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In Vitro Activities of Novel *trans*-3,5-Disubstituted Pyrrolidinylthio-1β-Methylcarbapenems with Potent Activities against Methicillin-Resistant *Staphylococcus aureus* and *Pseudomonas aeruginosa*

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The in vitro activities of the novel 1β-methylcarbapenems J-111,225, J-114,870, and J-114,871, which have a structurally unique side chain that consists of a *trans*-3,5-disubstituted 5-arylpyrrolidin-3-ylthio moiety at the C-2 position, were compared with those of reference antibiotics. Among isolates of both methicillin-resistant *Staphylococcus aureus* (MRSA) and methicillin-resistant coagulase-negative staphylococci (MRCoNS), 90% were inhibited by J-111,347 (prototype), J-111,225, J-114,870, and J-114,871 at concentrations of 2, 4, 4, and 4 μg/ml (MICs at which 90% of isolates are inhibited [MIC_{90}s]), respectively, indicating that these agents were 32- to 64-fold more potent than imipenem, which has an MIC_{90} of 128 μg/ml. Although these drugs were less active in vitro than vancomycin, which had MIC_{90}s of 1 and 2 μg/ml for MRSA and MRCoNS, respectively, the new carbapenems displayed better killing kinetics than vancomycin. The potent anti-MRSA activity was ascribed to the excellent affinities of the new carbapenems for penicillin-binding protein 2a of MRSA. Since the new carbapenems also exhibited good activity against gram-positive and -negative bacteria including clinically important pathogens such as penicillin-resistant *Streptococcus pneumoniae*, *Haemophilus influenzae*, members of the family *Enterobacteriaceae*, *Pseudomonas aeruginosa*, and *Clostridium difficile*, as well as MRSA, the novel carbapenems are worthy of further evaluation.

The emergence of multidrug-resistant microorganisms has caused serious concern about infectious diseases worldwide. Although more than three decades have passed since the first report of methicillin-resistant *Staphylococcus aureus* (MRSA), MRSA still presents a serious problem worldwide as a cause of nosocomial infections (7, 12). Vancomycin, a cyclic glycopeptide antibiotic, has been extensively used in the clinic to treat MRSA infections. However, it is not an ideal antibiotic because of the slow clinical response (6) and potential adverse effects (3). Furthermore, the emergence of MRSA strains with reduced susceptibility to vancomycin accelerated an urgent need for new chemotherapeutic agents for the treatment of MRSA infections (5).

Previously reported β-lactam antibiotics with activity against methicillin-resistant staphylococci (MRS) such as L-695,256 (2), SM-17466 (13), BO-3482 (8), TOC-39 (4), MC-02,479/RWJ-54428 (F. Malouin, C. Chan, S. Bond, S. Chamberland, and V. J. Lee, Abstr. 37th Intersci. Conf. Antimicrob. Agents Chemother., abstr. F-177, p. 176, 1997), Ro 63-9141 (P. Hohl, P. Angehrn, R. L. Then, P. Hebeisen, and I. Heinze-Krauss, Abstr. 38th Intersci. Conf. Antimicrob. Agents Chemother., abstr. F-24, p. 239, 1998), and L-786,392 (J. Huber, K. L. Dorso, J. Koheler, H. Kropp, H. Rosen, and L. L. Silver, Abstr. 38th Intersci. Conf. Antimicrob. Agents Chemother., abstr. F-30, p. 240, 1998) all had weak activities against gram-negative organisms and/or a lack of antipseudomonal activity.

In the course of our derivatization study of 1β-methylcarbapenems, a novel *trans*-3,5-pyrrolidinylthio-1β-methylcarbapenem, J-111,347, was identified as a broad-spectrum agent with activity against MRS, as well as gram-positive and -negative organisms, including *Pseudomonas aeruginosa*, that are usually covered by the marketed broad-spectrum carbapenems (A. Shimizu, Y. Sugimoto, S. Sakuraba, H. Imamura, H. Sato, N. Ohtake, R. Ushijima, S. Nakagawa, C. Suzuki, T. Hashizume, and S. Morishima, Abstr. 38th Intersci. Conf. Antimicrob. Agents Chemother., abstr. F-52, p. 246, 1998). Subsequently, we synthesized J-111,225, J-114,870, and J-114,871 (Fig. 1) (10). The stereochemistry of the side chains in these compounds is novel; known pyrrolidinylthio-1β-carbapenems like meropenem (14), S-4661 (S. Sasaki, K. Murakami, Y. Nishitani, and S. Kuwahara, Abstr. 34th Intersci. Conf. Antimicrob. Agents Chemother., abstr. F-33, p. 32, 1994), BO-2727 (9), MK-826 (formerly L-749,345) (L. Pelak, S. Gerckens, P. M. Scott, C. Gill, C. Pacholok, L. Lynch, K. Dorso, J. Kohler, D. Shungu, and H. Kropp, Abstr. 36th Intersci. Conf. Antimicrob. Agents Chemother., abstr. F-119, p. 120, 1996), and E1010 (formerly ER-35786) (11) share a *cis*-counterpart at the side chains.

In this paper, we describe the in vitro evaluation of the novel

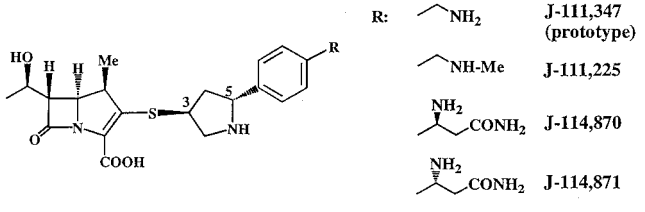

FIG. 1. Chemical structures of the *trans*-3,5-disubstituted 1β-methylcarbapenems J-111,347, J-111,225, J-114,870, and J-114,871. Me, methyl.

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TABLE 1. Comparative in vitro activities against clinical isolates

Organism (no. of isolates) and antibiotic	MIC (μg/ml)[a] Range	50%	90%
MRSA (26)			
J-111,347	0.5–4	2	2
J-111,225	0.5–4	2	4
J-114,870	1–4	2	4
J-114,871	0.5–4	2	4
Imipenem	16–128	64	128
Meropenem	8–64	32	64
Biapenem	16–128	64	64
Vancomycin	0.5–2	1	1
Staphylococcus aureus, methicillin susceptible (21)			
J-111,347	0.008–0.016	≤0.008	0.016
J-111,225	0.008–0.016	0.016	0.016
J-114,870	0.008–0.016	0.016	0.016
J-114,871	0.008–0.032	0.016	0.016
Imipenem	0.008–0.016	0.016	0.016
Meropenem	0.032–0.125	0.063	0.125
Biapenem	0.016–0.063	0.032	0.063
Ceftazidime	4–8	4	8
Vancomycin	0.5–1	0.5	0.5
Coagulase-negative staphylococci, methicillin resistant (25)			
J-111,347	0.125–4	0.5	2
J-111,225	0.125–4	1	4
J-114,870	0.125–4	1	4
J-114,871	0.125–4	1	4
Imipenem	0.125–128	16	128
Meropenem	1–128	16	64
Biapenem	0.5–128	16	128
Ceftazidime	8–>128	64	>128
Vancomycin	0.5–2	1	2
Streptococcus pyogenes (30)			
J-111,347	≤0.008	≤0.008	≤0.008
J-111,225	≤0.008	≤0.008	≤0.008
J-114,870	≤0.008	≤0.008	≤0.008
J-114,871	≤0.008	≤0.008	≤0.008
Imipenem	≤0.008	≤0.008	≤0.008
Meropenem	≤0.008	≤0.008	≤0.008
Biapenem	≤0.008	≤0.008	≤0.008
Ceftazidime	0.063–0.125	0.063	0.125
Penicillin G	≤0.008	≤0.008	≤0.008
Vancomycin	0.5	0.5	0.5
Streptococcus agalactiae (24)			
J-111,347	0.008–0.016	≤0.008	0.016
J-111,225	0.008–0.016	≤0.008	0.016
J-114,870	0.008–0.016	0.016	0.016
J-114,871	0.008–0.032	0.016	0.016
Imipenem	0.008–0.016	≤0.008	0.016
Meropenem	0.016–0.063	0.032	0.032
Biapenem	0.008–0.032	0.016	0.032
Ceftazidime	0.5–1	0.5	0.5
Penicillin G	0.032–0.125	0.032	0.063
Vancomycin	0.5–1	1	1
Streptococcus pneumoniae, including penicillin-resistant organisms (32)			
J-111,347	0.004–0.25	0.008	0.25
J-111,225	0.004–0.25	0.016	0.25
J-114,870	0.004–0.25	0.016	0.25
J-114,871	0.004–0.25	0.016	0.25
Imipenem	0.004–0.25	0.016	0.25
Meropenem	0.008–0.5	0.032	0.5
Biapenem	0.008–0.5	0.016	0.25
Ceftazidime	0.125–16	2	16
Penicillin G	0.008–2	0.125	2
Vancomycin	0.25–0.5	0.5	0.5
Enterococcus faecalis (26)			
J-111,347	1–8	2	8
J-111,225	1–16	4	8
J-114,870	1–8	4	8
J-114,871	2–16	4	8
Imipenem	0.5–4	1	4
Meropenem	4–32	8	16
Biapenem	2–16	8	16
Ceftazidime	>128	>128	>128
Ampicillin	0.5–4	1	4
Vancomycin	0.5–4	2	2
Enterococcus faecium (17)			
J-111,347	2–>128	32	>128
J-111,225	4–>128	32	>128
J-114,870	4–>128	64	>128
J-114,871	4–>128	64	>128
Imipenem	4–>128	64	>128
Meropenem	16–>128	128	>128
Biapenem	16–>128	>128	>128
Ceftazidime	>128	>128	>128
Ampicillin	1–>128	32	128
Vancomycin	1–4	2	4
Escherichia coli (29)			
J-111,347	0.016–0.032	0.016	0.032
J-111,225	0.016–0.063	0.032	0.032
J-114,870	0.016–0.032	0.016	0.016
J-114,871	0.016–0.063	0.032	0.032
Imipenem	0.032–0.125	0.063	0.125
Meropenem	0.016–0.016	0.016	0.016
Biapenem	0.016–0.063	0.032	0.063
Ceftazidime	0.063–16	0.063	0.125
Klebsiella pneumoniae (25)			
J-111,347	0.016–0.063	0.032	0.032
J-111,225	0.016–0.063	0.032	0.063
J-114,870	0.016–0.063	0.032	0.063
J-114,871	0.016–0.063	0.032	0.063
Imipenem	0.063–0.5	0.125	0.25
Meropenem	≤0.008–0.032	0.016	0.032
Biapenem	0.032–1	0.063	0.5
Ceftazidime	0.032–4	0.063	0.25
Enterobacter cloacae (27)			
J-111,347	0.032–0.5	0.063	0.5
J-111,225	0.032–1	0.063	0.5
J-114,870	0.032–2	0.063	1
J-114,871	0.016–1	0.032	0.5
Imipenem	0.063–1	0.5	1
Meropenem	0.016–4	0.032	2
Biapenem	0.032–2	0.125	1
Ceftazidime	0.125–>128	0.5	>128
Enterobacter aerogenes (23)			
J-111,347	0.032–0.5	0.063	0.125
J-111,225	0.032–1	0.125	0.25
J-114,870	0.016–1	0.063	0.25
J-114,871	0.032–1	0.125	0.5
Imipenem	0.25–2	0.5	1
Meropenem	0.032–1	0.063	0.25
Biapenem	0.063–1	0.25	1
Ceftazidime	0.063–>128	0.5	64
Citrobacter freundii (22)			
J-111,347	0.016–0.25	0.032	0.25
J-111,225	0.032–0.5	0.063	0.25

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TABLE 1—*Continued*

Organism (no. of isolates) and antibiotic	MIC (μg/ml)[a]		
	Range	50%	90%
J-114,870	0.016–0.5	0.032	0.5
J-114,871	0.016–0.5	0.032	0.5
Imipenem	0.063–1	0.25	1
Meropenem	≤0.008–2	0.016	1
Biapenem	0.032–1	0.125	0.5
Ceftazidime	0.063–>128	0.5	>128
Citrobacter diversus (17)			
J-111,347	0.016–0.125	0.032	0.063
J-111,225	0.016–0.25	0.032	0.063
J-114,870	0.016–0.125	0.032	0.063
J-114,871	0.016–0.25	0.032	0.063
Imipenem	0.063–0.5	0.125	0.25
Meropenem	≤0.008–0.5	0.016	0.125
Biapenem	0.016–0.125	0.032	0.125
Ceftazidime	0.032–16	0.063	0.5
Proteus mirabilis (24)			
J-111,347	0.063–1	0.125	0.25
J-111,225	0.063–1	0.25	0.5
J-114,870	0.032–1	0.125	0.25
J-114,871	0.063–1	0.25	0.5
Imipenem	0.5–4	2	4
Meropenem	0.032–0.125	0.032	0.063
Biapenem	1–4	2	4
Ceftazidime	0.016–0.063	0.032	0.032
Proteus vulgaris (24)			
J-111,347	0.032–0.25	0.125	0.25
J-111,225	0.063–0.5	0.125	0.25
J-114,870	0.032–0.25	0.125	0.25
J-114,871	0.063–0.5	0.125	0.25
Imipenem	0.5–4	2	2
Meropenem	0.032–0.063	0.032	0.063
Biapenem	0.125–2	1	2
Ceftazidime	0.016–16	0.063	4
Providencia rettgeri (25)			
J-111,347	0.032–0.25	0.125	0.25
J-111,225	0.032–0.5	0.125	0.25
J-114,870	0.032–0.25	0.125	0.25
J-114,871	0.063–0.5	0.125	0.25
Imipenem	0.25–2	1	2
Meropenem	0.016–0.125	0.032	0.063
Biapenem	0.125–1	0.5	1
Ceftazidime	0.016–0.5	0.032	0.063
Morganella morganii (21)			
J-111,347	0.125–1	0.25	0.5
J-111,225	0.125–1	0.5	1
J-114,870	0.25–2	0.5	2
J-114,871	0.125–2	0.25	1
Imipenem	1–4	4	4
Meropenem	0.063–2	0.125	0.125
Biapenem	1–4	1	4
Ceftazidime	0.032–128	0.125	16
Serratia marcescens (28)			
J-111,347	0.032–1	0.063	0.5
J-111,225	0.032–1	0.063	0.5
J-114,870	0.063–2	0.063	1
J-114,871	0.032–2	0.063	1
Imipenem	0.125–4	0.25	0.5
Meropenem	0.016–4	0.032	1
Biapenem	0.063–4	0.25	0.5
Ceftazidime	0.063–32	0.125	4
Moraxella catarrhalis (25)			
J-111,347	≤0.002–0.008	≤0.008	≤0.008
J-111,225	≤0.002–0.016	≤0.008	≤0.008
J-114,870	≤0.002–0.016	≤0.008	≤0.008
J-114,871	≤0.002–0.016	≤0.008	≤0.008
Imipenem	0.008–0.063	0.032	0.063
Meropenem	0.002–0.004	≤0.008	≤0.008
Biapenem	0.016–0.063	0.032	0.032
Ceftazidime	0.016–0.25	0.063	0.125
Haemophilus influenzae (20)			
J-111,347	0.032–0.25	0.063	0.125
J-111,225	0.063–0.5	0.125	0.125
J-114,870	0.063–0.5	0.125	0.125
J-114,871	0.063–0.5	0.125	0.125
Imipenem	0.25–2	1	1
Meropenem	0.032–0.125	0.063	0.063
Biapenem	0.25–4	1	1
Ceftazidime	0.032–0.25	0.125	0.125
Ampicillin	0.125–64	0.25	1
Pseudomonas aeruginosa, imipenem susceptible (MIC, ≤4) (34)			
J-111,347	0.125–8	0.5	4
J-111,225	0.125–16	1	4
J-114,870	0.125–8	1	4
J-114,871	0.125–8	1	4
Imipenem	0.5–4	2	4
Meropenem	0.125–16	1	4
Biapenem	0.25–4	1	2
Ceftazidime	0.5–>128	1	16
Pseudomonas aeruginosa, imipenem resistant (MIC, ≥8) (38)			
J-111,347	1–16	4	8
J-111,225	2–32	8	16
J-114,870	1–32	4	16
J-114,871	2–64	8	16
Imipenem	8–128	16	32
Meropenem	8–128	8	16
Biapenem	1–>128	8	16
Ceftazidime	1–>128	8	128
Acinetobacter spp. (21)			
J-111,347	≤0.008–0.25	0.032	0.125
J-111,225	≤0.008–0.5	0.032	0.125
J-114,870	0.032–0.5	0.063	0.25
J-114,871	0.016–0.5	0.032	0.125
Imipenem	0.016–0.25	0.125	0.125
Meropenem	0.032–0.25	0.125	0.25
Biapenem	0.016–0.25	0.125	0.25
Ceftazidime	0.5–32	2	8
Burkholderia cepacia (21)			
J-111,347	0.25–16	8	16
J-111,225	0.5–32	8	16
J-114,870	0.5–16	8	16
J-114,871	0.5–32	8	32
Imipenem	0.5–16	4	16
Meropenem	0.032–8	1	4
Biapenem	0.125–8	2	8
Ceftazidime	0.5–>128	2	16
Bacteroides fragilis (22)			
J-111,347	0.125–0.5	0.25	0.5
J-111,225	0.25–1	0.25	0.5
J-114,870	0.125–1	0.25	0.5
J-114,871	0.25–1	0.25	0.5

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TABLE 1—*Continued*

Organism (no. of isolates) and antibiotic	MIC (μg/ml)[a]			Organism (no. of isolates) and antibiotic	MIC (μg/ml)[a]		
	Range	50%	90%		Range	50%	90%
Imipenem	0.063–2	0.5	0.5	J-111,225	1–2	2	2
Meropenem	0.063–4	0.25	0.25	J-114,870	1–2	2	2
Biapenem	0.125–4	0.25	0.5	J-114,871	1–2	2	2
Ceftazidime	4–>128	16	>128	Imipenem	4–16	8	8
				Meropenem	0.5–4	1	2
Clostridium difficile (21)				Biapenem	4–16	8	16
J-111,347	0.5–1	1	1	Ceftazidime	32–>128	128	>128

[a] Determined by the broth microdilution method.

trans-3,5-disubstituted pyrrolidinylthio-1β-methylcarbapenems J-111,347 (the prototype), J-111,225, J-114,870, and J-114,871.

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MATERIALS AND METHODS

Antibiotics. All of the carbapenems used in this study except imipenem and vancomycin were synthesized at Banyu Tsukuba Research Institute, Tsukuba, Japan; imipenem was a product of Banyu Pharmaceutical Co., Ltd., Tokyo, Japan, and vancomycin was purchased from Sigma Chemical Co., St. Louis, Mo. The antibiotics were dissolved in 10 mM 3-(*N*-morpholino)propanesulfonic acid (MOPS) buffer (pH 7.0) on the day of use.

Organisms. The clinical isolates used in this study were collected from several hospitals in Japan over the past several years. All isolates were maintained in glycerol broth at −80°C.

Determination of MICs. MICs were determined by the twofold serial broth microdilution method with Mueller-Hinton broth (Difco Laboratories, Detroit, Mich.) unless stated otherwise. Susceptibility testing for streptococci was performed with Todd-Hewitt broth (Difco) supplemented with 5% hemolyzed horse blood. Brain heart infusion broth (Difco), brain heart infusion broth (Difco) supplemented with 5% Fildes enrichment (Difco), and GAM broth (Nissui Seiyaku Co., Ltd., Tokyo, Japan) were used for enterococci, *Haemophilus influenzae*, and anaerobes, respectively. The inoculum sizes of gram-positive or -negative bacteria and anaerobes were 10^5 and 10^6 CFU/ml, respectively. The MIC was defined as the lowest antibiotic concentration that completely prevented visible growth after incubation at 37°C for 20 h.

Killing kinetics. MRSA strain BB6226, a β-lactamase-negative homogeneously resistant strain, was used. After preincubation at 37°C for 2 h, an antibiotic was added and the test tubes were incubated in a water bath at 37°C with gentle shaking. The viable cells were counted on a Mueller-Hinton medium (Difco) plate after an aliquot of the culture was taken at the times indicated in Fig. 2. The limit of detection of viable counts was 10 CFU/ml.

Affinity of PBP 2a of MRSA. The affinity of PBP 2a of MRSA was determined by a competition assay with [^{14}C]benzylpenicillin (15). Briefly, membrane fractions were preincubated at 30°C for 10 min with nonlabeled antibiotics and were postincubated with [^{14}C]benzylpenicillin for 10 min. Binding affinity was expressed as the concentration of nonlabeled antibiotic that inhibited radiolabeling with [^{14}C]benzylpenicillin by 50% (IC_{50}) compared with the control in the absence of nonlabeled antibiotics.

RESULTS

In vitro activity. The comparative antibacterial activities of J-111,347, J-111,225, J-114,870, J-114,871, and the reference antibiotics against the clinical isolates are shown in Table 1. The new carbapenems showed clearly improved activities against the isolates of MRSA and methicillin-resistant coagulase-negative staphylococci (MRCoNS) compared with those of the older carbapenems imipenem, meropenem, and biapenem. J-111,347, J-111,225, J-114,870, and J-114,871 inhibited 90% of the MRSA isolates at concentrations of 2, 4, 4, and 4 μg/ml (MICs at which 90% of isolates are inhibited [MIC_{90}]), respectively, representing activity 32 to 64 times more potent than that of imipenem, which has an MIC_{90} of 128 μg/ml. The activity of J-111,347 was about half as potent as or equivalent to that of vancomycin against MRSA and MRCoNS in terms of the MIC_{90}. In general, J-111,225, J-114,870, and J-114,871 were almost as active or were slightly less active than J-111,347 against the MRS isolates. Thus, the new carbapenems are definitely differentiated from conventional carbapenems in terms of their anti-MRS activities.

The new carbapenems were as active as imipenem against methicillin-susceptible *S. aureus*, *Streptococcus pyogenes*, *Streptococcus agalactiae*, and penicillin-resistant *Streptococcus pneumoniae*. The MIC_{90}s of the new carbapenems against penicillin-resistant *S. pneumoniae* were uniformly 0.25 μg/ml, as were those of imipenem and biapenem, while the MIC_{90} of penicillin G was 16 μg/ml. Against *Enterococcus faecalis*, the new carbapenems were two to four times less active than imipenem, but they were two to four times more active than meropenem and biapenem. Imipenem-resistant *Enterococcus faecium* showed cross-resistance to the new carbapenems.

As for gram-negative isolates, J-111,347, J-111,225, J-114,870, and J-114,871 had improved activities compared to those of imipenem and biapenem against *Moraxella catarrhalis* and *H. influenzae*, with MIC_{90}s of ≤0.008 and 0.125 μg/ml, respectively (MIC_{90}s of imipenem, 0.063 and 1 μg/ml, respectively); however, their activities against these two gram-negative bacteria were similar to that of meropenem. The activities of the new compounds were similar to or greater than those of imipenem and biapenem against members of the family *Enterobacteriaceae* such as *Escherichia coli*, *Klebsiella pneumoniae*, *Enterobacter cloacae*, *Citrobacter freundii*, *Citrobacter diversus*, *Proteus mirabilis*, *Proteus vulgaris*, *Providencia rettgeri*, *Morganella morganii*, and *Serratia marcescens*, but meropenem was the most potent agent against the gram-negative bacteria tested. Some of these enteric bacteria showed high-level resistance to ceftazidime, probably due to the high level of expression of chromosomal AmpC β-lactamase (or extended spectrum β-lactamases). All of the new compounds were two to four times more active than imipenem against imipenem-susceptible *P. aeruginosa*, with the MIC_{50}s ranging from 0.5 to 1 μg/ml, whereas the MIC_{50} of imipenem was 2 μg/ml; likewise, the new compounds were more active than imipenem against imipenem-resistant *P. aeruginosa*.

As a whole, there was no significant difference in the antibacterial activities of J-111,225, J-114,870, and J-114,871, although these compounds were slightly less active than J-111,347. Considering the history of development of β-lactam antibiotics, it is interesting that the new agents have the advantage of dual activity against both MRS and *P. aeruginosa*.

Bactericidal activity. Bactericidal activity against MRSA was determined by constructing time-kill curves (Fig. 2). As expected, J-111,225, J-114,870, and J-114,871 showed bactericidal kinetics at concentrations above the MICs of 1 to 4 μg/ml, although substantial regrowth was observed after 24 h of incubation in the presence of two times the MICs of J-111,225, J-111,347, and imipenem. Vancomycin (MIC, 1 μg/ml) showed

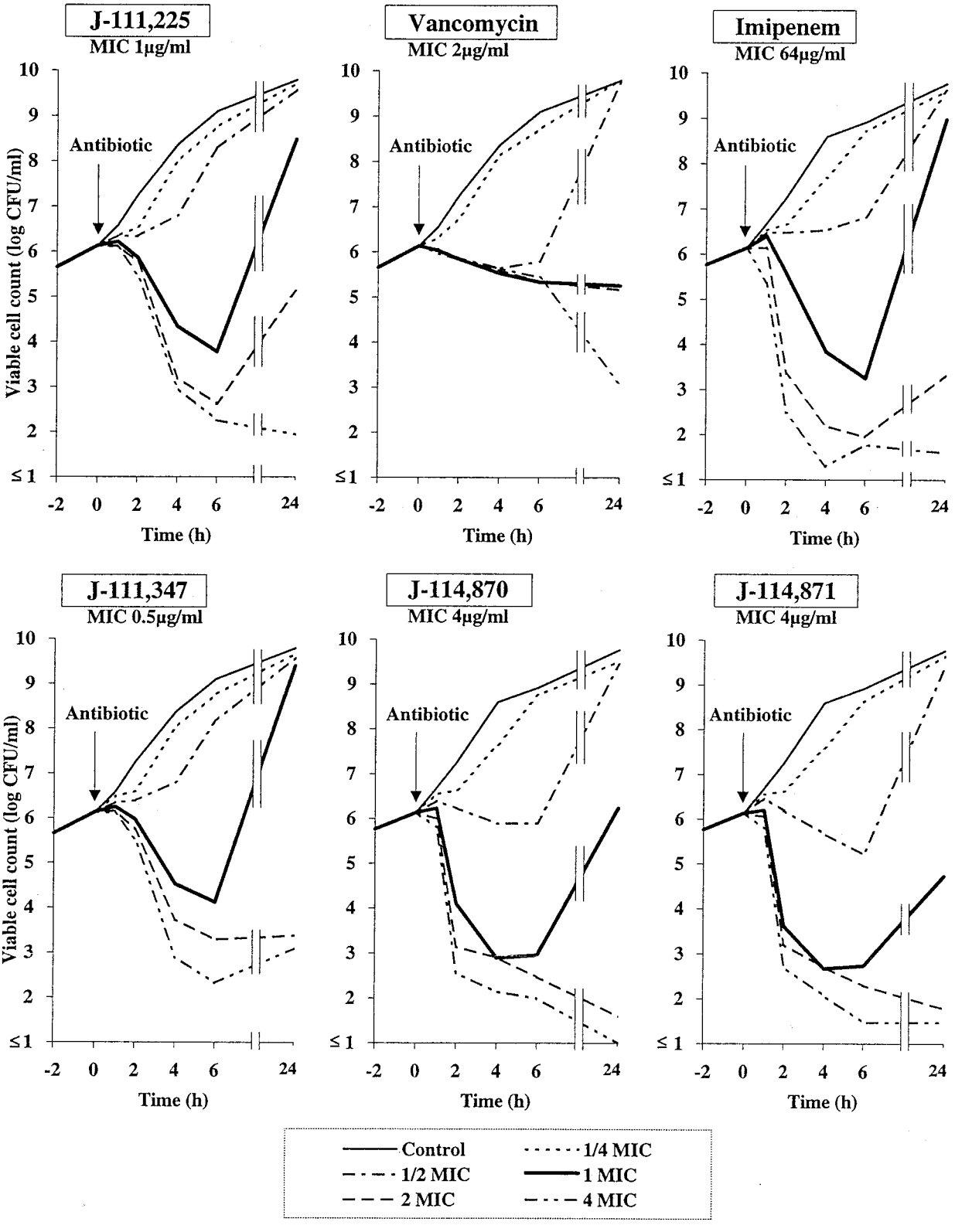

FIG. 2. Killing kinetic curves for *trans*-3,5-disubstituted 1β-methyl carbapenems, imipenem, and vancomycin against homogeneous MRSA.

TABLE 2. Comparative antibacterial activities against β-lactamase producers

Enzyme produced and organism	MIC (μg/ml)[a]										
	J-111,347	J-111,225	J-114,870	J-114,871	Imipenem	Biapenem	Meropenem	Aztreonam	Ceftazidime	Cefazolin	Ampicillin
Penicillinase											
E. coli ML4901 (TEM-1)	0.031	0.031	0.063	0.031	0.125	0.063	0.013	0.063	0.125	2	>128
E. coli ML4901 (PSE-1)	0.016	0.031	0.031	0.031	0.125	0.031	0.016	0.125	0.25	2	>128
E. coli ML4901 (OXA-1)	0.031	0.063	0.063	0.063	0.25	0.125	0.031	0.25	0.25	2	128
Cephalosporinase											
E. coli GN5482	0.016	0.031	0.031	0.031	0.125	0.031	0.016	2	2	64	64
M. morganii GN5407	0.125	0.125	0.125	0.063	2	0.5	0.031	0.008	0.125	128	128
C. freundii GN346	0.063	0.125	0.125	0.063	0.25	0.125	0.063	32	64	>128	>128
E. cloacae GN7471	0.063	0.125	0.125	0.125	0.5	0.5	0.063	8	32	>128	>128
P. aeruginosa GN10362	0.5	0.5	0.5	1	1	1	1	4	1	>128	>128
Oxyiminocephalosporinase											
Klebsiella oxytoca GN10650	0.063	0.063	0.063	0.063	0.25	0.063	0.031	8	0.125	>128	128
P. vulgaris GN7919	0.125	0.125	0.125	0.125	0.5	0.25	0.031	1	2	64	64
B. cepacia GN11164	2	4	8	8	8	2	4	2	0.5	>128	>128
Carbapenemase											
P. aeruginosa GN17203 (IMP-1)	16	32	32	64	128	>128	128	2	>128	>128	>128
Stenotrophomonas maltophilia GN12873 (L1, L2)	32	128	128	64	128	>128	32	>128	16	>128	128

[a] Determined by the broth microdilution method.

slow killing kinetics even at four times the MIC. Imipenem required high concentrations (over 64 μg/ml) to kill MRSA.

Activity against β-lactamase producers. The new carbapenems were active against various β-lactamase-producing bacteria except for *Burkholderia cepacia* and metallo-β-lactamase producers (Table 2). With the exceptions of *B. cepacia* and metallo-β-lactamase producers, the MICs of the new carbapenems for ampicillin- and/or cefazolin-resistant β-lactamase producers were within the susceptible range for other carbapenems, as were those of the reference carbapenems. In *E. coli*, there seemed to be no appreciable influence of β-lactamase producing strain on the activity since basal AmpC β-lactamase production had similar susceptibility to the respective new carbapenems (MIC, 0.031 μg/ml). It is noteworthy that the new drugs had greater activities than the marketed carbapenems against the IMP-1 β-lactamase-producing organism.

Mechanism of action against MRSA. Since PBP 2a, which has a low affinity for β-lactams, is the resistance determinant for β-lactam antibiotics in MRS, the binding affinities of J-111,347 and J-111,225 for PBP 2a were investigated. These two carbapenems had improved IC_{50}s of 2.6 and 2.5 μg/ml, respectively, compared with the IC_{50} of 85 μg/ml of imipenem in a competition assay with benzylpenicillin. There was a good correlation between anti-MRSA activity and the binding affinity for PBP 2a of the MRSA strain tested, indicating that the mechanism of anti-MRSA activity could be ascribed to the inhibition of PBP 2a.

DISCUSSION

MRS and gram-negative organisms including *P. aeruginosa* are common pathogens in serious infections, and vancomycin has been the agent of choice for the treatment of infections due to MRS, despite its association with a variety of drug-related side effects. Although a number of antibiotics that target MRSA and other resistant gram-positive organisms have been reported, no antibiotic so far provides potent anti-MRS activity while also providing the excellent broad-spectrum activity offered by carbapenems.

We conducted chemical modification studies with 1β-methylcarbapenem on the basis of the concept that a new analogue should provide coverage against MRS as well as other gram-negative organisms including *P. aeruginosa*. J-111,225, J-114,870, and J-114,871 are crystalline forms, and their structural features include a unique stereochemistry of the side chain, i.e., a phenyl ring directly attached to the pyrrolidine ring with a 3,5-*trans* configuration at the C-2 position of the carbapenem nucleus.

Since previously reported anti-MRS agents have limited activity against gram-negative bacteria, it was noted that the newly synthesized carbapenems with activity against MRSA showed improved activity against gram-negative organisms including *P. aeruginosa* and metallo-β-lactamase producers compared with the activity of imipenem. Metallo-β-lactamase or carbapenemase is known to confer high-level resistance to penicillins and cephems as well as to carbapenems (16). A recent problem is the spread of the transferable plasmid-mediated IMP-1 enzyme, especially in Japan (1). The mechanism underlying improved activity against IMP-1-producing organisms was explained by resistance to hydrolysis by this enzyme (R. Nagano, Y. Adachi, T. Hashizume, and S. Morishima, Abstr. 38th Intersci. Conf. Antimicrob. Agents Chemother., abstr. F-55, p. 247, 1998).

Although further evaluation of J-111,347 was suspended due to its epileptogenicity, this undesirable adverse effect was clearly eliminated by N methylation (J-111,225) or the introduction of carbamoylmethyl substituents (J-114,870 and J-114,871) at the α position of the benzylamino group.

The new carbapenems could be suited for use as monotherapy for serious polymicrobial infections associated with MRS. Furthermore, monotherapy with the new carbapenems would avoid unexpected adverse reactions due to combination therapy and the emergence of vancomycin-resistant MRS due to the intensive use of vancomycin. In conclusion, the novel *trans*-3,5-disubstituted pyrrolidinylthio-1β-methylcarbapenems J-111,347 (prototype), J-111,225, J-114,870, and

J-114,871 possess broad spectra of activity, with coverage of MRSA and *P. aeruginosa*, and are worthy of further evaluation.

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