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Dicationic Dithiocarbamate Carbapenems with Anti-MRSA Activity

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Abstract—A new class of 1β-methylcarbapenems bearing a doubly quaternarized 1,4-diazabicyclooctane (DABCO) substituted dithiocarbamate moiety at the C-2 side chain was prepared, and the biological profiles of the compounds, including in vitro and in vivo anti-MRSA activity and DHP-I susceptibility, were evaluated to identify a carbapenem derivative that was superior to BO-3482 (**1**). As a result, we discovered a 1β-methyl-2-[4-(4-carbamoylmethyl-1,4-diazabicyclo[2,2,2]octanediium-1-yl)methyl-1,2,3,6-tetrahydropyridinylthiocarbonylthio]carbapenem, **14a** showing greater than 2-fold better anti-MRSA activity in a mouse infection model and 3-fold better DHP-I susceptibility as compared with BO-3482 (**1**).

Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA) has been recognized as one of the major pathogens causing nosocomial infections. In spite of severe side effects, vancomycin,[1] a glycopeptide antibiotic, has been used as a first-line therapeutic agent for infections caused by MRSA. Alternative therapeutic agents with fewer side effects have been clinically desired. During the past decade, extensive synthetic efforts have been made to confer anti-MRSA activity on β-lactams such as a cephalosporin or a carbapenem. As a result, some cephalosporin[2] and carbapenem[3] derivatives with potent in vitro anti-MRSA activity were identified by introducing hydrophobic functional groups into the C-3 or C-7 side chain of the cephalosporin nucleus or the C-2 side chain of the carbapenem nucleus. However, clinical application of these anti-MRSA β-lactams has not been achieved because they may have unfavorable biological and physicochemical properties due to their increased hydrophobicity.

In a previous paper,[4] we reported that dithiocarbamate carbapenems showed potent in vitro and in vivo anti-MRSA activity. Ultimately, we selected BO-3482 (**1**) for further evaluation based on its balanced in vitro and in vivo anti-MRSA activity together with its low seizure potential. However, the in vivo anti-MRSA activity and susceptibility of BO-3482 (**1**) to renal dehydropeptidase-I (DHP-I) remain to be improved.

Derivatization of dithiocarbamate carbapenems by introducing hydrophobic side chains into the C-2 position resulted in excellent in vitro anti-MRSA activity, whereas in vivo anti-MRSA activity was unexpectedly reduced, probably due to high serum protein binding rates. These hydrophobic dithiocarbamate carbapenems showed higher susceptibility to DHP-I than did imipenem. Introduction of a quaternary ammonium moiety into the C-2 side chain is one way to reduce the protein binding rate and to improve DHP-I susceptibility. In fact, quaternarization of amine moieties of some dithiocarbamate carbapenems resulted in decreasing the protein binding rates to some extent and improving DHP-I susceptibility. Unfortunately, these quaternarized carbapenems displayed significant epileptogenicity in an intracerebroventricular rat head assay.

Recently, Merck scientists discovered two novel anti-MRSA carbapenems, L-742,728[5] and L-786,392,[6] which showed excellent in vitro and in vivo anti-MRSA activity together with good DHP-I stability. In particular,

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L-786,392 possessed extended antibacterial activity against vancomycin-resistant *Enterococci* (Fig. 1). We speculated that these carbapenems with highly hydrophobic C-2 side chains, such as a fluorenone and naphtylsultam group, became sufficiently hydrophilic after the introduction of a doubly quaternarized 1,4-diazabicyclooctane (DABCO) moiety, resulting in excellent in vivo anti-MRSA activity. On the based of this speculation, we tried to introduce a DABCO group into the side chains of representative dithiocarbamate carbapenems (**2**, **3** and **4**, see Fig. 2) to improve in vivo anti-MRSA activity. In this paper, we describe the synthesis of dithiocarbamate carbapenems with a DABCO moiety and their biological properties, including in vitro and in vivo anti-MRSA activity and DHP-I susceptibility.

Result and Discussion

Chemistry

Preparation of **10a** was outlined in Scheme 1 as a general procedure for dicationic dithiocarbamate carbapenems (Table 1). The benzyl alcohol moiety of a carbapenem intermediate **7**, derived from **5**,[7] was selectively activated with *n*-propanesulfonyl chloride and subsequently treated with sodium iodide to give an iodide **8**. Iodide **8** was substituted with *N*-substituted DABCO triflate salt **9a**[5a] to afford a protected doubly quaternarized carbapenem, which was deprotected by catalytic hydrogenation in a mixture of THF and sodium 3-morpholinopropanesulfonate buffer (MOPS, pH 7.0) at room temperature, giving the crude carbapenem. Purification and ion-exchange of the crude material was

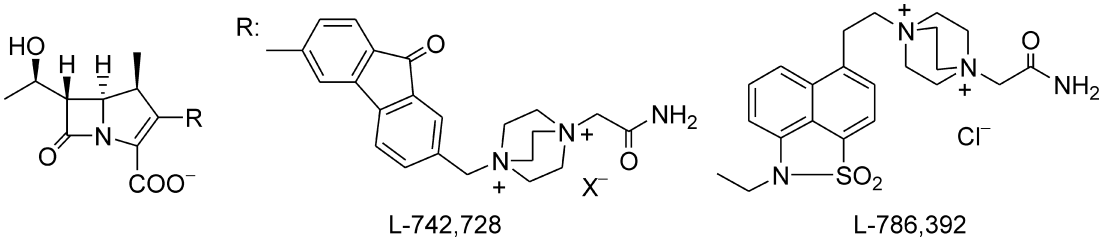


Figure 1. Chemical structure of Merck compounds.

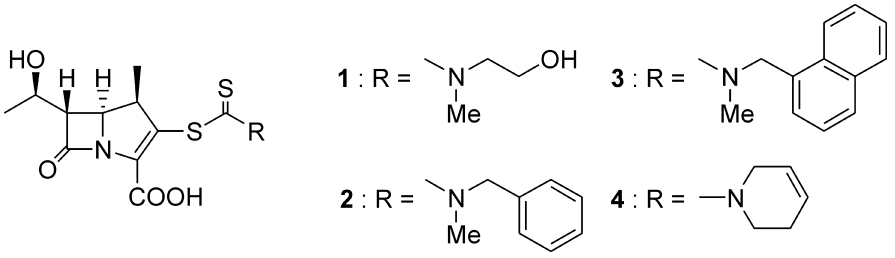
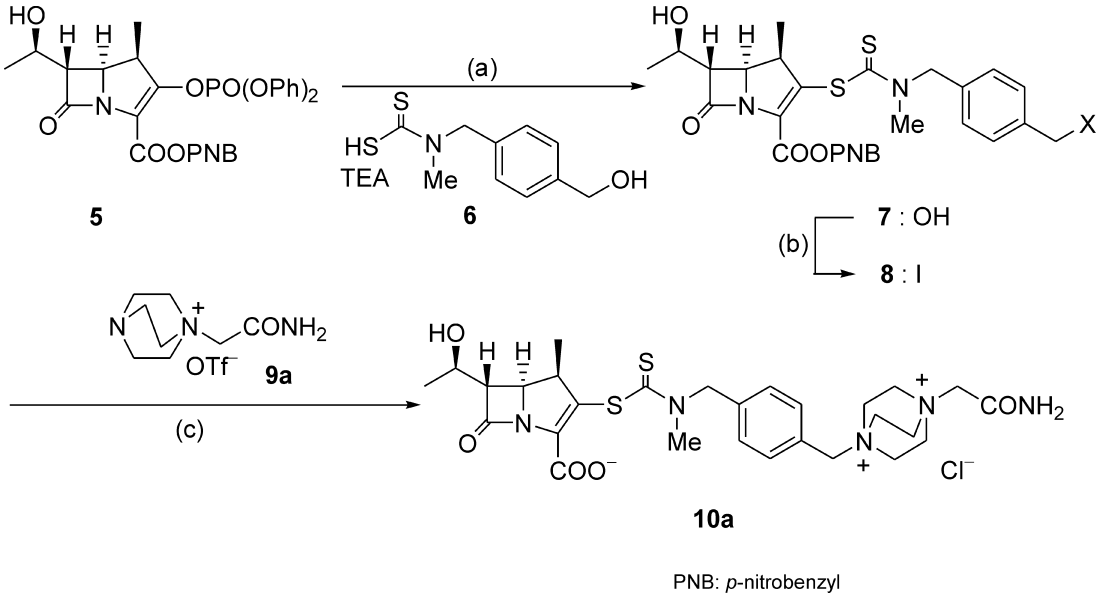
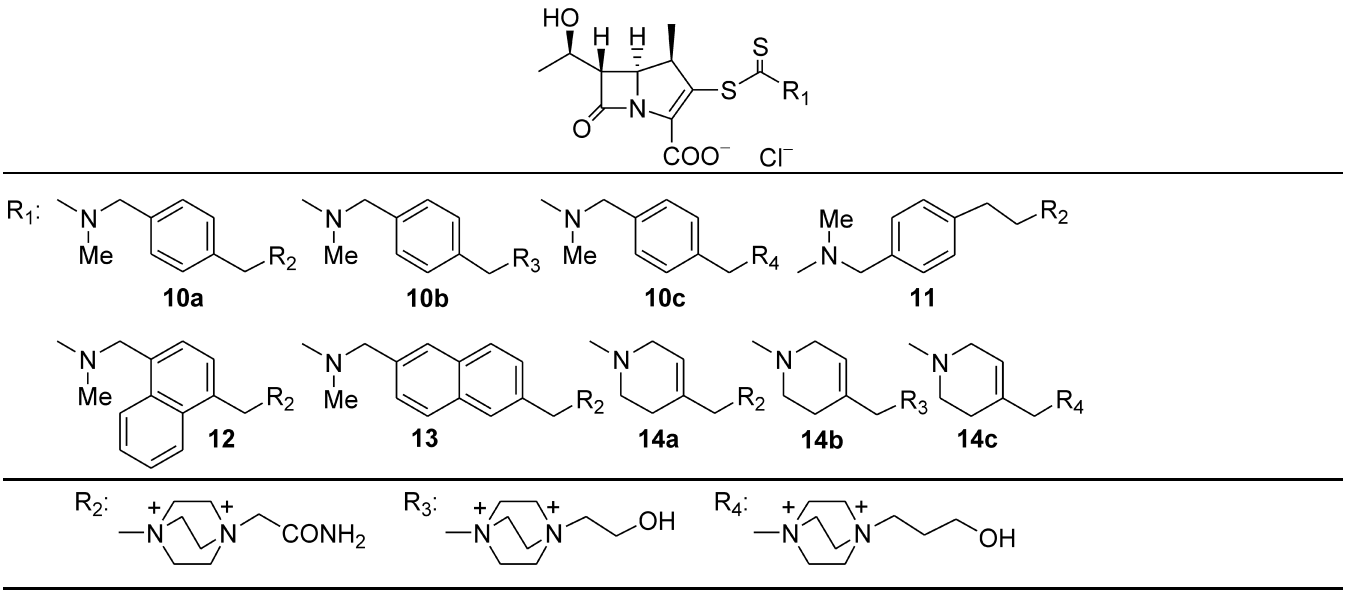


Figure 2. Dithiocarbamate carbapenems.



Scheme 1. Preparation of the dithiocarbamate carbapenem **10a**. Reagents: (a) *i*-Pr_2NEt, LiCl, THF, rt, (b) (i) 1-propanesulfonyl chloride, *i*-Pr_2NEt, THF, 0°C; (ii) Nal, acetone, 0°C, (c) (i) CH_3CN, rt; (ii) 10% Pd/C, H_2, THF–H_2O, rt.

Table 1. Dicationic dithiocarbamate carbapenems



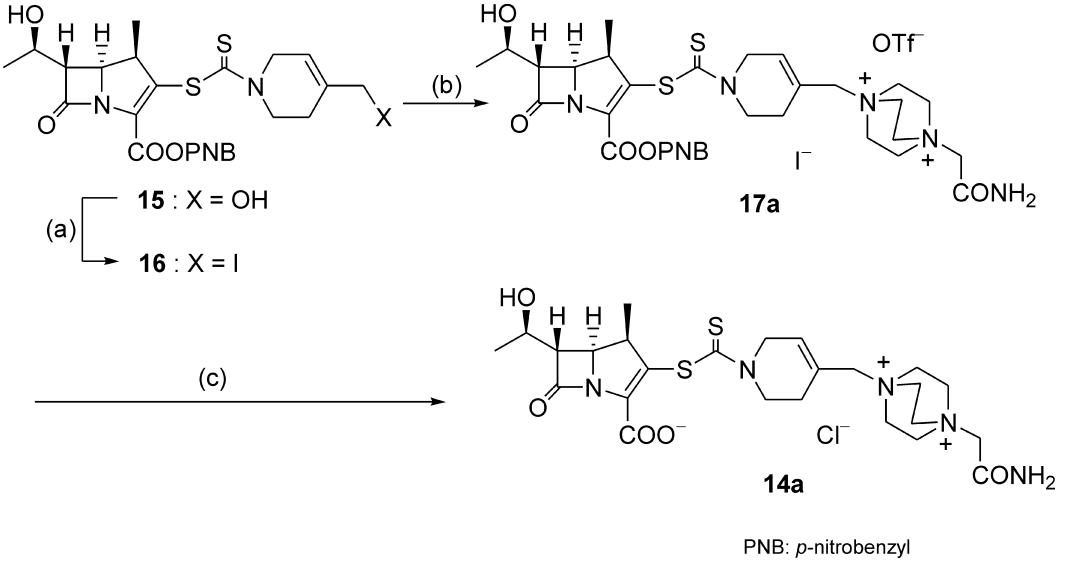
concurrently performed by reversed-phase column chromatography to produce **10a** as an amorphous powder after lyophilization.

A 100-g scale preparation of the selected compound **14a** is highlighted in Scheme 2. Protected doubly quaternarized carbapenem **17a** was prepared by reacting an iodide **16** with **9a** in 98% yield. Deprotection of **17a** was carried out by the Zinc reduction method[8] to give the crude carbapenem, which was purified, ion-exchanged by reversed-phase column chromatography, and crystallized from 80% EtOH–H_2O to produce **14a** as colorless prisms.

Biological properties

Table 2 shows the in vitro antibacterial activity and other biological properties, such as DHP-I susceptibility, human serum protein binding rates and epileptogenic potential, of the dicationic dithiocarbamate carbapenems (**10a–c**, **11**, **12**, **13** and **14a–c**) and the original compounds (**2**, **3** and **4**). Both imipenem (IPM) and vancomycin (VCM) were used as reference drugs.

First, we looked at the effects of the DABCO moiety in the dicationic derivatives (**10a**, **11**, **12**, **13**, and **14a**) on antibacterial activity against MRSA and methicillin-resistant *Staphylococcus epidermidis* (MRSE). Generally, these derivatives showed potent in vitro anti-MRSA activity and high affinity to PBP (penicillin-binding protein)-2′, similar to the effects of the original compounds (**2**, **3** and **4**). It should be noted that a DABCO moiety was effective in enhancing antibacterial activity against the MRSE strain in the case of the phenyl (**10a**) and 1,2,3,6-tetrahydropyridyl (**14a**) derivatives.



Scheme 2. Preparation of the dithiocarbamate carbapenem **14a**. Reagents: (a) (i) 1-propanesulfonyl chloride, *i*-Pr_2NEt, THF, 0°C; (ii) NaI, acetone, 0°C; (b) **9a**, CH_3CN, rt, (c) Zu dust, THF, phosphate buffer (pH 6.5), rt.

Table 2. In vitro activity[a] and biological properties of dithiocarbamate carbapenems

Organism	**10a**	**10b**	**10c**	**11**	**2**	**IPM**	**VCM**
S. aureus 209P NIHJ JC1	≤0.006	≤0.006	0.012	≤0.006	0.012	≤0.006	0.39
S. aureus BB6294[b]	1.56	3.13	6.25	3.13	1.56	100	1.56
S. aureus CSa1009[b]	1.56	3.13	6.25	6.25	1.56	100	1.56
S. epidermidis MB5181[b]	0.78	0.78	3.13	0.39	3.13	100	1.56
E. faecalis MB4966	3.13	3.13	6.25	1.56	1.56	0.78	1.56
P. aeruginosa MB5002	>25	>25	>25	12.5	>25	1.56	>25
DHP-I susceptibility[c]	1.76	1.67	1.71	2.55	1.94	1.00	—
PBP-2′ affinity (IC_{50}, μg/mL)	2.3	1.7	12	2.1	1.8	>25	—
Serum protein-binding (%)[d]	85	81	56	70	99	<5.0	41
Epileptogenicity (200 μg/rat head, $n=5$)	1/5	2/5	0/5	0/5	0/5	5/5	—
	12	**13**	**3**	**14a**	**14b**	**14c**	**4**
S. aureus 209P NIHJ JC1	≤0.006	≤.006	≤0.006	≤0.006	≤0.006	≤0.006	0.025
S. aureus BB6294[b]	1.56	1.56	0.78	3.13	3.13	3.13	1.56
S. aureus CSa1009[b]	3.13	1.56	1.56	3.13	6.25	6.25	3.13
S. epidermidis MB5181[b]	0.39	0.78	0.39	0.78	0.78	0.78	1.56
E. faecalis MB4966	1.56	0.78	1.56	3.13	3.13	3.13	6.25
P. aeruginosa MB5002	>25	>25	>25	>25	>25	>25	>25
DHP-I susceptibility[c]	2.37	2.45	4.41	0.51	0.57	0.46	2.63
PBP-2′ affinity (IC_{50}, μg/mL)	1.9	1.6	—	1.1	2.7	—	6.2
Serum protein-binding (%)[d]	90	93	99	55	—	59	98
Epileptogenicity (200 μg/rat head, $n=5$)	0/5	0/5	0/5	0/5	0/5	0/5	0/5

[a]MIC (μg/ml) determined by agar dilution method.
[b]Methicillin-resistant.
[c]Relative rate of hydrolysis to imipenem, porcine renal DNP-I.
[d]Binding rate for human serum.

Since the monocationic derivatives exhibited significant epileptogenicity,[4c] we were concerned that this class of dicationic dithiocarbamate carbapenems could also have epileptogenic potential. Fortunately, these dicationic derivatives showed far less epileptogenicity than did the monocationic derivatives in the rat head assay, suggesting that the dicationic DABCO moiety may not induce seizures as does the monocationic ammonium group.

Next, the effect of the DABCO moiety on the serum protein binding rate was examined. As expected, the DABCO moiety effectively contributed to reduce serum protein binding rates in the phenyl and 1,2,3,6-tetrahydropyridyl derivatives (**11** = 70%, **14a** = 55%). However, the naphtyl derivatives (**12** and **13**) still showed high protein binding rates (**12** = 90%, **13** = 93%) in spite of the introduction of the DABCO moiety.

With respect to DHP-I susceptibility, the 1,2,3,6-tetrahydropyridyl derivative (**14a**) showed approximately 5-fold improvement as compared with the original compound **4**. The other dicationic derivatives (**10a**, **11**, **12** and **13**) displayed DHP-I susceptibility comparable to that of the original compounds (**2** and **3**).

To examine the effects of the DABCO moiety on in vivo anti-MRSA activity, we evaluated the in vivo anti-MRSA activity of representative compounds (**10a**, **13** and **14a**) in a mouse systemic infection model by using homotypic MRSA BB6221 and compared our findings with the activity of the original compounds. In this infection model, the original hydrophobic dithiocarbamate carbapenems (**2**, **3** and **4**) showed ED_{50} values of >50, >50 and 7.5 mg/kg, respectively. By contrast, the corresponding dicationic derivatives (**10a**, **13** and **14a**) and vancomycin showed ED_{50} values of 6.0, 7.7, 1.9 and 5.5 mg/kg, respectively, suggesting that the DABCO moiety may play a key role in improving in vivo anti-MRSA activity. We assumed that the good in vivo anti-MRSA activity was related to the significant decrease in protein binding rates. Since the protein binding rate (99%) of **2**, for example, was improved up to 85% in the case of **10a**, the plasma concentration of the free carbapenem of **10a** was approximately 15-fold higher than that of **2**, so that **10a** showed approximately 10-fold improvement in in vivo efficacy in comparison with **2**.

Optimization of the carbamoylmethyl group on the quaternarized DABCO moiety of **10a** and **14a** was carried out by replacement with a hydroxyethyl (**10b** and **10c**) or hydroxypropyl group (**14b** and **14c**). In the phenyl derivatives (**10b** and **10c**), in vitro anti-MRSA activity was decreased compared with that of **10a**, while the 1,2,3,6-tetrahydropyridyl derivatives (**14b** and **14c**) retained the in vitro anti-MRSA activity to some extent (Table 2). Taken together, it was concluded that **14a** with a [4-(4-carbamoylmethyl-1,4-diazabicyclo[2,2,2]octanediium-1-yl)methyl-1,2,3,6-tetrahydropyridinyl-thiocarbonylthio] moiety seemed optimal in this class.

Table 3 shows the biological properties of the dicationic derivative **14a**, its original compound **4**, and BO-3482 (**1**). It is obvious that **14a** was superior to the original compound **4** in all respects. In addition, **14a** showed considerable improvement in vivo anti-MRSA activity and DHP-I susceptibility as compared with **1**. **14a** also possessed an excellent safety profile in a 48-h rabbit nephrotoxicity study (225 mg/kg), similar to that of **1**.

Table 3. Anti-MRSA activities and other biological properties of **14a**, **4** and **1**

Compound	**14a**	**4**	**1**
In vitro anti-MRSA activity[a] G-mean MIC (μg/mL)	5.36	2.02	4.36
In vivo anti-MRSA activity[b] ED_{50} (mg/kg)	1.86	7.50	4.80
Affinity to PBP-2′[c] IC_{50} (μg/mL)	1.1	6.2	3.8
DHP-I susceptibility[d]	0.51	2.63	1.65
Serum protein-binding (%)[e]	55	98	47
Epileptogenicity (200 μg/rat head, $n=5$)	0/5	0/5	0/5
48-Hour nephrotoxicity (225 mg/kg, single iv, $n=3$)	No change	NT[f]	No change

[a]High level MRSA (27 strains); geometric-mean MIC methicilin, 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]Relative rate of hydrolysis to imipenem, porcine renal DHP-I.
[e]Binding rate for human serum at a carbapenem concentration of 10 μg/mL.
[f]Not tested.

In summary, we prepared a new class of dicationic dithiocarbamate carbapenems in pursuit of a carbapenem derivative with improved in in vivo anti-MRSA activity and DHP-I susceptibility. As a result, **14a** was identified as having excellent in vivo anti-MRSA activity, favorable DHP-I susceptibility, and a good safety profile. Further biological properties of **14a** such as pharmacokinetic parameters will be reported in the near future.

Experimental

Determination of MIC

MICs were determined by a 2-fold serial agar dilution method with Mueller–Hinton medium (Difco Laboratories, Detroit, MI). An overnight culture was diluted to give a final concentration of approximately 10^6CFU/mL. A portion of the dilution was delivered onto a drug-containing agar surface with an inoculum apparatus (Microplanter: Sakuma Seisakusho Co., Ltd., Tokyo, Japan). The final inoculum size was approximately 10^4CFU per spot. The MIC was defined as the lowest concentration of antibiotics that completely prevented visible growth after incubation at 37 °C for 20 h.

Determination of susceptibility to renal dehydropeptidase-1 (DHP-I)

The relative hydrolysis rate of carbapenems by porcine renal DHP-I was determined, taking the initial hydrolysis rate of imipenem as 1.0. Partially purified porcine DHP-I (final concentration, 0.3 U/mL) was incubated with 50 μM carbapenem at 35 °C in 50 mM MOPS buffer, pH 7.0. The initial hydrolysis rate was monitored by the spectrophotometric method. One unit of activity was defined as the amount of enzyme hydrolyzing 1 μM of glycyldehydrophenylalanine per min when the substrate, 50 μM, was incubated at 35 °C in 50 mM MOPS buffer, pH 7.0.

Affinity for PBP-2′

The affinity for PBP-2′ of MRSA was determined by 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. Binding affinity was expressed as the inhibitory concentration for [^{14}C]benzylpenicillin binding by 50% (IC_{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

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

Determination of epileptogenicity

Male SD rats, 7 weeks of age, were cannulated in the right cerebroventricle before 1 week of drug administration. The carbapenems were dissolved in saline, adjusted to pH 7.0, and an aliquot of 10 μL/head was intracerebroventricularly injected ($n=5$). Convulsant behavior was monitored for 30 min after administration.

General methods

Melting points were measured on a Yanaco MP micro-melting point apparatus and were not corrected. The ^{1}H NMR spectra were recorded on a Varian VXR-300 spectrometer with tetramethylsilane (TMS) as an internal standard. IR absorption spectra were recorded with a Horiba FT-200 spectrometer. Specific rotations were measured with a Jasco DIP-370 polarimeter. UV spectra were taken on a SHIMAZU SPD-10A spectrometer in 0.1 M 3-morpholinopropanesulfonate (MOPS) buffer (pH 7.0). Mass spectra (MS) were measured on a JEOL JMS-SX102A spectrometer. TLC was performed with Merck Kieselgel F_{254} precoated plates. The silica gel used for column chromatography was WAKO gel C-300. Reversed-phase column chromatography was carried out on YMC-gel ODS-AQ 120-S50. All reactions involving air-sensitive reagents were performed under nitrogen using syringe-septum cap techniques.

General procedure for the preparation of cationic zwitterionic dithiocarbamate carbapenems

The experimental procedure for **10a** is described as a representative example.

***p*-Nitrobenzyl (1*R*,5*S*,6*S*)-6-[(*R*)-1-hydroxyethyl]-2-[*N*-(4-hydroxymethylbenzyl)-*N*-methyl]aminothiocarbonylthio]-1-methyl-1-carbapen-2-em-3-carboxylate 7.** To a stirred solution of *p*-nitrobenzyl (1*R*,5*S*,6*S*)-2-diphenoxyphosphoryloxy-6-[(*R*)-1-hydroxyethyl]-1-methyl-1-carbapen-2-em-3-carboxylate **5** (5.0 g, 8.4 mmol) in THF (120 mL) were added **6** (3.27 g, 10 mmol) and lithium chloride (428 mg, 10 mmol), and the mixture was stirred overnight at room temperature. The reaction mixture was poured into H_2O and extracted with EtOAc. The organic layer was washed with brine, dried over $MgSO_4$, and evaporated under reduced pressure. The residue was purified by silica gel column chromatography to give **7** (2.1 g, 42%): IR ν_{max} (KBr) 1778, 1546, 1468 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$) δ 1.16 (3H, d, $J=7.5$ Hz), 1.27 (3H, d, $J=6.4$ Hz), 2.43 (1.6H, s), 2.49 (1.4H, s), 3.42 (2H, s), 3.88 (2H, m), 4.18 (3H, m), 4.51 (2H, m), 4.43 (1H, m), 7.47 (2H, d, $J=8.3$ Hz), 7.78 (4H, m), 8.24 (2H, d, $J=8.3$ Hz); FAB-HRMS *m*/*z* calcd for $C_{27}H_{30}N_3O_7S_2$ $[M+H]^+$: 572.1525, found 572.1523.

(1*R*,5*S*,6*S*)-6-[(*R*)-1-Hydroxyethyl]-2-[*N*-[4-(4-carbamoylmethyl-1,4-diazabicyclo[2.2.2]octanediium-1-ylmethyl)-benzyl]-*N*-methylamino]thiocarbonylthio]-1-methyl-1-carbapen-2-em-3-carboxylate chloride 10a. To a solution of **7** (2.5 g, 4.4 mmol) in THF (40 mL) were added diisopropylamine (2.34 mL, 13.4 mmol) and *n*-propanesulfonylchloride (1.47 mL, 13.1 mmol) at 0 °C, and the mixture was stirred for 2 h. The reaction mixture was poured into H_2O and extracted with EtOAc. The organic layer was successively washed with 0.1 N HCl, 5% aqueous $NaHCO_3$ and brine, dried over $MgSO_4$, and evaporated under reduced pressure. To a solution of the residue in acetone (40 mL) was added sodium iodide (2.6 g, 17.5 mol) 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 evaporated under reduced pressure to give iodide **8**. To a solution of **8** in CH_3CN (57 mL) was added **9a** (2.09 g, 6.6 mmol) at 0 °C, and the mixture was stirred for 12 h at room temperature. The reaction mixture was evaporated under reduced pressure. To a solution of the residue in THF (113 mL) and 0.5 M sodium 3-morpholinopropanesulfonate buffer (pH 7.0, 113 mL) was added 10% palladium carbon catalyst (4.24 g), and the mixture was stirred overnight at room temperature under a hydrogen atmosphere. The catalyst was removed by filtration and the filtrate was subjected to reversed-phase column chromatography filled with saturated NaCl solution. Eluant was monitored by HPLC. The fractions eluted with 30% MeOH/H_2O containing the desired compound were combined and lyophilized to give **10a** (603 mg, 22%): IR ν_{max} (KBr) 3731, 2970, 1757, 1693, 1385, 1209, 1113 cm^{-1}; ^1H NMR (300 MHz, D_2O) δ 1.04 (3H, m), 1.23 (3H, d, $J=6.5$ Hz), 3.58 (5H, m), 4.18 (18H, m), 5.21 (2H, m), 7.49 (4H, m); FAB-HRMS *m*/*z* calcd for $C_{28}H_{38}N_5O_5S_2$ $[M]^+$: 588.2314, found 588.2300; UV λ_{max} 300 (ε 9920).

The following compounds (**10b**, **10c**, **11**, **12**, **13** **14b** and **14c**) were prepared as described for the preparation of **10a**.

(1*R*,5*S*,6*S*)-6-[(*R*)-1-Hydroxyethyl]-2-[*N*-[4-[4-(2-hydroxyethyl)-1,4-diazabicyclo[2.2.2]octanediium-1-ylmethyl]-benzyl]-*N*-methylamino]thiocarbonylthio]-1-methyl-1-carbapen-2-em-3-carboxylate chloride 10b. IR ν_{max} (KBr) 3423, 1749, 1691, 1606, 1387, 1214, 1101 cm^{-1}; ^1H NMR (300 MHz, D_2O) δ 1.06 (3H, m), 1.23 (3H, d, $J=6.3$ Hz), 3.37–3.74 (7H, m), 3.97–4.35 (18H, m), 5.05–5.45 (2H, m), 7.40–7.57 (4H, m); FAB–HRMS *m*/*z* calcd for $C_{28}H_{39}N_4O_5S_2$ $[M]^+$: 575.2362, found 575.2353; UV λ_{max}300 (ε 8640).

(1*R*,5*S*,6*S*)-6-[(*R*)-1-Hydroxyethyl]-2-[*N*-[4-[4-(3-hydroxypropyl)-1,4-diazabicyclo[2.2.2]octanediium-1-ylmethyl]-benzyl]-*N*-methylamino]thiocarbonylthio]-1-methyl-1-carbapen-2-em-3-carboxylate chloride 10c. IR ν_{max} (KBr) 3398, 1745, 1606, 1390, 1203, 1105 cm^{-1}; ^1H NMR (300 MHz, D_2O) δ 0.78–1.23 (6H, m), 1.95–2.05 (2H, m), 2.57–3.01 (2H, m), 3.30–3.74 (9H, m), 3.90–4.19 (14H, m), 4.85–5.70 (2H, m), 7.35–7.53 (4H, m); FAB-HRMS *m*/*z* calcd for $C_{29}H_{41}N_4O_5S_2$ $[M]^+$: 589.2518, Found: 589.2504; UV λ_{max}300 (ε 6420).

(1*R*,5*S*,6*S*)-6-[(*R*)-1-Hydroxyethyl]-2-[*N*-[4-(4-carbamoylmethyl-1,4-diazabicyclo[2.2.2]octanediium-1-ylethyl)benzyl]-*N*-methylamino]thiocarbonylthio]-1-methyl-1-carbapen-2-em-3-carboxylate chloride 11. IR ν_{max} (KBr) 3423, 1753, 1695, 1387, 1126, 1093 cm^{-1}; ^1H NMR (300 MHz, D_2O) δ 1.00 (1.5H, d, $J=7.3$ Hz), 1.09 (1.5H, d, $J=7.3$ Hz), 1.27 (3H, d, $J=6.3$ Hz), 3.18–3.30 (2H, m), 3.42 (1.5H, s), 3.46 (1.5H, s), 3.38–3.55 (1H, m), 3.63–3.93 (3H, m), 4.05–4.42 (14H, m), 4.49 (2H, s), 5.20 (2H, m), 7.29–7.44 (4H, m); FAB–HRMS *m*/*z* calcd for $C_{29}H_{40}N_5O_5S_2$ $[M]^+$: 602.2471, found 602.2446; UV λ_{max} 300 (ε 9550).

(1*R*,5*S*,6*S*)-6-[(*R*)-1-Hydroxyethyl]-2-[*N*-[4-(4-carbamoylmethyl-1,4-diazabicyclo[2.2.2]octanediium-1-ylmethyl)-1-naphtylmethyl]-*N*-methylamino]thiocarbonylthio]-1-methyl-1-carbapen-2-em-3-carboxylate chloride 12. IR ν_{max} (Nujol) 1754, 1697, 1598 cm^{-1}; ^1H NMR (300 MHz, D_2O) d 0.92 (3H, m), 1.11 (3H, m), 3.03–4.22 (18H, m), 4.98–6.02 (4H, m), 7.03–8.41 (4H, m); FAB–HRMS *m*/*z* calcd for $C_{32}H_{40}N_5O_5S_2$ $[M]^+$: 638.2471, found: 638.2452; UV λ_{max} 287 (ε 17,800).

(1*R*,5*S*,6*S*)-6-[(*R*)-1-Hydroxyethyl]-2-[*N*-[6-(4-carbamoylmethyl-1,4-diazabicyclo[2.2.2]octanediium-1-ylmethyl)-2-naphtylmethyl]-*N*-methylamino]thiocarbonylthio]-1-methyl-1-carbapen-2-em-3-carboxylate chloride 13. IR ν_{max} (Nujol) 1754, 1699, 1600 cm^{-1}; ^1H NMR (300 MHz, D_2O) δ 0.67 (1.2H, d, $J=7.4$ Hz), 0.81 (1.8H, d, $J=7.4$ Hz), 0.94 (1.8H, d, $J=6.4$ Hz), 1.03 (1.2H, d, $J=6.3$ Hz), 3.17 (1H, m), 3.23 (1.8H, s), 3.26 (1.2H, s), 3.42 (1H, m), 3.83–4.26 (16H, m), 5.01 (4H, m), 7.30 (1H, m), 7.41 (1H, m), 7.52 (1H, m), 7.83 (3H, m); FAB-HRMS *m*/*z* calcd for $C_{32}H_{40}N_5O_5S_2$ $[M]^+$: 638.2471, found 638.2507; UV λ_{max} 300 (ε 8920).

(1*R*,5*S*,6*S*)-6-[(*R*)-1-Hydroxyethyl]-2-[1,2,3,6-tetrahydro-4-[4-(2-hydroxyethyl)-1,4-diazabicyclo[2.2.2]octanediium-1-ylmethyl]-1-pyridyl]thiocarbonylthio-1-methyl-1-carbapen-2-em-3-carboxylate chloride 14b. IR ν_{max} (KBr) 1751, 1604, 1419, 1390 cm^{-1}; ^1H NMR (300 MHz, D_2O) d 1.08 (3H, d, $J=7.3$ Hz), 1.25 (3H, d, $J=6.3$ Hz), 2.40–2.70 (2H, m), 3.48–3.80 (4H, m), 3.98–4.18 (12H, m), 4.19–4.42 (5H, m), 5.55–4.70 (1H, m), 6.20–6.35 (1H, m); FAB–HRMS *m*/*z* calcd for $C_{25}H_{37}N_4O_5S_2$ $[M]^+$: 537.2205, found: 537.2233; UV λ_{max} 300 (ε 8620).

(1*R*,5*S*,6*S*)-6-[(*R*)-1-Hydroxyethyl]-2-[1,2,3,6-tetrahydro-4-[4-(3-hydroxypropyl)-1,4-diazabicyclo[2.2.2]octanediium-1-ylmethyl]-1-pyridyl]thiocarbonylthio-1-methyl-1-carbapen-2-em-3-carboxylate chloride 14c. IR ν_{max} (KBr) 1747, 1699, 1650, 1538 cm^{-1}; ^1H NMR (300 MHz, D_2O) δ 1.09 (3H, d, $J=7.3$ Hz), 1.26 (3H, d, $J=6.3$ Hz), 1.95–2.12 (2H, m), 2.40–2.72 (2H, m), 3.53 (1H, dd, $J=5.8$, 2.9 Hz), 3.58–3.78 (5H, m), 3.88–4.12 (13H, m), 4.20–4.48 (5H, m), 6.22–6.38 (1H, m); FAB–HRMS *m*/*z* calcd for $C_{26}H_{39}N_4O_5S_2$ $[M]^+$: 551.2362, found 551.2360; UV λ_{max} 300 (ε 9180).

***p*-Nitrobenzyl (1*R*,5*S*,6*S*)-6-[(*R*)-1-hydroxyethyl]-2-[(1,2,3,6-tetrahydro-4-iodomethyl)-1-pyridyl]thiocarbonylthio-1-methyl-1-carbapen-2-em-3-carboxylate 16.** To a solution of **15** (215 g, 403 mmol) in THF (1700 mL) were added diisopropylamine (91.3 mL, 524 mmol) and *n*-propanesulfonylchloride (49.9 mL, 443 mmol) at 0 °C, and the mixture was stirred for 2 h. 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 $MgSO_4$, and evaporated under reduced pressure. To a solution of residue in acetone (1100 mL) was added sodium iodide (181 g, 1.21 mol) 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 evaporated under reduced pressure to give **16** (236.7 g, 91%) as a solid: IR ν_{max} (KBr) 1771, 1522, 1435 cm^{-1}; ^1H 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); FAB–HRMS *m*/*z* calcd for $C_{24}H_{27}N_3O_6S_2I$ $[M+H]^+$: 644.0386, found 644.0369.

***p*-Nitrobenzyl (1*R*,5*S*,6*S*)-6-[(*R*)-1-hydroxyethyl]-2-[1,2,3,6-tetrahydro-4-(4-carbamoylmethyl-1,4-diazabicyclo[2.2.2]octanediium-1-ylmethyl)-1-pyridyl]thiocarbonylthio-1-methyl-1-carbapen-2-em-3-carboxylate iodide trifluoromethanesulfonate 17a.** To a solution of **16** (236 g, 367 mmol) in CH_3CN (960 mL) was added **9a** (128 g, 385 mmol) at 0 °C, and the mixture was stirred for 30 min at room temperature. The reaction mixture was evaporated under reduced pressure and the resulting precipitates were washed with EtOAc/THF/CH_3CN (10:3:1) to give **17** (349.2 g, 98%) as a solid: IR ν_{max} (KBr) 3403, 1772, 1693, 1608, 1523, 1467, 1029, 850, 638 cm^{-1}; ^1H NMR (300 MHz, DMSO-d_6) δ 1.13 (6H, m), 3.46 (3H, m), 3.83 (8H, m), 4.06 (8H, m), 4.19 (3H, m), 4.30 (3H, m), 4.59 (1H, m), 5.12 (1H, m), 5.33 (1H, d, $J=14.0$ Hz), 5.46 (1H, d, $J=14.0$ Hz), 6.18 (1H, m), 7.68 (2H, d, $J=8.8$ Hz), 8.22 (2H, d, $J=8.8$ Hz); FAB–HRMS *m*/*z* calcd for $C_{32}H_{42}N_6O_7S_2$ $[M]^+$: 686.2556, found 686.2547.

(1*R*,5*S*,6*S*)-6-[(*R*)-1-Hydroxyethyl]-2-[1,2,3,6-tetrahydro-4-(4-carbamoylmethyl-1,4-diazabicyclo[2.2.2]octanediium-1-ylmethyl)-1-pyridyl]thiocarbonylthio-1-methyl-1-carbapen-2-em-3-carboxylate chloride 14a. To a solution of **17** (488 g, 507 mmol) in THF (7 L) and 0.7 M phosphate buffer (pH 6.0, 10 L) was added zinc dust (2 kg), and the reaction mixture was stirred vigorously for 40 min. The reaction mixture was passed through a pad of Celite and the filtrate was evaporated under reduced pressure to about 3 L. The aqueous layer was subjected to reversed-phase column chromatography in which the column was filled with saturated NaCl solution. Eluant was monitored by HPLC. The fractions eluted with 5% CH_3CN/H_2O containing the desired compound were combined, lyophilized and crystallized from 80% $EtOH/H_2O$ to give **14a** as colorless prisms (101.5 g, 34%): IR ν_{max} (KBr) 1749, 1695, 1608, 1388 cm^{-1}; ^1H NMR (300 MHz, D_2O) δ 1.11 (3H, d, $J=7.2$ Hz), 1.28 (3H, d, $J=6.3$ Hz), 2.56 (2H, m), 3.56 (1H, dd, $J=5.6$, 2.9 Hz), 3.70 (1H, q, $J=7.6$ Hz), 4.08 (6H, m), 4.26 (12H, m), 4.38 (1H, dd, $J=9.7$, 2.7 Hz), 4.45 (2H, s), 4.68 (1H, m), 6.42 (1H, m); FAB-HRMS *m*/*z* calcd for $C_{25}H_{36}N_5O_5S_2$ $[M]^+$: 550.2158, found 550.2140. Anal. calcd for $C_{25}H_{36}N_5O_5S^+$ Cl^- $3H_2O$: C, 46.90; H, 6.61; N, 10.94; S, 10.02, found C, 46.91; H, 6.77; N, 10.95; S, 9.99; UV λ_{max} 300 (ε 6370).

1-Carbamoylmethyl-4-aza-1-azoniabicyclo[2.2.2]-octane trifluoromethanesulfonate 9a[5a]

To a solution of DABCO (659 g, 5.88 mol) in CH_3CN (7.0 L) was added a solution of 2-chloroacetamide (500 g, 5.35 mol) in CH_3CN (7.0 L) at 0 °C, and the reaction mixture was stirred for 16 h at room temperature. The resulting precipitates were collected, washed with CH_3CN and dried to give the chloride salt (943 g, 86%). To a solution of the chloride salt (943 g, 4.59 mol) in MeOH (6.0 L) was added a solution of AgOTf (1.12 kg, 4.36 mol) in CH_3CN (4.0 L) at room temperature. After being stirred for 30 min, the mixture was filtered and the filtrate was evaporated under reduced pressure. The residue was crystallized from EtOH to give **9a** (898 g, 65%): ^1H NMR (300 MHz, D_2O) δ 3.22 (6H, t, $J=7.5$ Hz), 3.65 (6H, t, $J=7.5$ Hz), 4.02 (2H, s). Anal. calcd for $C_9H_{16}N_3O_4F_3S$: C, 33.85; H, 5.05; N, 13.16, found C, 34.02; H, 5.02; N, 13.14.

9b and **9c** were prepared as described above by using 2-bromoethanol or 3-bromopropanol instead of chloroacetamide.

9b. ^1H NMR (300 MHz, D_2O) δ 3.14 (6H, m), 3.40 (8H, m), 3.65 (2H, t, $J=7.6$ Hz).

9c. ^1H NMR (300 MHz, D_2O) δ 1.96 (2H, m), 3.15 (6H, m), 3.38 (8H, m), 3.63 (2H, m).

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