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In Vitro Activity of JPC 2067 Alone and in Combination with Sulfamethoxazole against *Nocardia* Species

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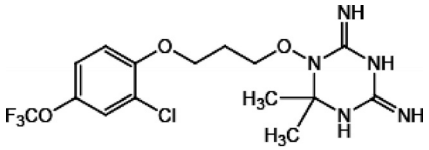
JPC 2067 is a novel dihydrotriazine dihydrofolate reductase inhibitor that is being developed as an antimalarial therapeutic. We evaluated the *in vitro* activity of JPC 2067 alone and in combination with sulfamethoxazole (SMX) against a panel of nocardia isolates. The MIC_{50}s and MIC_{90}s for JPC 2067, SMX, and the combination were 0.125 μg/ml and 4 μg/ml, 16 μg/ml and 32 μg/ml, and 0.03 μg/ml and 2 μg/ml, respectively. JPC 2067 alone and in combination with SMX should be evaluated further to understand its clinical potential.

Nocardiae are aerobic actinomycetes that are responsible for localized and disseminated infections of the skin, lungs, subcutaneous tissue, and central nervous system, usually in immunocompromised patients. Current treatment options for nocardial infections include amikacin, imipenem, ceftriaxone, or sulfamethoxazole (SMX) with the dihydrofolate reductase (DHFR) inhibitor trimethoprim (TMP) (3). SMX enhances the activity of TMP by inhibiting dihydropteroate synthase, which prevents the conversion of bacterial para-aminobenzoic acid to dihydrofolic acid (5). TMP-SMX has been used as an antibacterial agent in treatment of clinical nocardial lung infections (5). JPC 2067, a novel dihydrotriazine DHFR inhibitor (Fig. 1) being developed as an antimalarial agent, has demonstrated *in vitro* and *in vivo* activity against *Toxoplasma gondii* (4) and *in vitro* activity against *Mycobacterium tuberculosis and Mycobacterium kansasii* (data not shown). Previously, we demonstrated that PS-22 (a DHFR inhibitor), the cyclic metabolite of WR99120, a biguanide compound, had promising *in vitro* activity alone and in combination with SMX against *Nocardia* species (6). The purpose of this study was to evaluate the *in vitro* activities of JPC 2067 alone and in combination with SMX against a group of clinical nocardia isolates.

JPC 2067 was provided by Jacobus Pharmaceutical Co. (Princeton, NJ). SMX was purchased from Sigma Chemical Co. (St. Louis, MO). Each drug was dissolved in dimethyl sulfoxide (DMSO) at a final concentration of 1 mg/ml, and aliquots were frozen at −20°C. The drugs were thawed prior to testing and diluted in Mueller-Hinton (MH) broth (Becton Dickinson, Sparks, MD). JPC 2067 and SMX were tested alone at concentrations from 64 μg/ml to 0.06 μg/ml. For combination testing, SMX was evaluated at a fixed concentration of 1 μg/ml and JPC 2067 at concentrations from 16 μg/ml to 0.015 μg/ml. Twenty-eight nocardia isolates (from the American Type Culture Collection and clinical isolates from Barbara Body and Betty Ann Forbes) were used in the study.

An *in vitro* microtiter broth dilution method similar to that suggested by the Clinical and Laboratory Standards Institute (CLSI) (7) was utilized with some 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 2-fold diluted. The frozen bacterial cultures were thawed and diluted to a final concentration of about 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 μl of the working stock was added to each well. The microtiter plates were covered with SealPlate adhesive sealing film (Excel Scientific, Wrightwood, CA) prior to being incubated at 37°C in ambient air. The CLSI recommends a 4-day incubation period; however, some of our isolates grew slowly, requiring 7 days of incubation. For consistency, all plates were incubated for 7 days. CLSI recommends that 80% inhibition of growth, compared to the growth in the control well with no drug, be used as an endpoint for SMX. In this study, the MIC was defined as the lowest concentration of drug yielding no visible growth for both JPC 2067 and SMX. Each isolate was tested in duplicate.



JPC 2067 (dihydrotriazine)

FIG 1 JPC 2067.

The MIC_{50}s and MIC_{90}s for JPC 2067, SMX, and the combination (with the SMX concentration fixed at 1 μg/ml) were 0.125 μg/ml and 4 μg/ml, 16 μg/ml and 32 μg/ml, and 0.03 μg/ml and 2 μg/ml, respectively (Table 1). The MICs of JPC 2067 ranged from 0.015 μg/ml to 8 μg/ml. In future studies it would be useful to determine the species of nocardia isolates based on molecular taxonomy to determine if there are species-specific susceptibility patterns with JPC 2067 (2). The MICs of SMX ranged from 1 μg/ml to 64 μg/ml. The MICs of the combination of JPC 2067 + SMX ranged from 0.0038 μg/ml to 4 μg/ml.

These results indicate that JPC 2067 is active against *Nocardia*

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TABLE 1 MICs of JPC 2067 and SMX alone and in combination against various *Nocardia* spp.

Isolate or MIC type	MIC (μg/ml)		
	JPC 2067	SMX	JPC 2067 + SMX[a]
1964	8	16	2
2039	0.06	2	0.015
651	0.125	16	0.06
1170	0.125	8	0.015
1260	0.25	16	0.06
720	0.125	8	0.03
22	0.25	16	0.03
9	4	1	0.015
F71743	0.125	16	0.03
W34480	0.015	2	0.0038
S3840	0.125	32	0.03
13	2	8	2
4	4	16	4
3318	1	32	0.5
1	2	64	0.5
6	0.5	16	0.125
1276	0.5	4	0.03
11	0.06	8	0.03
8	0.25	16	0.06
7	0.125	8	0.015
2	4	16	4
10	0.125	8	0.015
5	0.125	16	0.06
14	0.125	8	0.03
12	0.125	16	0.03
2497	4	64	0.5
243	4	2	0.25
99	0.06	2	0.015
MIC_{50}	0.125	16	0.03
MIC_{90}	4	32	2

[a] The concentration of SMX when in combination with JPC-2067 was fixed at 1 μg/ml.

spp. In a previous study, the MICs of PS-22 against a similar panel of nocardia isolates ranged from 0.5 μg/ml to 16 μg/ml (6). The range of MICs for TMP against the same panel of nocardia isolates used in this study was 64 μg/ml to 256 μg/ml (data not shown). The combination of JPC 2067 and SMX (at 1 μg/ml) displayed enhanced activity against these isolates compared to JPC 2067 alone. In the prior study with the PS-22 metabolite (6), SMX used at a concentration of 10 μg/ml demonstrated greater enhancement of *in vitro* activity than we observed in the current study with JPC 2067 in combination with SMX at 1 μg/ml. It is likely that the activity of JPC 2067 in combination with SMX would be further augmented if the concentration of SMX was increased to 10 μg/ml, a readily achievable serum level for this agent (1). Additional testing of JPC 2067 alone and in combination with SMX should be explored *in vitro* and in animal models of nocardial infection to understand the clinical potential of these agents.

REFERENCES

1. **Braude AI.** 1976. Pharmacologic principles, p 68. *In* Antimicrobial Drug Therapy. WB Saunders, Philadelphia, PA.
2. **Brown-Elliott B, Brown JM, Conville PS, Williams RJ, Jr.** 2006. Clinical and laboratory features of the *Nocardia* spp. based on current molecular taxonomy. Clin. Microbiol. Rev. **19**:259–282.
3. **Lerner PI.** 1996. Nocardiosis. Clin. Infect. Dis. **22**:891–903.
4. **Mui EJ, et al.** 2008. Novel triazine JPC-2067-B Inhibits *Toxoplasma gondii in vitro* and *in vivo*. PLoS Negl. Trop. Dis. **2**(3):e190. doi:10.1371/journal.pntd. 0000190.
5. **Smego RA, Jr, Moeller MB, Gallis HA.** 1983. Trimethoprim-sulfamethoxazole therapy for Nocardia infections. Arch. Intern. Med. **143**: 711–718.
6. **Thielking DM, DeStefano MS, Cynamon MH, Yeo AET.** 2003. Enhanced *in vitro* activity of dihydrofolate reductase and dihydropteroase synthase inhibitors in combination against *Nocardia spp.* Antimicrob. Agents Chemother. **47**:1174.
7. **Woods GL, et al.** 2011. Susceptibility testing of mycobacteria, nocardiae, and other aerobic actinomycetes; approved standard—second edition. CLSI document M24–A2. CLSI, Wayne, PA.