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CP5484, a novel quaternary carbapenem with potent anti-MRSA activity and reduced toxicity

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Abstract—A new series of 1β-methyl carbapenems possessing a 6,7-disubstituted imidazo[5,1-*b*]thiazol-2-yl group directly attached to the C-2 position of the carbapenem nucleus was prepared, and the activities of these compounds against methicillin-resistant *Staphylococcus aureus* (MRSA) were evaluated. To study the effect of basic moieties on anti-MRSA activity, we introduced an amino, or imino, or amidino group at the 6-position of imidazo[5,1-*b*]thiazole in place of the carbamoylmethyl moiety of **CP5068**. Anti-MRSA activities of almost all basic group-substituted carbapenems were improved, though some of the compounds showed stronger acute toxicity in mice than IPM. In order to decrease the toxicity without decreasing the activity, we introduced various additional functionalities around the basic moiety. Finally, we obtained **CP5484**, which has excellent anti-MRSA activity and low acute toxicity.

1. Introduction

Various new drug-resistant pathogens have been emerging in spite of the development of new antibiotics. Among them, methicillin-resistant *Staphylococcus aureus* (MRSA) is one of the major pathogens causing nosocomial infections. After MRSA was first identified in England in 1961,[1] it became a serious clinical problem worldwide, so far, only a few marketed agents, such as arbekacin, vancomycin (VCM), and teicoplanin, are effective against MRSA. Moreover, glycopeptide-resistant strains have been emerging concomitantly with the increasing use of glycopeptide.[2] Recently Linezolid and daptomycin have also been available for MRSA infections. However, new resistant strains against many drugs including them have already been reported.[3] For these reasons, new anti-MRSA agents are highly desirable.

Recently, many research groups have reported anti-MRSA β-lactams, such as cephalosporins[4] and carbapenems.[5] Those β-lactams show high binding affinity to penicillin-binding protein 2a (PBP2a). We have already reported a potent anti-MRSA agent, **CP5068**,[6] which has as high a binding affinity for PBP2a as other anti-MRSA β-lactams.

Some β-lactams having an amino group, such as MC-02479[7] and J-111,347,[8] show strong anti-MRSA activity. Therefore, to study the effect of the basic moiety on the activity, we designed and synthesized novel carbapenem derivatives having a basic group (i.e., amine, imine, or amidine) in place of the carbamoylmethyl group at the 6-position of the imidazo[5,1-*b*]thiazole moiety of **CP5068** (Fig. 1). These compounds exhibited improved anti-MRSA activity, but showed strong acute toxicity in mice. To overcome this problem, we designed and synthesized further derivatives and finally found CP5484, which has reduced toxicity while retaining its potent anti-MRSA activity. Herein we report the synthesis and structure-activity relationships of **CP5484** and its derivatives having a basic moiety on the imidazo[5,1-*b*]thiazole ring, as well as the in vitro and in vivo efficacy, and pharmacokinetic profile.

Keywords: Carpabapenem; MRSA; **CP5484**; Toxicity; Anti-MRSA activity.
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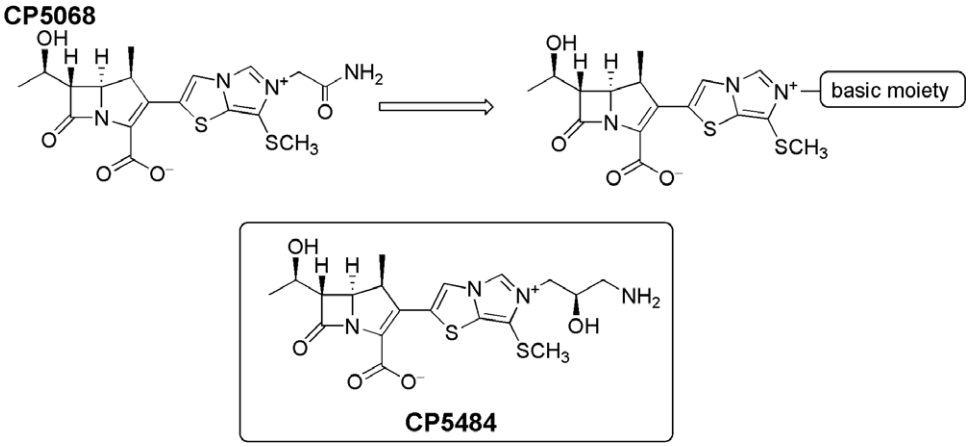


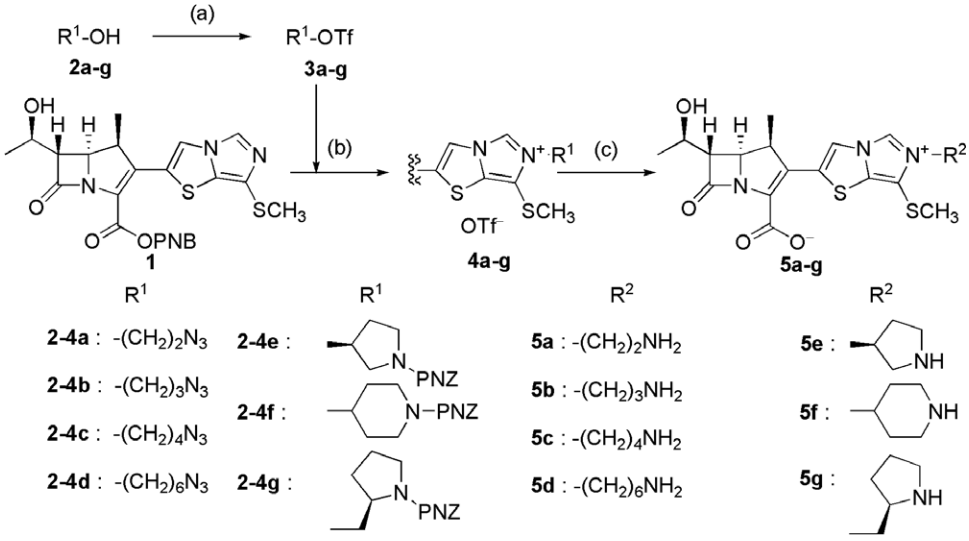
Figure 1. Strategy for synthesizing amino-substituted carbapenems.

2. Chemistry

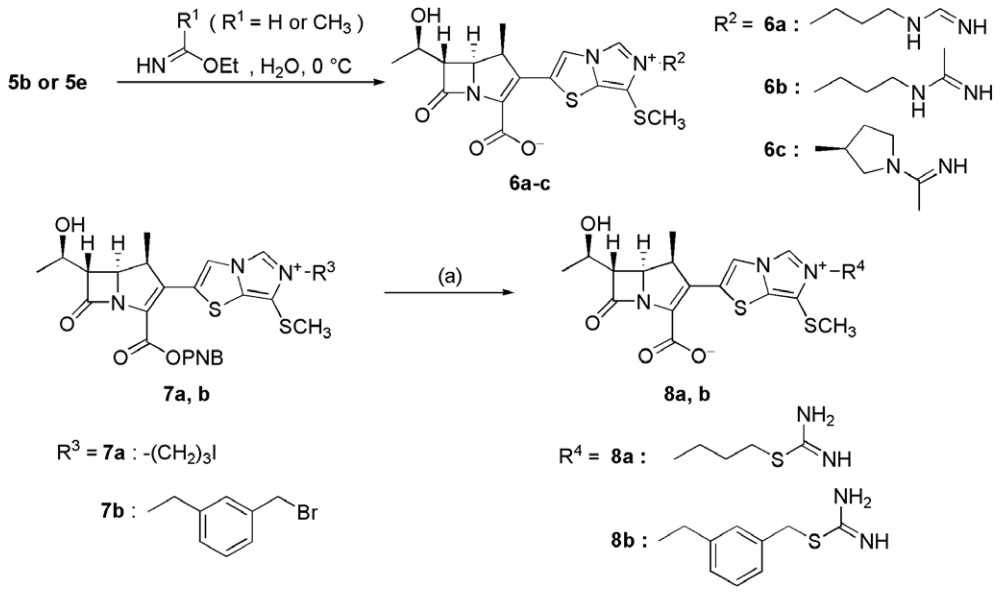
First, we synthesized carbapenem derivatives having a simple amino group. The synthetic routes are illustrated in Scheme 1. All derivatives were prepared from key intermediate **1**. Primary and secondary amines were introduced using azido groups (**2a**–**d**) and PNZ(=4-nitrobenzyloxycarbonyl)-protected amines (**2e**–**g**). Alcohols **2a**–**g** having amino precursors were converted to trifluoromethanesulfonates **3a**–**g** with 2,6-lutidine and trifluoromethanesulfonic anhydride. Reaction of **3a**–**g** and the key intermediate **1** provided the corresponding quaternary salts **4a**–**g**. Deprotection and reduction of azido group afforded novel amino-substituted carbapenems **5a**–**g**, each as an amorphous powder, by lyophilization after reversed-phase column chromatographic purification.

Preparation of amidine-substituted carbapenems is shown in Scheme 2. Reaction of ethyl formimidate or acetimidate and **5b** or **5e** afforded the corresponding amidine- substituted carbapenems **6a–c**. Introduction of thioamidine was also achieved by reaction of thiourea and **7a** or **7b**, derived from **1** and 3-iodopropyl trifluoromethanesulfonate or *m*-α,α′-dibromoxylene. Removal of the 4-nitrobenzyl group yielded the thioamidine-carbapenems **8a** and **8b**.

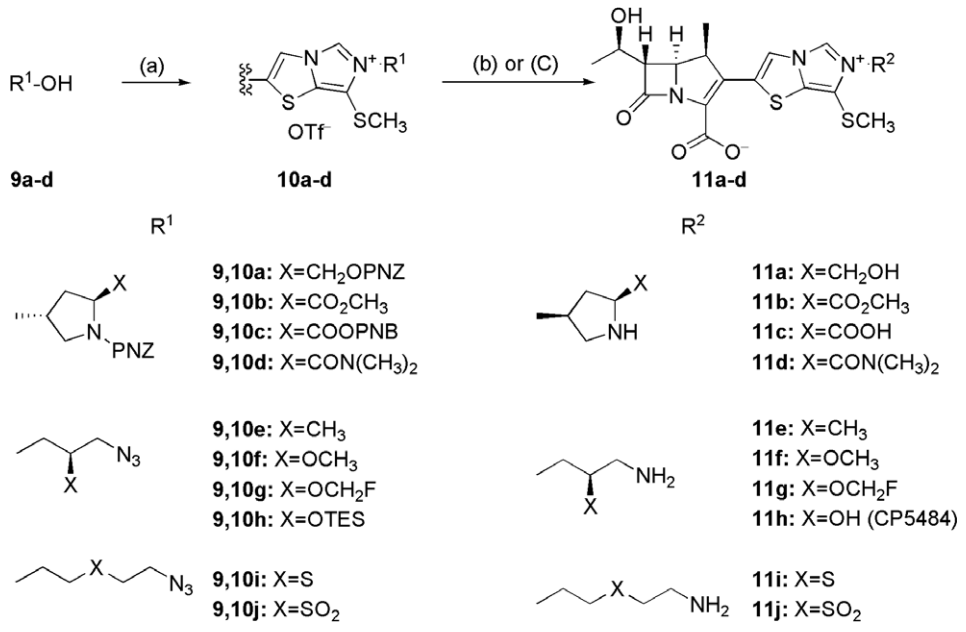
In Scheme 3, introduction of additional functional groups around the amino group is illustrated. All compounds were synthesized from **1** and the corresponding triflates, which were prepared in the same manner as **5a**–**g** in Scheme 1. Alcohols **9a**–**d** derived from L-hydroxyproline were converted to the corresponding carbapenems **11a**–**d**, which are derivatives of **5e** possessing a hydroxymethyl, methoxycarbonyl, carboxyl, or *N*,*N*′-dimethylcarbamoyl group, respectively, on the pyrrolidine ring. Compounds **11e**–**h**, which have a methyl, methoxyl, fluoromethoxyl or hydroxyl group on the aminopropyl chain of **5b** were similarly prepared from the alcohols **9e**–**h** and **1**. Insertion of sulfur atom or sulfone into the aminobutyl chain of **5c** was also performed through the reaction of **9i** or **9j** and **1** to form **11i** or **11j**, respectively.



Scheme 1. Synthesis of carbapenem derivatives (**1**). Reagents and conditions: (a) 2,6-lutidine, Tf_2O,CH_2Cl_2, −40 °C; (b) CH_2Cl_2, rt.; (c) Pd/C, H_2, THF–H_2O, rt.



Scheme 2. Synthesis of carbapenem derivatives (**2**). Reagents and conditions: (a)(i) thiourea, CH_3CN–DMF, 35 °C; (ii)Pd/C, H_2, THF–H_2O, rt.



Scheme 3. Synthesis of carbapenem derivatives (**3**). Reagents and conditions: (a) 2,6-lutidine, Tf_2O,CH_2Cl_2, −40 °C; (ii)1, CH_2Cl_2, rt; (b) Pd/C, H_2, THF–H_2O, rt. (for 11a–g, 11i–j) (c)(i)pH 2.2, THF–H_2O, rt. (ii)Pd/C, H_2, THF–H_2O, rt. (for 11h).

3. Biological activity

The antibacterial activities of the novel amino-carbapenems **5a**–**g**, together with those of CP5068, IPM, and VCM, are shown in Table 1. All compounds exhibited strong anti-MRSA activity equipotent to that of VCM. Introduction of an amino-group at the 6-position of imidazo[5,1-*b*]thiazole resulted in enhancement of the anti-MRSA activity compared with that of CP5068. In the aminoalkyl series (**5a**–**d**), the length of the alkyl chain is significant, because **5a**, having an aminoethyl moiety, showed weaker activity than **5b**–**d** with longer aminoalkyl chains. In the cyclic amine series (**5e**–**f**), introduction of pyrrolidine gave the best result (**5e**). These findings prompted us to investigate further derivatization of amino-substituted carbapenems.

Table 2 shows the anti-MRSA activities of amidine-carbapenems. Introduction of amidine (**6a**–**c**) slightly decreased the anti-MRSA activity compared with the precursor **5b** or **5e.** However, thioamidine improved the anti-MRSA activity (**8a**). In particular, the combination of thioamidine and the benzyl moiety resulted in improved anti-MRSA activity superior to VCM (**8b**). Most derivatives with a benzyl moiety at the 6-position of imidazo[5,1-*b*]thiazole showed poor water solubility, but this was not the case for **8b** which could avoid this problem because of the hydrophilic amidine moiety.

Table 1. Antibacterial activities of **5a–g** and reference compounds (MIC; μg/mL)

Test organism	*S. aureus* 209P JC-1	*S. aureus* M-126[a]	*S. aureus* M-126 HR[b]
5a	<0.006	0.78	3.13
5b	<0.006	0.78	1.56
5c	<0.006	0.78	1.56
5d	<0.006	0.78	1.56
5e	<0.006	0.78	1.56
5f	0.013	1.56	3.13
5g	<0.006	0.78	3.13
CP5068	<0.006	1.56	3.13
IPM	0.013	25	100
VCM	0.78	1.56	1.56

[a] MRSA.
[b] Carbapenem-resistant MRSA.

Table 2. Antibacterial activities of **6a–c**, **8a** and **8b** (MIC; μg/mL)

Test organism	*S. aureus* 209P JC-1	*S. aureus* M-126[a]	*S. aureus* M-126 HR[b]
5b	<0.006	0.78	1.56
6a	<0.006	0.78	3.13
6b	<0.006	0.78	3.13
6c	<0.006	1.56	3.13
8a	<0.006	0.39	1.56
8b	<0.006	0.39	0.78

[a] MRSA.
[b] Carbapenem-resistant MRSA.

The effects of additional functional groups introduced into the amino-substituted side chain at the 6-position of imidazo[5,1-*b*]thiazole on anti-MRSA activity are summarized in Table 3. Additional functional groups were not favorable for anti-MRSA activity among the pyrrolidine analogues (**11a–d**). Introduction of electron-withdrawing groups actually reduced anti-MRSA activity (**11b–d**), possibly via a reduction of basicity.

On the other hand, introduction of a second functional group on the aminopropyl side chain at the 2′ position did not decrease anti-MRSA activity. The substituted aminopropyl derivatives **11e–h** displayed almost the same activity as the unsubstituted compound **5b**. In these compounds, smaller substituents tended to keep the anti-MRSA activity of **5b**. Compound **11i** with a sulfur atom in the aminoalkyl side chain showed potent anti-MRSA activity, however, though oxidation of the sulfur atom **(11j)** resulted in a decline of the activity.

Table 3. Antibacterial activities of **11a–j** (MIC; μg/mL)

Test organism	*S. aureus* 209P JC-1	*S. aureus* M-126[a]	*S. aureus* M-126 HR[b]
5e	<0.006	0.78	1.56
11a	<0.006	0.78	3.13
11b	0.013	1.56	6.25
11c	0.025	3.13	6.25
11d	0.025	3.13	6.25
5b	<0.006	0.78	1.56
11e	<0.006	0.78	1.56
11f	<0.006	0.78	3.13
11g	<0.006	0.78	3.13
11h (CP5484)	<0.006	0.78	1.56
11i	<0.006	0.39	1.56
11j	<0.006	0.78	3.13

[a] MRSA.
[b] Carbapenem-resistant MRSA.

Next, we tested the acute toxicity in mice of the most potent antibacterially active compounds via intravenous administration (Table 4). All analogues having a simple amino group (**5a** and **5b** and **5d** and **e**) exhibited high toxicity. Thioamidine compounds **8a** and **8b** also showed high toxicity in spite of having the strongest anti-MRSA activity. Basic moieties might thus be associated with not only strong anti-MRSA activity, but also high acute toxicity in mice. Therefore, we designed and synthesized new aminoalkyl quaternary derivatives possessing additional functionalities (**11e–j**). Among them, the (2*R*)-2-methoxy-3-propyl **11f**, (2*R*)-2-hydroxy-3-propyl **11h** (**CP5484**), and 2-(2-aminoethanesulfonyl)ethyl **11j** compounds did not cause death of any mice at the dose of 500 mg/kg, so that they should be acceptable for drug use (their toxicities were similar to that of IPM). Furthermore, **CP5484** retained potent anti-MRSA activity, equal to that of VCM. Hence, it was subjected to further evaluation.

In vitro antibacterial activities of **CP5484** and IPM against various pathogens are shown in Table 5. **CP5484** showed superior activity to IPM against not

Table 4. Acute toxicity in mice and anti-MRSA activities

Compound	Toxicity	MIC (μg/mL)	
	Dose[a] (mg/kg)	MRSA M126	MRSA M126-HR
5a	500	0.78	3.13
5b	250	0.78	1.56
5d	250	0.78	1.56
5e	500	0.78	1.56
8a	250	0.39	1.56
8b	250	0.39	0.78
11e	500	0.78	1.56
11f	>500	0.78	3.13
11g	500	0.78	3.13
11h (CP5484)	>500	0.78	1.56
11j	>500	0.78	3.13

[a] The minimal lethal dose.

Table 5. Antibacterial activities of **CP5484** and IPM

Test organism	MIC (μg/mL)	
	CP5484	IPM
S. aureus 209P JC-1	<0.006	0.013
S. aureus M-126[a]	0.78	25
S. aureus M-126 HR[a]	1.56	100
S. pneumoniae PRC9[b]	0.05	0.39
E. coli NIHJ JC-2	0.05	0.1
K. pneumoniae PCI602	0.1	0.39
M. catarrhlis W-0500	0.05	0.05
H. influenzae PRC44[c]	0.78	3.13
P. aeruginosa PAO1	6.25	1.56

[a] MRSA.
[b] Penicillin-resistant.
[c] β-Lactamase-positive, amoxicillin/clavulanic acid-resistant.

Table 6. Anti-MRSA ($n = 54$) activities of **CP5484** and VCM

Compound	MIC (μg/mL)		
	50%	90%	Range
CP5484	0.78	1.56	<0.05–1.56
VCM	1.56	1.56	0.78–3.13

Table 7. Protective effects of **CP5484** and VCM on systemic infection caused by MRSA MF126

Compound	Challenge dose (CFU/mouse)	MIC (μg/ml)	ED_{50} (95% confidence limit) (mg/mouse/day)
CP5484	4.5×10^5($15LD_{50}$)	1.56	0.039 (0.022–0.071)
VCM		0.78	0.18 (0.1–0.51)

only MRSA, but also penicillin-resistant *Streptococcus pneumoniae* (PRSP), which often causes severe respiratory tract infections. **CP5484** also exhibited strong antibacterial activity against Gram-negative bacteria, such as *Escherichia coli* and *Klebsiella pneumoniae*. Table 6 summarizes the anti-bacterial activities of **CP5484** and VCM against 54 clinically isolated strains of MRSA. MIC_{50} and MIC_{90} of **CP5484** were 0.78 and 1.56 μg/mL, respectively, and those of VCM were 1.56 and 1.56 μg/mL, respectively. Thus the in vitro anti-MRSA activity of **CP5484** is slightly higher than that of VCM.

We also conducted an in vivo efficacy test. Table 7 shows the ED_{50} values of **CP5484** and VCM after subcutaneous administration in a systemic infection model caused by MRSA MF126 strain in mice. The therapeutic effect of **CP5484**was superior to that of VCM, even though **CP5484** had shown inferior antibacterial activity in vitro.

Pharmacokinetic parameters of **CP5484** after intravenous administration to mice are shown in Table 8. Plasma levels of **CP5484** were similar to those of imipenem,[9] and the protein binding rate in human was very low (12.2%).

4. Conclusion

We introduced various basic moieties at the 6-position of imidazo[5,1-*b*]thiazole in place of the carbamoylmethyl group of CP5068 in order to study structure-anti-MRSA activity relationships. Some carbapenems possessing an amino group showed superior anti-MRSA activity to VCM, though some also had higher acute toxicity in mice. We next introduced additional functionalities around the amino group. This yielded the (2*R*)-2-hydroxy-3-propyl compound **CP5484** (**11h**), which exhibited both potent anti-MRSA activity in vivo (superior to VCM) and low acute toxicity in mice (similar to IPM). Moreover, **CP5484** showed excellent antibacterial activities against various Gram- and Gram-negative bacteria, including PRSP. Further evaluation of **CP5484** is under way.

5. Experimental

5.1. Chemistry

^{1}H NMR spectra were measured by a JEOL JNM-LA400 NMR spectrometer for 400 MHz in D_2O. *H*OD (4.65 ppm) in D_2O was used as internal reference standard. Mass spectra were obtained on a JEOL JMS-700 mass spectrometer for FABMS and FABHRMS. Silica gel flash column chromatography was performed on Wako-gel C-300 and reversed phase column chromatography was carried out on Nacalai Tesque Cosmosil $40C_{18}$-PREP.

5.1.1. 4-Nitrobenzyl (1*S*,5*R*,6*S*)-2-[6-(2-azidoethyl)-7-methylthioimidazo[5,1-*b*]thiazolium-2-yl]-6-((1*R*)-1-hydroxyethyl)-1-methyl-1-carbapen-2-em-3-carboxylate trifluoromethane-sulfonate (4a). To a dichloromethane solution (10 mL) of 2-azidoethanol (220 mg, 2.52 mmol), 2,6-lutidine (0.323 mL, 2.78 mmol) and trifluoromethanesulfonic anhydride (0.446 mL, 2.65 mmol) were added at −60 °C. After 20 min, the mixture was quenched with water (30 mL) and then extracted with dichloromethane (30 mL), and the extract was dried over anhydrous magnesium sulfate and filtered. To the filtrate, compound **1** (1.05 g, 2.04 mmol) was added and then the solvent was removed under reduced pressure up to ca. 20 mL. After stirred at room temperature for 7 h, the reaction solution was added dropwise to ether (50 mL). The precipitated solid was collected by filtration to afford **4a** (1.47 g) as a crude product.

5.1.2. (1*S*,5*R*,6*S*)-2-[6-(2-Aminoethyl)-7-methylthioimidazo[5,1-*b*]thiazolium-2-yl]-6-((1*R*)-1-hydroxyethyl)-1-methyl-1-carbapen-2-em-3-carboxylate hydrochloride (5a). To a mixed solution of the crude product of **4a** (1.35 g) in THF (45 mL) and water (45 mL), 10% Pd–C (1.05 g) was added, and stirred under hydrogen atmosphere at room temperature for 3 h. After the catalyst was removed by filtration and washed with water, the aqueous filtrate was washed with ethyl acetate and concentrated under the reduced pressure up to about 10 mL. The residue was purified by column chromatography on Cosmosil $40C_{18}$-PREP (a 20% aqueous methanol solution) and subjected to column chromatography

Table 8. Pharmacokinetic parameters of CP5484[a] after intravenous administration in male mice

Compound	Dose (mg/kg)	$T_{1/2}$ (min)	$AUC_{0-\infty}$ (μgh/mL)	Cl_{tot} (L/h/kg)	V_{ss} (L/kg)	U.E.[b] (%)	Protein binding (%)	
							Mice	Human
CP5484	20	13.4	12.6	1.61	0.34	41.9 ± 10.6	16	12.2

[a] CP5484 was administered with 10 mg/kg cilastatin.
[b] Urinary excretion.

(water) on Amberlyst A-26 as an ion-exchange resin (chloride form) to give 448 mg (53%) of **5a**: ^{1}H NMR (D_2O) δ (*H*OD = 4.65 ppm): 1.13 (3H, d, J = 7.1 Hz), 1.17 (3H, d, J = 6.4 Hz), 2.29 (3H, s), 3.43 (1H, dd, J_1 = 5.8 Hz, J_2 = 2.7 Hz), 3.46–3.54 (3H, m), 4.10–4.18 (1H, m), 4.22 (1H, dd, J_1 = 9.3 Hz, J_2 = 2.7 Hz), 4.68 (2H, t, J = 6.2 Hz), 8.06 (1H, s); FABMS *m*/*z* 423 [(M+H)$^+$]; FABHRMS calcd for $C_{18}H_{23}N_4O_4S_2$ [(M+H)$^+$]: 423.1161, found: 423.1164.

5.1.3. Compounds 5b–5g. These compounds were prepared by a similar procedure as the preparation of **4a** and **5a**.

Compound **5b** was obtained in 43% yield from compound **1**: ^{1}H NMR (D_2O) δ (*H*OD = 4.65 ppm): 1.13 (3H, d, J = 7.3 Hz), 1.18 (3H, d, J = 6.3 Hz), 2.25 (2H, m), 2.29 (3H, s), 3.02 (2H, t, J = 7.7 Hz), 3.43 (1H, dd, J_1 = 5.8 Hz, J_2 = 2.7 Hz), 3.52 (1H, m), 4.15 (1H, m), 4.21 (1H, dd, J_1 = 9.5 Hz, J_2 = 2.7 Hz), 8.04 (1H, s), 9.25 (1H, s); FABMS *m*/*z* 437 [(M+H)$^+$]; FABHRMS calcd for $C_{19}H_{25}N_4O_4S_2$ [(M+H)$^+$]: 437.1317, found: 437.1321.

Compound **5c** was obtained in 11% yield from compound **1**: ^{1}H NMR (D_2O) δ (*H*OD = 4.65 ppm): 1.10 (3H, d, J = 7.3 Hz), 1.15 (3H, d, J = 6.3 Hz), 1.56–1.65 (2H, m), 1.84–1.93 (2H, m), 2.25 (3H, s), 2.91 (2H, t, J = 7.0 Hz), 3.39–3.43 (1H, m), 3.45–3.52 (1H, m), 4.09–4.21 (2H, m), 4.34 (2H, t, J = 7.2 Hz), 7.99 (1H, s), 9.19 (1H, s); FABMS *m*/*z* 451 [(M+H)$^+$]; FABHRMS calcd for $C_{20}H_{27}N_4O_4S_2$ [(M+H)$^+$]: 451.1474, found: 451.1472.

Compound **5d** was obtained in 34% yield from compound **1**: ^{1}H NMR (D_2O) δ (*H*OD = 4.65 ppm): 1.11 (3H, d, J = 7.3 Hz), 1.18 (3H, d, J = 6.4 Hz), 1.25–1.40 (4H, m), 1.51–1.61 (2H, m), 1.80–1.90 (2H, m), 2.25 (3H, s), 2.87 (2H, t, J = 7.7 Hz), 3.42 (1H, dd, J_1 = 5.9 Hz, J_2 = 2.9 Hz), 3.46–3.57 (1H, m), 4.11–4.19 (1H, m), 4.21 (1H, dd, J_1 = 9.5 Hz, J_2 = 2.7 Hz), 4.25–4.38 (2H, m), 7.98 (1H, s), 9.19 (1H, s); FABMS *m*/*z* 479 [(M+H)$^+$]; FABHRMS calcd for $C_{22}H_{31}N_4O_4S_2$ [(M+H)$^+$]: 479.1787, found: 479.1789.

Compound **5e** was obtained in 16% yield from compound **1**: ^{1}H NMR (D_2O) δ (*H*OD = 4.65 ppm): 1.11 (3H, d, J = 7.3 Hz), 1.15 (3H, d, J = 6.3 Hz), 2.28 (3H, s), 2.44–2.54 (1H, m), 2.70–2.81 (1H, m), 3.39–3.55 (3H, m), 3.56–3.68 (2H, m), 3.93–4.01 (1H, m), 4.08–4.15 (1H, m), 4.19 (1H, dd, J_1 = 9.3 Hz, J_2 = 2.9 Hz), 5.50–5.58 (1H, m), 8.05 (1H, s), 9.40 (1H, s); FABMS *m*/*z* 449 [(M+H)$^+$]; FABHRMS calcd for $C_{22}H_{31}N_4O_4S_2$ [(M+H)$^+$]: 449.1317, found: 449.1318.

Compound **5f** was obtained in 3% yield from compound **1**: ^{1}H NMR (D_2O) δ (*H*OD = 4.65 ppm): 1.17 (3H, d, J = 7.3 Hz), 1.24 (3H, d, J = 6.3 Hz), 2.04–2.11 (2H, m), 2.31–2.35 (5H, m), 2.98–3.08 (2H, m), 3.37–3.60 (4H, m), 4.17–4.30 (2H, m), 4.85–4.96 (1H, m), 8.07 (1H, s); FABMS *m*/*z* 463 [(M+H)$^+$]; FABHRMS calcd for $C_{23}H_{33}N_4O_4S_2$ [(M+H)$^+$]: 463.1474, found: 463.1478.

Compound **5g** was obtained in 15% yield from compound **1**: ^{1}H NMR (D_2O) δ (*H*OD = 4.65 ppm): 1.11 (3H, d, J = 7.3 Hz), 1.18 (3H, d, J = 6.4 Hz), 1.25–1.40 (4H, m), 1.51–1.61 (2H, m), 1.80–1.90 (2H, m), 2.25 (3H, s), 2.87 (2H, t, J = 7.7 Hz), 3.42 (1H, dd, J_1 = 5.9 Hz, J_2 = 2.9 Hz), 3.46–3.57 (1H, m), 4.11–4.19 (1H, m), 4.21 (1H, dd, J_1 = 9.5 Hz, J_2 = 2.7 Hz), 4.25–4.38 (2H, m), 7.98 (1H, s), 9.19 (1H, s); FABMS *m*/*z* 463 [(M+H)$^+$]; FABHRMS calcd for $C_{23}H_{33}N_4O_4S_2$ [(M+H)$^+$]: 463.1474, found: 463.1466.

5.1.4. (1*S*,5*R*,6*S*)-2-[6-(3-Formimidoylaminopropyl)-7-methylthioimidazo[5,1-*b*]thiazolium-2-yl]-6-((1*R*)-1-hydroxyethyl)-1-methyl-1-carbapen-2-em-3-carboxylate hydrochloride (6a). To an aqueous solution (3 mL) of compound **5b** (83 mg, 0.176 mmol), ethyl formimidate hydrochloride (140 mg, 1.27 mmol) was added portionwise under ice-cooling adjusting within pH 8–8.5 by 1 N NaOH aq. The mixture was stirred for 30 min, adjusted to pH 3.6 by the addition of a 1 N HCl, purified by reversed-phase column chromatography (a 20% aqueous methanol solution), and then subjected to column chromatography (water) on Amberlyst A-26 as an ion-exchange resin (chloride form) to yield 5 mg (6.1%) of **6a**: ^{1}H NMR (D_2O) δ (*H*OD = 4.65 ppm): 1.09 (3H, d, J = 7.3 Hz), 1.16 (3H, d, J = 6.4 Hz), 2.18 (2H, m), 2.25 (3H, s), 3.30 (2H, t, J = 6.9 Hz), 3.40 (1H, m), 3.46 (1H, m), 4.12 (1H, m), 4.18 (1H, m), 4.39 (2H, m), 7.73 (1H, brs), 7.98 (1H, s), 9.20 (1H, s); FABMS *m*/*z* 464 [(M+H)$^+$]; FABHRMS calcd for $C_{20}H_{26}N_5O_4S_2$ [(M+H)$^+$]: 464.1426, found: 464.1429.

5.1.5. Compounds 6b and 6c. These compounds were prepared by a similar procedure as the preparation of **6a**.

Compound **6b** was obtained in 62% yield from compound **5b**: ^{1}H NMR (D_2O) δ (*H*OD = 4.65 ppm): 1.11 (3H, d, J = 7.1 Hz), 1.16 (3H, d, J = 6.3 Hz), 2.08 (3H, s), 2.15 (2H, m), 2.26 (3H, s), 3.24 (2H, t, J = 6.8 Hz), 3.42 (1H, m), 3.49 (1H, m), 4.13 (1H, m), 4.20 (1H, m), 4.41 (2H, t, J = 6.8 Hz), 8.01 (1H, s), 9.23 (1H, s); FABMS *m*/*z* 478 [(M+H)$^+$]; FABHRMS calcd for $C_{21}H_{28}N_5O_4S_2$ [(M+H)$^+$]: 478.1583, found: 478.1581.

Compound **6c** was obtained in 62% yield from compound **5e**: ^{1}H NMR (D_2O) δ (*H*OD = 4.65 ppm): 1.10 (3H, d, J = 7.3 Hz), 1.15 (3H, d, J = 6.3 Hz), 2.18–2.23 (3H, m), 2.28 (3H, s), 2.44–2.56 (1H, m), 2.65–2.81 (1H, m), 3.38–3.42 (1H, m), 3.47–3.55 (1H, m), 3.60–3.99 (3H, m), 4.08–4.22 (2H, m), 4.27–4.33 (1H, m), 5.53–5.64 (1H, m), 8.02 (1H, s), 9.32 (1H, s); FABMS *m*/*z* 490 [(M+H)$^+$]; FABHRMS calcd for $C_{22}H_{28}N_5O_4S_2$ [(M+H)$^+$]: 490.1583, found: 490.1577.

5.1.6. (1*S*,5*R*,6*S*)-6-((1*R*)-1-Hydroxyethyl)-2-[6-(3-isothioureidopropyl)-7-methylthioimidazo[5,1-*b*]-thiazolium-2-yl]-1-methyl-1-carbapen-2-em-3-carboxylate hydrochloride (intramolecular salt) (8a). To a mixed solution of compound **7a** (85 mg, 0.102 mmol) in acetonitrile (2 mL) and methanol (0.5 mL), thiourea (10 mg, 0.123mmol) was added, and stirred at room temperature for 1 day. After the reaction solution was poured into ether (30 mL), the generated precipitates were collected

by filtration and treated in similar procedure as described for the preparation of **5a,** to give **8a** (15 mg) in 18% yield from compound **7a**: ^{1}H NMR (D_2O) δ (*H*OD = 4.65 ppm): 1.12 (3H, d, J = 7.3 Hz), 1.17 (3H, d, J = 6.4 Hz), 2.21–2.31 (5H, m), 3.12 (2H, t, J = 7.0 Hz), 3.40 (1H, dd, J_1 = 6.0 Hz, J_2 = 2.9 Hz), 3.51 (1H, m), 4.14 (1H, m), 4.22 (1H, dd, J_1 = 9.5 Hz, J_2 = 2.7 Hz), 4.47 (2H, t, J = 7.0 Hz), 8.00 (1H, s), 9.24 (1H, s); FABMS *m*/*z* 496 [(M+H)$^+$]; FABHRMS calcd for $C_{20}H_{26}N_5O_4S_3$ [(M+H)$^+$]: 496.1147, found: 496.1155.

Compound **8b** was prepared by a similar procedure as the preparation of **8a** in 45% yield from compound **7b**: ^{1}H NMR (D_2O) δ (*H*OD = 4.65 ppm): 1.11 (3H, d, J = 7.3 Hz), 1.17 (3H, d, J = 6.3 Hz), 1.89 (3H, s), 3.43 (1H, dd, J_1 = 5.9 Hz, J_2 = 2.7 Hz), 3.52 (1H, m), 4.15 (1H, m), 4.23 (1H, dd, J_1 = 9.5 Hz, J_2 = 2.7 Hz), 4.28 (2H, s), 5.51 (2H, s), 7.24–7.36 (4H, m), 7.99 (1H, s); FABMS *m*/*z* 558 [(M+H)$^+$]; FABHRMS calcd for $C_{25}H_{28}N_5O_4S_3$ [(M+H)$^+$]: 558.1303, found: 558.1301.

5.1.7. Compounds 11a–g, 11i and 11j. These compounds were prepared by a similar procedure as the preparation of **4a** and **5a**.

Compound **11a** was obtained in 9% yield from compound **1**: ^{1}H NMR (D_2O) δ (*H*OD = 4.65 ppm): 1.13 (3H, d, J = 7.3 Hz), 1.18 (3H, d, J = 6.3 Hz), 2.30 (3H, s), 2.31–2.41 (1H, m), 2.80–2.89 (1H, m), 3.43 (1H, dd, J_1 = 6.1 Hz, J_2 = 2.7 Hz), 3.47–3.63 (2H, m), 3.75–3.80 (1H, m), 3.89–3.98 (1H, m), 4.01–4.07 (1H, m), 4.11–4.18 (1H, m), 4.22 (1H, dd, J_1 = 9.5 Hz, J_2 = 2.7 Hz), 5.58–5.69 (1H, m), 8.08 (1H, s), 9.47 (1H, s); FABMS *m*/*z* 479 [(M+H)$^+$]; FABHRMS calcd for $C_{21}H_{27}N_4O_5S_2$[(M+H)$^+$]: 479.1423, found: 479.1427.

Compound **11b** was obtained in 5% yield from compound **1**: ^{1}H NMR (D_2O) δ (*H*OD = 4.65 ppm): 1.11 (3H, d, J = 7.3 Hz), 1.16 (3H, d, J = 6.3 Hz), 2.27 (3H, s), 2.43–2.52 (1H, m), 2.62–2.72 (1H, m), 3.14 (1H, dd, J_1 = 12.1 Hz, J_2 = 4.0 Hz), 3.42 (1H, dd, J_1 = 5.8 Hz, J_2 = 2.7 Hz), 3.47–3.56 (2H, m), 3.65 (3H, s), 4.10–4.23 (3H, m), 5.30–5.37 (1H, m), 8.01 (1H, s); FABMS *m*/*z* 507 [(M+H)$^+$]; FABHRMS calcd for $C_{22}H_{27}N_4O_6S_2$ [(M+H)$^+$]: 507.1372, found: 507.1365.

Compound **11c** was obtained in 17% yield from compound **1**: ^{1}H NMR (D_2O) δ (*H*OD = 4.65 ppm): 1.14 (3H, d, J = 7.1 Hz), 1.19 (3H, d, J = 6.2 Hz), 2.14–2.21 (1H, m), 2.29 (3H, s), 2.87–2.97 (1H, m), 3.26–3.32 (1H, m), 3.42–3.58 (1H, m), 3.76–3.82 (1H, m), 4.12–4.19 (1H, m), 4.23 (1H, dd, J_1 = 9.5 Hz, J_2 = 2.7 Hz), 5.36–5.43 (1H, m), 8.05 (1H, s); FABMS *m*/*z* 493 [(M+H)$^+$]; FABHRMS calcd for $C_{21}H_{25}N_4O_6S_2$[(M+H)$^+$]: 493.1216, found: 493.1207.

Compound **11d** was obtained in 19% yield from compound **1**: ^{1}H NMR (D_2O) δ (*H*OD = 4.65 ppm): 1.11 (3H, d, J = 7.1 Hz), 1.16 (3H, d, J = 6.4 Hz), 2.27 (3H, s), 2.48–2.54 (2H, m), 2.83 (3H, s), 2.96 (3H, s), 2.80–2.89 (1H, m), 3.11 (1H, dd, J_1 = 12.4 Hz, J_2 = 5.4 Hz), 3.42 (1H, dd, J_1 = 5.9 Hz, J_2 = 2.7 Hz), 3.45–3.55 (1H, m), 3.64 (1H, dd, J_1 = 12.4 Hz, J_2 = 6.6 Hz), 4.09–4.16 (1H, m), 4.20 (1H, dd, J_1 = 9.5 Hz, J_2 = 2.7 Hz), 4.41–4.46 (1H, m), 5.29–5.36 (1H, m), 8.02 (1H, s); FABMS *m*/*z* 520 [(M+H)$^+$]; FABHRMS calcd for $C_{23}H_{30}N_5O_5S_2$[(M+H)$^+$]: 520.1688, found: 520.1683.

Compound **11e** was obtained in 23% yield from compound **1**: ^{1}H NMR (D_2O) δ (*H*OD = 4.65 ppm): 0.96 (3H, d, J = 6.8 Hz), 1.14 (3H, d, J = 7.1 Hz), 1.19 (3H, d, J = 6.3 Hz), 2.29 (3H, s), 2.41–2.50 (1H, m), 2.83–3.07 (2H, m), 3.44 (1H, dd, J_1 = 5.9 Hz, J_2 = 2.7 Hz), 3.48–3.58 (1H, m), 4.11–4.19 (1H, m), 4.20–4.44 (3H, m), 8.04 (1H, s), 9.28 (1H, s); FABMS *m*/*z* 451 [(M+H)$^+$]; FABHRMS calcd for $C_{20}H_{27}N_4O_4S_2$ [(M+H)$^+$]: 451.1474, found: 451.1472.

Compound **11f** was obtained in 31% yield from compound **1**: ^{1}H NMR (D_2O) δ (*H*OD = 4.65 ppm): 1.13 (3H, d, J = 7.0 Hz), 1.18 (3H, d, J = 6.4 Hz), 2.29 (3H, s), 2.82 (1H, dd, J_1 = 13.4 Hz, J_2 = 9.0 Hz), 3.25 (1H, dd, J_1 = 13.4 Hz, J_2 = 3.2 Hz), 3.31 (3H, s), 3.44 (1H, dd, J_1 = 6.0 Hz, J_2 = 2.7 Hz), 3.53 (1H, m), 3.93 (1H, m), 4.14 (1H, m), 4.22 (1H, dd, J_1 = 9.5 Hz, J_2 = 2.9 Hz), 4.50–4.65 (2H, m), 8.01 (1H, s), 9.25 (1H, s); FABMS *m*/*z* 467 [(M+H)$^+$]; FABHRMS calcd for $C_{20}H_{27}N_4O_5S_2$[(M+H)$^+$]: 467.1423, found: 467.1416.

Compound **11g** was obtained in 39% yield from compound **1**: ^{1}H NMR (D_2O) δ (*H*OD = 4.65 ppm): 1.16 (3H, d, J = 7.0 Hz), 1.21 (3H, d, J = 6.3 Hz), 2.33 (3H, s), 3.06 (1H, dd, J_1 = 13.6 Hz, J_2 = 9.0 Hz), 3.38 (1H, dd, J_1 = 13.6 Hz, J_2 = 2.9 Hz), 3.47 (1H, dd, J_1 = 6.0 Hz, J_2 = 2.7 Hz), 3.55 (1H, m), 4.17 (1H, m), 4.25 (1H, dd, J_1 = 9.5 Hz, J_2 = 2.9 Hz), 4.45 (1H, m), 4.69 (2H, m), 5.12–5.35 (2H, m), 8.07 (1H, s), 9.32 (1H, s); FABMS *m*/*z* 485 [(M+H)$^+$]; FABHRMS calcd for $C_{20}H_{26}FN_4O_5S_2$[(M+H)$^+$]: 485.1329, found: 485.1322.

Compound **11i** was obtained in 7% yield from compound **1**: ^{1}H NMR (D_2O) δ (*H*OD = 4.65 ppm): 1.15 (3H, d, J = 7.1 Hz), 1.21 (3H, d, J = 6.3 Hz), 2.31 (3H, s), 2.70 (3H, s), 2.85 (2H, t, J = 6.5 Hz), 3.05 (2H, t, J = 6.5 Hz), 3.17 (2H, t, J = 6.5 Hz), 3.47 (1H, m), 3.55 (1H, m), 4.14–4.28 (2H, m), 4.59 (2H, m), 8.04 (1H, s), 9.31 (1H, s); FABMS *m*/*z* 483 [(M+H)$^+$]; FABHRMS calcd for $C_{20}H_{27}N_4O_4S_3$[(M+H)$^+$]: 483.1194, found: 483.1203.

Compound **11j** was obtained in 37% yield from compound **1**: ^{1}H NMR (D_2O) δ (*H*OD = 4.65 ppm): 1.16 (3H, d, J = 7.0 Hz), 1.21 (3H, d, J = 6.6 Hz), 2.34 (3H, s), 3.44–3.59 (4H, m), 3.67 (2H, t, J = 6.5 Hz), 3.97 (2H, t, J = 6.5 Hz), 4.18 (1H, m), 4.25 (1H, dd, J_1 = 9.5 Hz, J_2 = 2.9 Hz), 4.96 (2H, t, J = 6.5 Hz), 8.06 (1H, s); FABMS *m*/*z* 483 [(M+H)$^+$]; FABHRMS calcd for $C_{20}H_{27}N_4O_6S_3$[(M+H)$^+$]: 515.1093, found: 515.1085.

5.1.8. 4-Nitrobenzyl(1*S*,5*R*,6*S*)-2-[6-((2*R*)-3-azido-2- triethylsilyloxy)propyl-7-methylthioimidazo[5,1-*b*]thiazolium-2-yl]-6-((1*R*)-1-hydroxyethyl)-1-methyl-1-carbapen-2-em-3-carboxylate trifluoromethanesulfonate (10h). To a dichloromethane solution (7 mL) of (2*R*)-3-azido-2-triethylsi-

lyloxy-1-propanol (341 mg, 1.47 mmol), 2,6-lutidine (0.189 mL, 1.62 mmol) and trifluoromethanesulfonic anhydride (0.260 mL, 1.54 mmol) were added at −60°C. After stirred for 20 min, a 1/15 M sodium phosphate buffer (pH 6.8) (15 mL) was added to the reaction solution, and the mixture was extracted with dichloromethane (15 mL). The extract was dried over anhydrous magnesium sulfate and filtered. To the filtrate, compound **1** (600 mg, 1.16 mmol) was added and then the solvent was removed under reduced pressure up to 12 mL. After stirred at 35°C for 12 h, the reaction solution was added dropwise to the mixture of diethyl ether (30 mL) and hexane (30 mL). The precipitated solid was collected by filtration, to give **10 h** (970 mg) as a crude product.

5.1.9. (1*S*,5*R*,6*S*)-2-[6-((2*R*)-3-Amino-2-hydroxy)propyl-7-methylthioimidazo[5,1-*b*]thiazolium-2-yl]-6-((1*R*)-1-hydroxyethyl)-1-methyl-1-carbapen-2-em-3-carboxylate hydrochloride (11h, CP5484). To a mixed solution of the crude product of **10 h** (1.07 g) in THF (30 mL) and water (30 mL), 1 N HCl aq was added to adjust to pH 2.1. After stirred at room temperature for 5 h, 10% Pd–C (640 mg) was added and stirred under hydrogen atmosphere at room temperature for 2 h. The catalyst was removed by filtration on Celite, and washed with water. The filtrate was washed with ethyl acetate and then concentrated under the reduced pressure up to about 10 mL. The residue was purified by reversed phase column chromatography (a 20% aqueous methanol solution) and was subjected to column chromatography (water) on Amberlyst A-26 as an ion-exchange resin (chloride form) to give 271 mg (48% from compound **1**) of **11h (CP5484)**: ^{1}H NMR (D_2O) δ (*H*OD = 4.65 ppm): 1.12 (3H, d, J = 7.0 Hz), 1.16 (3H, d, J = 6.3 Hz), 2.26 (3H, s), 2.87–2.94 (1H, m), 3.20–3.26 (1H, m), 3.42 (1H, dd, J_1 = 6.0 Hz, J_2 = 2.7 Hz), 3.46–3.55 (1H, m), 4.10–4.23 (3H, m), 4.28–4.35 (1H, m), 4.60–4.63 (1H, m), 8.03 (1H, s); FABMS *m*/*z* 453 [(M+H)$^+$]; FABHRMS calcd for $C_{19}H_{25}N_4O_5S_2$ [(M+H)$^+$]: 453.1266, found: 453.1258.

5.2. Antimicrobial activity in vitro

Minimum inhibitory concentration (MIC) was determined by the agar plate dilution method. Test strains were subjected to seed culture using Sensitivity test broth (STB, Nissui Pharmaceutical). A 5 μL portion of cell suspension of test strains having about 10^6 CFU/mL was inoculated and incubated at 35 °C for 18 h. The MIC was then measured.

5.3. Efficacy in synthetic infection in mice

The protective effect of **CP5484** on systemic infection models caused by MRSA was compared with that of VCM. Eight male ICR mice were challenged intraperitoneally with 0.5 mL of bacterial suspension containing 2.5% gastric mucin. The animals were administered the test compounds subcutaneously, twice at 1 and 3 hours after the challenge. The 50% effective dose (ED_{50}) values were calculated by probit analysis from the number of mice still alive 7 days after infection.

5.4. Acute toxicity test in mice

Test compounds were administered intravenously to male ICR mice. Morality was observed for 24 h. Five-hundred milligrams per kilogram was set as highest dose level.

5.5. Pharmacokinetic parameters in mice

CP5484 was administered intravenously with 10 mg/kg cilastatin to two or three mice at a 20 mg/kg dose. The protein binding of **CP5484** was assessed in mice and human serum using the equilibrium dialysis method. The initial concentration of **CP5484** in serum was 10 μg/mL. The concentrations of **CP5484** in plasma, urine, and filtrate were determined by an HPLC method. The pharmacokinetic parameters were obtained using standard non-compartmental analysis.

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