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Syntheses and Pharmacokinetic Studies of Prodrug Esters for the Development of Oral Carbapenem, L-084

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Abstract We discovered an orally active carbapenem, L-084, through pharmacokinetic studies on various prodrug esters of (1*R*,5*S*,6*S*)-6-[(*R*)-1-hydroxyethyl]-1-methyl-2-[1-(1,3-thiazolin-2-yl)azetidin-3-yl]thio-1-carbapen-2-em-3-carboxylic acid (LJC11,036). L-084 showed a strong antimicrobial activity against Gram-positive and Gram-negative bacteria and exhibited the highest intestinal absorption among synthesized prodrugs of LJC11,036.

Keywords 1β-methylcarbapenem, prodrug ester, azetidine, oral administration, pharmacokinetics

Introduction

Carbapenems are well recognized to have broad antibacterial activities, and most of the carbapenem compounds have been developed for parenteral use such as imipenem [1], panipenem [2], meropenem [3], biapenem [4, 5], ertapenem [6] and doripenem [7]. The development of oral carbapenems is now expected in the clinical realm because oral administration is advantageous for patients. Recently several oral carbapenems have been developed as prodrug esters, GV-118819 [8], CS-834 [9] and DZ-2640 [10] and these compounds were under clinical trials.

For the development of a new oral carbapenem, we carried out a prodrug approach [11] to optimize the oral absorbability of LJC11,036 (**4**), which showed a strong antimicrobial activity against Gram-positive and Gram-negative bacteria. The potently active **4** also exhibited high stability to β-lactamase and human renal dehydropeptidase-I (DHP-I). After pharmacokinetic studies in rats and chemical stability tests, L-084 (**5**) was selected for further development among synthesized prodrug esters of **4**.

In this paper we describe the syntheses and pharmacokinetic studies of prodrug esters of **4** for the development of a new oral carbapenem.

Chemistry

The potent LJC11,036 (**4**) was synthesized by condensation of enolphosphate **2** [12] and thiol compound **1** and then hydrogenolysis (Scheme 1). This antimicrobial agent **4** has an azetidine moiety at the C-2 position of the carbapenem skeleton. Although a chiral pyrrolidine moiety was widely used as the C-2 side chain for the discovery of new carbapenems, the azetidine moiety has not been applied because of the difficulty in preparing it. So, we decided to comfort the challenge of using an achiral azetidine moiety instead of a chiral pyrrolidine moiety as the C-2 side chain on carbapenem skeleton. As shown in Scheme 2, thiol compound **1** was synthesized through a key intermediate, 3-hydroxyazetidine hydrochloride (**7**), that was prepared according to the literature in 48% yield from benzhydrylamine [13]. Compound **8** was obtained by the

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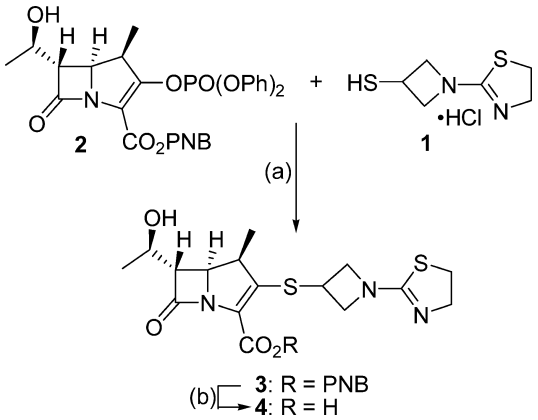
reaction of **7** and 2-methylthio-2-thiazoline in 92% yield. Converting the hydroxy group of **8** to a thiol group was conducted by a conventional method [14].

Various prodrug esters of **4** were prepared in order to evaluate the bioavailability in rats. The esterification of **4** was carried out by using several kinds of substituted-alkyl chloride (R-Cl) in the presence of a quaternary ammonium salt as shown in Scheme 3 [15]. Among ten synthesized derivatives, four compounds, **5**, **13**, **15** and **18**, were obtained as crystals that were greatly advantageous for further development because of being easy to handle and to formulate. Cyclohexyloxycarbonyloxyethyl ester **12** was obtained as a diastereo-mixture and was an amorphous powder. As compound **18** did not show high absorbability in rats (Table 2), it was eliminated from this development. Three of the prodrugs, **5**, **13** and **15**, were subjected to accelerated conditions to evaluate their chemical stability. Table 1 shows that each amorphous powder was less stable than corresponding crystalline powder, and the crystalline forms of **5** and **13** were more stable than that of **15**.

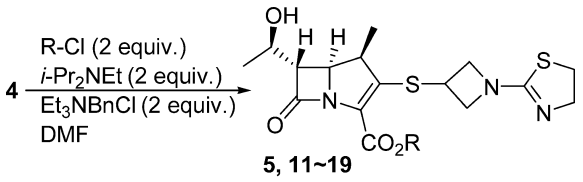
Biological Properties

As previously reported, LJC11,036 (**4**) exhibited strong and well-balanced activity against both Gram-positive and Gram-negative bacteria [16]. In addition to this, compound **4** showed higher stability to human renal dehydropeptidase-



Reagents and conditions: (a) **1** (1.1 equiv.), *i*-Pr_2NEt (2.2 equiv.), MeCN, −20°C, 2 hours, 94%; (b) 10% Pd-C, $NaHCO_3$ (0.5 equiv.), H_2O - *n*-BuOH, H_2 (400 kPa), 1.5 hours, 82%.

Scheme 1



5 (L-084): R = -CH_2OCOt-Bu (POM)

11: R = -CH_2OCO-(cyclohexyl)-Me

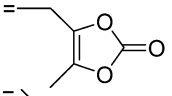
12: R = -CH(Me)OCOO-(cyclohexyl)

13: R = -CH_2OCO-(Me-cyclohexyl)

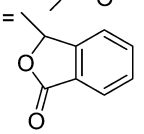
14: R = -CH_2OCOCH_2-(cyclohexyl)

15: R = -CH_2OCO-(cyclohexyl)

16: R =



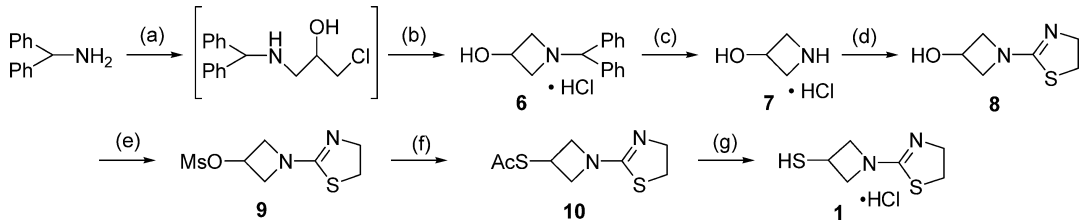
17: R =



18: R = -CH_2OCO-(Me-cyclohexyl)

19: R = -CH_2OCO-(Cl-cyclohexyl)

Scheme 3



Reagents and conditions: (a) epichlorohydrin (1.0 equiv.), MeOH, rt, 1 day; (b) DMSO, 50°C, 3 days, 48% (2 steps); (c) 5% Pd-C, H_2O-EtOH, H_2 (350 kPa), rt, 4 hours, 100%; (d) 2-methylthio-2-thiazoline (1.0 equiv.), $KHCO_3$ (0.7 equiv.), MeOH, reflux, 1 day, 92%; (e) MsCl (1.1 equiv.), Et_3N (1.2 equiv.), DMAP (0.01 equiv.), THF, 5°C, 0.5 hours, 93%; (f) AcSK (1.5 equiv.), DMF, 100°C, 5.5 hours, 88%; (g) KOH (1.1 equiv.), IPA, 5°C, 10 minutes then HCl (2.4 equiv.), 87%.

Scheme 2

Table 1 Remaining percentage of prodrug esters after storage under accelerated conditions

Storage conditions		40°C				40°C, 75% RH[a]	
Time (month)		0.5	1	2	3	0.5	1
5	amorphous	90	80	—	—	0	—
	crystal	100	98	—	—	99	98
13	amorphous	—	68	—	37	5	—
	crystal	—	99	—	99	—	99
15	amorphous	85	71	48	38	0	—
	crystal	100	99	97	94	97	74

[a] Relative humidity.

Table 2 Pharmacokinetic parameters after oral administration to rats at dose of 20 mg/kg as **4**

Compounds	**4**	**5**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**
Cmax (μg/ml)	0.3	14.8	9.5	13.6	15.3	12.3	10.3	2.0	7.3	8.4	7.9
AUC (μg·h/ml)	0.2	10.7	6.9	10.1	11.5	9.7	8.3	1.6	5.5	7.1	6.2
BA (%)[a]	0.8	38.1	24.6	36.0	41.2	34.9	29.6	5.8	19.5	25.5	22.3

[a] Bioavailability (BA) was calculated from AUC (28 μg·h/ml) after i.v. administration of **4** at a dose of 20 mg/kg.

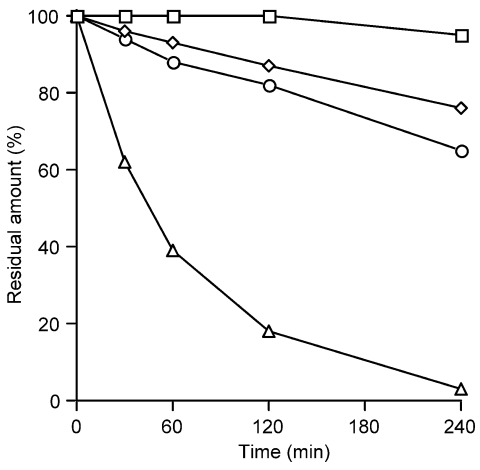


Fig. 1 Stability of LJC11,036 (**4**) to recombinant human DHP-I.

□, Biapenem; ◇, LJC11,036; ○, meropenem; △, imipenem.

I (DHP-I) than meropenem as shown in Figure 1 [17]. For the development of an orally active carbapenem, we focused on a prodrug ester of **4**, which exhibited a high maximum plasma level (Cmax) of **4** in rats.

Table 3 Pharmacokinetic parameters to various species

Species	Mouse	Dog	Monkey	Human
Dose (mg/kg)[a]	10	10	10	100 mg/man
Cmax (μg/ml)	38.1	5.7	5.8	4.1
AUC (μg h/ml)	87.8	11.1	5.9	3.5
Urinary recovery (%)	—	—	25	72
BA (%)	71	35	45	—

[a] L-084 was orally administered to each species at a dose as an active form.

In order to evaluate oral absorption based on Cmax, AUC and BA in rats, esters of **4** were prepared (Scheme 3). The results of pharmacokinetics in rats are shown in Table 2. The Cmax of prodrugs **5**, **12**~**15** was more than 10 μg/ml at an oral dose of 20 mg/kg as **4**. The areas under the blood concentration-time curve (AUC) of **5**, **12** and **13** were more than 10 μg·h/ml. The BA of **5**, **12** and **13** was more than 35%.

Based on these biological and chemical studies and cost of production, we chose **5** for further development. Table 3

shows pharmacokinetic parameters of **4** after dosing **5** to mice, dogs, monkeys and humans. Especially in humans, the prodrug ester **5** showed a high Cmax and high urine excretion level of the active metabolite **4** [18]. The cumulative urinary recoveries of **4** were in the range of 65 to 79% (average: 72%) when **5** was administered orally to healthy male volunteers at a dose corresponding to 100 mg of **4**.

Conclusion

We found a new oral carbapenem antibiotic L-084 (**5**), showing a high bioavailability and Cmax in humans, by a prodrug approach. The active metabolite **4** shows potent and well-balanced antibacterial activity and also shows higher stability to DHP-I than meropenem.

Experimental

General Methods

Melting points were determined using Yanagimoto micro-melting point apparatus. IR spectra were recorded on a JASCO FT-IR (VALOR-III) spectrometer. NMR spectra were performed on JEOL JNM-FX100 (100 MHz), JNM-EX270 (270 MHz) or Bruker Avance DPX400 (400 MHz) spectrometers using tetramethylsilane (TMS) or sodium 3-(trimethylsilyl)propionate-d_4 (TSP) as an internal standard. Mass spectra were recorded on JEOL JMS-DX300 or JMS-SX102A spectrometers. Elemental analysis was recorded on Yanako CHN-Corder. HPLC analysis was performed on Shimadzu LC system constructed from SPD-10AV and LC-10AS.

1-(1,3-Thiazolin-2-yl)azetidin-3-ol (8)

To a solution of 3-hydroxyazetidine hydrochloride (**7**, 39.5 g, 361 mmol) in MeOH (361 ml) were added potassium hydrogen carbonate (25.3 g, 253 mmol) and 2-methylthio-2-thiazoline (48.1 g, 361 mmol), and the mixture was refluxed for 23 hours. The reaction mixture was allowed to cool to 40°C, and additional potassium hydrogen carbonate (18.1 g, 180 mmol) was added. The mixture was stirred for 2 hours at 40°C. After removal of insoluble material by filtration, the filtrate was concentrated under reduced pressure to give **8** as colorless crystals (52.4 g, 92%).

Mp 114～117°C; IR (KBr) cm^{-1} 2939, 2743, 1610, 1452, 1371, 1128, 1014; ^{1}H-NMR (270 MHz, $CDCl_3$) δ 3.34 (2H, t, J=7.4 Hz), 3.85～3.90 (2H, m), 3.98 (2H, t, J=7.4 Hz), 4.17～4.23 (2H, m), 4.62～4.71 (1H, m); HRMS (EI) calcd for $C_6H_{10}N_2OS$ 158.0514, found m/z 158.0510 $(M)^+$; *Anal.* Calcd for $C_6H_{10}N_2OS$: C, 45.55; H, 6.37; N, 17.71. Found: C, 45.37; H, 6.38; N, 17.61.

3-Methanesulfonyloxy-1-(1,3-thiazolin-2-yl)azetidine (9)

1-(1,3-Thiazolin-2-yl)azetidin-3-ol (**8**, 27.7 g, 176 mmol) in THF (300 ml) was stirred for 0.5 hours with DMAP (214 mg, 1.76 mmol), Et_3N (21.3 g, 211 mmol) and methanesulfonyl chloride (22.2 g, 193 mmol) at 5°C, and the solvent was evaporated under reduced pressure. After the addition of AcOEt (250 ml) and a saturated aqueous solution of sodium hydrogen carbonate (400 ml) to the residue, the mixture was stirred for 10 minutes at room temperature. The organic layer was dried over $MgSO_4$, and the filtrate was concentrated under reduced pressure. The residue was triturated with THF (40 ml) and *n*-hexane (400 ml) for 1 hour at room temperature. The resulting precipitate was filtered and dried *in vacuo* to give **9** as colorless crystals (38.6 g, 93%).

Mp 85～86°C; IR (KBr) cm^{-1} 1613, 1343, 1193, 1126, 1041, 949; ^{1}H-NMR (270 MHz, $CDCl_3$) δ 3.07 (3H, s), 3.38 (2H, t, J=7.6 Hz), 4.03 (2H, t, J=7.6 Hz), 4.14～4.19 (2H, m), 4.30～4.37 (2H, m), 5.26～5.32 (1H, m); HRMS (EI) calcd for $C_7H_{12}N_2O_3S_2$ 236.0289, found m/z 236.0286 $(M)^+$.

3-Acetylthio-1-(1,3-thiazolin-2-yl)azetidine (10)

To DMF (44 ml) at 100°C were added 3-methanesulfonyloxy-1-(1,3-thiazolin-2-yl)azetidine (**9**, 5.14 g, 21.8 mmol) and potassium thioacetate (3.73 g, 32.7 mmol), and the mixture was stirred for 5.5 hours at 100°C. The reaction mixture was concentrated under reduced pressure, and then AcOEt (30 ml) and water (20 ml) were added to the residue. The AcOEt layer was separated, and the aqueous layer was extracted with AcOEt (8 ml) three times. The combined organic layer was washed with brine, dried over $MgSO_4$ and the filtrate was concentrated under reduced pressure to give **10** as yellow oil (4.1 g, 88%).

IR (KBr) cm^{-1} 2944, 2869, 1694, 1616, 1355, 1296, 1132, 952; ^{1}H-NMR (270 MHz, $CDCl_3$) δ 2.34 (3H, s), 3.36 (2H, t, J=7.4 Hz), 3.87～4.92 (2H, m), 4.00 (2H, t, J=7.4 Hz), 4.30～4.35 (1H, m), 4.40～4.46 (2H, m); HRMS (EI) calcd for $C_8H_{12}N_2OS_2$ 216.0391, found m/z 216.0380 $(M)^+$; *Anal.* Calcd for $C_8H_{12}N_2OS_2$: C, 44.42; H, 5.59; N, 12.95. Found: C, 44.17; H, 5.64; N, 12.84.

3-Mercapto-1-(1,3-thiazolin-2-yl)azetidine hydrochloride (1)

To a solution of 3-acetylthio-1-(1,3-thiazolin-2-yl)azetidine (**10**, 52.1 g, 241 mmol) in IPA (24.1 ml) was added 2.0 mol/liter KOH in MeOH (133 ml, 265 mmol) under 5°C. After being stirred for 10 minutes at 5°C, 2.0 M HCl in MeOH (289 ml, 578 mmol) was added and the mixture

was stirred for additional 15 minutes. The insoluble material was filtered off, and the filtrate was concentrated under reduced pressure. The residue was purified by crystallization from MeCN (48 ml) and THF (289 ml) to give **1** as colorless crystals (44.4 g, 87%).

Mp 134～136°C; IR (KBr) cm^{-1} 2960, 2430, 1639, 1135; ^{1}H-NMR (270 MHz, $CDCl_3$) δ 2.57 (1H, d, J=8.2 Hz), 3.59 (2H, t, J=7.4 Hz), 4.02～4.18 (4H, m), 4.63 (2H, t, J=7.4 Hz), 5.19～5.26 (1H, m), 12.19 (1H, s); HRMS (FAB) calcd for $C_6H_{11}N_2S_2$ 175.0364, found *m/z* 175.0376 $(M-Cl)^+$; *Anal.* Calcd for $C_6H_{11}ClN_2S_2$: C, 34.19; H, 5.26; N, 13.29. Found: C, 34.17; H, 5.18; N, 13.24.

4-Nitrobenzyl (1*R*,5*S*,6*S*)-6-[(*R*)-1-hydroxyethyl]-1-methyl-2-[1-(1,3-thiazolin-2-yl)azetidin-3-yl]thio-1-carbapen-2-em-3-carboxylate (3)

To a suspension of 3-mercapto-1-(1,3-thiazolin-2-yl)azetidine hydrochloride (**1**, 5.79 g, 27.5 mmol) and 4-nitrobenzyl (1*R*,5*R*,6*S*)-2-diphenylphosphoryloxy-6-[(*R*)-1-hydroxyethyl]-1-methyl-1-carbapen-2-em-3-carboxylate (**2**, 14.86 g, 25 mmol) in dry MeCN (100 ml) was added diisopropylethylamine (9.58 ml, 55 mmol) under −20°C. After being stirred for 2 hours at −20°C under a nitrogen atmosphere, the reaction mixture was quenched with water (60 ml) and stirred at 5°C for 0.5 hours. The resulting precipitate was filtered, washed successively with MeCN-water (1 : 1, 30 ml) and IPA (30 ml) and then dried *in vacuo* to obtain **3** as colorless crystals (12.3 g, 94%).

Mp 178～180°C; IR (KBr) cm^{-1} 2939, 1768, 1702, 1612, 1553, 1513, 1340, 1140; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 1.24 (3H, d, J=7.3 Hz), 1.36 (3H, d, J=6.3 Hz), 3.15～3.19 (1H, m), 3.27 (1H, dd, J=2.6, 6.8 Hz), 3.37 (2H, t, J=7.5 Hz), 3.94～3.98 (2H, m), 4.01 (2H, t, J=7.5 Hz), 4.11～4.15 (1H, m), 4.21～4.28 (2H, m), 4.35～4.40 (2H, m), 5.25 (1H, d, J=13.7 Hz), 5.51 (1H, d, J=13.7 Hz), 7.66 (2H, d, J=8.7 Hz), 8.23 (2H, d, J=8.7 Hz); HRMS (FAB) calcd for $C_{23}H_{27}N_4O_6S_2$ 519.1372, found *m/z* 519.1391 $(M+H)^+$; *Anal.* Calcd for $C_{23}H_{26}N_4O_6S_2$: C, 53.27; H, 5.05; N, 10.80. Found: C, 53.36; H, 5.15; N, 10.76.

(1*R*,5*S*,6*S*)-6-[(*R*)-1-Hydroxyethyl]-1-methyl-2-[1-(1,3-thiazolin-2-yl)azetidin-3-yl]thio-1-carbapen-2-em-3-carboxylic acid (4)

To a mixture of **3** (9.33 g, 18 mmol) and sodium hydrogen carbonate (756 mg, 9 mmol) in *n*-BuOH (126 ml) were added 10% Pd-C (1.4 g, dry reduced) and water (156 ml), and the mixture was stirred vigorously for 1.5 hours under 400 kPa pressure of hydrogen at room temperature. The catalyst was removed by filtration, and the pH of the filtrate was adjusted to 5.6 using 1.0 M aqueous HCl. After separation, the aqueous layer was poured into cold acetone (467 ml) at 5°C, and the mixture was stirred for 10 minutes. Additional acetone (467 ml) was added dropwise over 20 minutes and the mixture was stirred for 3 hours at 5°C. The resulting precipitate was filtered and dried *in vacuo* to give tetrahydrate of **4** as colorless crystals (6.79 g, 82%).

Mp 170°C (decomp.); IR (KBr) cm^{-1} 1736, 1649, 1573; ^{1}H-NMR (400 MHz, D_2O) δ 1.16 (3H, d, J=7.2 Hz), 1.28 (3H, d, J=6.4 Hz), 3.18 (1H, dq, J=7.2, 9.0 Hz), 3.42 (1H, dd, J=2.5, 6.2 Hz), 3.63 (2H, t, J=7.5 Hz), 3.99 (2H, t, J=7.5 Hz), 4.17～4.25 (4H, m), 4.31～4.38 (1H, m), 4.70～4.79 (2H, m); HRMS (FAB) calcd for $C_{16}H_{22}N_3O_4S_2$ 384.1052, found *m/z* 384.1045 $(M+H)^+$; *Anal.* Calcd for $C_{16}H_{21}N_3O_4S_2 \cdot 4H_2O$: C, 42.18; H, 6.37; N, 9.21. Found: C, 42.07; H, 6.11; N, 9.01.

Typical Procedure for Esterification of 4 (Scheme 3): Pivaloyloxymethyl (1*R*,5*S*,6*S*)-6-[(*R*)-1-hydroxyethyl]-1-methyl-2-[1-(1,3-thiazolin-2-yl)azetidin-3-yl]thio-1-carbapen-2-em-3-carboxylate (5)

To a solution of the tetrahydrate of **4** (445 mg, 1 mmol), benzyltriethylammonium chloride (456 mg, 2 mmol) and chloromethyl pivalate (301 mg, 2 mmol) in DMF (1 ml) was added diisopropylethylamine (0.44 ml, 2 mmol) and the mixture was stirred for 4 hours at 45°C. After cooling to 5°C, AcOEt (2 ml) and water (2 ml) were added and then the mixture was adjusted to pH 4 by using 1.0 M aqueous citric acid. After discarding organic layer, potassium hydrogen carbonate was added to adjust pH to 7.6. The mixture was extracted with AcOEt, and the organic layer was washed with water and brine, dried over $MgSO_4$ and concentrated under reduced pressure. The residue was purified by silica gel column chromatography (EtOH-CH_2Cl_2-Acetone, 1 : 20 : 40) to yield **5** as a yellow amorphous solid. The amorphous solid was triturated with AcOEt to give **5** as colorless crystals (388 mg, 78%).

Mp 140～142°C; IR (KBr) cm^{-1} 1778, 1753, 1609; ^{1}H-NMR (270 MHz, $CDCl_3$) δ 1.22 (3H, d, J=7.2 Hz), 1.22 (9H, s), 1.31 (3H, d, J=6.3 Hz), 3.17 (1H, dq, J=7.2, 9.2 Hz), 3.22 (1H, dd, J=2.7, 6.3 Hz), 3.37 (2H, t, J=7.2 Hz), 3.94 (2H, m), 4.00 (2H, m), 4.11～4.23 (3H, m), 4.35～4.43 (2H, m), 5.83 (1H, d, J=5.3 Hz), 5.96 (1H, d, J=5.3 Hz); HRMS (EI) calcd for $C_{22}H_{31}N_3O_6S_2$ 497.1654, found *m/z* 497.1660 $(M)^+$; *Anal.* Calcd for $C_{22}H_{31}N_3O_6S_2$: C, 53.10; H, 6.28; N, 8.44. Found: C, 53.08; H, 6.19; N, 8.41.

4-Methylcyclohexylcarbonyloxymethyl (1*R*,5*S*,6*S*)-6-[(*R*)-1-hydroxyethyl]-1-methyl-2-[1-(1,3-thiazolin-2-yl)-azetidin-3-yl]thio-1-carbapen-2-em-3-carboxylate (11)
Amorphous powder (80% yield), mp 78～79°C; IR (KBr) cm^{-1} 3382, 2928, 1774, 1615, 1545; ^{1}H-NMR (270 MHz, $CDCl_3$) δ 0.88 (0.9H, d, J=6.6 Hz), 0.89 (2.1H, d, J=6.6 Hz), 1.22 (3H, d, J=7.3 Hz), 1.34 (3H, d, J=6.3 Hz), 1.34～1.80 (7H, m), 1.96～2.05 (2H, m), 2.23～2.28 (0.3H, m), 2.45 (1H, br s), 2.57～2.59 (0.7H, m), 3.15～3.21 (1H, m), 3.23 (1H, dd, J=2.3, 6.6 Hz), 3.40 (2H, t, J=7.6 Hz), 4.00～4.05 (4H, m), 4.15～4.27 (3H, m), 4.43～4.51 (2H, m), 5.86 (0.3H, d, J=5.6 Hz), 5.87 (0.7H, d, J=5.6 Hz), 5.94 (0.3H, d, J=5.6 Hz), 5.95 (0.7H, d, J=5.6 Hz); HRMS (EI) calcd for $C_{25}H_{35}N_3O_6S_2$ 537.1967, found m/z 537.1974 $(M)^+$.

1-(Cyclohexyloxycarbonyloxy)ethyl (1*R*,5*S*,6*S*)-6-[(*R*)-1-hydroxyethyl]-1-methyl-2-[1-(1,3-thiazolin-2-yl)azetidin-3-yl]thio-1-carbapen-2-em-3-carboxylate (12)
Amorphous powder (82% yield, diastereo-mixture), mp 107～109°C; IR (KBr) cm^{-1} 3184, 2937, 1790, 1611, 1548; ^{1}H-NMR (270 MHz, $CDCl_3$) δ 1.20～1.95 (19H, m), 3.12～3.23 (2H, m), 3.36 (2H, t, J=7.6 Hz), 3.92～4.03 (4H, m), 4.10～4.23 (3H, m), 4.34～4.42 (2H, m), 4.63～4.69 (1H, m), 6.88 (1H, q, J=2.6 Hz); HRMS (FAB) calcd for $C_{25}H_{36}N_3O_7S_2$ 554.1995, found m/z 554.1964 $(M+H)^+$; *Anal.* Calcd for $C_{25}H_{35}N_3O_7S_2$: C, 54.23; H, 6.37; N, 7.59. Found: C, 53.72; H, 6.27; N, 7.99.

1-Methylcyclohexylcarbonyloxymethyl (1*R*,5*S*,6*S*)-6-[(*R*)-1-hydroxyethyl]-1-methyl-2-[1-(1,3-thiazolin-2-yl)-azetidin-3-yl]thio-1-carbapen-2-em-3-carboxylate (13)
Colorless crystals (88% yield from MeCN), mp 137～139°C; IR (KBr) cm^{-1} 3123, 2940, 1790, 1747, 1717, 1613 1550; ^{1}H-NMR (270 MHz, $CDCl_3$) δ 1.13～1.54 (8H, m), 1.19 (3H, s), 1.22 (3H, d, J=7.3 Hz), 1.33 (3H, d, J=6.3 Hz), 2.02～2.11 (2H, m), 2.41 (1H, br s), 3.13～3.25 (2H, m), 3.39 (2H, t, J=7.6 Hz), 3.97～4.05 (4H, m), 4.11～4.26 (3H, m), 4.42～4.49 (2H, m), 5.87 (1H, d, J=5.6 Hz), 5.97 (1H, d, J=5.6 Hz); HRMS (FAB) calcd for $C_{25}H_{36}N_3O_6S_2$ 538.2046, found m/z 538.2032 $(M+H)^+$; *Anal.* Calcd for $C_{25}H_{35}N_3O_6S_2$: C, 55.84; H, 6.56; N, 7.81. Found: C, 55.52; H, 6.47; N, 7.65.

Cyclohexylacetoxymethyl (1*R*,5*S*,6*S*)-6-[(*R*)-1-hydroxyethyl]-1-methyl-2-[1-(1,3-thiazolin-2-yl)azetidin-3-yl]thio-1-carbapen-2-em-3-carboxylate (14)
Amorphous powder (76% yield), mp 70～71°C; IR (KBr) cm^{-1} 3381, 2925, 1771, 1615, 1542; ^{1}H-NMR (270 MHz, $CDCl_3$) δ 0.81～1.00 (2H, m), 1.00～1.26 (3H, m), 1.15 (3H, d, J=7.3 Hz), 1.25 (3H, d, J=6.3 Hz), 1.55～1.80 (6H, m), 2.19 (2H, d, J=6.9 Hz), 3.10 (1H, quint, J=7.3 Hz), 3.15 (1H, dd, J=2.3, 6.9 Hz), 3.28 (1H, br s), 3.31 (2H, t, J=7.6 Hz), 3.87～3.91 (2H, m), 3.94 (2H, t, J=7.6 Hz), 4.04～4.16 (3H, m), 4.30～4.37 (2H, m), 5.79 (1H, d, J=5.6 Hz), 5.85 (1H, d, J=5.6 Hz); HRMS (EI) calcd for $C_{25}H_{35}N_3O_6S_2$ 537.1967, found m/z 537.1992 $(M)^+$.

Cyclohexylcarbonyloxymethyl (1*R*,5*S*,6*S*)-6-[(*R*)-1-hydroxyethyl]-1-methyl-2-[1-(1,3-thiazolin-2-yl)azetidin-3-yl]thio-1-carbapen-2-em-3-carboxylate (15)
Colorless crystals (71% yield from AcOEt), mp 120～122°C; IR (KBr) cm^{-1} 3195, 2931, 1785, 1695, 1611, 1541; ^{1}H-NMR (270 MHz, $CDCl_3$) δ 1.31 (3H, d, J=7.3 Hz), 1.41 (3H, d, J=6.3 Hz), 1.31～1.73 (6H, m), 1.81～1.85 (2H, m), 1.99～2.13 (2H, m), 2.40～2.51 (1H, m), 2.99 (1H, br s), 3.12～3.18 (1H, m), 3.20～3.27 (1H, m), 3.47 (2H, t, J=7.6 Hz), 4.03～4.13 (4H, m), 4.16～4.34 (3H, m), 4.51 (2H, t, J=7.6 Hz), 5.93 (1H, d, J=5.6 Hz), 6.03 (1H, d, J=5.6 Hz); HRMS (EI) calcd for $C_{24}H_{33}N_3O_6S_2$ 523.1811, found m/z 523.1791 $(M)^+$; *Anal.* Calcd for $C_{24}H_{33}N_3O_6S_2$: C, 55.05; H, 6.35; N, 8.02. Found: C, 55.00; H, 6.33; N, 7.91.

(5-Methyl-2-oxo-1,3-dioxolen-4-yl)methyl (1*R*,5*S*,6*S*)-6-[(*R*)-1-hydroxyethyl]-1-methyl-2-[1-(1,3-thiazolin-2-yl)-azetidin-3-yl]thio-1-carbapen-2-em-3-carboxylate (16)
Amorphous powder (54% yield), mp 115°C (decomp.); IR (KBr) cm^{-1} 3382, 2969, 1820, 1767, 1610, 1457; ^{1}H-NMR (270 MHz, $CDCl_3$) δ 1.22 (3H, d, J=7.3 Hz), 1.34 (3H, d, J=6.3 Hz), 2.22 (3H, s), 2.46 (1H, br s), 3.14～3.26 (2H, m), 3.39～3.45 (2H, m), 4.00～4.09 (4H, m), 4.13～4.28 (3H, m), 4.47～4.56 (2H, m), 4.98 (1H, d, J=3.9 Hz), 5.05 (1H, d, J=3.9 Hz); HRMS (FAB) calcd for $C_{21}H_{26}N_3O_7S_2$ 496.1212, found m/z 496.1240 $(M+H)^+$.

1,3-Dihydro-3-oxo-1-isobenzofuranyl (1*R*,5*S*,6*S*)-6-[(*R*)-1-hydroxyethyl]-1-methyl-2-[1-(1,3-thiazolin-2-yl)-azetidin-3-yl]thio-1-carbapen-2-em-3-carboxylate (17)
Amorphous powder (84% yield), mp 120°C (decomp.); IR (KBr) cm^{-1} 3421, 2969, 1782, 1611, 1541; ^{1}H-NMR (270 MHz, $CDCl_3$) δ 1.21 (1.5H, d, J=7.3 Hz), 1.24 (1.5H, d, J=6.9 Hz), 1.29 (3H, d, J=6.3 Hz), 2.74 (1H, br s), 3.18～3.28 (2H, m), 3.34～3.41 (2H, m), 3.94～4.04 (4H, m), 4.10～4.26 (3H, m), 4.39～4.49 (2H, m), 7.45 (0.5H, s), 7.51 (0.5H, s), 7.61～7.79 (3H, m), 7.89～7.94 (1H, m); HRMS (FAB) calcd for $C_{24}H_{26}N_3O_6S_2$ 516.1263, found m/z 516.1274 $(M+H)^+$.

4-Methylbenzoyloxymethyl (1*R*,5*S*,6*S*)-6-[(*R*)-1-hydroxyethyl]-1-methyl-2-[1-(1,3-thiazolin-2-yl)azetidin-3-yl]thio-1-carbapen-2-em-3-carboxylate (18)

Colorless crystals (84% yield from EtOH), mp 91~93°C; IR (KBr) cm^{-1} 3302, 2964, 1785, 1736, 1609, 1550; ^{1}H-NMR (270 MHz, $CDCl_3$) δ 1.21 (3H, d, J=7.3 Hz), 1.32 (3H, d, J=6.3 Hz), 2.41 (s, 3H), 2.50 (br s, 1H), 3.12~3.24 (2H, m), 3.38 (2H, t, J=7.6 Hz), 3.98~4.04 (4H, m), 4.11~4.26 (3H, m), 4.41~4.48 (2H, m), 6.11 (2H, d, J=5.6 Hz), 6.14 (1H, d, J=5.6 Hz), 7.24 (1H, d, J=8.3 Hz), 7.97 (2H, d, J=8.3 Hz); HRMS (FAB) calcd for $C_{25}H_{30}N_3O_6S_2$ 532.1576, found m/z 532.1584 $(M+H)^+$.

4-Chlorobenzoyloxymethyl (1*R*,5*S*,6*S*)-6-[(*R*)-1-hydroxyethyl]-1-methyl-2-[1-(1,3-thiazolin-2-yl)azetidin-3-yl]thio-1-carbapen-2-em-3-carboxylate (19)

Amorphous powder (81% yield), mp 80~82°C; IR (KBr) cm^{-1} 3370, 2969, 2869, 1740, 1612, 1542; ^{1}H-NMR (270 MHz, $CDCl_3$) δ 1.22 (3H, d, J=7.6 Hz), 1.32 (3H, d, J=6.3 Hz), 2.63 (1H, br s), 3.15~3.22 (1H, m), 3.23 (1H, dd, J=2.3, 6.6 Hz), 3.37 (2H, t, J=7.4 Hz), 3.97~4.04 (4H, m), 4.11~4.26 (3H, m), 4.40~4.46 (2H, m), 6.10 (1H, d, J=5.6 Hz), 6.16 (1H, d, J=5.6 Hz), 7.42 (2H, d, J=8.5 Hz), 8.02 (2H, d, J=8.5 Hz); HRMS (FAB) calcd for $C_{24}H_{27}ClN_3O_6S_2$ 552.1030, found m/z 552.0999 $(M+H)^+$.

Typical Assay Procedure for the Plasma Concentration of 4

Plasma (100 μl) was diluted with an equal volume of 1 M MOPS buffer (pH 7.0), and MeCN (400 μl) and $CHCl_3$ (800 μl) were added to the diluted plasma. The mixture was vortex-mixed and then centrifuged. A 20 μl sample of upper layer was injected onto the HPLC. HPLC conditions were as follows. Column: Develosil ODS-UG-3 (4.6 by 75 mm I.D., Nomura Chemical), mobile phase: 0.1 M sodium acetate buffer (pH 5.5) and MeCN (100 : 4), flow rate: 1.3 ml/minute, temperature: 35°C, wavelength: λ=300 nm.

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