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Short communication

Synthesis and antibacterial activities of new 1β-methylcarbapenems having aminopyrimidinylthioether moiety

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Abstract

The synthesis of new 1β-methylcarbapenems **1a**–**d** bearing aminopyrimidinylthioether moiety at C-5 position of pyrrolidine ring and their antibacterial activities are described. All the compounds exhibited potent antibacterial activity. Of these carbapenems, **1d** showed the best combination of antibacterial activity and stability to dehydropeptidase-I (DHP-I).

Keywords: 1β-Methylcarbapenems; Aminopyrimidinylthioether; Antibacterial activity; Stability to DHP-I

1. Introduction

By reason of the appearance of bacteria resistant to classical β-lactam antibiotics such as penicillins and cephalosporins, carbapenems have become the most important class of β-lactam antibiotics [1]. In spite of tremendous efforts to develop new synthetic carbapenems, only a few carbapenems were used clinically. Imipenem [2] and panipenem [3] must be co-administrated with enzyme inhibitors because of their instability to the renal dehydropeptidase-I (DHP-I). And also, owing to their convulsive side effect, they are used limitedly. Meropenem [4], which is a 1β-methylcarbapenem, has good stability to DHP-I and a well-balanced spectrum against both Gram-positive and Gram-negative bacteria including *Pseudomonas aeruginosa*. However, its half-life in human is relatively shorter than clinically used cephalosporins. Recently, ertapenem (Invanz®, Merck) were approved from US FDA for the treatment of mixed infections [5,6]. It exhibited excellent pharmacokinetic profiles, but its efficacy against *P. aeruginosa* is poor.

In our previous papers [7,8], we reported the synthesis and biological properties of 1β-methylcarbapenems containing heteroaromatic thioether moieties, such as tetrazole, triazole, thiadiazole, pyridine, and pyrimidine, at C-5 position of pyrrolidine. A series of 1β-methylcarbapenems having pyrimidine substituents showed better antibacterial activity against Gram-positive bacteria, but extremely poor activity against *P. aeruginosa*. In general, it has been well known that the introduction of basic site into the carbapenem molecule leads to improvement of the activity against *P. aeruginosa*. On the basis of these considerations, we further studied the effect of more basic aminopyrimidines compared with pyrimidines on the antibacterial activity and stability to DHP-I. Now we describe the synthesis and antibacterial activities of new 1β-methylcarbapenems **1a**–**d** having aminopyrimidinylthioether moiety at C-5 position of pyrrolidine ring as a side chain (Fig. 1).

2. Chemistry

2-Hydroxymethylpyrrolidin-4-thiol (**8**) was prepared by the sequence outlined in Scheme 1. *trans*-4-Hydroxy-L-proline (**2**) was treated with acetyl chloride in methanol to give methyl ester HCl salt **3**, which was protected with *p*-nitrobenzyl chloroformate to afford *N*-protected pyrrolidine **4**. Mesylation of hydroxyl group of **4** with MsCl followed by reduction of the resulting ester **5** with $LiBH_4$ provided the alcohol **6** by conventional methods [9,10]. Without protection of hydroxyl group, mesylate **6** was converted to thioacetate **7** with AcSK in

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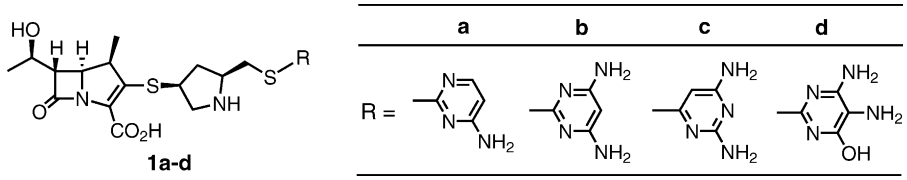
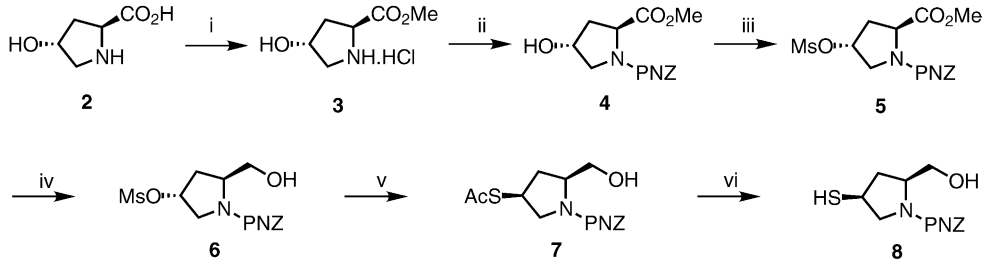


Fig. 1.



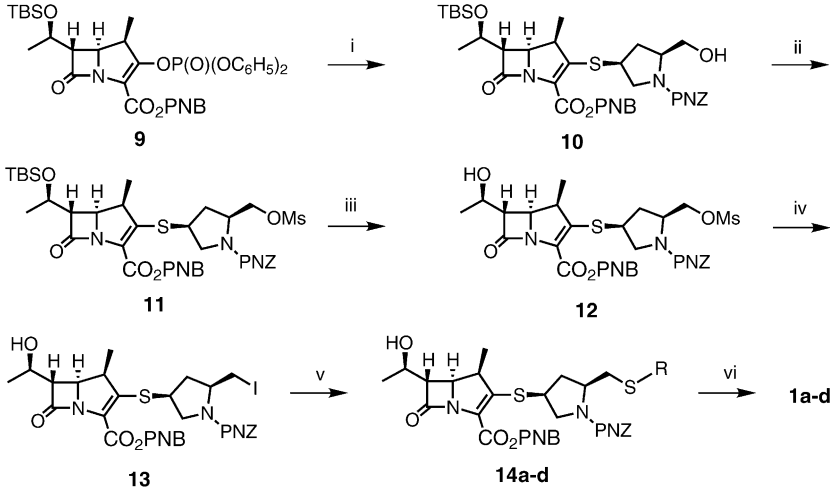
Scheme 1. *Reagents and reaction conditions*: (i) AcCl, MeOH, reflux, 8 h; (ii) *p*-nitrobenzyl chloroformate, Et_3N, CH_2Cl_2, 0 °C, 2 h; (iii) MsCl, Et_3N, CH_2Cl_2, 0 °C, 3 h; (iv) $NaBH_4$, LiCl, THF–EtOH, 0 °C to r.t., 4 h; (v) AcSK, CH_3CN, reflux, 8 h; (vi) 2*N*-NaOH, MeOH, 0 °C, 1 h.

CH_3CN. Deacetylation of **7** was performed with 2*N*-NaOH in MeOH to afford pyrrolidinethiol **8**.

Coupling reaction of enolphosphate **9** [11] with freshly prepared pyrrolidinethiol **8** was afforded the intermediate **10**. Iodo compound **13** was obtained by the mesylation and desilylation of **10** followed by substitution with KI. Treatment of iodide **13** with mercaptopyrimidines in the presence of K_2CO_3 in DMF gave protected carbapenems **14a**–**d**, which were deprotected by hydrogenolysis. The resulting 1β-methylcarbapenems **1a**–**d** were purified by column chromatography on Diaion HP-20 (**1d**: purity, 97.5%; Hewlett–Packard 1100 HPLC system; column, Capcell Pak C_{18} MG 4.6 mm i.d. × 250 mm (Shiseido Co. Ltd.); mobile phase, acetoniltrile-pH 5.0 phosphate buffer gradient (5:95 to 70:30); flow rate, 1.0 ml/min; detection, UV 260 nm) and lyophilized as an amorphous solid (Scheme 2).

3. Biological results and discussion

The in vitro antibacterial activity and DHP-I stability of new 1β-methylcarbapenems **1a**–**d** having aminopyrimidinylthioether moiety are shown in Table 1. The minimal inhibitory concentrations (MICs) of these compounds were compared with imipenem and meropenem as controls. All the compounds exhibited potent antibacterial activity. They showed improved activity against Gram-positive organisms than those of meropenem, but poorer against Gram-negative bacteria than meropenem. There was no significant difference between the activities of mono-aminopyrimidine **1a** and diaminopyrimidines **1b**–**c**. Among the carbapenems prepared, **1d** having an additional hydroxyl group showed well-balanced spectrum against both Gram-positive and Gram-



Scheme 2. *Reagents and reaction conditions*: (i) **8**, DIEA, CH_3CN, r.t., 3 h; (ii) MsCl, Et_3N, CH2Cl2, 0 °C, 1 h; (iii) $NH_4F.HF$, *N*-methylpyrrolidone-DMF, r.t., 48 h; (iv) KI, DMF, r.t., 24 h; (v) K2CO3, DMF, r.t., 2 h, 4-amino-2-mercaptopyrimidine for **14a**, 4,6-diamino-2-mercaptopyrimidine for **14b**, 2,4-diamino-6-mercaptopyrimidine for **14c**, 4,5-diamino-6-hydroxy-2-mercaptopyrimidine for **14d**; (iv) Pb/C, H_2, THF, EtOH, r.t., 2 h.

Table 1
In vitro antibacterial activity and DHP-I stability of **1a–d**

Organism	MIC (μg/ml)[a]					
	1a	**1b**	**1c**	**1d**	IPM[b]	MPM[c]
Streptococcus pyogenes 308A	0.004	0.004	0.004	0.013	0.004	0.007
Staphylococcus aureus SG 511	0.025	0.025	0.025	0.049	0.013	0.098
S. aureus 285	0.049	0.049	0.049	0.098	0.013	0.195
S. aureus 503	0.025	0.025	0.025	0.049	0.007	0.098
Escherichia coli 078	0.049	0.049	0.049	0.098	0.098	0.025
E. coli 1507E	0.049	0.049	0.098	0.098	0.098	0.025
P. aeruginosa 9027	3.125	6.250	3.125	0.781	0.391	0.195
P. aeruginosa 1771M	0.781	0.781	1.563	0.391	0.195	0.098
Salmonella typhimurium	0.098	0.098	0.098	0.195	0.781	0.049
Klebsiella aerogenes 1522E	0.098	0.098	0.098	0.195	0.195	0.049
Enterobacter cloacae 1321E	0.049	0.049	0.098	0.098	0.098	0.025
DHP-I stability[d]	0.98	0.87	1.18	1.30	0.18	1.00

[a] MIC was determined by agar dilution method using Mueller–Hinton.
[b] IPM: imipenem.
[c] MPM: meropenem.
[d] Relative $t_{1/2}$ of hydrolysis to meropenem by partially purified porcine renal DHP-I.

negative organisms including *P. aeruginosa*. In particular, **1d** was much more stable to DHP-I than meropenem.

4. Experimental

4.1. Chemistry

Melting points (m.p.) were measured with a Thomas–Hoover capillary m.p. apparatus and were uncorrected. ^{1}H NMR spectra were recorded on a Bruker Avance 300 spectrometer with tetramethylsilane as internal standard. Tetrahydrofuran (THF) and diethyl ether were dried by heating at reflux in presence of sodium metal and benzophenone followed by distillation immediately prior to use. Solvents and liquid reagents were transferred using hypodermic syringes. All other reagents and solvents used were reagent grade.

4.1.1. (2S,4R)-4-Hydroxy-2-methoxycarbonylpyrrolidine HCl salt (3)

To a suspension of *trans*-4-hydroxy-L-proline (**2**) (100.0 g, 0.76 mol) in methanol (1 l) was slowly added acetyl chloride 89.4 g, 1.14 mol) at room temperature (r.t.), and the reaction mixture was refluxed for 8 h. The mixture was cooled and concentrated in vacuo. The residue was recrystallized from diethyl ether to give **3** (137.0 g, 99%) as a white powder: m.p. 165–166 °C; ^{1}H NMR (DMSO-d_6) δ 9.95 (br s, 2H), 5.64 (br s, 1H), 3.78–4.46 (m, 2H), 3.72(s, 3H), 3.35 (m, 1H), 3.04 (m, 1H), 2.2 (m, 1H), 2.01 (m, 1H).

4.1.2. (2S,4R)-4-Hydroxy-2-methoxycarbonyl-1-(4-nitrobenzyloxycarbonyl)pyrrolidine (4)

To a solution of **3** (66.0 g, 0.36 mol) in CH_2Cl_2 (1 l) was slowly added triethylamine (109.3 g, 1.08 mol) at 0 °C under nitrogen atmosphere. To this solution was added dropwise a solution of *p*-nitrobenzyl chloroformate (94.0 g, 0.44 mol) in CH_2Cl_2 (400 ml), and then stirred for 2 h. The mixture was filtered, washed with water, dried over anhydrous Na_2SO_4, and concentrated in vacuo. The residue was purified by silica gel column chromatography (*n*-hexane/ethyl acetate = 1:3) to give **4** (109.1 g, 93%) as a pale yellow powder: m.p. 110–112 °C; ^{1}H NMR ($CDCl_3$) δ 8.21 (d, J = 8.7 Hz, 2H), 7.51 and 7.46 (d, J = 8.7 Hz, 2H), 5.10–5.30 (m, 2H), 4.50–4.56 (m, 2H), 3.75 and 3.67 (s, 3H), 3.55–3.78 (m, 2H), 2.66 (br s, 1H), 2.33 (m, 1H), 2.14 (m, 1H).

4.1.3. (2S,4R)-4-Methanesulfonyloxy-2-methoxycarbonyl-1-(4-nitrobenzyloxycarbonyl)pyrrolidine (5)

To a solution of **4** (109.0 g, 0.34 mol) in CH_2Cl_2 (1 l) was added triethylamine (40.8 g, 0.40 mol) and methanesulfonyl chloride (42.8 g, 0.37 mol) at 0 °C under nitrogen atmosphere, and the reaction mixture was stirred for 3 h at the same temperature. The mixture was diluted with CH_2Cl_2, washed with water and brine, dried over anhydrous Na_2SO_4, and concentrated in vacuo. The residue was recrystallized from CH_2Cl_2/methanol to give **5** (119.4 g, 88%) as a pale yellow powder: m.p. 85–86 °C; ^{1}H NMR ($CDCl_3$) δ 8.23 (d, J = 8.6 Hz, 2H), 7.53 and 7.48 (d, J = 8.6 Hz, 2H), 5.14–5.34 (m, 2H), 4.55 (m, 1H), 3.81–3.98 (m, 3H), 3.86 and 3.78 (s, 3H), 3.09 (s, 3H), 2.69 (m, 1H), 2.25 (m, 1H).

4.1.4. (2S, 4R)-2-Hydroxymethyl-4-methanesulfonyloxy-1-(4-nitrobenzyloxycarbonyl)pyrrolidine (6)

The compound **5** (17.4 g, 43.3 mmol) was dissolved in THF (50 ml) at 0 °C under nitrogen atmosphere. To this solution anhydrous lithium chloride (4.6 g, 108.3 mmol) and sodium borohydride (4.1 g, 108.3 mmol) were added, and the reaction mixture was stirred for 0.5 h at the same temperature. After addition of ethanol (200 ml), the mixture was stirred at r.t. for 4 h. The mixture was filtered, and then the filtrate was evaporated. The residue was treated with 25% aqueous ammonium chloride, and extracted with ethyl acetate. The organic layer was successively washed with brine, dried over anhydrous Na_2SO_4. Removal of solvent gave **6** (12.9 g, 80%) as a white powder: m.p. 98–100 °C; ^{1}H NMR ($CDCl_3$) δ 8.18 (d, J = 8.4 Hz, 2H), 7.45 (d, J = 8.4 Hz, 2H), 5.21 (s, 2H), 4.15 (m, 1H), 3.55–4.00 (m, 4H), 3.04 (s, 3H), 2.37 (m, 1H), 2.06 (m, 1H).

4.1.5. (2S,4S)-4-Acetylthio-2-hydroxymethyl-1-(4-nitrobenzyloxycarbonyl)pyrrolidine (7)

To a solution of **6** (18.3 g, 44.8 mmol) in CH_3CN (250 ml) was added potassium thioacetate (11.1 g, 97.6 mmol) at r.t. under nitrogen atmosphere, and the reaction mixture was heated under reflux for 8 h. After cooling, the mixture was concentrated in vacuo, diluted with ethyl acetate, washed with water and brine, dried over anhydrous Na_2SO_4, and evaporated under reduced pressure. The residue was purified by silica gel column chromatography (*n*-hexane/ethyl acetate = 1:1) to give **7** (14.1 g, 81%) as a pale brown powder: m.p. 128–130 °C; ^{1}H NMR ($CDCl_3$) δ 8.18 (d, J = 8.4 Hz, 2H), 7.49 (d, J = 8.4 Hz, 2H), 5.21 (s, 2H), 4.02–4.18 (m, 2H), 3.71–3.89 (m, 3H), 3.24 (m, 1H), 2.47 (m, 1H), 2.31 (s, 3H), 1.72 (m, 1H).

*4.1.6. (2S,4S)-2-Hydroxymethyl-5-mercapto-1-(4-nitrobenzyloxycarbonyl)pyrrolidine (**8**)*

To a solution of **7** (7.0 g, 19.6 mmol) in methanol (80 ml) was added 2*N*-NaOH (10.8 ml, 21.6 mmol) at 0 °C. The reaction mixture was stirred for 1 h, and treated with 1*N*-HCl (21.6 ml, 21.6 mmol) at the same temperature. The mixture was evaporated and diluted with ethyl acetate. The organic layer was washed with water and brine, dried over anhydrous Na_2SO_4, concentrated in vacuo to give **8** (5.8 g, 95%) as a brown oil which was used to next reaction without further purification.

*4.1.7. 4-Nitrobenzyl (1R,5S,6S)-6-[(1R)-1-(t-butyldimethylsilyoxy)ethyl]-2-[(3S,5S)-5-(hydroxymethyl)-1-(4-nitrobenzyloxycarbonyl)pyrrolidin-3-ylthio]-1-methylcarbapen-2-em-3-carboxylate (**10**)*

To a solution of enolphosphate **9** (14.1 g, 19.4 mmol) in CH_3CN (100 ml) was added Hunig's base (3.4 g, 26.4 mmol) at the 0 °C under nitrogen atmosphere. To this solution was added a solution of **8** (5.5 g, 17.7 mmol) in CH_3CN (50 ml), and the reaction mixture was stirred for 1 h at the same temperature. The mixture was warmed to r.t. and stirred for 2 h. The mixture was concentrated in vacuo, diluted with ethyl acetate, washed with water and brine, dried over anhydrous Na_2SO_4, and evaporated under reduced pressure. The residue was purified by silica gel column chromatography (*n*-hexane/ethyl acetate = 1:1) to give **10** (9.6 g, 70%) as a pale yellow powder: m.p. 86–87 °C; ^{1}H NMR ($CDCl_3$) δ 8.16 (d, J = 8.6 Hz, 4H), 7.62 (d, J = 8.6 Hz, 2H), 7.48 (d, J = 8.6 Hz, 2H), 5.18–5.46 (m, 4H), 4.25 (m, 1H), 3.98–4.11 (m, 3H), 3.62–3.71 (m, 3H), 3.24–3.34 (m, 3H), 2.51 (m, 1H), 1.72 (m, 1H), 1.25 (d, J = 7.0 Hz, 3H), 1.21 (d, J = 6.2 Hz, 3H), 0.81 (s, 9H), 0.04 (s, 6H).

*4.1.8. 4-Nitrobenzyl (1R,5S,6S)-6-[(1R)-1-(t-butyldimethylsilyoxy)ethyl]-2-[(3S,5S)-5-(methanesulfonyloxymethyl)-1-(4-nitrobenzyloxycarbonyl) pyrrolidin-3-ylthio]-1-methylcarbapen-2-em-3-carboxylate (**11**)*

To a solution of **10** (6.6 g, 8.6 mmol) in CH_2Cl_2 (100 ml) was added triethylamine (1.0 g, 10.3 mmol) and methanesulfonyl chloride (1.1 g, 9.4 mmol) at 0 °C under nitrogen atmosphere. After being stirred for 1 h at the same temperature, the reaction mixture was diluted with CH_2Cl_2, washed with water and brine, dried over anhydrous Na_2SO_4, concentrated in vacuo. The residue was purified by silica gel column chromatography (*n*-hexane/ethyl acetate = 1:1) to give **11** (6.6 g, 92%) as a pale yellow powder: m.p. 87–88 °C; ^{1}H NMR ($CDCl_3$) δ 8.18 (d, J = 8.0 Hz, 4H), 7.61 (d, J = 8.0 Hz, 2H), 7.49 (d, J = 8.0 Hz, 2H), 5.18–5.43 (m, 4H), 4.21–4.52 (m, 2H), 4.02 (m, 1H), 3.70 (m, 1H), 3.23–3.36 (m, 3H), 2.99 (s, 3H), 2.60 (m, 1H), 2.00 (m, 1H), 1.21 (m, 6H), 0.81 (s, 9H), 0.03 (s, 6H).

*4.1.9. 4-Nitrobenzyl (1R,5S,6S)-6-[(1R)-1-hydroxyethyl]-2-[(3S,5S)-5-(methanesulfonyloxymethyl)-1-(4-nitrobenzyloxycarbonyl)pyrrolidin-3-ylthio]-1-methylcarbapen-2-em-3-carboxylate (**12**)*

To a mixed solution (90 ml) of *N*-methylpyrrolidinone/DMF (1:3) of **11** (9.4 g, 11.1 mmol) was added ammonium hydrogen difluoride (2.5 g, 44.3 mmol) at r.t. The reaction mixture was stirred for 48 h, and then diluted with ethyl acetate, washed with water and brine, dried over anhydrous Na_2SO_4, concentrated in vacuo. The residue was purified by silica gel column chromatography (*n*-hexane/ethyl acetate = 1:3) to give **12** (7.9 g, 97%) as a pale yellow powder: m.p. 150–152 °C; ^{1}H NMR ($CDCl_3$) δ 8.18 (d, J = 8.2 Hz, 4H), 7.62 (d, J = 8.2 Hz, 2H), 7.49 (d, J = 8.2 Hz, 2H), 5.18–5.48 (m, 4H), 4.15–4.46 (m, 5H), 4.02 (m, 1H), 3.70 (m, 1H), 3.30 (m, 1H), 3.01 (s, 3H), 2.60 (m, 1H), 2.05 (m, 1H), 1.32 (d, J = 6.3 Hz, 3H), 1.25 (d, J = 7.1 Hz, 3H).

*4.1.10. 4-Nitrobenzyl (1R,5S,6S)-6-[(1R)-1-hydroxyethyl]-2-[(3S,5S)-5-(iodomethyl)-1-(4-nitrobenzyloxycarbonyl) pyrrolidin-3-ylthio]-1-methylcarbapen-2-em-3-carboxylate (**13**)*

To a solution of **12** (4.3 g, 5.9 mmol) in DMF (50 ml) was added potassium iodide (4.9 g, 29.3 mmol) at r.t. After being stirred for 24 h, the reaction mixture was quenched with water. The mixture was extracted with CH_2Cl_2, dried over anhydrous Na_2SO_4, concentrated in vacuo. The residue was purified by silica gel column chromatography (*n*-hexane/ethyl acetate = 1:3) to give **13** (3.3 g, 74%) as a yellow powder: m.p. 161–162 °C; ^{1}H NMR ($CDCl_3$) δ 8.24 (d, J = 8.4 Hz, 4H), 7.66 (d, J = 8.4 Hz, 2H), 7.52 (d, J = 8.4 Hz, 2H), 5.24–5.51 (m, 4H), 4.20–4.30 (m, 2H), 3.89–4.17 (m, 2H), 3.60–3.72 (m, 2H), 3.32–3.58 (m, 3H), 3.20 (m, 1H), 2.60 (m, 1H), 1.99 (m, 1H), 1.37 (d, J = 6.2 Hz, 3H), 1.28 (d, J = 7.0 Hz, 3H).

*4.1.11. General procedure for the synthesis of 4-nitrobenzyl (1R,5S,6S)-2-[(3S,5S)-5-(aminosubstituted pyrimidinyl-2-thiomethyl)-1-(4-nitrobenzyloxycarbonyl)pyrrolidin-3-ylthio]-6-[(1R)-1-hydroxyethyl]-1-methylcarbapen-2-em-3-carboxylates (**14a–d**)*

To a solution of the appropriate aminosubstituted 2-mercaptopyrimidine (0.6 mmol) in DMF (5 ml) was added

K_2CO_3 (0.07 g, 0.6 mmol) at r.t. The reaction mixture was added dropwise to a solution of **13** (0.40 g, 0.5 mmol) in DMF (3 ml) and stirred for 2 h. The reaction mixture was filtered, and then the filtrate was treated with water and ethyl acetate. The organic layer was dried over anhydrous Na_2SO_4, concentrated in vacuo. The residue was purified by silica gel column chromatography (*n*-hexane/ethyl acetate = 1:3) to give the title compound **14**. **14a**: Yield 56%; ^{1}H NMR ($CDCl_3$) δ 8.20 (d, J = 8.7 Hz, 4H), 7.93 (d, J = 5.7 Hz, 1H), 7.64 (d, J = 8.7 Hz, 2H), 7.25 (d, J = 8.7 Hz, 2H), 6.11 (d, J = 5.7 Hz, 1H), 5.11–5.50 (m, 4H), 3.99–4.30 (m, 4H), 3.25–3.35 (m, 6H), 2.50 (m, 1H), 1.89 (m, 1H), 1.36 (d, J = 5.8 Hz, 3H), 1.24 (d, J = 6.9 Hz, 3H). **14b**: Yield 71%; ^{1}H NMR ($CDCl_3$) δ 8.19 (d, J = 8.7 Hz, 4H), 7.63 (d, J = 8.7 Hz, 2H), 7.50 (d, J = 8.7 Hz, 2H), 6.05 (s, 1H), 5.20–5.50 (m, 4H), 3.90–4.25 (m, 5H), 3.26–3.65 (m, 5H), 2.55 (m, 1H), 2.20 (m, 1H), 1.33 (d, J = 6.1 Hz, 3H), 1.22 (d, J = 7.2 Hz, 3H). **14c**: Yield 42%; ^{1}H NMR ($CDCl_3$) δ 8.20 (d, J = 8.6 Hz, 4H), 7.63 (d, J = 8.6 Hz, 2H), 7.55 (d, J = 8.6 Hz, 2H), 5.23–5.49 (m, 5H), 4.24–4.41 (m, 3H), 4.12 (m, 1H), 3.27–3.68 (m, 6H), 2.55 (m, 1H), 2.05 (m, 1H), 1.36, (d, J = 6.2 Hz, 3H), 1.24 (d, J = 6.9 Hz, 3H). **14d**: Yield 42%; ^{1}H NMR ($CDCl_3$) δ 8.21–8.26 (m, 4H), 7.66 (d, J = 8.6 Hz, 2H), 7.53 (d, J = 8.6 Hz, 2H), 5.22–5.53 (m, 4H), 4.26–4.30 (m, 2H), 4.03–4.12 (m, 2H), 3.55–3.77 (m, 3H), 3.28–3.40 (m, 3H), 2.55 (m, 1H), 1.76 (m, 1H), 1.36 (d, J = 6.2 Hz, 3H), 1.30 (d, J = 7.2 Hz, 3H).

*4.1.12. General procedure for the synthesis of (1R,5S,6S)-2-[(3S,5S)-5-(aminosubstituted pyrimidinyl-2-thiomethyl) pyrrolidin-3-ylthio]-6-[(1R)-1-hydroxyethyl]-1-methylcarbapen-2-em-3-carboxylic acids (**1a–d**)*

The appropriate compound **14** (0.3 mmol) and 10% Pd/C (0.25 g) were suspended in a mixed solution (10 ml) of THF/EtOH (10:1), and the reaction mixture was hydrogenated at 50–60 psi at r.t. for 2 h. The mixture was filtered through celite pad, and then the filtrate was washed with ethyl acetate. The aqueous layer was lyophilized to give residue, which was purified on a Diaion HP-20 column by eluting with 3% THF in water to give the title carbapenem **1** as a white amorphous solid. **1a**: Yield 18%; ^{1}H NMR (D_2O) δ 7.80 (d, J = 6.1 Hz, 1H), 6.23 (d, J = 6.1 Hz, 1H), 4.08–4.15 (m, 2H), 3.87–3.91 (m, 2H), 3.19–3.48 (m, 6H), 2.64 (m, 1H), 1.73 (m, 1H), 1.17 (d, J = 6.3 Hz, 3H), 1.09 (d, J = 7.1 Hz, 3H); FABHRMS *m/z* Calcd. for $C_{19}H_{26}N_5O_4S_2$ $(M + H)^+$ 452.1426, Found 452.1424. **1b**: Yield 17%; ^{1}H NMR (D_2O) δ 5.93 (s, 1H) 4.13–4.63 (m, 2H), 3.93–3.95 (m, 2H), 3.27–3.56 (m, 6H), 2.69 (m, 1H), 1.82 (m, 1H), 1.22 (d, J = 6.4 Hz, 3H), 1.14 (d, J = 7.2 Hz, 3H); FABHRMS *m/z* Calcd. for $C_{19}H_{27}N_6O_4S_2$ $(M + H)^+$ 467.1535, Found 467.1526. **1c**: Yield 13%; ^{1}H NMR (D_2O) δ 5.47 (s, 1H), 4.12–4.19 (m, 2H), 3.90–3.92 (m, 2H), 3.25–3.41 (m, 5H), 2.67 (m, 1H), 1.79 (m, 1H), 1.22 (d, J = 6.3 Hz, 3H), 1.44 (d, J = 7.1 Hz, 3H); FABHRMS *m/z* Calcd. for $C_{19}H_{26}N_6NaO_4S_2$ $(M + Na)^+$ 489.1355, Found 489.1350. **1d**: Yield 16%; ^{1}H NMR (D_2O) δ 4.11–4.17 (m, 3H), 3.90 (m, 1H), 3.73 (m, 1H), 3.57 (m, 1H), 3.24–3.38 (m, 4H), 2.65 (m, 1H), 1.58 (m, 1H), 1.20 (d, J = 6.4 Hz, 3H), 1.12 (d, J = 7.2 Hz, 3H); FABHRMS *m/z* Calcd. for $C_{19}H_{27}N_6O_5S_2$ $(M + H)^+$ 483.1484, Found 483.1478.

4.2. Measurement of in vitro antibacterial activity

The in vitro antibacterial activity is given as minimum inhibitory concentration (MIC) in μg/ml as determined by an agar dilution method using Mueller–Hinton Broth. The culture grown at 37 °C for 18 h was diluted to 10^7 CFU/ml, and about 10^4 CFU/ml was spotted onto the agar plates containing serial twofold dilutions of antibiotics with a MIC-2000 multipin inoculator. The plates were incubated at 37 °C for 18 h.

4.3. Determination of susceptibility to renal DHP-I

Stability of carbapenems to hydrolysis by DHP-I was determined using partially purified porcine renal DHP-I. The carbapenems (50 μg/ml solution in 50 mM MOPS at pH 7.0) was challenged with DHP-I at 30 °C. Rates of hydrolysis were monitored on a spectrophotometer at time course between 0, 0.5, 1, 2, and 4 h. The stability was expressed as relative half life of hydrolysis, taking that of meropenem as 1.0.

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