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TP-6076 is Active against Carbapenem- and Polymyxin-resistant *Enterobacteriaceae* and *Acinetobacter baumannii* Isolates

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Abstract

TP-6076 is a novel, fully-synthetic antibiotic of the tetracycline class with *in vitro* activity against Gram-negative pathogens, including multidrug-resistant strains of *Acinetobacter baumannii* and *Enterobacteriaceae*. To further characterize its activity *in vitro*, TP-6076 was tested against new reference panels made available by the FDA and CDC for the testing of new antibacterial agents as well as various isolates expressing the polymyxin resistance gene *mcr*-1.

Using Clinical Laboratory Standards Institute methodology, minimal inhibitory concentration (MIC) values for antibiotics were determined for TP-6076 against isolates from the FDA-CDC Antimicrobial resistance bank, including the carbapenem breakpoint, carbapenemase detection, carbapenemase diversity, *Acinetobacter baumannii*, novel antibiotic resistance, and ceftolozane/tazobactam panels, and isolates expressing the *mcr*-1 polymyxin resistance gene.

The TP-6076 $MIC_{50/90}$ values and ranges against *Enterobacteriaceae* and *A. baumannii* isolates from all panels are shown in the Table. TP-6076 maintained an MIC_{90} value of less than or equal to 0.25 µg/mL against all *Enterobacteriaceae* and *Acinetobacter* isolates tested.

Panel	n	MIC_{50} (µg/mL)	MIC_{90} (µg/mL)	Range (µg/mL)
Breakpoint	31	0.031	0.25	0.004-0.5
Detection (ENT, AB subset)	68	0.063	0.5	0.004-1
Diversity	53	0.063	0.25	0.008-1
Acinetobacter baumannii	41	0.008	0.063	0.002-0.25
Novel Resistance	3	n/a	n/a	0.008-0.125
Ceftolozane/tazobactam (ENT subset)	20	0.016	0.25	0.002-0.5
***MCR*-1**	19	0.031	0.125	0.004-0.25

TP-6076 maintained potency against carbapenem-resistant *Enterobacteriaceae* and *A. baumannii* isolates across a wide diversity of resistance types.

Introduction

TP-6076 is a novel, fully-synthetic fluorocycline antibiotic of the tetracycline class in clinical development for treatment of infections caused by multidrug-resistant Gram-negative pathogens. TP-6076 has historically exhibited potent *in vitro* activity against Gram-negative pathogens, including extended-spectrum β-lactamase and carbapenemase-producing *Enterobacteriaceae* as well as multidrug-resistant strains of *Acinetobacter baumannii*. To further characterize this *in vitro* activity, TP-6076 was tested against reference panels made available by the FDA and CDC for the testing of new antibacterial agents, as well as a panel of *mcr*-1-positive *Enterobacteriaceae* from clinical and environmental sources.

Methods

Using Clinical and Laboratory Standards Institute (CLSI) methodology (1, 2), minimal inhibitory concentration (MIC) values for antibiotics were determined for isolates in the FDA-CDC Antimicrobial Resistance Isolate Bank panels: 1) *Enterobacteriaceae* Carbapenem Breakpoint Panel ("Breakpoint"), 2) Gram-Negative Carbapenemase Detection Panel ("Detection") 3) *Enterobacteriaceae* Carbapenemase Diversity Panel ("Diversity"), 4) *Acinetobacter baumannii* panel, 5) Novel resistance panel, 6) and a subset of *Enterobacteriaceae* from the ceftolozane-tazobactam panel. The Breakpoint panel contained 31 *Enterobacteriaceae* isolates, of which 7 were meropenem-resistant, including 5 bla_{KPC} isolates. The Detection panel was comprised of 80 isolates total; a 54 *Enterobacteriaceae* subset contained carbapenemase genes bla_{IMP} (n=2), bla_{KPC} (n=9), bla_{NDM} (n=10), bla_{OXA} (n=5), bla_{SME} (n=2), bla_{VIM} (n=3). The Diversity panel contained 53 ENT with carbapenemase genes bla_{IMI} (n=2), bla_{IMP} (n=1), bla_{KPC} (n=18), bla_{NDM} (n=20), bla_{OXA} (n=5), bla_{SME} (n=6), and bla_{VIM} (n=2). There were 55 *A. baumannii* isolates across all panels, of which 4 isolates expressed bla_{NDM} and 49 expressed bla_{OXA} genes. The novel resistance panel was comprised of two isolates carrying the *mcr*-1 polymyxin resistance gene and one bla_{KPC}-positive isolate resistant to ceftazidime-avibactam. Additional *mcr*-1 positive isolates were obtained from the laboratory of Dr. Patrice Nordmann (University of Fribourg).

Beta-lactam antibiotic resistance classifications are listed as reported by the CDC Antimicrobial Resistance Bank. Isolates with tetracycline resistance were screened for the presence of tetracycline-specific resistance genes by PCR using published methodology at Tetraphase Pharmaceuticals (3, 4). Further, isolates of *Klebsiella pneumoniae* with elevated tigecycline and/or tetracycline-class antibiotic MIC values were screened for sequence variations in genes known to be associated with tigecycline non-susceptibility, namely the regulatory genes *ramR*, *acrR*, and *oqxR*, and *rpsJ*, which encode ribosomal protein S10 (5 – 11).

Results

Table 1. Activity of TP-6076 and Comparators Against Gram-Negative Isolates from the CDC Antimicrobial Resistance Bank

Panel	Carbapenem Breakpoint (N = 31) MIC 50/90 (Range)	Carbapenemase Detection (including *P.aeruginosa*) (N = 80) MIC 50/90 (Range)	Carbapenemase Detection (*no P.aeruginosa*) (N = 68) MIC 50/90 (Range)	Carbapenemase Detection (*Enterobacteriaceae* only) (N = 54) MIC 50/90 (Range)	Carbapenemase Diversity (N = 53) MIC 50/90 (Range)	*Acinetobacter baumannii* (N = 41) MIC 50/90 (Range)	Novel Resistance (N = 3) MIC 50/90 (Range)	All *Enterobacteriaceae* (N = 141) MIC 50/90 (Range)	All Acinetobacter (N = 55) MIC 50/90 (Range)
TP-6076	0.031/0.25 (0.0039-0.5)	0.063/8 (0.004-8)	0.063/0.5 (0.004-1)	0.063/0.5 (0.004-1)	0.063/0.25 (0.008-1)	0.008/0.063 (0.001-0.25)	n/a (0.008-0.125)	0.063/0.5 (0.0039-1)	0.016/0.063 (0.001-0.25)
Tigecycline	0.25/1 (0.031-4)	1/32 (0.063->32)	0.5/4 (0.063-8)	1/4 (0.063-8)	0.5/2 (0.063-8)	2/4 (0.5-8)	n/a (0.125-4)	0.5/2 (0.031-8)	2/4 (0.25-8)
Minocycline	8/32 (0.5->32)	8/>32 (≤0.016->32)	4/>32 (≤0.016->32)	8/>32 (0.063->32)	4/>32 (0.5->32)	8/16 (0.125-32)	n/a (4-32)	8/>32 (0.5->32)	8/16 (0.016-32)
Tetracycline	8/>32 (0.5->32)	>32 (2->32)	16/>32 (2->32)	16/>32 (2->32)	16/>32 (0.5->32)	>32 (4->32)	n/a (>32)	16/>32 (0.5->32)	>32 (2->32)
Meropenem	0.063/32 (≤0.016->32)	16/>32 (≤0.016->32)	8/>32 (≤0.016->32)	4/>32 (≤0.016->32)	>32 (1->32)	>32 (4->32)	n/a (0.031->32)	8/>32 (≤0.016->32)	>32 (2->32)
Ceftazidime	1/>32 (0.125->32)	>32 (0.25->32)	>32 (0.25->32)	>32 (0.25->32)	>32 (0.063->32)	>32 (>32)	n/a (32->32)	>32 (0.063->32)	>32 (16->32)
Cefotaxime	0.5/>32 (0.031->32)	>32 (0.063->32)	>32 (0.063->32)	>32 (0.063->32)	>32 (0.125->32)	>32 (>32)	n/a (>32)	>32 (0.031->32)	>32 (>32)
Cefepime	0.5/>32 (0.063->32)	>32 (0.125->32)	>32 (0.125->32)	>32 (0.125->32)	>32 (0.125->32)	>32 (>32)	n/a (>32)	>32 (0.063->32)	>32 (>32)
Colistin	0.125/1 (0.063->32)	0.25/>32 (0.063->32)	0.25/>32 (0.063->32)	0.25/>32 (0.063->32)	0.25/>32 (0.125->32)	0.5/4 (0.125->32)	n/a (0.125-8)	0.25/>32 (0.063->32)	0.5/4 (0.063->32)
Gentamicin	1/>32 (0.25->32)	>32 (0.125->32)	>32 (0.125->32)	>32 (0.125->32)	16/>32 (0.25->32)	>32 (8->32)	n/a (4->32)	8/>32 (0.125->32)	>32 (8->32)
Levofloxacin	1/>32 (0.031->32)	32/>32 (0.031->32)	16/>32 (0.031->32)	16/>32 (0.031->32)	32/>32 (0.031->32)	16/>32 (4->32)	n/a (16->32)	16/>32 (0.031->32)	16/>32 (4->32)
Piperacillin-tazobactam	16/4 / >128/4 (1/4->128/4)	>128/4 (2/4->128/4)	>128/4 (2/4->128/4)	>128/4 (2/4->128/4)	>128/4 (1/4->128/4)	>128/4 (128/4->128/4)	n/a (2/4->128/4)	>128/4 (1/4->128/4)	>128/4 (128/4->128/4)
Aztreonam	4/>32 (≤0.016->32)	>32 (≤0.016->32)	>32 (≤0.016->32)	>32 (≤0.016->32)	>32 (≤0.016->32)	>32 (>32)	n/a (>32)	>32 (≤0.016->32)	>32 >32
Trimethoprim-sulfamethoxazole	0.5/9.5 / >8/152 (0.063/1.19->8/152)	>8/152 (0.063/1.19->8/152)	>8/152 (0.063/1.19->8/152)	>8/152 (0.063/1.19->8/152)	>8/152 (0.125/2.38->8/152)	>8/152 1/19->8/152	n/a (>8/152)	>8/152 (0.063/1.19->8/152)	>8/152 1/19->8/152

$MIC_{50/90}$ (µg/mL) and Range values (µg/mL) for TP-6076 and comparators against CDC Antimicrobial Resistance Bank panels. Data in "All *Enterobacteriaceae*" and "All *A. baumannii*" columns include all isolates of the given classifications across all CDC panels.

Overall, TP-6076 maintained potency against isolates of *Enterobacteriaceae* and *A. baumannii*. The $MIC_{50/90}$ values for TP-6076 against the Breakpoint and Diversity panels were 0.031/0.25 µg/ml and 0.063/0.25 µg/ml. Against the Detection panel TP-6076 maintained an $MIC_{50/90}$ of 0.063/0.5 µg/ml against isolates of *Enterobacteriaceae* alone and against the total pool of *Enterobacteriaceae* and *A. baumannii* isolates, however, TP-6076 MIC values against the *P. aeruginosa* isolates (n=12) increased the overall TP-6076 MIC_{90} to 8 µg/ml. Against the sum total of 141 *Enterobacteriaceae* and 55 *A. baumannii* TP-6076 maintained an $MIC_{50/90}$ of 0.063/0.5 and 0.016/0.063 µg/ml, respectively.

Table 2. Activity of TP-6076 and Comparators against *Enterobacteriaceae* with Defined Beta-Lactam and Tetracycline Resistance Mechanisms

	N=	TP-6076 MIC 50/90 µg/mL (Range)	Tigecycline MIC 50/90 µg/mL (Range)	Tetracycline MIC 50/90 µg/mL (Range)	Meropenem MIC 50/90 µg/mL (Range)
bla_{NDM}	30	0.063/0.5 (0.004-1)	1/4 (0.063-4)	>32 (0.5->32)	>32 (4->32)
bla_{KPC}	33	0.063/0.25 (0.004-1)	0.5/4 (0.063-8)	8/>32 (0.5->32)	8/>32 (0.25->32)
bla_{CTXM}	9	n/a (0.0078-1)	n/a (0.125-8)	n/a (4->32)	n/a (0.031->32)
bla_{OXA}	10	0.063/0.5 (0.031-0.5)	0.5/2 (0.5-2)	>32 (2->32)	2/16 (0.5->32)
bla_{TEM}	7	n/a (0.016-0.125)	n/a (0.125-1)	n/a (4->32)	n/a (0.031-8)
bla_{SHV}	4	n/a (0.016-0.5)	n/a (0.25-2)	n/a (4->32)	n/a (0.25-8)
bla_{TMA}	1	n/a (0.063)	n/a (0.5)	n/a (>32)	n/a (16)
bla_{CMY}	4	n/a (0.004-0.016)	n/a (0.063-0.25)	n/a (4->32)	n/a (0.031-0.5)
cAmpC	5	n/a (0.031-0.5)	n/a (0.25-2)	n/a (4->32)	n/a (≤0.016-0.5)
Porin-Loss	5	n/a (0.016-0.063)	n/a (0.25-0.5)	n/a (4->32)	n/a (0.5-32)
bla_{VIM}	5	n/a (0.031-1)	n/a (0.25-8)	n/a (2->32)	n/a (4->32)
bla_{IMI}	2	n/a (0.008-0.016)	n/a (0.125)	n/a (4)	n/a (32->32)
bla_{IMP}	3	n/a (0.016-0.031)	n/a (0.25-0.5)	n/a (2)	n/a (4-8)
bla_{SME}	8	n/a (0.031-0.125)	n/a (0.5-1)	n/a (8-32)	n/a (32->32)
ramR, acrR, oqxR mutation	17	0.25/1 (0.016-1)	2/4 (0.25-8)	16/>32 (4->32)	>32 (0.031->32)
tet Gene	49	0.063/0.5 (0.004-1)	0.5/4 (0.063-8)	>32 (4->32)	8/>32 (≤0.016->32)

As shown in Table 2, the presence of various beta-lactamase resistance mechanisms did not impact the activity of TP-6076 against isolates of *Enterobacteriaceae*. Across all *Enterobacteriaceae* carrying the bla_{NDM} (n=30) and bla_{KPC} (n=33) genes, two major carbapenem-resistance mechanisms, TP-6076 maintained $MIC_{50/90}$ values of 0.063/0.5 and 0.063/0.25 µg/ml, respectively. Similarly, against a total of 49 isolates carrying one or more of the tetracycline-specific efflux pump genes *tet*(A), *tet*(B), *tet*(C), or *tet*(D), TP-6076 showed an $MIC_{50/90}$ of 0.063/0.5 µg/ml, while in isolates of *K. pneumoniae* carrying sequence variants of genes known to be associated with reduced susceptibility to tigecycline (n=17), TP-6076 showed only modestly reduced susceptibility with an $MIC_{50/90}$ of 0.25/1 µg/ml.

Table 3. Activity of TP-6076 and Comparators against *A. baumannii* with Defined Beta-Lactam and Tetracycline Resistance Mechanisms

	N	TP-6076 MIC50/90 µg/mL (Range)	Tigecycline MIC50/90 µg/mL (Range)	Tetracycline MIC50/90 µg/mL (Range)	Meropenem MIC50/90 µg/mL (Range)
bla_{NDM}	4	n/a (0.004-0.031)	n/a (0.25-1)	n/a (2->32)	n/a (>32)
bla_{OXA}	49	0.016/0.063 (0.004-0.25)	2/4 (0.25-8)	>32 (4->32)	>32 (2->32)
tet Gene	28	0.016/0.063 (0.004-0.25)	2/4 (0.25-8)	>32 (16->32)	>32 (4->32)

Beta-lactam resistance mechanisms did not impact the activity of TP-6076 against isolates of *A. baumannii* with MIC values ranging between 0.004 to 0.031 µg/ml for isolates carrying bla_{NDM} (n=4), and 0.004 to 0.25 µg/ml for isolates positive for bla_{OXA} (n=49) and/or tetracycline-specific resistance genes (n=28).

Table 4. Activity of TP-6076 and Comparators against *Enterobacteriaceae* Carrying the *mcr*-1 polymyxin Resistance Gene

N = 19	MIC 50/90 µg/mL Range
TP-6076	0.031/0.125 (0.004-0.25)
Tigecycline	0.25/1 (0.063-2)
Colistin	8/16 (1->16)
Polymyxin B	4/8 (0.5-8)
Gentamicin	2/>32 (1->32)
Aztreonam	0.25/>32 (0.125->32)
Levofloxacin	8/>32 (0.0625->32)
Meropenem	0.031/0.063 (≤0.016-0.063)
Cefepime	0.5/>32 (0.125->32)

Polymyxin resistance mechanisms did not impact the activity of TP-6076 against isolates from various species of *Enterobacteriaceae*, with TP-6076 maintaining an $MIC_{50/90}$ of 0.031/0.25 µg/ml against 19 isolates.

Conclusions

TP-6076 maintained potency against carbapenem-resistant *Enterobacteriaceae* and *A. baumannii* isolates, including those containing carbapenemase or *mcr*-1 genes prevalent in contemporary isolates.

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