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Novel Dithiocarbamate Carbapenems with Anti-MRSA Activity

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Abstract—A new series of 1β-methyl carbapenems, in which a disubstituted-aminothiocarbonylthio moiety was attached to the C-2 position of the carbapenem nucleus, were prepared and evaluated for anti-MRSA activity. These derivatives showed good in vitro antibacterial activity against high-level MRSA, and the finding of good affinity for PBP-2′ supported these results. Some of the compounds having favorable protein-binding affinity showed excellent in vivo anti-MRSA activity.

Introduction

Nosocomial infections caused by methicillin-resistant *Staphylococcus aureus* (MRSA) are becoming difficult to treat because there are few therapeutic agents that are effective against MRSA. Vancomycin,[1] a potent anti-MRSA agent, is used as a first-line therapeutic agent, but its adverse effects restrict clinical use. Therefore, new and potent anti-MRSA agents with better safety profiles are highly desired.

It was demonstrated that MRSA strains produced a new penicillin-binding protein, PBP-2′ (PBP-2a),[2] which showed low affinity to various types of β-lactams such as penicillins, cephems, and carbapenems marketed so far, resulting in high resistance. However, it was recently disclosed that new β-lactams such as CP0467 (**I**),[3] LY-206763 (**II**),[4] SM-17466 (**III**),[5] and a Merck compound (**IV**)[6] showed good anti-MRSA activity and high affinity for the PBP-2′ protein of MRSA (see Figure 1). Coincidentally, these β-lactams had a thiazolothio moiety at the C-3 position of the cephem (or carbacephem) nucleus or at the C-2 position of the carbapenem nucleus, and this moiety was speculated to play an important role in improving the anti-MRSA activity of these drugs.

With the thiazolothio structural feature in mind, we envisioned a dithiocarbamate moiety at the C-2 side chain as in (**7**), which was arrived at by cleaving the 1–5 bond of the thiazole ring as shown in Figure 2. The targeted dithiocarbamate carbapenems (**7**)[7] were prepared via the coupling reaction of the carbapenem diphenylphosphate (**3**)[9] and dithiocarbamic acid salts (**2**). Compared with imipenem, they showed potent in vitro and in vivo anti-MRSA activity, and good affinity for PBP-2′.

A preliminary account of this work[8] has been presented previously, and herein we describe the synthesis and structure–activity relationships of these novel dithiocarbamate carbapenems and present, in depth, the biological profiles of the represented compounds.

Results and Discussion

Chemistry

Dithiocarbamate carbapenems (**7a**–**7z**) were prepared as shown in Scheme 1. The coupling reaction of the carbapenem diphenylphosphates (**3**, **4**)[9] and triethylammonium 1-pyrrolidinyldithiocarbamate (**2a**), easily derived from carbon disulfide and pyrrolidine (**1a**), did not proceed effectively in THF or DMF, probably due

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to the low nucleophilicity of **2a**. After adding lithium chloride to this system,[6] the reaction proceeded smoothly to give the desired products (**5a**, **6a**) in good yields. We speculated that lithium dithiocarbamate generated in situ during this reaction accelerated the production of the coupling products. The other coupling products (**5b**–**5z**, **6b**) were obtained by the above method. Deprotection of **5** and **6** was usually carried out by catalytic hydrogenation in the presence of $NaHCO_3$.

In the case of the compounds **7e**, **7u**, **7w**, which have double bonds in the side chains, deprotection was carried out by Zinc reduction method.[10] The crude carbapenem products (**7**, **8**) were purified by reversed-phase column chromatography followed by lyophilization to afford the final carbapenems.

The thiocarbamate carbapenems (**11a**, **11b**) were prepared via the acylation of 2-mercaptocarbapenem (**9**),[11]

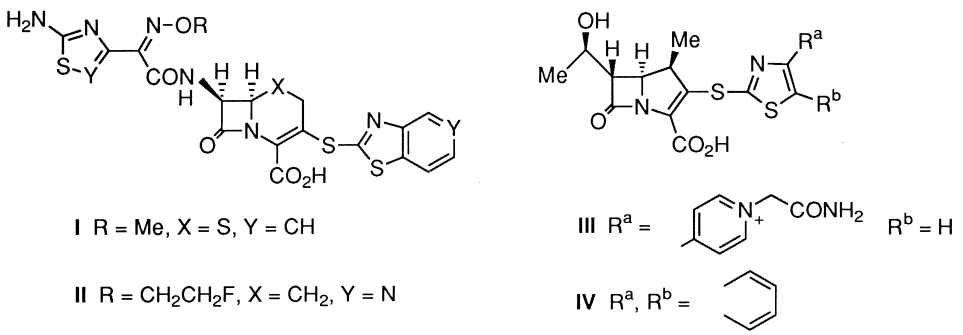


Figure 1. The structures of the anti-MRSA β-lactams.

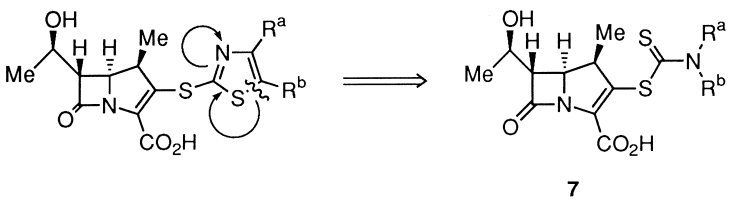
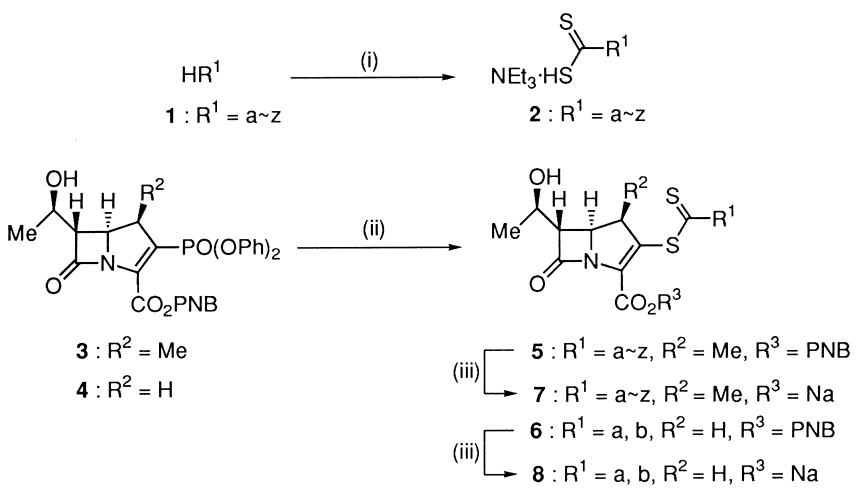


Figure 2. Dithiocarbamate structure envisioned from the thiazolothio moiety.



Scheme 1. Preparation of the dithiocarbamate carbapenems.

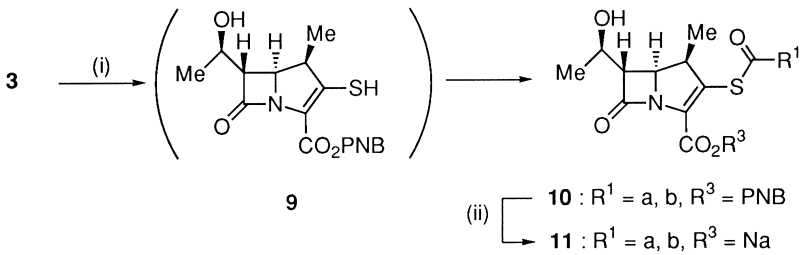
derived from **3** according to the published method as shown in Scheme 2.

Other dithiocarbamate carbapenems (**7u-2**–**7u-4**, **7z-2**–**7z-4**), including quaternized ammonium compounds, were prepared as shown in Scheme 3. The iodide (**12**), derived from the intermediate (**5u-1**) by selective propanesulfonylation of the allylic alcohol moiety and subsequent substitution with sodium iodide, was quaternized with appropriate amines such as triethylamine and pyridine. The quaternized compounds were subsequently deprotected by zinc reduction to give **5u-2**–**5u-4**.

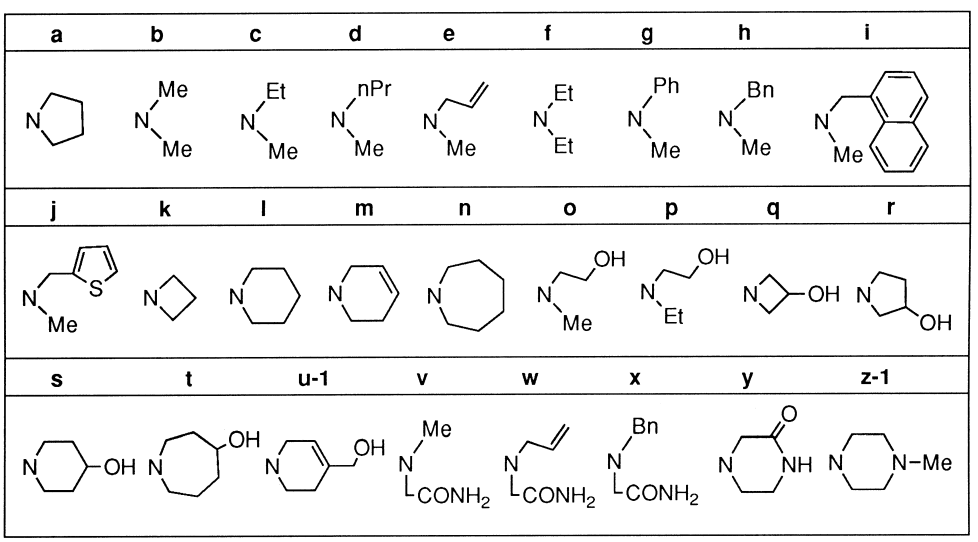
Quaternization of the coupling products (**5z-1**, **5z-2**) with iodomethane followed by deprotection by catalytic hydrogenation gave **7z-2**, **7z-3**.

Biological properties

Table 1 shows the comparative antibacterial activities of the dithiocarbamate carbapenems (**7a**, **7b**), their des 1β-methyl analogues (**8a**, **8b**), and their thiocarbamate derivatives (**11a**, **11b**) together with those of imipenem and vancomycin, against *S. aureus* including MRSA, *Escherichia coli*, and *Pseudomonas aeruginosa*. Table 1 also shows dehydropeptidase-I (DHP-1) susceptibilities. The dithiocarbamate carbapenems (**7a**, **7b**) showed good antibacterial activity against not only MSSA but also against high-level MRSA. Their anti-MRSA activity, which was superior to that of imipenem by 16 to 32-fold, was attributed to their good PBP-2′ affinity. The IC_{50}s of **7a**, **7b** and imipenem were 5.6, 9.6 and 125 μg/mL, respectively. The dithiocarbamate carbapenems also exhibited good-to-moderate activity against *Staphylococcus epidermidis*, *Enterococcus faecalis*, and *E. coli*, but were inactive against *P. aeruginosa*. It is evident that the des 1β-methylation of **7a** and **7b** affected the anti-staphylococcal activity and DHP-I susceptibility by a factor of 1/2 to 1/4, and that replacement of the thiocarbonyl moiety by a carbonyl moiety also reduced the activity to 1/4 to 1/8. The above findings indicate that the 1β-methyl substitution and thiocarbonyl moiety of the side chain are important to anti-MRSA activity.



Reagents: (i) $NaSH{\cdot}xH_2O$, DMF,-40°C, then, $EtN(i\text{-}Pr)_2$, $ClCOR^1$,0 °C, (ii) 10% Pd-C, THF, EtOH, H_2O, $NaHCO_3$, H_2, r.t. .

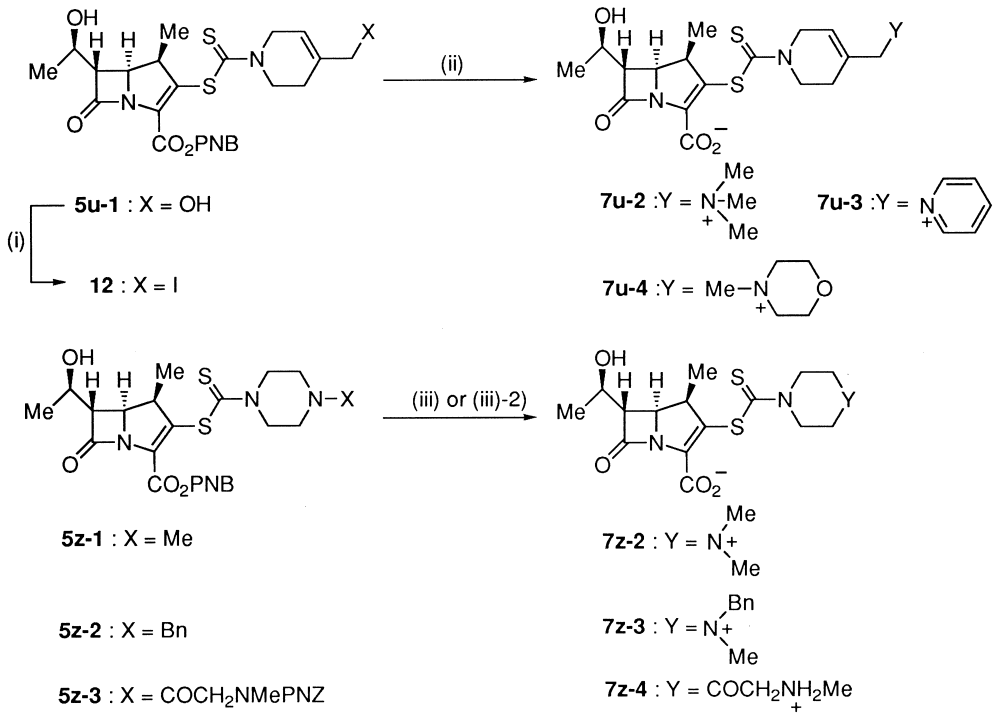


Scheme 2. Preparation of the thiocarbamate carbapenems.

These results prompted us to investigate further derivatization of dithiocarbamate carbapenem to identify a new anti-MRSA agent. Tables 2 and 3 show the antibacterial activities, DHP-I susceptibility, PBP-2′ affinity, and human protein-binding affinity of various types of dithiocarbamate carbapenems. All of the compounds listed in the Tables showed good anti-MRSA activity and PBP-2′ affinity as expected. The in vitro anti-MRSA activity improved as the hydrophobicity of the side chain moiety increased (**7b**→**7h**, **7k**→**7n**). However, the resulting derivatives (**7h**, **7n**) showed high protein-binding affinity [**7b** (88%)→**7h** (99%), **7k** (80%)→**7n** (99%)], which affected their in vivo activities (see Table 4).[4] Therefore, we introduced an appropriate hydrophilic group into the side chain in order to reduce this protein-binding affinity. Introducing amine (**7l**→**7z-1**) and quaternized amine (**7z-1**→**7z-3**, **7m**→**7u-3**) groups is one of the most effective methods available to reduce protein-binding. In addition, the introduction of the hydroxyl (**7c**→**7o**) and amide (**7a**→**7v**, **7e**→**7w**, **7l**→**7y**) moieties was found to reduce the protein-binding affinity. The piperidine derivative (**7z-4**), having both the amine and amide moieties, showed favorable protein-binding affinity without any loss of anti-MRSA activity.

As for DHP-I susceptibility, the dithiocarbamate carbapenems, except for the piperidinium derivatives (**7z-2**, **7z-3**) and amide derivatives (**7v**, **7w**, **7x**), were generally more susceptible than imipenem. Interestingly, the introduction of the amide group improved DHP-I susceptibility. Quaternization of the piperidine derivative improved the DHP-I stability greatly. However, the stability of other quaternary ammonium derivatives (**7u-2**, **7u-3**, **7u-4**) was not improved.[5c]

Among the synthesized dithiocarbamate carbapenems, 8 compounds (**7b**, **7h**, **7o**,[12] **7t**, **7w**, **7y**, **7z-2**, **7z-4**) were selected as representatives and further evaluated for their in vitro and in vivo anti-MRSA activity, epileptogenic potential by rat intraventricular assay, and pharmacokinetic parameters in mice, as shown in Table 4. Except for **7h**, these compounds showed good in vivo anti-MRSA activity that was comparable or superior to that of vancomycin against MRSA, reflecting their good in vitro anti-MRSA activity. The compound **7h** was inactive in vivo due to its high protein-binding affinity (99%). The compounds **7b** and **7o** showed better C_{max} and AUC values than the rest. On the contrary, the quaternary ammonium and amine derivatives (**7z-2**, **7z-4**) with relatively low protein-binding affinities, and **7h**



Scheme 3. Preparation of other dithiocarbamate carbapenems.

with high protein-binding affinity, showed low C_{max} and AUC values. The epileptogenicity of the selected compounds at 100 μg/rat head, as measured by rat intraventricular assay, suggested that **7z-4** had no epileptogenic potential, although the quaternary ammonium derivative (**7z-2**) showing high epileptogenicity. Other derivatives (**7u-2**–**7u-4**, **7z-3**) having cationic moiety on the side chain also showed high epileptogenicity.[13] This finding seems to be compatible with the results reported by Sunagawa et al.[14]

In conclusion, we have prepared a new series of dithiocarbamate carbapenems which showed good in vitro anti-MRSA activity. The biological properties of the selected compounds indicated that dithiocarbamate carbapenems deserve attention as potential anti-MRSA agents.

Experimental

General methods

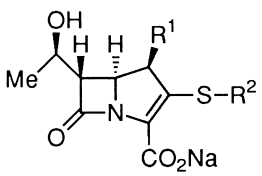
IR absorption spectra were recorded on a Horiba FT-200 spectrometer. ^{1}H NMR spectra were recorded on a Varian GEM-300 spectrometer (300 MHz), in the designated solvent, using tetramethylsilane (TMS) or residual DOH (δ 4.80) as an internal standard. Mass spectra (MS) were measured using a JEOL JMS-SX102A spectrometer. UV spectra were taken on a SHIMADZU SPD-10A spectrometer in 0.1 M 3-morpholinopropanesulfonate (MOPS) buffer (pH 7.0). TLC was performed with Merck Kieselgel F_{254} precoated plates. The silica gel for column chromatography was WAKO gel C-300. Reversed-phase column chromatography was carried out on YMC-gel ODS-AQ 120–S50.

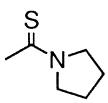
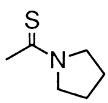
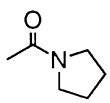
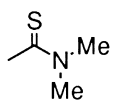
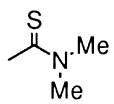
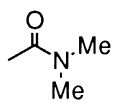
General procedure for the preparation of the dithiocarbamate carbapenems

The experimental procedure of **7a** was described as a representative example.

(1) Triethylammonium 1-pyrrolidinyldithiocarbamate (2a). To an ice-cooled solution of pyrrolidine (5.00 mL, 59.9 mmol) in THF (200 mL) was added triethylamine (25.0 mL, 180 mmol) and carbon disulfide (5.40 mL, 89.9 mmol), and the mixture was stirred for 30 min at

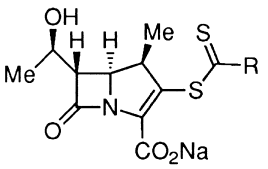
Table 1. Comparative antibacterial activities (MIC, μg/mL) and DHP-I susceptibilities of the dithiocarbamate carbapenems (**7a**, **7b**) and their analogues

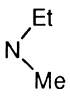
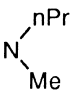
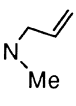
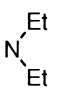
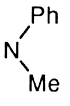
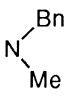
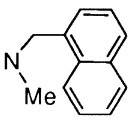
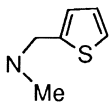


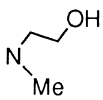
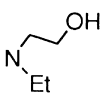
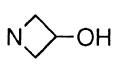
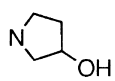
Compd		7a	8a	11a	7b	8b	11b		
	R[1]:	Me	H	Me	Me	H	Me		
Organism	R[2]:							IPM	VCM
S. aureus 209P NIHJ JC1		0.012	0.025	0.05	0.012	0.006	0.05	< 0.006	0.39
S. aureus BB6294[a]		3.13	6.25	25	3.13	12.5	25	100	1.56
S. aureus CSa1009[a,b]		6.25	25	25	6.25	> 25	> 25	100	1.56
S. epidermidis MB5181[a]		3.13	12.5	25	6.25	12.5	> 25	100	1.56
E. faecalis MB4966		12.5	12.5	6.25	12.5	12.5	12.5	0.78	1.56
E. coli NIHJ JC2		6.25	1.56	6.25	6.25	0.2	0.78	0.10	> 25
P. aeruginosa MB5002		> 25	> 25	> 25	25	> 25	> 25	1.56	> 25
DHP-I susceptibility[c]		3.18	11.7	0.65	2.12	9.99	0.62	1.00	—

[a]Methicillin-resistant strain.
[b]β-Lactamase-producing strain.
[c]Relative rate of hydrolysis to imipenem, porcine renal DHP-I.

Table 2. In vitro antibacterial activities (MIC, μg/mL) and biological properties of dithiocarbamate carbapenems

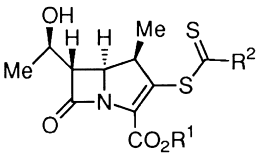


Compd	**7c**	**7d**	**7e**	**7f**	**7g**	**7h**	**7i**	**7j**
R: organism								
S. aureus 209P NIHJ JC1	0.025	0.025	0.012	0.025	0.05	0.012	< 0.006	< 0.006
S. aureus BB6294[a]	3.13	3.13	3.13	3.13	1.56	1.56	0.78	1.56
S. aureus CSa1009[a,b]	3.13	3.13	3.13	3.13	3.13	1.56	1.56	3.13
S. epidermidis MB5181[a]	3.13	3.13	3.13	3.13	1.56	3.13	0.39	0.78
E. faecalis MB4966	6.25	6.25	6.25	12.5	6.25	1.56	1.56	3.13
E. coli NIHJ JC2	6.25	25	12.5	25	> 25	12.5	25	12.5
P. aeruginosa MB5002	> 25	25	> 25	25	> 25	> 25	> 25	> 25
DHP-I susceptibility[c]	2.26	2.71	2.18	2.51	2.29	1.94	4.41	2.05
PBP–2′ affinity (IC_{50}, μg/mL)	3.4	NT	2.0	5.4	2.9	1.8	NT	0.2
Serum protein-binding (%)[d]	88	95	99	94	99	99	99	99

Compd	**7k**	**7l**	**7m**	**7n**	**7o**	**7p**	**7q**	**7r**
R: organism								
S. aureus 209P NIHJ JC1	0.025	0.025	0.025	0.012	0.025	0.025	0.025	0.025
S. aureus BB6294[a]	3.13	1.56	1.56	1.56	3.13	6.25	6.25	6.25
S. aureus CSa1009[a,b]	6.25	3.13	3.13	1.56	6.25	6.25	6.25	6.25
S. epidermidis MB5181[a]	6.25	3.13	1.56	1.56	6.25	3.13	3.13	3.13
E. faecalis MB4966	6.25	6.25	6.25	6.25	6.25	6.25	12.5	6.25
E. coli NIHJ JC2	3.13	25	12.5	> 25	0.78	6.25	0.78	0.78
P. aeruginosa MB5002	> 25	> 25	> 25	> 25	> 25	> 25	> 25	> 25
DHP-I susceptibility[c]	3.03	3.10	2.63	3.09	1.65	2.38	2.32	2.21
PBP–2′ affinity (IC_{50}, μg/mL)	12.0	2.0	6.2	NT	3.8	NT	8.0	NT
Serum protein-binding (%)[d]	80	98	98	99	47	84	69	65

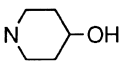
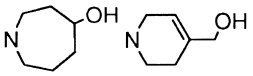
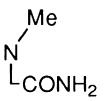
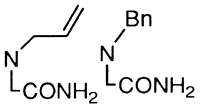
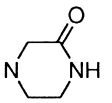
[*] MIC was determined by an agar dilution method using Mueller–Hinton medium (Difco).
[a]Methicillin-resistant strain.
[b]β-Lactamase-producing strain.
[c]Relative rate of hydrolysis to imipenem, porcine renal DHP-I.
[d]Binding rate for human serum.
NT; not tested.

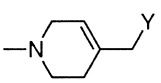
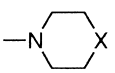
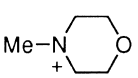
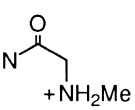
Table 3. In vitro antibacterial activities (MIC, μg/mL) and biological properties of dithiocarbamate carbapenems



7s~7y R^1 = Na

7u-2 to 7u-4, 7z-1 to 7z-4 R^1 = —

Compd	7s	7t	7u-1	7v	7w	7x	7y
R^2: organism							
S. aureus 209P NIHJ JC1	0.025	0.012	0.012	0.025	0.025	0.012	0.025
S. aureus BB6294[a]	6.25	3.13	3.13	3.13	6.25	3.13	3.13
S. aureus CSa1009[a,b]	6.25	3.13	3.13	6.25	12.5	6.25	6.25
S. epidermidis MB5181[a]	6.25	3.13	3.13	3.13	3.13	3.13	6.25
E. faecalis MB4966	6.25	3.13	6.25	6.25	3.13	3.13	6.25
E. coli NIHJ JC2	3.13	12.5	6.25	0.2	3.13	12.5	0.39
P. aeruginosa MB5002	> 25	> 25	> 25	25	> 25	> 25	> 25
DHP-I susceptibility[c]	2.23	2.88	3.43	0.82	0.64	0.46	1.05
PBP–2′ affinity (IC_{50}, μg/mL)	NT	1.9	2.8	2.5	2.9	1.9	2.6
Serum protein-binding (%)[d]	69	85	94	51	90	98	64

Compd	7u-2	7u-3	7u-4	7z-1	7z-2	7z-3	7z-4
R^2:							
Organism	Y: 			X: $^+$NHMe			
S. aureus 209P NIHJ JC1	0.012	< 0.006	0.025	0.025	0.012	< 0.006	0.006
S. aureus BB6294[a]	3.13	3.13	6.25	3.13	3.13	3.13	3.13
S. aureus CSa1009[a,b]	3.13	3.13	3.13	6.25	12.5	6.25	3.13
S. epidermidis MB5181[a]	3.13	3.13	6.25	3.13	3.13	1.56	1.56
E. faecalis MB4966	6.25	6.25	1.56	6.25	1.56	1.56	3.13
E. coli NIHJ JC2	0.78	1.56	1.56	1.56	0.1	1.56	0.39
P. aeruginosa MB5002	25	> 25	> 25	> 25	> 25	> 25	25
DHP-I susceptibility[c]	1.94	2.19	1.40	1.53	0.11	0.15	1.34
PBP–2′ affinity (IC_{50}, μg/mL)	2.2	NT	NT	2.1	1.0	NT	1.1
Serum protein-binding (%)[d]	87	54	51	91	30	60	58

*MIC was determined by an agar dilution method using Mueller–Hinton medium (Difco).
[a]Methicillin-resistant strain.
[b]β-Lactamase-producing strain.
[c]Relative rate of hydrolysis to imipenem, porcine renal DHP-I.
[d]Binding rate for human serum.
NT; not tested.

Table 4. Anti-MRSA activities and other biological properties of the representative dithiocarbamate carbapenems

Compd	7b	7h	7o	7t	7w	7y	7z-2	7z-4
In vitro anti-MRSA activity[a] G-mean MIC (μg/mL)	4.59	1.97	4.36	3.55	6.41	6.25	5.50	3.84
In vivo anti-MRSA activity[b] ED_{50} (μg/mL)	5.91	>50	4.80	2.88	4.90	2.74	7.30	2.19
Affinity to PBP-2′[c] IC_{50} (μg/mL)	9.6	1.8	3.8	1.9	2.9	2.6	1.0	1.1
DHP-I susceptibility	2.12	1.94	1.65	2.88	0.64	1.05	0.11	0.75
Serum protein-binding (%)[d]	88(69)	99(99)	47(58)	85(92)	90(77)	64(48)	30(24)	58(34)
Epileptogenicity (100 μg/rat head, $n=5$)	0/5	0/5	0/5	0/5	0/5	0/5	5/5	0/5
Pharmacokinetic parameters in mice[e]								
C_{max} (μg/mL)	21.6	5.1	24.5	13.1	11.6	10.4	5.6	4.4
$T_{1/2}$ (min)	27.6	12.5	22.1	18.8	13.7	13.1	9.5	12.4
AUC (μg/min/mL)	1061	134	1026	551	431	287	146	123
Urinary recovery (%)	38	1.1	38	26	37	25	48	29

[a]High-level MRSA (27 strains); geometric-mean MIC (μg/mL) of methicillin, imipenem, and vancomycin were 3000, 115 and 1.33, respectively.
[b]ED_{50}s (mg/kg) of imipenem and vancomycin were >200 and 5.56, respectively.
[c]IC_{50} (μg/mL) of imipenem was 125.
[d]Binding rate for human (mouse) serum at a carbapenem concentration of 10 μg/mL.
[e]The drugs were administered intraperitoneally in combination with 40 mg/kg of cilastatin.

the same temperature. The resulting precipitate was collected by filtration, washed with diisopropyl ether, and dried under reduced pressure to yield **2a** (13.5 g, 91%): ^{1}H NMR (300 MHz, D_2O) δ 1.26 (9H, d, $J=7.0$ Hz), 1.97 (4H, m), 3.18 (6H, q, $J=7.0$ Hz), 3.75 (4H, m); IR (KBr) cm^{-1} 1375, 1165, 1001, 943.

(2) *p*-Nitrobenzyl (1*R*,5*S*,6*S*)-6-[(*R*)-1-hydroxyethyl]-2-[(1-pyrrolidinyl)thiocarbonylthiol-1-methyl-1-carbapen-2-em-3-carboxylate 5a. To a stirred solution of *p*-nitrobenzyl (1R,5S,6S)-2-diphenoxyphosphoryloxy-6-[(R)-1-hydroxyethyl]-1-methyl-1-carbapen-2-em-3-carboxylate **3** (2.00 g, 3.36 mmol) in THF (50 mL) were added **2a** (1.00 g, 4.03 mmol) and lithium chloride (171 mg, 4.03 mmol), and the mixture was stirred overnight at room temperature under a nitrogen atmosphere. The reaction mixture was poured into H_2O and extracted with EtOAc. The organic layer was washed with brine, dried over $MgSO_4$, and concentrated in vacuo. Purification of the residue by silica gel column chromatography gave **5a** (1.21 g, 74%): ^{1}H NMR (300 MHz, $CDC1_3$) δ 1.16 (3H, d, $J=7.5$ Hz), 1.36 (3H, d, $J=6.3$ Hz), 1.90–2.20 (4H, m), 3.37 (1H, dd, $J=3.0$, 6.3 Hz), 3.73 (2H, m), 3.87 (2H, d, $J=3.0$, 9.9 Hz), 5.25 (1H, $J=14.0$ Hz), 5.48 (1H, d, $J=14.0$ Hz), 7.64 (2H, d, $J=9.0$ Hz), 8.22 (2H, d, $J=9.0$ Hz); IR (KBr) cm^{-1} 1772, 1522, 1437, 1346.

(3) Sodium (1*R*,5*S*,6*S*)-6-[(*R*)-1-hydroxyethyl]-2-[(1-pyrrolydinyl)thiocarbonylthio]-1-methyl-1-carbapen-2-em-3-carboxylate 7a. To a solution of **5a** (300 mg, 0.61 mmol) in THF (30 mL) and EtOH (5 mL) were added $NaHCO_3$ (51 mg, 0.61 mmol) in H_2O (30 mL) and 10% Pd-C (300 mg), and the mixture was stirred overnight at room temperature under a hydrogen atmosphere. The reaction mixture was passed through a pad of celite and the filtrate was concentrated under reduced pressure to ca. 20 mL. After the insoluble of the aqueous layer was removed by filtration, the filtrate was subjected to reversed-phase column chromatography, which was eluted with H_2O and then 20% MeOH–H_2O. The fractions detected by HPLC were combined and lyophilized to give **7a** (117 mg, 51%): ^{1}H NMR (300 MHz, D_2O) δ 1.11 (3H, d, $J=7.0$ Hz), 1.28 (3H, d, $J=6.0$ Hz), 1.90–2.20 (4H, m), 3.53 (1H, dd, $J=3.0$, 6.0 Hz), 3.60–3.90 (5H, m), 4.25 (1H, m), 4.36 (1H, dd, $J=3.0$, 10.0 Hz); IR (KBr) cm^{-1} 1749, 1608, 1437, 1389; FAB-HRMS *m/z* calcd for $C_{15}H_{20}N_2O_4S_2Na$ $(M+Na)^+$: 379.0763, found 379.0742; UV λ_{max} 276 (ε 15400).

The following compounds (**7b**–**7d**, **7f**–**7l**, **7n**–**7t**, **7v**–**7y**, **7z-1**, **7z-4**, **8a**, **8b**) were prepared as described for the preparation of **7a**.

7b. ^{1}H NMR (300 MHz, D_2O) δ 1.10 (3H, d, $J=7.3$ Hz), 1.28 (3H, d, $J=6.3$ Hz), 3.47 (3H, s), 3.48 (3H, s), 3.53 (1H, dd, $J=3.0$, 6.0 Hz), 3.71 (1H, m), 4.26 (1H, m), 4.36 (1H, dd, $J=3.0$, 10.0 Hz); IR (KBr) cm^{-1} 1749, 1603, 1387, 1248, 1147; FAB-HRMS *m/z* calcd for $C_{13}H_{17}N_2O_4S_2Na_2$ $(M+2Na\text{-}H)^+$: 375.0425, found 375.0430; UV λ_{max} 277 (ε 13700).

7c. ^{1}H NMR (300 MHz, D_2O) δ 1.11 (3H, d, $J=6.9$ Hz), 1.21 (3H, d, $J=6.9$ Hz), 1.28 (3H, d, $J=6.3$ Hz), 3.43 (3H, br s), 3.50–3.55 (1H, m), 3.65–3.79 (1H, m), 3.80–

4.12 (2H, m), 4.20–4.32 (1H, m), 4.33–4.40 (1H, m); IR (KBr) cm^{-1} 3425, 1757, 1603, 1389, 1282; FAB-MS *m/z* 389 $(M+2Na\text{-}H)^+$; UV λ_{max} 278 (ε 13000).

7d. ^1H NMR (300 MHz, D_2O) δ 0.89, 0.93 (3H, each d, $J=7.3$ Hz), 1.10 (3H, d, $J=7.3$ Hz), 1.28 (3H, d, $J=6.5$ Hz), 1.62–1.84 (2H, m), 3.44 (3H, s), 3.49–3.54 (1H, m), 3.66–3.79 (1H, m), 3.80–4.06 (2H, m), 4.20–4.31 (1H, m), 4.32–4.39 (1H, m); IR (KBr) cm^{-1} 3407, 1757, 1604, 1389, 1267; FAB-MS *m/z* 403 $(M+2Na\text{-}H)^+$; UV λ_{max} 279 (ε 15900).

7f. ^1H NMR (300 MHz, D_2O) δ 1.16 (3H, d, $J=7.3$ Hz), 1.26–1.43 (9H, m), 3.61 (1H, m), 3.73–4.18 (5H, m), 4.26–4.38 (1H, m), 4.39–4.45 (1H, m); IR (KBr) cm^{-1} 1768, 1606, 1558, 1379; FAB-MS *m/z* 403 $(M+2Na\text{-}H)^+$; UV λ_{max} 280 (ε 15200).

7g. ^1H NMR (300 MHz, D_2O) δ 1.01 (3H, m), 1.25 (3H, d, $J=6.4$ Hz), 3.46 (1H, m), 3.61 (1H, m), 3.75 (3H, s), 4.22 (2H, m), 7.50 (5H, m); IR (KBr) cm^{-1} 1749, 1603, 1363; FAB-MS *m/z* 437 $(M+2Na\text{-}H)^+$; UV λ_{max} 281 (ε 17800).

7h. ^1H NMR (300 MHz, D_2O) δ 1.02, 1.12 (3H, each d, $J=7.3$ Hz), 1.27 (3H, d, $J=6.3$ Hz), 3.44, 3.47 (3H, each s), 3.52 (1H, m), 3.74 (1H, m), 4.25 (1H, m), 4.35 (1H, m), 5.00–5.40 (2H, m), 7.20–7.50 (5H, m); IR (KBr) cm^{-1} 1749, 1608, 1387; FAB-MS *m/z* 451 $(M+2Na\text{-}H)^+$; UV λ_{max} 280 (ε 17300).

7i. ^1H NMR (300 MHz, D_2O) δ 0.64–0.92 (3H, m), 1.09–1.15 (3H, m), 3.04–3.74 (5H, m), 4.11–4.25 (2H, m), 4.83–5.63 (2H, m), 6.96–7.80 (7H, m); IR (KBr) cm^{-1} 3406, 1753, 1603, 1389; FAB-MS *m/z* 489 $(M+Na)^+$; UV λ_{max} 284 (ε 24000).

7j. ^1H NMR (300 MHz, D_2O) δ 1.08 (3H, d, $J=7.3$ Hz), 1.27 (3H, d, $J=6.3$ Hz), 3.38–3.56 (4H, m), 3.72 (1H, m), 4.18–4.42 (1H, m), 5.15–5.55 (2H, m), 7.02 (1H, m), 7.16 (1H, m), 7.41 (1H, m); IR (KBr) cm^{-1} 1747, 1612, 1392; FAB-MS *m/z* 435 $(M+Na)^+$; UV λ_{max} 281 (ε 16300).

7k. ^1H NMR (300 MHz, D_2O) δ 1.10 (3H, d, $J=7.3$ Hz), 1.27 (3H, d, $J=6.6$ Hz), 2.37 (4H, m), 3.5 3 (1H, dd, $J=3.0$, 6.0 Hz), 3.72 (1H, m), 4.20–4.40 (6H, m); IR (KBr) cm^{-1} 1751, 1601, 1489, 1394; FAB-MS *m/z* 387 $(M+2Na\text{-}H)^+$; UV λ_{max} 280 (ε 15600).

7l. ^1H NMR (300 MHz, D_2O) δ 1.11 (3H, d, $J=7.3$ Hz), 1.28 (3H, d, $J=6.5$ Hz), 1.71 (6H, m), 3.52 (1H, dd, $J=3.0$, 5.9 Hz), 3.69 (1H, m), 3.90–4.30 (5H, m), 4.36 (1H, dd, $J=3.0$, 9.8 Hz); IR (KBr) cm^{-1} 1749, 1603, 1227; FAB-MS *m/z* 393 $(M+Na)^+$; UV λ_{max} 280 (ε 18000).

7n. ^1H NMR (300 MHz, D_2O) δ 1.10 (3H, d, $J=7.0$ Hz), 1.26 (3H, d, $J=6.0$ Hz), 1.55 (4H, m), 1.83 (4H, m), 3.51 (1H, dd, $J=2.5$, 6.0 Hz), 3.73 (1H, m), 3.90–4.15 (4H, m), 4.24 (1H, m), 4.35 (1H, dd, $J=2.5$, 10.0 Hz); IR (KBr) cm^{-1} 1760, 1602; FAB-MS *m/z* 429 $(M+2Na\text{-}H)^+$; UV λ_{max} 282 (ε 16400).

7o. ^1H NMR (300 MHz, D_2O) δ 1.15, 1.17 (3H, each d, $J=7.3$ Hz), 1.33 (3H, d, $J=6.3$ Hz), 3.01–3.11 (1H, m), 3.55 (1H, d, $J=2.3$ Hz), 3.91–3.98 (2H, m), 4.09–4.15 (1H, m), 4.20–4.37 (2H, m), 4.38–4.45 (1H, m); IR (KBr) cm^{-1} 3430, 1749, 1608, 1506; FAB-HRMS *m/z* calcd for $C_{14}H_{19}N_2O_5S_2Na_2$ $(M+2Na\text{-}H)^+$: 405.0531, found 405.0523; UV λ_{max} 277 (ε 13,700).

7p. ^1H NMR (300 MHz, D_2O) δ 1.07, 1.09 (3H, each d, $J=5.9$ Hz), 1.22, 1.31 (3H, each d, $J=7.2$ Hz), 1.26 (3H, d, $J=6.4$ Hz), 3.51 (1H, m), 3.65–3.80 (1H, m), 3.85–4.18 (6H, m), 4.24 (1H, dq, $J=6.2$, 6.2 Hz), 4.31–4.39 (1H, m); IR (KBr) cm^{-1} 3347, 1753, 1604, 1386; FAB-MS *m/z* 419 $(M+2Na\text{-}H)^+$; UV λ_{max} 281 (ε 17100).

7q. ^1H NMR (300 MHz, D_2O) δ 1.10 (3H, d, $J=7.4$ Hz), 1.25 (3H, d, $J=6.3$ Hz), 3.52 (1H, dd, $J=2.9$, 5.7 Hz), 3.70 (1H, m), 4.00–4.30 (3H, m), 4.34 (1H, m), 4.40–4.80 (3H, m); IR (KBr) cm^{-1} 3400, 1755, 1604, 1489, 1390; FAB-MS *m/z* 381 $(M+Na)^+$; UV λ_{max} 279 (ε 15800).

7r. ^1H NMR (300 MHz, D_2O) δ 1.12 (3H, d, $J=7.0$ Hz), 1.29 (3H, d, $J=6.0$ Hz), 1.65 (2H, m), 2.02 (2H, m), 3.54 (1H, dd, $J=3.0$, 6.0 Hz), 3.65–3.90 (3H, m), 4.06 (1H, m), 4.26 (1H, m), 4.37 (1H, dd, $J=3.0$, 10.0 Hz); IR (KBr) cm^{-1} 1749, 1604, 1434, 1386; FAB-MS *m/z* 395 $(M+Na)^+$; UV λ_{max} 277 (ε 16100).

7s. ^1H NMR (300 MHz, D_2O) δ 1.08 (3H, d, $J=7.0$ Hz), 1.25 (3H, d, $J=6.5$ Hz), 1.62 (2H, m), 2.00 (2H, m), 3.50 (1H, dd, $J=2.5$, 5.5 Hz), 3.60–3.85 (4H, m), 4.03 (1H, m), 4.23 (1H, m), 4.35 (2H, m); IR (KBr) cm^{-1} 1749,1602, 1430, 1386; FAB-MS *m/z* 409 $(M+Na)^+$; UV λ_{max} 281 (ε 16400).

7t. ^1H NMR (300 MHz, D_2O) δ 1.08 (3H, d, $J=7.0$ Hz), 1.25 (3H, d, $J=6.5$ Hz), 1.55–2.30 (6H, m), 3.51 (1H, m), 3.71 (1H, m), 3.80–4.15 (4H, m), 4.23 (1H, m), 4.34 (1H, dd, $J=3.0$, 9.5 Hz); IR (KBr) cm^{-1} 3400, 1745, 1691, 1612, 1390; FAB-HRMS *m/z* calcd for $C_{17}H_{24}N_2O_5S_2Na$ $(M+Na)^+$: 423.1024, found 423.1018; UV λ_{max} 280 (ε 17700).

7v. ^1H NMR (300 MHz, D_2O) δ 1.12, 1.16 (3H, each d, $J=7.4$ Hz), 1.32 (3H, d, $J=6.3$ Hz), 3.56 (3H, s), 3.52–3.60 (1H, m), 3.67–3.80 (1H, m), 4.25–4.35 (1H, m), 4.41 (1H, dd, $J=3.0$, 9.7 Hz), 4.46–4.48 (2H, m); IR (KBr)

cm^{-1} 3391, 1751, 1680, 1605, 1390; FAB-HRMS *m/z* calcd for $C_{14}H_{19}N_3O_5S_2Na$ $(M+Na)^+$: 396.0664, found 396.0668; UV λ_{max} 280 (ε 8350).

7w. ^1H NMR (300 MHz, D_2O) δ 1.02–1.16 (3H, m), 1.26 (3H, d, *J* = 6.4 Hz), 3.48–3.58 (1H, m), 3.60–3.75 (1H, m), 4.19–4.30 (1H, m), 4.30–4.40 (1H, m), 4.42–4.73 (3H, m), 5.15–5.38 (2H, m), 5.75–5.98 (1H, m); IR (KBr) cm^{-1} 1749, 1683, 1604, 1394; FAB-MS *m/z* 422 $(M+Na)^+$; UV λ_{max} 281 (ε 14800).

7x. ^1H NMR (300 MHz, D_2O) δ 1.04 (3H, d, *J* = 7.3 Hz), 1.25 (3H, d, *J* = 6.4 Hz), 3.49–3.51 (1H, m), 3.64–3.73 (1H, m), 4.21–4.25 (1H, m), 4.34 (1H, d, *J* = 9.6 Hz), 4.53–4.64 (2H, m), 5.17 (2H, s), 7.32–7.45 (5H, m); IR (KBr) cm^{-1} 1764, 1751, 1681, 1608, 1392; FAB-MS *m/z* 494 $(M+2Na\text{-}H)^+$; UV λ_{max} 281 (ε 16600).

7y. ^1H NMR (300 MHz, D_2O) δ 1.09 (3H, d, *J* = 7.3 Hz), 1.26 (3H, d, *J* = 6.4 Hz), 3.42–3.58 (3H, m), 3.60–3.78 (1H, m), 4.15–4.32 (2H, m), 4.34–4.42 (1H, m); IR (KBr) cm^{-1} 1751, 1670, 1394; FAB-HRMS *m/z* calcd for $C_{15}H_{19}N_3O_5S_2Na$ $(M+Na)^+$: 375.0425, found 375.0430; UV λ_{max} 279 (ε 15300).

7z-1. ^1H NMR (300 MHz, D_2O) δ 1.12 (3H, d, *J* = 7.3 Hz), 1.29 (3H, d, *J* = 6.4 Hz), 2.32 (3H, s), 2.64 (4H, m), 3.55 (1H, dd, *J* = 3.0, 5.9 Hz), 3.69 (1H, m), 3.90–4.50 (6H, m); IR (KBr) cm^{-1} 1757, 1604, 1389, 1288; FAB-MS *m/z* 408 $(M+Na)^+$; UV λ_{max} 281 (ε 16100).

7z-4. ^1H NMR (300 MHz, D_20) δ 1.17 (3H, d, *J* = 7.2 Hz), 1.34 (3H, d, *J* = 6.5 Hz), 2.84 (3H, s), 3.61 (1H, dd, *J* = 2.8, 5.8 Hz), 3.68–3.85 (5H, m), 4.21 (2H, s), 4.20–4.42 (4H, m), 4.33 (1H, quint, *J* = 6.1 Hz), 4.44 (1H, dd, *J* = 2.8, 9.8 Hz), 4.66–4.72 (2H, m), 5.73–5.81 (1H, m); IR (KBr) cm^{-1} 1760, 1660, 1600, 1380, 1220; FAB-HRMS *m/z* calcd for $C_{18}H_{27}N_4O_5S_2$ $(M+H)^+$: 443.1423, found 443.1434; UV λ_{max} 281 (ε 19400).

8a. ^1H NMR (300 MHz, D_2O) δ 1.25 (3H, d, *J* = 6.5 Hz), 1.95–2.10 (4H, m), 2.99 (1H, dd, *J* = 9.5, 17.0 Hz), 3.45–3.52 (2H, m), 3.65–3.85 (4H,m), 4.15–4.35 (2H, m); IR (KBr) cm^{-1} 1764, 1600, 1430; FAB-MS *m/z* $(M+Na)^+$ 365; UV λ_{max} 275 (ε 12100).

8b. ^1H NMR (300 MHz, D_2O) δ 1.24 (3H, d, *J* = 6.5 Hz), 2.97 (1H, dd, *J* = 9.0, 15.0 Hz), 3.25–3.50 (8H, m), 4.15–4.35 (2H, m); IR (KBr) cm^{-1} 1801, 1600, 1373; FAB-MS *m/z* $(M+Na)^+$ 339; UV λ_{max} 275 (ε 12100).

Sodium (1*R*,5*S*,6*S*)-2-[dimethylaminocarbonylthio]-6-[(*R*)-1-hydroxyethyl]-methyl-1-carbapen-2-em-3-carboxylate (11b). (1) To a stirred solution of **3** (590 mg, 0.99 mmol) in DMF (5.9 mL) were added sodium hydrosulfide-hydrate (67.0 mg, 1.20 mmol) in DMF (2.0 mL) and diisopropylethylamine (0.41 mL, mmol) at −40 °C under a nitrogen atmosphere. After the mixture was stirred for 20 min at the same temperature, dimethylcarbamyl chloride (0.22 mL, 2.40 mmol) was added, and the resulting mixture was further stirred overnight at 4 °C. The reaction mixture was concentrated in vacuo and the residue was purified by silica gel column chromatography to yield **10b** (180 mg, 40%): ^1H NMR (300 MHz, $CDCl_3$) δ 1.17 (3H, d, *J* = 7.4 Hz), 1.34 (3H, d, *J* = 6.3 Hz), 3.01 (3H, brs), 3.08 (3H, brs), 3.37 (1H, dd, *J* = 3.0, 6.3 Hz), 4.05–4.13 (1H, m), 4.28 (1H, m), 4.37 (1H, d, *J* = 3.5, 10.3 Hz), 5.25 (1H, *J* = 13.5 Hz), 5.49 (1H, d, *J* = 13.5 Hz), 7.65 (2H, d, *J* = 8.9 Hz), 8.22 (2H, d, *J* = 8.9 Hz); IR (KBr) cm^{-1} 1772, 1668, 1522, 1348; FAB-MS *m/z* $(M+H)^+$ 449.

(2) To a solution of **10b** (170 mg, 0.38 mmol) in THF (5.1 mL) and EtOH (0.85 mL) were added $NaHCO_3$ (32 mg, 0.38 mmol) in H_2O (5.1 mL) and 10% Pd-C (50 mg), and the mixture was stirred for 4.5 h at room temperature under a hydrogen atmosphere. The reaction mixture was passed through a pad of celite and the filtrate was concentrated in vacuo to ca. 5 mL. After the insoluble of the aqueous layer was removed by filtration, the filtrate was subjected to reversed-phase column chromatography, which was eluted with H_2O and then 10% CH_2CN–H_2O. The fractions detected by HPLC were combined and lyophilized to give **11b** (40 mg, 31%): ^1H NMR (300 MHz, D_2O) δ 1.11 (3H, d, *J* = 7.0 Hz), 1.28 (3H, d, *J* = 6.0 Hz), 1.90–2.20 (4H, m), 3.53 (1H, dd, *J* = 3.0, 6.0 Hz), 3.60–3.90 (5H, m), 4.25 (1H, m), 4.36 (1H, dd, *J* = 3.0, 10.0 Hz); IR (KBr) cm^{-1} 1749, 1608, 1437, 1389; FAB-HRMS *m/z* calcd for $C_{13}H_{17}N_2O_5SNa_2$ $(M+2Na\text{-}H)^+$: 359.0654, found 359.0666; UV λ_{max} 286 (ε 8260).

The compound **11a** was prepared as described for the preparation of **11b**.

11a. ^{1} NMR (300 MHz, D_2O) δ 1.12 (3H, d, *J* = 7.0 Hz), 1.28 (3H, d, *J* = 6.0 Hz), 1.90 (4H, m), 3.30–3.70 (6H, m), 4.25 (2H, m); IR (KBr) cm^{-1} 3400, 1790, 1640, 1610, 1370; FAB-HRMS *m/z* calcd for $C_{15}H_{20}N_2O_5SNa$ $(M+Na)^+$: 363.0991, found 363.0973; UV λ_{max} 287 (ε 6480).

Sodium (1*R*,5*S*,6*S*)-2-(*N*-allyl-*N*-methylaminothiocarbonylthio)-6-[(*R*)-1-hydroxyethyl]-1-methyl-1-carbapen-2-em-3-carboxylate (7e). To a solution of **5e** (400 mg, 0.81 mmol) in THF (8.0 mL) and 0.35 M phosphate buffer (pH 6.0, 15 mL) was added zinc dust (1.2 g, mmol), and the mixture was stirred vigorously for 2 h. The reaction mixture was passed through a pad of celite and the filtrate was concentrated in vacuo to ca. 20 mL.

After the insoluble of the aqueous layer was removed by filtration, the filtrate was subjected to reversed phase column chromatography, which was eluted with 3% CH_3CN–H_2O. The fractions detected by HPLC were combined and lyophilized to give **7e** (132 mg, 42%): ^{1}H NMR (300 MHz, D_2O) δ 1.13, 1.15 (3H, each d, J = 6.9 Hz), 1.32 (3H, d, J = 6.3 Hz), 3.48, 3.50 (3H, each s), 3.52–3.60 (1H, m), 3.69–3.83 (1H, m), 4.24–4.35 (1H, m), 4.36–4.44 (1H, m), 4.52–4.65, 4.72–4.78 (2H, each m), 5.17–5.40 (2H, m), 5.80–6.01 (1H, m); IR (KBr) cm^{-1} 3431, 1759, 1606, 1367, 1267; FAB-MS m/z 401 $(M+2Na\text{-}H)^+$; UV λ_{max} 279 (ε 16600).

The following compounds (**7m**, **7u-1**) were prepared as described for the preparation of **7e**.

7m. ^{1}H NMR (300 MHz, D_2O) δ 1.18 (3H, d, J = 6.9 Hz), 1.35–1.37 (3H, m), 2.35–2.50 (2H, 3.59–3.62 (1H, m), 3.77 (1H, dq, J = 6.8, 7.3 Hz), 4.18–4.24, 4.60–4.7 (3H, each m), 4.28–4.37 (1H, m), 5.74–5.90 (1H, m), 6.03–6.13 (1H, m); IR (KBr) cm^{-1} 3396, 1753,1386; FAB-MS m/z 413 $(M+2Na\text{-}H)^+$; UV λ_{max} 281 (ε 18100).

7u-1. ^{1}H NMR (300 MHz, D_2O) δ 1.15–1.21 (3H, m), 1.35 (3H, d, J = 6.4 Hz), 2.32–2.46 (2H, m), 3.61 (1H, dd, J = 3.0, 5.9 Hz), 3.76 (1H, dq, J = 7.6, 9.8 Hz), 4.10–4.23 (4H, m), 4.28–4.33 (1H, quint, J = 6.1 Hz), 4.43 (1H, dd, J = 2.9, 9.6 Hz), 4.66–4.72 (2H, m), 5.72–5.82 (1H, m); IR (KBr) cm^{-1} 1754,1604, 1388; FAB-MS m/z 443 $(M+2Na\text{-}H)^+$; UV λ_{max} 280 (ε 17700).

(1*R*,5*S*,6*S*)-6-[(*R*)-1-Hydroxyethyl]-1-methyl-2-[4-(pyridinium)methyl-1,2,3,6-tetrahydropyridin-1-yl]thiocarbonylthio]-1-carbapen-2-em-3-carboxylate (7u-3). (1) To a solution of **5u-1** (5.90 g, 11.1 mmol) in TUF (47 mL) were added diisopropylethylamine (7.51 mL, 14.4 mmol) and *n*-propanesulfonylchloride (1.37 mL, 12.2 mmol), and the mixture was stirred for 2 h at 0 °C. The reaction mixture was poured into H_2O and extracted with EtOAc. The organic layer was washed with 0.1 N HCl, 5% aqueous $NAHCO_3$, and brine, dried over $MgSO_2$, and concentrated in vacuo. To a solution of the residue in acetone (30 mL) was added sodium iodide (4.99 g, 3.33 mmol) at 0 °C, and the mixture was stirred for 2 h at room temperature. The reaction mixture was poured into H_2O and extracted with EtOAc. The organic layer was washed with 5% aqueous $Na_2S_2O_3$, and brine, dried over $MgSO_4$, and concentrated in vacuo to give **12** (6.33 g, 89%): ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.15 (3H, d, J = 7.3 Hz), 1.36 (3H, d, J = 6.3 Hz), 2.38–2.53 (2H, m), 3.37 (1H, m), 3.92 (2H, s), 3.95–4.35 (3H, m), 4.42–4.65 (2H, m), 5.24 (1H, d, J = 13.8 Hz), 5.48 (1H, d, J = 13.8 Hz), 7.63 (2H, d, J = 8.2 Hz), 8.21 (2H, d, J = 8.2 Hz); IR (KBr) cm^{-1} 1771,1522, 1435.

(2) To a solution of **12** (700 mg, 1.08 mmol) in CH_3CN (26 mL) was added pyridine (90 mg, 1.14 mmol), and the mixture was stirred overnight at room temperature. The reaction mixture was concentrated in vacuo, and the residue was deprotected by a similar method for the preparation of **7e** to give **7u-3** (17%): ^{1}H NMR (300 MHz, D_2O) δ 1.07 (3H, d, J = 7.3 Hz), 1.26 (3H, d, J = 6.4 Hz), 2.16–2.31 (2H, m), 3.51 (1H, dd, J = 3.1, 6.0 Hz), 3.60–3.75 (1H, m), 4.00–4.20 (2H, m), 4.24 (1H, quint, J = 6.3 Hz), 4.33 (1H, dd, J = 2.5, 9.5 Hz), 4.39–4.71 (2H, m), 5.24 (2H, s), 5.88–5.98 (1H, m), 8.08 (2H, dd, J = 6.6, 8.8 Hz), 8.57 (1H, t, J = 7.8 Hz), 8.83 (2H, d, J = 5.5 Hz); IR (KBr) cm^{-1} 1756,1604, 1425, 1380; FAB-MS m/z 460 $(M+H)^+$; UV λ_{max} 280 (ε 17300).

The following compounds (**7u-2**, **7u-4**) were prepared as described for the preparation of **7u-3**.

7u-2. ^{1}H NMR (300 MHz, D_2O) δ 1.17 (3H, d, J = 7.2 Hz), 1.34 (3H, d, J = 6.3 Hz), 2.52–2.82 (2H, m), 3.17 (9H, s), 3.61 (1H, dd, J = 3.0, 5.9 Hz), 3.71–3.83 (1H, m), 4.04 (2H, s), 4.10–4.48 (4H, m), 4.61–4.99 (2H, m), 6.21–6.32 (1H, m); IR (KBr) cm^{-1} 1756,1604, 1419, 1384; FAB-MS m/z 440 $(M+H)^+$; UV λ_{max} 279 (ε 15100).

7u-4. ^{1}H NMR (300 MHz, D_2O) δ 1.14 (3H, d, J = 7.2 Hz), 1.31 (3H, d, J = 6.0 Hz), 2.52–2.75 (2H, m), 3.21 (3H, s), 3.42–3.50 (2H, m), 3.53–3.65 (3H, m), 3.71 (1H, dd, J = 7.8, 9.8 Hz), 4.02–4.20 (9H, m), 4.30 (1 H, quint, J = 3.0 Hz), 4.41 (1H, dd, J = 3.0, 9.9 Hz), 6.23–6.29 (1H, m); IR (KBr) cm^{-1} 1749, 1271; FAB-MS m/z 482 $(M+H)^+$; UV λ_{max} 274 (ε 19400).

(1*R*,5*S*,6*S*)-2-[(4,4-Dimethylpiperadinio)thiocarbonylthio]-6-[(*R*)-1-hydroxyethyl]-1-methyl-1-carbapen-2-em-3-carboxylate (7z-2). To an ice-cooled solution of **5z-1** (500 mg, 0.96 mmol) in acetone (10 mL) and THF (10 mL) was added iodomethane (0.3 mL, 4.8 mmol) and the mixture was stirred for 2 h at the same temperature. After being stirred for additional 2 h at room temperature, the reaction mixture was concentrated in vacuo. To a solution of the residue in THF (25 mL) and EtOH (5.0 mL) were added $NaHCO_3$ (80 mg, 0.95 mmol) in H_2O (25 mL) and 10% Pd-C (500 mg), and the mixture was stirred overnight at room temperature under a hydrogen atmosphere. The reaction mixture was passed through a pad of celite and the filtrate was concentrated in vacua to ca. 20 mL. After the insoluble of the aqueous layer was removed by filtration, the filtrate was subjected to reversed-phase column chromatography, which was eluted with H_2O and then 10% MeOH–H_2O. The fractions detected by HPLC were combined and lyophilized to give **7z-2** (48 mg, 13%): ^{1}H NMR (300 MHz, D_2O) δ 1.09 (3H, d, J = 7.3 Hz), 1.27 (3H, d, J = 6.8 Hz), 3.28 (6H, s), 3.50–3.70 (7H, m), 4.25 (1H, m), 4.38 (1H,

dd, $J=3.0$, 10.0 Hz), 4.50 (3H, brs); IR (KBr) cm^{-1} 1749, 1603, 1387; FAB-MS m/z 400 $(M+H)^+$; UV λ_{max} 280 (ε 13500).

The compound **7z-3** was prepared as described for the preparation of **7z-2**.

7z-3. ^1H NMR (300 MHz, D_2O) δ 1.08 (3H, d, $J=7.3$ Hz), 1.26 (3H, d, $J=6.3$ Hz), 3.15 (3H, s), 3.46–3.80 (6H, m), 4.08–4.42 (4H, m), 7.45–7.62 (5H, m); IR (KBr) cm^{-1} 1766, 1456; FAB-MS m/z 476 $(M+H)^+$; UV λ_{max} 280 (ε 15200).

Determination of MIC. MIC was determined by agar dilution method using Mueller–Hinton medium (Difco). The bacterial suspension was inoculated at ca. 10^4 cells per spot on the agar plates. The MIC was defined as the lowest concentration which inhibited visible growth after incubation at 37 °C for 18 h. In the case of methicillin-resistant staphylococci (MRS), 2% NaCl was supplemented in the medium, and inoculated agar plates were incubated at 35 °C for 48 h. MRS strains used in this study were isolated recently at different hospitals in Japan.

Susceptibility to renal dehydropeptidase-I (DHP-I). The relative hydrolysis rate was determined by using partially purified porcine DHP-I, taking the hydrolysis rate of imipenem as 1.0. The hydrolysis was monitored spectrophotometrically under the condition of 50 μM of substrate in 50 mM MOPS buffer at pH 7.0 and 35 °C.

Affinity to PBP-2′. The PBP-2′ affinity was determined by the competition assay with [^{14}C]benzylpenicillin using membrane isolated from MRSA BB6294 strain. The membrane fraction was incubated with carbapenems at 30 °C for 10 min, and further incubated radio-labeled benzylpenicillin for 10 min. Binding affinity was expressed as the inhibitory concentration (IC_{50}) for [^{14}C]benzylpenicillin binding by 50%, which was determined by Bio-Imaging Analyzer (BAS2000, Fuji Photo Film Co., Ltd., Tokyo, Japan) after exposure of dried gel film to the imaging plate.

Systemic infection. ICR mice (4 week old, male) were infected intraperitoneally with homotypic MRSA BB6221 suspended in 5% gastric mucin. Each agent was administered subcutaneously at 1 h after infections in combination with cilastatin at a dose of 40 mg/kg. The ED_{50} values were calculated by Probit method.

Determination of antibiotic levels in mouse plasma and urine. Groups of three mice each were injected subcutaneously with 10 mg of each carbapenem per kg of body weight in combination with cilastatin at a dose of 40 mg/kg. The levels of carbapenems were determined by biological assay with a paper disk method using Bacillus subtilis ATCC 12432 as the indicator organism. The inoculated agar plates (antibiotic medium No. 1; Difco) were incubated at 37 °C for 16 h. The contents of the disk were calculated from a standard curve.

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