kg)	95% Confidence Limits
PRSP DR9	TR-701 (DA-7218)	0.125	i.v.	4.89	2.95 - 8.04
	Linezolid	0.5	p.o.	5.70	3.45 - 9.38
PRSP DR10	TR-701 (DA-7218)	0.125	i.v.	31.84	18.16 - 59.08
	Linezolid	0.5	p.o.	11.06	6.73 - 18.22
PRSP DR11	TR-701 (DA-7218)	0.125	i.v.	3.52	1.82 - 6.50
	Linezolid	0.5	p.o.	3.19	1.63 - 5.94
PRSP DR14	TR-701 (DA-7218)	0.125	i.v.	17.62	9.51 - 34.24
	Linezolid	0.5	p.o.	6.38	3.48 - 11.55
PRSP DR11	TR-701 (DA-7218)	0.125	i.v.	10.19	6.38 - 16.14
	Linezolid	0.5	p.o.	7.63	4.81 - 12.09
PRSP DR14	TR-701 (DA-7218)	0.125	i.v.	30.83	18.93 - 52.29
	Linezolid	0.5	p.o.	14.85	6.38 - 16.14
PRSP DR14	TR-701 (DA-7218)	0.125	i.v.	10.01	3.56 - 28.53
	Linezolid	0.5	p.o.	11.53	3.90 - 32.77
PRSP DR14	TR-701 (DA-7218)	0.125	i.v.	39.53	9.98 - 233.33
	Linezolid	0.5	p.o.	12.98	4.65 - 37.90

\* The MIC reported are for TR-700 the active antibacterial agent formed *in vivo* when TR-701 was administered

## Conclusions

- TR-700 (DA-7157) showed 4-fold more potent antibacterial activity than linezolid against penicillin-resistant *S. pneumoniae*.
- TR-700 (DA-7157) exhibited bacteriostatic activity against penicillin-resistant *S. pneumoniae*.
- TR-701 (DA-7218), a prodrug of TR-700 (DA-7157), had excellent *in vivo* efficacy against pneumococcal systemic infections.