NEW PENEM COMPOUNDS WITH 5'-SUBSTITUTED PYRROLIDINYLTHIO GROUP AS A C-2 SIDE CHAIN; COMPARISON OF THEIR BIOLOGICAL PROPERTIES WITH THOSE OF CARBAPENEM COMPOUNDS

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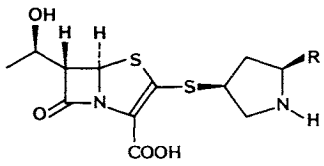
A series of new penem compounds with a C-2 side chain consisting of a pyrrolidin-3'-ylthio group substituted with various aminocarbonyl groups at the C-5' position have been prepared. Antibacterial activity, stability to renal dehydropeptidase-I and affinity to penicillin-binding proteins of these compounds were investigated and compared with the corresponding carbapenem compounds.

In our search for novel carbapenem compounds, we found that (1) carbapenems bearing 5'-aminocarbonylpyrrolidinylthio group as a C-2 side chain possessed potent and well balanced antibacterial activities including anti-pseudomonal activity[1] and (2) introduction of a methylene spacer between the aminocarbonyl group and the pyrrolidine ring at the C-5' position resulted in enhanced stability to renal dehydropeptidase-I (DHP-I)[2]. The structure-activity relationships of several penem derivatives with various 5'-substituted pyrrolidinylthio side chains have been reported by the Sandoz group[3]. However there has been little study done on correlation of the biological activities between penem and carbapenem derivatives having the same C-2 side chain. We were interested in comparing the biological properties of both derivatives described above. In this paper, *in vitro* antibacterial activity, stability to DHP-I and affinity to penicillin-binding proteins (PBPs) of new penem compounds were compared with the corresponding carbapenems.

Chemistry

The penem derivatives examined here were prepared according to the conventional procedures[3,4] of displacing the sulfinyl groups in penem-*S*-oxides with various mercaptans as shown in Scheme 1. In this pathway, we chose the penem-*S*-oxide (**2**)[5] as the key intermediate, which was prepared from (3*R*,4*R*)-4-acetoxy-3-[(*R*)-1-*tert*-butyldimethylsilyloxyethyl]-2-azetidinone. The mercaptan derivatives

Fig. 1. Penem and carbapenem compounds having 5'-substituted pyrrolidinylthio side chain.



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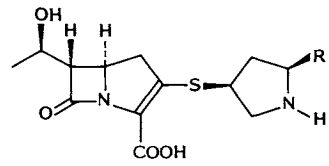

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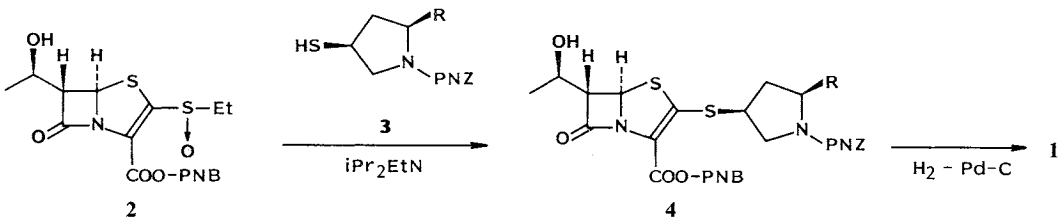
a R=CONMe$_2$, **b** R=CONH$_2$, **c** R = , **d** R=CH$_2$CONMe$_2$, **e** R=CH$_2$CH$_2$CONMe$_2$.

Scheme 1.



PNB: *p*-Nitrobenzyl.
PNZ: *p*-Nitrobenzyloxycarbonyl.

Table 1. Antibacterial activity and DHP-I stability of penem compounds having 5′-substituted pyrrolidinylthio group at the C-2 position.

Organism	MIC (μg/ml)						
	1a	**1b**	**1c**	**1d**	**1e**	**5a**	**5b**
S.a. FDA 209P	<0.013	<0.013	<0.013	0.05	0.05	<0.013	<0.013
S.p. Cook	<0.013	<0.013	<0.013	0.025	<0.013	<0.013	<0.013
E.c. NIHJ JC-2	0.025	0.025	0.025	0.39	0.39	<0.013	<0.013
K.p. ATCC 10031	<0.013	<0.013	0.025	0.39	0.20	<0.013	<0.013
P.m. GN 2425	0.10	0.10	0.10	0.78	0.78	0.10	0.05
P.a. IFO 3451	6.25	6.25	6.25	25	25	0.78	1.56
S.m. X 100	0.05	0.05	0.05	0.78	0.78	0.05	0.05
E.c. ML 1410/RP4[a]	0.05	0.05	0.05	0.78	1.56	0.05	0.05
E.c. GN 5482[a]	0.025	0.025	0.025	0.39	0.20	<0.013	<0.013
P.v. GN 7919[a]	0.10	0.10	0.05	1.56	1.56	0.10	0.10
S.m. GN 6473[a]	0.10	0.10	0.05	0.78	0.78	0.10	0.10
DHP-I stability[b]	5.6	11.7	5.3	42.6	16.0	1.0	1.4

[a] β-Lactamase producing strain.
[b] DHP-I stability is given relative to compound **5a**.
Abbreviations: *S.a.*, *Staphylococcus aureus*; *S.p.*, *Streptococcus pyogenes*; *E.c.*, *Escherichia coli*; *K.p.*, *Klebsiella pneumoniae*; *P.m.*, *Proteus mirabilis*; *P.a.*, *Pseudomonas aeruginosa*; *S.m.*, *Serratia marcescens*; *P.v.*, *Proteus vulgaris*.

(**3a**～**3e**) were prepared from 4-hydroxyproline as reported previously by us[1,2]. The penem-*S*-oxide (**2**) was treated with mercaptan (**3**) in acetonitrile in the presence of diisopropylethylamine to give the protected penem (**4**). The *p*-nitrobenzyl and *p*-nitrobenzyloxycarbonyl groups of **4** were removed by hydrogenolysis over Pd-C and the target penem (**1**) having 5′-substituted pyrrolidinylthio side chain was purified by column chromatography on Diaion CHP-20P.

Biological Properties

MICs and stability to DHP-I of these new penem derivatives (**1a**～**1e**) are shown in Table 1, together with those of the reference carbapenems (**5a** and **5b**)[1].

The 5′-aminocarbonyl derivatives (**1a**～**1c**) exhibited potent antibacterial activities against Gram-positive and Gram-negative bacteria comparable to the corresponding carbapenems. The introduction of methylene spacer $(CH_2)_n$ between the aminocarbonyl group and the pyrrolidine ring at the C-5′ position resulted in overall reduction of the antibacterial activities, as seen in penem derivatives (**1d** and **1e**).

As for the activity against *Pseudomonas aeruginosa*, penem derivatives (**1a**～**1c**) showed poorer activity

Table 2. Affinity of penem and carbapenem compounds for PBPs of *Pseudomonas aeruginosa* NCTC 10490.

Compounds	ID_{50} (μg/ml)						MIC (μg/ml)
	1A	1B	2	3	4	5	
Penem **1a**	0.17	1.9	0.3	0.17	0.019	>10	0.20
Carbapenem **5a**	0.49	2.6	0.87	0.38	0.054	>10	1.56

ID_{50} indicates the concentration of β-lactams required to reduce [^{14}C]benzylpenicillin binding by 50%.

than carbapenem derivatives (**5a** and **5b**). However, it was interestingly noted that there was no difference of anti-pseudomonal activity among three penems (**1a**~**1c**), which have the different 5'-aminocarbonyl substituents, although in the case of carbapenems the dimethylaminocarbonyl derivative (**5a**) was superior to the carbamoyl derivative (**5b**).

In order to further study the anti-pseudomonal activity, affinities of penem **1a** and carbapenem **5a** to PBPs of *P. aeruginosa* NCTC 10490 were compared as shown in Table 2. The affinity pattern of **1a** was similar to that of **5a** and higher affinities of PBPs 1A, 2, 3 and 4 were observed in **1a** compared with **5a**. Their affinities of PBPs had a good correlation with the activity against *P. aeruginosa* NCTC 10490. On the other hand, the activity of **1a** against *P. aeruginosa* IFO 3451 was lower than that of **5a** (see Table 1). This discrepancy might be due to the different membrane permeability to β-lactam compounds between *P. aeruginosa* NCTC 10490 and IFO 3451. Penem **1a** seems to have lower membrane permeability than carbapenem **5a** in *P. aeruginosa* IFO 3451.

Carbapenems are highly sensitive to DHP-I, while penems are generally more stable to DHP-I judging from the results reported previously[6,7]. Similar tendency was observed in comparison between penems (**1a** and **1b**) and carbapenems (**5a** and **5b**). The DHP-I stability was significantly enhanced by the introduction of a methylene spacer not only in carbapenems but also in penems. However, the elongation of spacer led to a reduced stability.

In this series of 5'-substituted pyrrolidinylthio side chain, penem compounds essentially possess potent and well balanced antibacterial activities similar to those of the corresponding carbapenem compounds. A diminution of anti-pseudomonal activity in the penem derivatives is presumably attributed to low membrane permeability. Against DHP-I, the penem compounds have greater stability compared with the corresponding carbapenems.

Experimental

General Methods

IR spectra were recorded on a Hitachi 260-10 IR spectrophotometer. ^{1}H NMR spectra were taken with Jeol FX-90Q (90 MHz) or GX270 (270 MHz) spectrometers, in the designated solvent, using tetramethylsilane or residual HOD (δ 4.80) as an internal reference. UV spectra were recorded on a Hitachi 330 UV-VIS spectrophotometer. Mass spectra were obtained on a Hitachi M-80B spectrometer. Optical rotations were determined on a Jasco DIP-181 digital polarimeter. Preparative TLC was carried out on Silica gel 60 F_{254} TLC plates (E. Merck).

p-Nitrobenzyl (5*R*,6*S*)-2-[(3*S*,5*S*)-5-Dimethylaminocarbonyl-1-(*p*-nitrobenzyloxycarbonyl)pyrrolidin-3-ylthio]-6-[(*R*)-1-hydroxyethyl]penem-3-carboxylate (**4a**)

To a stirred solution of *p*-nitrobenzyl (5*R*,6*S*)-2-ethylsulfinyl-6-[(*R*)-1-hydroxyethyl]penem-3-carboxylate (**2**) (45 mg, 0.106 mmol) in dry CH_3CN (0.8 ml) was added a solution of diisopropylethylamine (30 mg, 0.23 mmol) in dry CH_3CN (0.3 ml) at −40°C under nitrogen atmosphere. After stirring for 5

minutes, a solution of (2*S*,4*S*)-2-dimethylaminocarbonyl-4-mercapto-1-*p*-nitrobenzyloxycarbonylpyrrolidine (**3a**)[1] (81 mg, 0.23 mmol) in CH_3CN (0.6 ml) was added to this mixture at the same temperature and followed by stirring for 10 minutes. The reaction mixture was diluted with EtOAc, washed with brine and dried over anhydr Na_2SO_4. Evaporation of the solvents *in vacuo* gave an oily residue which was purified by preparative TLC (EtOAc - Me_2CO, 3 : 1) to give **4a** (69 mg, 93%) as a colorless powder: $[\alpha]_D^{29}$ +52° (*c* 0.48, $CHCl_3$); IR ($CHCl_3$) cm^{-1} 1788, 1700, 1660, 1607; ^1H NMR ($CDCl_3$) δ 1.38 (3H, d, J=6.0 Hz), 1.98 (1H, m), 2.78 (1H, m), 2.93 (1/3H, s), 2.97 (1/3H, s), 2.99 (2/3H, s), 3.10 (2/3H, s), 3.76 (1H, dd, J=1.5 and 6.0 Hz), 4.74 (1H, m), 5.24 (2H, s), 5.30 (2H, s), 5.71 (1H, d, J=1.5 Hz), 7.44 (1H, d, J=8.5 Hz), 7.52 (1H, d, J=8.5 Hz), 7.61 (2H, d, J=8.5 Hz), 8.22 (4H, d, J=8.5 Hz).

The following compounds (**4b**~**4e**) were prepared from **2** as described for the preparation of **4a**, in 77%, 84%, 68% and 78% yields, respectively.

4b: $[\alpha]_D^{33}$ +32° (*c* 0.22, THF); IR (Nujol) cm^{-1} 1780, 1680, 1608; ^1H NMR ($CDCl_3$) δ 1.18 (3H, d, J=6.0 Hz), 5.22 (2H, s), 5.79 (1H, s).

4c: IR ($CHCl_3$) cm^{-1} 1790, 1706, 1653, 1612; ^1H NMR ($CDCl_3$) δ 1.36 (3H, d, J=6.0 Hz), 5.22 (2H, s), 5.75 (1H, d, J=1.5 Hz).

4d: IR (neat) cm^{-1} 1793, 1703, 1641; ^1H NMR ($CDCl_3$) δ 1.37 (3H, d, J=6.0 Hz), 2.91 (18/5H, s), 2.95 (12/5H, s), 5.22 (2H, s), 5.43 (1H, d, J=14.0 Hz), 5.70 (1H, d, J=1.0 Hz).

4e: IR (neat) cm^{-1} 1780, 1702, 1635; ^1H NMR ($CDCl_3$) δ 1.37 (3H, d, J=6.0 Hz), 2.91 (3H, s), 2.94 (3H, s), 5.21 (2H, s), 5.44 (1H, d, J=13.5 Hz), 5.71 (1H, d, J=1.0 Hz), 8.20 (4H, d, J=8.5 Hz).

(5*R*,6*S*)-2-[(3*S*,5*S*)-5-Dimethylaminocarbonylpyrrolidin-3-ylthio]-6-[(*R*)-1-hydroxyethyl]penem-3-carboxylic Acid (**1a**)

To a solution of **4a** (68 mg, 0.097 mmol) in THF (7.7 ml) and 0.1 M phosphate buffer (pH 6.86, 5.1 ml) was added 5% Pd-C (204 mg) which was activated in EtOH (3.8 ml) and water (3.8 ml) before use for hydrogenolysis. The mixture was stirred under hydrogen atmosphere for 3 hours at room temperature. The catalyst was filtered off and washed with 0.1 M phosphate buffer (2 ml). THF was evaporated *in vacuo* from the filtrate to give aqueous solution, which was washed with EtOAc. The aqueous layer was concentrated briefly to remove any residual organic solvents *in vacuo* and then subjected to column chromatography on Diaion CHP-20P (Mitsubishi Chemical Industries, Ltd.) which was successively eluted with water and water containing 1% of THF. The fractions having UV absorption at 320 nm were combined and lyophilized to give **1a** (13.5 mg, 36%): IR (KBr) cm^{-1} 1765, 1645, 1580; ^1H NMR (D_2O) δ 1.29 (3H, d, J=6.5 Hz), 1.94~2.08 (1H, m), 2.93~3.15 (1H, m), 2.98 (3H, s), 3.05 (3H, s), 3.53~3.62 (1H, m), 3.83~3.93 (1H, m), 3.94 (1H, dd, J=1.5 and 6.0 Hz), 4.06~4.30 (3H, m), 5.71 (1H, d, J=1.5 Hz); UV $\lambda_{max}^{H_2O}$ nm 255, 322; SI-MS *m/z* 410 (M+Na).

The following compounds (**1b**~**1e**) were prepared from **4b**~**4e** as described for the preparation of **1a**.

1b: IR (KBr) cm^{-1} 1762, 1650, 1577; ^1H NMR (D_2O) δ 1.29 (3H, d, J=6.5 Hz), 1.75~1.89 (1H, m), 2.62~2.87 (1H, m), 3.00~3.09 (1H, m), 3.38~3.48 (1H, m), 3.65~3.92 (2H, m), 3.90 (1H, dd, J=1.5 and 6.0 Hz), 4.17~4.30 (1H, m), 5.68 (1H, d, J=1.5 Hz); UV $\lambda_{max}^{H_2O}$ nm 255, 322; SI-MS *m/z* 360 (M+H).

1c: IR (KBr) cm^{-1} 1765, 1636, 1582; ^1H NMR (D_2O) δ 1.29 (3H, d, J=6.5 Hz), 1.80~2.08 (5H, m), 2.88~3.05 (1H, m), 3.34~3.61 (5H, m), 3.64~3.74 (1H, m), 3.93 (1H, dd, J=1.5 and 6.0 Hz), 4.04 (1H, m), 4.24 (1H, m), 5.70 (1H, d, J=1.5 Hz); UV $\lambda_{max}^{H_2O}$ nm 255, 322; SI-MS *m/z* 436 (M+Na), 414 (M+H).

1d: IR (KBr) cm^{-1} 1772, 1630, 1595; ^1H NMR (D_2O) δ 1.31 (3H, d, J=6.5 Hz), 2.93 (3H, s), 3.05 (3H, s), 5.72 (1H, d, J=1.5 Hz); UV $\lambda_{max}^{H_2O}$ nm 256, 321.

1e: IR (KBr) cm^{-1} 1768, 1623, 1585; ^1H NMR (D_2O) δ 1.31 (3H, d, J=6.5 Hz), 2.93 (3H, s), 3.07 (3H, s), 5.72 (1H, d, J=1.5 Hz); UV $\lambda_{max}^{H_2O}$ nm 256, 323.

Measurement of *In Vitro* Antibacterial Activity

MICs were determined by the 2-fold agar dilution method using Sensitivity Test Agar (Nissui). An appropriate dilution (10^6 cells/ml) of a fresh overnight culture of the test organism was prepared as an inoculum. The inoculated plates were incubated at 37°C for 18 hours. The MIC (μg/ml) was determined as the lowest concentration of the test compound that inhibited the development of visible growth of the test microorganism.

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Stability Test to DHP-1

The DHP-1 stability was determined by spectrophotometric method in a spectrophotometer controlled at 37°C. Renal DHP-1 was partially purified from swine kidney according to CAMBELL's method[8]. The $T_{1/2}$ (minute) of enzyme-catalized hydrolysis of the test compound (200 μM) was measured in the presence of the limited amount of enzyme by the decrease in absorbance at a wavelength around 300 nm in 50 mM of MOPS buffer (pH 7.2), where the test compound shows the maximum absorption.

Analysis of PBPs

The method used for PBPs analysis, a competition assay with [^{14}C]benzylpenicillin, was described by NOGUCHI *et al.*[9] and FUKASAWA *et al.*[10]. After *P. aeruginosa* membranes (30 μl) were incubated with the test compound (3 μl) at various concentrations for 10 minutes at 30°C, [^{14}C]benzylpenicillin (50 μCi/ml; 3 μl, a final concentration of 22.5 μg/ml) was added and the incubation was continued for another 10 minutes. Reactions were terminated by an addition of excess non-radioactive benzylpenicillin (a final concentration of 2,600 μg/ml) and sarkosyl (a final concentration, 1%). After removal of sarkosyl-insoluble fractions by centrifugation, the supernatant was used for sodium dodesylsulfate-polyacrylamide gel electrophoresis (SDS-PAGE).

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