

***In Vitro* Activity of PTK 0796 (BAY 73-6944) Against Gram-Positive and Gram-Negative Organisms**

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ABSTRACT

Background To address the problem of antibiotic resistance, Paratek scientists have produced PTK 0796 (7-dimethylamino, 9-(2,2-dimethyl-propyl)-aminomethylcyclohexane), a novel antibacterial agent of the tetracycline family with potent and enhanced activity against resistant gram-positive and gram-negative pathogens.

Methods The present study compared the *in vitro* activity of PTK 0796 and 10 agents including, vancomycin, tetracycline, and ciprofloxacin against >200 strains of recent gram-positive and gram-negative clinical isolates including methicillin sensitive *S. aureus* (MSSA), methicillin resistant *S. aureus* (MRSA), vancomycin resistant *E. faecium* (EFA VRE), *E. faecalis* (EF), penicillin resistant *S. pneumoniae* (SPN PENR), Groups A and B *Beta hemolytic Streptococci* (BHS), *E. coli* (EC), and other pathogens. Microdilution MIC tests were performed according to NCCLS guidelines.

Results As shown in the Table, PTK 0796 exhibited potent activity particularly against antibiotic resistant gram-positive isolates.

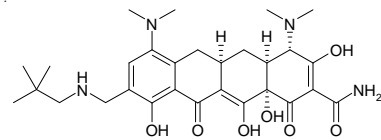
Susceptibility <i>in vitro</i> (MIC 90s ug/ml)				
Strains	PTK-0796	Vancomycin	Tetracycline	Ciprofloxacin
MSSA (n=16)	0.125	0.5	0.125	0.5
MRSA (n=41)	0.5	1.0	64.0	>64
EFA VRE (n=19)	0.5	>64	64.0	>64
EF (n=31)	0.5	2.0	64.0	64.0
SPN PENR (n=23)	<=0.06	0.25	32.0	1.0
BHS (n=48)	0.25	0.5	64.0	0.5
EC (n=23)	2.0	-	>64	8.0

Conclusions PTK 0796 is highly active *in vitro* against a broad spectrum of pathogens including multiple antibiotic-resistant pathogens, most notably methicillin resistant *Staphylococcus aureus*, vancomycin resistant *Enterococcus*, and penicillin resistant *S. pneumoniae*. The activity of PTK 0796 is not affected by the presence of resistance to tetracycline.

INTRODUCTION

- PTK 0796 (BAY 73-6944) is a novel aminomethylcyclohexane (AMC), 7-dimethylamino, 9-(2,2-dimethyl-propyl)-aminomethylcyclohexane.
- In this study, the activities of PTK 0796 (BAY 73-6944) were determined against a variety of aerobic gram-positive and select gram-negative bacteria.

Structure of PTK 0796 (BAY 73-6944)



METHODS

Strains

- Recent clinical isolates obtained from 7 laboratories within the United States.
- Appropriate quality control strains were included in each test.

MIC Determination

- MICs were determined using current NCCLS-recommended microdilution methods:
 - Cation-adjusted Mueller Hinton broth for staphylococci, enterococci, and gram-negative rods
 - Cation-adjusted Mueller Hinton broth supplemented with 5% horse blood for streptococci
 - Haemophilus Test Medium for *Haemophilus influenzae*
- Organisms were grown (or direct inoculum for fastidious organisms) to a 0.5 McFarland standard. Turbidity was measured using a Microscan turbidity meter
- Incubation at 35°C for 18 – 24 hr depending on the organisms tested

RESULTS

Gram-Positive Aerobic Bacteria (Table 1)

PTK 0796 inhibited all strains of

- Methicillin-sensitive *Staphylococcus aureus* (MIC₉₀ = 0.125 µg/ml)
- Methicillin-resistant *Staphylococcus aureus* (MIC₉₀ = 0.5 µg/ml)
- Methicillin-resistant, and multi-resistant (multiR) *Staphylococcus aureus* (MIC₉₀ = 0.5 µg/ml)
- Enterococcus faecium*, including vancomycin-resistant and vancomycin-resistant, multiR strains (MIC₉₀ = 0.5 µg/ml)
- Enterococcus faecalis*, including vancomycin-resistant and vancomycin-resistant, multiR strains (MIC₉₀ = 0.5 µg/ml)
- Streptococcus pneumoniae* including penicillin-resistant and penicillin-resistant, multiR strains (MIC₉₀ = <= 0.06 µg/ml)
- Streptococcus pyogenes* (MIC₉₀ = 0.25 µg/ml)
- Streptococcus agalactiae* (MIC₉₀ = 0.125 µg/ml)

PTK 0796 was the most active compound tested against all gram-positive bacteria

Select Gram-Negative Aerobic Bacteria (Table 2)

PTK 0796 inhibited all strains of

- Escherichia coli* (MIC₉₀ = 2.0 µg/ml)
- Klebsiella pneumoniae* (MIC₉₀ = 4.0 µg/ml)
- Haemophilus influenzae* (MIC₉₀ = 2.0 µg/ml)

Table 1 Comparative <i>in vitro</i> activity of PTK 0796 vs. aerobic Gram-positive bacteria					
Organism	N	Compound	MIC ug/ml		
			Range	MIC50	MIC90
<i>S. aureus</i>	55	PTK 0796	<=0.06 - 1	0.125	0.5
		Tetracycline	<=0.06 - 64	0.125	64
		Doxycycline	<=0.06 - 8	<=0.06	8
		Minocycline	<=0.06 - 16	0.125	8
		Cefotaxime	1 - >64	32	>64
		Vancomycin	0.25 - 2	0.5	1
		Levofloxacin	<=0.06 - >64	4	32
		Linezolid	0.5 - 2	2	2
		Azithromycin	0.25 - >64	>64	>64
		Clindamycin	<=0.06 - >64	0.125	>64
Methicillin Resistant	39	PTK 0796	0.125 - 1	0.25	0.5
		Tetracycline	<=0.06 - 64	0.25	64
		Doxycycline	<=0.06 - 8	0.125	8
		Minocycline	<=0.06 - 16	0.25	8
		Cefotaxime	4 - >64	>64	>64
		Vancomycin	0.25 - 2	0.5	1
		Levofloxacin	0.5 - >64	8	32
		Linezolid	0.5 - 2	2	2
		Azithromycin	0.5 - >64	>64	>64
		Clindamycin	<=0.06 - >64	>64	>64
<i>S. aureus</i>	16	PTK 0796	<=0.06 - 0.25	0.125	0.125
		Tetracycline	<=0.06 - 16	<=0.06	0.125
		Doxycycline	<=0.06 - 1	<=0.06	<=0.06
		Minocycline	<=0.06 - 0.125	<=0.06	0.125
		Cefotaxime	1 - 2	2	2
		Vancomycin	0.25 - 0.5	0.5	0.5
		Levofloxacin	<=0.06 - 4	0.125	0.125
		Linezolid	1 - 2	1	2
		Azithromycin	0.25 - 32	0.5	0.5
		Clindamycin	<=0.06 - 0.125	<=0.06	0.125

Table 1 (continued) Comparative <i>in vitro</i> activity of PTK 0796 vs. aerobic Gram-positive bacteria					
<i>S. aureus</i>	10	PTK 0796	0.25 - 0.5	0.5	0.5
		Tetracycline	32 - >64	>64	>64
		Doxycycline	2 - 8	8	8
		Minocycline	2 - 16	8	8
		Cefotaxime	32 - 64	>64	>64
		Vancomycin	0.5 - 1	1	1
		Levofloxacin	8 - 32	8	32
		Linezolid	0.5 - 2	1	2
		Azithromycin	>64	>64	>64
		Clindamycin	>64	>64	>64
<i>E. faecium</i>	24	PTK 0796	0.125 - 0.5	0.25	0.5
		Tetracycline	0.125 - >64	32	64
		Doxycycline	<=0.06 - 16	2	8
		Minocycline	0.125 - 32	8	16
		Vancomycin	0.5 - >64	>64	>64
		Levofloxacin	1 - >64	64	>64
		Linezolid	0.5 - 4	2	2
		Azithromycin	4 - >64	>64	>64
		Clindamycin	<=0.06 - >64	>64	>64
		<i>E. faecium</i>	19	PTK 0796	0.125 - 0.5
Tetracycline	0.125 - >64			32	64
Doxycycline	<=0.06 - 8			2	4
Minocycline	0.25 - 32			8	16
Vancomycin	64 - >64			>64	>64
Levofloxacin	1 - >64			64	>64
Linezolid	0.5 - 4			2	2
Azithromycin	>64			>64	>64
Clindamycin	>64			>64	>64
multiR and Vancomycin Resistant	12			PTK 0796	0.125 - 0.5
		Tetracycline	32 - >64	32	>64
		Doxycycline	2 - 8	32	4
		Minocycline	4 - 16	8	16
		Vancomycin	>64	>64	>64
		Levofloxacin	8 - >64	32	>64
		Linezolid	0.5 - 2	1	2
		Azithromycin	>64	>64	>64
		Clindamycin	>64	>64	>64
		<i>E. faecalis</i>	31	PTK 0796	0.125 - 0.5
Tetracycline	0.125 - >64			32	64
Doxycycline	<=0.06 - 16			4	16
Minocycline	0.125 - 16			8	16
Vancomycin	0.5 - 8			1	2
Levofloxacin	0.5 - 64			1	32
Linezolid	1 - 4			1	2
Azithromycin	1 - >64			8	>64
Clindamycin	2 - >64			32	>64
<i>E. faecalis</i>	3			PTK 0796	0.25 - 0.5
		Tetracycline	32 - 64	32	64
		Doxycycline	4	4	4
		Minocycline	8 - 16	8	16
		Vancomycin	0.5 - 8	0.5	8
		Levofloxacin	16 - 64	32	64
		Linezolid	1	1	1
		Azithromycin	>64	>64	>64
		Clindamycin	>64	>64	>64

Table 1 (continued) Comparative <i>in vitro</i> activity of PTK 0796 vs. aerobic Gram-positive bacteria					
<i>S. pneumoniae</i>	41	PTK 0796	<=0.06 - 0.25	<=0.06	0.125
		Tetracycline	<=0.06 - 64	16	32
		Doxycycline	<=0.06 - 4	2	4
		Minocycline	<=0.06 - 8	2	8
		Cefotaxime	<=0.06 - 8	1	2
		Vancomycin	<=0.06 - 0.5	0.25	0.25
		Levofloxacin	0.25 - 1	0.5	1
		Penicillin-G	<=0.06 - 8	2	4
		Linezolid	0.25 - 2	1	1
		Azithromycin	<=0.06 - >64	2	>64
<i>S. pneumoniae</i>	23	PTK 0796	<=0.06	<=0.06	<=0.06
		Tetracycline	<=0.06 - 64	32	32
		Doxycycline	<=0.06 - 4	4	4
		Minocycline	0.125 - 8	8	8
		Cefotaxime	0.5 - 8	1	8
		Vancomycin	0.125 - 0.25	0.25	0.25
		Levofloxacin	0.5 - 1	0.5	1
		Penicillin-G	2 - 8	4	8
		Linezolid	0.5 - 2	1	1
		Azithromycin	<=0.06 - >64	4	>64
<i>S. pneumoniae</i>	18	PTK 0796	<=0.06	<=0.06	<=0.06
		Tetracycline	16 - 64	32	32
		Doxycycline	2 - 4	4	4
		Minocycline	4 - 8	8	8
		Cefotaxime	0.5 - 8	1	8
		Vancomycin	0.125 - 0.25	0.125	0.25
		Levofloxacin	0.5 - 1	0.5	1
		Penicillin-G	2 - 8	4	8
		Linezolid	0.5 - 2	1	1
		Azithromycin	<=0.06 - >64	>64	>64
<i>S. pyogenes</i>	30	PTK 0796	<=0.06 - 0.5	0.125	0.25
		Tetracycline	<=0.06 - 64	<=0.06	64
		Doxycycline	<=0.06 - 8	<=0.06	8
		Minocycline	0.125 - 8	0.25	8
		Cefotaxime	<=0.06	<=0.06	<=0.06
		Vancomycin	0.25	0.25	0.25
		Levofloxacin	0.25 - 1	0.25	1
		Linezolid	0.5 - 1	1	1
		Azithromycin	<=0.06 - >64	<=0.06	8
		Clindamycin	<=0.06 - >64	<=0.06	<=0.06
<i>S. agalactiae</i>	18	PTK 0796	<=0.06 - 0.25	0.125	0.125
		Tetracycline	<=0.06 - 64	32	64
		Doxycycline	<=0.06 - 16	8	8
		Minocycline	0.125 - 32	16	16
		Cefotaxime	<=0.06	<=0.06	<=0.06
		Vancomycin	0.125 - 0.5	0.25	0.5
		Levofloxacin	0.125 - 0.5	0.5	0.5
		Linezolid	1	1	1
		Azithromycin	<=0.06 - 8	<=0.06	0.125
		Clindamycin	<=0.06	<=0.06	<=0.06

Table 2 Comparative <i>in vitro</i> activity of PTK 0796 vs. <i>Escherichia coli</i> , <i>Klebsiella pneumoniae</i> , and <i>Haemophilus influenzae</i>							
Organism	N	Compound	MIC ug/ml				
			Range	MIC50	MIC90		
<i>E. coli</i>	23	PTK 0796	0.5 - 2	1	2		
		Tetracycline	0.5 - >64	2	>64		
		Doxycycline	0.5 - 64	1	64		
		Cefotaxime	<=0.06 - 0.5	<=0.06	0.125		
		Levofloxacin	<=0.06 - 16	<=0.06	4		
		Minocycline	0.5 - 16	1	8		
		Ampicillin	2 - >64	>64	>64		
		Gentamicin	<=0.06 - 64	1	8		
		Ciprofloxacin	<=0.06 - 32	<=0.06	8		
		<i>K. pneumoniae</i>	14	PTK 0796	1 - 8	2	4
Tetracycline	0.5 - >64			2	>64		
Doxycycline	1 - 64			2	32		
Cefotaxime	<=0.06 - >64			<=0.06	32		
Levofloxacin	<=0.06 - 64			<=0.06	32		
Minocycline	2 - >64			2	64		
Gentamicin	0.5 - 32			0.5	32		
Ciprofloxacin	<=0.06 - >64			<=0.06	>64		
<i>H. influenzae</i>	53			PTK 0796	0.5 - 8	1	2
				Tetracycline	0.125 - 64	2	32
		Doxycycline	0.125 - 8	0.5	4		
		Cefotaxime	<=0.06 - 1	<=0.06	<=0.06		
		Levofloxacin	<=0.06 - 64	<=0.06	<=0.06		
		Ampicillin	<=0.06 - >64	64	>64		
		Azithromycin	0.25 - 4	1	2		

CONCLUSIONS

PTK 0796 (BAY 73-6944) was highly active *in vitro* against a broad spectrum of pathogens most notably:

- Methicillin-resistant *Staphylococcus aureus*
- Vancomycin-resistant *Enterococcus faecium*
- Penicillin-resistant *Streptococcus pneumoniae*
- Escherichia coli*
- Klebsiella pneumoniae*
- Haemophilus influenzae*

PTK 0796 (BAY 73-6944) was active *in vitro* against organisms which are resistant to multiple classes of antibiotics including:

- Methicillin-resistant *Staphylococcus aureus* and vancomycin