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In Vitro Antimicrobial Activities and Spectra of U-100592 and U-100766, Two Novel Fluorinated Oxazolidinones

RONALD N. JONES,* DAVID M. JOHNSON, AND MERIDITH E. ERWIN

Medical Microbiology Division, Department of Pathology, University of Iowa College of Medicine, Iowa City, Iowa

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Two new fluorinated oxazolidinones, U-100592 and U-100766, were evaluated against more than 659 gram-positive and -negative organisms and compared with glycopeptides, erythromycin, clindamycin, clinafloxacin, and chloramphenicol. U-100592 and U-100766 were usually equally potent, but the MICs at which 90% of the isolates are inhibited (MIC_{90}s) of U-100592 for some staphylococci and enterococci were slightly lower than those of U-100766 (1 versus 2 μg/ml). The MIC_{90} of U-100592 and U-100766 for oxacillin-resistant *Staphylococcus aureus* was 2 μg/ml, the same as observed for oxacillin-susceptible strains. The oxazolidinone MICs for other *Staphylococcus* spp. were ≤2 μg/ml (MIC_{50}, 0.5 to 1 μg/ml). All enterococci were inhibited by ≤4 and ≤2 μg of U-100592 and U-100766 per ml, respectively. Against 152 vancomycin-resistant enterococci (five species), both compounds had a narrow range of MICs (0.25 to 2 μg/ml) and a MIC_{90} of 1 μg/ml. *Corynebacterium jeikeium*, *Bacillus* spp., and all tested streptococci were inhibited (≤4 μg/ml). Members of the family *Enterobacteriaceae* and other gram-negative bacilli were not susceptible (MIC_{50}, >64 μg/ml) to either oxazolidinone. Three potencies of U-100592 and U-100766 disks were tested (5, 15, and 30 μg), and acceptable correlations (r = 0.81 to 0.90) with the measured MICs were observed. Best discrimination of the tentatively susceptible organisms (MICs, ≤4 μg/ml) was demonstrated with the 30-μg disk concentration. The oxazolidinones demonstrated a dominant bacteristatic action. These oxazolidinones (U-100592 and U-100766) appear promising for treatment of gram-positive organisms that demonstrate resistance to contemporary therapeutic agents.

The oxazolidinone class of antimicrobial agents was initially investigated in the late 1980s (3–5, 13–15), particularly the compounds designated DuP 105, DuP 721, E-3556-2, E-3656-5, E-2709-5, S-6123, and XA-043. These drugs are protein synthesis inhibitors (2, 4) focused at the step preceding the interaction of fMet-$tRNA^{fMet}$ and the 30S ribosomal subunits with the initiator codon (5). This mechanism of action does not inhibit the peptide elongation phase (14) and these drugs are generally believed to be bacteristatic (3, 4, 14), but concentration-dependent killing has been described for some species (13).

The spectrum of activity for oxazolidinones includes the staphylococci (oxacillin susceptible and resistant), *Streptococcus* spp., enterococci, diphtheroids, some *Bacteroides fragilis* group anaerobes, and mycobacteria (3, 4, 13, 14). Oral bioavailability has been documented, with concentrations in serum potentially allowing clinical use against important gram-positive cocci (15). In vivo protection model results indicate an activity comparable to that of vancomycin, but a greater efficacy than that of cefoxitin against intra-abdominal infections was observed (14).

Some Upjohn Co. (Kalamazoo, Mich.) oxazolidinone compounds were originally described by Brickner et al. (2) and Kilburn et al. (7). Similar fluorinated drugs (U-100592 and U-100766) share the class spectrum of activity (1) against clinically important gram-positive species and mycobacteria (Fig. 1). U-100592 and U-100766 were studied against a large variety of clinical isolates from patients at the University of Iowa Hospitals and Clinics, Iowa City. Spectrum-of-activity comparisons with representative antimicrobial agents from the glycopeptides (teicoplanin and vancomycin), macrolides (erythromycin), fluoroquinolones (clinafloxacin), clindamycin, chloramphenicol, and representative penicillins (penicillin G and ampicillin), where appropriate, were made.

* Corresponding author. Mailing address: Department of Pathology, 5232 RCP, University of Iowa College of Medicine, Iowa City, IA 52242. Phone: (319) 356-2990. Fax: (319) 356-4916.

MATERIALS AND METHODS

Antimicrobial agents. The tested compounds were obtained from the following sources: U-100592, U-100766, and clindamycin from The Upjohn Co.; erythromycin and vancomycin from Eli Lilly & Co. (Indianapolis, Ind.); teicoplanin from Marion Merrell Dow (Kansas City, Mo.); clinafloxacin from Parke-Davis/Warner Lambert (Detroit, Mich.); and all other drugs from Sigma Chemicals (St. Louis, Mo.). Antimicrobial agent-containing disks were prepared by the investigators at concentrations of 5, 15, and 30 μg per disk.

Bacterial strains. All strains were derived from the clinical organism collection of the University of Iowa College of Medicine. The vast majority of strains were isolated from patients, usually with bloodstream infections (1993 to 1995). The distributions of tested strains are listed below (see Tables 1 [255 staphylococci, 44 enterococci, 10 *Bacillus cereus* strains, 10 *Corynebacterium jeikeium* strains, and 84 *Streptococcus* spp.], 2 [152 vancomycin-resistant enterococci] [6], and 3 [52 anaerobic bacteria, 10 *Haemophilus influenzae* strains, 14 gonococci, and 28 other gram-negative bacilli]). Among the staphylococci, 111 strains (44%) were resistant to oxacillin. Most of these isolates were also resistant to macrolides, tetracyclines, fluoroquinolones, clindamycin, and aminoglycosides.

Susceptibility testing. All testing utilized methods described in National Committee for Clinical Laboratory Standards documents (8–11). Modifications of media for fastidious organisms that were recommended for strict anaerobic bacteria (10), streptococci (9), *H. influenzae* (9), and *Neisseria* spp. (9) were applied. A subset of organisms (491 strains) was used to determine the optimal disk antimicrobial agent concentrations for these oxazolidinones, assuming a susceptible breakpoint concentration of approximately 4 μg/ml. Three concentrations (5, 15, and 30 μg) of antimicrobial agents per disk were prepared by National Committee for Clinical Laboratory Standards guidelines (11). The results of broth microdilution and agar dilution MICs were compared, and the broth microdilution MIC was compared with the zones of inhibition (diameters) around each potency disk. The statistical method of least squares was utilized for regression analyses (calculated by computer programs), and the error rate bounding method was also applied (11).

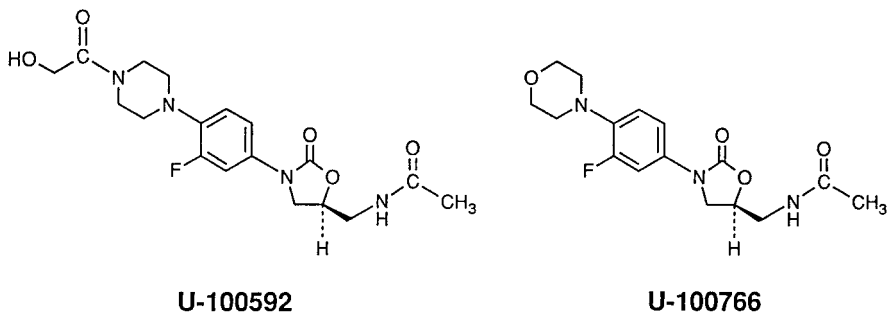

FIG. 1. Structures of U-100592 and U-100766.

RESULTS

Antimicrobial activity against gram-positive cocci. Table 1 lists the comparative antimicrobial activities of U-100592 and U-100766 against 403 isolates of staphylococci, enterococci, streptococci, and some other species. Against *Staphylococcus aureus* isolates, the two oxazolidinones were equally potent (MIC at which 90% of the isolates are inhibited [MIC_{90}], 2 μg/ml) and exhibited a narrow MIC range (0.5 to 4 μg/ml) regardless of the oxacillin resistance pattern. The glycopeptides and clinafloxacin were the most active agents tested. Similarly, the oxazolidinones were active against the coagulase-negative staphylococci (MIC_{90}s, 1 to 2 μg/ml). No species-specific variation in the activity was identified.

The MICs of U-100592 and U-100766 for vancomycin-susceptible enterococci (44 strains) ranged from 0.5 to 4 and 0.5 to 2 μg/ml, respectively. Only the glycopeptides and oxazolidinones demonstrated a broad spectrum of action against these *Enterococcus faecalis* and *Enterococcus faecium* strains.

All pathogenic streptococci tested were inhibited (MICs, ≤4 μg/ml) by the investigational oxazolidinones. Generally, U-100592 was twofold more active than U-100766 against *Streptococcus pneumoniae* and some beta-hemolytic streptococci. No difference in oxazolidinone potency was identified among strains of streptococci that possessed resistance to erythromycin and/or penicillin (data not shown). *C. jeikeium* was more susceptible to U-100592 (MIC_{90}, 0.25 μg/ml) than U-100766, and both oxazolidinones were active against *B. cereus* strains (MIC_{90}, 1 μg/ml).

Table 2 shows the oxazolidinones' activities against 152 enterococci derived from a collection of strains isolated in more

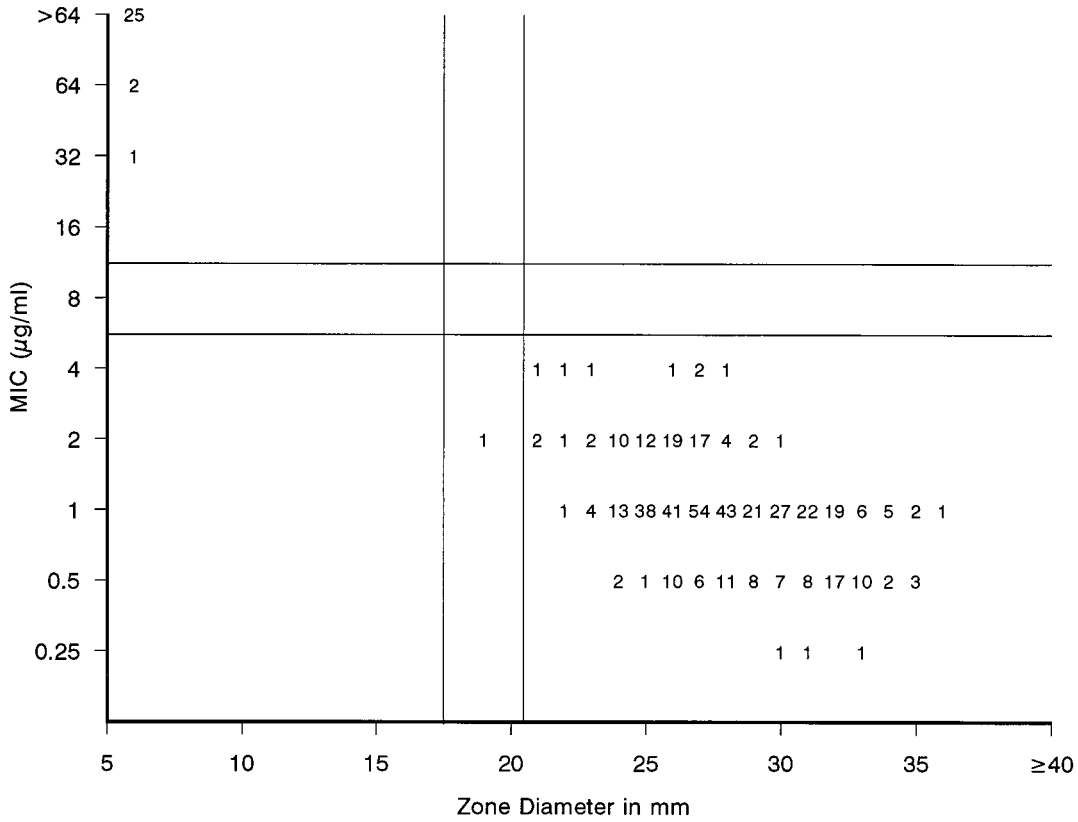

FIG. 2. Scattergram comparing the U-100592 MIC with zones of inhibition around 30-μg disks. Regression formula: $y = 16.5 - 0.27x$; $r = 0.90$. Numbers of isolates with the indicated MIC or zone diameter are shown.

TABLE 1. Antimicrobial activities of two oxazolidinones (U-100592 and U-100766) compared with those of six other compounds against 403 gram-positive cocci and bacilli

Organism (no. tested)	Antimicrobial agent	MIC (μg/ml)		
		50%	90%	Range
Staphylococcus aureus				
Oxacillin susceptible (102)	U-100592	1	2	0.5–4
	U-100766	2	2	1–4
	Vancomycin	<0.5	≤0.5	≤0.5–1
	Teicoplanin	≤0.5	≤0.5	≤0.5–1
	Erythromycin	1	2	0.5–>2
	Clindamycin	0.25	0.5	≤0.06–>2
	Clinafloxacin	≤0.12	≤0.12	≤0.12
	Chloramphenicol	8	16	4–16
Oxacillin resistant (53)	U-100592	2	2	1–4
	U-100766	2	2	1–4
	Vancomycin	≤0.5	≤0.5	≤0.5–1
	Teicoplanin	≤0.5	≤0.5	≤0.5–1
	Erythromycin	>2	>2	1–>2
	Clindamycin	>2	>2	0.25–>2
	Clinafloxacin	≤0.12	0.5	≤0.25–1
	Chloramphenicol	16	16	4–16
Staphylococcus epidermidis				
Oxacillin susceptible (22)	U-100592	1	1	0.5–1
	U-100766	1	2	0.5–2
	Vancomycin	1	1	≤0.5–2
	Teicoplanin	1	1	≤0.5–4
	Erythromycin	1	>2	0.5–>2
	Clindamycin	0.25	>2	≤0.12–>2
	Clinafloxacin	≤0.12	≤0.12	≤0.12
	Chloramphenicol	8	8	4–>16
Oxacillin resistant (28)	U-100592	1	1	0.5–1
	U-100766	1	2	0.5–2
	Vancomycin	1	1	1–2
	Teicoplanin	≤0.5	1	≤0.5–1
	Erythromycin	>2	>2	1–>2
	Clindamycin	>2	>2	0.25–>2
	Clinafloxacin	≤0.12	≤0.12	≤0.12–0.25
	Chloramphenicol	8	16	4–>16
Staphylococcus haemolyticus				
Oxacillin susceptible (10)	U-100592	0.5	1	0.25–1
	U-100766	0.5	1	0.25–1
	Vancomycin	≤0.5	1	≤0.5–4
	Teicoplanin	2	8	≤0.5–16
	Erythromycin	1	>2	0.5–>2
	Clindamycin	0.25	>2	≤0.06–>2
	Clinafloxacin	≤0.12	≤0.12	≤0.12–0.25
	Chloramphenicol	4	8	1–8
Oxacillin resistant (10)	U-100592	1	1	0.5–1
	U-100766	1	1	0.5–2
	Vancomycin	1	2	1–4
	Teicoplanin	>16	>16	8–>16
	Erythromycin	>2	>2	>2
	Clindamycin	>2	>2	1–>2
	Clinafloxacin	≤0.12	0.5	≤0.12–0.5
	Chloramphenicol	4	8	4–16
Coagulase-negative staphylococci, other (30)[a]	U-100592	0.5	1	0.5–2
	U-100766	1	1	0.5–2
	Vancomycin	1	1	≤0.5–2
	Teicoplanin	0.5	2	≤0.5–8
	Erythromycin	2	>2	0.5–>2
	Clindamycin	0.25	>2	0.12–>2
	Clinafloxacin	≤0.12	0.25	≤0.12–0.5
	Chloramphenicol	4	>16	4–>16
Enterococcus faecalis (29)[b]	U-100592	1	1	0.5–2
	U-100766	1	1	0.5–2
	Vancomycin	≤0.5	1	≤0.5–2

Continued on following page

TABLE 1—*Continued*

Organism (no. tested)	Antimicrobial agent	MIC (μg/ml)		
		50%	90%	Range
	Teicoplanin	≤0.5	≤0.5	≤0.5
	Erythromycin	>2	>2	1–>2
	Clindamycin	>2	>2	>2
	Clinafloxacin	2	>2	≤0.12–>2
	Chloramphenicol	8	>16	4–>16
Enterococcus faecium (15)[b]	U-100592	1	1	0.5–4
	U-100766	1	2	1–2
	Vancomycin	≤0.5	≤0.5	≤0.5
	Teicoplanin	≤0.5	≤0.5	≤0.5
	Erythromycin	>2	>2	>2
	Clindamycin	>2	>2	0.12–>2
	Clinafloxacin	2	>2	0.12–>2
	Chloramphenicol	4	8	4–16
Bacillus cereus (10)	U-100592	1	1	0.5–1
	U-100766	1	1	0.5–1
	Vancomycin	≤0.5	≤0.5	≤0.5
	Teicoplanin	≤0.5	≤0.5	≤0.5
	Erythromycin	>2	>2	0.5–>2
	Clindamycin	1	>2	1–>2
	Clinafloxacin	≤0.12	≤0.12	≤0.12
	Chloramphenicol	2	4	2–8
Corynebacterium jeikeium (10)	U-100592	0.25	0.25	0.25
	U-100766	2	2	0.25–2
	Penicillin	>8	>8	8–>8
Streptococcus pyogenes (20)	U-100592	1	2	1–2
	U-100766	2	4	2–4
	Penicillin	≤0.03	≤0.03	≤0.03–0.06
Streptococcus agalactiae (20)	U-100592	0.5	1	0.5–1
	U-100766	1	2	0.5–2
	Vancomycin	≤0.5	≤0.5	≤0.5
	Teicoplanin	≤0.5	≤0.5	≤0.5–1
	Erythromycin	0.25	0.25	0.12–>2
	Clindamycin	0.12	0.12	≤0.06–0.25
	Clinafloxacin	≤0.12	≤0.12	≤0.12
	Chloramphenicol	2	2	1–2
Streptococcus group C, F, G (15)	U-100592	2	2	1–2
	U-100766	2	2	1–4
	Penicillin	≤0.03	0.06	≤0.03–0.06
Streptococcus pneumoniae (29)[c]	U-100592	1	1	0.5–1
	U-100766	2	2	1–2
	Penicillin	≤0.03	0.25	≤0.03–2

[a] Includes 20 oxacillin-resistant strains (nine species).
[b] All strains were vancomycin susceptible.
[c] Includes 10 penicillin-intermediate and -resistant strains.

than a dozen U.S. medical centers (6). The most common glycopeptide-resistant species was *E. faecium*, and the most prevalent resistance phenotype was VanA. In contrast, the *E. faecalis* strains were usually VanB in their resistance pattern. Regardless of the glycopeptide resistance phenotype or species, the U-100592 and U-100766 activities were similar, with a MIC range of 0.25 to 2 μg/ml. Less than 50% of strains tested were susceptible to teicoplanin, erythromycin, and clinafloxacin. Only chloramphenicol remained active (MIC, ≤8 μg/ml) against >90% of these vancomycin-resistant isolates.

Antimicrobial activity against gram-negative organisms and anaerobes. Table 3 lists the activities of U-100592 and U-100766 with selected comparators tested against anaerobic organisms and representative gram-negative fastidious species. The anaerobic organisms were inhibited by the investigational oxazolidinones but over a wide range of concentrations. The greatest potency was found for U-100592 against *Peptostreptococcus* spp. (MIC_{90}, 1 μg/ml), and the highest MICs were observed for the gram-negative species (*Bacteroides fragilis* group and *Prevotella* spp.).

Gram-negative, fastidious aerobic species were less susceptible to U-100592 and U-100766. The oxazolidinone MIC_{50}s for *H. influenzae* and the gonococci were ≥8 μg/ml (Table 3). The oxazolidinone MICs for all tested members of the family

TABLE 2. Activities of U-100592 and U-100766 against 152 isolates of vancomycin-resistant enterococci

Organism (no. tested)	Antimicrobial agent	MIC (μg/ml)		
		50%	90%	Range
E. faecalis (15)	U-100592	1	1	0.5–1
	U-100766	1	1	1–2
	Teicoplanin	≤0.5	≤0.5	≤0.5–>16
	Erythromycin	>2	>2	>2
	Clinafloxacin	2	>2	0.5–>2
	Chloramphenicol	8	8	4–>16
E. faecium (116)	U-100592	1	1	0.5–2
	U-100766	1	2	0.5–2
	Teicoplanin	>16	>16	≤0.5–>16
	Erythromycin	>2	>2	>2
	Clinafloxacin	>2	>2	≤0.12–>2
	Chloramphenicol	4	8	4–16
Enterococcus spp., other (21)[a]	U-100592	1	1	0.25–2
	U-100766	1	1	0.25–2
	Teicoplanin	>16	>16	≤0.5–>16
	Erythromycin	>2	>2	>2
	Clinafloxacin	0.5	>2	≤0.12–>2
	Chloramphenicol	8	16	4–>16
All enterococci tested (152)	U-100592	1	1	0.25–2
	U-100766	1	1	0.25–2
	Teicoplanin	>16	>16	≤0.5–>16
	Erythromycin	>2	>2	2–>2
	Clinafloxacin	2	>2	≤0.12–>2
	Chloramphenicol	8	8	4–>16

[a] Includes *Enterococcus casseliflavus*, *Enterococcus durans*, and *Enterococcus gallinarum.*

TABLE 3. Antimicrobial activities of two oxazolidinones (U-100592 and U-100766) compared with those of other compounds tested against 104 anaerobic bacteria and gram-negative bacteria

Organism (no. tested)	Antimicrobial agent	MIC (μg/ml)		
		50%	90%	Range
Bacteroides fragilis (3)	U-100592	1		0.5–8
	U-100766	2		0.5–4
	Clindamycin	≤0.5		≤0.5
	Metronidazole	4		1–8
Clostridium spp. (20)[a]	U-100592	2	2	0.5–4
	U-100766	2	2	0.5–4
	Clindamycin	1	4	≤0.5–8
	Metronidazole	2	4	1–4
Peptostreptococcus spp. (17)[b]	U-100592	1	1	0.5–1
	U-100766	1	2	0.5–2
	Clindamycin	1	2	≤0.5–>16
	Metronidazole	1	2	≤0.5–4
Prevotella spp. (12)[c]	U-100592	4	8	1–8
	U-100766	2	2	1–4
	Clindamycin	≤0.5	≤0.5	≤0.5
	Metronidazole	4	4	2–8
Haemophilus influenzae (10)[d]	U-100592	8	8	4–8
	U-100766	16	16	8–16
	Ampicillin	2	16	0.25–>16
Neisseria gonorrhoeae (14)[e]	U-100592	8	>16	2–>16
	U-100766	8	16	4–>16
	Penicillin	4	>8	0.03–>8
Gram-negative bacilli (28)[f]	U-100592	>64	>64	64–>64
	U-100766	>64	>64	64–>64
	Vancomycin	>16	>16	>16
	Teicoplanin	>16	>16	>16
	Erythromycin	>2	>64	>64
	Clindamycin	>2	>2	>2
	Clinafloxacin	≤0.12	≤0.12	≤0.25
	Chloramphenicol	4	8	2–16

[a] Includes *Clostridium butyricum*, *C. perfringens*, *C. tertium*, and *Clostridium* sp. strain NOS.
[b] Includes *Peptostreptococcus micros*, *P. magnus*, *P. asaccharolyticus*, *P. tetradius*, and *P. anaerobius.*
[c] Includes *Prevotella disiens* and *P. bivius.*
[d] Includes six ampicillin-resistant strains.
[e] Includes nine penicillin-resistant strains.
[f] Includes two strains each of *Citrobacter freundii*, *Enterobacter aerogenes*, *Enterobacter cloacae*, *Escherichia coli*, *Klebsiella oxytoca*, *Klebsiella pneumoniae*, *Morganella morganii*, *Proteus mirabilis*, *Providencia rettgeri*, *Serratia marcescens*, *P. aeruginosa*, *Acinetobacter* spp., and *S. maltophilia*. Single strains of *A. xylosoxidans* and *F. meningosepticum* were also tested.

Enterobacteriaceae, *Stenotrophomonas maltophilia*, *Acinetobacter* spp., *Alcaligenes xylosoxidans*, and *Pseudomonas aeruginosa* strains were ≥64 μg/ml. Marginal activity was observed for both compounds (MIC, 2 μg/ml) against *Moraxella catarrhalis*, and U-100766 was marginally active against a single strain of *Flavobacterium meningosepticum* (MIC, 8 μg/ml; data not shown).

Measurements of bactericidal action. Several experiments attempted to determine the antimicrobial action of the oxazolidinones. Table 4 lists the variation in the initial inoculum concentration over 24 h for five bacterial strains tested in the presence of 4 μg of drug per ml. The measured MIC of both oxazolidinones for all strains was 2 μg/ml. A static effect (0.0 to -1.7 $\log_{10}$ CFU/ml variation) was observed (Table 4); however, in a series of MBC determinations for similar *Staphylococcus* sp. and *Enterococcus* sp. strains, all MBCs were ≤4-fold higher than the MIC. The addition of 4 μg of gentamicin per ml to either oxazolidinone (4 μg/ml) did not enhance the activity against enterococci (five strains tested). Further studies testing staphylococcal and enterococcal strains with U-100592 and U-100766 concentrations at the MIC and 2- to sixteen-fold the MIC failed to exhibit significant concentration-dependent bactericidal action.

Effects of variations in test conditions on oxazolidinone MICs. Table 5 shows the results of testing 25 gram-positive cocci (10 *S. aureus* strains [five oxacillin resistant], 5 coagulase-negative staphylococci [four species], 5 *E. faecalis* strains, and 5 *E. faecium* strains) by the agar dilution method (9). Technical parameters of the test were altered, and the U-100592 and U-100766 MICs were determined and compared with the MICs obtained with an inoculum of 10^4 CFU per spot, pH 7.2, Mueller-Hinton unsupplemented agar, and overnight incubation at 35°C (9). No specific-species-based change was observed in these experiments. Elevating or lowering the inoculum concentrations (CFU per milliliter) by ≥10-fold resulted in a twofold MIC change (Table 5). Changes in medium pH, incubation, sheep blood, or magnesium supplement had minimal (≤2-fold MIC variation) effects.

The broth microdilution and agar dilution results (8, 9) for both new compounds were determined. Twenty-five strains (15 staphylococci and 10 enterococci) were selected for testing by both reference methods. U-100592 agar dilution MIC results averaged only 0.1 $\log_2$ dilution greater than broth test results, with 14 strains having identical values determined by the two methods. Similarly, U-100766 agar and broth dilution MICs

TABLE 4. Changes in the initial inoculum for five organisms tested in the presence of 4 μg of U-100592 and U-100766 per ml[a]

Organism	Drug	Log_2 dilution change at:	
		6 h	24 h
S. aureus			
Oxacillin susceptible	U-100592	0.1	−0.1
	U-100766	0.0	−0.1
Oxacillin resistant	U-100592	0.0	−1.0
	U-100766	0.0	−0.9
Staphylococcus auricularis[b]	U-100592	0.0	−0.2
	U-100766	0.0	−0.2
E. faecalis	U-100592	−0.5	−1.7
	U-100766	−0.4	−1.6
E. faecium	U-100592	−0.2	−0.2
	U-100766	0.0	0.0

[a] Twofold the MIC. The MIC for all tested strains was 2 μg/ml. From these results, both drugs were interpreted as having bacteristatic action.
[b] Oxacillin susceptible.

were nearly identical, with the broth microdilution MICs being minimally increased (also 0.1 log_2 dilution).

Disk diffusion tests with oxazolidinones. The MICs of U-100592 and U-100766 were compared with zones of inhibition around 5-, 15-, and 30-μg disks (491 strains). Preliminary pharmacokinetic investigations in humans and animals (12) indicate that dosing could be adjusted to approximately 1,000 mg orally (maximum concentration of drug in serum, 5.73 μg/ml) or administered by parenteral route to validate a susceptibility breakpoint concentration of ≤2 or ≤4 μg/ml. This tentative interpretive criterion (4 μg/ml) would predict susceptibility of all tested gram-positive aerobic and anaerobic organisms to these oxazolidinone agents (Tables 1 through 3).

Table 6 shows the correlation statistics of the comparisons for each oxazolidinone disk concentration. The best correlations (r = 0.85 to 0.90) between the MIC and zone diameters were achieved with the 15- and 30-μg disk concentrations. The occurrence of interpretive error when the cited correlate zone diameter criteria were used was very low (absolute categorical agreement, 98.2 to 99.8%; nil false-susceptible error). It was necessary to use gram-negative bacteria (28 strains) to assess the risk of the false-susceptible, very major errors. Proposed interpretive criteria minimize error between methods and also conform to the regression formula-calculated breakpoint zone.

Figure 2 shows a typical oxazolidinone scattergram for the U-100592 MIC and zone diameter (30-μg disk). The regression equation was $y = 16.5 - 0.27x$ ($r = -0.90$), and the total interpretive error rate was only 0.2%. The 30-μg disk was proposed for future studies with each drug, and zone diameter breakpoints of ≥21 mm (MIC, ≤4 μg/ml) for susceptible and ≤17 mm (MIC, ≥16 μg/ml) for resistant were recommended.

TABLE 5. Effects of modifying in vitro testing conditions on the MICs (agar dilution) of two oxazolidinones

Drug	Change in log_2 dilution for[a]:								
	Inoculum concn (CFU/spot)			5% SB[b]	pH		10× Mg^{2+}	Incubation	
	10^3	10^5	10^6		6	8		In 5% CO_2	Anaerobic
U-100592	−1.4	0.3	1.0	−0.2	−0.4	−0.4	−0.8	−0.2	−1.0
U-100766	−1.0	0.8	1.1	0.5	0.5	−0.3	−0.2	0.3	0.3

[a] Compared with MIC determined with 10^4 CFU per spot, pH 7.2, ambient air, and appropriate divalent cation content (8).
[b] SB, 5% sheep blood agar.

TABLE 6. Proposed oxazolidinone disk test interpretive criteria using three different disk drug concentrations and a tentative MIC breakpoint of ≤4 μg/ml (491 strains)

Oxazolidinone	Disk drug concn (μg)	Zone diam (mm)		Error rate (%)			Correlation coefficient
		Susceptible	Resistant	Very major	Major	Minor	
U-100592	5	≥15	≤11	0.0	0.4	1.4	0.84
	15	≥18	≤14	0.0	0.2	0.0	0.88
	30	≥21	≤17	0.0	0.0	0.2	0.90
U-100766	5	≥15	≤11	0.0	0.4	1.2	0.81
	15	≥18	≤14	0.0	0.0	0.4	0.85
	30	≥21	≤17	0.0	0.0	0.2	0.87

DISCUSSION

Investigations of oxazolidinone antimicrobial agents have suggested an activity focused against important gram-positive species, including *S. aureus*, coagulase-negative staphylococci, *Enterococcus* spp., and streptococci (3–5, 14). Recently, U-100592 and U-100766 were selected for further study from a series of novel 3-(3-fluorophenyl)-2-oxazolidinone analogs (1, 2, 7, 12). These compounds possess nearly identical activities and spectra against gram-positive species, anaerobes, and some gram-negative species (*M. catarrhalis* and *F. meningosepticum*). Complete inhibition by U-100592 and U-100766 was achieved against the tested oxacillin-resistant staphylococci, vancomycin-resistant enterococci, and gram-positive strains from several species having demonstrated resistance to macrolides, aminoglycosides, and various beta-lactams (for example, penicillin-resistant *S. pneumoniae*).

U-100592 and U-100766 antimicrobial action appears to be bacteristatic, and no concentration-dependent killing could be demonstrated for staphylococci or enterococci. Previously, concentration-dependent killing was observed for some other oxazolidinones (13) against mycobacteria; this contrasts to the more frequent finding of a bacteristatic effect against gram-positive species (3, 4, 14). The addition of an aminoglycoside to U-100592 or U-100766 did not enhance the activity against enterococcal strains.

All tested gram-positive strains were inhibited by ≤4 μg of these investigational oxazolidinones per ml. This in vitro observation, coupled with earlier reports on drugs in this class (3–5, 13–15) and reports by Upjohn scientists (1, 2, 7, 12), indicates a potential therapeutic role for these compounds for the eradication of organisms demonstrating resistance to glycopeptides (6), penicillinase-resistant penicillins (staphylococci), penicillin (streptococci), macrolides, and other classes of antimicrobial agents.

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