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# TP-434 is a Novel Broad-Spectrum Fluorocycline

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## Abstract

**Background:** Semi-synthetic tetracyclines with C-9 modifications have been reported that overcome resistance by efflux and ribosomal protection. Using a total synthesis approach, systematic modification of the tetracycline D-ring led to the discovery of **TP-434**, a novel broad-spectrum fluorocycline.

**Method:** Compounds were synthesized *via* a tandem Michael-Dieckmann reaction with systematic modification of the tetracycline D-ring to optimize potency and pharmacology. Protein synthesis inhibition was confirmed in a coupled transcription/translation assay and antibacterial activities were evaluated using CLSI guidelines. *In vivo* efficacy after IV dosing was determined in a mouse systemic infection model challenged with ESBL$^+$ *E. coli* or in a neutropenic thigh model infected with *S. aureus* SA191 *tet*(M).

**Results:** Among the fluorocyclines, **TP-434** had the best overall properties. **TP-434** was an equipotent inhibitor of protein synthesis ± ribosomal protection (TetM). Molecular modeling of **TP-434** into the 30S ribosomal crystal structure indicates key binding interactions in the A-site.

| Compound | MIC (μg/mL) | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | SA101 | SA161 | SA158 | EF159 | SP160 | EC155 | EC133 | KP153 | KP457 | AB250 | PM112 |
| | ATCC 29213 | MRSA *tet*(M) | *tet*(K) | *tet*(M) | *tet*(M) | *tet*(A) | ESBL | *tet*(A) | ESBL | *tet*(B) | ATCC 35659 |
| TP-434 | 0.016 | 0.016 | 0.016 | 0.016 | 0.016 | 1 | 0.13 | 0.5 | 0.5 | 2 | 0.25 |
| Tetracycline | 0.5 | 32 | > 32 | > 32 | > 32 | > 32 | > 32 | > 32 | 4 | > 32 | 32 |
| Tigecycline | 0.063 | 0.13 | 0.063 | 0.063 | 0.016 | 0.5 | 0.13 | 1 | 1 | 4 | 1 |
| Levofloxacin | 0.13 | 16 | 0.25 | 2 | 0.5 | 1 | > 32 | 2 | 64 | 32 | 0.031 |

SA: *S. aureus*; SP: *S. pneumoniae*; EF: *E. faecium*; EC: *E. coli*; AB: *A. baumannii*; KP: *K. pneumoniae*; PM: *P. mirabilis*; MRSA: methicillin-resistant *S. aureus*; ESBL: extended-spectrum β-lactamase producing

The mouse septicemia $PD_{50}$ challenged with *E. coli* was 1.2 mg/kg for **TP-434** versus 3.5 mg/kg for tigecycline. In the *S. aureus* neutropenic thigh model, **TP-434** provided a 3 $\log_{10}$ reduction in bioburden at a dose of 3 mg/kg while tigecycline and vancomycin required 17 and 10 mg/kg, respectively.

**Conclusion:** The *in vitro* antibacterial activity against MDR gram-positive and gram-negative pathogens and the efficacy in established animal models of infection warrant development of **TP-434** as a single therapy treatment option.

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## Methods

**Bacterial Strains.** Strains with defined tetracycline-resistant mechanisms were obtained from M. Roberts (Univ. Washington, Seattle, WA). *E. coli* EC133 was obtained from CMI (Wilsonville, OR) or Micromyx (Kalamazoo, MI; *S. aureus* SA161).

***In vitro* Susceptibility.** Compounds were dissolved in water and assayed in microtiter plates according to CLSI standards.

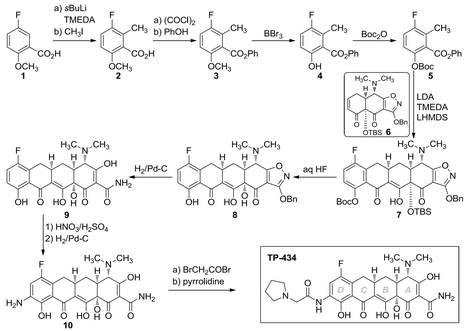
***In vitro* coupled *E. coli* transcription/translation assay.** Anti-translational activity ($IC_{50}$ values) was assessed in an *E. coli in vitro* coupled transcription/translation assay (TnT) with a firefly luciferase readout (Promega, Madison, WI). Purified TetM (2-3 μM) was added to TnT reactions to evaluate ribosome protection effects *in vitro*.

**Mouse Systemic Infection Studies.** Mice received treatment *via* intravenous (IV) injection 1 hour post intraperitoneal (IP) infection. At termination of study, percent survival was calculated and the dose (mg/kg) affecting 50% survival, the protective dose 50% ($PD_{50}$), was reported along with 95% confidence intervals as calculated by Probit analysis using GraphPad Prism version 4.03 (GraphPad Software).

**Mouse Thigh Infection.** Mice were rendered neutropenic through two consecutive IP injections of cyclophosphamide of 150 and 100 mg/kg on days -4 and -1, respectively. Mice were infected with approximately 5 x $10^5$ CFU/mL of bacteria in a 0.1 mL volume into the right thigh. At 1.5 hours post-infection mice received treatment *via* IV injection. One group of infected mice were euthanized and thighs processed for bacterial titers to serve as T = 0 controls. Twenty-four hours post-treatment, the remaining mice were euthanized, thighs aseptically removed, weighed, homogenized, serially diluted and CFUs per gram of thigh were calculated. The amount of test article required to achieve 1, 2, and 3 $\log_{10}$ reductions from 24 hour control thighs was determined.

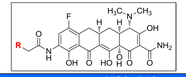
**Materials.** Fluorocycline **TP-434** was synthesized from the D-ring precursor **5** and the mechanisms were obtained from M. Roberts (Univ. Washington, bicyclic enone **6**[1] *via* a Michael-Dieckmann annulation according to Scheme 1. The prepared according to published procedures.[2] Other marketed antibiotics were from Sigma-Aldrich or USP.

### Scheme 1. Synthesis of TP-434




## Results

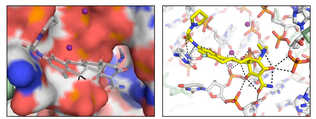
**Table 1. *In vitro* Activity of TP-434 and Analogs**



| ID | R | MIC (μg/mL) | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | SA101 ATCC 29213 | SA100 ATCC 13709 | SA161 MRSA *tet*(M) | SA158 *tet*(K) | EF103 ATCC 29212 | EF159 *tet*(M) | SP106 ATCC 49619 | SP160 *tet*(M) | EC107 ATCC 25922 | EC155 *tet*(A) | AB110 ATCC 19606 | PA111 ATCC 27853 | EC108 ATCC 13047 | KP109 ATCC 13883 | KP153 *tet*(A) |
| **TP-772** |  | 0.063 | 0.13 | 0.25 | 1 | 0.063 | 0.13 | 0.016 | 0.016 | 0.25 | 16 | 2 | 8 | 0.5 | 1 | 8 |
| **TP-435** |  | 1 | 1 | 2 | 4 | 2 | 4 | 0.5 | 1 | 16 | >32 | 2 | >32 | >32 | 32 | >32 |
| **TP-221** |  | 0.13 | 0.25 | 0.25 | 0.063 | 0.063 | 0.13 | 0.016 | 0.016 | 0.25 | 2 | 0.5 | 16 | 1 | 1 | 2 |
| **TP-715** |  | 4 | 4 | >32 | >32 | 8 | >32 | 0.25 | 2 | 16 | >32 | 8 | >32 | >32 | 32 | >32 |
| **TP-535** |  | 0.016 | 0.5 | 0.13 | 0.25 | 0.016 | 0.031 | 0.016 | 0.016 | 0.13 | 8 | 0.13 | 8 | 1 | 0.5 | 8 |
| **TP-434** |  | 0.016 | 0.016 | 0.016 | 0.016 | 0.016 | 0.016 | 0.016 | 0.016 | 0.016 | 1 | 0.031 | 8 | 0.13 | 0.13 | 0.5 |
| **TP-921** |  | 8 | 8 | 16 | 32 | 8 | 16 | 2 | 8 | >32 | >32 | >32 | >32 | >32 | >32 | >32 |
| **TP-561** |  | 0.5 | 0.5 | 1 | 0.5 | 0.25 | 0.5 | 0.016 | 0.016 | 1 | 4 | 0.13 | 32 | 4 | 2 | 4 |
| **TP-002** |  | 0.063 | 0.063 | 0.5 | 1 | 0.25 | 0.5 | 0.031 | 0.13 | 1 | >32 | 0.5 | 32 | 8 | 4 | 32 |
| **Tigecycline** | | 0.063 | 0.063 | 0.13 | 0.063 | 0.031 | 0.063 | 0.016 | 0.016 | 0.031 | 0.5 | 0.25 | 8 | 0.25 | 0.13 | 1 |

AB: *Acinetobacter baumannii*; EC: *Escherichia coli*; ECI: *Enterobacter cloacae*; EF: *Enterococcus faecalis*; KP: *Klebsiella pneumoniae*; PA: *Pseudomonas aeruginosa*; SA: *Staphylococcus aureus*; SP: *Streptococcus pneumoniae*.

**Figure 1. Molecular Modeling of TP-434 in the 30S Ribosomal A-site**



**TP-434** is docked into the binding pocket of the 30S ribosomal A-site, assuming a similar binding mode to tetracycline. The ball-and-stick model on the right pane illustrates potential hydrogen bonds and participation of one of the Mg cations (purple spheres) in the hydrogen-bonding network.

**Figure 2. TP-434 Inhibits Protein Synthesis in the Presence of Tet(M)**

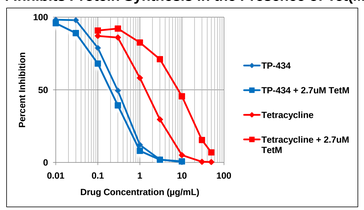



**Table 2. *In vitro* Activity of TP-434 and Comparators against *ESKAPE* Pathogens[3]**

| Organism | N | MIC range (μg/mL) MIC$_{50}$/MIC$_{90}$ (μg/mL) TP-434 | Carbapenem[a] | Fluoroquinolone[b] | 3rd Gen Ceph[c] | Gentamicin | Piperacillin/ Tazobactam | Tigecycline |
|---|---|---|---|---|---|---|---|---|
| ***Klebsiella pneumoniae*** | 208 | 0.13-16 | 0.063->32 | ≤0.016->32 | ≤0.016->64 | ≤0.25->32 | 1->128 | 0.13-16 |
| | | 0.5/2 | 0.5/16 | 1/>32 | 32/>32 | 4/>32 | 8/>128 | 0.5/4 |
| **ESβL$^+$ *Klebsiella pneumoniae*** | 91 | 0.13-8 | 0.03->32 | 0.03->32 | 0.13->64 | ≤0.25->32 | 2->128 | 0.13-8 |
| | | 0.5/1 | 0.5/>32 | 8/>32 | >32/>64 | >8/>32 | >64/>128 | 1/4 |
| **Carbapenem-resistant *K. pneumoniae*** | 19 | 0.13-16 | 4->32 | 4->32 | 32->32 | 2->32 | >128->128 | 0.25-16 |
| | | 0.5/1 | 32/>32 | >32/>32 | >32/>32 | 16/>32 | >128/>128 | 1/1 |
| ***Acinetobacter baumannii*** | 89 | ≤0.016-4 | 0.12->32 | 0.02->32 | 0.12->16 | 0.5->32 | 1->128 | ≤0.016-8 |
| | | 0.5/2 | 1/>32 | 8/>16 | >16/>16 | 32/>32 | >128/>128 | 1/4 |
| ***Pseudomonas aeruginosa*** | 88 | 1->64 | 0.12->32 | 0.06->2 | 1->16 | 0.12->32 | 1->128 | 1->16 |
| | | 8/16 | 1/16 | 025/>2 | >16/>16 | 2/16 | 8/>128 | 16/>16 |
| ***Enterobacter cloacae*** | 134 | 0.03-4 | 0.06-32 | 0.008->32 | 0.03->64 | ≤0.25->32 | 0.5->128 | 0.06-8 |
| | | 0.5/2 | 0.5/4 | 0.25/>4 | >16/>64 | 0.5/>8 | >64/>128 | 0.5/4 |
| ***Enterobacter aerogenes*** | 30 | 0.25-2 | ≤1-2 | ≤0.25-0.5 | ≤0.5->64 | ≤0.25-1 | ≤0.5->64 | 0.25-4 |
| | | 0.25/0.25 | ≤1/≤1 | ≤0.25/≤0.25 | ≤0.5/16 | ≤0.25/0.5 | 2/16 | 0.5/0.5 |

**Green boxes indicate that the MIC$_{90}$ of TP-434 < MIC$_{90}$ of tigecycline**
[a]meropenem, ertapenem, imipenem; [b]levofloxacin, ciprofloxacin; [c]cetazidime, ceftriaxone; ESβL$^+$ = extended spectrum β-lactamase producing isolates

**Table 3. *In vitro* Activity of TP-434 and Comparators against *ESKAPE* Pathogens[3] *-continued***

| Organism | N | MIC range (μg/mL) MIC$_{50}$/MIC$_{90}$ μg/mL) TP-434 | Linezolid | Daptomycin | Vancomycin | Levofloxacin | Tigecycline |
|---|---|---|---|---|---|---|---|
| ***E. faecium* Vancomycin-Susceptible** | 51 | 0.03-0.5 | 1-4 | 2-8 | 0.5-2 | 0.25->32 | 0.03-0.25 |
| | | 0.06/0.12 | 2/2 | 4/8 | 1/1 | >32/>32 | 0.06/0.12 |
| ***E. faecium* Vancomycin-Resistant** | 43 | 0.03-0.12 | 2-4 | 2-16 | >64->64 | >32->32 | 0.03-0.12 |
| | | 0.06/0.06 | 4/4 | 8/16 | >64->64 | >32/>32 | 0.06/0.06 |
| **MRSA** | 137 | ≤0.015-0.5 | 1-4 | 0.5-1 | 0.5-1 | 0.12->32 | 0.06-0.5 |
| | | 0.06/0.12 | 2/4 | 1/1 | 1/1 | >32/>32 | 0.12/0.12 |

**Figure 3. Activity of TP-434 in a Murine Septicemia Model Challenged with Extended-spectrum β-lactamase (SHV) Producing *E. coli* EC133 *tet*(B)**

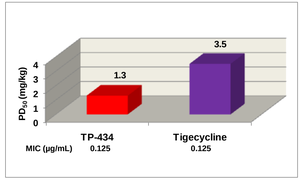
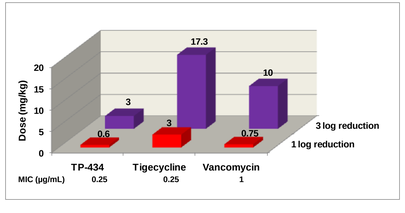



**Figure 4. Activity of TP-434 in a Neuropenic Thigh Model Challenged with MRSA SA191 *tet*(M)**




## Conclusions

- **TP-434 belongs to a diverse collection of novel tetracyclines generated by Tetraphase's fully synthetic platform and is the first fluorocycline selected for development as a broad spectrum antibacterial agent**
- **TP-434 is an equipotent inhibitor of protein synthesis in the presence or absence of Tet(M)**
- **TP-434 is broadly active against all ESKAPE pathogens (except *Pseudomonas*), including ESBL-producing and carbapenem-resistant *Enterobacteriaceae***
- **TP-434 is currently undergoing clinical trials**

## References

1) M.G. Charest, C.D. Lerner, J.D. Brubaker, D.R. Siegel, A.G. Myers, *Science*, **308**, 395 (2005).
2) P.-E. Sum, V.J. Lee, R.T. Testa, J.J. Hlavka, G.A. Ellestad, J.D. Bloom, Y. Gluzman, F.P. Tally, *J. Med. Chem.*, **37**, 184 (1994).
3) L.B. Rice, *J. Infect.* Dis., **197**, 1079 (2008).