

Target- and Resistance-Based Mechanistic Studies with TP-434, a Novel Fluorocycline Antibiotic

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TP-434 is a novel, broad-spectrum fluorocycline antibiotic with activity against bacteria expressing major antibiotic resistance mechanisms, including tetracycline-specific efflux and ribosomal protection. The mechanism of action of TP-434 was assessed using both cell-based and *in vitro* assays. In *Escherichia coli* cells expressing recombinant tetracycline resistance genes, the MIC of TP-434 (0.063 $\mu\text{g/ml}$) was unaffected by *tet(M)*, *tet(K)*, and *tet(B)* and increased to 0.25 and 4 $\mu\text{g/ml}$ in the presence of *tet(A)* and *tet(X)*, respectively. Tetracycline, in contrast, was significantly less potent (MIC \geq 128 $\mu\text{g/ml}$) against *E. coli* cells when any of these resistance mechanisms were present. TP-434 showed potent inhibition in *E. coli in vitro* transcription/translation (50% inhibitory concentration [IC₅₀] = 0.29 \pm 0.09 $\mu\text{g/ml}$) and [³H]tetracycline ribosome-binding competition (IC₅₀ = 0.22 \pm 0.07 μM) assays. The antibacterial potencies of TP-434 and all other tetracycline class antibiotics tested were reduced by 4- to 16-fold, compared to that of the wild-type control strain, against *Propionibacterium acnes* strains carrying a 16S rRNA mutation, G1058C, a modification that changes the conformation of the primary binding site of tetracycline in the ribosome. Taken together, the findings support the idea that TP-434, like other tetracyclines, binds the ribosome and inhibits protein synthesis and that this activity is largely unaffected by the common tetracycline resistance mechanisms.

Antibiotic-resistant bacteria are spreading at an alarming rate in both hospital and community settings, severely limiting the utility of all classes of antibiotics and prompting initiatives toward new antibiotic development (19, 20, 29, 35). The bacterial translation pathway is essential and highly conserved across bacteria. It is the target of a large number of many distinct classes of clinically successful antibiotics (33, 43). The attractiveness of this pathway as an antibacterial target is also reflected in the large number of translation-targeting antibiotics in current clinical development (6, 37). The majority of antibiotics that inhibit bacterial translation target the 70S ribosome complex, arresting translation at various stages, spanning initiation, elongation, termination, and recycling. Tetracycline antibiotics bind to the bacterial 30S ribosomal subunit and prevent chain elongation by blocking aminoacyl-tRNA binding to the A site (3, 21, 27). Structures of tetracycline complexed in its primary binding site with the 30S ribosomal subunit of *Thermus thermophilus* confirm this mechanism of action (3, 27); biochemical studies (1, 24) such as cross-linking and chemical modification experiments implicate other binding sites that are not as fully occupied in crystallographic studies. Consistent with crystallographic characterization of the primary tetracycline binding site, mutations within helices h31 and h34 of 16S rRNA have been reported to confer tetracycline resistance in *Helicobacter pylori* and *Propionibacterium acnes* (31, 40).

Widespread use of tetracyclines for over 60 years increased the spread of acquired tetracycline-specific resistance mechanisms among clinically important bacterial pathogens, severely limiting the utility of “legacy” tetracyclines, such as tetracycline, doxycycline, and minocycline (30). These tetracycline resistance genes are generally present on transmissible genetic elements which can rapidly disseminate throughout diverse bacterial populations. To date, 27 genes encoding efflux pumps, 12 genes encoding ribosomal protection proteins (RPPs), 3 distinct genes encoding mod-

ification enzymes, and 1 unknown mechanism, *tet(U)*, have been reported (<http://faculty.washington.edu/marilynr/>). The tetracycline-specific efflux pumps present in clinical pathogens are members of the major facilitator superfamily of bacterial efflux pumps, energized by proton motive force across the inner membrane (39). The most clinically prevalent tetracycline-specific efflux mechanisms include *tet(A)* and *tet(B)* in Gram-negative bacteria (e. g., *Escherichia coli*, *Klebsiella* spp.) and *tet(K)* and *tet(L)* in Gram-positive bacteria (e.g., *Streptococcus* spp., *Staphylococcus* spp.). RPPs bear significant homology to bacterial elongation factors (12, 32) and are thought to destabilize the interaction of tetracycline with the 30S ribosomal subunit via ribosome-stimulated GTP hydrolysis (5, 11, 39). The *tet(M)* and *tet(O)* genes are the most prevalent RPP mechanisms, commonly found in aerobic and anaerobic Gram-negative bacteria (e.g., *Enterobacteriaceae*, *Bacteroides* spp.) and Gram-positive bacteria (e.g., *Streptococcus* spp., *Enterococcus* spp., *Staphylococcus* spp.) (4) (<http://faculty.washington.edu/marilynr/>). *Tet(X)*, the first tetracycline-inactivating mechanism identified, is encoded on a transposon in the anaerobe *Bacteroides fragilis* (34). *Tet(X)* is a flavin-dependent monooxygenase capable of covalently inactivating tetracycline

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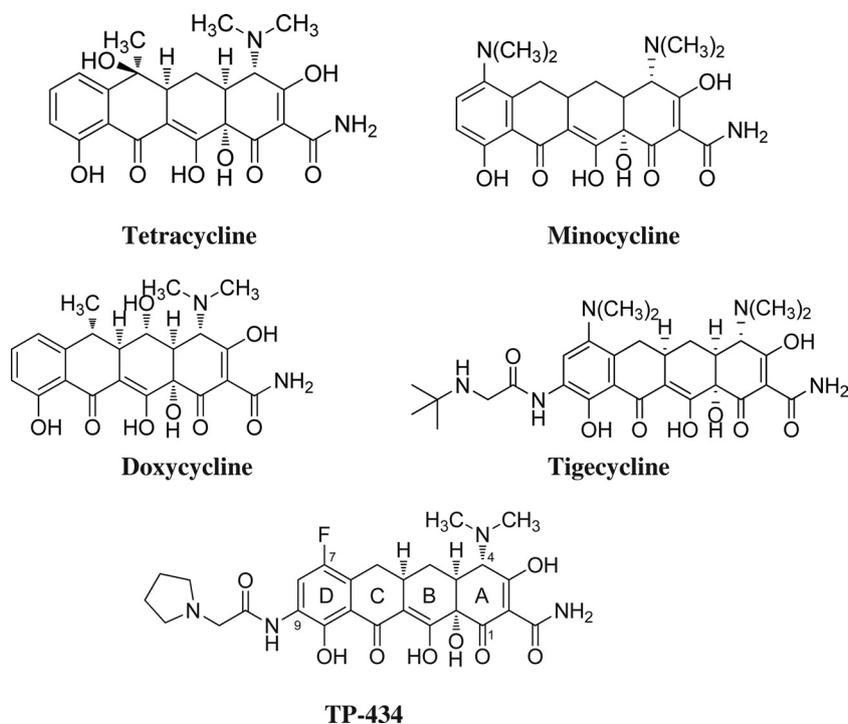


FIG 1 Structures of TP-434 and comparator tetracyclines. The TP-434 structure is labeled using the convention for tetracycline carbon numbering and ring letter assignments.

(45); genes encoding similar enzymatic activities have also been found in oral flora and environmental bacteria (13, 15).

To date, the evolution of the tetracycline antibiotic class has been driven by chemical modification of natural product precursors. Semisynthetic tetracycline derivatives, such as doxycycline and minocycline (Fig. 1), show improved activity versus tetracycline against tetracycline-specific efflux but remain susceptible to resistance by RPPs (39). The parenteral glycylicycline tigecycline, another semisynthetic derivative, was developed to have improved activity against both tetracycline-specific efflux and RPP mechanisms (26) and is a more potent inhibitor of protein synthesis. More recently, TP-434, a novel fluorocycline antibiotic, was made by total synthesis using methodology first described by Charest et al. (7) and further developed at Tetrphase Pharmaceuticals (8, 18, 44). TP-434 contains the tetracyclic core scaffold common to other tetracycline antibiotics, with two unique modifications: a fluorine atom at position C-7 and a pyrrolidinoacetamido group at C-9 on the tetracyclic D ring (Fig. 1). Here, we show that TP-434 is a potent translation inhibitor, binds to the ribosome, and has well-balanced antibacterial activity against strains expressing all three classes of acquired tetracycline-specific resistance. These properties impart to TP-434 the potential to address a wide variety of serious infections caused by drug-resistant pathogens (14, 23, 38).

MATERIALS AND METHODS

Antibiotics. Tetracycline (catalog number T9823), doxycycline (catalog number D9891), penicillin G (catalog number P3032), and erythromycin (catalog number E0774) were purchased from Sigma-Aldrich, St. Louis, MO. Linezolid (catalog number 70412) was from AK Scientific, Union City, CA; tigecycline was from Wyeth Laboratories (Pfizer), Pearl River, NY. TP-434 was synthesized at Tetrphase Pharmaceuticals (8, 44).

***E. coli*-coupled *in vitro* transcription/translation assay.** Antitranslational activity was assessed in an *E. coli*-coupled *in vitro* transcription/translation assay with a firefly luciferase readout (Promega, Madison, WI). Purified Tet(M) [final concentration, 2.75 μ M; preparation of Tet(M) is described in reference 22] was added to reaction mixes evaluating ribosome protection effects *in vitro*. Serial 3-fold compound dilutions spanning the activity range of each compound (0.01 to 10 μ g/ml for TP-434 and tigecycline, 0.03 to 30 μ g/ml for linezolid, 0.1 to 40 μ g/ml for tetracycline) were pipetted into Costar black 96-well assay plates (catalog no. 3915; Corning, Lowell, MA), followed by the addition of reaction mixture with or without Tet(M). Reaction mixtures were run in a total volume of 22 μ l for 1 h at 37°C and stopped by placing on ice for 5 min followed by the addition of 25 μ l/well of luciferase substrate (Promega, Madison, WI). Plates were read on a LUMIStar Optima luminometer (BMG Labtech, Ortenberg, Germany) with gain set to 3,600, 0.2-s read, 0 s between wells. Percent luminescence was plotted against 50% inhibitory concentration (IC_{50}), compared to untreated controls. Results were reported as averages from at least two independent experiments.

3H tetracycline competition assay. Binding of all compounds to empty ribosomes was examined using a competition assay with radiolabeled [3H]tetracycline (Perkin Elmer, Waltham, MA) as described previously for erythromycin (36). Briefly, all reaction mixtures contained 0.4 μ M *E. coli* 70S ribosomes and 8 μ M [3H]tetracycline in binding buffer (10 mM HEPES/KOH [pH 7.8], 30 mM MgCl₂, 150 mM NH₄Cl, 6 mM β -mercaptoethanol), which equated to 80% binding from the saturation curve (data not shown). To measure the IC_{50} for each of the compounds, reactions were performed in the absence or presence of increasing concentrations of the competing compounds. Tigecycline and TP-434 were both tested at concentrations ranging from 0.01 to 10 μ M. Tetracycline and erythromycin were tested at concentrations ranging from 0.01 to 75 μ M and 0.01 to 100 μ M, respectively. After incubation at room temperature for 2 h, reactions were passed through nitrocellulose filters, type HA, 0.45- μ m pore size (Millipore, Billerica, MA). Filters were washed three times with binding buffer, and radioactivity was determined using a scin-

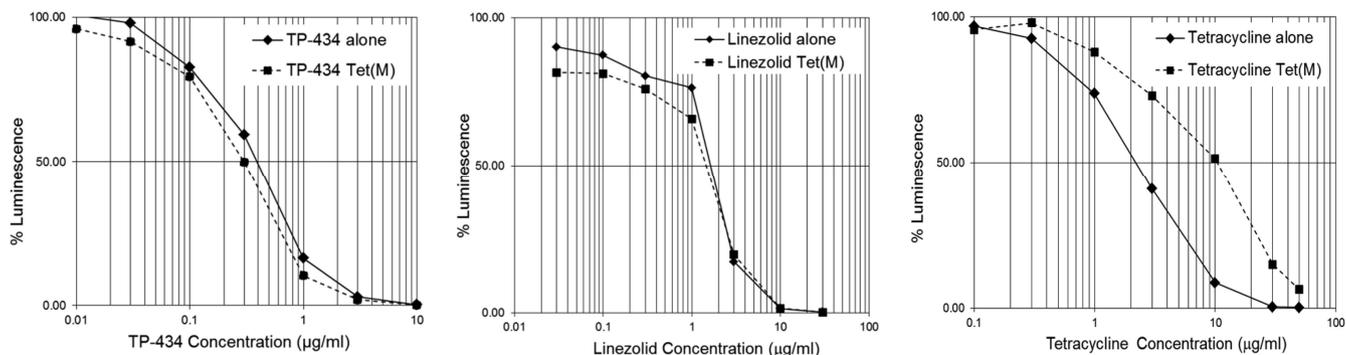


FIG 2 *E. coli*-coupled *in vitro* transcription/translation assays in the presence and absence of purified Tet(M). Compound titrations were assayed for inhibition of translation of a firefly luciferase gene, in the presence and absence of Tet(M) protein, as described in Materials and Methods.

tillation counter in the presence of Filtersafe (Zinsser Analytic, Frankfurt, Germany) scintillant. Results were graphed and IC_{50} s were determined. Results presented are averages from at least two independent experiments.

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(K)*, *tet(M)*, *tet(X)*, and the *E. coli* β -galactosidase gene (*lacZ*) as negative controls 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)*, AP010961; *tet(K)*, AJ888003; *tet(M)*, X90939; *tet(X)*, AB097942).

MIC assays were performed essentially as described by the Clinical and Laboratory Standards Institute (9) with the modification that ampicillin was present at a constant concentration of 50 μ g/ml in each well to maintain plasmids, and inocula were preinduced with L-arabinose (see below). To verify that the constant presence of ampicillin and L-arabinose had no effect on assay results, all antibiotics used in this study were tested against DH10B cells containing the *lacZ* plasmid (in the presence of 50 μ g/ml ampicillin plus 1% L-arabinose) versus DH10B cells (without ampicillin and L-arabinose); MIC values obtained under both conditions were within 2-fold for all antibiotics tested, confirming noninterference of ampicillin and L-arabinose on assay results (data not shown).

Cells were grown in cation-adjusted Mueller-Hinton broth (MHB; BBL/BD, Franklin Lakes, NJ) containing 50 μ g/ml ampicillin and preinduced for 30 min 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. Optimal preinduction conditions producing tetracycline MIC values of ≥ 128 μ g/ml were identified for each strain in preliminary experiments, in which temperatures and L-arabinose concentrations were varied. MIC assays with drug concentrations ranging from 0.016 to 32 μ g/ml were incubated at 35°C for 18 to 20 h, and results were read visually. Assays

were performed at least twice, and results from a representative experiment are reported in Table 3.

***Propionibacterium acnes* susceptibility testing.** Strains were obtained from the American Type Culture Collection (ATCC 6919) or Stephen Shapiro (16). As described by Heller et al. (16), strain SW101 CDA contains an *erm(X)* gene encoding a 23S rRNA methylase conferring erythromycin resistance. Strain SW101T contains a 23S rRNA A2058G mutation (*E. coli* rRNA numbering; conferring macrolide-lincosamide-streptogramin B resistance) and a 16S rRNA G1058C mutation (*E. coli* rRNA numbering; conferring tetracycline resistance). Strain P:413 contains a 16S rRNA G1058C mutation (conferring tetracycline resistance). *P. acnes* isolates were grown for 3 days at 37°C on CDC anaerobic blood agar plates (BBL/BD, Franklin Lakes, NJ) in a Mitsubishi 2.5-liter rectangular anaerobic box (Remel, Lenexa, KS) using an anaerobic gas pack (Remel, Lenexa, KS) with an anaerobic indicator strip (BBL/BD, Franklin Lakes, NJ). Prior to assay, all strains were restreaked for isolation onto CDC anaerobic blood agar and grown for 3 days. Two-fold serial dilutions of test compounds were prepared in a 96-well round-bottom plate format in 50 μ l Wilkins-Chalgren broth (General Laboratory Products, Bolingbrook, IL). Individual *P. acnes* colonies were picked and suspended in 2 ml of 0.9% sterile saline to a turbidity equal to that of a 0.5 McFarland standard. The bacterial suspension (50 μ l) was aseptically transferred to 10 ml Wilkins-Chalgren broth, and 50 μ l/ml of bacterial suspension was added to each well of the 96-well plate containing compound dilutions. Plates were incubated in an anaerobic box at 37°C for 48 h. MIC readings were recorded visually following CLSI guidelines (10).

TABLE 1 Inhibition of *E. coli*-coupled *in vitro* transcription/translation in the presence and absence of Tet(M)

Antibiotic	IC_{50} (μ g/ml) \pm SD	
	With Tet(M)	Without Tet(M)
TP-434	0.29 \pm 0.09	0.27 \pm 0.16
Tigecycline	0.08 \pm 0.01	0.09 \pm 0.04
Tetracycline	1.26 \pm 0.48	6.50 \pm 3.30
Linezolid	1.30 \pm 0.28	1.08 \pm 0.74

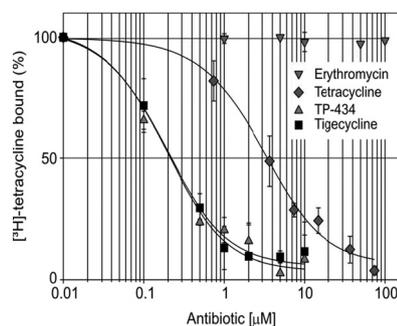


FIG 3 Ribosome binding competition assays with [3 H]tetracycline. Compound titrations were incubated with empty ribosomes in the presence a fixed concentration of [3 H]tetracycline, and the concentration of compound required to compete for 50% of [3 H]tetracycline binding was determined as described in Materials and Methods.

TABLE 2 Susceptibility of *Propionibacterium acnes* rRNA mutants to TP-434 and comparators

Antibiotic	MIC ($\mu\text{g/ml}$) ^a			
	ATCC 6919 (wild type; Ery ^s Cl ^s Tet ^s)	SW101 CDA [<i>erm(X)</i> ; Ery ^r Cl ^r Tet ^s]	SW101T (23S rRNA A2058G; 16S rRNA G1058C; Ery ^r Cl ^r Tet ^r)	P:413 (16S rRNA G1058C; Ery ^s Cl ^s Tet ^r)
TP-434	0.063	≤0.016	1	1
Tigecycline	0.5	0.5	2	2
Tetracycline	1	0.125	16	16
Doxycycline	1	0.125	8	8
Erythromycin	0.125	>32	32	0.063
Penicillin	≤0.016	≤0.016	≤0.016	≤0.016

^a Genotypes are as described in reference 15. 23S rRNA A2058G confers MLS_B resistance; 16S rRNA G1058C confers tetracycline resistance. Ery^r or Ery^s, erythromycin resistant or sensitive; Cl^r or Cl^s, clindamycin resistant or sensitive; Tet^r or Tet^s, tetracycline resistant or sensitive. rRNA base numbering is derived from *E. coli*.

RESULTS AND DISCUSSION

TP-434 inhibits translation and is unaffected by Tet(M). The ability of TP-434 to inhibit translation was tested in an *E. coli*-coupled *in vitro* transcription/translation assay with a luminescent readout. The concentration of TP-434 showing 50% inhibition (IC₅₀) of luminescence compared to that of the untreated controls was 0.29 ± 0.09 $\mu\text{g/ml}$. Tetracycline was ~4.5-fold less potent, with an IC₅₀ of 1.26 ± 0.48 $\mu\text{g/ml}$ (Fig. 2 and Table 1). When purified Tet(M) was added to the transcription/translation reaction, the IC₅₀s of both TP-434 and tigecycline were unaffected (0.27 ± 0.09 $\mu\text{g/ml}$ and 0.09 ± 0.04 $\mu\text{g/ml}$, respectively), whereas the IC₅₀ for tetracycline increased 5-fold (6.50 ± 3.3 $\mu\text{g/ml}$). Results were consistent with earlier reports showing that inhibition of translation by tigecycline and related glycylycylines, compared to that by tetracycline, is unaffected by RPPs (2, 28). The IC₅₀ for linezolid, a nontetracycline control, was unaffected by the addition of Tet(M), substantiating that the RPP mechanism was specific to tetracycline.

TP-434 binds to the ribosome. TP-434 was tested for its ability to compete with labeled tetracycline for binding to purified 70S ribosomes. Increasing concentrations of drug were incubated with purified ribosomes in the presence of [³H]tetracycline, and the amount of compound required to compete for 50% of [³H]tetracycline binding was determined as described in Materials and Methods. TP-434 competed with [³H]tetracycline for ribosome binding (Fig. 3), with an IC₅₀ of 0.22 ± 0.07 μM . Tigecycline had a similar IC₅₀ (0.22 ± 0.08 μM), and tetracycline was ~14-fold less potent (IC₅₀ = 3.00 ± 1.15 μM), consistent with earlier find-

ings that the 9-*t*-butylglycylamido modification at C-9 in tigecycline strengthens its interaction with the ribosome compared to that of tetracycline (25). These results imply that TP-434, tigecycline, and tetracycline bind to at least partially overlapping sites on the ribosome. Erythromycin tested up to 100 μM failed to compete with [³H]tetracycline, consistent with the location of the erythromycin-binding site on the 50S ribosomal subunit.

TP-434 and comparators tigecycline, tetracycline, doxycycline, erythromycin, and penicillin were tested against unrelated *P. acnes* clinical isolates. Results showed that both isolates, SW101T and P:413, containing the G1058C mutation exhibited elevated MIC values for all tetracycline antibiotics compared to those of strains ATCC 6919 and SW101CDA, which have no mutations impacting the tetracycline binding region of 16S rRNA (Table 2). This finding is consistent with at least a partial overlap of the binding sites of TP-434, tigecycline, and doxycycline. Mutations in 16S rRNA within helices h31 and h34 have been reported to confer resistance to tetracycline antibiotics in *P. acnes* and *H. pylori* (31, 40). Consistent with this genetic data, direct contacts between these helices and tetracycline are also observed in the 30S cocomplex crystal structures (3, 27). Although G1058 is not part of the primary binding site for tetracycline, mutation to cytosine likely causes a conformational change, reducing the binding affinity of tetracycline for the 30S ribosomal subunit. As expected from previous studies with these isolates (16), erythromycin MIC values were elevated in SW101 CDA (MIC > 32 $\mu\text{g/ml}$) and SW101T (MIC = 32 $\mu\text{g/ml}$) compared to those in strains ATCC 6919 (MIC = 0.125 $\mu\text{g/ml}$) and P:413 (MIC = 0.063 $\mu\text{g/ml}$), and all strains were similarly susceptible to penicillin (MIC ≤ 0.016 $\mu\text{g/ml}$; Table 2).

TP-434 is active against the major tetracycline-specific resistance mechanisms. To evaluate the effects of individual tetracycline resistance mechanisms in the absence of other clinical antibiotic resistance phenotypes, TP-434 was tested against an isogenic set of *E. coli* strains expressing each of the following genes: *tet(M)*, *tet(K)*, *tet(A)*, *tet(B)*, *tet(X)*, and *lacZ*, the negative control. Results from a representative assay are shown in Table 3. The MIC of TP-434 (0.063 $\mu\text{g/ml}$) was unaffected by overexpression of *tet(K)* and *tet(B)*; however, a 4-fold increase in TP-434 MIC (0.25 $\mu\text{g/ml}$) was observed in the presence of *tet(A)* expression, indicating that TP-434 is recognized to some extent by the Tet(A) pump. Consistent with findings in the TNT assay with purified Tet(M), the activity of TP-434 was also immune to the cell-based overexpression of *tet(M)*. Susceptibility to tigecycline (MIC = 0.063 $\mu\text{g/ml}$) was unaffected by *tet(M)*, *tet(K)*, and *tet(B)*; however, a 16-fold increase (MIC = 1 $\mu\text{g/ml}$) was observed in the presence of *tet(A)* for tigecycline, indicating that tigecycline is a substrate for Tet(A) efflux.

Reduced tigecycline activity in the presence of *tet(A)* is consis-

TABLE 3 Susceptibility of TP-434 and comparators to *E. coli* DH10B expressing recombinant major tetracycline resistance genes

Antibiotic	MIC ($\mu\text{g/ml}$) for <i>E. coli</i> strain expressing:					
	<i>lacZ</i>	<i>tet(M)</i>	<i>tet(K)</i>	<i>tet(A)</i>	<i>tet(B)</i>	<i>tet(X)</i>
TP-434	0.063	0.063	0.031	0.25	0.063	4
Tigecycline	0.063	0.13	0.063	1	0.063	2
Doxycycline	2	64	4	32	32	16
Minocycline	0.5	64	1	8	16	4
Tetracycline	2	128	128	>128	>128	128
Ceftriaxone	0.063	0.13	0.063	0.13	0.13	0.13

tent with previous reports by Tuckman et al. and Hentschke et al. describing amino acid sequence variations in Tn1721-associated *tet(A)* genes which were suggested to confer reduced susceptibility to minocycline and glycylcyclines (17, 41). The *tet(A)* gene expressed in the present study had the same variations as the Tn1721-associated *tet(A)* gene, where the amino acids are Ala-201, Ser-202, and Phe-203 rather than those of the *tet(A)* sequence from *E. coli* plasmid RP1, Ser-201, Phe-202, and Val-203 (GenBank accession number X00006) (42). These residues reside in the largest cytoplasmic loop of the pump and are thought to impact the selective gating of tetracyclines recognized by the pump (41). While tigecycline is reported to be unaffected by the presence of *tet(A)* carried by plasmid RP1, this finding may be specific to the RP1 *tet(A)* sequence. A GenBank database search revealed that plasmid- and chromosomally associated *tet(A)* genes containing the sequence found in Tn1721 are present in a wide variety of Gram-negative organisms, and examples include *Shigella sonnei*, *E. coli*, *Pseudomonas aeruginosa*, *Salmonella* spp., *Aeromonas salmonicida*, and *Acinetobacter baumannii* (17, 41) (<http://faculty.washington.edu/marilynr/>). Thus, strains harboring *tet(A)* genes with reduced sensitivity to tigecycline may be more widespread than originally reported. Overexpression of *tet(X)* increased MIC values for TP-434 and tigecycline to 4 and 2 $\mu\text{g/ml}$, respectively. Consistent with tetracycline's known vulnerability to these mechanisms, a ≥ 64 -fold increase in MIC value, relative to the *lacZ* control strain, was associated with overexpression of each of the resistance genes. The MIC of ceftriaxone, a nontetracycline control, remained unaffected by the overexpression of any of the tetracycline resistance mechanisms.

In summary, novel modifications at positions C-7 and C-9 on the tetracyclic D ring of TP-434 appear to confer superior target-binding potency compared to that of legacy tetracyclines in the presence or absence of a major tetracycline ribosomal protection mechanism Tet(M). Further, TP-434 has improved activity against major tetracycline resistance mechanisms and is 4-fold more potent than tigecycline in *E. coli* expressing a widespread tetracycline efflux pump, Tn1721-associated *tet(A)*. These properties impart to TP-434 a broad spectrum of activity against multidrug-resistant Gram-positive and Gram-negative pathogens, including tetracycline-resistant *Enterobacteriaceae* producing extended-spectrum β -lactamase(s) or carbapenemases (14, 38), making it a promising new agent for the potential treatment of serious hospital- and community-acquired infections.

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ERRATUM

Erratum for Grossman et al., Target- and Resistance-Based Mechanistic Studies with TP-434, a Novel Fluorocycline Antibiotic

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Volume 56, no. 5, p. 2559–2564, 2012. Page 2561: In [Table 1](#), the labeling of the columns should be reversed. The table should appear as shown below.

Antibiotic	IC ₅₀ (μg/ml) ± SD	
	Without Tet(M)	With Tet(M)
TP-434	0.29 ± 0.09	0.27 ± 0.16
Tigecycline	0.08 ± 0.01	0.09 ± 0.04
Tetracycline	1.26 ± 0.48	6.50 ± 3.30
Linezolid	1.30 ± 0.28	1.08 ± 0.74

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