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In Vitro Activity of TP-271 against *Mycobacterium abscessus*, *Mycobacterium fortuitum*, and *Nocardia* Species

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The *in vitro* activities of TP-271, a novel fluorocycline antimicrobial, against 22 isolates of *Mycobacterium abscessus*, 22 isolates of *Mycobacterium fortuitum*, and 19 isolates of *Nocardia* spp. were studied by a microtiter broth dilution method. The MIC_{90}s for *M. abscessus*, *M. fortuitum*, and *Nocardia* spp. were 0.5 μg/ml, 0.03 μg/ml, and 8 μg/ml, respectively. TP-271 was significantly more active than the respective control drug in virtually all tests.

Mycobacterium abscessus and *Mycobacterium fortuitum* are rapidly growing mycobacteria that are associated primarily with opportunistic infections in immunocompromised subjects (2). Patients with bronchiectasis, especially those with cystic fibrosis, are at increased risk of *M. abscessus* infection. Other manifestations of *M. abscessus* infection include localized skin infections, postoperative wound infections, and infection of implanted medical devices. *M. fortuitum* is a relatively rare pathogen, even in immunocompromised individuals, but has been associated with skin infections and hospital-acquired postoperative infections (2). The most common clinical form of *Nocardia* infection is pulmonary nocardiosis, with and without dissemination, followed by skin and soft tissue infection (5).

Infections due to these microorganisms are occurring with increasing frequency and are often difficult to treat, especially the mycobacterial infections. Successful treatment is often hindered by the need for combination therapy, resistance to multiple drugs, necessity of long treatment duration, and lack of sufficiently active oral drugs (2, 3, 6).

While *Nocardia* remains susceptible to trimethoprim-sulfamethoxazole in the majority of cases, effective alternative oral therapy is lacking. Mortality from pulmonary nocardiosis remains high, in the range of 15 to 40% (5, 11).

TP-271 is a novel, fully synthetic fluorocycline antimicrobial related to tetracycline (Fig. 1) (1, 10). It has been shown to have potent broad-spectrum *in vitro* and *in vivo* activity against multiple community-acquired organisms, including *Staphylococcus* spp., *Streptococcus* spp., *Haemophilus influenzae*, *Moraxella catarrhalis*, *Legionella pneumophila*, and *Acinetobacter baumannii*, as well as biothreat organisms (*Bacillus anthracis*, *Francisella tularensis*, *Burkholderia pseudomallei*, and *Burkholderia mallei*) (4). The activity of TP-271 was shown to be unaffected by the Gram-positive tetracycline-specific pump *tet*(K) and ribosomal protection mechanism *tet*(M) and minimally affected by the most common Gram-negative efflux mechanisms, *tet*(A) and *tet*(B) (7). Promising oral activity was demonstrated by TP-271 in neutropenic mouse models of pneumonia caused by methicillin-resistant *Staphylococcus aureus* and *Streptococcus pneumoniae* and in immunocompetent models of pneumonia caused by *S. pneumoniae* in mice and *H. influenzae* in rats (4).

As a first step in its assessment as a novel therapy to treat infections caused by *M. abscessus*, *M. fortuitum*, and *Nocardia* spp., we evaluated the *in vitro* activity of TP-271 in comparison to those of several standard agents against clinical isolates.

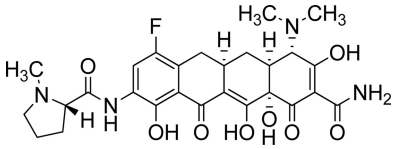

FIG 1 Structure of TP-271.

TP-271 was obtained from Tetraphase Pharmaceuticals, Inc. (Watertown, MA), moxifloxacin (MOX) from Bayer Pharmaceuticals (West Haven, CT), and amikacin (AMK) from (Bristol-Myers Squibb, Princeton, NJ). Doxycycline (DOX) and tetracycline (TET) were purchased from Sigma Chemical Co. (St. Louis, MO).

M. abscessus isolates were provided by Barbara Body (LabCorp, Burlington, NC) and Barbara Brown-Elliott (University of Texas Health Science Center, Tyler, TX). *M. fortuitum* isolates were provided by Barbara Brown-Elliott, and *Nocardia* isolates were from the American Type Culture Collection (Manassas, VA), Barbara Body, and Betty Ann Forbes (Virginia Commonwealth University, Richmond, VA). The compounds were initially dissolved in water to make concentrated stock solutions that were frozen at −20°C. Stock solutions were subsequently diluted in Mueller-Hinton (MH) broth (Becton, Dickinson, Sparks, MD) prior to their use. An *in vitro* microtiter broth dilution method similar to that suggested by the Clinical and Laboratory Standards Institute (CLSI) (9) was utilized, with several modifications. Polystyrene 96-well round-bottom plates (Corning Inc., Corning, NY) were prepared with 50 μl of MH broth per well. The compounds were prepared at 4 times the maximum concentration at which they were tested and were added to the first well prior to being serially diluted 2-fold. The bacterial isolates were grown in MH broth for 3 to 5 days and then diluted in MH broth with 20% glycerol to 100 Klett units (equivalent to 5×10^7 CFU/ml) (Photoelectric Colorimeter; Manostat Corp., New York, NY) and stored at −70°C until used. The bacterial cultures were thawed and diluted to a

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TABLE 1 MICs of TP-271, moxifloxacin, doxycycline, and tetracycline against M*ycobacterium abscessus*

Isolate	MIC (μg/ml)			
	TP-271	Moxifloxacin	Tetracycline	Doxycycline
BB2	0.5	8	>64	>64
BB1	0.5	8	>64	
BB3	0.06	1	8	
BB4	0.5	4	64	
BB6	0.06	1	>64	
BB8	0.5	1	>64	
MC6005	0.5	8	>64	
MC6025	0.25	8	>64	
B87	0.06	0.5		
MC5812	0.125	2		
MC5922	0.06	0.5		
MC5785	0.06	0.25		
MC5960	1	0.25		4
MC6031	0.06	1		8
MC5605	0.125	4		
MC5908	0.125	4		
MC5901	0.06	1		
MC5931	0.5	4		
BB10	0.5	0.5		2
MC6111	0.5	16		>64
MC6136	1	>16		>64
MC6153	0.5	8		>64
MIC_{50}	0.25	2		
MIC_{90}	0.50	8		

TABLE 2 MICs of TP-271, doxycycline, and amikacin against *Mycobacterium fortuitum*

Isolate	MIC (μg/ml)		
	TP-271	Doxycycline	Amikacin
3349	0.015	32	1
3107	0.03	256	2
3499	0.0038	£0.25	0.125
3357	0.03	64	0.25
3488	0.03	256	0.5
3126	0.015	64	0.25
3480	0.03	128	0.25
3316	0.015	32	0.06
3490	0.015	64	0.125
3276	0.06	2	0.25
3579	0.03	16	0.25
3481	0.06	≤0.25	0.25
3315	0.015	64	0.25
3484	0.015	64	2
3489	≤0.001	1	0.125
3491	0.0019	≤0.25	0.5
2797	0.015	128	0.5
3442	0.125	≤0.25	2
3423	0.125	≤0.25	2
3502	0.015	128	2
3562	0.0075	≤0.25	0.25
2491	0.015	128	1
MIC_{50}	0.015	32	0.25
MIC_{90}	0.06	128	2

final concentration of approximately 1×10^5 CFU/ml (working stock) in MH broth. The inocula used were measured by titration and plated on MH II agar (Becton, Dickinson, Sparks, MD). Fifty microliters of the working stock was added to each well containing compound. The microtiter plates were covered with SealPlate adhesive sealing film (Exel Scientific, Wrightwood, CA) prior to being incubated at 37°C in ambient air prior to reading (4 days for *M. abscessus* and 2 to 3 days for M. *fortuitum*). Each isolate was tested in duplicate. The CLSI recommends a 4-day incubation period for *Nocardia*; however, some of our isolates grew slowly, requiring 7 days of incubation. For consistency, all *Nocardia* plates were incubated for 7 days. The MIC was defined as the lowest concentration of antimicrobial agent yielding no visible turbidity.

The MIC_{50} and MIC_{90} of TP-271 and MOX against *M. abscessus* isolates were 0.25 μg/ml and 0.5 μg/ml for TP-271 and 2 μg/ml and 8 μg/ml for MOX, respectively; the MIC range for TP-271 was 0.06 to 1 μg/ml, and that for MOX was 0.25 to >16 μg/ml (Table 1). TET and DOX were tested against subsets of these organisms and were found to be much less active than TP-271. The MIC ranges for TET and DOX were 8 μg/ml to >64 μg/ml ($n = 8$) and 2 μg/ml to 64 μg/ml ($n = 7$), respectively.

The MIC_{50} and MIC_{90} for TP-271, DOX, and AMK against *M. fortuitum* isolates were 0.015 μg/ml and 0.06 μg/ml, 32 μg/ml and 128 μg/ml, and 0.25 μg/ml and 2 μg/ml, respectively (Table 2). The MIC range for TP-271 was ≤0.001 to 0.125 μg/ml, the range for DOX was ≤0.25 to 256 μg/ml, and the range for AMK was 0.06 to 2 μg/ml.

The MIC_{50} and MIC_{90} for TP-271 and TET against *Nocardia* spp. were 2 μg/ml and 8 μg/ml and 32 μg/ml and 64 μg/ml, respectively (Table 3). The MIC range for TP-271 was 0.06 to 16 μg/ml, and the range for DOX was 2 to 64 μg/ml.

Our results indicate that TP-271 has promising *in vitro* activity against *M. abscessus* and *M. fortuitum* isolates, with somewhat less

TABLE 3 MICs of TP-271 and tetracycline against *Nocardia* spp.

Isolate	MIC (μg/ml)	
	TP-271	Doxycycline
6	4	16
1260	2	32
7	16	64
651	2	16
2	0.06	64
16	2	16
8	2	32
2039	1	16
3840	1	16
12	4	32
1276	1	32
14	4	32
9B	1	8
71743	8	32
5	8	32
5	8	64
32	4	32
9A	1	2
11	8	64
MIC_{50}	2	32
MIC_{90}	8	64

activity against *Nocardia* spp. The activity of TP-271 was much better than those of other orally available tetracycline-related compounds (8). Infections due to these organisms are not common. Treatment of these infections is challenging, particularly for *M. abscessus* due to the lack of effective agents and the immunosuppressed population affected. It appears that further evaluation of TP-271 in animal studies should be performed to assess its potential *in vivo* activity.

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