

Abstract

Background: The total synthesis of tetracycline derivatives increases the scope of accessible chemical diversity in this important antibiotic class. TP-120 and TP-787 are two examples of novel synthetic 8-aza tetracyclines. *In vitro* and *in vivo* activities were evaluated.

Method: Susceptibility testing was performed according to CLSI guidelines. Mitochondrial protein synthesis inhibition by test articles was assessed in HepG2 cells using the Mito-Tox™ Mito-Biogenesis Kit. *In vivo* efficacy was determined after intravenous (IV) dosing in a mouse systemic infection model against *S. aureus* ATCC 13709 or *E. coli*/ATCC 25922. PK was evaluated following a single dose at 1 mg/kg IV or 10 mg/kg orally in Sprague-Dawley rats.

Results: The *in vitro* activity:

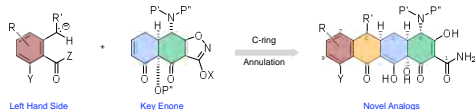
Test Article	MIC (µg/ml)						
	<i>S. aureus</i> ATCC 13709	<i>S. aureus</i> ATCC 29213	<i>S. pneumoniae</i> ATCC 49619	<i>E. coli</i> ATCC 25922	<i>E. cloacae</i> ATCC13047	<i>K. pneumoniae</i> ATCC13883	<i>P. aeruginosa</i> ATCC 27853
TP-120	0.125	0.03	0.03	0.125	1	0.5	8
TP-787	0.5	0.25	0.016	0.5	2	2	32
Tetracycline	1	0.5	0.25	2	2	4	32
Tigecycline	0.5	0.25	0.03	0.25	1	1	16

TP-120 and TP-787 decreased mitochondrial protein synthesis by 40% and 29%, respectively, while linezolid decreased synthesis by 63%. The PD₅₀ of TP-120 and TP-787 in the mouse septicemia model was <0.3 and 0.36 mg/kg against *S. aureus* and 4.3 and 17.0 mg/kg against *E. coli*, respectively. The following PK parameters for TP-120 were obtained after IV dosing: AUC 1087 ng*hr/mL, Cl 919 mL/h/kg, Vz 4177 mL/kg, and T_{1/2} 3 h. The oral bioavailability in the rat was 13%.

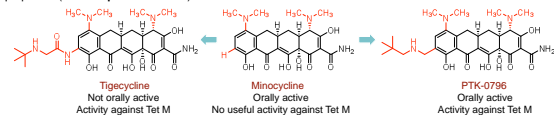
Conclusions: TP-120 and TP-787 exhibit excellent activity against gram-positive and gram-negative bacteria. Exposure to TP-120 and TP-787 result in less inhibition of mitochondrial protein synthesis than linezolid in an *in vitro* assay. TP-120 and TP-787 are efficacious in murine systemic infections and show favorable PK parameters in the rat.

Background

- The total synthetic approach discovered in Andrew Myers' lab (Charest, MG, et al. *Science* 2005, 308, 395-398) has reinvigorated the field of tetracycline chemistry allowing for novel nuclei, including heterocyclic tetracyclines.
- Convergent synthesis and adequate sourcing enables total synthesis of novel tetracyclines to be cost competitive.
- Historically, the majority of chemistry has been at the C7 and C9 positions.



- Small changes can profoundly affect spectrum, ability to evade resistance mechanisms, and pharmacokinetic properties (also see poster F1-1514).



Results

Table 1. MICs (µg/ml) against screening panel of bacterial pathogens.

Compound	Structure	Staphylococcus aureus ATCC 29213	Staphylococcus aureus ATCC 13709	MRSA (n6M)	Staphylococcus aureus BA156 (n6M)	Enterococcus faecalis ATCC 29212	Enterococcus faecalis EF159 (n6M)	Streptococcus pneumoniae ATCC 49619	Streptococcus pneumoniae SP160 (n6M)	Escherichia coli ATCC 25922	Escherichia coli EC155 (n6A)	Acinetobacter baumannii AB110	Pseudomonas aeruginosa ATCC 27853	Enterobacter cloacae ATCC 13047	Klebsiella pneumoniae ATCC 13883	Klebsiella pneumoniae KP153 (n6A)
TP-120		0.03	0.13	32	1	1	32	0.03	8	0.13	>32	0.5	8	1	0.5	>32
TP-787		0.25	0.5	2	0.125	0.25	2	0.016	0.125	0.5	8	2	32	2	2	8
Tetracycline		0.5	1	32	>32	16	>32	0.25	>32	2	>32	1	32	2	4	>32
Doxycycline		0.5	0.13	8	2	4	8	0.25	8	1	32	2	>32	4	2	32

Table 2. Pharmacokinetics of TP-120 and TP-787 in Sprague-Dawley rats.

Parameter	TP-787, IV (n=3)		TP-787, PO (n=3)		TP-120, IV (n=5)		TP-120, PO (n=5)	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Half-life (hr)	4.30	0.10	5.21	0.42	3.14	0.51	4.51	2.59
Tmax (hr)	0.22	0.24	0.83	0.29	0.08	0.00	1.40	0.55
Cl _{obs} (mL/hr/kg)	202.34	104.07	--	--	918.65	68.61	--	--
Vz _{obs} (mL/kg)	1256.14	102.39	--	--	4176.97	861.89	--	--
AUC _{0-∞} (hr*mg/mL)	4872.33	359.13	394.91	92.72	1086.51	86.55	1247.57	206.78
Cmax (ng/mL)	2404.67	1534.79	69.77	4.88	602.80	286.03	192.20	32.22
%F			0.80%				12.74%	

Note that the oral bioavailability of tetracycline in rats is 12.1%.

Methods

Susceptibility testing. MIC, MIC₅₀ and MIC₉₀ values were determined according to CLSI methodology. *S. pneumoniae* and *H. influenzae* isolates were obtained from Eurofins-Medinet and are recent clinical isolates. *S. aureus* strains were collected from various geographical sources.

Mitochondrial toxicity. Inhibition of mitochondrial protein synthesis was assessed in HepG2 cells using the Mito-Tox™ Mito-Biogenesis Kit.

PK analysis. The pharmacokinetic properties of TP-120 and TP-787 were determined in Sprague-Dawley rats (n=5 and 3, respectively) after a single dose of 1 mg/kg IV/10 mg/kg PO using WinNonlin, version 5.2.

Murine models of infection. Groups of 6 CD-1 mice (18-20 grams) per dosing concentration were used to assess the efficacy of compounds.

***S. aureus* septicemia model.** *S. aureus* ATCC 13707 (Smith) was mixed with 5% mucin and inoculated by intraperitoneal injection at 2.1 x 10⁸ cfu/mouse. One hour post-challenge, mice received intravenous treatment with either TP-120, TP-787, tetracycline or tigecycline at concentrations ranging from 0.05 – 10 mg/kg. The PD₅₀ in mg/kg was calculated as survival after 48 hours.

***E. coli* septicemia model.** *E. coli* ATCC 25922 was mixed with 5% mucin and inoculated by intraperitoneal injection at 2.0 x 10⁷ cfu/mouse. One hour post-challenge, mice received treatment with TP-120, TP-787, tetracycline or tigecycline in concentrations ranging from 30 to 0.3 mg/kg. Survival was assessed after 48 hours and PD₅₀ values were calculated.

Table 3. MIC₅₀ and MIC₉₀ analysis.

Compound	<i>S. aureus</i> ATCC 13709			<i>S. pneumoniae</i> (n=20)			<i>H. influenzae</i> (n=12)		
	MIC range	MIC ₅₀	MIC ₉₀	MIC range	MIC ₅₀	MIC ₉₀	MIC range	MIC ₅₀	MIC ₉₀
TP-120	<0.016 - 1	<0.016	<0.016	0.063 - 8	2	4	0.063 - 4	0.5	2
TP-787	0.063 - 0.5	0.13	0.25	<0.016 - 0.063	<0.016	0.063	0.25 - 2	1	1
Tetracycline	0.06 - 32	0.13	0.25	0.13 - >32	>32	>32	0.25 - 16	0.5	16
Doxycycline	ND	ND	ND	<0.016 - 16	8	8	0.5 - 4	1	4
Oxacillin/ Amoxicillin	0.06 - 64	8	64	<0.016 - 8	8	8	2 - 32	4	16

Table 4. IV efficacy in murine septicemia models.

Compound	<i>S. aureus</i> ATCC 13709			<i>E. coli</i> ATCC 25922		
	MIC (µg/ml)	PD ₅₀ (mg/kg)	95% C.I.	MIC (µg/ml)	PD ₅₀ (mg/kg)	95% C.I.
TP-120	0.25	<0.30	--	0.13	4.3	4.1 - 4.6
TP-787	0.5	0.36	0.36 - 0.56	0.25	17.0	4.1 - 30
Tetracycline	0.5	0.35	0.34 - 0.37	1	17.0	7.3 - 26.8
Tigecycline	0.13	0.35	0.24 - 0.47	0.13	2.1	1.8 - 2.4

Conclusions

- Novel 8-aza tetracycline analogs TP-120 and TP-787 illustrate the versatility of the Tetraphase chemistry platform for modulating essential antibiotic properties including resistance coverage and pharmacokinetic exposure (see F1-1514 for more examples).
- TP-120 has oral bioavailability in rats equivalent to tetracycline.
- TP-787 has improved activity against isolates containing ribosomal protection mechanisms [e.g., tet(M)].
- Both compounds protected when given intravenously in murine septicemia models.
- The platform is highly versatile and has the potential to produce compounds with improved antibacterial spectrum, potency, resistance coverage, and favorable IV/oral pharmacokinetic properties.