TP-6076 is efficacious in a mouse pneumonia model with carbapenem-resistant *Acinetobacter baumannii* (CRAB) and retains potency against common tetracycline-resistance mechanisms

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Introduction

TP-6076 is a novel, fully synthetic, broad-spectrum tetracycline-class antibiotic that is being developed for the treatment of serious and life-threatening bacterial infections, including those caused by pathogens otherwise resistant to current treatment options. It is highly active against difficult-to-treat Gram-negative pathogens in nonclinical studies, including carbapenemase-producing Enterobacteriaceae (CRE) and carbapenemase-producing *A. baumannii* (CRAB). TP-6076 was previously shown to have potent activity in vitro against CRE and CRAB and efficacy against carbapenem-resistant *K. pneumoniae* in a mouse pneumonia model [1, 2, 3]. Here we show that TP-6076 retained activity *in vitro* against Enterobacteriaceae and *A. baumannii* expressing tetracycline-specific resistance, and demonstrated efficacy against CRAB in a mouse pneumonia model.

Methods

Clinical isolates. Recent clinical isolates possessing diverse demographic, genotypic and phenotypic characterizations were obtained from Eurofins Medinet (Chantilly, VA) or International Health Management Associates, Inc. (IHMA; Schaumburg, IL). Quality control (QC) strains were used to ensure laboratory standards as guided by Clinical Laboratory Standards Institute (CLSI) [4] and the QC strains were obtained from the American Type Culture Collection (Manassas, VA). TP-6076 and comparators was tested against a set of *A. baumannii* strains with characterized *adeAB* expression [6] and a set of well-characterized *K. pneumoniae* mutant strains to further evaluate the impact of *ramA* over-expression on susceptibility [7]; both strain sets were obtained from Wyeth (now Pfizer). The *ramR* gene of *K. pneumoniae* clinical isolates with elevated tigecycline MIC values was PCR amplified and sequenced by standard methods using a forward primer (GCGCCTTACCAAAACCGGCA) and a reverse primer (CATGGTGATCATAAAGATGCG), and sequence variations were identified by comparing to the reference sequence Genbank No. CP003999.1

Minimum inhibitory concentration (MIC) assays with clinical isolates were performed essentially as described by the CLSI [4]. Resistant phenotypes were determined using CLSI breakpoints [5] except for tigecycline, where FDA breakpoints were used [8].

Susceptibility testing against *E. coli* DH10B expressing recombinant tetracycline-resistance genes. To determine the impact of specific tetracycline-resistance genes in isogenic *E. coli* strains, sequences encoding *tet*(A), *tet*(B), *tet*(D), *tet*(K), *tet*(M), *tet*(Q), *tet*(X) and *E. coli* β-galactosidase (*lacZ*) as a negative control were amplified by PCR from clinical isolates confirmed by prior sequencing to have these tetracycline-resistance determinants. Genes were cloned into an L-arabinose inducible expression system without any affinity tags (pBAD-Myc-His, Invitrogen, Carlsbad, CA). Plasmids were transformed into *E. coli* DH10B cells (Invitrogen, Carlsbad, CA). Cloned inserts were verified by sequencing and comparing with reported sequences in GenBank (accession numbers: *tet*(A), AJ419171; *tet*(B), *tet*(D), AF467074; AP010961; *tet*(K), AJ888003; *tet*(M), X90939; *tet*(Q), Z21523; *tet*(X), AB097942). For MIC assays, ampicillin was present at a constant concentration of 50 μg/mL in each well to maintain plasmids; cells were grown in cation-adjusted Mueller Hinton Broth (MHB; BBL/ BD, Franklin Lakes, NJ) containing ampicillin, 50 μg/ml, and pre-induced for 30 minutes with 1% arabinose (*tet*(A), *tet*(B), *tet*(M), *tet*(X)) or 0.1% arabinose (*tet*(K)) at 30°C prior to use as inocula in MIC assays.

Mouse lung infection model. Female mice (n=6) were rendered neutropenic with 150 mg/kg cyclophosphamide IP on day -4 and 100 mg/kg on day -1, prior to intranasal challenge with 6 – 7 $\log_{10}$ CFU of *A. baumannii* AB1709 (bla_{OXA}, bla_{TEM}, $bla_{ADC\text{-}30\text{-}like}$). TP-6076 (5, 15, or 40 mg/kg) or tigecycline (50 mg/kg) was administered IV every 12 hours, starting at 2 hrs postchallenge for 72 hours. Animals were euthanized at 24, 48 and 72 hours and lung CFUs were enumerated.

Results

Table 1. TP-6076 is Potent *In Vitro* against Difficult-to-Treat Gram-Negative Pathogens

Organism		TP-6076	Tetracycline[a]	Tigecycline	Carbapenem[f]	3rd Gen Ceph[g]	Pip/Tazo	Colistin	Fluoroquinolone[h]	Aminoglycoside[i]
Acinetobacter baumannii	MIC 50/90	≤0.016/0.063	0.5/>4	1/4	4/>32	32/>64	64/>128	0.5/1	>4/>32	>8/>32
	Range	(≤0.001-0.13)	(≤0.002-8)	(≤0.016-8)	(0.13->32)	(2->64)	(≤0.063->128)	(0.063-2)	(0.063->32)	(0.5->32)
	n	62	62	62	62	62	51	62	62	62
Acinetobacter baumannii 3rd Gen Ceph-R[b]	MIC 50/90	0.031/0.063	4/>4	2/4	32/>32	>64/>64	>128/>128	0.5/0.5	8/>32	>32/>32
	Range	(≤0.002-0.13)	(0.063-8)	(0.25-8)	(2->32)	(32->64)	(64->128)	(0.063-1)	(4->32)	(1->32)
	n	30	30	30	30	30	19	30	30	30
Acinetobacter baumannii CP-R[c]	MIC 50/90	0.031/0.063	4/>4	2/4	32/>32	>64/>64	>128/>128	0.5/0.5	8/>32	>32/>32
	Range	(≤0.002-0.13)	(0.063-8)	(0.25-8)	(4->32)	(32->64)	(16->128)	(0.063-2)	(4->32)	(1->32)
	n	30	30	30	30	30	19	30	30	30
Acinetobacter baumannii 3rd Gen Ceph-R, CP-R, AG-R[d]	MIC 50/90	0.031/0.13	2/>4	2/8	32/>32	>64/>64	>128/>128	0.5/0.5	8/>32	>32/>32
	Range	(≤0.002-0.13)	(0.063->4)	(0.25-8)	(4->32)	(32->64)	(64->128)	(0.13-1)	(4->32)	(>8->32)
	n	23	23	23	23	23	14	23	23	23
Acinetobacter baumannii 3rd Gen Ceph-R, CP-R, FQ-R[e]	MIC 50/90	0.031/0.13	4/>4	2/8	32/>32	>64/>64	>128/>128	0.5/0.5	8/>32	>32/>32
	Range	(≤0.002-0.13)	(0.063-8)	(0.25-8)	(4->32)	(32->64)	(64->128)	(0.063-1)	(4->32)	(1->32)
	n	27	27	27	27	27	16	27	27	27
Enterobacter cloacae	MIC 50/90	≤0.016/0.25	4/16	0.25/4	0.031/1	0.5/>32	2/128	0.25/>32	0.063/8	1/2
	Range	(≤0.016-0.5)	(2->32)	(0.13-4)	(≤0.016-2)	(0.063->32)	(1->128)	(0.063->32)	(0.031-16)	(0.5->32)
	n	36	36	36	36	36	15	36	36	36
Escherichia coli	MIC 50/90	≤0.016/0.031	>32/>32	0.13/0.5	0.031/0.063	0.5/>32	4/>64	0.25/0.5	0.5/32	2/>32
	Range	(≤0.002-0.25)	(1->64)	(0.031-2)	(≤0.016->32)	(0.06->64)	(≤0.5->128)	(0.063->32)	(≤0.016->32)	(0.25->32)
	n	253	253	253	253	253	159	203	253	177
Escherichia coli 3rd Gen Ceph-R	MIC 50/90	≤0.016/0.031	>32/>32	0.25/0.5	0.031/0.25	>32/>32	8/128	0.25/0.5	16/32	4/>32
	Range	(≤0.002-0.25)	(1->32)	(0.063-2)	(≤0.016->32)	(0.25->64)	(1->128)	(0.063-4)	(0.031->32)	(0.5->32)
	n	101	101	101	101	101	101	91	101	101
Klebsiella pneumoniae	MIC 50/90	0.063/0.5	8/>32	0.5/4	0.063/32	32/>32	32/>128	0.25/1 (0.063->32)	2/>32	8/>32
	Range	(≤0.016-8)	(1->32)	(0.063-16)	(≤0.016->32)	(0.031->64)	(0.5->128)		(0.031-64)	(0.25->64)
	n	235	235	235	235	235	198	190	235	198
Klebsiella pneumoniae 3rd Gen Ceph-R	MIC 50/90	0.063/0.5	16/>32	0.5/4	0.063/>32	>32/>32	128/>128	0.25/1 (0.063->32)	32/>32	32/>32
	Range	(≤0.016-2)	(1->32)	(0.13-16)	(0.031->32)	(0.5->64)	(2->128)		(0.031-64)	(0.25->64)
	n	143	143	143	143	143	143	130	143	143
Klebsiella pneumoniae CP-R	MIC 50/90	0.063/0.25	16/>32	1/2	32/>32	>32/>32	>128/>128	0.25/8 (0.063->32)	>32/>32	32/>32
	Range	(≤0.016-2)	(2->32)	(0.25-16)	(0.063->32)	(2->32)	(4->128)		(0.13->32)	(0.25->64)
	n	51	51	51	51	51	51	48	51	51
Proteus mirabilis	MIC 50/90	0.25/0.5	>32/>32	4/8	0.13/0.5	0.063/0.25	0.5/1	>32/>32	0.13/32	2/32
	Range	(≤0.016-1)	(8->64)	(0.5-16)	(≤0.016-1)	(≤0.016-4)	(≤0.5-2)	(>32->32)	(≤0.016->64)	(0.5->64)
	n	51	51	51	51	51	42	41	51	51
Pseudomonas aeruginosa	MIC 50/90	8/16	>32/>32	16/32	4/32	4/>32	16/>128	1/1	1/>32	2/>32
	Range	(0.5->32)	(8->32)	(1->32)	(0.5->32)	(1->32)	(0.5->128)	(0.25-2)	(0.25->32)	(0.25->32)
	n	75	75	75	75	75	75	75	75	75
Stenotrophomonas maltophilia	MIC 50/90	0.031/0.5	16/32	0.5/4	>32/>32	>32/>32	64/>128	>32/>32	1/16	>32/>32
	Range	(≤0.016-4)	(1->32)	(0.031-4)	(2->32)	(2->32)	(8->128)	(0.5->32)	(0.13-32)	(2->32)
	n	24	24	24	24	24	24	24	24	24

MIC and range values in μg/mL. [a] minocycline was used for *A. baumannii*; [b] 3rd Gen Ceph-R: resistant to cefotaxime, ceftazidime or ceftriaxone as per CLSI interpretive criteria; [c]CP-R: resistant to imipenem, ertapenem (Enterobacteriaceae-only), or meropenem as per CLSI interpretive criteria; [d]AG-R, resistant to gentamicin, amikacin or tobramycin as per CLSI interpretive criteria; [e]FQ-R, resistant to levofloxacin or ciprofloxacin as per CLSI interpretive criteria; [f]Carbapenem: imipenem, ertapenem, or meropenem; [g]3rd Gen Ceph: cefotaxime, ceftazidime or ceftriaxone; [h]fluoroquinolone: levofloxacin or ciprofloxacin; [i]aminoglycoside: gentamicin, amikacin or tobramycin.

Table 2. TP-6076 Is Active *In Vitro* against Mutant *A. baumannii* with Altered AdeABC Efflux Expression

Mutant Strain	Parent Strain and Phenotype	TP-6076	Tetracycline	Tigecycline	Meropenem	Levofloxacin	Gentamicin
AB991	G4904, wt parent of G5139	0.008	32	1	2	16	>32
AB992	G5141, wt parent of G5140 and GC7951	0.008	32	1	2	>32	>32
AB993	G5140, overexpressing *adeAB*	0.13	32	8	2	32	>32
AB994	G5139, overexpressing *adeAB*	0.13	32	8	2	32	>32
AB995	GC7951, knockout of *adeB*	0.016	>32	2	2	16	32

MIC values in μg/mL.

Table 3. TP-6076 is Active *In Vitro* against Tigecycline-Resistant *K. pneumoniae* Clinical Isolates

Compound	MIC 50/90	Range
TP-6076	1/4	(0.25 - 8)
Tigecycline	8/16	(8 - >16)
Carbapenem	16/>16	(0.031 - >32)
3rd Gen Ceph	>16/>16	(0.5->32)

MIC, $MIC_{50/90}$. Range values in μg/mL; 3rd Gen Ceph: cefotaxime, ceftazidime or ceftriaxone; carbapenem: imipenem, ertapenem, or meropenem; n=47 clinical isolates

Table 4. TP-6076 Retains Potency *In Vitro* against Clinical Isolates Containing Tetracycline-Specific Resistance Genes

Organism		TP-6076	Tetracycline[a]	Tigecycline	3rd Gen Ceph[b]	Carbapenem[c]	Colistin	Fluoroquinolone[d]	Aminoglycoside[e]
Acinetobacter baumannii tet(A)	MIC 50/90	NA/NA	NA/NA	NA/NA	NA/NA	NA/NA	NA/NA	NA/NA	NA/NA
	Range	(0.063-0.063)	(1-2)	(2-2)	(32->64)	(32-32)	(0.5-0.5)	(8-8)	(>32->32)
	n	3	3	3	3	3	3	3	3
Acinetobacter baumannii tet(B)	MIC 50/90	0.031/0.13	>4/>4	2/4	>64/>64	32/>32	0.5/1	16/>32	>32/>32
	Range	(0.004-0.13)	(1-8)	(0.5-8)	(32->64)	(1->32)	(0.063-2)	(4->32)	(1->32)
	n	22	22	22	18	22	22	22	22
Escherichia coli tet(A)	MIC 50/90	≤0.016/0.031	>32/>32	0.25/0.5	8/>32	≤0.016/0.13	0.13/0.25	>4/>32	2/>32
	Range	(≤0.016-0.13)	(>32->32)	(0.063-1)	(0.06->32)	(≤0.016->32)	(0.063-4)	(0.03->32)	(0.25->32)
	n	52	52	52	46	52	28	46	43
Escherichia coli tet(B)	MIC 50/90	≤0.016/0.063	>32/>32	0.13/0.25	16/>32	0.031/0.063	0.25/0.5	8/32	2/>32
	Range	(≤0.004-0.5)	(32->32)	(0.063-1)	(0.063->64)	(≤0.016->32)	(0.063-0.5)	(≤0.016->32)	(0.5->32)
	n	78	78	78	76	78	73	76	35
Escherichia coli tet(D)	MIC 50/90	NA/NA (≤0.016-≤0.016)	NA/NA	NA/NA	NA/NA	NA/NA	NA/NA	NA/NA	NA/NA
	Range		(>32->32)	(0.13-0.25)	(0.25->32)	(≤0.016-0.063)	(0.13-0.25)	(0.031->32)	(4->32)
	n	3	3	3	3	3	3	3	3
Escherichia coli tet(M)	MIC 50/90	NA/NA	NA/NA	NA/NA	NA/NA	NA/NA	NA/NA	NA/NA	NA/NA
	Range	(≤0.016-0.13)	(>8->32)	(0.063-1)	(8->32)	(≤0.016-0.063)	(0.25-0.25)	(0.063-32)	(2->8)
	n	5	5	5	3	5	1	3	2
Klebsiella pneumoniae tet(A)	MIC 50/90	0.13/1 (0.031-2)	>32/>32	1/4	>32/>32	0.13/16	0.25/0.25	8/>32	>32/>32
	Range		(32->32)	(0.25-8)	(0.063->64)	(≤0.016->32)	(0.13->32)	(0.031->32)	(0.25->32)
	n	41	41	41	34	41	21	34	31
Klebsiella pneumoniae tet(B)	MIC 50/90	NA/NA (≤0.016-0.13)	NA/NA	NA/NA	NA/NA	NA/NA	NA/NA	NA/NA	NA/NA
	Range		(4->32)	(0.063-0.5)	(0.06->32)	(0.031-4)	(0.13-0.5)	(0.063-4)	(0.5->32)
	n	5	5	5	5	5	5	5	2
Klebsiella pneumoniae tet(D)	MIC 50/90	0.063/0.5	>32/>32	0.5/2	>32/>32	0.063/1	0.25/1	2/32	>32/>32
	Range	(≤0.016-2)	(2->32)	(0.13-4)	(0.13->32)	(≤0.016->32)	(0.13-32)	(0.063-32)	(0.25->32)
	n	37	37	37	34	37	34	34	9

MIC and range values in μg/mL. [a] minocycline was used for *A. baumannii*; [b]3rd Gen Ceph: cefotaxime, ceftazidime or ceftriaxone; [c]carbapenem: imipenem, ertapenem (Enterobacteriaceae only), or meropenem; [d]fluoroquinolone: levofloxacin or ciprofloxacin; [e]aminoglycoside: gentamicin, amikacin or tobramycin. NA, not available.

Figure 1. TP-6076 Retains Potency *In Vitro* in the Presence of Tetracycline-Specific Resistance Mechanisms

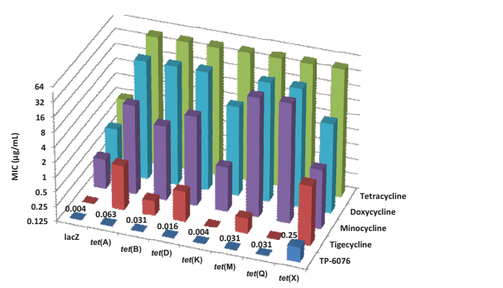
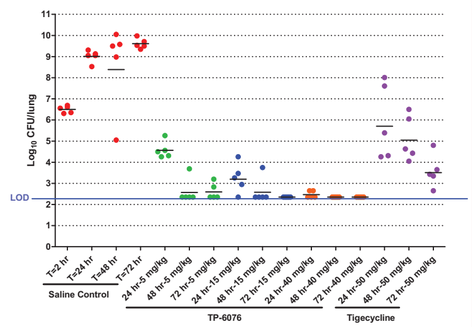


Table 5. *In Vitro* Antibacterial Activity of TP-6076 and Comparators against Molecularly Characterized *K. pneumoniae* Differentially Expressing *ramA* and Clinical Isolates Containing RamR Sequence Variations

Strain:	KP987	KP986	KP989	KP988	KP990	*K. pneumoniae* Clinical Isolates Containing RamR Sequence Variations (n=31)
	ramA on Chromosome			*ramA* on Plasmid		
Genotype:	wt *ramA*	Upregulated *ramA*	KP986 With Deleted *ramA*	KP987 With Overexpressed *ramA*	KP989 With Overexpressed *ramA*	$MIC_{50/90}$; (range)
TP-6076	0.031	1	≤0.016	2	2	0.5/2; (0.031 - 8)
Tigecycline	0.5	4	0.25	16	16	4/8; (0.25 - 16)
Tetracycline	4	32	4	32	>32	>32/>32; (2 - >32)
Levofloxacin	0.063	1	0.063	1	2	16/>32; (0.13 - >32)

MIC, $MIC_{50/90}$. Range values in μg/mL; wt = wildtype.

Figure 2. TP-6076 is Efficacious against Carbapenem-resistant (OXA) *A. baumannii* AB1709 in a Mouse Pneumonia Model



Compound	MIC (μg/mL)
TP-6076	0.008
Tigecycline	1

Conclusions

- TP-6076 retained activity in vitro against difficult-to-treat Gram-negative pathogens, including those expressing tetracycline-specific and intrinsic resistance mechanisms affecting the tetracycline-class antibiotics.
- In a mouse pneumonia model challenged with carbapenem-resistant *A. baumannii*, TP-6076 administered IV at 5 mg/kg BID, reduced bioburden in lung by ≥ 4-$\log_{10}$ CFUs (the limit of detection) at 48 hours versus the start of dosing. Higher doses of 15 and 40 mg/kg BID reduced lung CFUs to, or near, the lower limit of detection by 24 hours, whereas 50 mg/kg of tigecycline BID was much less efficacious.
- TP-6076 continues to demonstrate its potential as a promising antibiotic for the treatment of infections caused by drug-resistant Gram-negative pathogens including carbapenem-resistant Enterobacteriaceae and *A. baumannii*.

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Presented at the 26th ECCMID, April 9 - 12, 2016, Amsterdam, Netherlands