Synthesis and Structure-activity Relationships of a Novel Oral Carbapenem, CS-834

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We have studied an ester prodrug of a carbapenem to develop a potent orally active β-lactam antibiotic. A variety of 1β-methylcarbapenem derivatives have been synthesized. We have found that some derivatives having an amide group in the C-2 side chain show potent and well balanced antibacterial activities as well as high stability against dehydropeptidase-I. Oral absorption of derivatives has been optimized by modifying the C-3 ester promoiety. Pivaloyloxymethyl (1R,5S,6S)-6-[(R)-1-hydroxyethyl]-1-methyl-2-[(R)-5-oxopyrrolidin-3-ylthio]-1-carbapen-2-em-3-carboxylate, CS-834, has been selected as the most promising compound for further evaluation.

An orally active antibiotic with potent activity is of much interest in the clinical realm because oral administration and lower dosage are advantageous for patients.

Carbapenems are the most potent β-lactam antibiotics which have a broad antibacterial spectrum and potent bactericidal activities against both Gram-positive and-negative organisms.1,2) They are highly resistant to hydrolysis by a variety of β-lactamases. So far, imipenem,3) panipenem4) and meropenem5) have been launched on the market, and several compounds are currently under clinical evaluation.6~8) However, most compounds have been developed for parenteral use, and none for the practical purpose of oral administration. Currently, tricyclic β-lactam antibiotics, GV-104326 and its ester GV-118819, have been developed. GV-118819 is now under clinical study as an oral antiinfective drug.9~11)

The chemical instability of carbapenem has been recognized as a serious problem for the development of oral carbapenem, although 1β-methyl substitution of carbapenem improved its stability to some extent.12) We expected that 1β-methylcarbapenems could be stable enough for oral administration by preferable contribution of the 1β-methyl group, and that the dosage could be reduced as a result of their powerful antibacterial effects.

We have synthesized a variety of 1β-methylcarbapenems derivatized at the C-2 side chain and evaluated them for antibacterial activities and other biological properties. We have found that some derivatives having an amide group in the C-2 side chain show potent and well balanced antibacterial activity as well as high stability against dehydropeptidase-I. These compounds have been submitted for prodrug approach to optimize oral absorption. Through the investigation we have successfully obtained an orally active carbapenem, pivaloyloxymethyl (1R,5S,6S)-6-[(R)-1-hydroxyethyl]-1-methyl-2-[(R)-5-oxopyrrolidin-3-ylthio]-1-carbapen-2-em-3-carboxylate (CS-834).13)

In this paper we will describe the synthesis and structure-activity relationship of CS-834 and related compounds.

Chemistry

The C-2 substituted derivatives of carbapenems were synthesized through the general procedure shown in Scheme 1. It includes condensation of the carbapenem-2-yl diphenylphosphate 112~14) with appropriate thiol 3a~n designed for the C-2 side chain to give carbapenem ester 4a~n, and the following deprotection of the 4-nitrobenzyl group by catalytic hydrogenation using 10% palladium on charcoal. The resulting carbapenem derivatives 6a~61 will be reported elsewhere. The sodium salt 6m (R-83201) was converted to the corresponding free acid (R-95867) by treatment with 1N HCl in water.

Optically active (R)- and (S)-4-mercapto-2-oxopyrrolidine 3m and 3n were prepared starting from (S)- and (R)-4-hydroxy-2-oxopyrrolidine 8,17) respectively, as...
Scheme 1.

1: $R^1 = \text{CH}_3$
2: $R^1 = \text{H}$
3 - 7: $R^2 = a - n$

a
b

c

d

e
f

$R^1 = \text{CH}_3$
$R^1 = \text{H}$

4a-n: $R^1 = \text{CH}_3$
5m: $R^1 = \text{H}$
6a-n: $R^1 = \text{CH}_3$
7m: $R^1 = \text{H}$

Scheme 2.

Scheme 3.
shown in Scheme 2.

Several acyloxyalkyl and alkoxy carbonyloxyalkyl groups were selected as potential ester promoieties with effective absorption from the intestinal tract following hydrolysis to the parent compound. Ester prodrugs 11~16 were prepared from carbapenems 6m, 6n and 7m in order to give them enough lipophilicity for oral absorption as shown in Scheme 3.

Among the ester derivatives 11~16, pivaloyloxy-methyl ester of the (R)-isomer 11 was obtained as a crystalline form, although the pivaloyloxymethyl ester of the (S)-isomer 15 was an amorphous powder. The crystalline form of 11 is of greater value for practical use because it is stable in storage and easy to handle and formulate.

The chemical stability of the ester in solution is also an important factor because an orally administered compound dissolves in the gastrointestinal fluid prior to absorption. The chemical stability of carbapenem ester 11 was examined in a phosphate buffer solution of pH 6.86 at 37°C. The carbapenem 11 decomposed apparently according to first order kinetics at a rate constant of 0.03 1 hour^{-1}.18) The carbapenem showed higher stability than various cephalosporins, of which we have examined the chemical stability under similar conditions.19) This finding is contrary to the conventional view that carbapenems are unstable even at neutral pH.

Biological Properties

The antimicrobial activity (MICs) of carbapenems are shown in Tables 1 and 2.

First of all, the antimicrobial activity of aromatic and heteroaromatic derivatives which have a carboxamide group in the C-2 side chain were examined. The carbapenem 6a, which has only a phenylthio group, showed excellent activity against Gram-positive bacteria such as Staphylococcus aureus, but it was inactive against Gram-negative bacteria. However, the aromatic and heteroaromatic derivatives 6b~f having a carboxamide group showed improved activity against Gram-negative bacteria compared to 6a as well as excellent activity against Gram-positive bacteria. The aryl and heteroarylthio derivatives 6e and 6f were more active against Gram-negative bacteria than the corresponding aryl and heteroarylthiol compounds 6b and 6c, respectively.

Secondly, aliphatic carboxamide or carbamate derivatives were examined. The derivatives 6g~j having an alkyl carboxamide were potent against Gram-negative bacteria, but they were less active against Gram-positive bacteria. The stereochemistry of the methyl group on the carbon next to the sulfur atom influenced the antibacterial activity. The (R)-isomer 6i showed better antibacterial activity than the (S)-isomer 6j. The carbamate derivative 6k showed potent activity similar to the carboxamides 6g and 6h.

Finally, in order to promote higher activity against Gram-positive bacteria, a 5-membered cyclic amide system was introduced. Although 2-oxopyrrolidin-3-ylthio derivative 6l became less active, the 5-oxopyrrolidin-3-ylthio derivatives 6m and 6n exhibited well balanced and potent antimicrobial activity against

<table>
<thead>
<tr>
<th>Table 1. Antibacterial activity (MIC, µg/ml) of carbapenems 6a~k and urinary recovery (%) in mice.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Staphylococcus aureus 209P</td>
</tr>
<tr>
<td>S. aureus 56R</td>
</tr>
<tr>
<td>S. aureus 535 (MRSA)</td>
</tr>
<tr>
<td>Enterococcus fecalis 681</td>
</tr>
<tr>
<td>E. coli 609</td>
</tr>
<tr>
<td>Salmonella enteritidis</td>
</tr>
<tr>
<td>Klebsiella pneumoniae 806</td>
</tr>
<tr>
<td>K. pneumoniae 846</td>
</tr>
<tr>
<td>Enterobacter cloacae 903</td>
</tr>
<tr>
<td>Serratia marcescens 1184</td>
</tr>
<tr>
<td>Proteus vulgaris 1420</td>
</tr>
<tr>
<td>Morganella morganii 1510</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa 1001</td>
</tr>
<tr>
<td>Urinary recovery (%)</td>
</tr>
<tr>
<td>(s.c.)</td>
</tr>
</tbody>
</table>

a. MIC was determined by agar dilution method with an inoculum of 10^3 cfu/ml.
b. Urinary recovery (%) was determined by disk method using Bacillus subtilis ATCC 6633 as a test strain after subcutaneous administration of compound (50 mg/kg) in SPF ddY mice (n=5, 0~24 hours).
Table 2. Antibacterial activity (MIC, μg/ml) of carbapenems 6l~n and 7m and urinary recovery (%) in mice.

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>MIC (μg/ml)</th>
<th>Urinary recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Staphylococcus aureus 209P</td>
<td>0.2</td>
<td>55</td>
</tr>
<tr>
<td>S. aureus 56R</td>
<td>0.4</td>
<td>75</td>
</tr>
<tr>
<td>S. aureus 535 (MRSA)</td>
<td>25</td>
<td>46</td>
</tr>
<tr>
<td>Enterococcus faecalis 681</td>
<td>12.5</td>
<td>8</td>
</tr>
<tr>
<td>Escherichia coli NIHJ</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>E. coli 609</td>
<td>0.2</td>
<td>0.2</td>
</tr>
<tr>
<td>Salmonella enteritidis</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>Klebsiella pneumoniae 806</td>
<td>1.5</td>
<td>12.5</td>
</tr>
<tr>
<td>K. pneumoniae 846</td>
<td>6.2</td>
<td>0.8</td>
</tr>
<tr>
<td>Enterobacter cloacae 903</td>
<td>0.4</td>
<td>0.2</td>
</tr>
<tr>
<td>Serratia marcescens 1184</td>
<td>0.2</td>
<td>0.2</td>
</tr>
<tr>
<td>Proteus vulgaris 1420</td>
<td>0.4</td>
<td>0.2</td>
</tr>
<tr>
<td>Morganella morganii 1510</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa 1001</td>
<td>0.02</td>
<td>0.2</td>
</tr>
</tbody>
</table>

Urinary recovery (%) = 55 75 46 8 87 91 (s.c.)

a) MIC was determined by agar dilution method with an inoculum of 10^7 cfu/ml.
b) Urinary recovery (%) was determined by disk method using Bacillus subtilis ATCC 6633 as a test strain after subcutaneous administration of compound (50 mg/kg) in SPF ddY mice (n = 5, 0~24 hours).
c) GV104326 was synthesised according to Glaxo's patent procedure.

both Gram-positive and -negative bacteria. As for the configuration at the C-3 position of 5-oxopyrrolidine, the (R)-isomer 6m was slightly more active than the (S)-isomer 6n against S. aureus, E. faecalis and some Gram-negative bacteria.

The 1-H carbapenem 7m which has the same C-2 side chain as compound 6m showed similar activity to 6m. The antimicrobial activity of 6m is superior to that of cepodoxime (CPDX) and almost equal to that of parenteral carbapenems such as imipenem and panipenem. However, they are less active against Pseudomonas aeruginosa. A new tricyclic Mactam, GV-104326, seems to be inferior to compound 6m in antibacterial activity against Gram-negative bacteria.

The urinary recovery of carbapenems was determined after subcutaneous administration to mice in order to estimate stability of carbapenems under biological conditions. Derivatives having an alkyl or cyclic amide were recovered in moderate amounts (40~75%) as shown in Tables 1 and 2.

Some stereoselective effects of the chiral center in the C-2 side chain were observed in urinary recovery. As to the alkyl amides 6i and 6j, recovery of the (R)-isomer 6j was less than that of the (S)-isomer 6i although 6j had better antibacterial activity. With respect to the cyclic amides 6m and 6n, urinary recovery was highly dependent on the configuration at the C-3 position of 5-oxopyrrolidine. The recovery of the (R)-isomer 6m was 75%, although the recovery of the (S)-isomer 6n was only 46%.

Table 3. Effect of cilastatin on urinary recovery of carbapenems in mice.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Urinary recoverya (%)</th>
<th>DHP-I Degradation rate ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>CS (--)</td>
<td>CS (+)</td>
<td></td>
</tr>
<tr>
<td>1β-Methylcarbapenem</td>
<td>75 84</td>
<td>1.0</td>
</tr>
<tr>
<td>6m (R)</td>
<td>46 78</td>
<td>3.3</td>
</tr>
<tr>
<td>1-H Carbapenem</td>
<td>8 64</td>
<td>14.2</td>
</tr>
</tbody>
</table>

| Carboxyls (50 mg/kg) were subcutaneously administered to mice (n = 5) with or without cilastatin (CS: 50 mg/kg as sodium salt). Urinary recovery was determined by the same method described in Table 1. Mouse renal dehydropeptidase-I (DHP-I) was used. The figures are relative to the degradation rate of 6m.

Simultaneous administration of cilastatin, an inhibitor of dehydropeptidase-I, with the (S)-isomer 6n resulted in improved urinary recovery equal to that of the (R)-isomer 6m. In addition, the degradation rate of 6m with mouse dehydropeptidase-I (DHP-I) was slower than that of 6n as listed in Table 3. These results clearly demonstrate that the (R)-isomer 6m is reliable in the biological system due to higher resistance against DHP-I in mice.

The recovery of the 1-H carbapenem 7m was only 8%, which was in accordance with the fast degradation by DHP-I as listed in Table 3. The urinary recovery of 7m was increased to 64% by coadministration with cilastatin. This result shows that the 1-H carbapenem 7m
is very labile in mice due to degradation by DHP-I.

From these experiments the 1\(\beta\)-methylcarbapenem 6m which has an (R)-5-oxopyrrolidin-3-ylthio substitution at the C-2 position has been selected as the best parent compound for the following prodrug approach.

In order to optimize the oral absorption of the carbapenem 6m, several ester derivatives were prepared as described above. The oral absorption of a prodrug can be estimated from its urinary recovery as the parent compound. The urinary recoveries after oral administration of prodrugs to mice are shown in Table 4. Improved urinary recovery was observed in all of the derivatives (11 ~ 14). Among them, especially, the pivaloyloxymethyl ester 11 and the 1-methylcyclohexylcarbonyloxymethyl ester 12 had excellent urinary recoveries of 47% and 55%, respectively. Pivaloyloxymethyl ester 15 prepared from the (S)-isomer 6n showed lowered urinary recovery than ester 11 of the (R)-isomer 6m. These results reflect the better biological stability of the (R)-isomer.

The pivaloyloxymethyl ester 16 of the 1-H carbapenem 7m was hardly recovered in urine probably due to the chemical and biological instability of 7m and 16.

In order to find the best ester promoiety, the pharmacokinetics of prodrugs 11 and 12 were compared with each other in dogs. Results are listed in Table 5. The pivaloyloxymethyl ester 11 showed better profiles than the 1-methylcyclohexylcarbonyloxymethyl ester 12. The maximum plasma level (Cmax) for 11 was 6.1 \(\mu\)g/ml and the plasma half life (T1/2) was 0.90 hours at an oral dose of 10 mg/kg as the parent carbapenem 6m. The absolute oral bioavailability of 11 was approximately twice that of 12.

Based on good oral absorption and potent antibacterial activity, the prodrug 11 showed excellent therapeutic efficacy against systemic infections in mice. It was highly effective especially against infections caused by resistant strains of bacteria, to which oral cephalosporins had been inactive.22)

Through these experiments the pivaloyloxymethyl ester 11 was selected as the most promising compound for further evaluation.

### Table 4. Urinary recovery of ester derivatives after oral administration in mice.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Urinary recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/(\beta)-Methylcarbapenem</td>
<td></td>
</tr>
<tr>
<td>11 (R) (CS-834)</td>
<td>46</td>
</tr>
<tr>
<td>12 (R)</td>
<td>55</td>
</tr>
<tr>
<td>13 (R)</td>
<td>44</td>
</tr>
<tr>
<td>14 (R)</td>
<td>21</td>
</tr>
<tr>
<td>15 (S)</td>
<td>24</td>
</tr>
<tr>
<td>1-H Carbapenem</td>
<td></td>
</tr>
<tr>
<td>16 (R)</td>
<td>0.3</td>
</tr>
<tr>
<td>GV-118819(^b)</td>
<td>41</td>
</tr>
<tr>
<td>CPDX-PR</td>
<td>67</td>
</tr>
</tbody>
</table>

\(^a\) Compounds (50 mg/kg) were orally administered to mice \((n = 5)\) and urinary recovery was determined by the same method described in Table 1.

\(^b\) GV-118819 was synthesized according to Glaxo’s patent procedure.

### Table 5. Pharmacokinetic parameters of carbapenems in dogs (Beagle, \(n = 3\)) after intravenous injection of 6m or oral administration of its esters 11 and 12\(^a\).

<table>
<thead>
<tr>
<th></th>
<th>6m (R-83201)</th>
<th>11 (CS-834)</th>
<th>12</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cmax ((\mu{g}/m{l}))</td>
<td>38.4(38.4)</td>
<td>6.1</td>
<td>2.6</td>
</tr>
<tr>
<td>Tmax (hour)</td>
<td>—</td>
<td>1.00</td>
<td>0.83</td>
</tr>
<tr>
<td>T1/2 (hour)</td>
<td>0.75</td>
<td>0.90</td>
<td>0.94</td>
</tr>
<tr>
<td>AUC ((\mu{g}/h)/m{l}))</td>
<td>26.3</td>
<td>13.1</td>
<td>6.3</td>
</tr>
<tr>
<td>Absolute bioavailability (%)</td>
<td>100</td>
<td>50</td>
<td>24</td>
</tr>
</tbody>
</table>

\(^a\) The parent compound (6m) was intravenously injected to dogs (Beagle, \(n = 3\)) at a dose of 10 mg/kg. Esters (11 and 12) were orally administered at a dose of 10 mg/kg as 6m.
Conclusion

We have successfully obtained an oral carbapenem, pivaloyloxymethyl (1R,5S,6S)-6-[(1R)-1-hydroxyethyl]-1-methyl-2-[(7R)-5-oxopyrrolidin-3-ylthio]-1-carbapen-2-em-3-carboxylate 11 (CS-834) which shows potent and well balanced antibacterial activity and both chemical and biological reliability. CS-834 is now under clinical evaluation.

Experimental

General Methods

IR spectra were recorded on a Nicolet NIC FT-1R (SSXC) spectrometer. NMR spectra were determined on a Jeol GX-270 (270 MHz) or GX-400 (400 MHz) spectrometer using tetramethylsilane (TMS) or sodium 3-(trimethylsilyl)propionate-d4 (TSP) as an internal standard. The mp was determined using a Yanagimoto micro-melting point apparatus and was not corrected. UV spectra were recorded on a Shimadzu UV-3100 spectrometer. Optical rotations were determined on a Perkin-Elmer digital polarimeter. Column chromatography was carried out on Silica gel 60 (230~400 mesh, Art.9385, Merck), Cosmosil 75C18 PREP (75/μm, Nacalai tesque) or MCI GEL CHP-20P (75~150/μm, Mitsubishi Kasei Corporation).

Preparation of (R)-4-Mercapto-2-oxopyrrolidine (3m)

i) (R)-4-Methanesulfonyloxy-2-oxopyrrolidine 9
To a solution of (S)-4-hydroxy-2-oxopyrrolidine 8 (1.90 g, 18.8 mmol) in pyridine (100 ml), methanesulfonyl chloride (2.26 g, 19.7 mmol) was added dropwise under ice-cooling. The mixture was stirred at room temperature for 1.5 hours, after which the reaction mixture was concentrated by evaporation under reduced pressure. Aqueous NaHCO₃ was then added to the mixture, and the mixture was again concentrated to dryness by evaporation under reduced pressure. EtOAc was added to the residue, and insoluble matter was removed by filtration. The filtrate was concentrated by evaporation under reduced pressure. The residue obtained from the soluble portion was chromatographed through silica gel (Merck 9385, 150 ml) with EtOAc-MeOH (from 9:1 to 4:1) as the eluent to give a crystalline residue. The residue was recrystallized from EtOAc-MeOH to obtain the title compound 3m as crystals (275 mg, 2.34 mmol, 100% yield); mp 117.1, 115.9, 963; 1H NMR (400 MHz, DMSO-d6, TMS) δ 2.28 (1H, dd, J = 17.6 and 1.8 Hz), 2.71 (1H, dd, J = 17.6 and 6.3 Hz), 3.24 (3H, s), 3.37 (1H, d, J = 11.9 Hz), 3.66 (1H, dd, J = 11.9 and 5.3 Hz), 5.31~5.34 (1H, m), 7.85 (1H, br s).

ii) (R)-4-Acetylthio-2-oxopyrrolidine 10
To a solution of the compound (S)-9 (896 mg, 5.00 mmol) in CH₃CN (90 ml) was added potassium thioacetate (857 mg, 7.50 mmol), and the mixture was then refluxed for 2 hours. Insoluble material was removed by filtration, and the filtrate was concentrated by evaporation under reduced pressure. The residue was chromatographed on a silica gel column with EtOAc-MeOH (from 1:0 to 96:4) as the eluent to give a crystalline residue (593 mg). The residue was recrystallized from EtOAc-cyclohexane to give the compound (R)-10 as crystals (455 mg, 2.85 mmol, 57% yield); mp 59~60°C; [α]° +47.3° (c 1.33, MeOH); IR (KBr) cm⁻¹ 1689, 1125; 1H NMR (400 MHz, CDCl₃, TMS) δ 2.30 (1H, dd, J = 17.4 and 6.0 Hz), 2.35 (3H, s), 2.80 (1H, dd, J = 17.4 and 9.1 Hz), 3.31 (1H, dd, J = 10.2 and 5.1 Hz), 3.89 (1H, dd, J = 10.2 and 7.2 Hz), 4.15~4.23 (1H, m), 7.27 (1H, br s).

iii) (S)-4-Mercapto-2-oxopyrrolidine 3n
To a solution of the compound (R)-10 (375 mg, 2.35 mmol) in MeOH (5 ml) was added 1N NaOMe in MeOH (2.35 ml, 2.35 mmol) under ice-cooling, and the mixture was stirred for 20 minutes. To the mixture was added 1N aq. HCl (2.35 ml, 2.35 mmol), and then the mixture was concentrated to dryness by evaporation under reduced pressure. EtOAc was added to the residue and insoluble matter was removed by filtration. The filtrate was concentrated by evaporation under reduced pressure to give the title compound 3n as crystals (275 mg, 2.34 mmol, 100% yield); mp 69.5~70°C; [α]° +36.5° (c 1.18, MeOH); IR (KBr) cm⁻¹ 2539, 1699, 1683; 1H NMR (400 MHz, CDCl₃, TMS) δ 1.96 (1H, d, J = 7.2 Hz), 2.32 (1H, dd, J = 17.2 and 6.8 Hz), 2.80 (1H, dd, J = 17.2 and 8.2 Hz), 3.32 (1H, dd, J = 10.2 and 5.6 Hz), 3.62~3.70 (1H, m), 3.81 (1H, dd, J = 10.2 and 7.3 Hz), 7.27 (1H, br s).

(S)-4-Mercapto-2-oxopyrrolidine (3n)
The (S)-isomer 3n was prepared from (R)-4-hydroxy-2-oxopyrrolidine 8 as described for the preparation of the (R)-isomer 3m. MP, IR and 1H NMR spectra of the (S)-isomer 3n were identical to those of the (R)-isomer 3m. [α]° +36.8° (c 1.04, MeOH).
Preparation of Sodium (1R,5S,6S)-6-[(R)-1-Hydroxyethyl]-1-methyl-2-[(R)-5-oxopyrrolidin-3-ylthio]-1-carbapen-2-em-3-carboxylate (6m, R-83201)

i) 4-Nitrobenzyl (1R,5S,6S)-6-[(R)-1-Hydroxyethyl]-1-methyl-2-[(S)-5-oxopyrrolidin-3-ylthio]-1-carbapen-2-em-3-carboxylate 4m

To a solution of 4-nitrobenzyl (1R,5S,6S)-2-diphenylphosphoryloxy-6-[(R)-1-hydroxyethyl]-1-methyl-1-carbapen-2-em-3-carboxylate 1 (1000 mg, 1.68 mmol) was added a solution of the mercaptan 3m (197 mg, 1.68 mmol) in CH3CN and diisopropylethylamine (296 µl, 1.68 mmol). The mixture was stirred for 1 hour under ice-cooling and then left to stand overnight at 4°C. The crystalline compound which precipitated from the reaction mixture was collected by filtration and dried to give the compound 4m as crystals (672 mg, 1.45 mmol, 86% yield); mp 219~221°C; Anal Calcd for C21H23N3O7S: C 54.66, H 5.02, N 9.11. Found: C 54.72, H 5.12, N 8.97.

IR (KBr) cm⁻¹ 1756, 1684, 1521, 1338, 1148; 'HNMR (270MHz, DMSO-d6, TMS) δ 1.16 (3H, d, J=6.3Hz), 1.17 (3H, d, J=7.3Hz), 2.13 (1H, dd, J=17.1 and 4.4Hz), 2.79 (1H, dd, J=17.1 and 7.8Hz), 3.10 (1H, dd, J=10.8 and 3.4Hz), 3.16-3.35 (1H, m), 3.40-3.51 (1H, m), 3.70 (1H, dd, J=10.7 and 7.3Hz), 3.95-4.12 (2H, m), 4.25 (1H, dd, J=9.3 and 2.5Hz), 5.07 (1H, d, J=5.4Hz), 5.30 (1H, d, J=14.2Hz), 5.46 (1H, d, J=14.2Hz), 7.71 (2H, d, J=8.8Hz), 8.23 (2H, d, J=8.8Hz).

ii) Sodium (1R,5S,6S)-6-[(R)-1-Hydroxyethyl]-1-methyl-2-[(S)-5-oxopyrrolidin-3-ylthio]-1-carbapen-2-em-3-carboxylate 6m

To a solution of the compound 4m (390 mg, 0.78 mmol) in a mixture of THF (19 ml) and 0.1M aq. phosphate buffer (pH 7.0) (18 ml) was added 10% Pd-charcoal (300 mg), and the mixture was stirred vigorously at 30°C in an atmosphere of hydrogen for 2.5 hours. The catalyst was removed by filtration from the reaction mixture, and the nitrate was washed twice with Et2O. The aqueous layer was then concentrated by evaporation under reduced pressure, and the resulting residue was chromatographed through MCI GEL CHP-20P (Mitsubishi Kasei Corporation, 75~150 µm, 50 ml) developed with water. The desired fraction was concentrated to 10 ml, and then lyophilized to give the title compound 6m as a colorless powder (225 mg, 0.65 mmol, 83% yield); Anal Calcd for C14H17N2O5SNa-2H2O: C 43.75, H 5.51, N 7.29. Found: C 43.31, H 5.25, N 7.25.

IR (KBr) cm⁻¹ 1749, 1687, 1596, 1393, 1295; UV (H2O) nm 299; 'H NMR (270 MHz, D2O, TSP) δ 1.02 (3H, d, J=7.3Hz), 1.10 (3H, d, J=6.6Hz), 2.22 (1H, dd, J=17.6 and 4.4Hz), 2.77 (1H, dd, J=17.6 and 8.4Hz), 3.08~3.25 (2H, m), 3.25 (1H, dd, J=5.9 and 2.6Hz), 3.68 (1H, dd, J=11.4 and 6.4Hz), 3.84~3.96 (1H, m), 4.00~4.12 (2H, m).

Preparation of Sodium (1R,5S,6S)-6-[(R)-1-Hydroxyethyl]-1-methyl-2-[(S)-5-oxopyrrolidin-3-ylthio]-1-carbapen-2-em-3-carboxylic acid (R-95867)

To a solution of the sodium salt 6m (500 mg, 1.44 mmol) in water (2.5 ml) was added 1 N HCl (1.44 ml, 1.44 mmol) under ice-cooling, and then the mixture was stirred for 30 minutes. The crystal which precipitated from the mixture was taken by filtration and dried to give the title compound (446 mg, 1.37 mmol, 95% yield); mp 204~206°C; Anal Calcd for C14H18N2O5S H2O: C 48.83, H 5.85, N 8.13, S 9.31. Found: C 48.78, H 5.83, N 7.99, S 9.29.

IR (KBr) cm⁻¹ 3502, 3464, 3293, 3143, 3033, 2980, 1782, 1698, 1650, 1545, 1487, 1450, 1416, 1377, 1281, 1258, 1226, 1201, 1183, 1141, 1128, 1101, 1083, 1069, 1047; 'H NMR (270 MHz, DMSO-d6, TSP) δ 1.13 (3H, d, J=7.3Hz), 1.15 (3H, d, J=6.1Hz), 2.10 (1H, dd, J=16.9 and 4.3Hz), 2.77 (1H, dd, J=16.9 and 7.7Hz), 3.08 (1H, dd, J=10.8 and 3.9Hz), 3.20 (1H, dd, J=6.5 and 2.7Hz), 3.15~3.60 (4H, m), 3.69 (1H, dd, J=10.8 and 7.5Hz), 3.91~4.03 (2H, m), 4.17 (1H, dd, J=9.3 and 2.7Hz), 5.03 (1H, br s), 7.82 (1H, s).

Preparation of Sodium (1R,5S,6S)-6-[(R)-1-Hydroxyethyl]-1-methyl-2-[(S)-5-oxopyrrolidin-3-ylthio]-1-carbapen-2-em-3-carboxylate (6n)

i) 4-Nitrobenzyl (1R,5S,6S)-6-[(R)-1-Hydroxyethyl]-1-methyl-2-[(S)-5-oxopyrrolidin-3-ylthio]-1-carbapen-2-em-3-carboxylate 4n

To a solution of 4-nitrobenzyl (1R,5S,6S)-2-diphenylphosphoryloxy-6-[(R)-1-hydroxyethyl]-1-methyl-1-carbapen-2-em-3-carboxylate 1 (939 mg, 1.58 mmol) was added a solution of the mercaptan 3n (185 µl, 1.58 mmol) in CH3CN and diisopropylethylamine (330 µl, 1.89 mmol). The mixture was stirred for 1.5 hours under ice-cooling and then left to stand overnight at 4°C. The mixture was diluted with EtOAc and washed with water and brine, and then concentrated under reduced pressure. The residue was chromatographed through silica gel (30 g) with EtOAc-MeOH (from 1:0 to 9:1) as the eluent to give the compound 4n as a colorless powder.
(654 mg, 1.42 mmol, 89% yield); IR (KBr) cm\(^{-1}\) 1771, 1701, 1524, 1408; \(^1H\) NMR (270 MHz, DMSO-\(d_6\), TMS) δ 1.16 (3H, d, \(J = 6.0\) Hz), 1.18 (3H, d, \(J = 7.3\) Hz), 2.02 (1H, dd, \(J = 17.1\) and 4.9 Hz), 2.72 (1H, dd, \(J = 17.1\) and 8.3 Hz), 3.15 (1H, dd, \(J = 10.6\) and 3.3 Hz), 3.29 (1H, dd, \(J = 6.2\) and 2.5 Hz), 3.40 \~ 3.48 (1H, m), 3.74 (1H, dd, \(J = 10.7\) and 6.3 Hz), 3.94 \~ 4.05 (3H, m), 4.24 (1H, dd, \(J = 9.8\) and 2.9 Hz), 5.06 (1H, d, \(J = 4.9\) Hz), 5.30 (1H, d, \(J = 14.2\) Hz), 5.46 (1H, d, \(J = 14.2\) Hz), 7.71 (2H, d, \(J = 8.8\) Hz), 7.81 (1H, s), 8.23 (2H, d, \(J = 8.8\) Hz).

ii) Sodium (5R,6S)-6-[(R)-1-Hydroxyethyl]-1-methy1-2-[(5S)-5-oxopyrrolidin-3-ylthio]-1-carbapen-2-em-3-carboxylate 6n

To a solution of the compound 4n (648 mg, 1.40 mmol) in a mixture of THF (33 ml) and 0.1 M aq. phosphate buffer (pH 7.0) (33 ml) was added 10% Pd-charcoal (700 mg), and the mixture was stirred vigorously at 22°C in an atmosphere of hydrogen for 1.5 hours. The catalyst was removed by filtration from the reaction mixture, and the filtrate was washed twice with Et\(_2\)O. The aqueous layer was then concentrated by evaporation under reduced pressure, and the resulting residue was chromatographed through Cosmosil 75C\(_{18}\) PREP (60 ml) developed with water. The desired fraction was concentrated to 10 ml, and then lyophilized to give the title compound 6n as a colorless powder (246 mg, 0.70 mmol, 50% yield);

**Anal Calcd for C\(_{14}\)H\(_{17}\)N\(_2\)O\(_5\)SNa \(\cdot\) 2H\(_2\)O:**

C 43.75, H 5.51, N 7.29, S 8.34.

**Found:** C 43.40, H 5.48, N 6.96, S 8.14.

IR (KBr) cm\(^{-1}\) 1725, 1683, 1591, 1372, 1302; UV (H\(_2\)O) nm 299; \(^1H\) NMR (270 MHz, D\(_2\)O, TSP) δ 1.14 (3H, d, \(J = 6.2\) Hz), 2.15 (1H, dd, \(J = 17.1\) and 5.2 Hz), 2.74 (1H, dd, \(J = 17.1\) and 8.4 Hz), 3.21 (1H, dd, \(J = 10.6\) and 4.1 Hz), 3.25 \~ 3.39 (2H, m), 3.69 (1H, dd, \(J = 10.6\) and 7.1 Hz), 3.91 \~ 4.21 (3H, m), 5.10 (1H, d, \(J = 5.0\) Hz), 5.30 (1H, d, \(J = 14.2\) Hz), 5.43 (1H, d, \(J = 14.2\) Hz), 7.70 (2H, d, \(J = 8.6\) Hz), 7.85 (1H, s), 8.23 (2H, d, \(J = 8.6\) Hz).

Preparation of Sodium (5R,6S)-6-[(R)-1-Hydroxyethyl]-1-methy1-2-[(5S)-5-oxopyrrolidin-3-ylthio]-1-carbapen-2-em-3-carboxylate 7m

i) 4-Nitrobenzyl (5R,6S)-6-[(R)-1-Hydroxyethyl]-2-[(5S)-5-oxopyrrolidin-3-ylthio]-1-carbapen-2-em-3-carboxylate 5m

To a solution of 4-nitrobenzyl (5R,6S)-6-[(R)-1-hydroxyethyl]-2-oxo-1-carbapenam-3-carboxylate (2230 mg, 6.59 mmol) and diphenylphosphorylchloride (1.43 ml, 6.91 mmol) in CH\(_2\)CN (20 ml) was added diisopropylamine (1.3 ml, 7.54 mmol) under ice-cooling, and the mixture was stirred for 2 hours. The mixture was added a solution of mercaptan 3m (750 mg, 6.28 mmol) and diisopropylethylene (1.3 ml, 7.54 mmol) in CH\(_2\)CN (20 ml). The mixture was stirred for 1 hour under ice-cooling. The crystalline compound which precipitated from the reaction mixture during the period was collected by filtration and dried to give the title compound 5m as crystals (1.97 g, 4.40 mmol, 67% yield);

**Anal Calcd for C\(_{20}\)H\(_{21}\)N\(_3\)O\(_7\)S:**

C 53.69, H 4.73, N 9.39, S 7.17.

**Found:** C 53.87, H 4.69, N 9.32, S 7.09.

IR (KBr) cm\(^{-1}\) 1781, 1697, 1682, 1520, 1350, 1335, 1280, 1133; \(^1H\) NMR (270 MHz, DMSO-\(d_6\), TMS) δ 1.14 (3H, d, \(J = 6.2\) Hz), 2.15 (1H, dd, \(J = 17.1\) and 5.2 Hz), 2.74 (1H, dd, \(J = 17.1\) and 8.4 Hz), 3.21 (1H, dd, \(J = 10.6\) and 4.1 Hz), 3.25 \~ 3.39 (2H, m), 3.69 (1H, dd, \(J = 10.6\) and 7.1 Hz), 3.91 \~ 4.21 (3H, m), 5.10 (1H, d, \(J = 5.0\) Hz), 5.30 (1H, d, \(J = 14.2\) Hz), 5.43 (1H, d, \(J = 14.2\) Hz), 7.70 (2H, d, \(J = 8.6\) Hz), 7.85 (1H, s), 8.23 (2H, d, \(J = 8.6\) Hz).

ii) Sodium (5R,6S)-6-[(R)-1-Hydroxyethyl]-2-[(R)-5-oxopyrrolidin-3-ylthio]-1-carbapen-2-em-3-carboxylate 7m

To a solution of the compound 5m (1000 mg, 2.23 mmol) and NaHCO\(_3\) (187 mg, 2.23 mmol) in the mixture of THF (30 ml) and H\(_2\)O (25 ml) was added 10% Pd-charcoal (1000 mg), and the mixture was stirred vigorously at 30°C in an atmosphere of hydrogen for 1.5 hours. The catalyst was removed from the reaction mixture by filtration, and the filtrate was washed twice with EtOAc. The aqueous layer was then concentrated by evaporation under reduced pressure, and the resulting residue was chromatographed through Cosmosil 75C\(_{18}\) PREP (20 g) developed with water. The desired fraction was concentrated to 10 ml, and then lyophilized to give the title compound 7m as a colorless powder (622 mg, 1.86 mmol, 83% yield);

**Anal Calcd for C\(_{14}\)H\(_{15}\)N\(_2\)O\(_5\)SNa \(\cdot\) H\(_2\)O:**

C 44.32, H 4.86, N 7.95.

**Found:** C 44.37, H 4.81, N 7.95.

IR (KBr) cm\(^{-1}\) 1755, 1686, 1593, 1394, 1296, 1252; \(^1H\) NMR (400 MHz, D\(_2\)O, TSP) δ 1.29 (3H, d, \(J = 6.4\) Hz), 2.43 (1H, dd, \(J = 16.7\) and 4.9 Hz), 2.97 (1H, dd, \(J = 16.7\) and 8.6 Hz), 3.16 (1H, dd, \(J = 17.4\) and 8.6 Hz), 3.24 (1H, dd, \(J = 17.4\) and 9.6 Hz), 3.38 \~ 3.43 (2H, m), 3.89 (1H, dd, \(J = 11.3\) and 7.6 Hz), 4.09 (1H, m), 4.20 \~ 4.27 (2H, m).
Pivaloyloxymethyl (1R,5S,6S)-6-[(R)-1-Hydroxyethyl]-1-methyl-2-[(S)-5-oxopyrrolidin-3-ylthio]-1-carbapen-2-em-3-carboxylate (11, CS-834)

To a solution of 6m (4.76 g, 13.6 mmol) in N,N-dimethylacetamide (35 ml) was added pivaloyloxymethyl iodide (3.60 g, 14.8 mmol) under ice-cooling, and the mixture was stirred for 30 minutes. The mixture was diluted with EtOAc and washed with water and brine. The EtOAc layer was dehydrated using anhydrous Na2SO4, and then concentrated by evaporation under reduced pressure. The resulting residue in the form of an amorphous powder (4.54 g) was dissolved in EtOH-CH2Cl2 (1:1), and then the CH2Cl2 was evaporated under reduced pressure. Crystals were formed during the evaporation. The resulting crystals were collected by filtration and dried to give the title compound 11 (3.68 g, 8.35 mmol, 61% yield): mp 189°C; Anal Calcd for C20H28N2O7S:
C 54.53, H 6.41, N 6.36, S 7.28.

Found: C 54.48, H 6.54, N 6.32, S 7.38.

IR (KBr) cm⁻¹ 3336, 1764, 1751, 1717, 1691, 1542, 1347, 1213, 1160, 1114, 995; UV (CH3CN) nm 324; ¹H NMR (400 MHz, DMSO-d₆, TMS) δ 1.10-1.18 (15H, m), 2.11 (1H, dd, J = 17.0 and 4.3 Hz), 2.78 (1H, dd, J = 17.0 and 7.7 Hz), 3.09 (1H, dd, J = 10.9 and 3.9 Hz), 3.25 (1H, dd, J = 6.2 and 2.5 Hz), 3.44-3.48 (1H, m), 3.71 (1H, dd, J = 10.9 and 7.6 Hz), 3.94-4.00 (1H, m), 4.04-4.09 (1H, m), 4.23 (1H, dd, J = 9.5 and 2.5 Hz), 5.08 (1H, d, J = 5.1 Hz), 5.73 (1H, d, J = 5.9 Hz), 5.88 (1H, d, J = 5.9 Hz), 7.84 (1H, s).

1-Methylcyclohexylcarbonyloxymethyl (1R,5S,6S)-6-[(R)-1-Hydroxyethyl]-1-methyl-2-[(S)-5-oxopyrrolidin-3-ylthio]-1-carbapen-2-em-3-carboxylate (12)

Compound 12 was prepared from compound 6m and 1-methylcyclohexylcarbonyloxymethyl iodide as described above, mp 160°C; Anal Calcd for C23H32N2O7S:

Found: C 57.25, H 6.86, N 5.80, S 6.38.

IR (KBr) cm⁻¹ 1761, 1764, 1751, 1717, 1542, 1347, 1213, 1160, 1114, 995; UV (CH3CN) nm 324; ¹H NMR (270 MHz, DMSO-d₆, TMS) δ 1.10-1.17 (6H, m), 1.18-1.52 (6H, m), 1.58-1.70 (2H, m), 1.80-1.90 (2H, m), 2.14 (1H, dd, J = 17.0 and 4.4 Hz), 2.78 (1H, dd, J = 17.0 and 7.8 Hz), 3.10 (1H, dd, J = 10.7 and 3.9 Hz), 3.25 (1H, dd, J = 6.3 and 2.9 Hz), 3.42-3.55 (1H, m), 3.71 (1H, dd, J = 11.0 and 7.8 Hz), 3.90-4.00 (1H, m), 4.02-4.13 (1H, m), 4.23 (1H, dd, J = 9.3 and 2.4 Hz), 4.53-4.62 (1H, m), 5.07 (1H, d, J = 5.4 Hz), 5.72 (1H, d, J = 6.3 Hz), 5.83 (1H, d, J = 6.3 Hz), 7.84 (1H, s).

1-(Isopropoxycarbonyloxy) ethyl (1R,5S,6S)-6-[(R)-1-Hydroxyethyl]-1-methyl-2-[(S)-5-oxopyrrolidin-3-ylthio]-1-carbapen-2-em-3-carboxylate (14)

Compound 14 was prepared from compound 6m and 1-(isopropoxycarbonyloxy) ethyl iodide as described above. IR (KBr) cm⁻¹ 1759, 1693, 1273, 1071; ¹H NMR (270 MHz, DMSO-d₆, TMS) δ 1.12-1.17 (6H, m), 1.20-1.26 (6H, m), 1.44-1.48 (3H, m), 2.08-2.20 (1H, m), 2.79 (1H, dd, J = 17.1 and 7.8 Hz), 3.10 (1H, dd, J = 11.2 and 3.9 Hz), 3.21-3.26 (1H, m), 3.39-3.50 (1H, m), 3.71 (1H, dd, J = 10.7 and 7.8 Hz), 3.90-4.00 (1H, m), 4.01-4.10 (1H, m), 4.19-4.20 (1H, m), 4.70-4.85 (1H, m), 5.05-5.08 (1H, m), 6.66-6.75 (1H, m), 7.83 (1H, s).

Pivaloyloxymethyl (1R,5S,6S)-6-[(R)-1-Hydroxyethyl]-1-methyl-2-[(S)-5-oxopyrrolidin-3-ylthio]-1-carbapen-2-em-3-carboxylate (15)

Compound 15 was prepared from compound 6n and pivaloyloxymethyl iodide as described above. IR (KBr) cm⁻¹ 1756, 1700, 1346, 1280, 1116; ¹H NMR (400 MHz, CDCl₃, TMS) δ 1.23 (9H, s), 1.29 (3H, d, J = 7.1 Hz), 1.34 (3H, d, J = 6.4 Hz), 2.33 (1H, dd, J = 17.5 and 6.0 Hz), 2.78 (1H, dd, J = 17.5 and 8.7 Hz), 3.25-3.33 (2H, m), 3.38 (1H, dd, J = 10.0 and 4.5 Hz), 3.80 (1H, dd, J = 10.0 and 7.2 Hz), 3.96-4.05 (1H, m), 4.22-4.28 (2H, m), 5.78 (1H, brs), 5.83 (1H, d, J = 5.7 Hz), 5.97 (1H, d, J = 5.7 Hz).

Cyclohexyloxycarbonyloxymethyl (1R,5S,6S)-6-[(R)-1-Hydroxyethyl]-1-methyl-2-[(R)-5-oxopyrrolidin-3-ylthio]-1-carbapen-2-em-3-carboxylate (13)

Compound 13 was prepared from compound 6m and cyclohexyloxycarbonyloxymethyl iodide as described above. IR (KBr) cm⁻¹ 1763, 1695, 1277; ¹H NMR (270 MHz, DMSO-d₆, TMS) δ 1.13-1.17 (6H, m), 1.18-1.52 (6H, m), 1.58-1.70 (2H, m), 1.80-1.90 (2H, m), 2.14 (1H, dd, J = 17.0 and 4.4 Hz), 2.78 (1H, dd, J = 17.0 and 7.8 Hz), 3.10 (1H, dd, J = 10.7 and 3.9 Hz), 3.25 (1H, dd, J = 6.3 and 2.9 Hz), 3.42-3.55 (1H, m), 3.71 (1H, dd, J = 11.0 and 7.8 Hz), 3.90-4.00 (1H, m), 4.02-4.13 (1H, m), 4.23 (1H, dd, J = 9.3 and 2.4 Hz), 4.53-4.62 (1H, m), 5.07 (1H, d, J = 5.4 Hz), 5.72 (1H, d, J = 6.3 Hz), 5.83 (1H, d, J = 6.3 Hz), 7.84 (1H, s).
Pivaloyloxymethyl (5R,6S)-6-[(R)-1-Hydroxyethyl]-2-[(R)-5-oxopyrroloidin-3-ylthio]-l-carbapen-2-em-3-carboxylate (16)

Compound 16 was prepared from compound 7m and pivaloyloxymethyl iodide as described above, mp 189~190°C; IR (KBr) cm⁻¹ 1772, 1759, 1701, 1540, 1336, 1280, 1202, 1157, 1111; ¹H NMR (270 MHz, DMSO-d₆, TMS) δ 1.14 (9H, s), 2.14 (1H, dd, J = 17.0 and 5.2 Hz), 2.74 (1H, dd, J = 17.0 and 8.2 Hz), 3.22 (1H, dd, J = 10.6 and 8.2Hz), 3.69 (1H, dd, J = 10.6 and 7.3 Hz), 3.89 ~ 4.00 (1H, m), 4.02 ~ 4.20 (2H, m), 5.09 (1H, d, J = 4.8 Hz), 5.74 (1H, d, J = 5.8 Hz), 5.84 (1H, d, J = 5.8 Hz), 7.85 (1H, s).

Measurement of Antibacterial Activity
MICs were measured on Nutrient agar (Eiken Chemical Ltd.) by the twofold dilution method. The inoculum size of the bacteria was one-loopful of 10⁷ cfu/ml.

Stability of Carbapenems against Renal DHP-I of Mice
The relative hydrolysis rate was determined by using 20% homogenate of kidney cortex from mice. An equi-volume of homogenate was added to 50 mM MOPS buffer (pH 7.0) which containing compounds of tested (2 mM, final concentration: 1 mM) and the mixture was incubated at 37°C. At appropriate time intervals, portion of mixture was transferred to another test tube that contained 3-times volume of methanol to stop enzymatic reaction. After vortex and centrifugation, the supernatants were injected to HPLC. The HPLC conditions are as follows: Column; A-312 ODS (6 mm i.d. x 150 mm length) from YMC, eluent; 20 mM MES buffer (pH 6.0)-methanol (8 : 2), flow rate; 1.0 ml/minute and UV detection at 300 nm. The hydrolysis rates of compounds were determined from plots of peak areas of the compounds against time of samplings and expressed as relative hydrolysis rate against compound 6m. DHP-I activity of the homogenate was confirmed by the method of Campbell²³) using glycyldehydrophenylalanine as a substrate.

Urinary Recovery of Carbapenems in Mice

i) Administration
Carbapenems 6a ~ n and 7m (dose: 50 mg/kg as sodium salt) were dissolved in water and then subcutaneously administered to mice (n = 5, SPF ddY strain). A mixture of cilastatin sodium and carbapenems 6m, 6n and 7m (dose: 50 mg/kg each as sodium salt) was dissolved in water and then subcutaneously administered to mice (n = 5, SPF ddY strain). Carbapenem esters 11 ~ 16 (dose: 50 mg/kg as 6m, 6n and 7m) suspended in 0.5% tragacanth were orally administered to mice (n = 5, SPF ddY strain).

Pharmacokinetic Parameters of Carbapenems in Dogs

i) Administration
Carbapenem 6m (dose: 10 mg/kg) dissolved in water was intravenously administered to dogs (n = 3, Beagle). Carbapenem esters 11 and 12 (dose: 10 mg/kg as 6m) dissolved in dimethylacetamide - polyethylene glycol 400 - water (1 : 4 : 5) were orally administered to dogs (n = 3, Beagle).

ii) Determination of Pharmacokinetic Parameters
The concentration of R-95867 in blood was measured by HPLC method at suitable intervals after administration. Pharmacokinetic parameters were determined by the simulation program.

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References
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