AAC Accepts, published online ahead of print on 12 November 2012
Antimicrob. Agents Chemother. doi:10.1128/AAC.01051-12

Novel Carbapenem Antibiotics for Parenteral and Oral Applications: In Vitro and In Vivo Activities of 2-Aryl Carbapenems and Their Pharmacokinetics in Laboratory Animals

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Running title: Novel Parenteral and Oral 2-Aryl Carbapenems

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ABSTRACT

SM-295291 and SM-369926 are new parenteral 2-aryl carbapenems with strong activity against major causative pathogens of community-acquired infections such as methicillin-susceptible *Staphylococcus aureus*, *Streptococcus pneumoniae* including penicillin-resistant strains, *Streptococcus pyogenes*, *Enterococcus faecalis*, *Klebsiella pneumoniae*, *Moraxella catarrhalis*, *Haemophilus influenzae* including β-lactamase negative ampicillin-resistant strains, and *Neisseria gonorrhoeae* including ciprofloxacin -resistant strains, with MIC for 90% of isolates of ≤1 μg/ml. Unlike tebipenem (MIC for 50% of isolates [MIC_{50}], 8 μg/ml), SM-295291 and SM-369926 had no activity against hospital pathogens such as *Pseudomonas aeruginosa* (MIC_{50}, ≥128 μg/ml). The bactericidal activity of SM-295291 and SM-369926 against penicillin-resistant *S. pneumoniae* and β-lactamase negative ampicillin-resistant *H. influenzae* was equal or superior to tebipenem and greater than cefditoren. Therapeutic efficacy of intravenous administration of SM-295291 and SM-369926 against experimentally induced infections in mice caused by penicillin-resistant *S. pneumoniae* and β-lactamase negative ampicillin-resistant *H. influenzae* was equal or superior to tebipenem and greater than cefditoren, respectively, reflected their *in vitro* activity. SM-295291 and SM-369926 showed similar intravenous pharmacokinetics to meropenem in terms of

half-life in monkeys (0.4 h) and were stable against human dehydropeptidase-I. SM-368589 and SM-375769 which are medoxomil esters of SM-295291 and SM-369926, respectively, showing good oral bioavailability in rats, dogs, and monkeys (4.2-62.3%). Thus, 2-aryl carbapenems are promising candidates that show an ideal broad spectrum for the treatment of community-acquired infections, including infections caused by penicillin-resistant *S. pneumoniae* and β-lactamase negative ampicillin-resistant *H. influenzae*, have low selective pressure on antipseudomonal carbapenem-resistant nosocomial pathogens, and allow parenteral, oral, and switch therapy.

INTRODUCTION

Community-acquired infections caused by extended-spectrum β-lactamase (ESBL)-producers, quinolone-resistant pathogens, penicillin-resistant *S. pneumoniae* (PRSP), and β-lactamase negative ampicillin-resistant *H. influenzae* (BLNAR) are of great concern (19). In moderate or severe cases, inpatient parenteral antibiotic therapy is needed, and carbapenems are often used to treat infections refractory to parenteral penicillin or cephalosporin therapy (e.g., ESBL-producer, PRSP, and BLNAR infections); however, current practices in antipseudomonal carbapenem use are a risk

50 factor for the emergence of carbapenem-resistant nosocomial pathogens, especially
51 *Pseudomonas aeruginosa* (16). Non-antipseudomonal carbapenem, ertapenem (ERM) is
52 used for the treatment of community-acquired infections, but it has little or moderate
53 activity against *P. aeruginosa* (8), implying that there is a risk of selection for resistance
54 to antipseudomonal carbapenem in *P. aeruginosa* (2, 9). In the case of tebipenem
55 (TBM)-pivoxil, oral carbapenem, because there may be concern about the development
56 of cross-resistance to existing parenteral carbapenems in nosocomial pathogens,
57 TBM-pivoxil has been only used as salvage therapy for pediatric patients who are
58 expected to be refractory to another oral antimicrobial agent.

59 Therefore, there is a need to develop a new class of carbapenems that have adequate
60 antibacterial properties for the treatment of community-acquired infections and low
61 selective pressure on conventional carbapenem-resistant bacteria based on structural
62 differences from the existing carbapenems. Combinational development of parenteral
63 and oral formulations of the same new class carbapenem, allowing a switch from
64 parenteral to oral treatment, could contribute to early hospital discharge, decrease the
65 cost of treatment (7, 17, 18), and reduce the risk of selection for cross-resistance to
66 existing parenteral carbapenems in nosocomial pathogens.

67 We previously reported that 2-aryl carbapenems, which are desmethyl-carbapenems

with a structurally unique C2 side chain, exhibited well-balanced antibacterial activities against important pathogens of community-acquired infections (26).

Based on structure-activity relationships studies, we identified an attractive 2-aryl carbapenems, SM-295291, (5*R*,6*S*)-6-[(*R*)-1-hydroxyethyl]-3-{4-[(methoxycarbonylamino)methyl]phenyl}-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid, and SM-369926, (5*R*,6*S*)-6-[(*R*)-1-hydroxyethyl]-3-{4-[(methylcarbamoyloxy)methyl]phenyl}-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid (Fig. 1), and in this study, we investigated their *in vitro* antibacterial activities, compared with those of TBM, cefditoren (CDN), faropenem (FRM), clarithromycin (CLR), and levofloxacin (LVX). We evaluated the therapeutic efficacy of intravenous administration of SM-295291 and SM-369926 in several PRSP and BLNAR infection models, and their pharmacokinetics in various animals. Esterification of β-lactams is one of the ways to improve their oral absorption (12); therefore, we synthesized medoxomil esters of SM-295291 and SM-369926 (SM-368589 and SM-375769, respectively) (Fig. 1), and assessed their oral bioavailability in various animals.

(This study was presented in part at the 51st Interscience Conference on Antimicrobial Agents and Chemotherapy, Chicago, IL, September 2011 [abstr. F1-139 and F1-140].)

MATERIALS AND METHODS

Organisms. Clinical isolates used in this study were collected from different patients in various hospitals in Japan from 1996 to 2009. The β-lactamase-producing organisms were from our bacterial collections (28).

Antibacterial agents. SM-295291, SM-369926, SM-368589, SM-375769, TBM, FRM, and meropenem (MEM) were synthesized in our laboratories. Imipenem (IPM) and cilastatin were prepared from TIENAM (MSD K.K., Tokyo, Japan), and CDN, CLR, and LVX were prepared from MEIACT MS (Meiji Seika Pharma Co., Ltd., Tokyo, Japan), Klaricid (Abbott Japan Co., Ltd., Tokyo, Japan), and CRAVIT (Daiichi Sankyo Company, Limited, Tokyo, Japan), respectively, in our laboratories. ERM (INVANZ; Merck & Co., Inc., Whitehouse Station, NJ) was purchased.

Animals. Male ICR mice (Japan SLC, Inc., Shizuoka, Japan)**,** male Sprague-Dawley (SD) rats (Charles River Laboratories Japan, Kanagawa, Japan), male New Zealand White rabbits (Kitayama Labes, Co., Ltd., Nagano, Japan), and male beagle dogs and cynomolgus monkeys (Japan Laboratory Animal, Inc., Tokyo, Japan) were used. All animal procedures were performed in accordance with the institution's guidelines for the humane handling, care, and treatment of research animals in Dainippon Sumitomo

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Susceptibility testing. MICs were determined by the twofold serial agar dilution method recommended by the Japanese Society of Chemotherapy and the Clinical and Laboratory Standards Institute guidelines (14) with Mueller-Hinton agar (MHA) (Becton, Dickinson and Company, Tokyo, Japan) unless otherwise specified. Susceptibility testing was performed with MHA supplemented with 5% defibrinated horse blood for streptococci and Mueller-Hinton chocolate agar (5% defibrinated horse blood) for *H. influenzae* and *Neisseria gonorrhoeae*. Modified GAM agar (Nissui Pharmaceutical Co., Ltd., Tokyo, Japan) was used for the culture of anaerobic bacteria. The final inocula comprised approximately 10^6 CFU/ml. Agar plates were incubated at 35°C for 18 and 48 h for aerobic and anaerobic bacteria, respectively. Incubation was carried out anaerobically for anaerobes and in an atmosphere of 5% CO_2 for streptococci, *H. influenzae*, and *N. gonorrhoeae*. The MIC was defined as the lowest drug concentration that completely prevented visible growth.

Determination of MBC. MIC tests were performed by the broth microdilution method, with the organism grown in Mueller-Hinton broth. Minimal bactericidal concentration (MBC) was determined by aspirating the antibiotic-containing medium in MIC assay well, and adding antibiotic-free MHA. The MBC was defined as the lowest

antibiotic concentration that reduced the number of viable cells by >99.9%.

Time-kill curves. An aliquot of 4.5 ml bacterial suspension in medium (about 10^6 CFU/ml) was mixed with 0.5 ml drug solution in medium and incubated at 35°C in an atmosphere of 5% CO_2. Viable cell counts were determined by a general plating method 2, 4, and 6 h after drug addition.

In vitro evaluation of the emergence of carbapenem-resistant *P. aeruginosa.* *P. aeruginosa* PAO1 was incubated in Mueller-Hinton broth (MHB) (Becton, Dickinson and Company) containing various concentrations of drugs at 37°C for 1 day. MIC was defined as the lowest concentration of drugs that resulted in no visible growth in the broth. Serial passages of *P. aeruginosa* PAO1 were done daily for 14 days in MHB in the presence of SM-295291 or SM-369926 at 8 μg/ml. In the case of TBM and ERM, *P. aeruginosa* PAO1 in MHB containing the highest concentration of drug in which the optical density was almost the same as drug-free control was transferred to the fresh medium containing various drug concentrations. This passage was performed daily for 14 days.

Stability against hydrolysis by DHP-I. The stability of carbapenems to hydrolysis by DHP-I was determined with purified rat and dog renal DHP-I and recombinant human DHP-I, as reported previously (3, 28). The activity of DHP-I was

spectrophotometrically determined by measuring the hydrolysis of glycyldehydrophenylalanine as a substrate (28). The relative rate of hydrolysis was also calculated as a ratio against the hydrolysis rate for IPM or MEM, which was assigned a value of 1.

Stability in human plasma. An aliquot of 1 ml human plasma (Rockland Immunochemicals Inc., Gilbertsville, PA; Cat. No. D519-06) was mixed with 10 µl drug solution in 1/15 M phosphate buffer (pH 7.4) at a concentration of 30 µg/ml. The plasma sample was kept at 37°C for 4 h. The sample was mixed with three volumes of methanol, vortex-mixed and centrifuged at 10,000 g for 10 min at 4°C. The supernatant was collected. The levels of drugs in human plasma were determined by high-pressure liquid chromatography (HPLC)-UV detection assay method consisting LC-2010C and CLASS-VP workstation (Shimadzu Co., Kyoto, Japan). Chromatography was performed using Xterra MS C_{18} reverse-phase column (3.5 µm, 4.6 × 20 mm; Nihon Waters K.K., Tokyo, Japan). The mobile phase consisted of 5 mM phosphate buffer (pH 7.0) and methanol. The flow rate was 1.5 ml/min. The wavelength for drug detection was 302 nm.

Protein binding. Percent binding to rat, dog, and human plasma protein was determined by the ultrafiltration method (24). The degree of binding was measured

158 using a drug concentration of 30 μg/ml. The concentration of each drug in the
159 flow-through fraction was measured by HPLC.

160 **Murine PRSP and BLNAR infection models.** Male ICR mice were used at 4 weeks
161 of age. At each administration, 100 mg/kg cilastatin, a DHP-I inhibitor, was
162 administered subcutaneously 5 min before administration of SM-295291, SM-369926,
163 and TBM in the murine infection models, because the hydrolyzing activities of DHP-I
164 for a carbapenem differ greatly among animal species.

165 **(i) Systemic infection.** Mice were inoculated intraperitoneally with 0.5 ml of 5%
166 mucin (Nacalai Tesque Inc., Kyoto, Japan) suspension of PRSP 18280 (1.4×10^4
167 CFU/mouse). Drugs in saline were administered intravenously 1 and 3 h after infection.

168 **(ii) Pulmonary infection.** To induce neutropenia, cyclophosphamide was
169 administered intraperitoneally 4 days before (200 mg/kg/day, PRSP and BLNAR
170 infection models) and 4 h before (100 mg/kg/day, BLNAR infection model only)
171 infection. For airway impairment, 50 μl influenza virus A/PR8 suspension was instilled
172 intranasally into mice 5 days before BLNAR infection. Fifty microliters of PRSP 18280
173 (1.7×10^6 CFU/mouse) or BLNAR 17051 (4.4×10^7 CFU/mouse) suspension was
174 inoculated intranasally. Drugs in saline were administered intravenously thrice daily at 1
175 and 2 days after infection.

176 **(iii) Meningitis.** Mice were challenged intracerebrally with 2×10^4 CFU of PRSP
177 18280. Drugs in saline were administered intravenously at 5 h after infection and twice
178 daily at 1 and 2 days after infection.

179 **Pharmacokinetic study.** Male SD rats, beagle dogs, and cynomolgus monkeys were
180 used at 8 weeks, 20 months, and 2 years of age, respectively. Three animals were used
181 for each group. SM-295291 or SM-369926 in saline at a dose of 1 mg/kg was
182 administered intravenously to fasted rats given cilastatin at 100 mg/kg, fasted dogs and
183 fasted monkeys. SM-368589 or SM-375769 in 50% propylene glycol at a dose of 1
184 mg/kg was administered intraduodenally to fasted rats with cilastatin at 100 mg/kg, and
185 orally to fasted dogs and monkeys with omeprazole at 1 mg/kg. The plasma drug
186 concentrations were determined by the liquid chromatography-mass spectrometric
187 (LC-MS/MS) method consisting of API2000 (AB Sciex, Tokyo, Japan) with Agilent
188 1100 series (Agilent Technologies, Santa Clara, CA). Chromatography was performed
189 using Atlantis dC_{18} columns (5.0 μm particle size, 4.6 × 50 mm, Waters K.K.). The
190 mobile phase consisted of 10 mM ammonium acetate and acetonitrile. The flow rate
191 was 0.2 ml/min. Plasma samples were deproteinized using acetonitrile prior to
192 LC-MS/MS analysis. Compound was detected by selective reaction monitoring under
193 the positive ionization electrospray mode. The pharmacokinetic parameters were

194 calculated according to the moment analysis.

195 **Testing of convulsant activity.** The convulsant stability of carbapenems was
196 determined as reported previously (25). Seven-week-old male ICR mice were
197 intracerebroventricularly injected with each dose (50 to 400 μg/mouse) of drugs
198 dissolved in 5 μl phosphate-buffered saline. Immediately after injection, incidences of
199 clonic and tonic convulsion were recorded for 30 min.

200 **Assessment of renal nephrotoxicity.** SM-295291 at a dose of 100 mg/kg was
201 administered intravenously to two rabbits. Blood and urine were collected at 1 (urine
202 only), 2, and 4 days after administration. The kidneys were removed 4 days after the
203 dose. The following parameters were investigated: blood urea nitrogen, blood creatinine,
204 urinary glucose, urinary protein, urinary pH, renal weight, macroscopic examination of
205 kidneys, and histopathological examination of renal sections. Because the synthetic
206 quantity of SM-369926 was insufficient, we were not able to evaluate the
207 nephrotoxicity of SM-369926.

208 **Statistical analysis.** The 50% effective dose (ED_{50}) and the convulsant activity (ED_{50})
209 were calculated by probit analysis. Dunnett's test for multiple comparisons were used to
210 assess significant differences in the bacterial burden. All analyses were performed using
211 the Statistical Analysis System for Windows (SAS Institute Inc., Cary, NC).

212

213 **RESULTS**

214 **In vitro antimicrobial activity.** Strong antibacterial activity is required for oral
215 antibiotics because of a relatively low achievable concentration in blood compared to a
216 parental drug. Therefore, to determine whether our 2-aryl carbapenems could be
217 attractive candidates for alternative oral antibiotics, antimicrobial activity of
218 SM-295291 and SM-369926 was compared with those of conventional oral antibiotics
219 (TBN, CDN, FRM, CLR, and LVX) (Tables 1 and 2).

220 The MICs of SM-295291 and SM-369926 against methicillin-susceptible
221 staphylococci ranged from ≤0.0313 to 0.25 μg/ml and were lower than all other
222 comparators except TBM. Against the streptococci (except PRSP), the maximum MIC
223 observed for SM-295291 and SM-369926 was 0.0313 μg/ml, which was lower than all
224 comparators except TBM (0.0156 μg/ml). SM-295291 and SM-369926 had the lowest
225 MICs against *E. faecalis* of any tested comparators. SM-295291 and SM-369926
226 exhibited low to moderate activity against *E. faecium*, and MIC_{50} and MIC_{90} for this
227 organism were 32 and 64 μg/ml, respectively.

228 SM-295291 and SM-369926 demonstrated strong activity with MIC_{90}s ≤1 μg/ml
229 against most Gram-negative species, *E. coli*, *K. pneumoniae*, *H. influenzae*, *M.*

catarrhalis, and *N. gonorrhoeae*. Unlike TBM (MIC_{50}, 8 μg/ml), SM-295291 and SM-369926 had no activity against *P. aeruginosa* (MIC_{50}, ≥128 μg/ml). SM-295291 and SM-369926 showed very poor activity against *Acinetobacter* spp.

SM-295291 and SM-369926 showed potent activity against peptostreptococci and *Bacteroides fragilis*, with an MIC_{90} of ≤2 μg/ml, which was similar to TBM and lower than CDN, CLR, and LVX.

SM-295291 and SM-369926 were less active against methicillin-resistant staphylococci (MIC_{90}, ≥128 μg/ml). The SM-295291 and SM-369926 MIC_{90} value of 0.125 μg/ml for PRSP was comparable to TBM and ≥8-hold more potent than FRM, CDN, CLR, and LVX. The MIC_{90}s of SM-295291 and SM-369926 against BLNAR were 0.25 and 1 μg/ml, respectively, which were less than LVX but comparable to TBM and CDN and ≥16-hold more potent than FRM and CLR. Against the ciprofloxacin-resistant isolates of *N. gonorrhoeae*, the *in vitro* activities of SM-295291 and SM-369926 were higher than those of FRM and LVX and comparable to that of CLR, but were lower than those of TBM and CDN. Against ciprofloxacin- and ceftazidime-resistant *E. coli*, the *in vitro* activities of SM-295291 and SM-369926 were similar to FRM and higher than the other comparators, with the exception of TBM. As shown in Table 3, SM-295291 and SM-369926 maintained activity against *E. coli* and *K.*

248 *pneumoniae* producing ESBL, although the MICs of SM-295291 and SM-369926 were
249 higher than IPM. For these ESBL-producing isolates, no inoculum effect was observed
250 for SM-295291 and SM-369926 as well as IPM.
251
252 **Bactericidal activity.** SM-295291 and SM-369926 were bactericidal against *S. aureus*,
253 *E. coli*, and *K. pneumoniae* in terms of an MBC/MIC ratio of 1 (Table 4).
254 In time-kill assays, SM-295291 caused a 2-log reduction in the CFU of *S. pneumoniae*
255 18280 (PRSP) at more than the MIC until 2 h, whereas the killing rate of CDN was
256 relatively low (Fig. 2A). After 6 h of incubation, SM-295291 and TBM resulted in a
257 4-log reduction at more than the MIC and 2 × MIC, respectively. For *H. influenzae*
258 17051 (BLNAR), four times the MIC of SM-295291 caused a 2-log reduction after 6 h;
259 its killing kinetics was similar to those of IPM (Fig. 2B). The killing rate of SM-295291
260 was higher than TBM. The killing rate of CDN was lower than SM-295291 until 4 h,
261 although CDN only achieved a 3-log reduction at 4 × MIC 6 h after incubation.
262
263 **In vivo efficacy against PRSP and BLNAR.** Prior to all murine experiments, we
264 determined the pharmacokinetics of SM-295291, SM-369926, and MEM administered
265 intravenously at a dose of 10 mg/kg with 2 mg cilastatin in ICR male mice. The $C_{5\ min}$s

of SM-295291, SM-369926, and MEM were 49.8, 53.6, and 21.0 μg/ml, respectively. The area under the serum concentration-time curve (AUC) for SM-295291, SM-369926, and MEM were 1383, 1942, and 481 μg·min/ml, respectively. The $t_{1/2}$s of SM-295291, SM-369926, and MEM were 21.5, 30.5, and 10.9 min, respectively. SM-295291 and SM-369926 exhibited better pharmacokinetics than MEM in mice.

The MICs of SM-295291, SM-369926, TBM, FRM, CDN, CLR, and LVX against PRSP 18280 were 0.125, 0.0625, 0.0625, 0.5, 0.5, 2, and 1 μg/ml, respectively.

In a mouse systemic and meningitis infection models with PRSP 18280, the ED_{50} of SM-295291 and SM-369926 were comparable to TBM and much lower than CDN (Table 5). In a murine pneumonia model, the bacterial count in the lungs of untreated mice on day 3 after infection was 7.19 log CFU (Fig. 3A). SM-295291 and SM-369926 dose-dependently reduced bacterial numbers in the lungs following six intravenous injections of 0.32, 1, and 3.2 mg/kg/dose and caused >5-log reduction of bacterial numbers at 3.2 mg/kg/dose.

The MICs of SM-295291, SM-369926, TBM, and CDN against BLNAR 17051 were 0.125, 0.125, 0.5, and 0.25 μg/ml, respectively. The bacterial count in the lungs of untreated mice on day 3 after infection was 7.06 log CFU (Fig. 3B). Dose-dependent effects of SM-295291 and SM-369926 were observed: treatment with 1, 5, and 20

mg/kg resulted in 2-log, 3-log, and 4-log reduction of bacterial numbers in the lungs, respectively.

In vitro serial passage of *P. aeruginosa*. We evaluated the risk of the emergence of carbapenem-resistant *P. aeruginosa* after the clinical use of our 2-aryl carbapenem with oral application. The MICs of SM-295291, SM-369926, TBM, ERM, IPM, and MEM against *P. aeruginosa* PAO1 were 128, 64, 2, 4, 1, and 0.25 μg/ml, respectively. Since the blood concentration of oral antibiotics generally achieves less than 8 μg/ml, serial passages of *P. aeruginosa* PAO1 were done in the presence of SM-295291 (8 μg/ml), SM-369926 (8 μg/ml), TBM (initial concentration, 1 μg/ml), or ERM (initial concentration, 2 μg/ml). Against *P. aeruginosa* PAO1, the MICs of SM-295291 and SM-369926 against were always within 2-fold of the initial values during 14 daily passages, whereas the MICs of TBM and ERM increased 32 fold from 2 to 64 μg/ml and 16 fold from 4 to 64 μg/ml, respectively (Fig. 4). Exposure to SM-295291 and SM-369926 had little to no impact on the MICs of IPM and MEM (1 and 0.5 μg/ml, respectively); in contrast, passages in sub-inhibitory levels TBM resulted in cross-resistance development to IPM and MEM (both MICs were 16 μg/ml). The exposure to ERM sub-inhibitory concentrations showed a 2-fold increase in IPM MIC (2 μg/ml) and an 8-fold increase in MEM MIC (2 μg/ml).

Pharmacokinetics in laboratory animals. SM-295291 and SM-369926 were more stable to hydrolysis by human DHP-I than IPM: the relative hydrolysis rates of SM-295291 and SM-369926 were 0.55 and 0.46, respectively, compared to which the rate of IPM was assigned a value of 1. The stability of SM-295291 and SM-369926 to hydrolysis by human DHP-I was equal to that of MEM: the relative hydrolysis rates of SM-295291 and SM-369926 were 0.85 and 0.99, respectively, compared to which the rate of MEM was assigned a value of 1. In contrast to human and dog (0.29, ratio of susceptibility compared with IPM) DHP-I, rat DHP-I rapidly hydrolyzed SM-295291, and the ratio of susceptibility was 9.24.

To avoid this species-specific effect by rat DHP-I on the metabolism of carbapenems in rats, SM-295291 and SM-369926 were administered with DHP-I inhibitor, cilastatin, to rats in subsequent pharmacokinetic analysis. The $AUC_{0\text{-}3h}$s and $t_{1/2}$s of SM-295291 and SM-369926 at a dose of 1 mg/kg were 1.13 to 1.69 μg·h/ml and 0.39 to 0.56 h, respectively, in dogs and monkeys (Table 6). The $AUC_{0\text{-}3h}$ (6.41 and 5.35 μg·h/ml) and $t_{1/2}$ (0.85 and 0.45 h) of SM-295291 and SM-369926 in rats were higher than or equal to those in dogs and monkeys, probably due to the co-administration of cilastatin and/or higher rat plasma protein binding (87.3% in rats versus 32.2% in dogs for SM-295291).

Sumita *et al*. reported that the $t_{1/2}$s of MEM at a dose of 20 mg/kg in dogs and monkeys were 0.68 and 0.52 h, respectively (23), indicating they were almost equal to those of SM-295291 and SM-369926.

SM-295291 and SM-369926 were relatively stable after 4-h incubation at 37°C in human plasma, although their residual percentages (44% and 22%, respectively) were lower than those of IPM and MEM (60% and 70%, respectively). The consideration of the half-life of IPM and MEM in humans (1 h) (15), pharmacokinetics of SM-295291 and SM-369926 in humans may not be greatly influenced by their stability in human plasma over 4 h. SM-295291 and SM-369926 were not highly bound to human plasma protein (43% and 64%, respectively), although the protein binding rates of IPM and MEM were low (2% and 16%, respectively).

These results suggested that SM-295291 and SM-369926 may show similar pharmacokinetics to MEM in humans.

Pharmacokinetic parameters following intraduodenal or oral administration of ester prodrugs. Since carbapenems are very slightly lipophilic and are hardly orally absorbed from the gastrointestinal tract (27), no non-prodrug carbapenems are being developed for use as oral therapy. Based on our previous study of a suitable series of

ester prodrug, we selected medoxomil ester, because of good oral absorption and the risk of formaldehyde generation from a pivoxil ester (13), although pivoxil esters of 2-aryl carbapenems also showed good oral absorption.

Because SM-295291 and SM-369926 were unstable at normal gastric pH, SM-368589 and SM-375769, which are medoxomil esters of SM-295291 and SM-369926, were administered intraduodenally to rats with cilastatin, and orally to dogs and monkeys with omeprazole, which inhibits gastric acid secretion. The oral bioavailabilities of SM-368589 were 8.0, 62.3, and 12.9%, and of SM-375769 were 17.1, 34.2, and 4.2% in rats, dogs, and monkeys, respectively (Table 6).

Toxicity study. Since carbapenems have been suggested to induce convulsive side effects and have nephrotoxicity in experimental animals and humans (4, 15), preliminary toxicity studies of SM-295291 and SM-369926 were carried out. Intracerebroventricular administration of 50 μg IPM resulted in convulsions in all mice. The administration of 200, 280, and 400 μg SM-295291 or SM-369926 resulted in incidence rates of 0, 20, and 70%, or 0, 0, and 20%, respectively. The administration of 50, 100, and 200 μg TBM resulted in incidence rates of 10, 80, and 90%, respectively. The ED_{50}s of SM-295291 and SM-369926, which induced convulsions in 50% of mice,

were 348.8 and >400 μg/mouse, respectively, and were higher than that of TBM (82.2 μg/mouse), suggesting that SM-295291 and SM-369926 had low CNS toxicity. SM-295291 at 100 mg/kg did not change blood urea nitrogen, blood creatinine, urinary pH, and kidney weight to body weight ratio (SM-295291 versus pre-treatment, 15 versus 20.5 mg/dl, 0.65 versus 0.70 mg/dl, 8.5 versus 8.5, and 0.5%, respectively). Urinary glucose, urinary protein, macroscopic abnormalities, and histopathological abnormalities were not detected in rabbit administered SM-295291 at 100 mg/kg. Therefore, no renal toxicity was seen with SM-295291 at a dose of at least 100 mg/kg in rabbits. In our general safety assessments, all studies indicated that SM-295291 and SM-369926 had no major adverse effects.

DISCUSSION

SM-295291 and SM-369926 have ideal drug properties for the treatment of community-acquired infections, because these compounds show strong (bactericidal), broad-spectrum antibacterial activity against important pathogens of community-acquired infections such as staphylococci, streptococci including PRSP, *E. faecalis*, *M. catarrhalis*, *H. influenzae* including BLNAR, *Enterobacteriaceae* including ESBL-producers, *N. gonorrhoeae* including ciprofloxacin-resistant strains, and

anaerobes, but no activity against non-target nosocomial pathogens such as *P. aeruginosa* and *Acinetobacter* spp. These profiles were due to construction from a unique carbapenem skeleton (desmethyl-carbapenems) and a unique C2 side chain (having 2-aryl moiety).

The excellent antimicrobial activities of SM-295291 and SM-369926 could be confirmed in PRSP and BLNAR infection models. Therapeutic efficacy of SM-295291 and SM-369926 was equal or superior to TBM, which is the only oral carbapenem agent on the market, and were greater than CDN, which is a representative oral cephalosporin, in PRSP and BLNAR infection models, suggesting that SM-295291 and SM-369926 could be effective in clinical infections due to these resistant bacteria. The *in vivo* activities of SM-295291, SM-369926, TBM, and CDN against PRSP and BLNAR showed a correlation with their MIC and *in vitro* early bactericidal activity.

The unbound fraction of the drug (non-protein-bound) is only available for inhibiting bacterial cell growth, and thus the protein-binding properties of antibiotics need to be considered in order to predict their clinical efficacy (1). Since SM-295291 and SM-369926 had a moderate degree of protein binding (43-64%), the effect of 4% human serum albumin on their MICs were assessed against the type strains of Gram-positive and -negative bacteria. The presence of 4% human serum albumin had a

small effect; the MICs of SM-295291 and SM-369926 against *S. aureus* ATCC 6538p, *E. coli* ATCC 25404, *K. pneumoniae* ATCC 10031, *S. pneumoniae* ATCC 6305, and *H. influenzae* ATCC 9334 were within one dilution except for MIC of SM-369926 against *S. aureus* ATCC 6538p (two dilutions). Although CDN is a highly protein bound antibiotics (about 90%), it shows clinical efficacy in respiratory tract infection (20). Theses observations suggest that the protein binding rates of SM-295291 and SM-369926 may not significantly affect their clinical antimicrobial activities.

Our study suggests that SM-295291 and SM-369926 with parenteral application could have similar pharmacokinetics to the existing carbapenems in humans. In the ester prodrug approach, SM-368589 and SM-375769 showed good oral bioavailability in all animals, although the oral bioavailability of SM-368589 and SM-375769 differed among animal species. Another groups of investigators reported that the bioavailabilities of TBM-pivoxil were 59, 35, and 45%, and of cefcapene-pivoxil were 14, 6, and 21% in rats, dogs, and monkeys, respectively (5, 6). For CDN-pivoxil, these were 20 and 10% in rats and dogs, respectively (10). Based on these literature data for the bioavailability of existing ester prodrug of β-lactam agents in animals, it could be expected that SM-368589 and SM-375769 will show oral absorption in humans.

We found that SM-295291 and SM-369926 had good safety profiles. IPM and

410 panipenem (PAM) at a higher dose cause acute renal injuries in animals (4). These renal
411 injuries are closely related to the high intracellular concentration of these agents in renal
412 tubules (4). To inhibit IPM and PAM uptake into the tubular epithelium and prevent
413 their nephrotoxicity, IPM and PAM are co-administered with an anion transport
414 inhibitor, cilastatin and betamipron, respectively (4). Besides the higher stability against
415 human DHP-I, SM-295291 had low renal toxicity in rabbits; therefore,
416 co-administration of cilastatin or betamipron may not be necessary with SM-295291 in
417 humans.

418 Biologically active β-lactam antibiotics in the gut lumen can affect the intestinal
419 microbial flora, causing postantibiotic diarrhea (11, 21). SM-368589 and SM-375769
420 may have no antibacterial activity before their ester bond is hydrolyzed; this may occur
421 either during its passage through the small-intestine wall (12, 21, 22). In addition to
422 improved oral bioavailability, esterification of SM-295291 and SM-369926 would make
423 them likely to have little impact on the intestinal microbial flora compared with
424 non-prodrug agents.

425 Owing to a simple synthetic route, 2-aryl carbapenems are expected to have markedly
426 low manufacturing costs compared to carbapenems with a thiol side chain and
427 1β-methyl, for example, MEM and TBM. Besides a good safety and pharmacokinetic

428 profile because of a unique carbapenem skeleton and a unique side chain, this economic
429 advantage is also a key point in the development of oral antibiotics.

430 Since the adequate antibacterial properties, with typically ≤1 μg/ml MIC_{90} against
431 clinical important pathogens, safety properties, and pharmacokinetic properties of our
432 2-aryl carbapenems seem favorable for not only parenteral formulation but also oral
433 formulation, we believe that combinational development of these formulations is the
434 best way to effective use of their properties. Hospitalized patients with severe
435 community-acquired infections should be treated initially with parenteral agents, and
436 could be switched to oral therapy when the clinical status improves. This switch therapy
437 is gaining acceptance as a means of facilitating early discharge of patients from the
438 hospital and reducing the overall costs of antimicrobial therapy (7, 17, 18). In the case
439 of our 2-aryl carbapenems, this treatment strategy for community acquired infections
440 could also contribute to preserve the therapeutic efficacy of existing antipseudomonal
441 carbapenems, which are key antibiotics for hospital-acquired infections. Our serial
442 passage study suggests that there is a low risk of selection of antipseudomonal
443 carbapenem-resistant *P. aeruginosa* after the clinical use of our 2-aryl carbapenem with
444 oral application, and supports the above expectation. However, because our 2-aryl
445 carbapenems may be hydrolyzed by carbapenemase of *Enterobacteriaceae* such as KPC

and OXA-48-like, there is a possibility of selection of carbapenem resistant *P. aeruginosa* via carbapenemase-producing *Enterobacteriaceae* due to use of our 2-aryl carbapenem.

We are continuing preclinical investigations of SM-295291, SM-369926, and their ester-prodrugs for development into potential therapeutic agents of community-acquired infections.

In conclusion, a new category of antibiotic, 2-aryl carbapenems showed an ideal broad spectrum for the treatment of community-acquired infections, including infections caused by conventional antibiotic-resistant pathogens, but no activity against hospital pathogens such as *P. aeruginosa*, and had a good safety and pharmacokinetic profile. These results suggest that these new 2-aryl carbapenems are promising candidates as novel therapeutic agents for parenteral, oral, and switching from parenteral to oral treatment of community-acquired infections.

ACKNOWLEDGMENTS

We thank K. Kubota for suggestions and acknowledge the excellent technical assistance of K. Urasaki and Y. Hirai.

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broad-spectrum parenteral carbapenem. Antimicrob. Agents Chemother. **49:**4185-4196.

558 TABLE 1 *In vitro* activity of SM-295291, SM-369926, and selected antimicrobial
559 agents against clinical isolates

Organism	*n*	Antimicrobial agent	MIC (μg/ml)		
			Range	50%	90%
Staphylococcus aureus	50	SM-295291	0.125−0.25	0.125	0.125
(MSSA)	26	SM-369926	≤0.0313−0.125	0.0625	0.0625
	50	Tebipenem	≤0.0313−0.0625	≤0.0313	≤0.0313
	50	Cefditoren	0.5−2	1	1
	50	Clarithromycin	0.25−>128	0.25	>128
	50	Levofloxacin	0.125−16	0.25	0.5
Staphylococcus	50	SM-295291	0.0625−0.125	0.0625	0.125
epidermidis	27	SM-369926	≤0.0313−0.0625	0.0625	0.0625
(MSSE)	50	Tebipenem	≤0.0313	≤0.0313	≤0.0313
	50	Cefditoren	0.125−0.5	0.25	0.25
	50	Clarithromycin	0.125−>128	0.25	0.25
	50	Levofloxacin	0.125−4	0.25	0.5
Streptococcus	48	SM-295291	≤0.0039−0.0156	0.0078	0.0078
pneumoniae	27	SM-369926	≤0.0039−0.0156	≤0.0039	0.0078
(PSSP; penicillin, ≤0.06)	48	Tebipenem	≤0.0039−0.0156	≤0.0039	≤0.0039
	48	Faropenem	0.0078−0.125	0.0156	0.0313
	48	Cefditoren	0.0078−0.25	0.0625	0.125
	48	Clarithromycin	0.0625−>128	2	128
	48	Levofloxacin	0.5−16	1	2
Streptococcus pyogenes	48	SM-295291	≤0.0039−0.0078	0.0078	0.0078
	24	SM-369926	≤0.0039−0.0078	≤0.0039	0.0078
	48	Tebipenem	≤0.0039	≤0.0039	≤0.0039
	48	Cefditoren	≤0.0039−0.0156	0.0078	0.0156
	48	Clarithromycin	0.0313−>128	0.0625	4
	48	Levofloxacin	0.25−2	0.5	2
Streptococcus	49	SM-295291	≤0.0039−0.0313	0.0156	0.0156
agalactiae	24	SM-369926	≤0.0039−0.0313	0.0156	0.0156
	49	Tebipenem	≤0.0039−0.0156	0.0078	0.0156
	49	Cefditoren	0.0156−0.125	0.0313	0.0313
	49	Clarithromycin	0.0625−>128	0.125	>128
	49	Levofloxacin	0.5−>16	1	>16

560 TABLE 1-Continued

Organism	*n*	Antimicrobial agent	MIC (μg/ml)		
			Range	50%	90%
Enterococcus faecalis	51	SM-295291	0.125−2	0.5	1
	27	SM-369926	0.0625−1	0.25	0.5
	51	Tebipenem	0.25−8	1	4
	51	Cefditoren	8−>128	>128	>128
	51	Clarithromycin	0.25−>128	>128	>128
	51	Levofloxacin	1−64	2	64
Enterococcus faecium	41	SM-295291	2−>128	32	64
	27	SM-369926	1−128	32	64
	41	Tebipenem	4−>128	128	>128
	41	Cefditoren	128−>128	>128	>128
	41	Clarithromycin	0.125−>128	>128	>128
	41	Levofloxacin	1−>128	32	128
Moraxella catarrhalis	44	SM-295291	≤0.0313−0.25	0.125	0.25
	24	SM-369926	≤0.0313−0.125	≤0.0313	0.0625
	44	Tebipenem	≤0.0313−0.0625	≤0.0313	0.0625
	44	Cefditoren	≤0.0313−2	0.25	1
	44	Clarithromycin	0.0625−1	0.125	0.5
	44	Levofloxacin	≤0.0313−4	0.0625	0.125
Haemophilus influenzae	41	SM-295291	0.0625−0.5	0.0625	0.25
	27	SM-369926	0.0313−1	0.0625	0.5
	41	Tebipenem	0.0313−1	0.125	0.5
	41	Faropenem	0.25−16	1	8
	41	Cefditoren	0.0078−0.5	0.0313	0.125
	41	Clarithromycin	4−32	8	16
	41	Levofloxacin	0.0078−16	0.0156	0.0313
Klebsiella pneumoniae	47	SM-295291	0.125−2	0.25	0.5
	26	SM-369926	0.125−2	0.25	0.5
	47	Tebipenem	≤0.0313−0.0625	≤0.0313	≤0.0313
	47	Cefditoren	0.125−1	0.25	0.5
	47	Clarithromycin	32−>128	128	128
	47	Levofloxacin	0.0625−2	0.125	0.125

561

562 TABLE 1-Continued

Organism	*n*	Antimicrobial agent	MIC (μg/ml)		
			Range	50%	90%
Escherichia coli	50	SM-295291	0.25−8	0.5	1
	26	SM-369926	0.125−8	0.5	1
	50	Tebipenem	≤0.0313−1	≤0.0313	≤0.0313
	50	Cefditoren	0.125−>128	0.25	0.5
	50	Clarithromycin	16−>128	64	>128
	50	Levofloxacin	≤0.0313−64	0.0625	16
Enterobacter cloacae	48	SM-295291	1−16	4	8
	27	SM-369926	1−16	4	8
	48	Tebipenem	≤0.0313−0.125	≤0.0313	0.125
	48	Cefditoren	0.25−>128	1	64
	48	Clarithromycin	64−>128	128	128
	48	Levofloxacin	≤0.0313−8	0.0625	1
Enterobacter aerogenes	50	SM-295291	0.125−16	4	8
	26	SM-369926	0.0625−8	2	8
	50	Tebipenem	≤0.0313−0.125	≤0.0313	0.0625
	50	Cefditoren	0.125−128	1	32
	50	Clarithromycin	32−>128	128	>128
	50	Levofloxacin	≤0.0313−1	0.125	0.125
Pseudomonas aeruginosa	50	SM-295291	64−>128	>128	>128
	27	SM-369926	32−>128	128	>128
	50	Tebipenem	1−128	8	64
	50	Cefditoren	16−>128	64	>128
	50	Clarithromycin	32−>128	>128	>128
	50	Levofloxacin	0.125−>128	2	64
Acinetobacter spp.	27	SM-295291	2−64	16	32
	27	SM-369926	1−64	16	32
	27	Tebipenem	0.25−16	4	4
	27	Faropenem	1−64	16	32
	27	Cefditoren	4−64	32	32
	27	Clarithromycin	2−>128	16	32
	27	Levofloxacin	0.0625−16	0.125	8

563

564 TABLE 1-Continued

Organism	*n*	Antimicrobial agent	MIC (μg/ml)		
			Range	50%	90%
Neisseria gonorrhoeae	35	SM-295291	0.0313−1	0.5	1
	14	SM-369926	0.0156−0.25	0.25	0.25
	35	Tebipenem	0.0156−0.5	0.25	0.25
	35	Faropenem	0.0156−2	2	2
	35	Cefditoren	≤0.0039−0.25	0.0313	0.125
	35	Clarithromycin	≤0.0313−64	0.5	4
	35	Levofloxacin	0.0156−8	4	8
Peptostreptococcus sp.	38	SM-295291	≤0.0313−1	0.0625	0.125
	26	SM-369926	≤0.0313−1	≤0.0313	0.0625
	38	Tebipenem	≤0.0313−0.25	≤0.0313	0.125
	38	Cefditoren	≤0.0313−32	0.25	8
	38	Clarithromycin	≤0.0313−>128	0.5	>128
	38	Levofloxacin	0.5−128	4	64
Bacteroides fragilis	45	SM-295291	≤0.0313−32	0.25	2
	27	SM-369926	0.0625−16	0.5	2
	45	Tebipenem	0.0625−32	0.25	2
	45	Cefditoren	1−>128	2	64
	45	Clarithromycin	0.5−>128	2	>128
	45	Levofloxacin	0.5−32	2	8

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566

567 TABLE 2 *In vitro* activity of SM-295291, SM-369926, and selected antimicrobial
568 agents against drug-resistant clinical pathogens

Organism	*n*	Antimicrobial agent	MIC (μg/ml)		
			Range	50%	90%
S. aureus	49	SM-295291	0.5–>128	64	128
(MRSA; oxacillin, ≥4)	27	SM-369926	0.5−128	64	128
	49	Tebipenem	0.5−16	4	16
	49	Cefditoren	8–>128	128	>128
	49	Clarithromycin	0.25–>128	>128	>128
	49	Levofloxacin	0.25–>128	16	>128
S. epidermidis	36	SM-295291	0.5–>128	64	>128
(MRSE; oxacillin, ≥0.5)	27	SM-369926	1−128	16	128
	36	Tebipenem	0.25−16	8	16
	36	Cefditoren	1−128	64	128
	36	Clarithromycin	0.25–>128	128	>128
	36	Levofloxacin	0.25−32	4	16
S. pneumoniae	54	SM-295291	0.0625−0.25	0.0625	0.125
(PRSP; penicillin, ≥2)	54	SM-369926	0.0313−0.25	0.0625	0.125
	54	Tebipenem	0.0313−0.25	0.0625	0.125
	54	Faropenem	0.25−2	0.5	1
	54	Cefditoren	0.5−8	1	2
	54	Clarithromycin	0.0625–>128	2	>128
	54	Levofloxacin	0.5–>16	1	2
H. influenzae	22	SM-295291	0.125−0.5	0.25	0.25
(BLNAR; ampicillin, ≥2)	22	SM-369926	0.125−1	0.25	1
	22	Tebipenem	0.0625−2	0.5	1
	22	Faropenem	2−16	8	16
	22	Cefditoren	0.0313−1	0.25	0.5
	22	Clarithromycin	4−16	8	16
	22	Levofloxacin	0.0156−0.0313	0.0156	0.0313

569

570 TABLE 2-Continued

Organism	*n*	Antimicrobial agent	MIC (μg/ml)		
			Range	50%	90%
E. coli	9	SM-295291	1−16	−	−
(Ciprofloxacin and	9	SM-369926	1−16	−	−
ceftazidime resistant;	9	Tebipenem	0.0156−1	−	−
ciprofloxacin, ≥4;	9	Faropenem	1−16	−	−
ceftazidime, ≥16)	9	Cefditoren	4−>128	−	−
	9	Clarithromycin	32−>128	−	−
	9	Levofloxacin	8−64	−	−
N. gonorrhoeae	16	SM-295291	0.0313−1	−	−
(Ciprofloxacin resistant;	16	SM-369926	0.0156−1	−	−
ciprofloxacin, ≥1)	16	Tebipenem	≤0.0039−0.25	−	−
	16	Faropenem	0.0078−2	−	−
	16	Cefditoren	≤0.0039−0.125	−	−
	16	Clarithromycin	0.25−2	−	−
	16	Levofloxacin	2−32	−	−

571

572

573 TABLE 3 *In vitro* antibacterial activity of SM-295291, SM-369926, and IPM against
574 extended-spectrum β-lactamase producing bacteria

Organism	β-lactamase	MIC (μg/ml)					
		SM-295291		SM-369926		IPM	
		10^{6} [a]	10^{8} [a]	10^{6} [a]	10^{8} [a]	10^{6} [a]	10^{8} [a]
E. coli TL-3138	CTX-M-44	1	1	1	1	0.062	0.125
E. coli TL-3135	CTX-M-14	1	2	1	2	0.125	0.125
E. coli TL-3141	CTX-M-1	2	2	2	2	0.125	0.25
E. coli TL-3180	SHV-12	0.5	0.5	0.25	0.5	0.062	0.125
K. pneumoniae TL-3139	CTX-M-1	1	1	1	1	0.062	0.125
K. pneumoniae TL-3149	SHV	2	4	2	4	0.5	1

575 [a] inoculum size (CFU/ml)
576
577
578 TABLE 4 MICs, MBCs, and MBC/MIC ratios of SM-295291, SM-369926, and TBM

Strain		SM-295291	SM-369926	TBM
S. aureus ATCC6538p	MIC (μg/ml)	0.0313	0.0156	0.0039
	MBC (μg/ml)	0.0313	0.0156	0.0156
	[MBC/MIC ratio]	[1]	[1]	[4]
E. coli ATCC25404	MIC (μg/ml)	0.5	0.5	0.0156
	MBC (μg/ml)	0.5	0.5	0.0313
	[MBC/MIC ratio]	[1]	[1]	[2]
K. pneumoniae ATCC10031	MIC (μg/ml)	0.0625	0.0313	0.0156
	MBC (μg/ml)	0.0625	0.0313	0.0313
	[MBC/MIC ratio]	[1]	[1]	[2]

579

580 TABLE 5 *In vivo* efficacy of SM-295291, SM-369926, TBM, and CDN against PRSP

581 18280 systemic infection and meningitis in mice

Antimicrobial agent	ED_{50} [95% confidence intervals] (mg/kg)	
	Systemic infection [a]	Meningitis [b]
SM-295291	0.20 [0.083−0.48]	0.72 [0.14−1.72]
SM-369926	Not tested	1.01 [0.45−2.25]
TBM	0.34 [0.15−0.68]	1.01 [0.45−2.25]
CDN	5.42 [not determined]	3.23 [0.95−10.2]

582 [a] Mice were inoculated intraperitoneally with 1.4×10^4 CFU of PRSP 18280. Antimicrobial agents
583 were administered intravenously 1 and 3 h after infection ($n = 8$).
584 [b] Mice were challenged intracerebrally with 2×10^4 CFU of PRSP 18280. Antimicrobial agents were
585 administered intravenously 5 h after infection and twice daily 1 and 2 days after infection ($n = 8$).
586 Cilastatin at a dose of 100 mg/kg was administered subcutaneously 5 min before carbapenem
587 treatment.
588 ED_{50} was calculated from survival rates 7 days after infection.

589

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591 TABLE 6 Pharmacokinetic parameters of intravenous administration of SM-295291 and

592 SM- 369926 and intraduodenal or oral administration of their ester prodrugs in animals

Carbapenem[a]	Parameter	Rat	Dog	Monkey
SM-295291	$C_{5\ min}$ (μg/ml)	9.64	2.73	3.90
	$AUC_{0\text{-}3h}$[b] (μg·h/ml)	6.41	1.49	1.59
	$t_{1/2}$ (h)	0.85	0.56	0.40
	Vd_{ss}[c] (l/kg)	0.12	0.33	0.21
SM-368589	C_{max} (μg/ml)	0.42	0.96	0.18
(ester prodrug)	$AUC_{0\text{-}3h}$[b] (μg·h/ml)	0.51	0.93	0.21
	F[d] (%)	8.0	62.3	12.9
SM-369926	$C_{5\ min}$ (μg/ml)	7.91	2.82	3.23
	$AUC_{0\text{-}3h}$[b] (μg·h/ml)	5.35	1.69	1.13
	$t_{1/2}$ (h)	0.45	0.56	0.39
	Vd_{ss}[c] (l/kg)	0.11	0.32	0.20
SM-375769	C_{max} (μg/ml)	0.78	0.63	0.05
(ester prodrug)	$AUC_{0\text{-}3h}$[b] (μg·h/ml)	0.93	0.57	0.05
	F[d] (%)	17.1	34.2	4.2

593 [a] Three animals in each group were administered carbapenem at 1 mg/kg.

594 [b] Area under the concentration-time curve from 0 h to 3 h.

595 [c] Volume of distribution at steady state.

596 [d] Bioavailability.

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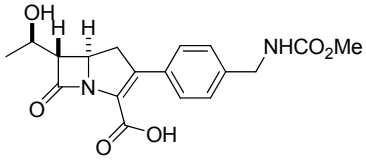

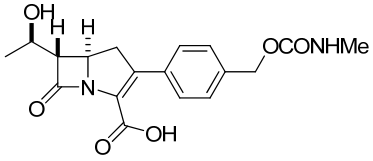

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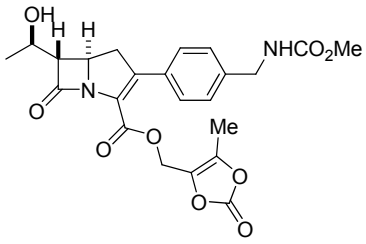
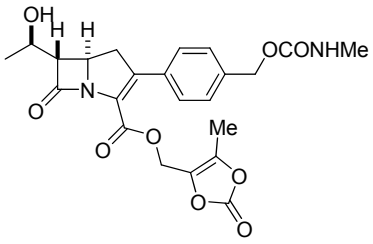
600 SM-295291 SM-369926





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602 SM-368589 SM-375769

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604 FIG 1 Chemical structures of 2-aryl carbapenems

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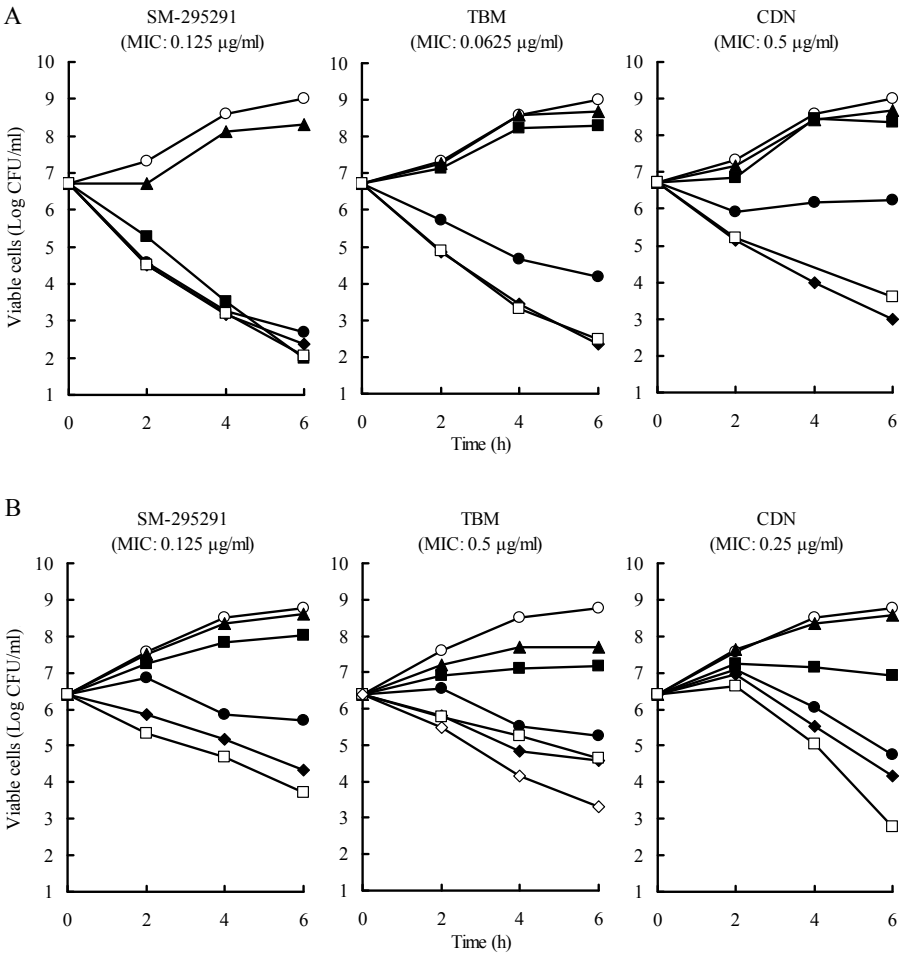

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609 FIG 2 Bactericidal activity of SM-295291 and reference antimicrobial agents against

610 (A) PRSP 18280 and (B) BLNAR 17051. Symbols: ○, control; ▲, 1/4 × MIC; ■, 1/2 ×

611 MIC; ●, 1 × MIC; ♦, 2 × MIC; □, 4 × MIC; ◊, IPM 32 μg/ml.

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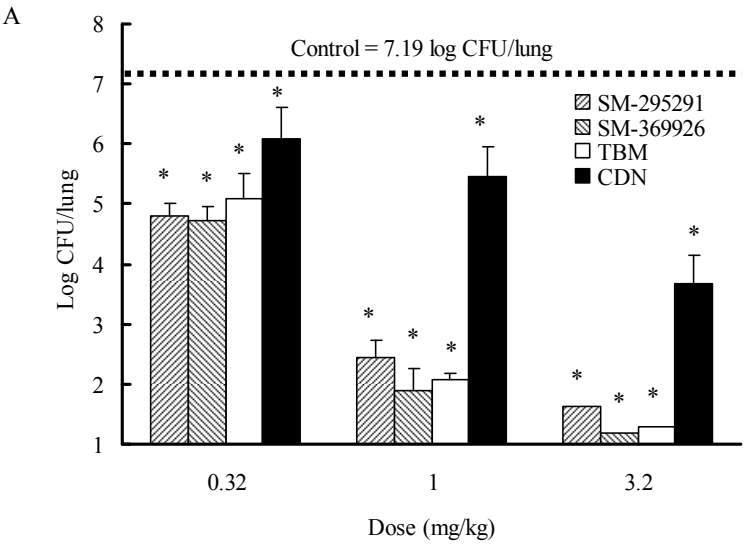

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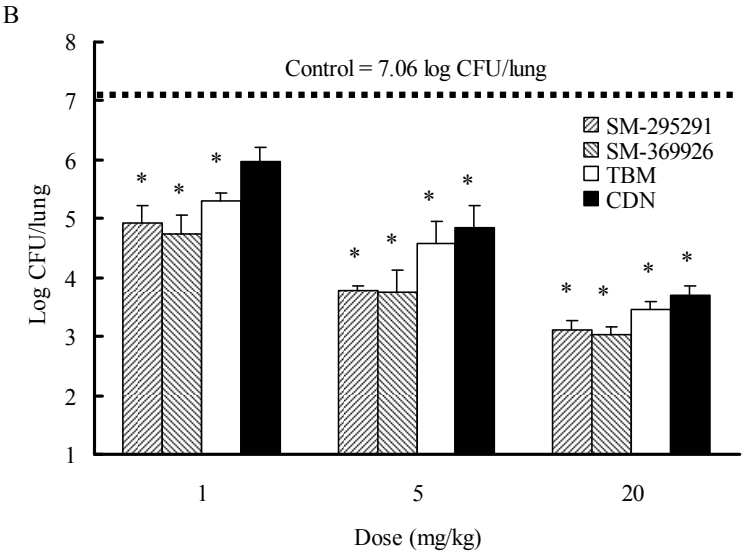

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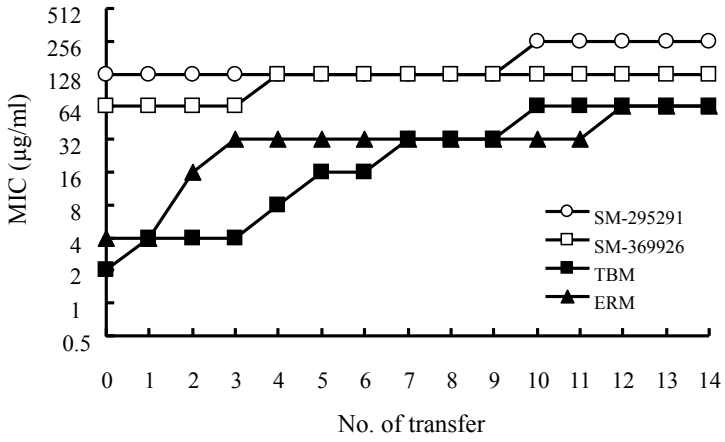
615 FIG 3 Effects of antimicrobial agents on the bacterial burden in the lungs of mice

616 infected intranasally with (A) PRSP 18280 at 1.7×10^6 CFU/mouse and (B) BLNAR

17051 at 4.4×10^7 CFU/mouse. Antibacterial agents were administered intravenously thrice daily 1 day and 2 days after infection ($n = 6$). Cilastatin at a dose of 100 mg/kg was administered subcutaneously 5 min before carbapenem treatment. The lungs were removed 3 days after infection. The values represent the mean and standard deviation. Dotted line represents the mean bacterial burden in the lungs for the control group. *Significantly different from control ($P < 0.01$ by Dunnett's test for multiple comparisons).



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626 FIG 4 *In vitro* serial passage study of *P. aeruginosa* PAO1. Symbols: ○, SM-295921; □,

627 SM-369926; ■, TBM; ▲, ERM.

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