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In Vitro Activity of Fluorocycline TP-434 Against Panels of Recent Bacterial Clinical Isolates

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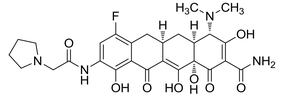
Revised Abstract

Background: TP-434 is a novel fluorocycline antibiotic being developed by Tetraphase Pharmaceuticals. TP-434 and multiple comparator agents were tested against panels of recent clinical strains of aerobic, facultative, and anaerobic gram-negative and gram-positive pathogens primarily from the United States.
Methods: The activity of the compounds was assessed using standard CLSI methodology.
Results: Panels of recent clinical isolates were tested, with most isolates from blood, wound/abscess, sputum, and urine. TP-434 had MIC_{90} values ≤0.5 µg/ml against all gram-positive isolates; all gram-positive pathogens were susceptible to comparators linezolid, vancomycin, daptomycin, and tigecycycline. Resistance to levofloxacin was 7% in MSSA, 83% in MRSA, 45% in CA-MRSA, 23% in MSSE, 80% in MRSE, 39-80% in VSE, 100% in VRE, 2.4% in pneumococci, and 0% in streptococci. TP-434 had MIC_{90} values ≤ 1 µg/ml against *Bacteroides fragilis*, *B. vulgatus*, *Peptoniphilus asaccharolyticus*, *Peptostreptococcus micros*, *Peptostreptococcus anaerobius*, and *Clostridium difficile*. The $MIC_{50/90}$ values for *B. thetaiotaomicron* and *B. ovatus* were 1/4, 0.25/1 for *Prevotella* spp., and 1/2 for *C. perfringens*, with a 2-and 4-fold advantage versus tigecycline against *C. perfringens* and *Bacteroides* spp., respectively. Comparators imipenem, metronidazole, and vancomycin had MIC_{90} ranges of 0.03->16, 2->16, and 0.5->16 µg/ml, respectively, against all anaerobes. TP-434 had MIC_{90} values against *E. coli* (including ESβL-producing isolates), *Acinetobacter lwoffii, Enterobacter aerogenes, Salmonella* spp., *Shigella* spp., M. catarrhalis, and *H. influenzae* of ≤0.5 µg/ml, MIC_{90} values of 1 µg/ml against ESβL-and carbapenem-resistant isolates of *K. pneumoniae*, *K. oxytoca, S. marcescens,* and *S. maltophilia*, and MIC_{90} values of 2 µg/ml against other multidrug-resistant gram-negatives, excepting *Pseudomonas aeruginosa* and *Proteus mirabilis*.
Conclusion: TP-434, a broad-spectrum antibiotic, has the *in vitro* potency to potentially become an important part of the antibiotic armamentarium.

Background

TP-434, a novel fully synthetic tetracycline-class antibiotic, was designed to have a broad antibacterial spectrum with potent activity against problematic multidrug-resistant gram-negative bacteria. It provides coverage of all gram-positive pathogens, including methicillin-resistant *Staphylococcus aureus*, streptococci, and vancomycin-resistant enterococci. TP-434 is unaffected by resistance mechanisms to other antibiotics and is specifically active against tetracycline-resistant strains (see posters F1-2155, F1-2157, F1-2160). TP-434 shows efficacy at low doses in animal models of infection, including those challenged with gram-negatives intraperitoneally (see poster F1-2161). The potency, spectrum, and tissue distribution (see posters A1-027, A1-028) of TP-434 provides an opportunity for treating serious hospital infections empirically including intra-abdominal , skin and respiratory infections.

TP-434



Methods

TP-434 was tested against panels of recent clinical aerobic and anaerobic isolates, including quality control strains according to methods published by Clinical and Laboratory Standards Institute (CLSI) (1, 2). Recent clinical isolate collections include strains from Micromyx (Kalamazoo, MI), Eurofins Medinet (Chantilly, VA) and IHMA (Schaumburg, IL). PCR-characterization of extended spectrum β-lactamases was done at IHMA or by standard PCR methodology at Tetraphase Pharmaceuticals using published primers (3).

References

1) Clinical and Laboratory Standards Institute(CLSI). *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; Approved Standard—Eighth Edition. CLSI document M07-A8 [ISBN 1-56238-689-1]. Clinical and Laboratory Standards Institute, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898 USA, 2009.*
2) Clinical and Laboratory Standards Institute (CLSI). *Methods for Antimicrobial Susceptibility Testing of Anaerobic Bacteria; Approved Standard—Seventh Edition. CLSI document M11-A7 [ISBN 1-56238-626-3]. Clinical and Laboratory Standards Institute, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898 USA, 2007.*
3) Dallenne, C., A. Da Costa, D. Decre, C. Favier, G. Arlet. 2010. *Development of a set of multiplex PCR assays for the detection of genes encoding important β-lactamases in Enterobacteriaceae.* J. Antimicrob. Chemother. 65:490-495.

Results

Table 1. Activity of TP-434 and comparators against gram-negative pathogens

Organism	N	MIC range (µg/ml) MIC$_{50}$/MIC$_{90}$ (µg/ml)						
		TP-434	Carbapenem	Fluoroquinolone	3rd Gen Ceph	Gentamicin	Piperacillin/ Tazobactam	Tigecycline
Escherichia coli	142	≤0.016-2 0.25/0.5	0.06-8 0.5/≤1	0.008->32 >2/32	0.03->64 >16/>64	≤0.25->32 2/>32	≤0.5->128 4/>64	0.06->8 0.25/1
ESβL+ *Escherichia coli*	97	0.03-2 0.25/0.5	0.06-8 ≤1/1	0.03->32 >2/32	0.25->64 >32/>64	≤0.25->32 4/>32	≤0.5->128 8/>128	0.06->8 0.25/2
Klebsiella pneumoniae	208	0.13-16 0.5/2	0.063->32 0.5/16	≤0.016->32 1/>32	≤0.016->64 32/>32	≤0.25->32 4/>32	1->128 8/>128	0.13-16 0.5/4
ESβL+ *Klebsiella pneumoniae*	91	0.13-8 0.5/1	0.13->32 ≤1/32	0.03->32 8/>32	0.13->64 >32/>64	≤0.25->32 >8/>32	2->128 >64/>128	0.13-8 1/4
Carbapenem-resistant *K. pneumoniae*	19	0.13-16 0.5/1	4->32 32/>32	4->32 >32/>32	32->32 >32/>32	2->32 16/>32	>128->128 >128/>128	0.25-16 1/1
Klebsiella oxytoca	41	0.03-2 0.25/1	≤1-≤1 ≤1/≤1	≤0.25->4 ≤0.25/>4	≤0.5->32 ≤0.5/>32	≤0.25->32 0.5/>32	≤0.5->64[a] 2/8	0.06-4 0.5/2
Proteus mirabilis	68	0.5-8 2/4	2->32 4/32	0.02-32[b] 0.063/>2	≤0.02-64 ≤0.02/4	0.5->64 2/16	≤0.13-8[c] 0.25/2	1-16 4/8
Providencia stuartii	50	0.12-8 1/2	0.25-16 2/4	0.02->2 >2/>2	≤0.02->16 0.25/16	0.5->32 4/>32	≤0.02->16 4/>128	0.06-16 1/2
Proteus vulgaris	29	0.5-2 1 /2	≤1-≤1 ≤1/≤1	≤0.25-1 ≤0.25/1	0.25->64 ≤0.25/>64	≤0.25->8 1/>8	≤0.5-4 ≤0.5/2	0.5-4 2/4
Morganella morganii	30	0.5-2 1/2	≤1-≤1 ≤1/≤1	≤0.25->4 ≤0.25/4	≤0.5-16 ≤0.5/4	0.5->8 1/>8	≤0.5-2 ≤0.5/1	0.25-8 2/4
Serratia marcescens	30	0.5-2 1/1	≤1-≤1 ≤1/≤1	≤0.25->4 ≤0.25/2	≤0.5->64 ≤0.5/>0.5	0.5-8 0.5/1	1-32 2/8	0.5-2 1/2
Citrobacter freundii	50	0.12-2 0.5/2	0.12->32 1/8	0.008->2 0.06/>2	0.06->16 4/>16	0.25->32 5/>32	0.25->128 16/>128	0.12-8 0.5/2
Enterobacter cloacae	134	0.03-4 0.5/2	0.06-32 0.5/4	0.008->32 0.25/>4	0.03->64[d] >16/>64	≤0.25->32 0.5/>8	0.5->128[e] >64/>128	0.06-8 0.5/4
Enterobacter aerogenes	30	0.25-2 0.25/0.25	≤1-2 ≤1/≤1	≤0.25-0.5 ≤0.25/≤0.25	≤0.5->64 ≤0.5/>16	≤0.25-1 ≤0.25/0.5	≤0.5->64 2/16	0.25-4 0.5/0.5
Salmonella spp.	30	0.12-0.5 0.25/0.25	≤1-8 ≤1/≤1	≤0.25->4 ≤0.25/≤0.25	≤0.5-≤0.5 ≤0.5/≤0.5	≤0.25->8 0.5/1	1-64 2/4	0.12-1 0.25/0.5
Shigella spp.	30	0.06-1 0.12/0.5	≤1-≤1 ≤1/≤1	≤0.25-1 ≤0.25/0.5	≤0.5-2 ≤0.5/≤0.5	≤0.25->8 1/1	≤0.5-4 2/2	0.12-1 0.25/0.5
Stenotrophomonas maltophilia	29	0.12-4 0.25/1	8->8 >8/>8	≤0.25->4 ≤0.25/>4	1->64 8/>64	≤0.25->8 >8/>8	8->64 32/>64	0.25-8 0.5/2
Acinetobacter lwoffii	29	0.03-0.25 0.12/0.25	≤1->8 ≤1/4	≤0.25-2 ≤0.25/≤0.25	≤0.5->64 1/16	≤0.25-8 ≤0.25/1	≤0.5-16 ≤0.5/8	0.06-0.5 0.12/0.5
Acinetobacter baumannii	89	≤0.016-4 0.5/2	0.12->32 1/>32	0.02->32 8/>16	0.12->16[f] >16/>16	0.5->32[h] 32/>32	1->128[i] >128/>128	≤0.016-8 1/4
Pseudomonas aeruginosa	88	1->64 8/16	0.12->32 1/16	0.06->2 025/>2	1->16 >16/>16	0.12->32 2/16[h]	1->128 8/>128[i]	1->16 16/>16
Haemophilus influenzae	15	0.06-0.25 0.13/0.2	ND[j]	≤0.016-0.13 ≤0.016/0.03	≤0.016-0.6 ≤0.016/≤0.016	ND	ND	0.13-1 0.5/0.5
Moraxella catarrhalis	15	≤0.016-0.063 ≤0.016/0.063	ND	0.03-0.25 -/-63/0.063	ND	ND	ND	≤0.016-0.063 0.03/0.063
Legionella pneumophila	70	0.016-2 1/2	ND	ND	ND	ND	ND	ND

Green boxes indicate that the MIC_{90} of TP-434 < MIC_{90} of tigecycline
Carbapenem = meropenem, ertapenem, or imipenem; Fluoroquinolone = levofloxacin or ciprofloxacin; 3rd Gen Ceph = third generation cephalosporin (either cefotaxime or ceftazidime); Piperacillin/tazobactam (only the piperacillin MIC is shown in the presence of 4 µg/ml of tazobactam); ESβL+ = extended spectrum β-lactamase producing isolates
[a]30 isolates tested against PTZ;
[b]55 tested with ciprofloxacin; 13 with levofloxacin;
[c]55 isolates tested with PTZ; 13 not tested;
[d]97 isolates tested, 68 isolates tested with cefotaxime and 29 isolates tested with ceftazidime;
[e]81 isolates tested; [f]29 isolates tested with cefotaxime and 60 isolates not tested; [g]29 isolates tested with PTZ and 60 not tested; [h]36 isolates tested with tobramycin and 52 isolates tested with gentamicin; [i]52 isolates with PTZ and 36 isolates not tested; [j]ND = Not done

Table 2. Activity of TP-434 and comparators against anaerobic pathogens

Organism	N	MIC range (µg/ml) MIC$_{50}$/MIC$_{90}$ (µg/ml)				
		TP-434	Imipenem	Metronidazole	Vancomycin	Tigecycline
Bacteroides fragilis	10	0.12-1 0.25/0.5	0.12-1 0.12/1	0.5- >16 1/2	16- >16 >16/>16	0.12-4 0.5/2
Bacteroide fragilis[a]	20	0.12-2 0.5/1	ND	0.25-1 1/1	ND	0.25-8 1/4
Bacteroides vulgatus	10	0.12-1 0.25/0.25	0.25-1 0.25/0.5	0.5-1 0.5/1	16->16 >16/>16	0.12-4 0.5/0.5
Bacteroides thetaiotaomicron	10	0.12-4 1/4	0.12-4 0.25/1	0.5->16 1/2	16->16 >16/>16	0.25-16 4/16
Bacteroides ovatus	10	0.015-8 1/4	0.03- >16 0.25/1	0.12->16 1/2	8- >16 >16/>16	0.06-32 0.5/16
Porphyromonas asaccharolytica	10	0.015-0.12 0.03/0.06	≤0.008-0.03 0.015/0.03	0.5-4 1/2	0.12-1 0.25/0.5	0.03-0.12 0.06/0.06
Prevotella spp.[b]	20	0.03-1 0.25/1	≤0.008- >16 0.06/0.5	≤0.008- >16 1/>16	1->16 >16/>16	0.06-4 0.5/1
Prevotella bivia	10	0.12-1 1/1	ND	1-4 2/4	ND	0.03-2 1/2
Prevotella buccae	10	0.03-0.12 0.06/0.12	ND	0.25-1 0.5/1	ND	0.06/0.25 0.12/0.12
Prevotella disiens	10	0.06-0.25 0.12/0.25	ND	0.5-2 1/1	ND	0.12-0.5 0.25/0.5
Prevotella intermedia	10	0.03-0.12 0.06/0.12	ND	0.25-1 0.5/0.5	ND	0.12-0.25 0.25/0.25
Prevotella melaninogenica	5	0.12-1	ND	0.25-1	ND	0.12-1
Peptostreptococcus anaerobius	10	0.015-0.25 0.06/0.25	0.03-1 0.06/1	0.25-2 1/2	0.5->16 0.5/2	0.015-0.5 0.06/0.25
Peptostreptococcus micros	10	0.015-0.5 0.015/0.25	≤0.008-0.03 0.015/0.03	≤0.008- >16 0.25/>16	0.5-2 1/1	0.015-1 0.03/0.25
Clostridium perfringens	10	0.5-2 1/2	0.06-1 0.12/0.5	2- >16 4/16	0.5->16 1/>16	0.12-8 1/4
Clostridium difficile	10	0.03-0.12 0.06/0.12	0.25-8 4/8	0.5-2 1/1	0.5-4 1/2	0.06-0.12 0.12/0.12
Anaerococcus spp.[c]	10	0.03-0.25 0.12/0.12	ND	0.5-4 2/2	ND	0.06-0.25 0.12/0.25
Actinomyces spp.[d]	5	0.25	ND	4->16	ND	0.25-0.5
Finegoldia magna	10	0.12-0.5 0.25/0.5	ND	≤0.12-1 0.5/1	ND	0.12-0.25 0.25/0.25
Fusobacterium spp.[e]	5	0.12-0.25	ND	≤0.12	ND	0.12-0.5
Parabacteroides distasonis	10	0.25-1 0.5/1	ND	0.5-1 1/1	ND	0.25-4 1/2
Peptoniphilus asaccharolyticus	10	0.03-0.12 0.06/0.12	ND	0.5-2 1/2	ND	0.06-0.25 0.12/0.25
Propionibacterium acnes	5	0.12	ND	>16	ND	0.12

Green boxes indicate that the MIC_{90} of TP-434 < MIC_{90} of tigecycline
[a] All known to be cefinase positive.
[b] 8 *P. melaninogenica*, 1 *P. corporis*, 1 *P. denticola*, 2 *P. disiens*, 3 P. bivia, 5 *Prevotella* spp.
[c] 5 *A. prevotii* and 5 *A. tetradius* isolates
[d] 2 *A. georgiae*, 2 *A. odontolyticus*, and 1 *A. meyeri*
[e] 3 *F. mortiferum* and 2 *F. varium*

Table 3. Activity of TP-434 and comparators against gram-positive pathogens

Organism[a]	N	MIC range (µg/ml) MIC$_{50}$/MIC$_{90}$ (µg/ml)					
		TP-434	Linezolid	Daptomycin	Vancomycin	Levofloxacin	Tigecycline
MSSA	56	0.12-0.25 0.25/0.25	2-4 4/4	0.5-1 1/1	0.5-2 1/1	0.12->32 1/1	0.12-0.25 0.12/0.25
MRSA	107	0.06-0.5 0.06/0.12	2-4 2/4	0.5-1 1/1	0.5-1 1/1	0.12->32 8/>32	0.06-0.5 0.12/0.25
MRSA PVL+	30	≤0.015-0.03 0.03/0.03	1-2 1/1	0.5-1 0.5/0.5	1-1 1/1	0.25->2 0.25/>2	0.06-0.12 0.12/0.12
MSSE	52	0.06-1 0.12/0.5	1-2 2/2	0.5-2 1/1	1-2 1/2	0.12->32 0.25/16	0.06-0.5 0.12/0.25
MRSE	54	0.06-1 0.06/0.5	1-2 2/2	0.25-2 1/1	0.5-2 2/2	0.25->32 8/>32	0.06-0.5 0.12/0.5
Streptococcus pneumoniae	82	0.008-0.03 0.015/0.03	0.5-2 1/1	0.25-2 0.25/0.5	0.12-0.5 0.25/0.5	0.5-8 1/2	≤0.015-0.12 0.03/0.03
Streptococcus anginosus	20	≤0.008-0.03 0.015/0.015	≤0.25-1 0.5/1	≤0.03-0.5 0.25/0.25	≤0.06-1 0.5/0.5	≤0.25-2 0.5/0.5	≤0.008-0.06 0.03/0.06
Streptococcus intermedius	30	0.015-0.06 0.015/0.06	≤0.25-1 1/1	≤0.03->1 0.5/1	≤0.06-0.5 0.5/0.5	≤0.25->4 1/2	≤0.008-0.25 0.03/0.12
Streptococcus mitis	29	0.015-0.06 0.015/0.06	0.5-1 1/1	0.5-1 0.5/1	≤0.06-0.5 0.5/0.5	0.5-2 1/2	≤0.008-0.25 0.03/0.12
Streptococcus sanguis	18	≤0.008-0.06 0.015/0.03	≤0.25-1 0.5/1	≤0.03-1 0.5/1	≤0.06-1 0.5/1	≤0.25-2 0.5/2	≤0.008-0.12 0.03/0.06
Streptococcus pyogenes	54	0.015-0.12 0.03/0.03	1-2 2/2	≤0.06-0.25 0.12/0.12	0.25-0.5 0.25/0.5	0.25-2 0.5/1	≤0.015-0.12 0.03/0.06
Streptococcus agalactiae	44	0.03-0.06 0.06/0.06	1-2 2/2	0.5-1 0.5/0.5	0.25-0.5 0.5/0.5	0.5-1 1/1	0.03-0.12 0.06/0.12
VSE	102	0.03-0.5 0.06/0.12	1-4 2/2	0.5-8 2/8	0.5-4 1/2	0.25->32 >32/>32	0.03-0.25 0.12/0.12
VRE	88	0.03-0.12 0.06/0.12	1-8 2/4	0.5-16 2/8	>64 >64/>64	32->32 >32/>32	0.03-0.12 0.12/0.12

Green boxes indicate that the MIC_{90} of TP-434 < MIC_{90} of tigecycline
[a]MSSA=methicillin-susceptible *S. aureus*;
MRSA=methicillin-resistant *S. aureus*;
MRSA PVL+= MRSA isolates confirmed to contain Panton-Valentine leukocidin;
MSSE=methicillin-susceptible *Staphylococcus epidermidis*;
MRSE=methicillin-resistant *S. epidermidis*;
VSE=vancomycin-susceptible *E. faecalis* and *E. faecium*;
VRE=vancomycin-resistant *E. faecalis* and *E. faecium*.

Conclusions

- **TP-434 demonstrated potent, broad-spectrum gram-negative antibacterial activity, with MIC_{90} values generally lower than or equivalent to comparators.**
- **TP-434 was active against *Enterobacteriaceae* isolates expressing one or more of the major groups of ESBLs, including strains confirmed by PCR to contain genes encoding TEM, SHV, CTX-M, OXA, DHA, FOX, CMY, ACT β-lactamases and KPC carbapenemases.**
- **TP-434 was two-fold more active than tigecycline against a set of *A. baumannii* isolates that was 44% resistant to carbapenems, 53% resistant to tetracycline and 64% resistant to fluoroquinolones. Nearly every *A.baumannii* isolate was two-fold more susceptible to TP-434 than to tigecycline.**
- **TP-434 has excellent activity against a wide range of gram-positive and gram-negative anaerobes; vancomycin- and metronidazole-resistance had no impact on TP-434 susceptibility.**
- **TP-434, a broad-spectrum antibiotic, has the *in vitro* potency to potentially become an important part of the antibiotic armamentarium.**