

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 (GCGCCTTACCAAAACGGCA) 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 μ g/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 μ g/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 log₁₀ CFU of *A. baumannii* AB1709 (*bla*_{OXA}, *bla*_{TEM}, *bla*_{ADC-30}_{HD}). 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 ^b	3 rd Gen CepH ^c	Pip/Tazo	Colistin	Fluoroquinolone ^d	Aminoglycoside ^e	
<i>Acinetobacter baumannii</i>	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 n	(≤0.001-0.13) 62	(≤0.002-8) 62	(≤0.016-8) 62	(0.13-32) 62	(2-64) 62	(0.063-2) 62	(0.063-2) 62	(0.5-5) 62	(0.5-32) 62
<i>Acinetobacter baumannii</i> 3rd Gen CepH-R ^b	MIC _{50/90}	0.031/0.063	4/4	2/4	32/32	>64/64	8	0.5/0.5	8/32	>32/32
	Range n	(≤0.002-0.13) 30	(0.063-8) 30	(0.25-8) 30	(2-32) 30	(32-64) 30	(64-128) 30	(0.063-1) 30	(4-32) 30	(1-32) 30
<i>Acinetobacter baumannii</i> CP-R ^b	MIC _{50/90}	0.031/0.063	4/4	2/4	32/32	>64/64	8	0.5/0.5	8/32	>32/32
	Range n	(≤0.002-0.13) 30	(0.063-8) 30	(0.25-8) 30	(4-32) 30	(32-64) 30	(16-128) 30	(0.063-2) 30	(4-32) 30	(1-32) 30
<i>Acinetobacter baumannii</i> 3rd Gen CepH-R, CP-R, AG-R ^b	MIC _{50/90}	0.031/0.13	2/4	2/8	32/32	>64/64	8	0.5/0.5	8/32	>32/32
	Range n	(≤0.002-0.13) 23	(0.063-4) 23	(0.25-8) 23	(4-32) 23	(32-64) 23	(64-128) 23	(0.13-1) 23	(4-32) 23	(8-32) 23
<i>Acinetobacter baumannii</i> 3rd Gen CepH-R, CP-R, FQ-R ^b	MIC _{50/90}	0.031/0.13	4/4	2/8	32/32	>64/64	8	0.5/0.5	8/32	>32/32
	Range n	(≤0.002-0.13) 27	(0.063-8) 27	(0.25-8) 27	(4-32) 27	(32-64) 27	(64-128) 27	(0.063-1) 27	(4-32) 27	(1-32) 27
<i>Enterobacter cloacae</i>	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 n	(≤0.016-0.5) 36	(2-32) 36	(0.13-4) 36	(≤0.016-2) 36	(0.063-32) 36	(1-128) 36	(0.063-32) 36	(0.031-16) 36	(0.5-32) 36
<i>Escherichia coli</i>	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 n	(≤0.002-0.25) 253	(1-64) 253	(0.031-2) 253	(≤0.016-32) 253	(0.06-64) 253	>128 253	>32 253	(≤0.016-32) 253	(0.25-32) 177
<i>Escherichia coli</i> 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 n	(≤0.002-0.25) 101	(1-32) 101	(0.063-2) 101	(≤0.016-32) 101	(0.25-64) 101	(1-128) 101	(0.063-32) 101	(0.031-32) 101	(0.5-32) 101
<i>Klebsiella pneumoniae</i>	MIC _{50/90}	0.063/0.5	8/32	0.5/4	0.063/32	32/32	32/128	0.5/1	2/32	8/32
	Range n	(≤0.016-8) 235	(1-32) 235	(0.063-16) 235	(≤0.016-32) 235	(0.031-64) 235	(0.5-128) 235	(0.063-32) 235	(0.031-64) 235	(0.25-64) 198
<i>Klebsiella pneumoniae</i> 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	32/32	32/32
	Range n	(≤0.016-2) 143	(1-32) 143	(0.13-16) 143	(0.031-32) 143	(0.5-64) 143	(2-128) 143	>32 143	(0.031-64) 143	(0.25-64) 143
<i>Klebsiella pneumoniae</i> CP-R	MIC _{50/90}	0.063/0.25	16/32	1/2	32/32	>32/32	8	0.25/8	>32/32	32/32
	Range n	(≤0.016-2) 51	(2-32) 51	(0.25-16) 51	(0.063-32) 51	(2-32) 51	(4-128) 51	>32 51	(0.13-32) 51	(0.25-64) 51
<i>Proteus mirabilis</i>	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 n	(≤0.016-1) 51	(8-64) 51	(0.5-16) 51	(≤0.016-1) 51	(≤0.016-4) 51	(≤0.5-2) 51	>32-32 51	(≤0.016-64) 51	(0.5-64) 51
<i>Pseudomonas aeruginosa</i>	MIC _{50/90}	8/16	>32/32	16/32	4/32	4/32	16/128	1/1	1/32	2/32
	Range n	(0.5-32) 75	(8-32) 75	(1-32) 75	(0.5-32) 75	(1-32) 75	(0.5-128) 75	(0.25-2) 75	(0.25-32) 75	(0.25-32) 75
<i>Stenotrophomonas maltophilia</i>	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 n	(≤0.016-4) 24	(1-32) 24	(0.031-4) 24	(2-32) 24	(2-32) 24	(8-128) 24	(0.5-32) 24	(0.13-32) 24	(2-32) 24

MIC and range values in μ g/mL. ^aminocycline was used for *A. baumannii*. ^b3rd Gen CepH-R: resistant to cefotaxime, ceftazidime or ceftiofloxacin as per CLSI interpretive criteria. ^cCP-R: resistant to imipenem, eropenem (Enterobacteriaceae-only), or meropenem as per CLSI interpretive criteria; ^dAG-R: resistant to gentamicin, amikacin or tobramycin as per CLSI interpretive criteria. ^eFQ-R: resistant to levofloxacin or ciprofloxacin as per CLSI interpretive criteria; ^fCarbapenem: imipenem, eropenem, or meropenem; ^g3rd Gen CepH: cefotaxime, ceftazidime or ceftiofloxacin; ^hfluoroquinolone: levofloxacin or ciprofloxacin; ⁱaminoglycoside: 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 GCT951	0.008	32	1	2	>32	>32
AB993	G5140, overexpressing adeA6	0.13	32	8	2	32	>32
AB994	G5139, overexpressing adeA6	0.13	32	8	2	32	>32
AB995	GCT951, knockout of adeB	0.016	>32	2	2	16	32

MIC values in μ g/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)
3 rd Gen CepH	>16/16	(0.5-32)

MIC, MIC_{50/90}. Range values in μ g/mL; 3rd Gen CepH: cefotaxime, ceftazidime or ceftiofloxacin; carbapenem: imipenem, eropenem, 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	3 rd Gen CepH ^c	Carbapenem ^b	Colistin	Fluoroquinolone ^d	Aminoglycoside ^e	
<i>Acinetobacter baumannii</i> tet(A)	MIC _{50/90}	NA/NA	NA/NA	NA/NA	NA/NA	NA/NA	NA/NA	NA/NA	
	Range n	(0.063-0.063) 3	(1-2) 3	(2-2) 3	(32-64) 3	(32-32) 3	(0.5-0.5) 3	(8-8) 3	(-32-32) 3
<i>Acinetobacter baumannii</i> 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 n	(0.004-0.13) 22	(1-8) 22	(0.5-8) 22	(32-64) 22	(32-32) 22	(0.063-2) 22	(0.063-2) 22	(4-32) 22
<i>Escherichia coli</i> 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 n	(≤0.016-0.13) 52	(-32-32) 52	(0.063-1) 52	(0.06-32) 52	(≤0.016-32) 52	(0.063-4) 52	(0.03-32) 52	(0.25-32) 52
<i>Escherichia coli</i> 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 n	(≤0.004-0.5) 78	(2-32) 78	(0.063-1) 78	(0.063-64) 78	(≤0.016-32) 78	(0.063-0.5) 78	(≤0.016-32) 78	(0.5-32) 78
<i>Escherichia coli</i> tet(D)	MIC _{50/90}	NA/NA	NA/NA	NA/NA	NA/NA	NA/NA	NA/NA	NA/NA	NA/NA
	Range n	(≤0.016-≤0.016) 3	(-32-32) 3	(0.13-0.25) 3	(0.25-32) 3	(≤0.016-0.063) 3	(0.13-0.25) 3	(0.031-32) 3	(4-32) 3
<i>Escherichia coli</i> tet(M)	MIC _{50/90}	NA/NA	NA/NA	NA/NA	NA/NA	NA/NA	NA/NA	NA/NA	NA/NA
	Range n	(≤0.016-0.13) 5	(-8-32) 5	(0.063-1) 5	(8-32) 5	(≤0.016-0.063) 5	(0.25-0.25) 5	(0.063-32) 5	(2-8) 5
<i>Klebsiella pneumoniae</i> tet(A)	MIC _{50/90}	0.13/1	>32/32	1/4	>32/32	0.13/16	0.25/0.25	8/32	>32/32
	Range n	(0.031-2) 41	(32-32) 41	(0.25-8) 41	(0.063-64) 41	(0.13-16) 41	(0.13-32) 41	(0.031-32) 41	(0.25-32) 41
<i>Klebsiella pneumoniae</i> tet(B)	MIC _{50/90}	NA/NA	NA/NA	NA/NA	NA/NA	NA/NA	NA/NA	NA/NA	NA/NA
	Range n	(≤0.016-0.13) 5	(4-32) 5	(0.063-0.5) 5	(0.06-32) 5	(0.031-4) 5	(0.13-0.5) 5	(0.063-4) 5	(0.5-32) 5
<i>Klebsiella pneumoniae</i> 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 n	(≤0.016-2) 37	(2-32) 37	(0.13-4) 37	(0.13-32) 37	(≤0.016-32) 37	(0.13-32) 37	(0.063-32) 37	(0.25-32) 37

MIC and range values in μ g/mL. ^aminocycline was used for *A. baumannii*; ^b3rd Gen CepH: cefotaxime, ceftazidime or ceftiofloxacin; ^ccarbapenem: imipenem, eropenem (Enterobacteriaceae only), or meropenem; ^dfluoroquinolone: levofloxacin or ciprofloxacin; ^eaminoglycoside: gentamicin, amikacin or tobramycin. NA, not available.

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

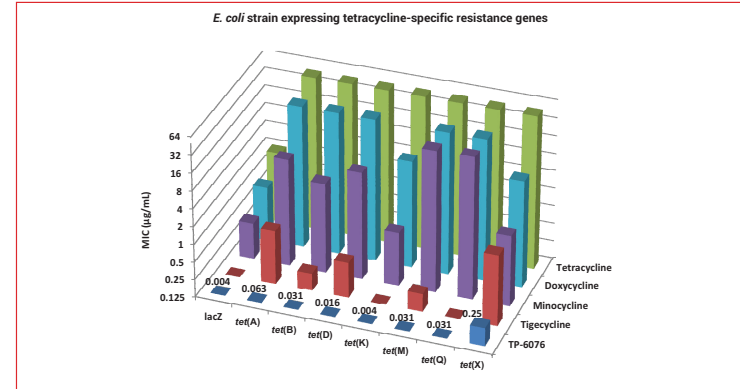


Table 5. *In Vitro* Antibacterial Activity of TP-6076 and Comparators against Molecularly Characterized *K. pneumoniae* Differentially Expressing *ramA* and Clinical Isolates Containing RamR Sequence Variations

Strain:	ramA on Chromosome			ramA on Plasmid		<i>K. pneumoniae</i> Clinical Isolates Containing RamR Sequence Variations (n=31)
	KP987	KP986	KP989	KP988	KP990	
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.					