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In Vitro and *In Vivo* Activities of LCB01-0371, a New Oxazolidinone▽

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LCB01-0371 is a new oxazolidinone with cyclic amidrazone. *In vitro* activity of LCB01-0371 against 624 clinical isolates was evaluated and compared with those of linezolid, vancomycin, and other antibiotics. LCB01-0371 showed good activity against Gram-positive pathogens. *In vivo* activity of LCB01-0371 against systemic infections in mice was also evaluated. LCB01-0371 was more active than linezolid against these systemic infections. LCB01-0371 showed bacteriostatic activity against *Staphylococcus aureus*.

The emergence of multidrug-resistant (MDR) pathogens, such as methicillin-resistant *Staphylococcus aureus* (MRSA), methicillin-resistant coagulase-negative staphylococci (MRCNS), penicillin-resistant *Streptococcus pneumoniae* (PRSP), and vancomycin-resistant enterococci (VRE), has generated worldwide concern in the medical community (11). The requirement for effective new antimicrobial agents to treat infections caused by Gram-positive organisms is becoming urgent as resistance to existing agents arises and spreads around the world.

The oxazolidinones, a totally synthetic class of novel antibiotics, have strong activity against nearly all Gram-positive organisms, including those resistant to other agents (1, 10). They inhibit protein synthesis by binding to domain V of the 23S rRNA and thereby blocking formation of the initiation complex (6). Linezolid is the first member of the oxazolidinone class approved by the FDA in the United States. The success of linezolid and the occurrence of strains resistant to linezolid in clinical isolates of *Enterococcus faecium* (4, 5) and *S. aureus* (12) have inspired further efforts toward developing new oxazolidinones with improved safety and antibacterial activity.

LCB01-0371 (Fig. 1), a novel oxazolidinone with cyclic amidrazone, was synthesized by LegoChem BioSciences Inc. (Daejeon, Republic of Korea). In this study, *in vitro* activity of LCB01-0371 was compared with those of eight different antibacterial agents against 624 clinical isolates that were collected from several general hospitals in the Republic of Korea. *In vivo* activity of LCB01-0371 against systemic infections in mice and time-kill studies of LCB01-0371 against *S. aureus* giorgio (methicillin-susceptible *S. aureus* [MSSA]) and *S. aureus* p125 (MRSA) were also investigated.

(This study was presented in part at the 49th Annual Interscience Conference on Antimicrobial Agents and Chemotherapy, San Francisco, CA, 2009 [7].)

In vitro MICs were determined by the 2-fold agar dilution method as described by the Clinical and Laboratory Standards Institute (CLSI) (3). Mueller-Hinton agar (MHA) medium was used for testing aerobic and facultative organisms. *Streptococcus pneumoniae*, *Streptococcus pyogenes*, and *Moraxella catarrhalis* were grown on Mueller-Hinton agar supplemented with 5% defibrinated sheep blood (Hanil Komed Ltd., Sungnam City, Republic of Korea). Mueller-Hinton agar supplemented with 3% Fildes enrichment (Oxoid Ltd., Basingstoke, Hampshire, England) was used for *Haemophilus influenzae*. Bacteria (10^4 to 10^5 CFU) were spotted onto plates containing the appropriate concentration of drug. Plates were incubated at 35°C for 18 h and examined for growth. The MIC was considered to be the lowest concentration that completely inhibited growth on agar plates, disregarding a single colony or a faint haze caused by the inoculum.

Time-kill studies were performed by the M26-A method of the NCCLS (8). Test organisms incubated on tryptic soy agar (TSA) for 18 h at 37°C were diluted with fresh Mueller-Hinton broth to ~10^5 CFU/ml, and the diluted cultures were preincubated for 2 h. Each drug was added to the cultures at concentrations of 0.25×, 0.5×, 1×, 2×, 4×, and 8× MIC. Aliquots (0.1 ml) of the cultures were removed at 0, 2, 4, 6, and 24 h of incubation, and serial 10-fold dilutions were prepared in saline as needed. Drug carryover effects were reduced by 100-fold dilution of the sample with agar. The numbers of viable cells on drug-free MHA plates after 24 h of incubation were determined. The compound was considered bactericidal at the concentration that reduced the original inoculum by 3 $\log_{10}$ CFU/ml (99.9%) at each of the time periods or considered

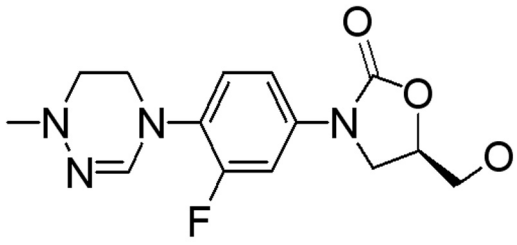

FIG. 1. Chemical structure of LCB01-0371.

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TABLE 1. *In vitro* antibacterial activities of LCB01-0371 against clinical isolates

Microorganism (no. of strains) and compound	MIC (μg/ml)		
	Range	50%	90%
MSSA (69)			
LCB01-0371	~0.5–2	1	2
Linezolid	~2–4	2	2
Oxacillin	~0.06–1	0.25	0.5
Erythromycin	~0.125–>64	0.25	>64
Ciprofloxacin	~0.06–>64	0.25	0.5
Moxifloxacin	~0.015–64	0.06	0.125
Gemifloxacin	~0.008–64	0.015	0.06
Vancomycin	~0.25–2	1	1
Quinupristin-dalfopristin	~0.125–0.5	0.25	0.5
MRSA (202)			
LCB01-0371	~0.5–4	1	2
Linezolid	~2–2	2	2
Oxacillin	~2–>64	>64	>64
Erythromycin	~0.25–>64	>64	>64
Ciprofloxacin	~0.125–>64	32	>64
Moxifloxacin	~0.03–>64	4	64
Gemifloxacin	~0.008–>64	2	64
Vancomycin	~0.5–4	1	2
Quinupristin-dalfopristin	~0.125–1	0.5	1
MSCNS (20)			
LCB01-0371	~0.5–1	0.5	0.5
Linezolid	~1–2	1	2
Oxacillin	~0.03–1	0.125	1
Erythromycin	~0.06–>64	0.25	>64
Ciprofloxacin	~0.06–8	0.125	8
Moxifloxacin	~0.03–4	0.125	4
Gemifloxacin	~0.008–0.5	0.015	0.5
Vancomycin	~1–4	2	4
Quinupristin-dalfopristin	~0.125–1	0.25	1
MRCNS (33)			
LCB01-0371	~0.5–1	0.5	0.5
Linezolid	~1–2	1	1
Oxacillin	~2–>64	>64	>64
Erythromycin	~0.06–>64	>64	>64
Ciprofloxacin	~0.06–64	8	32
Moxifloxacin	~0.06–16	2	8
Gemifloxacin	~0.008–8	0.5	1
Vancomycin	~1–4	2	2
Quinupristin-dalfopristin	~0.125–8	0.25	1
S. pneumoniae (97)			
LCB01-0371	~0.125–2	0.5	1
Linezolid	~0.5–1	1	1
Oxacillin	~0.008–>64	8	16
Erythromycin	~0.008–>64	64	>64
Ciprofloxacin	~0.5–32	1	2
Moxifloxacin	~0.06–4	0.25	0.5
Gemifloxacin	~0.008–0.25	0.03	0.06
Vancomycin	~0.25–1	0.5	1
Quinupristin-dalfopristin	~0.5–4	1	2
S. pyogenes (46)			
LCB01-0371	~0.5–2	1	2
Linezolid	~1–2	2	2
Oxacillin	~0.03–16	0.5	8
Erythromycin	~0.008–8	0.06	2
Ciprofloxacin	~0.5–2	1	2
Moxifloxacin	~0.125–0.25	0.125	0.25
Gemifloxacin	~0.015–0.125	0.03	0.06
Vancomycin	~0.5–1	1	1
Quinupristin-dalfopristin	~0.25–4	1	2
E. faecalis (109)			
LCB01-0371	~1–2	2	2
Linezolid	~1–2	2	2
Oxacillin	~8–>64	16	>64
Erythromycin	~0.125–>64	>64	>64
Ciprofloxacin	~0.06–>64	2	64
Moxifloxacin	~0.06–64	1	32
Gemifloxacin	~0.008–16	0.125	4
Vancomycin	~0.5–64	2	4
Quinupristin-dalfopristin	~0.25–16	4	16
E. faecium (29)			
LCB01-0371	~1–2	2	2
Linezolid	~1–2	2	2
Oxacillin	~16–>64	>64	>64
Erythromycin	~0.125–>64	>64	>64
Ciprofloxacin	~1–64	4	64
Moxifloxacin	~0.25–>64	4	32
Gemifloxacin	~0.03–64	2	16
Vancomycin	~0.5–8	1	2
Quinupristin-dalfopristin	~0.25–32	0.5	4
VRE (16)			
LCB01-0371	~1–1	1	1
Linezolid	~2–2	2	2
Oxacillin	~32–>64	>64	>64
Erythromycin	~>64–>64	>64	>64
Ciprofloxacin	~0.5–4	4	4
Moxifloxacin	~0.25–4	2	4
Gemifloxacin	~0.015–2	0.5	2
Vancomycin	~>64–>64	>64	>64
Quinupristin-dalfopristin	~0.5–2	2	2
M. catarrhalis (20)			
LCB01-0371	~2–8	4	8
Linezolid	~4–8	8	8
Oxacillin	~0.25–32	8	16
Ciprofloxacin	~<0.008–0.06	0.03	0.06
Moxifloxacin	~0.015–0.06	0.06	0.06
Gemifloxacin	~<0.008–0.03	<0.008	0.015
Vancomycin	~64–>64	64	>64
Quinupristin-dalfopristin	~0.5–2	1	1
H. influenzae (13)			
LCB01-0371	~2–16	8	16
Linezolid	~8–32	16	32
Oxacillin	~>32–>32	>32	>32
Erythromycin	~0.5–8	2	8
Ciprofloxacin	~<0.008–<0.008	<0.008	<0.008
Moxifloxacin	~0.008–0.015	0.008	0.008
Gemifloxacin	~<0.008–<0.008	<0.008	<0.008
Vancomycin	~>64–>64	>64	>64
Quinupristin-dalfopristin	~2–8	4	8

bacteriostatic if the inoculum was reduced by ~0 to 3 $\log_{10}$ CFU/ml.

In vivo activity of LCB01-0371 against systemic infections caused by *S. aureus* giorgio (MSSA), *S. aureus* p125 (MRSA), *Enterococcus faecalis* u810, *S. pneumoniae* ATCC 6305, and *Haemophilus influenzae* hd2 in mice was determined. Four-week-old male ICR mice weighing 18 to 22 g (Daehan Bio Link Co., Ltd., Eum-sung Gun, Republic of Korea) were used for

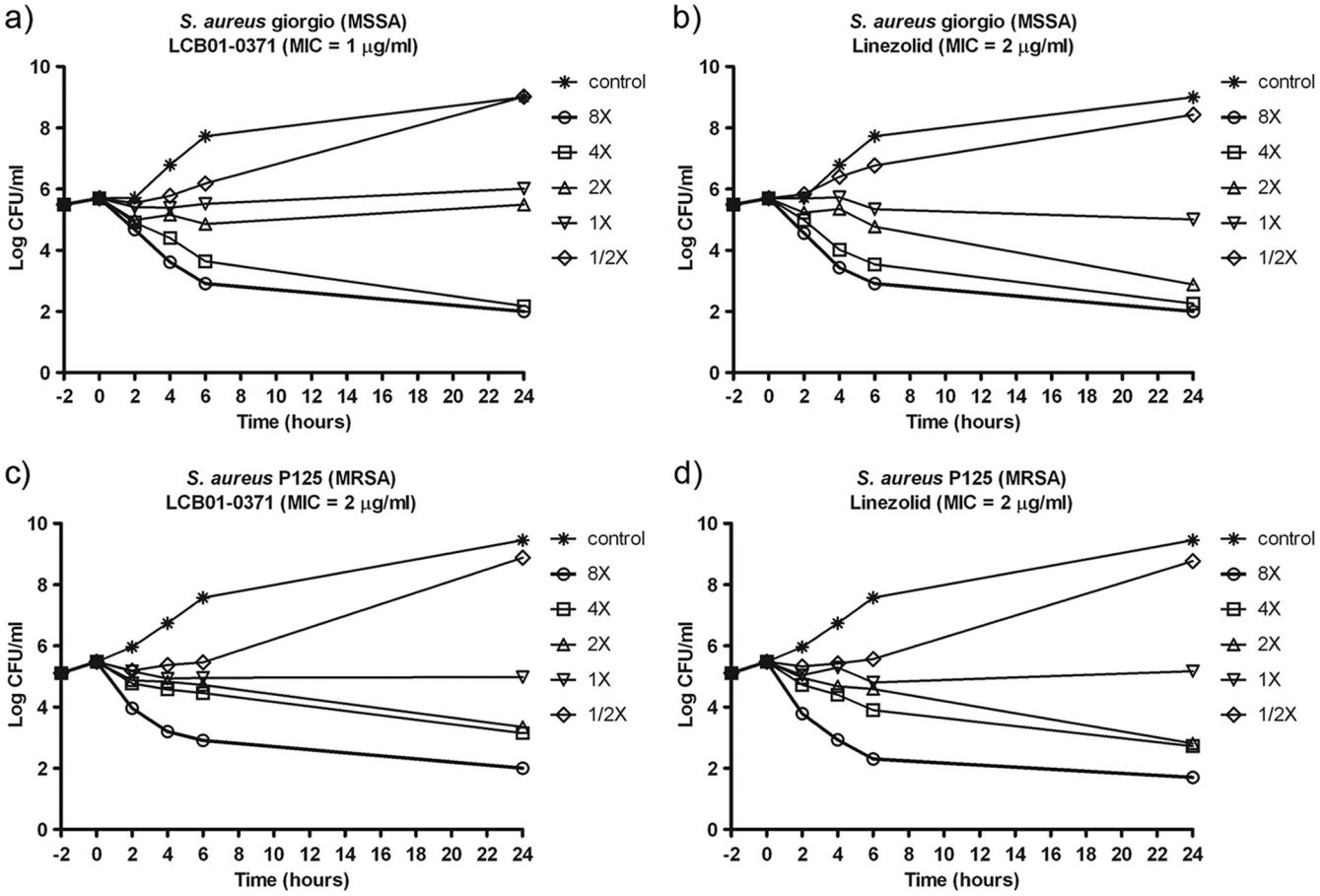

FIG. 2. Time-kill curves of LCB01-0371 and linezolid against *S. aureus* giorgio (MSSA) and *S. aureus* p125 (MRSA).

the systemic infection model. They were maintained in animal rooms kept at 23 ± 2°C with 55% ± 20% relative humidity. Test organisms for infection were cultured in Mueller-Hinton agar medium (Difco) at 37°C for 18 h. For *S. pneumoniae*, Muller-Hinton agar medium was supplemented with 5% defibrinated sheep blood. For use as inocula, bacterial strains were suspended in 0.9% saline solution containing 5% gastric mucin (Sigma), except for *S. pneumoniae*, which was suspended in 0.9% saline solution. Mice were used in groups of six for each dose and were challenged intraperitoneally with a single 0.5-ml portion of the bacterial suspension, corresponding to an inoculum range of 10 to 100 times the minimal lethal dose of bacteria. Four dose levels were used for each antibiotic, depending on the *in vitro* antimicrobial activity of the compound. Antibiotics at various dose regimens were administered orally twice, at 1 and 4 h postinfection. Mortality was recorded for 7 days, and the median effective dose needed to protect 50% of the mice (ED_{50}) was calculated by the Probit method (2). The challenge inoculum was sufficient to kill 100% of the untreated control mice, which died within 48 h postinfection.

All animal experiments were approved by the Ethics Review Committee of Handong Global University, Republic of Korea.

The comparative *in vitro* antibacterial activities of LCB01-0371 are shown in Table 1. The MIC_{90} of LCB01-0371 for MSSA and MRSA was 2 μg/ml. LCB01-0371 was as active as linezolid. Against methicillin-susceptible coagulase-negative staphylococci (MSCNS) (MIC_{90}, 0.5 μg/ml) and MRCNS (MIC_{90}, 0.5 μg/ml), LCB01-0371 was at least 2-fold more active than linezolid. LCB01-0371 was equally active irrespective of whether the strains were methicillin susceptible or resistant. Against *S. pneumoniae* (MIC_{90}, 1 μg/ml) and *S. pyogenes* (MIC_{90}, 2 μg/ml), LCB01-0371 showed antibacterial activity comparable to that of linezolid. LCB01-0371 was as active as linezolid against *E. faecalis* (MIC_{90}, 2 μg/ml) and *E. faecium* (MIC_{90}, 2 μg/ml). Against VRE (MIC_{90}, 1 μg/ml), LCB01-0371 was 2-fold more active than linezolid. LCB01-0371 showed weak activity against the fastidious Gram-negative aerobes *H. influenzae* and *M. catarrhalis.* Against *H. influenzae*, LCB01-0371 yielded a MIC_{90} of 16 μg/ml, while slightly better activity against *M. catarrhalis* (MIC_{90}, 8 μg/ml) was seen. The MIC_{90} of LCB01-0371 against *H. influenzae* was 2-fold lower than that of linezolid, but the MICs of LCB01-0371 against *H. influenzae* and *M. catarrhalis* were too high for clinical efficacy.

The time-kill analyses of LCB01-0371 against *S. aureus* giorgio (MSSA) and *S. aureus* p125 (MRSA) are presented in Fig. 2. LCB01-0371 and linezolid showed similar patterns of the time-kill effect irrespective of whether the strain was methicillin susceptible or resistant. LCB01-0371, at concentrations of 1× MIC and 2× MIC, had bacteriostatic activity against MSSA and MRSA after 24 h. At concentrations of 4× MIC

TABLE 2. *In vivo* activities of LCB01-0371 against systemic infection in mice

Microorganism (inoculum, CFU/mouse[a])	Antimicrobial agent[b]	MIC (μg/ml)	ED_{50}, mg/kg (95% confidence limit)
S. aureus giorgio, MSSA (1×10^7)	LCB01-0371	1	4.53 (~2.26–7.87)
	Linezolid	2	8.05 (~4.70–13.85)
S. aureus p125, MRSA (1×10^8)	LCB01-0371	1	2.96 (~0.00–5.81)
	Linezolid	2	4.84 (~0.01–12.66)
E. faecalis u810 (2×10^8)	LCB01-0371	2	4.53 (~2.26–7.87)
	Linezolid	2	5.97 (~2.23–7.87)
S. pneumoniae ATCC 6305 (1×10^4)	LCB01-0371	0.5	2.28 (~0.00–4.49)
	Linezolid	1	9.10 (~4.92–23.72)
H. influenzae hd2 (7.5×10^8)	LCB01-0371	8	9.96 (~4.26–16.75)
	Linezolid	16	21.43 (~9.99–450.60)

[a] Bacterial strains were suspended in 0.9% saline solution containing 5% mucin, except for *S. pneumoniae* ATCC 6305, which was suspended in 0.9% saline solution.
[b] Antimicrobial agents were administered orally at 1 and 4 h postinfection.

and 8× MIC, LCB01-0371 showed bacteriostatic activity, but there was no regrowth at concentrations of 4× MIC and 8× MIC after 24 h of incubation.

The protective efficacy of LCB01-0371 against systemic infections in mice was compared with that of linezolid (Table 2). When administered orally, LCB01-0371 showed more-potent protective effects than linezolid against systemic infections caused by Gram-positive and Gram-negative bacteria. Against infection caused by *S. aureus* giorgio (MSSA), the ED_{50}s of LCB01-0371 and linezolid were 4.53 and 8.05 mg/kg of body weight, respectively. Against *S. aureus* p125 (MRSA), LCB01-0371 (ED_{50}, 2.96 mg/kg) was more active than linezolid (ED_{50}, 4.84 mg/kg). Against *E. faecalis* u810, the ED_{50}s of LCB01-0371 and linezolid were 4.53 and 5.97 mg/kg, respectively. LCB01-0371 (ED_{50}, 2.28 mg/kg) was also more active than linezolid (ED_{50}, 9.10 mg/kg) against *S. pneumoniae* ATCC 6305. Against *H. influenzae* hd2, the ED_{50}s of LCB01-0371 and linezolid were 9.96 and 21.43 mg/kg, respectively. In general, the ED_{50}s of LCB01-0371 were well correlated with *in vitro* MICs.

Although linezolid has been recognized as an effective antibiotic against infections with Gram-positive bacteria, such as MRSA and VRE, it produced side effects, such as myelosuppression and peripheral neuropathy, in long-term applications (9). Therefore, it is important to develop new oxazolidinones with good safety profiles, broad antibacterial spectrum, improved pharmacokinetic (PK) parameters, and good water solubility for parenteral administration. LCB01-0371 showed good *in vitro* and *in vivo* activities against Gram-positive bacteria and had high aqueous solubility and good absorption, distribution, metabolism, excretion, and toxicity (ADMET) and PK profiles (7). In view of its improved antibacterial activities against Gram-positive bacteria and good pharmacokinetic profiles in animals, the clinical usefulness of LCB01-0371 should be established by further studies.

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