P0243 *In vitro* Activities of Novel Tetracyclines Against Molecularly Characterized Carbapenem-resistant Gram-negative Pathogens

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Revised Abstract

Objective: The US Centers for Disease Control (CDC) has declared carbapenem-resistant Enterobacteriaceae (CRE) and multidrug-resistant *Acinetobacter* urgent and serious threats, respectively. TP--6076, TP-138, and TP-600 are novel synthetic tetracycline antibiotics in development for use against multidrug-resistant Gram-negative pathogens, including those resistant to multiple classes of antibiotics including carbapenems. These compounds were tested against panels of molecularly characterized CRE (including *Escherichia coli*, *Enterobacter aerogenes*, *Enterobacter cloacae*, and *Klebsiella pneumoniae*), *Acinetobacter baumannii,* and *Pseudomonas aeruginosa* to determine their activities against these difficult-to-treat pathogens. **Methods:** Minimal inhibitory concentration (MIC) assays against panels of carbapenem-resistant isolates were performed according to Clinical Laboratory Standards Institute guidelines at IHMA. A total of 334 unique carbapenem-resistant isolates were screened. Among the 178 Enterobacteriaceae isolates, the following classes of extended-spectrum beta-lactamase and carbapenemase enzymes were represented: ACT (n=6), CTX-M (n=54), DHA (n=4), KPC (n=128), MOX/CMY (n=4), NDM (n=11), OXA (n=24), SHV (148), TEM (n=108), VEB-1 (N=1), and VIM (n=12). All *P. aeruginosa* isolates were confirmed carbapenemase producers and the following classes of enzymes were represented in the panel: GES (n=18), IMP (n=5), KPC (n=8), PER-1 (n=7), SPM-1 (n=1), TEM (n=4), VIM (n=29), VEB (n=7). All 80 *A. baumannii* isolates contained an OXA gene. **Results:** TP--6076, TP-138, and TP-600 were highly active against panels of CRE and *A. baumannii* (see Table). TP-6076, TP-138, and TP-600 had $MIC_{50/90}$ values of 0.06/0.125, 0.125/0.25, and 0.25/4 µg/mL, respectively, against in comparison to $MIC_{50/90}$ values of 4/8, >16/>16 and >16/>16 µg/mL for tigecycline, ceftazidime, and imipenem, respectively. All three compounds showed greatly reduced MIC values versus comparators against Enterobacteriaceae, with $MIC_{50/90}$ values of 0.25/1, 0.25/1, and 4/4 µg/mL for TP-6076, TP-138 and TP-600, respectively, versus $MIC_{50/90}$ values of 4/8, >16/>16 and >16/>16 µg/mL for tigecycline, ceftazidime and imipenem, respectively. The $MIC_{50/90}$ values of TP-6076, TP-138, and TP-600 were 8/16, 4/16, and 16/16 µg/mL, respectively, against *P. aeruginosa*, showing greater potency than all 3 comparators ($MIC_{50/90}$ = >16/>16 µg/mL). **Conclusions:** TP-6076, TP-138, and TP-600 show promising potential as next generation tetracycline antibiotics for the treatment of serious multidrug-resistant Gram-negative infections.

Background

Infections caused by multidrug-resistant and, in some cases, pan-resistant Gram-negative microorganisms, are increasingly common problems in hospital or nursing home settings. In a 2013 report [1], the CDC estimated over 9000 healthcare-associated infections, and over 600 deaths were caused by *Klebsiella* spp. and *Escherichia coli* . The majority of these cases (85%) were due to *Klebsiella* spp.. Carbapenem-resistant Enterobacteriaceae (CRE) are of such concern that the CDC has designated CRE as an *urgent threat.*

TP-6076, TP-138, TP-600 are novel, fully synthetic, tetracycline-class antibiotics with unique heterocyclic substituents at C8, along with novel modifications at C4 and C7 [2]. TP-6076, TP-138 and TP-600 showed excellent activity *in vitro* against a screening panel of CRE [2] and all three compounds were more efficacious than tigecycline in a mouse pneumonia model with a KPC-expressing *Klebsiella pneumoniae* isolate [3]. TP-6076, the leading member of this set of compounds, also demonstrated potent anti-biofilm activity against uropoathogenic *E. coli* (see poster P0054).

In the present study we show that TP-6076, TP-138 and TP-600 have potent antibacterial activity *in vitro* against molecularly characterized CRE collected by International Health Management Associates, Inc. (IHMA) in 2012 from worldwide locations and diverse infection types.

Methods and References

MIC determinations were performed at IHMA Europe by broth microdilution in line with CLSI susceptibility testing standards with frozen 96-well MIC panels prepared at IHMA according to M07-A9 (4) & M100-S23 (5).

References

1) **Centers for Disease Control and Prevention,** 2013. Antibiotic resistant threats in the United States, 2013.
2) **Sun C, Hunt D, Clark R, Fyfe C, Kerstein K, Grossman T, Sutcliffe J, Xiao, X.** In vitro potency of novel totally synthetic tetracycline against MDR Gram-negative pathogens including carbapenem-resistant Enterobacteriaceae, Abstr. P0299, 24th ECCMID, 10-13 May, 2014, Barcelona, Spain
3) **Grossman T, Fyfe C, Kerstein K, Sun C, Hunt D, Clark R, Xiao X, Sutcliffe J**. In vivo efficacy of novel, fully synthetic tetracyclines in a murine lung infection model challenged with KPC-producing Klebsiella pneumoniae, Abstr. P0300, 24th ECCMID, 10-13 May, 2014, Barcelona, Spain
4) **CLSI.** 2012. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; approved standard - ninth edition. CLSI document M07-A9. CLSI, 940 West Valley Road, Suite 1400, Wayne, PA.
5) **CLSI,** 2013. Performance standards for antimicrobial susceptibility testing: Twenty-third informational supplement. CLSI document M100-S23. Wayne, PA:

Results

Table 1. Novel tetracyclines are potent against panels of carbapenem-resistant Gram-negative pathogens

Organism	n	MIC 50/90 (range)					
		TP-6076	TP-138	TP-600	Tigecycline	Ceftazidime	Imipenem
Enterobacteriaceae	178	0.25/1 (0.03-8)	0.25/1 (0.06-8)	4/4 (0.06-16)	4/8 (0.5->16)	>16/>16 (1->16)	>16/>16 (4->16)
Enterobacter spp.	12	0.25/2 (0.125-4)	0.25/2 (0.125-8)	4/4 (0.25-8)	2/>16 (2->16)	>16/>16 (4->16)	16/>16 (4->16)
Escherichia coli	4	na (0.03-2)	na (0.06-2)	na (0.06-4)	na (0.5-16)	na (16->16)	na (4-8)
Klebsiella pneumoniae	162	0.25/0.5 (0.06-8)	0.25/1 (0.06-8)	4/4 (0.125-16)	4/8 (1->16)	>16/>16 (1->16)	>16/>16 (4->16)
Acinetobacter baumannii	80	0.06/0.125 (≤0.008-1)	0.125/0.25 (0.015-0.5)	0.25/4 (0.03-4)	4/8 (0.5-16)	>16/>16 (8->16)	>16/>16 (16->16)
Pseudomonas aeruginosa	76	8/16 (1->16)	4/16 (0.5->16)	16/16 (4->16)	>16/>16 (4->16)	>16/>16 (4->16)	>16/>16 (8->16)

na=not applicable

Table 2. ESBL and carbapenemase enzymes present in screening panels

Organism	# Isolates	Enzyme															carbapenemase-positive	carbapenemase-negative
		SHV	TEM	CTX-M	CMY/MOX	IMP	ACT/MIR	GES	PER	SPM	DHA	VEB	VIM	KPC	NDM	OXA		
Enterobacteriaceae	178	148	108	54	4	1	6				4	1	12	128	11	24	171	7
E. coli	4		2	2										2			2	2
Enterobacter spp.	12	5	7	5	1		6				1		4	4	3	1	11	1
K. pneumoniae	162	143	99	47	3	1					3	1	8	122	8	23	158	4
A. baumannii	80															80	77[a]	
P. aeruginosa	76		4			5		18	7	1		7	29	8			76	

[a] 3 isolates had unknown carbapenemase production status

Conclusions

- **TP-6076, TP-138 and TP-600 all showed potent antibacterial activity *in vitro* against recent panels of CRE, *A. baumannii,* and *P. aeruginosa* expressing a diverse array of ESBL and carbapenemase enzymes. Antibacterial activity appeared to be unaffected by these resistance mechanisms.**
- **All three compounds were more potent than tigecycline, imipenem and ceftazidime against all pathogen subpanels, with TP-6076 and TP-138 showing greater overall potency than TP-600.**
- **Novel modifications of the tetracycline scaffold have lead to the discovery of three new antibacterial candidates that are potent against recent clinical isolates of the most difficult-to-treat Gram-negative pathogens.**

Figure 1. MIC distributions[a] for TP-6076, TP-138, and TP-600 and comparators

All Enterobacteriaceae

Compound	MIC (µg/mL)											Total # of isolates
	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	>16	
TP-6076	1	5	32	89	31	13	3	3	1	0	0	178
TP-138	0	6	43	77	29	10	10	0	3	0	0	178
TP-600	0	1	6	24	36	2	7	95	6	1	0	178
imipenem	0	0	0	0	0	0	0	10	22	44	102	178
tigecycline	0	0	0	0	1	5	51	80	27	10	4	178
ceftazidime	0	0	0	0	0	4	0	1	1	5	167	178

Enterobacter spp.

Compound	MIC (µg/mL)								Total # of isolates
	0.12	0.25	0.5	1	2	4	8	16	>16	
TP-6076	3	3	1	2	2	1	0	0	0	12
TP-138	3	3	1	2	2	0	1	0	0	12
TP-600	0	3	0	1	0	7	1	0	0	12
imipenem	0	0	0	0	0	1	2	5	4	12
tigecycline	0	0	0	0	6	0	2	2	2	12
ceftazidime	0	0	0	0	0	1	0	0	11	12

E. coli

Compound	MIC (µg/mL)											Total # of isolates
	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	>16	
TP-6076	1	1	0	1	0	0	1	0	0	0	0	4
TP-138	0	1	1	1	0	0	1	0	0	0	0	4
TP-600	0	1	1	0	1	0	0	1	0	0	0	4
imipenem	0	0	0	0	0	0	0	2	2	0	0	4
tigecycline	0	0	0	0	1	1	0	1	0	1	0	4
ceftazidime	0	0	0	0	0	0	0	0	0	1	3	4

K. pneumoniae

Compound	MIC (µg/mL)									Total # of isolates
	0.06	0.12	0.25	0.5	1	2	4	8	16	>16	
TP-6076	4	29	85	30	11	0	2	1	0	0	162
TP-138	5	39	73	28	8	7	0	2	0	0	162
TP-600	0	5	21	35	1	7	87	5	1	0	162
imipenem	0	0	0	0	0	0	7	18	39	98	162
tigecycline	0	0	0	0	4	45	79	25	7	2	162
ceftazidime	0	0	0	0	4	0	0	1	4	153	162

A. baumannii

Compound	MIC (µg/mL)													Total # of isolates
	≤0.008	0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	>16	
TP-6076	1	7	17	37	13	2	2	1	0	0	0	0	0	80
TP-138	0	1	6	15	36	20	2	0	0	0	0	0	0	80
TP-600	0	0	1	5	14	31	17	0	1	11	0	0	0	80
imipenem	0	0	0	0	0	0	0	0	0	0	0	1	79	80
tigecycline	0	0	0	0	0	0	1	5	14	36	20	4	0	80
ceftazidime	0	0	0	0	0	0	0	0	0	1	4	75		80

P. aeruginosa

Compound	MIC (µg/mL)							Total # of isolates
	0.5	1	2	4	8	16	>16	
TP-6076	0	1	2	14	35	20	4	76
TP-138	1	1	6	31	25	10	2	76
TP-600	0	0	0	1	32	40	3	76
imipenem	0	0	0	0	5	16	55	76
tigecycline	0	0	0	1	3	11	61	76
ceftazidime	0	0	0	1	3	1	71	76

[a] blue numbers represent the number of isolates at a given MIC for a given compound