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In Vivo Efficacy of Novel, Fully Synthetic Tetracyclines in a Murine Lung Infection Model Challenged with KPC-producing *Klebsiella pneumoniae*

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Abstract

Objective: To examine the *in vivo* efficacy of novel, fully synthetic tetracyclines with potent *in vitro* activity against multidrug-resistant (MDR) Gram-negative pathogens (including carbapenem-resistant *Enterobacteriaceae*) in a mouse lung infection model challenged with *Klebsiella pneumoniae* carbapenemase (KPC)-producing *K. pneumoniae.*

Methods: Minimal inhibitory concentration (MIC) assays were conducted according to CLSI guidelines. Groups of six female CD-1 mice were made neutropenic by administration of 150 mg/kg cytoxan intraperitoneally on Day -4. On Day 0, mice were inoculated intranasally with KPC-producing *K. pneumoniae* KP1906 (~6-7 $\log_{10}$ colony forming unit (CFU)/mouse in 0.05 mL). **TP-076**, **TP-138**, and **TP-600** were administered intravenously (IV) at 5, 15, and 40 mg/kg twice at 2 and 12 hr post-infection. Tigecycline was administered IV at 80 mg/kg twice at 2 and 12 hr post-infection and colistin was administered subcutaneously at 10 mg/kg once at 2 hr post-infection. At 2 hour (untreated control only) and 24 hr (untreated control and treated) post-infection, mice were euthanized via CO_2 inhalation and their lungs aseptically removed, homogenized, diluted, and plated for CFU determination.

Results: The MIC values of **TP-076**, **TP-138**, **TP-600**, tigecycline, and colistin against KP1906 were 0.0625, 0.0625, 0.125, 1, and 0.5 µg/mL, respectively. As compared to the 24 hr untreated controls, at doses of 5, 15 and 40 mg/kg, respectively, **TP-076** showed CFU reductions of 0.33, 2.79, and 3.18 $\log_{10}$,**TP-138** showed CFU reductions of 0.38, 1.99, and 2.81 $\log_{10}$, and **TP-600** showed CFU reductions of 1.88, 2.58, and 3.21 $\log_{10}$. Tigecycline had a modest effect at 80 mg/kg, producing only a 1.23 $\log_{10}$ CFU reduction. Subcutaneous administration of a single 10 mg/kg dose of colistin resulted in counts comparable to the untreated control group.

Conclusions: Novel tetracyclines, **TP-076**, **TP-138**, and **TP-600**, demonstrated potent *in vivo* efficacy in a mouse lung infection model challenged with KPC-producing *Klebsiella pneumoniae.* Additional studies are in progress to further develop these and related new tetracycline analogs to combat serious and life-threatening Gram-negative infections.

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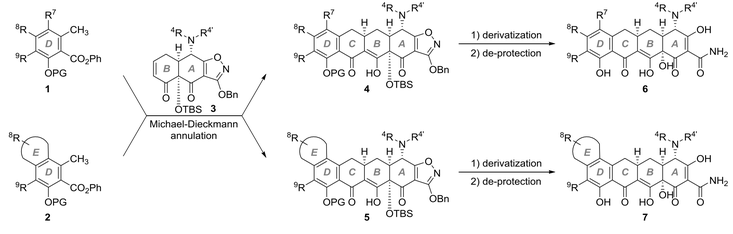
Materials and Methods

Compounds. Novel, fully synthetic tetracyclic and polycyclic tetracycline analogs such as **6** and **7** were prepared by coupling a D-ring precursor **1** or a DE-ring precursor **2** to the AB-ring enone **3**[1] via a Michael-Dieckmann annulation to form polycyclic intermediates **4** and **5**, followed by post-annulation derivatization and global de-protection, as shown in the following general synthetic scheme. Lead compounds **TP-076**, **TP-138**, and **TP-600** in this study belong to general scaffolds **6** or **7**.



Bacterial strains. *Klebsiella pneumoniae* KP1906 (KPC-producing strain UNT-203-1) was from the laboratory of William J. Weiss at UNT Health Science Center, Fort Worth, TX. It is resistant to gentamicin, levofloxacin, cefotaxime/ceftazidime, tetracycline, and meropenem by Clinical and Laboratory Standards Institute (CLSI) breakpoints, but susceptible to tigecycline and colistin.

***In vitro* susceptibility.** Compounds were tested against *Klebsiella pneumoniae* KP1906 (KPC-producing strain) and quality control strains according to methods published by CLSI (Clinical and Laboratory Standards Institute, 2012, Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically, Approved Standard—9th Edition, CLSI document M07-A9, CLSI, Wayne, PA).

Lung infection model. Female CD-1 mice (22 ± 2 g, Harlan laboratories) were pretreated with cytoxan (150 mg/kg, IP) on day -4 for partial neutropenia (based on previous virulence studies). Mice (n = 6) were anesthetized by IP injection of 0.15 mL of a mixture of ketamine-HCl (40 mg/kg b.w.) and xylazine (6 mg/kg b.w.) and intranasally (IN) inoculated with 0.05 mL of the designated inoculum (final infective dose of approx. 6-7 $\log_{10}$ CFU/mouse). For IN inoculation, inoculum drops were placed onto the external nares for inhalation. After inoculation, mice were placed back into cages and monitored for recovery. Dosing was initiated at +2 hr post-infection with a second dose administered at 12 hours for each dose group. For determining lung bioburden at 2 and 24 hours post-infection, animals were euthanized via CO_2 inhalation, their lungs aseptically removed, homogenized, diluted, and plated for colony forming units (CFU) determination.

Results

Figure 1. *In Vivo* Activity in a Murine Lung Infection Model Challenged with Carbapenem-resistant *Klebsiella pneumoniae* KP1906

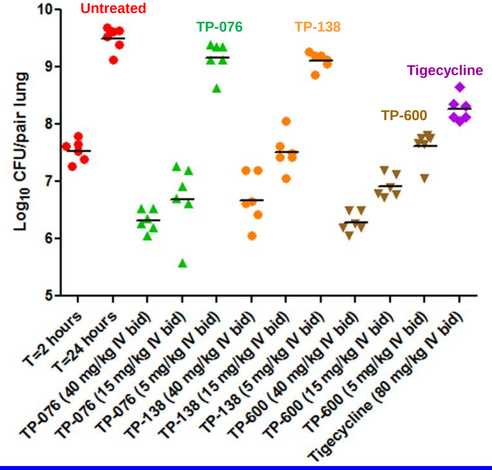


Table 1. MIC Values against Carbapenem-resistant *Klebsiella pneumoniae* KP1906

Compound	MIC (µg/mL)
TP-076	0.0625
TP-138	0.0625
TP-600	0.125
Tigecycline	1
Imipenem	16
Cefotaxime/Ceftazidime	>32

Conclusions

- **The potent *in vitro* antibacterial activity of novel, fully synthetic tetracyclines (TP-076, TP-138, and TP-600) against serious Gram-negative pathogens translated to superior *in vivo* efficacy in a murine lung infection model challenged with KPC-producing *K. pneumoniae*, as compared to tigecycline.**
- ***In vivo* efficacy observed was dose proportional.**
- **Further studies are in progress to develop these new, potent tetracycline agents for treating life-threatening infections, especially those caused by MDR Gram-negative bacteria including carbapenem-resistant *Enterobacteriaceae* (CRE).**

References & Acknowledgements

1) M.G. Charest, C.D. Lerner, J.D. Brubaker, D.R. Siegel, A.G. Myers, A Convergent Enantioselective Route to Structurally Diverse 6-Deoxytetracycline Antibiotics, *Science*, **308**, 395 (2005).
2) We thank Dr. William Weiss and colleagues for conducting the *in vivo* experiments.