ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Jan. 2007, p. 361–365 Vol. 51, No. 1
0066-4804/07/$08.00+0 doi:10.1128/AAC.01017-06

In Vitro Antibacterial Activity of the Pyrrolopyrazolyl-Substituted Oxazolidinone RWJ-416457[▽]

Barbara D. Foleno,[1]* Darren Abbanat,[1] Raul M. Goldschmidt,[1] Robert K. Flamm,[2]† Steve D. Paget,[1] Glenda C. Webb,[1] Ellyn Wira,[1] Mark J. Macielag,[1] and Karen Bush[1]

Johnson & Johnson Pharmaceutical Research & Development, L.L.C., Raritan, New Jersey 08869,[1] and Focus Bio-Inova, Inc., Herndon, Virginia 20171[2]

Received 14 August 2006/Returned for modification 12 September 2006/Accepted 30 October 2006

RWJ-416457, an investigational pyrrolopyrazolyl-substituted oxazolidinone, inhibited the growth of linezolid-susceptible staphylococci, enterococci, and streptococci at concentrations of ≤4 μg/ml, generally exhibiting two- to fourfold-greater potency than that of linezolid. Time-kill studies demonstrated bacteriostatic effects for both RWJ-416457 and linezolid.

Gram-positive bacteria are a frequent cause of infection among patients in the hospital and community (4). The emergence of vancomycin-resistant *Staphylococcus aureus* and the increasing incidence of community-acquired methicillin-resistant *S. aureus* (MRSA) (6), penicillin- and macrolide-resistant *Streptococcus pneumoniae* (2, 8), and vancomycin-resistant enterococci are indications of the increasing resistance of gram-positive bacteria to available antimicrobial agents and provide an impetus to discover novel or more efficacious antimicrobial agents (2–4).

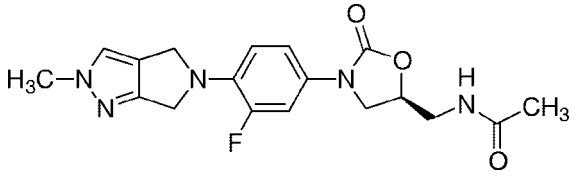
Oxazolidinones are one of the newest classes of antibacterial agents, with linezolid as the only member of this class to gain regulatory approval thus far (14). Linezolid is currently approved to treat skin and respiratory infections caused by gram-positive pathogens, including multidrug-resistant staphylococci, enterococci, and streptococci (14). It is currently the only drug that can be used both orally and parenterally to treat infections caused by MRSA and vancomycin-resistant enterococci. In the quest for a new oxazolidinone with enhanced characteristics compared with linezolid, RWJ-416457 (Fig. 1), an investigational, orally active, oxazolidinone, was identified (7a, 11a).

This study describes the in vitro activity of RWJ-416457 against clinically important bacteria using linezolid and other relevant agents as comparators. Targeted organisms included susceptible and multidrug-resistant staphylococci, enterococci, and pneumococci, *Haemophilus influenzae*, and *Moraxella catarrhalis* from the Johnson & Johnson Pharmaceutical Research & Development culture collection and the atypical intracellular respiratory tract pathogens *Chlamydia pneumoniae*, *Legionella pneumophila*, and *Mycoplasma pneumoniae* from Focus Bio-Inova.

MICs were determined by the CLSI (formerly NCCLS) broth microdilution procedure for susceptibility testing for bacteria that grow aerobically (11). *C. pneumoniae* susceptibility testing was performed in HEp-2 cells by the method of Hammerschlag et al. (7). Frozen aliquots of *C. pneumoniae* were diluted in antibiotic-free *Chlamydia* overlay medium (COM) and inoculated into a 72-h monolayer of HEp-2 cells. After preincubation, COM plus antibiotic were serially diluted in twofold steps. Following a 72-h incubation at 35°C, the monolayer was fixed and stained. The MIC end point was determined as the lowest concentration that showed significant or complete inhibition of inclusion-forming units. *M. pneumoniae* susceptibility was determined by the microbroth method of Waites et al. (13). Serial 10-fold dilutions of each isolate were prepared in SP4 glucose broth (Remel) to determine the number of color changing units (CCU). Microtiter plates were prepared with antibiotics serially diluted in antibiotic-free SP4 glucose broth, inoculated (10^4 CCU/ml), and incubated at 35°C until color changed in control wells. The MIC end point was the lowest concentration at which growth was inhibited on the basis of color change. *L. pneumophila* susceptibility was performed by broth microdilution. Briefly, the inoculum was suspended in buffered yeast extract broth and transferred to microtiter wells containing serial dilutions of antibiotic and incubated aerobically at 34°C to 36°C for 48 h. The MIC end point was determined as the lowest concentration that inhibited visible growth.

Time-kill experiments with RWJ-416457 and linezolid were performed at four times the MIC by CLSI methodology (10) against *S. pneumoniae* ATCC 6305 and OC 4446, *Enterococcus faecalis* ATCC 29212 and ATCC 51299, and *S. aureus* ATCC 29213 and MRSA OC 2878. The limit of detection was 100 CFU/ml.

Oxazolidinones have a unique mechanism of action, inhib-



RWJ-416457

FIG. 1. Structure of RWJ-416457.

* Corresponding author. Mailing address: Johnson & Johnson Pharmaceutical Research & Development, L.L.C., Raritan, NJ 08869. Phone: (908) 704-4212. E-mail: bfoleno@prdus.jnj.com.

† Present address: Johnson & Johnson Pharmaceutical Research & Development, L.L.C., Raritan, NJ 08869.

▽ Published ahead of print on 13 November 2006.

TABLE 1. In vitro activities of RWJ-416457 and comparators against gram-positive bacteria and respiratory tract pathogens

Organism	No. of isolates	Compound	MIC range (μg/ml)	MIC_{50} (μg/ml)	MIC_{90} (μg/ml)
S. aureus					
All	191	RWJ-416457	0.25–16	1	2
	191	Linezolid	0.5–>32	4	4
	181	Vancomycin	0.5–8	1	4
Methicillin-susceptible	72	RWJ-416457	0.5–4	1	2
		Linezolid	2–4	2	4
		Vancomycin	1–2	1	2
Methicillin-resistant (hospital-associated MRSA, linezolid-susceptible)[a]	102	RWJ-416457	0.25–4	1	2
		Linezolid	0.5–4	4	4
		Vancomycin	0.5–8	1	4
		Oxacillin	4–>32	>32	>32
Vancomycin-intermediate (MRSA)[a]	13	RWJ-416457	0.25–2	0.5	2
		Linezolid	0.5–4	1	4
		Vancomycin	2–8	4	8
		Oxacillin	4–>32	>32	>32
Linezolid-resistant (MRSA)[a]	4	RWJ-416457	4–16	NA[b]	NA
		Linezolid	8–>32	NA	NA
		Vancomycin	1–2	NA	NA
		Oxacillin	>32	NA	NA
Community-acquired MRSA	13	RWJ-416457	1–2	1	2
		Linezolid	2–4	4	4
		Vancomycin	1	1	1
		Oxacillin	16–32	32	32
Coagulase-negative staphylococci					
All	67	RWJ-416457	0.25–1	0.5	1
		Linezolid	0.5–2	2	2
		Vancomycin	0.5–4	2	4
		Oxacillin	0.12–>16	>16	>16
Methicillin-susceptible	14	RWJ-416457	0.25–1	0.5	0.5
		Linezolid	1–2	1	2
		Vancomycin	1–4	2	4
		Oxacillin	0.12–0.25	0.12	0.25
Methicillin-resistant	53	RWJ-416457	0.25–1	0.5	1
		Linezolid	0.5–2	2	2
		Vancomycin	0.5–4	2	4
		Oxacillin	0.5–>16	>16	>16
E. faecalis					
Linezolid-susceptible	42	RWJ-416457	0.5–2	0.5	1
		Linezolid	1–4	2	2
		Vancomycin	1–>16	4	>16
Linezolid-resistant	1	RWJ-416457	32	NA	NA
		Linezolid	64	NA	NA
		Vancomycin	>128	NA	NA
E. faecium					
Linezolid-susceptible	36	RWJ-416457	0.25–1	1	1
		Linezolid	0.5–4	2	4
		Vancomycin	1–>16	>16	>16
Linezolid-resistant	2	RWJ-416457	16	NA	NA
		Linezolid	32–64	NA	NA
		Vancomycin	>128	NA	NA
Enterococcus gallinarum (linezolid-resistant)	1	RWJ-416457	16	NA	NA
		Linezolid	64	NA	NA
		Vancomycin	>128	NA	NA

Continued on facing page

TABLE 1—*Continued*

Organism	No. of isolates	Compound	MIC range (μg/ml)	MIC_{50} (μg/ml)	MIC_{90} (μg/ml)
Streptococcus pyogenes (37 macrolide-resistant isolates)	39	RWJ-416457	0.25–0.5	0.5	0.5
		Linezolid	1–2	1	2
		Erythromycin	0.03–>16	8	>16
S. pneumoniae All	108	RWJ-416457	0.06–1	0.5	0.5
		Linezolid	0.25–2	1	1
Penicillin-resistant	31	RWJ-416457	0.25–1	0.5	0.5
		Linezolid	0.5–2	1	1
		Penicillin	2–4	2	4
Macrolide-resistant	51	RWJ-416457	0.25–1	0.5	0.5
		Linezolid	0.5–2	1	1
		Erythromycin	1–>16	4	>16
Fluoroquinolone-resistant	20	RWJ-416457	0.06–1	0.5	0.5
		Linezolid	0.5–2	0.5	1
		Levofloxacin	8–>32	16	>16
H. influenzae	4	RWJ-416457	8–>16	NA	NA
		Linezolid	4–16	NA	NA
		Erythromycin	2–8	NA	NA
M. catarrhalis	12	RWJ-416457	2–4	2	4
		Linezolid	4–8	8	8
		Erythromycin	0.12–0.25	0.25	0.25
L. pneumophila	17	RWJ-416457	2–64	4	8
		Linezolid	1–64	4	16
		Levofloxacin	0.03–0.5	0.12	0.5
M. pneumoniae	17	RWJ-416457	16–32	16	32
		Linezolid	64–>64	>64	>64
		Levofloxacin	0.5–1	0.5	1
C. pneumoniae	3	RWJ-416457	16–64	NA	NA
		Linezolid	64–>64	NA	NA
		Levofloxacin	0.5	NA	NA

[a] Isolates received through the Network on Antimicrobial Resistance in *S. aureus* (NARSA) program.
[b] NA, not applicable.

iting bacterial protein synthesis by binding at the peptidyl transferase center of the 50S ribosomal subunit; studies suggest this binding interferes with ribosomal assembly and formation of the initial peptide bond (1, 5, 9, 12). In a bacterial cell-free transcription-dependent translation assay system, RWJ-416457 inhibited protein synthesis at a concentration twofold lower than linezolid, with 50% inhibitory concentrations of 1.4 and 2.9 μM, respectively (D. Montenegro and A. M. Queenan, unpublished data).

The in vitro antibacterial activities of RWJ-416457 and linezolid against *S. aureus* and coagulase-negative staphylococci (CoNS) are shown in Table 1. RWJ-416457 had MIC_{90} values of ≤2 μg/ml against staphylococci (including methicillin-susceptible *Staphylococcus aureus* [MSSA], MRSA, vancomycin-intermediate *S. aureus*, and CoNS) (Table 1), consistently twofold lower than those of linezolid. Against a single vancomycin-resistant *S. aureus* isolate, RWJ-416457 was equipotent to linezolid, with an MIC of 1 μg/ml (B. Bozdogan and P. C. Appelbaum, personal communication). Against vancomycin-susceptible and -resistant *E. faecalis* and *Enterococcus faecium*, RWJ-416457 had MIC_{90} values of 1 μg/ml, two- and fourfold lower than those of linezolid, respectively (Table 1). As illustrated by the MIC distributions of RWJ-416457 and linezolid against *S. aureus* (MSSA and MRSA), *E. faecium*, and *E. faecalis* (Fig. 2), individual MICs of RWJ-416457 were generally two- to fourfold lower than those of linezolid against these pathogens.

Against linezolid-resistant staphylococci and enterococci, RWJ-416457 MICs ranged from 4 to 32 μg/ml, values that were again two- to fourfold lower than those of linezolid. Because concentrations of 16 and 32 μg/ml are not likely to be achieved clinically on the basis of the anticipated drug levels and pharmacodynamic profile (6a), this compound is not expected to have utility against most linezolid-resistant isolates.

When tested against respiratory pathogens, the MIC_{90} of RWJ-416457 for penicillin-, macrolide-, and fluoroquinolone-resistant *S. pneumoniae* was 0.5 μg/ml, twofold lower than that of linezolid. RWJ-416457 MICs were 2 to >16 μg/ml against the gram-negative pathogens *H. influenzae* and *M. catarrhalis*. RWJ-416457 and linezolid had MIC_{90} values of 8 and 16 μg/ml, respectively, against *L. pneumophila* and MICs of ≥16 and ≥64 μg/ml, respectively, against *M. pneumoniae* and *C. pneu-*

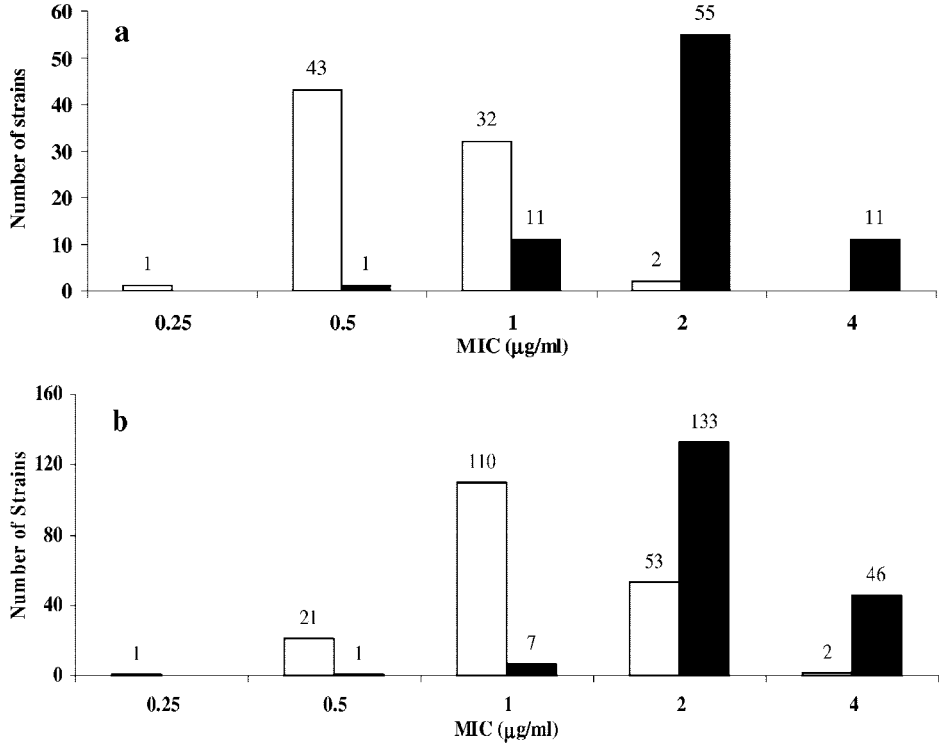


FIG. 2. (a) MIC distribution of RWJ-416457 and linezolid against enterococci (36 *E. faecium* isolates and 42 *E. faecalis* isolates). (b) MIC distribution of RWJ-416457 and linezolid against *S. aureus* (72 MSSA isolates, 102 hospital-associated MRSA isolates, and 13 community-acquired MRSA isolates). The white bars depict RWJ-416457 values, and the black bars depict linezolid values. The numbers of isolates are indicated above the bars.

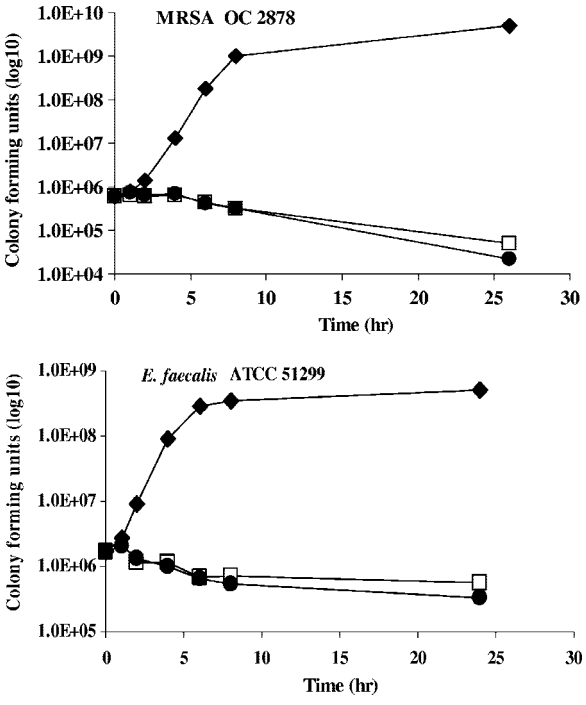


FIG. 3. Time-kill analysis of isolates treated with RWJ-416457 or linezolid at four times the MIC. Isolates were grown in control medium (◆), medium with RWJ-416457 (□), and medium with linezolid (●). Against MRSA OC 2878, the RWJ-416457 MIC was 1 μg/ml and the linezolid MIC was 2 μg/ml; against *E. faecalis* ATCC 51922, the RWJ-416457 MIC was 1 μg/ml and the linezolid MIC was 4 μg/ml.

moniae. Thus, although RWJ-416457 generally retained at least a twofold advantage in potency over linezolid against the atypical respiratory tract pathogens, MICs of both agents frequently exceeded 8 μg/ml.

Time-kill experiments demonstrated that both RWJ-416457 and linezolid were bacteriostatic against the six *S. pneumoniae*, *S. aureus*, and *E. faecalis* strains tested at four times the MIC levels (Fig. 3 for representative time-kill curves). These observations are consistent with the protein synthesis inhibitory mechanism of action for both agents.

In conclusion, the phase 1 investigational oxazolidinone RWJ-416457 demonstrated inhibitory activity against many bacteria resistant to vancomycin, macrolides, and fluoroquinolones. RWJ-416457 generally had two- to fourfold-lower MICs than those of linezolid against most pathogens tested. Its in vitro microbiological activity warrants further investigation to determine an appropriate dosing regimen, in addition to its safety and efficacy in human trials.

We thank Deborah Montenegro and Anne Marie Queenan for conducting protein synthesis assays.

REFERENCES

1. **Barbachyn, M. R., and C. W. Ford.** 2003. Oxazolidinone structure-activity relationships leading to linezolid. Angew. Chemie **42:**2010–2023.
2. **Bush, K.** 2004. Antibacterial drug discovery in the 21st century. Clin. Microbiol. Infect. **10:**(Suppl. 4):10–17.
3. **Bush, K.** 2004. Why it is important to continue antibacterial drug discovery. ASM News **70:**282–286.
4. **Clark, N. M., E. Hershberger, M. J. Zervosc, and J. P. Lynch III.** 2003. Antimicrobial resistance among gram-positive organisms in the intensive care unit. Curr. Opin. Crit. Care **9:**403–412.
5. **Colca, J. R., W. G. McDonald, D. J. Waldon, L. M. Thomasco, R. C.**

Gadwood, E. T. Lund, G. S. Cavey, W. R. Mathews, L. D. Adams, E. T. Cecil, J. D. Pearson, J. H. Bock, J. E. Mott, D. L. Shinabarger, L. Xiong, and A. S. Mankin. 2003. Cross-linking in the living cell locates the site of action of oxazolidinone antibiotics. J. Biol. Chem. **278:**21972–21979.

6. **Diederen, B. M. W., and J. Kluytmans.** 2006. The emergence of infections with community-associated methicillin resistant *Staphylococcus aureus*. J. Infect. **52:**157–168.

6a. **Drusano, G. L., W. Liu, K. Bush, M. R. Deziel, M. Drusano, and A. Louie.** 2005. Pharmacodynamics (PD) of RWJ-416457, a new oxazolidinone antibiotic, in a neutropenic mouse thigh model of *Staphylococcus aureus* (SA) infection, abstr. F-1248, p. 196. Abstr. 45th Intersci. Conf. Antimicrob. Agents Chemother. American Society for Microbiology, Washington, DC.

7. **Hammerschlag, M. R., K. K. Qumei, and P. M. Roblin.** 1992. In vitro activities of azithromycin, clarithromycin, L-ofloxacin, and other antibiotics against *Chlamydia pneumoniae*. Antimicrob. Agents Chemother. **36:**1573–1574.

7a. **Hilliard, J. J., J. Melton, J. Fernandez, S. Paget, M. Macielag, R. Goldschmidt, and K. Bush.** 2005. In vivo activity of the novel oxazolidinone RWJ-416457, abstr. F-1247, p. 195–196. Abstr. 45th Intersci. Conf. Antimicrob. Agents Chemother. American Society for Microbiology, Washington, DC.

8. **Klugman, K. P., and J. R. Lonks.** 2005. Hidden epidemic of macrolide-resistant pneumococci. Emerg. Infect. Dis. **11:**802–807.

9. **Livermore, D. M.** 2003. Linezolid in vitro: mechanism and antibacterial spectrum. J. Antimicrob. Chemother. **51:**(Suppl. S2)ii9–ii16.

10. **National Committee for Clinical Laboratory Standards.** 1999. Methods for determining bactericidal activity of antimicrobial agents. Approved guideline M26-A. National Committee for Clinical Laboratory Standards, Wayne, PA.

11. **National Committee for Clinical Laboratory Standards.** 2003. Methods for dilution antimicrobial susceptibility testing for bacteria that grow aerobically. Approved standard, 6th ed. M7-A6. National Committee for Clinical Laboratory Standards, Wayne, PA.

11a. **Paget, S. D., C. M. Boggs, B. D. Foleno, R. M. Goldschmidt, M. Weidner-Wells, E. Wira, K. Bush, and M. Macielag.** 2005. Synthesis and in vitro antibacterial activity of pyrrolopyrazolyl-substituted oxazolidinones, abstr. F-1242, p. 194. Abstr. 45th Intersci. Conf. Antimicrob. Agents Chemother. American Society for Microbiology, Washington, DC.

12. **Shinabarger, D. L., K. R. Marotti, R. W. Murray, A. H. Lin, E. P. Melchior, S. M. Swaney, D. S. Dunyak, W. F. Demyan, and J. M. Buysse.** 1997. Mechanism of action of oxazolidinones: effects of linezolid and eperezolid on translation reactions. Antimicrob. Agents Chemother. **41:**2132–2136.

13. **Waites, K. B., L. B. Duffy, T. Schmid, D. Crabb, M. S. Pate, and G. H. Cassell.** 1991. In vitro susceptibilities of *Mycoplasma pneumoniae*, *Mycoplasma hominis*, and *Ureaplasma urealyticum* to sparfloxacin and PD 127391. Antimicrob. Agents Chemother **35:**1181–1185.

14. **Wilcox, M. H.** 2005. Update on linezolid: the first oxazolidinone antibiotic. Expert Opin. Pharmacother. **6:**2315–2326.