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# The Fluorocycline TP-271 is Potent Against Major Complicated Community-Acquired Bacterial Pneumonia (CABP) Pathogens

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## Abstract

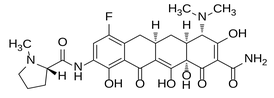
**Objective:** TP-271 is a novel, fully synthetic fluorocycline antibiotic in preclinical development for IV/oral treatment of respiratory infections caused by susceptible and multidrug-resistant (MDR) public health and biothreat pathogens. **Method:** In vitro susceptibility testing against recent isolates of *Streptococcus* spp., *Staphylococcus aureus*, *Haemophilus influenzae, Moraxella catarrhalis* and *Mycoplasma pneumoniae* was performed according to CLSI guidelines. TP-271 was tested against a total of 70 *Legionella pneumophila* isolates including serogroups 1 – 6 by agar dilution using buffered yeast extract agar. For *Chlamydophila pneumoniae*, the minimal inhibitory concentration (MIC) was assessed in HEp-2 cell line without passage and was defined as the lowest concentration of test compound that resulted in reduction of inclusions. Time-kill assays with 3 to 4 clinical isolates per organism were performed as per CSLI guidelines in cation-adjusted Mueller Hinton broth (caMHB; *S. aureus, M. catarrhalis*), caMHB + 5% lysed horse blood (*Streptococcus* spp.), or *Haemophilus* Test Medium (*H. influenzae*). **Results:** TP-271 showed good activity against all key respiratory pathogens, including atypical organisms *L. pneumophila, C. pneumoniae* and *M. pneumoniae.* $MIC_{90}$ values for TP-271 were 0.03 μg/mL for all streptococci, regardless of resistance phenotype. TP-271 was also active ($MIC_{90}$ values 0.13 – 0.25 μg/mL) against methicillin-susceptible (MSSA) and -resistant (MRSA) *S. aureus*, including MRSA expressing Panton-Valentine leukocidin (PVL) toxin. Against *H. influenzae* and *M. catarrhalis*, TP-271 showed $MIC_{90}$ values of 0.13 and ≤0.016 μg/mL, respectively. At concentrations of 4X and 8X MIC, TP-271 was bacteriostatic with 3 of 4 *S. pneumoniae* isolates, all 3 *S. pyogenes* isolates, all 4 MRSA isolates, and all 3 *M. catarrhalis* isolates. TP-271 was bactericidal against all 3 *H. influenzae* isolates and at a top concentration of 2 μg/mL TP-271, bactericidal activity was seen against 1 of 3 *S. pneumoniae*, 2 of 3 *S. pyogenes*, 1 of 4 MRSA, all 3 *H. influenzae*, and all 3 *M. catarrhalis* isolates. **Conclusions:** TP-271 displayed excellent potency against major CABP pathogens and was unaffected by pre-existing tetracycline-specific, quinolone-resistant, or macrolide-resistant drug resistance phenotypes. TP-271 was also found to be bactericidal against some CABP isolates, particularly at 2 μg/mL. TP-271 shows promise as a new antibiotic for the treatment of complicated CABP.

## Introduction

A greater than 60-year track record of proven safety and clinical success validates the tetracyclines as a valuable class of broad-spectrum antibiotics worthy of continued drug development. Tetracyclines arrest the growth of bacteria by blocking the binding of aminoacyl-tRNA to the A site of the 30S ribosomal subunit. While antibacterial activity is generally considered bacteriostatic, it has been shown that tetracyclines can be bactericidal in some cases, with some organisms [1, 2].

TP-271 is a novel, fully synthetic fluorocycline antibiotic in preclinical development for IV/oral treatment of moderate to severe respiratory infections caused by susceptible and multidrug-resistant (MDR) public health and biothreat pathogens [3].

**TP-271**



[1] **Bantar, C.,** et al. 2008. Comparative time-kill study of doxycycline, tigecycline, sulbactam, and imipenem against several clones of *Acinetobacter baumanii.* Diag. Microbiol. Infect. Dis. 61:309-314

[2] **Petersen, P.,** et al. 2007. In vitro antibacterial activities of tigecycline and comparative agents by time-kill studies in fresh Mueller Hinton broth. Diag. Microbiol. Infect. Dis. 59:347-349.

[3] **Grossman, T., et al.** 2012. TP-271 is a Potent, Broad-spectrum fluorocycline with activity against community-acquired bacterial respiratory and biothreat pathogens. Abstr F-1525. 52nd Intersci. Conf. Antimicrob. Agents Chemother., American Society for Microbiology, Washington, DC.

## Methods

**Susceptibility testing of aerobic pathogens.** All minimal inhibitory concentration (MIC) assays were performed as per CLSI guidelines [1]. All isolates used in susceptibility testing were recent (less than 3 years old) from Eurofins Medinet, IHMA and the Canadian Bacterial Surveillance Network and demographically diverse. Testing was performed at Tetraphase Pharmaceuticals, Inc., International Health Management Associates (IHMA), and Mount Sinai Hospital (Toronto, ON).

**Susceptibility testing of atypical pathogens:**

- ***L. pneumophila.*** *L. pneumophila* strains isolated from the respiratory tract from 1992 to 2010, were identified by standard methods described by Murray et al. (2). Seventy *L. pneumophila* isolates from six serogroups were tested by agar dilution using Buffered Yeast extract (BYE; with original *Legionella* BCYE Growth supplement).
- ***M. pneumoniae.*** Low passaged clinical isolates and type strains of *M. pneumoniae* were tested by microbroth dilution using standardized procedures as described by CLSI (3).
- ***C. pneumoniae.*** Ten human isolates of *C. pneumoniae* were utilized for screening. The isolates were obtained through the University of Washington (Seattle, WA), University of Alabama at Birmingham (Birmingham, AL) and ATCC. The strains have no known drug resistance. Each *C. pneumoniae* isolate was propagated and screened for susceptibility using the cell line HEp-2 (ATCC, Manassas, VA), an adherent epithelial cell line originating in humans (4). The following controls were included: i) non-infected cells, ii) cells infected with each organism without drug treatment (negative control) and iii) infected cells treated with doxycycline and azithromycin (positive controls). The minimal inhibitory concentration (MIC) is defined as the lowest concentration of test compound that results in >50% reduction in the number of inclusions after 3 days of treatment with compound.

**Time-kill assays.** Time-kill assays were performed as described by CLSI guidelines (5), with the following modifications: five milliliter cultures inoculated to a final starting density of ~1 x $10^5$ – 1 x $10^6$ colony forming units (CFU) /mL were shaken vigorously (300 rpm for all organisms except *H. influenzae* and *S. pneumoniae* which were shaken at 150 rpm) at 35°C in 50 mL polypropylene conical tubes. Cultures were sampled at various time points, serially diluted in sterile saline, and plated on tryptic soy agar, chocolate agar (*H. influenzae*), and blood agar (*S. pneumoniae* and *S. pyogenes*). Colonies were counted and growth/killing curves were plotted, with a lower limit of detection per organism of 100 CFU/mL. A bactericidal response was defined at 24h as a 3 $log_{10}$ reduction in CFU/mL versus the starting inoculum (CFU/mL) for a given culture. Assays were performed in duplicate or triplicate and representative results are shown in Figure 1. Generally 4x and 8x multiple concentrations were tested for each species and in some cases, a top concentration of 2 μg/mL was evaluated.

[1]**Clinical and Laboratory Standards Institute (CLSI).** Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; Approved Standard—Ninth Edition. CLSI document M07-A9. Clinical and Laboratory Standards Institute, Wayne, Pennsylvania, 2012.

[2]Murray et al., Manual of Clinical Microbiology, 9rd ed., 2007, A.S.M. Chap. 53; 835-849.

[3]**Clinical and Laboratory Standards Institute (CLSI).** Methods for antimicrobial susceptibility testing for human mycoplasmas; approved guideline. CLSI document M43-A. Clinical and Laboratory Standards Institute, Wayne, PA. 2011

[4]L.A. Campbell and C. Kuo. 2009. Cultivation and Laboratory Maintenance of *C. pneumoniae*. Curr. Pro Protoc. Micro. 12:11B.1.1-11B.1.23.

[5]**Clinical and Laboratory Standards Institute (CLSI).** Methods for determining bactericidal activity of antimicrobial agents; approved guideline. CLSI document M26-A , Clinical and Laboratory Standards Institute, Wayne, PA. 1999.

## Results

### Table 1. Determination of $MIC_{50}$ and $MIC_{90}$ values for CABP Pathogens

| Organism | N | $MIC_{50}/MIC_{90}$ (range) TP-271 | Tetracycline[l] | Tigecycline | Macrolide[a] | Fluoroquinolone[b] | Linezolid | Vancomycin |
|---|---|---|---|---|---|---|---|---|
| *Streptococcus pneumoniae* | 267 | ≤0.016/0.03 (≤0.016-0.03) | 32/>32[c] (≤0.016->32) | ≤0.016/≤0.016[d] (≤0.016-≤0.016) | >32/>32 (≤0.016->32) | 1/1 (0.25-32) | 1/1[c] (0.13-2) | 0.5/0.5[c] (≤0.016-0.5) |
| *S. pneumoniae* penicillin-R[m] | 125 | ≤0.016/0.03 (≤0.016-0.03) | 32/>32 (0.031->32) | ≤0.016/≤0.016[e] (≤0.016-≤0.016) | >32/>32 (≤0.016->32) | 1/1 (0.5-8) | 1/1 (0.25-2) | 0.5/0.5 (0.25-0.5) |
| *S. pneumoniae* macrolide-R | 209 | ≤0.016/0.03 (≤0.016-0.03) | 32/>32[f] (0.03->32) | ≤0.016/≤0.016[h] (≤0.016-≤0.016) | >32/>32 (1->32) | 1/1[g] (0.25-32) | 1/1[f] (0.25-2) | 0.5/0.5[f] (0.13-0.5) |
| *Streptococcus pyogenes* | 100 | ≤0.016/0.03 (≤0.016-0.03) | 0.5/>32 (0.13->32) | ≤0.016/≤0.016[i] (≤0.016-0.063) | 0.063/>32 (≤0.016->32) | 0.5/1 (0.25-2) | 1/2 (0.5-2) | 0.5/0.5 (0.25-0.5) |
| *Staphylococcus aureus* | 155 | 0.06/0.25 (≤0.03-1) | ≤2/32 (0.063->32) | 0.12/0.25 (≤0.016-0.5) | >4/>4 (0.25->4) | >4/>4 (≤0.13->4) | 2/4 (0.5-64) | 1/1 (≤0.5-8) |
| *S. aureus* (MRSA) | 124 | 0.063/0.13 (≤0.016-1) | ≤2-32 (0.063->32) | 0.13/0.25 (≤0.016-0.5) | >4/>4 (0.25->4) | >4/>4 (≤0.13->4) | 2/4 (1-64) | 1/1 (≤0.5-8) |
| *S. aureus* (MRSA) PVL+ | 25 | 0.063/0.13 (0.063-0.13) | ≤2/≤2 (≤2-16) | 0.12/0.12 (0.063-0.25) | >4/>4 (1->4) | 2/>4 (≤0.13->4) | 2/2 (1-4) | 1/1 (≤0.5-1) |
| *S. aureus* (MSSA) | 31 | 0.12/0.25 (≤0.031-0.25) | ≤2/≤2 (≤2-32) | 0.12/0.25 (0.03-0.25) | 1/>4 (0.5->4) | 0.25/0.5 (≤0.13->4) | 2/4 (0.5-4) | 1/1 (≤0.5-1) |
| *Haemophilus influenzae* | 65 | 0.031/0.13 (≤0.016-0.25) | 0.5/4 (0.13-16) | 0.063/0.25 (≤0.016-0.5) | 8/8 (0.063-16) | ≤0.016/0.031 (≤0.016-0.13) | 8/16 (4-32) | >32/>32[j] (16->32) |
| *Moraxella catarrhalis* | 57 | ≤0.016/≤0.016 (≤0.016-0.031) | 0.5/32 (0.13->32) | ≤0.016/0.031 (≤0.016-0.13) | 0.063/0.25 (≤0.016-4) | 0.031/0.063 (0.031-0.13) | 8/8 (2-32) | >32/>32[k] (16->32) |
| *Legionella pneumophila* | *70* | 0.25/1 (≤0.004-2) | 4/8 (0.5-8) | ND | 0.25/0.5 (0.06-1) | ND | ND | ND |
| *Chlamydophila pneumoniae* | 10 | 4/4 (2-4) | 0.25/0.25 (0.13-0.25) | ND | 0.25/0.25 (0.13-0.25) | ND | ND | ND |
| *Mycoplasma pneumoniae* | 20 | 0.001/0.004 (0.0005-0.008) | 0.063/0.13 (0.032-0.13) | 0.031/0.031 (0.016-0.125) | 0.000063/8 (0.000032-16) | 0.5/0.5 (0.25-0.5) | ND | ND |

[a]erythromycin, azithromycin or clarithromycin; [b]ciprofloxacin or levofloxacin; [c]256 *S. pneumoniae* isolates; [d]137 *S. pneumoniae* isolates; [e]58 *S. pneumoniae* isolates; [f]201 *S. pneumoniae* isolates; [g]185 *S. pneumoniae* isolates; [h]82 *S. pneumoniae* isolates; [i]64 *S. pyogenes* isolates; [j]51 *H. influenzae* isolates; [k]43 *M. catarrhalis* isolates ; [l]doxycycline for *C. pneumoniae* and *M. pneumoniae*; [m]penicillin MIC ≥2 μg/ml

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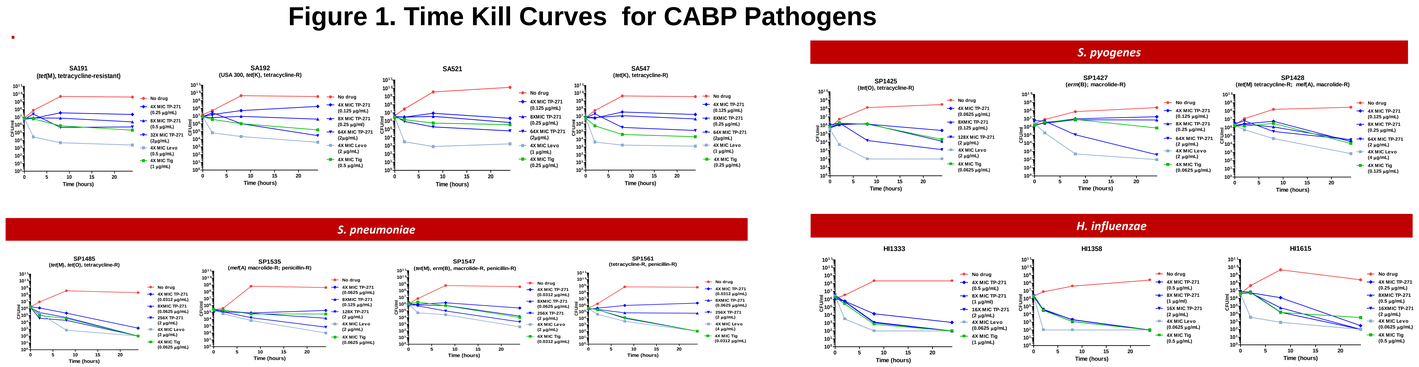
### Figure 1. Time Kill Curves for CABP Pathogens




### Table 2. Media used in *C. pneumoniae* and *L. pneumophila* assays interferes with TP-271 activity

Microtiter Assays in CA-MHB vs. EMEM (*C. pneumoniae* media)

| Compound | *E. coli* ATCC 25922 MIC (μg/mL) CA-MHB no FBS | CA-MHB 10% FBS | EMEM no FBS | EMEM 10% FBS |
|---|---|---|---|---|
| TP-271 | ≤0.016 | 0.0312 | 0.5 | 0.5 |
| Tigecycline | 0.0312 | 0.0625 | 0.25 | 0.5 |
| Tetracycline | 1 | 2 | 4 | 4 |
| Doxycycline | 1 | 1 | 2 | 2 |

Agar Dilution Assays in MHB vs. BYE (*L. pneumophila* media) or ModBYE (w/o ferric pyrophosphate)

| Compound | *E. coli* ATCC 25922 MIC (μg/mL) 24 hrs MHB | Mod BYE | BYE |
|---|---|---|---|
| TP-271 | 0.12 | 0.5 | 2 |
| Tetracycline | 1 | 0.5 | 16 |

## Conclusions

- **TP-271, a novel fully synthetic tetracycline, exhibited excellent potency against key MDR public health respiratory pathogens, including *S. pneumoniae,* MRSA, *S. pyogenes*, *H. influenzae* and *M. catarrhalis.***
- **TP-271 showed good activity against atypical pathogens *M. pneumoniae, L. pneumophila,* and *C. pneumoniae,* however, *in vitro* activity against the latter two organisms was likely limited by media interference, and thus potency is underestimated in these assays.**
- **TP-271 was generally bacteriostatic against MRSA, *S. pneumoniae, S. pyogenes*, and *M. catarrhalis* at 4X and 8X MIC. At 2 μg/mL, an estimation of the $C_{max}$ in man, TP-271 showed bactericidal activity against some isolates.**
- **TP-271 was bactericidal against *H. influenzae* at all concentrations tested.**
- **TP-271 shows promise as a new antibiotic for the empiric treatment of moderate-to-severe CABP.**