

**PTK 0796 (BAY 73-6944) and Other Novel Tetracycline
Derivatives Exhibiting Potent *In vitro* and *In vivo*
Activities Against Antibiotic Resistant
Gram-Positive Bacteria**

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PTK 0796 and Other Novel Tetracycline Derivatives Exhibiting Potent *In vitro* and *In vivo* Activities Against Antibiotic Resistant Gram-Positive Bacteria

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ABSTRACT

Background Antibiotic resistant bacteria pose a significant health risk because antibiotic treatment is ineffective against infections caused by these organisms. In an effort to combat resistance, Paratek Pharmaceuticals has undertaken a medicinal chemistry effort to create novel tetracycline derivatives as potential agents against multiple antibiotic resistant gram-positive bacteria.

Methods 7-position and 7,9 position derivatives of sancycline were synthesized and tested for activity *in vitro* against MRSA, VRE, *E. faecalis* (Ef), and *S. pneumoniae* (Spn). Serum effects and COS-1 and CHO-K1 cell cytotoxicity were obtained to aid in the selection of compounds for efficacy testing *in vivo* using a mouse systemic Spn. infection model.

Results *In vitro* MIC screening *in vitro* identified four 7-dimethylamino-9-aminomethylcyclines (AMCs) and four 7-aryl or heteroaryl sancyclines with potent activity (MIC range ≤ 0.06 -2.0 $\mu\text{g/mL}$). Both novel series were more potent than the antibiotic standards (MIC range 16-64 $\mu\text{g/mL}$) against one or more of the resistant strains. The AMCs were minimally or moderately affected by serum as indicated by the activity for MRSA in the presence (MIC range 1.0-4.0 $\mu\text{g/mL}$) or absence (MIC range 0.25-0.50 $\mu\text{g/mL}$) of 50% mouse and human serum. These compounds also exhibited low cytotoxicity ($\text{IC}_{50} > 100 \mu\text{g/mL}$). When tested in an infection model *in vivo*, the compounds demonstrated good efficacy (PD_{50} range 0.35-0.63 mg/kg) against susceptible Spn.

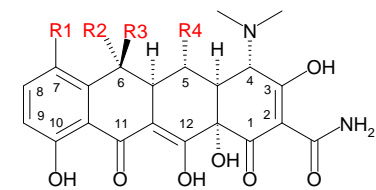
Conclusions This study identified two classes of novel tetracyclines which exhibited potent *in vitro* activity against resistant gram-positive bacteria. The antibacterial activity, absence of cytotoxicity, and demonstrated efficacy *in vivo* highlights the potential of the AMCs as a novel class of antibacterials, one of which, PTK 0796 has been chosen for development.

INTRODUCTION

The tetracyclines are a group of *Streptomyces* natural products previously used as broad spectrum antibiotics since the late 1940's (Figure 1). The emergence of antibiotic resistance in the last fifty years has limited the use of tetracyclines to the treatment of only a few conditions, such as acne, Lyme disease, rickettsia, chlamydia and periodontal disease. Since tetracyclines typically display a broad spectrum of antibacterial activity and low toxicity, Paratek Pharmaceuticals has undertaken a medicinal chemistry effort to develop novel tetracycline derivatives with activity against multiple antibiotic resistant bacteria.

Figure 1.

Structure of some clinically used tetracyclines



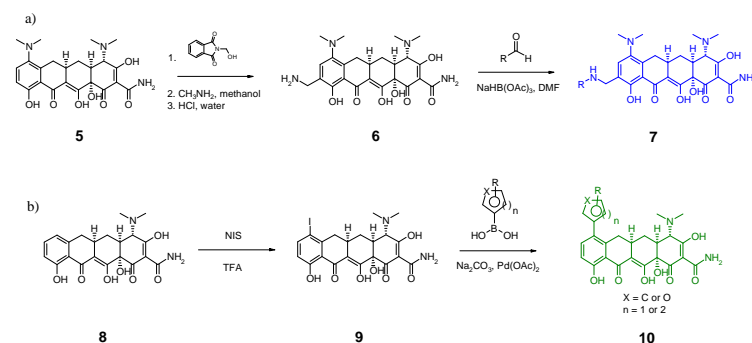
Compound	R1	R2	R3	R4
chlorotetracycline	Cl	CH ₃	OH	H
doxycycline	H	CH ₃	H	OH
minocycline	N(CH ₃) ₂	H	H	H
oxytetracycline	H	CH ₃	OH	OH
tetracycline	H	CH ₃	OH	H

METHODS

- Several substituted 7-dimethylamino-9-aminomethylcyclines (AMC, 7) and 7-aryl or heteroaryl sancyclines (10) were prepared as potential agents against antibiotic resistant gram-positive bacteria (Scheme 1).
- Novel analogs were screened for MIC values against methacillin-resistant *Staphylococcus aureus* MRSA5, vancomycin-resistant *Enterococcus faecium* 494, resistant *Enterococcus faecalis* JH2-2 pMV158, susceptible *Streptococcus pneumoniae* 157E and tetracycline resistant *Streptococcus pneumoniae* ATCC 700905. Microdilution MICs were performed according to current NCCLS guidelines.
- Serum MICs against *Staphylococcus aureus* MRSA5 and cytotoxicity data against monkey fibroblasts COS-1 and hamster ovary cells CHO-K1 were obtained to aid in the selection of compounds for efficacy testing *in vivo*.
- The efficacy *in vivo* of selected compounds was determined using a systemic intraperitoneal (IP) challenge model in mice infected with 10^9 mouse of susceptible *S. pneumoniae* 157E. Single doses of compound were administered IV, one hr post-infection. Survival of animals was then monitored for 7 days and PD_{50} 's calculated.

Scheme 1. Preparation of novel tetracycline analogs.

- Synthesis of 7-dimethylamino-9-aminomethylcyclines (7).
- Synthesis of 7-aryl or heteroaryl sancyclines (10).



RESULTS

- Both series of novel tetracycline analogs demonstrated good activity *in vitro* for all antibiotic resistant gram-positive bacteria tested (MICs 0.06-2 $\mu\text{g/mL}$) (Table 1).
- All analogs were more potent than the antibiotic standards (MIC range 16-64 $\mu\text{g/mL}$) (Table 1, 1-4) against one or more of the resistant strains.
- The MIC values for the AMC series 7a-d were not affected, slightly affected, or moderately affected by the presence of serum (Table 2). However, the presence of serum greatly affected the observed MICs for the 7-aryl or heteroaryl sancyclines 10a-d (serum MICs >64 $\mu\text{g/mL}$).
- The AMCs 7a-d demonstrated low cytotoxicity *in vitro* ($\text{IC}_{50} > 100 \mu\text{g/mL}$), whereas the 7-aryl and heteroaryl sancyclines 10a-d displayed significant cytotoxicity against monkey fibroblast COS-1 cells and Chinese hamster ovary (CHO-K1) cells (IC_{50} 's of <1.5-9.6 $\mu\text{g/mL}$) (Table 3).

Table 1. MICs ($\mu\text{g/mL}$) for novel tetracycline analogs (7a-d and 10a-d) and comparators (1-4) against antibiotic resistant gram-positive bacteria. MIC values in RED indicate resistance.

No.	Name	Structure	MRSA	VRE	Ef	Spn
7						
7a	PTK 0796	R1 =	0.25	0.5	0.5	≤ 0.06
7b	P001221		0.5	1	0.25	≤ 0.06
7c	P002352		0.5	0.5	1	≤ 0.06
7d	P001207		0.5	0.5	1	≤ 0.06
10						
10a	P001075	R2 =	1	1	≤ 0.06	$\leq 0.06 / 0.5$
10b	P001036		0.25	2	≤ 0.06	$\leq 0.06 / 1$
10c	P000538		0.25	0.5	≤ 0.06	$\leq 0.06 / 0.25$
10d	P000642		0.5	0.5	≤ 0.06	$\leq 0.06 / 0.5$
1	vancomycin		1	>64	1	0.25 / 0.25
2	ciprofloxacin		16	1	1	0.5 / 1
3	minocycline		4	16	0.5	$\leq 0.06 / 8$
4	doxycycline		8	16	4	$\leq 0.06 / 4$

MRSA: Methacillin-resistant *Staphylococcus aureus* MRSA5.
VRE: Vancomycin-resistant *Enterococcus faecium* 494.
Ef: *Enterococcus faecalis* JH2-2pMV158.
Spn: *Streptococcus pneumoniae* 157E (tet susceptible) / ATCC 700905 (tet resistant).

Table 2. Serum MICs ($\mu\text{g/mL}$) of novel tetracyclines against MRSA in the absence and presence of 50% human and mouse serum.

No.	Name	No Serum	Human	Mouse
7a	PTK 0796	0.5	0.5	0.25
7b	P001221	0.5	16	8
7c	P002352	0.5	4	2
7d	P001207	0.5	2	1
10a	P001075	1	>64	>64
10b	P001036	0.25	>64	>64
10c	P000538	0.25	>64	>64
10d	P000642	0.5	>64	>64

Table 3. IC_{50} values ($\mu\text{g/mL}$) of novel tetracyclines for cytotoxicity against monkey fibroblast COS-1 and hamster ovary CHO-K1 cells.

No.	Name	COS-1	CHO-K1
4	doxycycline	>25	>25
7a	PTK 0796	>100	>100
7b	P001221	>100	>100
7c	P002352	>100	>100
7d	P001207	>100	>100
10a	P001075	2.9	4.6
10b	P001036	9.6	23
10c	P000538	<1.6	<1.6
10d	P000642	<1.6	<1.6

- The low or moderate serum MICs and low cytotoxicity of the AMC series 7a-d was used to select these novel tetracycline analogs for efficacy testing *in vivo*.
- The efficacy *in vivo* of all AMCs 7a-d tested was significantly better against susceptible *S. pneumoniae* than the antibiotic standard, doxycycline. (Table 4). In particular, compound 7a (PTK 0796) demonstrated PD_{50} almost 7-fold lower than doxycycline.

Table 4. PD_{50} (mg/kg) efficacy of AMCs 7a-d *in vivo* using a systemic *S. pneumoniae* 157E (susceptible) infection model in mice.

Compound	Name	PD_{50}
4	doxycycline	2.3
7a	PTK 0796	0.35
7b	P001221	0.63
7c	P002352	0.43
7d	P001207	0.58

CONCLUSIONS

- The two novel classes of tetracycline derivatives presented in this study demonstrated potent activity *in vitro* against antibiotic resistant gram-positive bacteria.
- The low serum MICs, low cytotoxicity and good efficacy *in vivo* of the 7-dimethylamino-9-aminomethylcyclines (AMCs, 7a-d) highlight the potential of these derivatives as novel agents to combat antibiotic resistance.
- As a result of this study, the AMC derivative, PTK 0796 (BAY 73-6944) (7a), was chosen for further development.