Evaluation of PTK 0796 (BAY 73-6944) in Experimental Models of Infections Caused by Gram-Positive and Gram-Negative Pathogens

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ABSTRACT

Background With the emergence of resistance to all commonly used antibiotics in the treatment of infectious diseases, the development of novel antibiotics has become of major importance. PTK 0796 (7-dimethylamino, 9-(2,2-dimethyl-propyl)-aminomethylcycline) is a novel antibacterial agent of the tetracycline family that has broad and enhanced antibacterial activity including multi-antibiotic resistant gram-positive bacteria. We have evaluated PTK 0796 in various infection models with gram-positive and gram-negative pathogens in both immunocompromised and immunocompetent mice.

Methods Models (all treated intravenously): systemic *S. aureus* (immunocompetent, IP inoculation, survival endpoint); MRSA Thigh Wound (neutropenic, intramuscular inoculation, bacterial burden endpoint); systemic *E. faecalis* (IV inoculation, kidney bacterial burden endpoint); *E. coli* UTI (intrabladder inoculation, kidney burden endpoint).

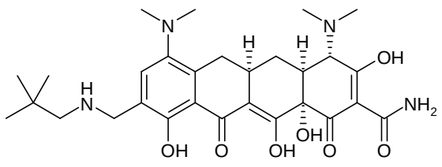
Results In the systemic *S. aureus* infection, PTK 0796 was equally or more efficacious (PD50 0.4 mg/kg) when compared to minocycline (1.0mg/kg), vancomycin (0.4 mg/kg) and linezolid (3.5 mg/kg). The efficacy of PTK 0796 in the Thigh Wound caused by MRSA was superior to the comparators tested, with a more than 5-fold decrease in the ED50 when compared to vancomycin, linezolid, and minocycline. In the systemic *E. faecalis* model, PTK 0796 treatment had an ED50 of 4.5 mg/kg compared to >40 mg/kg for linezolid and over >100 mg/kg for both vancomycin and minocycline. In addition to gram-positive infections, we also evaluated PTK 0796 in an *E. coli* UTI model. When compared to minocycline (ED50 = 11.0mg/kg), PTK 0796 had an ED50 of 4.8 mg/kg.

Conclusions Overall, PTK 0796 performed better than or equal to the marketed therapeutic agents tested in all the models investigated in this study.

INTRODUCTION

We evaluated PTK 0796 (BAY 73-6944) in four Gram-positive models of infection, using both immunocompetent and immuncompromised hosts. In addition, we evaluated the efficacy of PTK 0796 (BAY 73-6944) in an *E. coli* urinary tract infection model.

Structure of PTK 0796 (BAY 73-6944)



- Emergence of resistance to all commonly used antibiotics including Vancomycin has created the need for the development of new antibiotics.
- PTK 0796 (BAY 73-6944) is highly active against resistant Gram-positive and Gram-negative pathogens *in vitro*.
- MIC90 for PTK 0796 (BAY 73-6944) against susceptible and resistant *S. aureus* is 0.5 µg/ml (range of ≤0.06 – 1.0) and for susceptible and resistant *E. faecalis* is 0.5 µg/ml (range of 0.125- 0.5).
- Efficacy was determined in systemic and tissue based animal infection models.

METHODS

Animals and Strains Systemic and UTI models of infection: Male CD-1 mice (18-25g) were used. Female ICR (20–30g) mice were used for the neutropenic thigh model. Neutropenia was established by injecting cyclophosphamide IP at 150 and 100mg/kg doses on days -3 and 0, respectively.

Systemic models: *S. aureus* ATCC 29213 was used in these studies. For the thigh wound model a multiply resistant strain of *S. aureus*, MRSA5, was chosen. *E. faecalis* ATCC 29212 was selected for the systemic infection model. *E. coli* C189P4, a cystitis isolate, was used for the UTI model of infection.

Acute *S. aureus* Systemic Infection Model The LD50 of S. aureus ATCC 29213 was found to be 10^5CFU per mouse. 10^7CFU per mouse (100 x LD50) was used as the inoculum for the IP challenge model. S. aureus ATCC 29213 was grown in an overnight broth one day prior to infection. 10^9CFU/ml of the culture was diluted in 5 % bacteriological mucin and mice infected with 10^7 CFU per mouse. Increasing single doses of PTK 0796 or comparators were then administered IV, 1h post-infection. Survival of animals was then monitored for 7d and PD50s calculated.

MRSA Thigh Wound Model For the thigh wound model, MRSA5 was grown in an overnight broth one day prior to infection to 10^8CFU/ml and was diluted in PBS. Mice were infected with 10^5CFU IM in the thigh and treated IV with increasing doses of PTK 0796 and comparators 2h post-infection. Bacterial burdens of the thighs were evaluated at 24h post-infection. An ED50 was determined as the dose that reduced the number of bacteria in the thighs by 2log10 CFU/thigh.

Systemic *E. faecalis* IV Infection Model *E. faecalis* ATCC 29212 was streaked out onto TSA plates containing 5% Sheep Blood and incubated overnight at 37ºC. The bacteria were harvested and placed into PBS at an OD_{650nm} of 1.0 and then diluted to give an inoculum of ~ 1.0 x 10^8 CFU/ml. Male, CD1 mice were infected IV with 10^7CFU per mouse. Mice were then treated IV with increasing doses of PTK 0796 and comparators at 1, 5, 24, and 48h post-infection. At 72h post-infection the mice were sacrificed and their kidneys removed for evaluation of bacterial burden. An ED50 was calculated as the dose that reduced the number of bacteria in the kidneys by 2log10/gram.

Urinary Tract Infection Model In the UTI infection model, male, mice were placed on a restricted diet for 7days and were given 5% glucose water to induce diuresis. *E. coli* C189P4 from a –80ºC stock was inoculated into 2ml of LB broth and incubated overnight at 37ºC. On day 1, 1ml of the cells were centrifuged out of the broth and reconstituted into 1ml of PBS. A 1:25 dilution was made into PBS in order to achieve an OD_{650nm} of ~0.2 (~ 1 x 10^8 CFU/ml) of which 100µl were used to infect the mice. The bladders of the mice were isolated and excess urine removed. Following this, mice were then infected directly into the bladder with 10^7CFU per mouse. After infection, mice were treated IV with increasing single doses of PTK 0796 and comparators on day 4 post-infection. After 5 days the mice were sacrificed and their kidneys removed for evaluation of bacterial burden. The dose that reduced the number of bacteria in the kidneys by 2log10/gram (ED50) was determined.

RESULTS

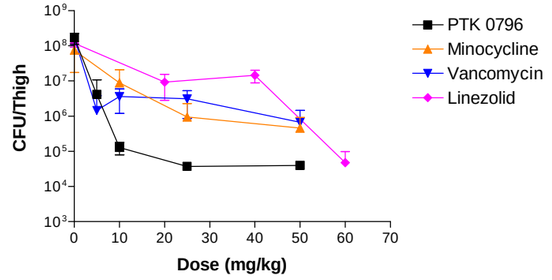
Table 1. Acute *S. aureus* Systemic Infection Model

The PD50s obtained for PTK 0796 and comparators in the systemic IP challenge infection model of immunocompetent mice with *S. aureus* ATCC 29213.

Compound	MIC (*S. aureus* 29213)	PD50 (mg/kg)
PTK 0796	**0.5**	**0.4**
Minocycline	0.5	1.0
Linezolid	2.0	3.5
Vancomycin	0.5	0.4

PTK 0796 showed greater efficacy in the *S. aureus* systemic IP challenge model compared to Minocycline and Linezolid. PTK 0796 also showed equal efficacy when compared to Vancomycin.

FIGURE 1. MRSA Thigh Wound Model - Efficacy of PTK 0796, Minocyline, Linezolid and Vancomycin in the treatment of a thigh wound model in immunocompromised mice caused by *S. aureus* MRSA5 strain.



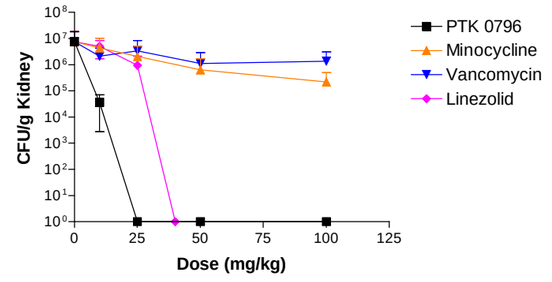
PTK 0796 caused greater reduction in the numbers of CFU in the thighs of neutropenic mice infected with MRSA5 at 10, 25 and 50mg/kg compared to all comparators tested in this study.

Table 2. The ED50s obtained in the thigh wound model infecting immunocompromised mice with *S. aureus* MRSA5

Compound	MIC (*S. aureus* MRSA5)	ED50 (mg/kg)
PTK 0796	**0.5**	**5.9**
Minocycline	2.0	35.2
Linezolid	1.0	47.7
Vancomycin	1.0	30.4

PTK 0796 showed far superior ED50s in the MRSA thigh wound model compared to all other comparators tested.

FIGURE 2. Systemic *E. faecalis* IV Infection Model - Efficacy of PTK 0796, Minocyline, Linezolid and Vancomycin in the treatment of a systemic IV infection model in immunocompetent mice caused by *E. faecalis* ATCC 29212 strain



In the systemic *E. faecalis* model, PTK 0796 caused greater reduction in CFU/g kidney at 10mg/kg up to 100mg/kg doses when compared to Minocycline and Vancomycin and was better at reducing CFU/g kidney than Linezolid at 10 and 25mg/kg.

Table 3. Systemic *E. faecalis* IV Infection Model. The ED50s obtained in the systemic IV infec-

Compound	MIC (*E. faecalis* 29212)	ED50 (mg/kg)
PTK 0796	**0.5**	**4.5**
Minocycline	2.0	71.0
Linezolid	1.0	14.3
Vancomycin	2.0	70.3

The ED50 of PTK 0796 illustrated that the compound was efficacious at a much lower dose than any of the comparators tested in this *E. faecalis* systemic infection study.

Table 4. Systemic *E. faecalis* IV Infection Model. The number of animals that showed below detectable levels of bacteria CFU obtained in the systemic IV infection model infecting immunocompetent mice with *E. faecalis* ATCC 29212.

Compound	Number of animals cleared infection (n=5)				
	100mg/kg	50mg/kg	25mg/kg	10mg/kg	5mg/kg
PTK 0796	**5 (100%)**	**5 (100%)**	**5 (100%)**	**5 (100%)**	**3 (60%)**
Vancomycin	3 (60%)	2 (40%)	0 (0%)	0 (0%)	0 (0%)
Minocycline	3 (60%)	2 (40%)	0 (0%)	0 (0%)	0 (0%)

PTK 0796 completely cleared infections down to a dose of 10mg/kg. The comparators however only showed 60% clearance at 100mg/kg. This finding clearly demonstrates the improved efficacy of PTK 0796 compared to the comparators tested in this model of infection.

Table 5. Urinary Tract Infection Model. The ED50s obtained in the UTI model infecting diuresed male, mice bladders with *E. coli* C189P4.

Compound	MIC (*E. coli* C189P4)	ED50 (mg/kg)
PTK 0796	**0.5**	**4.3**
Minocycline	0.5	4.5
Ciprofloxacin	≤0.06	<1.0

PTK 0796 performed equally as well as Minocycline in the *E. coli* UTI, but not as welll as Ciprofloxacin.

CONCLUSIONS

PTK 0796 (BAY 73-6944)

- When tested in neutropenic mice challenged in the thigh with MRSA, was superior to the comparators studied.
- In a systemic, *E. faecalis* model showed greater efficacy than Minocycline, Vancomycin and Linezolid.
- Was equally efficacious against *S. aureus* compared to the other comparators tested in a systemic model of infection in immunocompetent mice.
- Showed equal efficacy to Minocycline in an *E. coli* urinary tract infection model.

In summary, PTK 0796 (BAY 73-6944) performed better than or equal to the reference therapeutic agents tested in all the models investigated in these studies.