J. Antibiot. 58(12): 787–803, 2005

ORIGINAL ARTICLE

THE JOURNAL OF ANTIBIOTICS

Orally Active Carbapenem Antibiotics I

Antibacterial and Pharmacokinetic Potential of 2-Phenyl and 2-Thienylcarbapenems

Makoto Sunagawa, Yutaka Ueda, Shin-ichiro Okada, Shoji Watanabe, Takahiko Hashizuka, Seiji Hori, Akira Sasaki, Yoshiro Eriguchi, Katsunori Kanazawa

Received: September 27, 2005 / Accepted: December 2, 2005

Abstract In order to design orally active carbapenem antibiotics effective against β-lactam-resistant pathogens, such as penicillin-resistant *Streptococcus pneumoniae* (PRSP) and β-lactamase non-producing ampicillin-resistant *Haemophilus influenzae* (BLNAR), a series of novel 2-phenylcarbapenems and some 2-thienyl derivatives were synthesized and tested for antibacterial activities. These compounds were highly active against PRSP, BLNAR, and major Gram-positive and Gram-negative bacteria that cause community-acquired infections. Their pivaloyloxymethylester-type prodrug exhibited good oral absorption in mice, suggesting that this series of carbapenems were promising as a prototype of novel orally active β-lactams.

Keywords carbapenem antibiotic, structure-activity relationship, antibacterial activity, orally active agent

Introduction

The history of antimicrobial chemotherapy has seen the development of many orally active agents including β-lactams, quinolones, and macrolides, most of which are used to treat outpatients with bacterial infections. However, the recent remarkable increase in pathogens resistant to such agents has become a serious problem [1]. In particular, the incidence of community acquired respiratory infections caused by penicillin-intermediate/resistant *Streptococcus pneumoniae* (PISP/PRSP) and β-lactamase-non-producing ampicillin-resistant *Haemophilus influenzae* (BLNAR) has increased at an alarming rate and the lack of efficacy of traditional "oral penicillin" is a great concern [2]. Although newly available oral cephalosporins show broader antibacterial spectra than penicillins, their efficacy against these resistant bacteria also appears to be insufficient [3]. The development of a new oral agent that is more effective against these key pathogens is therefore urgently needed.

There are several parenteral carbapenems currently on the market. These agents have been qualified as the most potent β-lactam antibiotics available based on their antibacterial properties compared with penicillins and cephalosporins, *e.g.* a broader antibacterial spectrum covering resistant bacteria including PRSP and BLNAR, stronger bactericidal activity, high resistance against hydrolysis by various β-lactamases, and so on [4]. Given its highly intrinsic potentials, carbapenem is considered to be an attractive target for developing new orally active agents. Consequently, a number of carbapenem compounds have been disclosed as orally active by research groups, however, the required profile of an "orally active carbapenem" has not been extensively discussed.

In the present paper, we report the design and synthesis of 2-phenyl and 2-thienylcarbapenems taking into consideration the profile required of new orally active carbapenems. Subsequently, structure-activity relationships (SARs) on antibacterial activity were clarified. In addition, stability in human plasma and rate of hydrolysis by human dehydropeptidase-I (DHP-I), and oral absorption in mice

M. Sunagawa (Corresponding author)**, Y. Ueda, S. Okada, S. Watanabe, T. Hashizuka, S. Hori, A. Sasaki, Y. Eriguchi, K. Kanazawa:** Sumitomo Pharmaceuticals Research Division, 1-98, Kasugade Naka 3-Chome, Konohana-ku, Osaka 554-0022, Japan, E-mail: makoto-sunagawa@ds-pharma.co.jp

were also investigated to assess the potential of each of the compounds as an orally active agent.

Results and Discussion

Profile of Orally Active Carbapenems

The remarkable increase in pathogens resistant to both oral and parenteral cephalosporins such as PRSP and BLNAR has been blamed on the development of orally active cephalosporins similar in their antibacterial spectrum and also chemical structure to the parenteral drugs and on unsuitable clinical use [5]. Because of a relatively low achievable concentration in blood compared to the parenteral drug, there is a high risk of bacterial resistance not only to the oral agent per se, but also to the parenteral drug.

Therefore, for the design of an orally active carbapenem, the following profile is thought to be required, i) Sufficient antibacterial activity against a wide range of pathogens, for which the clinical efficacy of existing oral β-lactams has been established. ii) Potent activity against bacteria resistant to existing β-lactams, especially against PRSP and BLNAR. iii) Little or no activity against bacteria causing nosocomial infections, especially *Pseudomonas aeruginosa*. Considering the clinical value of parenteral carbapenems for treating pseudomonal infections, lowering the risk of cross-resistance to the parenteral carbapenems is the most important issue in the design of orally active carbapenems. In this context, iv) an unique chemical structure differing from the parenteral one should reduce the risk of bacteria developing resistance. Studies of six orally active carbapenems have already been reported (Fig. 1) [6～11]. But only sanfetrinem was designed not to be similar in terms of the spectrum of its antibacterial activity and chemical structure to the parenteral carbapenems.

Besides antibacterial activity, v) good tolerability and oral absorption comparable with existing orally active β-lactams, and vi) a simple synthetic route from the viewpoint of economic competition, are also essential.

Design and Synthesis of 2-Phenyl and 2-Thienylcarbapenem

We previously reported that 2-phenylpenem derivatives, similar in chemical structure to 2-phenylcarbapenems, were highly active against Gram-positive bacteria and that their pivaloyloxymethyl esters were significantly well absorbed in mice when administrated orally [12]. It was also reported by Cama *et al.* that 2-phenylcarbapenem derivatives were highly active against Gram-positive bacteria and showed an appropriate spectrum of antibacterial activity, although the levels of activity against PRSP and BLNAR were not elucidated [13, 14]. In addition, 2-phenylcarbapemens were highly resistant to hydrolysis by DHP-I, an enzyme involved in the metabolism of carbapenems in animals, without the 1β-methyl structure indicating an economic advantage in the synthesis of orally active carbapenems. The information described above suggested that 2-phenylcarbapenem might be a promising candidate for a novel orally active carbapenem, however a detailed investigation has not been reported so far. Therefore, we focused on 2-phenylcarbapenem and also 2-thienylcarbapenem, which is closely related structurally to the phenyl derivative, to assess the potential for oral application.

The strategy proposed for the preparation of 2-phenyl and 2-thienylcarbapenems is based on the procedures used by the Merck group [13, 14] in Fig. 2.

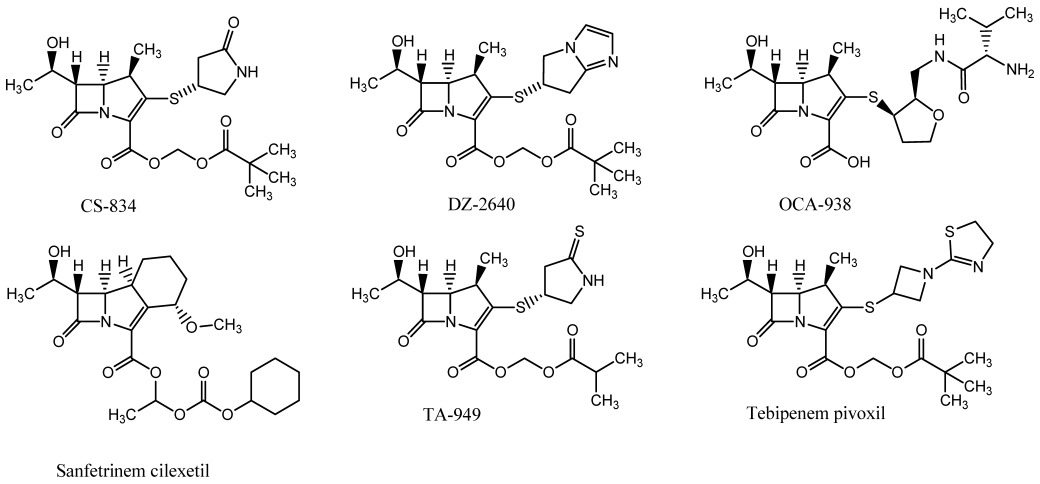


Fig. 1 Orally active carbapenems reported previously.

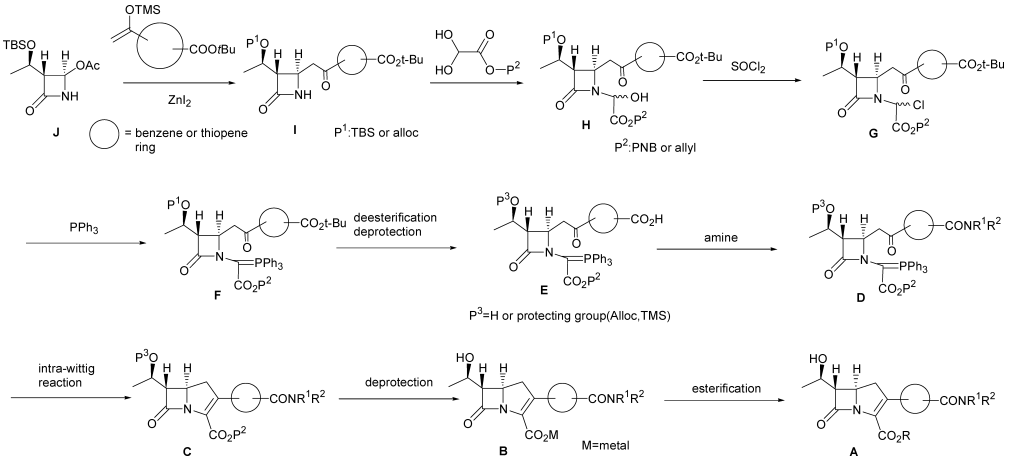


Fig. 2 Synthesis of 2-phenyl and 2-thienylcarbapenems (Scheme 1).

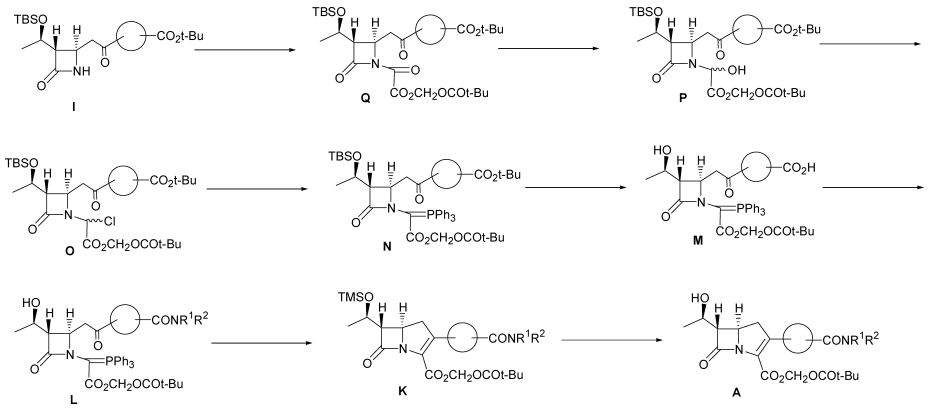


Fig. 3 Synthesis of 2-phenyl and 2-thienylcarbapenems (Scheme 2).

The desired prodrug-ester **A** was prepared from (2*R*, 3*R*)-3-((1*R*)-1-{[*tert*-butyl(dimethyl)silyl]oxy}ethyl)-4-oxo-2-azetidinyl acetate (**J**) as the starting material. The commercially available compound (**J**) and the corresponding sillylenol-ether were reacted in the presence of zinc iodide to give compound (**I**). The compound (**I**) and glyoxylate monohydroxylate were heated under dehydrating conditions in an inert solvent to give a corresponding hemiacetal compound (**H**), which was changed into the chloride (**G**) and treated with triphenylphosphine to give the phoshorane (**F**). The phosphorane (**F**) was deesterified and amidated to the corresponding amide (**D**). The critical cyclization to the carbapenem nucleus, **C**, was accomplished by the internal Wittig reaction of a keto phosphorane (**D**) with refluxing in toluene or xylene. Subsequent deprotection and purification of **C** gave the desired product (**B**). Esterification of **B** with pivaloyloxymethyl iodide gave the prodrug-ester **A**.

Protecting group P^1, P^2, and P^3 was *t*-butyldimethylsilyl (TBS) or an allyloxycarbonyl group, an allyl or *p*-nitrobenzylester, and a trimethylsillyl or allyloxycarbonyl group, respectively.

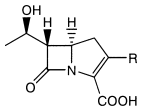
Some prodrug-esters could also be prepared from the phosporane **M** that had the pivaloyloxymethyl ester moiety [15, 16] as shown in Fig. 3.

Biological Properties

The MICs of 14 derivatives of 2-arylcarbapenem against Gram-positive and Gram-negative bacteria are listed in Tables 1～3. For reference, the MICs of a parenteral carbapenem, imipenem (IPM), and typical orally active β-lactams such as cefditoren (CDTR), which is an active form of cefditoren - pivoxil, faropenem (FRPM), and ampicillin - sulbactam (ABPC-SBT), were also determined (Table1).

2-Phenyl-6-(2-hydroxyethyl)carbapenem (**1**), which has the simplest structure in this series of compounds was highly active against both PRSP and BLNAR, the two most important targets of new orally active antibiotics as described above. The MIC_{90} against 27 clinical isolates of

Table 1 Antibacterial activity of 2-phenyl-6-(2-hydroxyethyl)carbapenem and reference β-lactam antibiotics



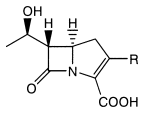
Organism	R–	MIC (μg/ml)				
		1 	IPM	CDTR	FRPM	ABPC/SBT
Staphylococcus aureus 209P		0.063	≦0.016	0.25	0.063	0.125s
Staphylococcus epidermidis IAM1296		0.25	0.5	4	2	0.25
Enterococcus faecalis ATCC19433		0.5	1	>128	1	2
Escherichia coli NIHJ JC-2		0.125	0.125	0.063	0.25	2
Klebsiella pneumoniae ATCC10031		≦0.016	0.125	≦0.016	0.063	2
Proteus mirabilis GN2425		0.063	0.25	0.063	0.5	2
Proteus vulgaris OX-19		0.25	1	0.063	0.5	0.25
Pseudomonas aeruginosa IFO3451		64	1	32	>128	64
Serratia marcescens χ100		0.25	0.25	0.5	2	8
Enterobacter cloacae GN7471		2	0.125	64	1	32
Citrobacter freundii GN346		1	0.5	16	2	16
Moraxella catarrhalis ATCC3077		≦0.016	≦0.016	≦0.016	≦0.016	≦0.016
MIC_{90} against *Streptococcus pneumoniae* (PRSP, n=27)		0.125	0.125	1	1	16
MIC_{90} against *Haemophilus influenzae* (BLNAR, n=24)		0.25	32	0.5	8	32

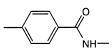
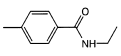
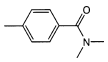
PRSP was 0.125 μg/ml, which was the same as that of IPM and more than 8-fold lower than those of any comparator tested, and the MIC_{90} against 24 clinical isolates of BLNAR was 0.25 μg/ml, which was more than 2-fold lower than that of other β-lactams. The antibacterial activity of **1** against *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Enterococcus faecalis*, *Escherichia coli*, *Klebsiella pneumoniae*, and *Proteus* spp., which are also important causative pathogens in community-acquired infections, was very potent and the MICs were comparable or superior to those of the reference antibiotics. Compound **1** still maintained potent activities against *Enterobacter cloacae*, *Serratia marsescence*, and *Citrobacter freundii*, but the MICs were significantly higher than those of IPM. Moreover, the antipseudomonal activity of **1** was much weaker than that of IPM and considered to be ineffective when **1** was administered orally. Thus, the preferable antibacterial profile of 2-phenylcarbapenem as an orally active agent, *e.g.* a broad spectrum of antibacterial activity covering PRSP, BLNAR, and other major pathogens causing community-acquired infections and remarkably low antipseudomonal activity, was confirmed.

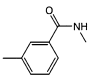
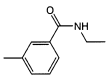
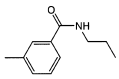
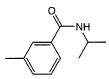
Next, we investigated the effect of substituents in the phenyl ring at the C-2 side chain (Table 2). The introduction of a *p*-aminocarbonyl group significantly increased the activity against most of the bacterial strains tested (**1** *vs.* **2**), however, the introduction of a *m*-aminocarbonyl group (**6**) was not effective, and actually reduced the antibacterial activity of **1**.

A comparison of **2**, **3**, **4** and **6**, **7**, **8** revealed that the introduction of an alkyl group into the aminocarbonyl moiety gave different SARs for the *para*- and the *meta*-substitution. In the case of *p*-aminocarbonyl derivatives (**2**∼**5**), replacing the amino residue with methylamine (**3**), ethylamine (**4**), and dimethylamine (**5**) tended to reduce the antibacterial activity. Unlike the *p*-substitution, the introduction of a methyl (**7**), ethyl (**8**), *n*-propyl (**9**), or *i*-propyl (**10**) group into the aminocarbonyl moiety of **6** did not significantly reduce the antibacterial activity, and in fact increased the activity against most of the bacteria tested. Among these *m*-substituted derivatives, compound **9** had the strongest antibacterial activity. Thus, based on the difference in the effect of the subsutituent among the regioisomers, it was thought that the geometry and the size of the substituent in the aminocarbonyl moiety affect the antibacterial activity of 2-phenylcarbapenems. Moreover,

Table 2 Antibacterial activity of derivatives of 2-(-4-aminocarbonylphenyl)-6-(2-hydroxyethyl)carbapenem



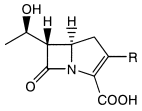
Organism	R–	MIC (μg/ml)			
		2	**3**	**4**	**5**
					
Staphylococcus aureus 209P		0.031	0.031	0.031	0.125
Staphylococcus epidermidis IAM1296		0.125	0.25	0.25	0.5
Enterococcus faecalis ATCC19433		0.125	0.25	0.25	0.5
Escherichia coli NIHJ JC-2		0.031	≦0.016	0.031	0.25
Klebsiella pneumoniae ATCC10031		≦0.016	≦0.016	≦0.016	0.063
Proteus mirabilis GN2425		0.031	0.031	0.063	0.125
Proteus vulgaris OX-19		0.031	0.031	0.063	0.125
Pseudomonas aeruginosa IFO3451		16	32	32	>128
Serratia marcescens χ100		0.063	0.125	0.125	1
Enterobacter cloacae GN7471		0.5	1	2	8
Citrobacter freundii GN346		0.25	0.5	1	8
Moraxella catarrhalis ATCC3077		≦0.016	≦0.016	≦0.016	≦0.016
MIC_{90} against *Streptococcus pneumoniae* (PRSP, n=27)		0.063	0.063	0.063	0.063
MIC_{90} against *Haemophilus influenzae* (BLNAR, n=24)		1	1	2	1

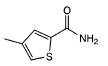
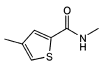
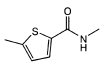
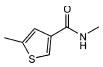
Organism	R–	**6**	**7**	**8**	**9**	**10**
						
Staphylococcus aureus 209P		0.125	0.125	0.063	0.063	0.125
Staphylococcus epidermidis IAM1296		1	1	0.5	0.25	0.5
Enterococcus faecalis ATCC19433		1	1	0.5	0.25	0.5
Escherichia coli NIHJ JC-2		0.125	0.063	0.031	0.031	0.063
Klebsiella pneumoniae ATCC10031		0.063	0.031	≦0.016	≦0.016	≦0.016
Proteus mirabilis GN2425		0.25	0.125	0.063	0.031	0.063
Proteus vulgaris OX-19		0.25	0.125	0.063	0.031	0.063
Pseudomonas aeruginosa IFO3451		128	64	64	64	64
Serratia marcescens χ100		0.25	0.25	0.125	0.125	0.25
Enterobacter cloacae GN7471		8	8	8	8	16
Citrobacter freundii GN346		1	2	1	2	4
Moraxella catarrhalis ATCC3077		≦0.016	≦0.016	≦0.016	≦0.016	≦0.016
MIC_{90} against *Streptococcus pneumoniae* (PRSP, n=27)		0.125	0.25	0.25	0.25	0.25
MIC_{90} against *Haemophilus influenzae* (BLNAR, n=24)		1	1	1	0.5	1

the dimethylaminocarbonyl derivative **5** had significantly less antibacterial activity than the ethylaminocarbonyl derivative (**4**), which has the same molecular weight, suggesting the importance of the hydrogen atom on the amino residue for the antibacterial activity.

Focusing on the slight geometric difference between the *para*- and *meta*-positions of the thienyl ring and those of the phenyl ring, we investigated the antibacterial

Table 3 Antibacterial activity of derivatives of 2-aminocarbonylthienyl-6-(2-hydroxyethyl)carbapenem



Organism	R–	MIC (μg/ml)			
		11	**12**	**13**	**14**
					
Staphylococcus aureus 209P		≦0.016	≦0.016	0.125	0.031
Staphylococcus epidermidis IAM1296		0.25	0.25	1	0.25
Enterococcus faecalis ATCC19433		0.125	0.125	1	0.25
Escherichia coli NIHJ JC-2		0.063	0.031	0.063	0.031
Klebsiella pneumoniae ATCC10031		0.031	≦0.016	0.063	≦0.016
Proteus mirabilis GN2425		0.063	0.031	0.25	0.063
Proteus vulgaris OX-19		0.063	0.031	0.25	0.063
Pseudomonas aeruginosa IFO3451		32	128	128	32
Serratia marcescens χ100		0.125	0.125	0.25	0.25
Enterobacter cloacae GN7471		0.5	1	1	1
Citrobacter freundii GN346		0.25	0.5	0.5	0.5
Moraxella catarrhalis ATCC3077		≦0.016	≦0.016	≦0.016	≦0.016
MIC_{90} against *Streptococcus pneumoniae* (PRSP, n=27)		0.063	0.063	0.125	0.125
MIC_{90} against *Haemophilus influenzae* (BLNAR, n=24)		1	1	0.25	1

activity of thienyl derivatives (Table 3). Consistent with their structural and physicochemical similarity, compound **11** exhibited remarkable potent activity similar to the corresponding 2-phenylcarbapenem (**2**). Also, the introduction of a methyl group into the aminocarbonyl moiety on the thiophene ring had a significant impact on the antibacterial activity of **11**. In addition, the effect of the methyl group differed among the regioisomers (**12**, **13**, **14**), suggesting that a slight geometric change of substituents might influence the antibacterial activity.

Taken together, our SAR study suggested that this series of 2-phenyl and 2-thienyl carbapenems has a favorable antibacterial spectrum for orally active carbapenems and could be optimized for antibacterial activity and hopefully for other pharmacological, pharmacokinetic, and toxicological properties through chemical modification of the C-2 side chain.

In order to assess the pharmacokinetic potential of 2-phenyl and 2-thienylcarbapenems, stability in human plasma and susceptibility to hydrolysis by human DHP-I were investigated. As shown in Table 4, 2-phenyl and thienylcarbapenems were relatively unstable compared with IPM, especially the unsubstituted **1**, which was rapidly decomposed in human plasma. The introduction of substituents at the benzene ring significantly improved the stability of 2-phenylcarbapenems in human plasma. Although a significant reduction in the stabilizing effect of substituent was observed for **8**, **10** and **12**, a clear SAR was not seen with this limited number of compounds.

As expected, most of the 2-phenyl and thienyl carbapenems were highly resistant to hydrolysis by human DHP-I in comparison with IPM: the rates of hydrolysis were not notably changed among the compounds tested, suggesting that the substituent effect was not obvious. However, in contrast to human DHP-I, mouse DHP-I rapidly hydrolyzed compounds **3**, **6**, **7**, and **12** but not IPM (Table 4). Although a limited number of compounds have been tested, this result suggests that these carbapenems might rapidly disappear after their administration in mice. To avoid this species-specific effect by mouse DHP-I, we decided to pre-treat mice with a DHP-I inhibitor, cilastatin, in the subsequent pharmacokinetic analysis. Mice were subcutaneously injected with 2 mg of cilastatin, which based on preliminary experiments was considered an excess amount to inhibit DHP-I activity in mice, 5 minutes before being administered 10 mg/kg of compound.

Table 4 Pharmacokinetic study of 2-phenyl and 2-thienyl-6-(2-hydroxyethyl)carbapenems

Compound No.	Stability in human plasma	Relative hydrolysis rate by		Mouse pharmacokinetic parameters		
	Residual % after 4 hours at 37°C	Human DHP-I	Mouse DHP-I	F (%)	Cmax (μg/ml)	AUC (μg·h/ml)
1	b.q.l.	0.34	—	—	—	—
2	46	0.38	—	2	1.0	0.17
3	40	0.27	8.03	14	3.1	0.91
4	40	0.43	—	11	3.8	1.12
5	60	0.70	—	23	4.9	1.77
6	52	0.20	6.94	3	1.6	0.22
7	33	0.47	7.93	23	5.7	1.81
8	11	0.31	—	34	20.7	4.89
10	7	0.54	—	13	5.1	0.87
12	b.q.l.	0.47	9.01	11	2.7	0.40
13	36	0.24	—	36	6.9	1.43
14	40	0.68	—	21	4.4	0.88
IPM	60	1.00	1.00	—	—	—

b.q.l., below quantitation limit.

Based on our previous study of the pharmacokinetics of 2-phenylpenem derivatives, we synthesized ester-type prodrugs possessing a pivaloyloxymethyl (POM) esterized carboxyl acid at the C-3-position to improve oral absorption. As expected, after the administration of POM esters of **2**∼**8**, **10**, **12**∼**14**, the corresponding active metabolites were detected in mouse serum (Table 4). Their bioavailability (%F) varied from 2% to 36% and the maximum serum concentration (Cmax) and area under blood concentration curve (AUC) also differed among these compounds. Although the bioavailability seems to correlate with the lipophilicity and/or the geometry of the substituents, further investigation will be necessary to clarify fine SAR.

Conclusion

In conclusion, the SAR of a series of 2-phenyl and thienylcarbapenems was investigated. The derivatives were highly active against clinical isolates of both PRSP and BLNAR in comparison with the other β-lactams tested. In addition, the spectrum of the antibacterial activity seems favorable for an orally active agent targeting mainly community-acquired infections. POM esters of these carbapenems showed significant oral absorption, indicating that this series of carbapenems is highly promising as a prototype of an orally active carbapenems. The substituent in the phenyl and the thienyl ring affected both antibacterial activity and oral absorption. Further modification and optimization of this series of compounds could result in novel orally active carbapenems.

Experimental

General Analytical Methods

IR spectra were recorded on a Perkin Elmer 1600 or JASCO 4200 FT-IR spectrometer. NMR spectra were recorded on a JEOL JNM-LA300 or Bruker 400 MHz spectrometer and chemical shifts are reported in δ values (ppm) relative to internal standard TMS for organic solvents, and unreferenced in D_2O. The high-resolution mass studies were conducted on a QSTAR mass spectrometer. Optical rotations were recorded on a Jasco DIP-370 at 20±2°C in the stated solvent; $[\alpha]_D$ values are given in deg·ml·g^{-1}·dm^{-1}.

***tert*-Butyl 4-{[(2*R*,3*S*)-3-((1*R*)-1-{[*tert*-Butyl(dimethyl)silyl]oxy}ethyl)-4-oxo-2-azetidinyl]acetyl}benzoate (I_2)**

To a solution of (2*R*,3*R*)-3-((1*R*)-1-{[*tert*-butyl(dimethyl)-silyl]oxy}ethyl)-4-oxo-2-azetidinyl acetate (**J**) (14.37 g) and *tert*-butyl 4-{[(trimethylsilyl)oxy]vinyl}benzoate (about 50 mmol) in dry methylene chloride (90 ml) was added zinc iodide (15.96 g, 50 mmol) at room temperature, and the mixture was stirred overnight at rt. The reaction mixture was diluted with aqueous $NaHCO_3$, and extracted with chloroform. The organic layer was washed successively with an aqueous sodium thiosulfate solution, a saturated brine, and dried over anhydrous magnesium sulfate. The solvent was evaporated under reduced pressure, and the residue was purified by silica gel column chromatography (chloroform/ethyl acetate) to give $\mathbf{I_2}$ (5.32 g). ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.08 (3H, s), 0.09 (3H, s), 0.88 (9H, s), 1.26 (3H, d, J=6.2 Hz), 1.62 (9H, s), 2.90 (1H, dd, J=2.3 Hz and 5.3 Hz), 3.13～3.25 (1H, m), 3.43～3.53 (1H, m), 4.08～4.18 (1H, m), 4.18～4.28 (1H, m), 6.13 (1H, s), 7.97 (2H, d, J=8.2 Hz), 8.09 (2H, d, J=8.3 Hz).

***tert*-Butyl 3-{[(2*R*,3*S*)-3-((1*R*)-1-{[*tert*-Butyl(dimethyl)silyl]oxy}ethyl)-4-oxo-2-azetidinyl]acetyl}benzoate (I_6)**

$\mathbf{I_6}$ (7.831 g) was obtained from **J** (11.78 g) in a similar manner to the preparation method of $\mathbf{I_2}$.

^{1}H NMR (400 MHz, $CDCl_3$) δ 0.08 (3H, s), 0.09 (3H, s), 0.88 (9H, s), 1.26 (3H, d, J=6.2 Hz), 1.62 (9H, s), 2.91 (1H, dd, J=2.3 Hz and 5.2 Hz), 3.14～3.28 (1H, m), 3.45～3.57 (1H, m), 4.10～4.19 (1H, m), 4.19～4.29 (1H, m), 6.13 (1H, s), 7.52～7.61 (1H, m), 8.08～8.15 (1H, m), 8.18～8.26 (1H, m), 8.49～8.55 (1H, m).

***tert*-Butyl 3-{[(2*R*,3*S*)-3-((1*R*)-1-{[(Allyloxy)carbonyl]oxy}ethyl)-4-oxo-2-azetidinyl]acetyl}benzoate (I_7)**

To the solution of $\mathbf{I_6}$ (2.24 g) obtained above in THF (20 ml) was added drop wise acetic acid (2.9 ml) and a solution of *tetra-n*-butylammonium fluoride (4.58 g) in THF (18 ml) at room temperature. The mixture was stirred at rt for 3 days, and diluted with ethyl acetate. The solution was washed with a cold aqueous $NaHCO_3$ and brine. The organic layer was dried over anhydrous magnesium sulfate. The solvent was evaporated under reduced pressure to give *tert*-butyl 3-({(2*R*,3*S*)-3-[(1*R*)-1-hydroxyethyl]-4-oxo-2-azetidinyl}-acetyl)benzoate, which was used in the subsequent reaction without further purification.

To the residue obtained above and 4-dimethylaminopyridine (1.34 g) in dry methylene chloride (20 ml) was added drop wise allyl chloroformate (1.21 g) under ice-cooling. The mixture was warmed gradually to room temperature, and stirred for 5 hours. The reaction mixture was diluted with ethyl acetate, and washed with cold aqueous $KHSO_4$ twice, brine, an aqueous $NaHCO_3$, and brine.

The organic layer was dried over anhydrous magnesium sulfate. The solvent was removed under reduced pressure, and the resulting residue was purified by silica gel column chromatography (hexane/ethyl acetate) to give $\mathbf{I_7}$ (1.126 g). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.49 (3H, d, J=6.3 Hz), 1.63 (9H, s), 3.10 (1H, dd, J=2.3 Hz and 8.2 Hz), 3.16～3.28 (1H, m), 3.52～3.62 (1H, m), 4.07～4.16 (1H, m), 4.57～4.70 (2H, m), 5.09～5.20 (1H, m), 5.22～5.41 (2H, m), 5.87～6.00 (1H, m), 6.20 (1H, s), 7.52～7.60 (1H, m), 8.07～8.14 (1H, m), 8.18～8.25 (1H, m), 8.47～8.54 (1H, m).

***tert*-Butyl 4-{[(2*R*, 3*S*)-3-((1*R*)-1-{[*tert*-Butyl-(dimethyl)silyl]oxy}ethyl)-4-oxoazetidin-2-yl]-acetyl}thiophene-2-carboxylate(I_{11})**

$\mathbf{I_{11}}$ was obtained from **J** and *tert*-butyl 4-{[(trimethylsilyl)oxy]vinyl}thiophene-2-carboxylate, which was obtained from *tert*-butyl 4-acetylthiophene-2-carboxylate by the well-known method disclosed in the literature [17] in a similar manner to the preparation method of $\mathbf{I_2}$. IR (KBr) 2968, 2930, 1760, 1712, 1686, 1534, 1370, 1275, 1256, 1157, 836, 778 cm^{-1}.

***tert*-Butyl 5-{[(2*R*,3*S*)-3-((1*R*)-1-{[*tert*-Butyl(dimethyl)silyl]oxy}ethyl)-4-oxo-2-azetidinyl]-acetyl}- 2-thiophenecarboxylate (I_{13})**

$\mathbf{I_{13}}$ was obtained from **J** in a similar manner to the preparation method of $\mathbf{I_2}$. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.07 (3H, s), 0.08 (3H, s), 0.88 (9H, s), 1.24 (3H, d, J=6.2 Hz), 1.59 (9H, s), 2.89 (1H, dd, J=2.3 Hz and 5.2 Hz), 3.07～3.18 (1H, m), 3.34～3.43 (1H, m), 4.08～4.16 (1H, m), 4.17～4.27 (1H, m), 6.08 (1H, s), 7.61～7.73 (2H, m).

***tert*-Butyl 5-{[(2*R*,3*S*)-3-((1*R*)-1-{[*tert*-Butyl(dimethyl)silyl]oxy}ethyl)-4-oxoazetidin-2-yl]-acetyl}thiophene-3-carboxylate (I_{14})**

$\mathbf{I_{14}}$ was obtained from *tert*-butyl 5-{1-[(trimethylsilyl)-oxy]vinyl}thiophene-3-carboxylate, which was obtained by the well-known method disclosed in the literature [17], and **J** in a similar manner to the preparation method of $\mathbf{I_2}$. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.07 (s, 3H), 0.08 (s, 3H), 0.87 (s, 9H), 1.24 (d, 3H, J=6.2 Hz), 1.59 (s, 9H), 2.89～2.91 (m, 1H), 3.10～3.16 (m, 1H), 3.37～3.42 (m, 1H), 4.09～4.13 (m, 1H), 4.21～4.23 (m, 1H), 6.09 (s, 1H), 8.01 (d, 1H, J=1.2 Hz), 8.29 (d, 1H, J=1.2 Hz).

***tert*-Butyl 4-({(2*R*,3*S*)-3-((1*R*)-1- {[*tert*-Butyl-(dimethyl)silyl]oxy}ethyl)-1-[2- [(4-nitrobenzyl)oxy]-2-oxo-1-(triphenylphosphoranylidene)ethyl]-4-oxo-2-azetidinyl}acetyl)benzoate (F_3)**

p-Nitrobenzyl glyoxylate monohydrate (1.477 g) was dissolved in toluene (50 ml), and the mixture was subjected to azeotropic dehydration under heating with reflux. To the solution was cooled to room temperature once, and added I_2 (2.238 g). The mixture was subjected to azeotropic dehydration under stirring with reflux. After the starting materials were consumed, the solvent was evaporated under reduced pressure. The residue was dissolved in dry THF (20 ml), and thereto was added 2,6-lutidine (809 mg). The mixture was cooled to −20 to −30°C. To the mixture was added dropwise thionyl chloride (898 mg) at the same temperature. The insoluble materials were separated by filtration, and washed with dry THF. The filtrate was concentrated under reduced pressure at a temperature below 35°C. The residue was dissolved in dry 1,4-dioxane (100 ml), and thereto were added triphenylphosphine (2.83 g) and 2,6-lutidine (1.179 g). The mixture was stirred at room temperature for one hour, and further stirred with heating at a bath temperature of 60°C for 3.5 hours. The mixture was cooled to room temperature, and diluted with cold aqueous citric acid solution. The mixture was extracted with ethyl acetate. The organic layer was washed successively with cold aqueous citric acid solution (twice), a saturated brine, an aqueous sodium hydrogen carbonate solution, and a saturated brine, dried over anhydrous magnesium sulfate, and the solvent was evaporated under reduced pressure. The residue was purified by silica gel column chromatography (hexane/ethyl acetate) to give F_3 (2.870 g). IR (KBr) 1746, 1716, 1688, 1625, 1522, 835, 774, 751, 719 cm^{-1}.

***tert*-Butyl 4-{[(2*R*,3*S*)-1-[2-(Allyloxy)-2-oxo-1-(triphenylphosphoranylidene)ethyl]-3-((1*R*)-1- {[*tert*-butyl(dimethyl)silyl]oxy}ethyl)-4-oxoazetidin-2-yl]acetyl}benzoate (F_2)**

To a solution of I_2 (15.06 g) in toluene (300 ml) was added allylglyoxylate monohydrate (8.89 g) and the mixture was refluxed for 8 hours. The solvent was evaporated under reduced pressure. The residue (25.5 g) was dissolved in dry THF (230 ml), and added 2,6-lutidine (5.76 g). To the mixture was added dropwise thionyl chloride (5.20 g) at −20°C. The insoluble materials were separated by filtration, and washed with dry THF. The filtrate was concentrated under reduced pressure at a temperature below 35°C. The residue was dissolved in dry 1,4-dioxane (230 ml), and added triphenylphosphine (19.74 g) and 2,6-lutidine (8.21 g). The mixture was stirred at 60°C for 3.5 hours. The mixture was cooled to room temperature, and diluted with ethyl acetate and cold 5%$KHSO_4$ aqueous solution. The mixture was extracted and separated. The organic layer was washed successively with cold saturated $NaHCO_3$ aqueous solution and brine, and dried over anhydrous sodium sulfate. The solvent was evaporated under reduced pressure. The residue was purified by silica gel column chromatography (hexane/ethyl acetate) to give F_2 (15.90 g). IR (KBr) 1748, 1715, 1685, 1624, 1294, 1256, 1224, 1107, 834, 693 cm^{-1}.

***tert*-Butyl 3-({(2*R*,3*S*)-3-((1*R*)-1-{[*tert*-Butyl(dimethyl)silyl]oxy}ethyl)-1-[{[(2,2-dimethyl-propanoyl)oxy]methoxy}(oxo)acetyl]-4-oxo-2-azetidinyl}acetyl)benzoate (Q_6)**

I_6 (2.24 g) and triethylamine (1.11 g) were dissolved in dry methylene chloride (10 ml), and thereto was added drop wise a solution of [(2-chloro-2-oxoacetyl)oxy]methyl pivalate (10 mmol) in dry methylene chloride (10 ml) under ice-cooling. The reaction was quenched by addition of saturated aqueous ammonium chloride solution. The mixture was extracted with ethyl acetate. The organic layer was washed successively with an aqueous sodium hydrogen carbonate solution and brine, dried over anhydrous magnesium sulfate, and the solvent was removed under reduced pressure. The residue was purified by silica gel column chromatography (hexane/ethyl acetate) to give Q_6 (3.152 g). ^1H NMR (400 MHz, $CDCl_3$) δ 0.03 (3H, s), 0.08 (3H, s), 0.85 (9H, s), 1.24 (9H, s), 1.29 (3H, d, J=6.4 Hz), 1.62 (9H, s), 3.15～3.31 (1H, m), 3.35～3.53 (1H, m), 3.89～4.04 (1H, m), 4.28～4.47 (1H, m), 4.77～5.41 (1H, m), 5.92 (2H, s), 7.49～7.64 (1H, m), 8.05～8.18 (1H, m), 8.18～8.30 (1H, m), 8.52 (1H, broad s).

***tert*-Butyl 3-({(2R, 3S)-3-((1R)-1-{[*tert*-Butyl-(dimethyl)silyl]oxy}ethyl)-1-[2-{[(2,2-dimethyl-propanoyl)oxy]methoxy}-2-oxo-1-(triphenylphosphoranylidene)ethyl]-4-oxo-2-azetidinyl}acetyl)benzoate (N_6)**

Q_6 (1.90 g) was dissolved in acetic acid (10 ml) and methylene chloride (10 ml), and thereto was added zinc powder (5.88 g) under ice-cooling. The mixture was vigorously stirred at the same temperature for 15 minutes, and the reaction solution was filtered on cerite and washed with chloroform. The filtrate and washings were combined and washed with a cold aqueous sodium hydrogen carbonate solution (three times) and brine (once), and dried over anhydrous magnesium sulfate. The solvent was removed under reduced pressure to give a corresponding hemiacetal compound (1.923 g), which was further treated in a similar manner to the preparation method of F_3 to give

N_6 (1.706 g). IR (KBr) 1748, 1718, 1689, 1641, 834, 753, 693 cm^{-1}.

***tert*-Butyl 3-({(2*R*,3*S*)-3-((1*R*)-1-{[(Allyloxy)carbonyl]oxy}ethyl)-1-[2-(allyloxy)-2-oxo-1-(triphenylphosphoranylidene)ethyl]-4-oxo-2-azetidinyl}acetyl)benzoate (F_6)**

F_6 (945 mg) was obtained from **I_7** (1.026 g) in a similar manner to the preparation method of **F_3**. IR (KBr) 1749, 1715, 1687, 755, 693 cm^{-1}.

***tert*-Butyl 5-{[(2*R*,3*S*)-1-[2-(Allyloxy)-2-oxo-1-(triphenylphosphoranylidene)ethyl]-3-((1*R*)-1-{[*tert*-butyl(dimethyl)silyl]oxy}ethyl)-4-oxoazetidin-2-yl]acetyl}thiophene-2-carboxylate (F_{13})**

F_{13} was obtained from **I_{13}** (1.026 g) in a similar manner to the preparation method of **F_3**. IR (KBr) 1748, 1711, 1665, 1439, 1371, 1295, 1257, 1230, 1163, 1103, 833, 754, 694 cm^{-1}.

4-({(2*R*,3*S*)-3-[(1*R*)-1-Hydroxyethyl]-1-[2-[(4-nitrobenzyl)oxy]-2-oxo-1-(triphenylphosphoranylidene)ethyl]- 4-oxo-2-azetidinyl}acetyl)benzoic Acid (E_3)

To **F_3** (2.703 g, 3.00 mmol) was added trifluoroacetic acid (9 ml) under ice-cooling, and the mixture was further stirred at room temperature for 30 minutes. The solvent was evaporated under reduced pressure, and the residue was dissolved again in chloroform/toluene, and concentrated again under reduced pressure. To the residue was added hexane/diethyl ether, and the mixture was subjected to decantation (three times), and the resulting solid was collected by filtration, washed with hexane, and dried under reduced pressure to give **E_3** (2.187 g). IR (KBr) 3428 (broad), 1719, 1680, 1523, 750, 721 cm^{-1}.

4-({(2*R*,3*S*)-1-[2-(Allyloxy)-2-oxo-1-(triphenylphosphoranylidene)ethyl]-3-[(1*R*)-1-hydroxyethyl]-4-oxoazetidin-2-yl}acetyl)benzoic Acid (E_2)

To **F_2** (7.5 g) was added trifluoroacetic acid (15 ml) under ice-cooling and dissolved it, and the mixture was further stirred at room temperature for 50 minutes. The solvent was evaporated under reduced pressure with toluene to give **E_2** (11.26 g). IR (KBr) 3448 (broad), 1773, 1701, 1685, 1676, 1648, 1202, 722, 690 cm^{-1}.

5-({(2*R*,3*S*)-1-[2-(Allyloxy)-2-oxo-1-(triphenylphosphoranylidene)ethyl]-3-[(1*R*)-1-hydroxyethyl]-4-oxoazetidin-2-yl}acetyl)thiophene-2-carboxylic Acid (E_{13})

E_{13} was obtained from **F_{13}** in a similar manner to the preparation method of **E_2**. IR (KBr) 3439 (broad), 1772, 1708, 1663, 1441, 1378, 1192, 1108, 749, 724, 690 cm^{-1}.

4-Nitrobenzyl [(2*R*,3*S*)-3-[(1*R*)-1-Hydroxyethyl]-2-(2-{4-[(methylamino)carbonyl]phenyl}-2-oxoethyl)-4-oxo-1-azetidinyl](triphenylphosphoranylidene)acetate (D_3)

E_3 (2.137 g) was dissolved in dry THF (24 ml), and thereto was added dropwise a solution of triethylamine (354 mg) in dry THF (3 ml) at −30°C. Subsequently, to the mixture was added dropwise a solution of ethyl chloroformate (348 mg) in dry THF (3 ml). In addition, to the mixture was added dropwise a solution of triethylamine (354 mg) in THF (3 ml), and then thereto was added dropwise a solution of monomethylamine in 40% methanol (249 mg). The mixture was warmed to 0°C, and to the reaction solution were added ethyl acetate and ice-water. The mixture was extracted, and the organic layer was washed successively with an aqueous sodium hydrogen carbonate solution, brine, diluted hydrochloric acid and an aqueous sodium hydrogen carbonate solution, dried over anhydrous magnesium sulfate, and the solvent was removed under reduced pressure to give **D_3** (1.909 g). IR (KBr) 3388 (broad), 1742, 1649, 1607, 1521, 753, 720 cm^{-1}.

Allyl {(2*R*,3*S*)-2-{2-[4-(Aminocarbonyl)phenyl]-2-oxoethyl}-3-[(1*R*)-1-hydroxyethyl]-4-oxo-1-azetidinyl}(triphenylphosphoranylidene)acetate (D_2)

D_2 (945 mg) was obtained from **E_2** (1.026 g) in a similar manner to the preparation method of **D_3**. IR (KBr) 3418 (broad), 1744, 1675, 1619, 1202, 751, 720, 692 cm^{-1}.

Allyl ((2*R*,3*S*)-3-((1*R*)-1-{[(Allyloxy)carbonyl]oxy}ethyl)-2-{2-[3-(aminocarbonyl)phenyl]-2-oxoethyl}-4-oxo-azetidinyl)(triphenylphosphoranylidene)acetate (D_6)

3-({(2*R*,3*S*)-3-((1*R*)-1-{[(Allyloxy)carbonyl]oxy}ethyl)-1-[2-(allyloxy)-2-oxo-1-(triphenylphosphoranylidene)ethyl]-4-oxo-2-azetidinyl}acetyl)benzoic acid (**E_6**) was obtained from **F_6** (889 mg) in a similar manner to the preparation method of **E_3**, which was used in the subsequent reaction without further purification.

D_6 (324 mg) was obtained from **E_6** and a 28% aqueous ammonia solution in a similar manner to the preparation method of **D_3**. IR ($CHCl_3$) 3413, 1746, 1679, 1614, 1260 cm^{-1}.

{[2-{(2*R*,3*S*)-2-{2-[3-(Aminocarbonyl)phenyl]-2-oxoethyl}-3-[(1*R*)-1-hydroxyethyl]-4-oxo-1-azetidinyl}-2-(triphenylphosphoranylidene)acetyl]oxy}methyl Pivalate (L_6)

L_6 was obtained from **N_6** in a similar manner to the preparation method of **D_3**. IR ($CHCl_3$) 1744, 1676 cm^{-1}.

Allyl {(2*R*,3*S*)-2-{2-[4-(Ethylaminocarbonyl)phenyl]-2-oxoethyl}-3-[(1*R*)-1-hydroxyethyl]-4- oxo-1-azetidinyl}(triphenylphosphoranylidene)acetate (D_4)

D_4 was obtained from E_2 in a similar manner to the preparation method of D_3. IR (KBr) 3408 (broad), 1733, 1641, 1106, 754, 720, 693 cm^{-1}.

Allyl [(2*R*,3*S*)-3-((1*R*)-1-{[(Allyloxy)carbonyl]oxy}ethyl)-2-(2-{3-[(methylamino)carbonyl]phenyl}-2-oxoethyl)-4-oxo-1-azetidinyl](triphenylphosphoranylidene)acetate (D_7)

D_7 was obtained from a solution of E_6 and monomethylamine in 40% methanol in a similar manner to the preparation method of D_3. IR ($CHCl_3$) 1746, 1661 cm^{-1}.

{[2-[(2*R*,3*S*)-3-[(1*R*)-1-Hydroxyethyl]-2-(2-{3-[(methylamino)carbonyl]phenyl}-2-oxoethyl)-4-oxo-1-azetidinyl]-2-(triphenylphosphoranylidene)acetyl]oxy} methyl Pivalate (L_7)

L_7 was obtained from M_6 in a similar manner to the preparation method of D_3. IR ($CHCl_3$) 1745, 1660 cm^{-1}.

Allyl {(2*R*,3*S*)-2- (2-{3-[(Ethylamino)carbonyl]phenyl}-2-oxoethyl)-3- [(1*R*)-1- hydroxyethyl]-4-oxazetidin-1-yl}(triphenylphosphoranylidene)acetate (D_8)

D_8 was obtained from I_6 in a similar manner to the preparation method of D_2. IR (KBr) 3394 (broad), 1740, 1640, 1543, 1438, 1302, 1257, 1226, 1106, 753, 721, 694 cm^{-1}.

Allyl [(3*S*,4*R*)-3-[(1*R*)-1-Hydroxyethyl]-2-oxo-4-(2-oxo-2-{3-[(propylamino)carbonyl]phenyl}ethyl)azetidin-1-yl](triphenylphosphoranylidene)acetate (D_9)

D_9 was obtained from I_6 in a similar manner to the preparation method of D_2. IR (KBr) 3372 (broad), 2969, 2931, 1735, 1640, 1542, 1438, 1304, 1255, 1227, 1107, 752, 720, 694 cm^{-1}.

Allyl [(2*R*,3*S*)-3-[(1*R*)-1-Hydroxyethyl]-2-(2-{3-[(isopropylamino)carbonyl]phenyl}-2-oxoethyl)-4-oxoazetidin-1-yl](triphenylphospho-ranylidene)acetate (D_{10})

D_{10} was obtained from I_6 in a similar manner to the preparation method of D_2.

IR (KBr) 3340 (broad), 2975, 2932, 1739, 1636, 1540, 1438, 1256, 1228, 1107, 753, 719, 693 cm^{-1}.

Allyl {(2*R*,3*S*)-2-{2-[5-(Aminocarbonyl)thien-3-yl]-2-oxoethyl}-3-[(1*R*)-1-hydroxyethyl]-4-oxoazetidin-1-yl}(triphenylphosphoranylidene)acetate (D_{11})

D_{11} was obtained from I_{11} in a similar manner to the preparation method of D_2. IR (KBr) 3412 (broad), 2973, 1735, 1668, 1612, 1439, 1107, 754, 694 cm^{-1}.

Allyl [(2*R*,3*S*)-3-[(1*R*)-1-Hydroxyethyl]-2-(2-{5-[(methylamino)carbonyl]thien-3-yl}-2-oxoethyl)-4-oxoazetidin-1-yl](triphenylphosphoranylidene)acetate (D_{12})

D_{12} was obtained from I_{11} in a similar manner to the preparation method of D_2. IR (KBr) 3426 (broad), 2934, 1734, 1634, 1558, 1438, 1412, 1307, 1255, 1107, 754, 719, 694 cm^{-1}.

Allyl ((2*R*,3*S*)-2-(2-{5-[(Methylamino)carbonyl]-2-thienyl}-2-oxoethyl)-4-oxo-3-{(1*R*)-1-[(trimethylsilyl)-oxy]ethyl}-1-azetidinyl)(triphenylphosphoranylidene)-acetate (D_{13})

D_{13} was obtained from I_{13} in a similar manner to the preparation method of D_2.

Allyl [(2*R*,3*S*)-3-[(1*R*)-1-Hydroxyethyl]-2-(2-{4-[(methylamino)carbonyl]thien-2-yl}-2-oxoethyl)-4-oxoazetidin-1-yl](triphenylphosphoranylidene)acetate (D_{14})

D_{14} was obtained from I_{14} in a similar manner to the preparation method of D_2. IR (KBr) 3335 (broad), 3083, 1734, 1651, 1560, 1438, 1296, 1258, 1192, 753, 693 cm^{-1}.

4-Nitrobenzyl (5*R*,6*S*)-6-[(1*R*)-1-Hydroxyethyl]-3-{4-[(methylamino)carbonyl]phenyl}-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate (C_3)

To a solution of D_3 (844 mg) in THF (20 ml) was added N,O-bis (trimethylsilyl)acetamide (1.38 g) and 2,6-di-*tert*-butyl-*p*-cresol (25 mg) at room temperature, and the mixture was heated with stirring at bath temperature of 80°C for 2 hours. The solvent was evaporated under reduced pressure to give 4-nitrobenzyl((2*R*,3*S*)-2-(2-{4-[(methyl amino)carbonyl]phenyl}-2-oxoethyl)-4-oxo-3-{(1*R*)-1-[(trimethylsilyl)oxy]ethyl}-1-azetidinyl)(triphenyl-phosphoranylidene) acetate, which was used in the subsequent step without further purification.

To 4-nitrobenzyl ((2*R*,3*S*)-2-(2-{4-[(methylamino)carbonyl]phenyl}-2-oxoethyl)-4-oxo-3-{(1*R*)-1-[(trimethylsilyl)oxy]ethyl}-1-azetidinyl)(triphenylphosphoranylidene) acetate obtained in the above Step was added toluene (70 ml), and the mixture was heated with stirring at bath temperature of 120°C for 2 hours. The solvent was evaporated under reduced pressure, and the resulting

residue was dissolved again in ethyl acetate (100 ml). To the solution was added 0.1 N hydrochloric acid (0.5 ml) under ice-cooling, and the mixture was vigorously stirred. After the starting materials were consumed, an aqueous sodium hydrogen carbonate solution was added to the reaction mixture for neutralization, and the mixture was separated. The organic layer was washed with a saturated brine, and dried over anhydrous magnesium sulfate. The solvent was evaporated under reduced pressure, and the resulting residue was purified by silica gel column chromatography (chloroform/acetone) to give **C_3** (204 mg). ^{1}H NMR (400 MHz, $CDCl_3$/CD_3OD=8/1) δ 1.36 (3H, d, J=6.3 Hz), 2.98 and 2.99 (combined 3H, each s), 3.18～3.41 (3H, m), 4.16～4.26 (1H, m), 4.35 (1H, dt, J=2.8 Hz and 9.4 Hz), 5.15～5.40 (2H, m), 7.39 (2H, d, J=8.5 Hz), 7.45 (2H, d, J=8.8 Hz), 7.74 (2H, d, J=8.5 Hz), 8.16 (2H, d, J=8.8 Hz).

Allyl (5*R*,6*S*)-6-((1*R*)-1-{[(Allyloxy)carbonyl]oxy}ethyl)-3-[3-(aminocarbonyl)phenyl]-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate (C_6)

To **D_6** (324 mg) and 2,6-di-*tert*-butyl-*p*-cresol (10 mg) was added toluene (30 ml), and the mixture was heated with stirring at bath temperature of 100°C for 2 hours, and then further heated with stirring at 130°C for 2 hours. The solvent was evaporated under reduced pressure, and the resulting residue was purified by silica gel thin layer chromatography (ethyl acetate) to give **C_6** (251 mg). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.49 (3H, d, J=6.3 Hz), 3.19～3.37 (2H, m), 3.43 (1H, dd, J=2.8 Hz and 8.3 Hz), 4.31 (1H, dt, J=2.9 Hz and 9.7 Hz), 4.58～4.77 (4H, m), 5.11～5.44 (5H, m), 5.80～6.02 (2H, m), 7.74～7.80 (1H, m), 7.86～7.92 (1H, m).

[(2,2-Dimethylpropanoyl)oxy]methyl(5*R*,6*S*)-3-[3-(aminocarbonyl)phenyl]-7-oxo-6-{(1R)-1-[(trimethylsilyl)oxy]ethyl}-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate (K_6)

L_6 (34.7 mg) and chlorotrimethylsilane (21 mg) were dissolved in dry THF (1.5 ml), and thereto was added dropwise triethylamine (20 mg) under ice-cooling. After the starting materials were consumed, the reaction mixture was diluted with aqueous sodium hydrogen carbonate solution,and extracted with ethyl acetate. The organic layer was washed with brine, and dried over anhydrous sodium sulfate. The solvent was evaporated under reduced pressure to give {[2-((2*R*,3*S*)-2-{2-[3-(aminocarbonyl)phenyl]-2-oxoethyl}-4-oxo-3-{(1*R*)-1-[(trimethylsilyl)oxy]ethyl}-1-azetidinyl)-2-(triphenylphosphoranylidene)acetyl]oxy} methyl pivalate, which was used in the subsequent reaction without further purification.

K_6 (11 mg) was obtained from the compound obtained above in a similar manner to the preparation method of **C_6**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.15 (9H, s), 1.18 (9H, s), 1.29 (3H, d, J=6.2 Hz), 3.17～3.27 (2H, m), 3.28～3.38 (1H, m), 4.18～4.29 (2H, m), 5.80 (2H, s), 7.40～7.52 (2H, m), 7.76～7.85 (2H, m).

Allyl (5*R*,6*S*)-6-((1*R*)-1-{[(Allyloxy)carbonyl]oxy}ethyl)-3-{3-[(methylamino)carbonyl]phenyl}-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate (C_7)

C_7 (214 mg) was obtained from **D_7** (312 mg) in a similar manner to the preparation method of **C_6**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.49 (3H, d, J=6.3 Hz), 2.97～3.06 (3H, m), 3.19～3.37 (2H, m), 3.43 (1H, dd, J=2.8 Hz and 8.3 Hz), 4.30 (1H, dt, J=2.9 Hz and 9.4 Hz), 4.57～4.77 (4H, m), 5.11～5.43 (5H, m), 5.80～6.01 (2H, m), 7.51～7.59 (2H, m), 7.78～7.84 (1H, m).

Allyl (5*R*,6*S*)-6-[(1*R*)-1-Hydroxyethyl]-3-{5-[(methylamino)carbonyl]-2-thienyl}-7-oxo-1-azabicyclo-[3.2.0]hept-2-ene-2-carboxylate (C_{13})

C_{13} was obtained from **E_{13}** in a similar manner to the preparation method of **C_3**. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.39 (3H, d, J=6.3 Hz), 2.99 and 3.00 (total 3H, each s), 3.26 (1H, dd, J=2.8 Hz and 6. 8 Hz), 3.32～3.52 (2H, m), 4.22～4.34 (2H, m), 4.71～4.93 (2H, m), 5.25～5.53 (2H, m), 5.93～6.08 (2H, m), 7.44～7.55 (2H, m).

(5*R*,6*S*)-6-[(1*R*)-1-Hydroxyethyl]-3-{4-[(methylamino)-carbonyl]phenyl}-7-oxo-1-azabicyclo[3.2.0]hept- 2-ene-2-carboxylic acid sodium salt (3)

C_3 (60 mg) was dissolved in THF (6 ml) and ion-exchange water (4.7 ml), and thereto was added 0.1 N aqueous sodium hydrogen carbonate solution (1.3 ml). To the mixture was added 10% palladium on carbon (120 mg), and the mixture was subjected to hydrogenolysis for 30 minutes under atmospheric pressure at room temperature. The catalyst was removed by filtration, and washed with water, and chloroform.

The mixture was extracted with water(three times). The organic solvent in the aqueous layer was removed under reduced pressure, and the resultant was purified by polymer chromatography (CHP-20P). The fractions eluted with 0～2% aqueous THF solution were combined and lyophilized to give **3** (18 mg). ^{1}H NMR (400 MHz, D_2O) δ 1.23 (3H, d, J=6.4 Hz), 2.83 (3H, s), 2.93～3.10 (1H, m), 3.29～3.43 (1H, m), 3.45 (1H, dd, J=2.8 Hz and 5.9 Hz), 4.07～4.33 (2H, m), 7.35 (2H, d, J=8.5 Hz), 7.60 (2H, d, J=8.5 Hz). $[\alpha]_D$=−37.31° (c=0.304 , EtOH).

(5*R*,6*S*)-3-[3-(Aminocarbonyl)phenyl]-6-[(1*R*)-1-hydroxyethyl]-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic Acid Sodium Salt (6)

C_6 (80 mg) and aniline (339 mg) were dissolved in monochlorobenzene (4 ml), and thereto was added tetrakistriphenylphosphine palladium (11 mg) under ice-cooling. The mixture was stirred at the same temperature for 1 hour, and thereto were added 0.1 N aqueous sodium hydrogen carbonate solution (10 ml). The mixture was washed with chloroform twice. The organic solvent contained in the aqueous layer was removed under reduced pressure, and the resultant was purified by polymer chromatography (CHP-20P). The fractions eluted with water were combined, and lyophilized to give **6** (8 mg). ^{1}H NMR (400 MHz, D_2O) δ 1.23 (3H, d, J=6.4 Hz), 2.99～3.09 (1H, m), 3.34～3.44 (1H, m), 3.46 (1H, dd, J=2.8 Hz and 6.0 Hz), 4.13～4.29 (2H, m), 7.36～7.44 (1H, m), 7.46～7.52 (1H, m), 7.60～7.67 (2H, m). HRMS calcd for $C_{16}H_{16}N_2O_5Na$ 339.0951, found 339.0942.

(5*R*,6*S*)-3-[4-(Aminocarbonyl)phenyl]-6-[(1*R*)-1-hydroxyethyl]-7-oxo-1-azabicyclo[3.2.0] hept-2-ene-2-carboxylic Acid Sodium Salt (2)

2 was obtained from **D_2** in a similar manner to the preparation method of **6**. ^{1}H NMR (400 MHz, D_2O) δ 1.23 (3H, d, J=6.4 Hz), 2.78～2.93 (1H, m), 3.34～3.44 (1H, m), 3.46 (1H, dd, J=2.7 Hz and 5.8 Hz), 4.12～4.30 (2H, m), 7.37 (2H, d, J=8.4 Hz), 7.68 (2H, d, J=8.4 Hz). HRMS calcd for $C_{16}H_{16}N_2O_5Na$ 339.0951, found 339.0974. $[\alpha]_D$=−51.41° (c=0.229, EtOH).

(5*R*,6*S*)- 3-[4-(Ethylaminocarbonyl)phenyl]-6-[(1*R*)-1-hydroxyethyl]-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic Acid Sodium Salt (4)

4 was obtained from **D_4** in a similar manner to the preparation method of **6**. ^{1}H NMR (400 MHz, D_2O) δ 1.12 (3H, t, J=7.3 Hz), 1.23 (3H, d, J=6.4 Hz), 2.94～3.06 (1H, m), 3.25～3.41 (3H, m), 3.44 (1H, dd, J=2.8 Hz and 5.9 Hz), 4.13～4.26 (2H, m), 7.34 (2H, d, J=8.4 Hz), 7.59 (2H, d, J=8.4 Hz). HRMS calcd for $C_{18}H_{20}N_2O_5Na$ 367.1264, found 367.1276.

(5*R*,6*S*)-3-[4-(Dimethylaminocarbonyl)phenyl]-6-[(1*R*)-1-hydroxyethyl]-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic Acid Sodium Salt (5)

5 was obtained from **D_5** in a similar manner to the preparation method of **6** except that sodium 2-ethylhexanoate was used instead of aniline. ^{1}H NMR (400 MHz, D_2O) δ 1.23 (3H, d, J=6.4 Hz), 2.94 (3H, s), 2.96～3.08 (1H, m), 3.01 (3H, s), 3.33～3.43 (1H, m), 3.46 (1H, dd, J=2.8 Hz and 6.0 Hz), 4.11～4.29 (2H, m), 7.27～7.42 (4H, m).

(5*R*,6*S*)-6-[(1*R*)-1-Hydroxyethyl]-3-{3-[(methylamino)carbonyl]phenyl}-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic Acid Sodium Salt (7)

7 (20 mg) was obtained from **C_7** (70 mg) in a similar manner to the preparation method of **6**. ^{1}H NMR (400 MHz, D_2O) δ 1.23 (3H, d, J=6.4 Hz), 2.83 (3H, s), 2.97～3.07 (1H, m), 3.33～3.43 (1H, m), 3.45 (1H, dd, J=2.8 Hz and 6.0 Hz), 4.13～4.29 (2H, m), 7.34～7.42 (1H, m), 7.43～7.49 (1H, m), 7.53～7.60 (2H, m). HRMS calcd for $C_{17}H_{18}N_2O_5Na$ 353.1107, found 353.1118.

(5*R*,6*S*)-3-{3-[(Ethylamino)carbonyl]phenyl}-6-[(1*R*)-1-hydroxyethyl]-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic Acid Sodium Salt (8)

8 was obtained from **D_8** in a similar manner to the preparation method of **5**. ^{1}H NMR (400 MHz, D_2O) δ 1.13 (t, 3H, J=7.3 Hz), 1.23 (d, 3H, J=6.4 Hz), 3.02～3.06 (m, 1H), 3.29～3.47 (m, 4H), 4.13～4.28 (m, 2H), 7.36～7.56 (m, 4H). HRMS calcd for $C_{18}H_{20}N_2O_5Na$ 367.1264, found 367.1266. $[\alpha]_D$=−4.01°(c=0.311, EtOH).

(5*R*,6*S*)-6-[(1*R*)-1-Hydroxyethyl]-7-oxo-3-{3-[(propylamino)carbonyl]phenyl}-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic Acid Sodium Salt (9)

9 was obtained from **D_9** in a similar manner to the preparation method of **5**. ^{1}H NMR (400 MHz, D_2O) δ 0.86 (t, 3H, J=7.4 Hz), 1.23 (d, 3H, J=6.4 Hz), 1.51～1.56 (m, 2H), 2.99～3.06 (m, 1H), 3.26 (t, 2H, J=7.0 Hz), 3.36～3.46 (m, 2H), 4.17～4.24 (m, 2H), 7.36～7.40 (m, 1H), 7.44～7.47 (m, 1H), 7.55～7.56 (m, 2H). HRMS calcd for $C_{19}H_{22}N_2O_5Na$ 381.1420, found 381.1420.

(5*R*,6*S*)-6-[(1*R*)-1-Hydroxyethyl]-3-{3-[(isopropylamino)-carbonyl]phenyl}-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic Acid Sodium Salt (10)

10 was obtained from **D_{10}** in a similar manner to the preparation method of **5**. ^{1}H NMR (400 MHz, D_2O) δ 1.56 (d, 6H, J=6.6 Hz), 2.23 (d, 3H, J=6.4 Hz), 2.99～3.06 (m, 1H), 3.56～3.42 (m, 2H), 4.03～4.07 (m, 1H), 4.17～4.26 (m, 2H), 7.35～7.39 (m, 1H), 7.44～7.46 (m, 1H), 7.52～7.54 (m, 2H). HRMS calcd for $C_{19}H_{22}N_2O_5Na$ 381.1420, found 381.1427.

(5*R*,6*S*)-3-[5-(Aminocarbonyl)thien-3-yl]-6-[(1*R*)-1-hydroxyethyl]-7-oxo-1-azabicyclo[3.2.0]hept-2-ene- 2-carboxylic Acid Sodium Salt (11)

11 was obtained from **D_{11}** in a similar manner to the preparation method of **5**. ^{1}H NMR (400 MHz, D_2O) δ 1.21

(d, 3H, J=6.4 Hz), 3.03～3.10 (m, 1H), 3.22～3.29 (m, 1H), 3.38～3.40 (m, 1H), 4.13～4.20 (m, 2H), 7.58 (d, 1H, J=1.4 Hz), 7.77 (d, 1H, J=1.4 Hz). HRMS calcd for $C_{14}H_{14}N_2O_5S_1Na$ 345.0515, found 345.0523.

(5*R*,6*S*)-6-[(1*R*)-1-Hydroxyethyl]-3-{5-[(methylamino)-carbonyl]thien-3-yl}-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic Acid Sodium Salt (12)

12 was obtained from $\mathbf{D_{12}}$ in a similar manner to the preparation method of **5**. ^{1}H NMR (400 MHz, D_2O) δ 1.22 (d, 3H, J=6.4 Hz), 2.81 (s, 3H), 3.04～3.11 (m, 1H), 3.24～3.41 (m, 1H), 3.39～3.41 (m, 1H), 4.15～4.18 (m, 2H), 7.54 (d, 1H, J=1.4 Hz), 7.70 (d, 1H, J=1.4 Hz).

(5*R*,6*S*)-6-[(1*R*)-1-Hydroxyethyl]- 3-{5-[(methylamino)carbonyl]-2-thienyl}7-oxo-1-azabicyclo-[3.2.0]hept-2-ene-2-carboxylic Acid Sodium Salt (13)

13 was obtained from $\mathbf{C_{13}}$ in a similar manner to the preparation method of **6**. ^{1}H NMR (400 MHz, D_2O) δ 1.20 (3H, d, J=6.4 Hz), 2.78 (3H, s), 3.11～3.33 (2H, m), 3.41 (1H, dd, J=2.9 Hz and 5.8 Hz), 4.08～4.24 (2H, m), 7.10 (1H, d, J=4.0 Hz), 7.42 (1H, d, J=4.1 Hz).

(5*R*,6*S*)-6-[(1*R*)-1-Hydroxyethyl]-3-{4-[(methylamino)-carbonyl]thien-2-yl}-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic Acid Sodium Salt (14)

14 was obtained from $\mathbf{D_{14}}$ in a similar manner to the preparation method of **6**. ^{1}H NMR (400 MHz, D_2O) δ 1.22 (d, 3H, J=6.4 Hz), 2.79 (s, 3H), 3.16～3.30 (m, 2H), 3.38～3.40 (m, 1H), 4.13～4.19 (m, 2H), 7.33 (d, 1H, J=1.5 Hz), 7.86 (d, 1H, J=1.5 Hz). HRMS calcd for $C_{15}H_{16}N_2O_5S_1Na$ 359.0672, found 359.0687. $[\alpha]_D$=−532.43° (c=0.311, EtOH).

[(2,2-Dimethylpropanoyl)oxy]methyl(5*R*,6*S*)-6-[(1*R*)-1-hydroxyethyl]-3-{4-[(methylamino)carbonyl]phenyl}-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate ($\mathbf{A_3}$)

To a solution of **3** (5 mg) in dry dimethylformamide (1 ml) was added pivaloyloxymethyl iodide (53 mg) under ice-cooling. The mixture was stirred at the same temperature for 1 hour. To the reaction mixture were added ice-water, and extracted with ethyl acetate. The organic layer was washed successively with a saturated brine (four times), an aqueous sodium hydrogen carbonate solution, an aqueous sodium thiosulfate solution, and a saturated brine, and dried over anhydrous magnesium sulfate. The solvent was evaporated under reduced pressure, and the residue was purified by silica gel thin layer chromatography (chloroform/methanol=9/1) to give $\mathbf{A_3}$ (27 mg). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.18 (9H, s), 1.37 (3H, d, J=6.3 Hz), 3.02 and 3.03 (combined 3H, each s), 3.15～3.41 (3H, m), 4.20～4.39 (2H, m), 5.70～5.90 (2H, m), 6.19 (1H, d, J=4.8 Hz), 7.39 (2H, d, J=8.4 Hz), 7.72 (2H, d, J=8.5 Hz). HRMS calcd for $C_{23}H_{29}N_2O_7$ 445.1969, found 445.1964.

[(2,2-Dimethylpropanoyl)oxy]methyl(5*R*,6*S*)-3-[3-(aminocarbonyl)phenyl]-7-oxo-6-{(1*R*)-1-[(trimethylsilyl)oxy]ethyl}-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate ($\mathbf{A_6}$)

$\mathbf{K_6}$ (11 mg) was dissolved in ethyl acetate (2 ml) and THF (2 ml), and thereto was added 0.1 N hydrochloric acid under ice-cooling to adjust the pH value thereof to the range of from pH 2 to pH 3. The mixture was vigorously stirred for 20 minutes, and the reaction solution was diluted with ethyl acetate, and washed with a cold aqueous sodium hydrogen carbonate solution. The organic layer was dried over anhydrous magnesium sulfate, and evaporated under reduced pressure. The residue was purified by silica gel thin layer chromatography (ethyl acetate/acetone=4/1) to give $\mathbf{A_6}$ (9 mg). ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.17 (9H, s), 1.36 (3H, d, J=6.3 Hz), 3.17～3.42 (3H, m), 4.20～4.38 (2H, m), 5.80 (2H, s), 7.40～7.52 (2H, m), 7.77～7.87 (2H, m). HRMS calcd for $C_{22}H_{27}N_2O_7$ 431.1812, found 431.1823.

[(2,2-Dimethylpropanoyl)oxy]methyl(5*R*,6*S*)-3-[4-(aminocarbonyl)phenyl]-6-[(1*R*)-1-hydroxyethyl]-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate ($\mathbf{A_2}$)

$\mathbf{A_2}$ was obtained from **1** in a similar manner to the preparation method of $\mathbf{A_3}$. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.18 (9H, s), 1.38 (3H, d, J=6.3 Hz), 3.16～3.42 (3H, m), 4.21～4.39 (2H, m), 5.73～5.88 (2H, m), 7.42 (2H, d, J=8.4 Hz), 7.79 (2H, d, J=8.4 Hz). HRMS calcd for $C_{22}H_{27}N_2O_7$ 431.1812, found 431.1815. $[\alpha]_D$=−53.64° (c=0.095, $CHCl_3$).

[(2,2-Dimethyl propanoyl)oxy]methyl(5*R*,6*S*)-3-[4-(ethylaminocarbonyl)phenyl]-6-[(1*R*)-1-hydroxyethyl]-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate ($\mathbf{A_4}$)

$\mathbf{A_4}$ was obtained from **4** in a similar manner to the preparation method of $\mathbf{A_3}$. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.18 (9H, s), 1.26 (3H, t, J=7.3 Hz), 1.37 (3H, d, J=6.3 Hz), 3.15～3.41 (3H, m), 3.43～3.57 (2H, m), 4.22～4.38 (2H, m), 5.72～5.89 (2H, m), 6.12 (1H, broad s), 7.39 (2H, d, J=8.5 Hz), 7.73 (2H, d, J=8.4 Hz). HRMS calcd for $C_{22}H_{27}N_2O_7$ 431.1812, found 431.1815.

(2,2-Dimethylpropanoyl)oxy]methyl(5*R*,6*S*)-6-[(1*R*)-1-hydroxyethyl]-3-{3-[(methylamino)carbonyl]phenyl}-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate ($\mathbf{A_7}$)

$\mathbf{A_7}$ (1.9 mg) was obtained from $\mathbf{L_7}$ (32 mg) in a similar manner to the preparation method of $\mathbf{A_6}$. ^{1}H NMR

(400 MHz, $CDCl_3$) δ 1.18 (9H, s), 1.38 (3H, d, J=6.3 Hz), 3.02 and 3.03 (combined 3H, each s), 3.19～3.39 (3H, m), 4.23～4.37 (2H, m), 5.79 (2H, s), 7.39～7.48 (2H, m), 7.71～7.81 (2H, m). HRMS calcd for $C_{23}H_{29}N_2O_7$ 445.1969, found 445.1976.

[(2,2-Dimethylpropanoyl)oxy]methyl(5*R*,6*S*)-3-{3-[(ethylamino)carbonyl]phenyl}-6-[(1*R*)-1-hydroxyethyl]-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate (A_8)

A_8 was obtained from **8** in a similar manner to the preparation method of A_3. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.17 (s, 9H), 1.25 (dt, 3H, J=7.2 and 2.0 Hz), 1.37 (d, 3H, J=6.3 Hz), 3.20～3.36 (m, 3H), 3.47～3.54 (m, 2H), 4.25～4.34 (m, 2H), 5.76～5.81 (m, 2H), 6.41 (br-s, 1H), 7.41～7.44 (m, 1H), 7.73～7.76 (m, 1H). HRMS calcd for $C_{24}H_{31}N_2O_7$ 459.2125, found 459.2124. $[\alpha]_D$=−3.03° (c=0.312, $CHCl_3$)

[(2,2-Dimethylpropanoyl)oxy]methyl(5*R*,6*S*)-6-[(1*R*)-1-hydroxyethyl]-7-oxo-3-{3-[(propylamino)-carbonyl]phenyl}-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate (A_9)

A_9 was obtained **9** in a similar manner to the preparation method of A_3. ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.99 (t, 3H, J=7.4 Hz), 1.17 (s, 9H), 1.37 (d, 3H, J=6.3 Hz), 1.63～1.70 (m, 2H), 3.20～3.36 (m, 3H), 3.40～3.45 (m, 2H), 4.25～4.34 (m, 2H), 5.76～5.81 (m, 2H), 6.42 (br-s, 1H), 7.40～7.45 (m, 2H), 7.73～7.75 (m, 2H). HRMS calcd for $C_{25}H_{33}N_2O_7$ 473.2282, found 473.2276.

[(2,2-Dimethylpropanoyl)oxy]methyl(5*R*,6*S*)-6-[(1*R*)-1-hydroxyethyl]-3-{3-[(isopropylamino)carbonyl]phenyl}-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate (A_{10})

A_{10} was obtained from **10** in a similar manner to the preparation method of A_3. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.16 (s, 9H), 1.27 (d, 6H, J=6.6 Hz), 1.37 (d, 3H, J=6.3 Hz), 2.96～3.36 (m, 3H), 4.24～4.34 (m, 3H), 5.75 (d, 1H, J=5.5 Hz), 5.82 (d, 1H, J=5.5 Hz), 6.13 (br-d, 1H, J=8.0 Hz), 7.41～7.45 (m, 2H), 7.69～7.73 (m, 2H). HRMS calcd for $C_{25}H_{33}N_2O_7$ 473.2282, found 473.2278.

[(2,2-Dimethylpropanoyl)oxy]methyl(5*R*,6*S*)-3-[5-(aminocarbonyl)thien-3-yl]-6-[(1*R*)-1-hydroxyethyl]-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate (A_{11})

A_{11} was obtained from **11** in a similar manner to the preparation method of A_3. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.22 (s, 9H), 1.37 (d, 3H, J=6.3 Hz), 1.96 (br-d, 1H, J=4.3 Hz), 3.22～3.25 (m, 1H), 3.34 (d, 2H, J=9.4 Hz), 4.23～4.29 (m, 2H), 5.88 (d, 1H, J=5.6 Hz), 5.91 (d, 1H, J=5.6 Hz), 7.72 (d, 1H, J=1.3 Hz), 7.95 (d, 1H, J=1.3 Hz). HRMS calcd for $C_{20}H_{25}N_2O_7S_1$ 437.1387, found 437.1384.

[(2,2-Dimethylpropanoyl)oxy]methyl(5*R*,6*S*)-6-[(1*R*)-1-hydroxyethyl]-3-{5-[(methylamino)carbonyl]thien-3-yl}-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate (A_{12})

A_{12} was obtained from **12** in a similar manner to the preparation method of A_3. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.23 (s, 9H), 1,36 (d, 3H, J=6.3 Hz), 3.01 (d, 3H, J=4.8 Hz), 3.22～3.24 (m, 1H), 3.34 (d, 2H, J=9.4 Hz), 4.22～4.30 (m, 2H), 5.88～5.92 (m, 2H), 6.91 (br-d, 1H, J=4.5 Hz), 7.65 (d, 1H, J=1.3 Hz), 7.87 (d, 1H, J=1.3 Hz). HRMS calcd for $C_{21}H_{27}N_2O_7S_1$ 451.1533, found 451.1541.

[(2,2-Dimethylpropanoyl)oxy]methyl(5*R*,6*S*)-6-[(1*R*)-1-hydroxyethyl]-3-{5-[(methyl amino)carbonyl]-2-thienyl}-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate (A_{13})

A_{13} (7.9 mg) was obtained from **13** (11 mg) in a similar manner to the preparation method of A_3. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.23 (9H, s), 1.37 (3H, d, J=6.3 Hz), 2.99 and 3.00 (combined 3H, each s), 3.25 (1H, dd, J=2.8 Hz and 6.8 Hz), 3.31～3.53 (2H, m), 4.22～4.33 (2H, m), 5.89～6.07 (2H, m), 7.47～7.54 (2H, m).

[(2,2-Dimethylpropanoyl)oxy]methyl(5*R*,6*S*)-6-[(1*R*)-1-hydroxyethyl]-3-{4-[(methylamino)carbonyl]thien-2-yl}-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate (A_{14})

A_{14} was obtained from **14** in a similar manner to the preparation method of A_3. ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.23 (s, 9H), 1.36 (d, 3H, J=6.3 Hz), 2.99 (d, 3H, J=4.8 Hz), 3.22～3.24 (m, 1H), 3.33～3.50 (m, 2H), 4.23～4.28 (m, 2H), 5.91 (d, 1H, J=5.6 Hz), 5.98 (d, 1H, J=5.6 Hz), 6.51～6.52 (m, 1H), 7.87 (d, 1H, J=1.3 Hz), 8.01 (d, 1H, J=1.3 Hz). HRMS calcd for $C_{21}H_{27}N_2O_7S_1$ 451.1533, found 451.1534. $[\alpha]_D$=22.06° (c=0.312, $CHCl_3$).

Other Antibiotics

Imipenem (IPM) and cilastatin were prepared from Thienam® (Banyu Pharmaceutical Co., Ltd., Tokyo, Japan). Cefditoren (CDTR) was prepared from Meiact® (Meiji Seika Kaisha Ltd., Tokyo, Japan). Faropenem (FRPM) and ampicillin - sulbactam (ABPC/SBT) were obtained from commercial sources.

Bacterial Strains

Standard strains (ATCC, IFO, and GN) and clinical isolates of PRSP and BLNAR from Japanese, American and European hospitals were from bacterial collection in our laboratory. They were identified by standard methods and kept as stock cultures at −70°C or below.

Determination of *In Vitro* Antibacterial Activity

MICs were determined by the twofold serial agar dilution

method, with Mueller-Hinton agar (MHA; Nippon BD Company Ltd., Tokyo, Japan) unless otherwise specified. Susceptibility testing was performed with MHA supplemented with 5% defibrinated horse blood for streptococci and with 5% Fildes enrichment (BBL Microbiology Systems, Cockeysville, Md.) for *H. influenzae*. Cells of test strains were grown in appropriate medium at 37°C overnight, and diluted with phosphate buffered saline supplemented with 0.01% gelatin to give a final concentration of approximately 10^6 CFU/ml. A portion (5 μl) of the dilution was placed onto a drug-containing agar surface with a Microplanter® (Sakuma Seisakusho, Tokyo). The plates were incubated at 37°C overnight. The MIC was defined as the lowest antibiotic concentration that completely prevented visible growth.

Resistance to Hydrolysis by DHP-I

The stability of carbapenems against DHP-Is was determined with purified mouse renal DHP-I [18] and recombinant human DHP-I which was prepared according to the method reported by Adachi [19]. The activity of DHP-I was spectrophotometrically determined by measuring the hydrolysis of glycyldehydrophenyl alanine as a substrate [20]. The relative rate of hydrolysis was also calculated as a ratio against the rate for IPM, which was assigned a value of 1.00.

Oral Absorption in Mice

Three-week-old male slc:ICR mice weighing 11 g to 13 g were purchased from Japan SLC, Inc. (Shizuoka, Japan), and adapted to standardized environmental conditions (temperature, 23±2°C; humidity, 55±10%) for 1 week before the experiments. All animal procedures were performed in accordance with the institution's guidelines for the humane handling, care, and treatment of research animals. The mice (22～24g) were supplied only with water containing 40% glucose and 5% casamino acid for 20 hours then subcutaneously injected with 2 mg of cilastatiin 5 minutes before drug administration. Next, 10 mg/kg of pivaroyloxymethylester suspended with 0.5% methocel containing 5% dimethylsulfoxide, was administered orally and then blood samples were collected at 5, 15, 30, and 60 minutes (n=3). The level of biologically active carbapenems in plasma was determined by a bioassay method with *Bacillus subtillis* ATCC6633. Meanwhile, the corresponding active metabolites of ester prodrugs were dissolved with saline containing 5 mM MOPS and intravenously administered *via* the tail vein in mice. The pharmacokinetic parameters were calculated according to the moment analysis.

Acknowledgements We acknowledge the excellent technical assistance of K. Urasaki and Y. Hirai.

References

1. Nicolau D. Clinical and economic implications of antimicrobial resistance for the management of community-acquired respiratory tract infections. J Antimicrob Chemother 50: 61–70 (2002)
2. Ubukata K. Problems associated with high prevalence of multidrug-resistant bacteria in patients with community-acquired infections. J Infect Chemother 9: 285–291 (2003)
3. Aguilar L, Gimenez MJ, Garcia-Rey C, Martin JE. New strategies to overcome antimicrobial resistance in *Streptococcus pneumoniae* with β-lactam antibiotics. J Antimicrob Chemother 50: 93–100 (2002)
4. Edwards JR, Betts MJ. Carbapenems: the pinnacle of the β-lactam antibiotics or room for improvement? J Antimicrob Chemother 45: 1–4 (2000)
5. Dancer SJ. The problem with cephalosporins. J Antimicrob Chemother 48: 463–478 (2001)
6. Bitha P, Li Z, Lin YI. Convergent syntheses of oral THF 1β-methylcarbapenems. J Antibiot 52: 643–648 (1999)
7. Hikida M, Itahashi K, Igarashi A, Shiba T, Kitamura M. *In vitro* antibacterial activity of LJC 11,036, an active metabolite of L-084, a new oral carbapenem antibiotic with potent antipneumococcal activity. Antimicrob Agents Chemother 43: 2010–2016 (1999)
8. Seki M, Yamanaka T, Kondo K. Practical synthesis of (*R*)-4-mercaptopyrrolidine-2-thione from L-aspartic acid. Preparation of a novel orally active 1-β-methylcarbapenem, TA-949. J Org Chem 65: 517–522 (2000)
9. Tamura S, Miyazaki S, Tateda K, Ohno A, Ishii Y, Matsumoto T, Furuya N, Yamaguchi K. *In vivo* antibacterial activities of sanfetrinem cilexetil, a new oral tricyclic antibiotic. Antimicro Agents Chemother 42: 1858–1861 (1998)
10. Tanaka M, Hohmura M, Nishi T, Sato K, Hayakawa I. Antimicrobial activity of DU-6681a, a parent compound of novel oral carbapenem DZ-2640. Antimicrob Agents Chemother 41: 1260–1268 (1997)
11. Yamaguchi K, Domon H, Miyazaki S, Tateda K, Ohno A, Ishii K, Matsumoto T, Furuya N. *In vitro* and *in vivo* antibacterial activities of CS-834, a new oral carbapenem. Antimicrob Agents Chemother 42: 555–563 (1998)
12. Sunagawa M, Inoue T. Japan, Kokai Tokkyo Koho, JP88-208591
13. Cama LD, Wildonger KJ, Guthikonda R, Ratcliffe RW, Christensen BG. Total synthesis of thienamycin analogs-III. Tetrahedron 39: 2531–2549 (1983)
14. Guthikonda RN, Cama LD, Quesada M, Woods MF, Salzmann TN, Christensen BG. Structure-activity relationships in the 2-arylcarbapenem series: synthesis of 1-methyl-2-arylcarbapenems. J Med Chem 30: 871–80 (1987)

15. Sakurai O., Ogiku T, Takahashi M, Hayashi M, Yamanaka T, Horikawa H, Iwasaki T. A new synthesis of 1β-alkylcarbapenems utilizing Eschenmoser sulfide contraction of the novel thiazinone intermediates. J Org Chem 61: 7889–7894 (1996)
16. Mori M, Oida S. A short-step synthesis of orally active carbapenem antibiotic CS-834. Chem Pharm Bull 48: 126–130 (2000)
17. Rasmussen JK. *O*-Silylated Enolates - Versatile Intermediates for Organic Synthesis. Synthesis 91–110 (1977)
18. Fukasawa M, Sumita Y, Harabe ET, Tanio T, Nouda H, Kohzuki T, Okuda T, Matsumura H, Sunagawa M. Stability of meropenem and effect of 1β-methyl substitution on its stability in the presence of renal dehydropeptidase I. Antimicrob Agents Chemother 36: 1577–1579 (1992)
19. Adachi H, Katayama T, Inuzuka C, Oikawa S, Tsujimoto M, Nakazato H. Identification of membrane anchoring site of human renal dipeptidase and construction and expression of a cDNA for its secretory form. J Biol Chem 265: 15341–15345 (1990)
20. Campbell BJ. Renal dipeptidase. Methods Enzymol 19: 722–729 (1970)