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In Vitro Activity of a New Antibiotic, NVP-PDF386 (VRC4887), against *Chlamydia pneumoniae*

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The in vitro activity of NVP-PDF386 (VRC4887), a novel new peptide deformylase inhibitor, and those of levofloxacin and clarithromycin were tested against 21 isolates of *Chlamydia pneumoniae*. The MIC at which 90% of the isolates were inhibited and the minimal bactericidal concentration at which 90% of the isolates were killed by NVP-PDF386 for all isolates of *C. pneumoniae* were 0.008 μg/ml (range, 0.008 to 0.015 μg/ml) compared to 0.25 and 0.06 μg/ml for levofloxacin and clarithromycin, respectively.

Chlamydia pneumoniae is a frequent cause of community-acquired respiratory tract infection, including pneumonia and bronchitis, in adults and children (2). Peptide deformylase (PDF) is a necessary enzyme in bacterial protein synthesis (4). PDF deformylates the *N*-formylmethionine of newly synthesized polypeptides and represents a novel target for antibacterial chemotherapy. The gene encoding PDF (*def*) is essential for protein synthesis in a variety of pathogenic bacteria but is not required for mammalian protein synthesis (4). Preliminary studies have shown that PDF inhibitors are active against a wide range of bacterial respiratory pathogens, including *Streptococcus pneumoniae*, *Streptococcus pyogenes*, and *Moraxella catarrhalis* (3; C. Hackbarth, S. Lopez, W. Wang, J. Jacobs, R. Rain, Z. J. Ni, J. Trias, D. Chen, G. Withers, D. V. Patel, and Z. Yuan, Abstr. 40th Intersci. Conf. Antimicrob. Agents Chemother., abstr. 2173, 2000). Data on activity of these compounds against respiratory pathogens responsible for atypical pneumonia, including *C. pneumoniae* and *Mycoplasma pneumoniae*, are limited (3). We compared the activity of NVP-PDF386 (VRC4887), a new PDF inhibitor, and those of levofloxacin and clarithromycin against *C. pneumoniae.*

Isolates of *C. pneumoniae* tested included two reference isolates, TW183, obtained from the Washington Research Foundation (Seattle, Wash.), and AR39 (ATCC VR-2282); CM-1, a clinical isolate from the Centers for Disease Control and Prevention (ATCC VR-1360); J21, an isolate from Japan; and 17 recent clinical isolates from adults enrolled in a multicenter community-acquired pneumonia treatment study conducted in the United States (isolates 25001, 21001, 21002, 08002, 808016, 22012A, 22012, 24013A, 24013, 493, 453, 124, 490, 600, 912, and 109). NVP-PDF386 (Novartis, Summit, N.J.), levofloxacin (Ortho Pharmaceuticals, Raritan, N.J.), and clarithromycin (Abbott Laboratories, Abbott Park, Ill.) were supplied as drug substances in powder form and were solubilized in accordance with the manufacturers' instructions. Susceptibility testing of *C. pneumoniae* was performed in cell culture by using HEp-2 cells grown in 96-well microtiter plates as previously described (1, 2). Each experiment was set up in duplicate plates. Each well was inoculated with 0.1 ml of the test organism diluted to yield 10^3 to 10^4 inclusion-forming units/ml for a multiplicity of infection of 1:1; each well was centrifuged at 1,700 × *g* for 1 h and incubated at 35°C for 1 h. Wells were then aspirated and overlaid with 0.2 ml of medium containing 1 μg of cycloheximide per ml and serial twofold dilutions of the test drug. After incubation at 35°C for 72 h, the cultures in one plate were fixed and stained for inclusions with fluorescein-conjugated antibody to the lipopolysaccharide genus antigen (Pathfinder; Bio-Rad Labs, Hercules, Calif.). The MIC was the lowest antibiotic concentration at which no inclusions were seen. The minimal bactericidal concentration (MBC) was determined by aspirating the antibiotic-containing medium of the second plate, washing wells twice with phosphate-buffered saline, and adding antibiotic-free medium. Cultures were frozen at −70°C, thawed, passed onto fresh new cells, incubated for 72 h, and then fixed and stained as above. The MBC was the lowest antibiotic concentration that resulted in no inclusions after passage. Three replicates were conducted for each assay.

The MICs and MBCs for *C. pneumoniae* are shown in Table 1. The MIC at which 90% of the strains are inhibited (MIC_{90}) and MBC_{90} for NVP-PDF386 were 0.008 μg/ml compared to 0.25 and 0.06 μg/ml for levofloxacin and clarithromycin, respectively. The MICs obtained for NVP-PDF386 against *C. pneumoniae* in the present study were very consistent, especially in view of the wide geographic distribution of the isolates tested.

PDF is an interesting bacterial target that was discovered more than 30 years ago but has only recently been exploited. Over the past several years, a number of compounds have been

TABLE 1. Activity of NVP-PDF 386, levofloxacin, and clarithromycin against 21 isolates of *C. pneumoniae*

Compound or drug	MIC (μg/ml)			MBC (μg/ml)	
	Range	50%	90%	Range	90%
NVP-PDF 386	0.008–0.015	0.008	0.008	0.008–0.015	0.008
Levofloxacin	0.125–0.25	0.25	0.25	0.125–0.25	0.25
Clarithromycin	0.015–0.06	0.03	0.06	0.015–0.06	0.06

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evaluated in vitro and in animal studies. Recently, Wise et al. (3) evaluated the in vitro activity of six PDF compounds, namely, BB-3497, BB-83698, BB-83857, BB-84416, and BB-84518. The MIC_{90}s against *S. pneumoniae* and *Haemophilus influenzae* ranged from 0.5 to $\geq$ 6 μg/ml and from 2 to $\geq$16 μg/ml, respectively. The compounds were more active against *M. catarrhalis*, with MIC_{90}s ranging from 0.06 to 0.25 μg/ml. They also tested these compounds against one isolate of *C. pneumoniae*, TW-183, and two strains of *Chlamydia trachomatis.* The MICs for *C. pneumoniae* and *C. trachomatis* ranged from 0.5 to 2 μg/ml and 0.25 to 4 μg/ml, respectively. BB-83857 appeared to be the most consistently active compound. There are no published data on the activity of NVP-PDF386 against respiratory tract bacteria, although the activity of several related compounds against *S. pneumoniae*, *M. catarrhalis*, and *H. influenzae* appears to be similar to the results reported by Wise et al. (3; Hackbarth et al., 40th ICAAC). In contrast, NVP-PDF386 was extremely active against *C. pneumoniae*, with an MIC_{90} of 0.008 μg/ml, which was 7 to 30 times more active than clarithromycin and levofloxacin and 60 to 250 times more active against chlamydia than the PDF compounds studied by Wise et al. (3). The activity of NVP-PDF386 against *C. pneumoniae* was similar to that found previously with the ketolide ABT-773 and the des-quinolone BMS-284756 (MIC_{90} for both compounds, 0.015 μg/ml) (1, 2). Preliminary data have also shown that NVP-PDF386 has similar high activity against *M. pneumoniae* (C. Hackbarth and Z. Yuan, Versicor, personal communication). These data suggest that PDF inhibitors may have a potential role in the treatment of respiratory infections due to *C. pneumoniae* and other causes of atypical pneumonia.

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