NOVEL QUATERNARY AMMONIUM CARBAPENEMS: 1β-METHYL-2-(5'-SUBSTITUTED PYRROLIDINYLTHIO) CARBAPENEMS

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In the previous papers[1,2], we reported that carbapenem derivatives having a 5'-substituted pyrrolidinylthio group at the C-2 position exhibited broad and strong antibacterial activity. Some of them also showed high stability to renal dehydropeptidase-I (DHP-I). It is known that the plasma half lives ($T_{1/2}$'s) of the carbapenems, which are in clinical use or trials, are almost the same (*ca.* 1 hour) in humans[3~5]. In order to improve the pharmacokinetic properties (especially, $T_{1/2}$) without loss of the excellent antimicrobial activity and DHP-I stability, we have continued our studies on modifications of the substituent on the pyrrolidine ring to change the physico-chemical properties in the series described above. Recently, many types of carbapenem compounds which have quaternary heteroaromatic groups at the C-2 position were prepared, and their properties were well examined[6~11]. However, there were few reports on the effects of the substitution by quaternized aliphatic heterocycles[6,12,13]. We wish to describe here the synthesis of a new series of quaternary ammonium carbapenems (**2**~**4**) (Fig. 1) and the effects of the quaternization on the antimicrobial activity, DHP-I stability and pharmacokinetic parameters.

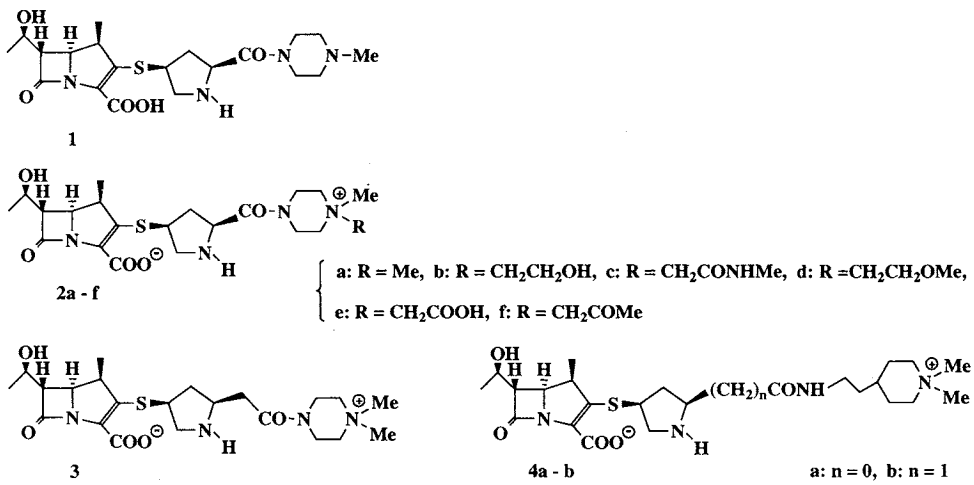
Chemistry

The synthetic routes employed for the title compounds are similar to those reported before[1,2] and the typical procedure is shown in the Scheme. Treatment of the enolphosphate (**5**)[14] with the freshly prepared mercaptan (**6a**) afforded the 2-substituted carbapenem ester (**7a**). After quaternization with methyl iodide, the obtained ammonium salt was deprotected by catalytic hydrogenolysis in aqueous tetrahydrofuran to give the desired carbapenem derivative (**2a**), which could be purified by column chromatography on Dianion CHP-20P. **2a**: IR (KBr) cm^{-1} 3440, 1745, 1640; ^1H NMR (270 MHz, D_2O) δ 1.21 (3H, d, J=7.3 Hz), 1.29 (3H, d, J=6.6 Hz), 1.72 (1H, m), 2.78 (1H, m), 3.11 (1H, dd, J=4.0 and 12.5 Hz), 3.27 (6H, s), 3.20~3.60 (9H, m), 3.80~4.20 (5H, m), 4.23 (3H, m); UV λ_{max} (H_2O) nm 299. The mercaptans (**6**) used in this work were prepared starting from *trans*-4-hydroxy-L-proline in similar procedures as described in the preceding papers[1,2].

Biological Studies

The *in vitro* antibacterial activities (MIC's) and the stabilities to DHP-I of the title carbapenems are shown in Table 1. In the piperazinium series (**2a**~**2f**), all compounds exhibited well balanced and potent antimicrobial activities comparable to

Fig. 1.



Scheme 1.

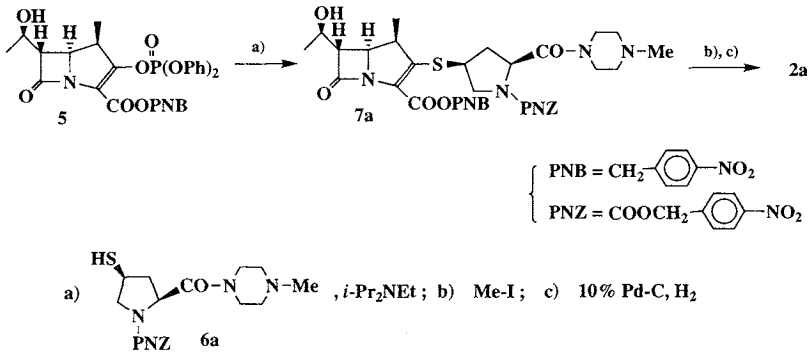


Table 1. Antimicrobial activity and DHP-I stability of carbapenem compounds.

Organism	Compound No.	MIC (μg/ml) 1	2a	2b	2c	2d
S.a. FDA 209P		0.10	0.10	0.05	0.10	0.10
S.p. Cook		<0.013	<0.013	<0.013	<0.013	<0.013
E.c. NIHJ JC-2		0.05	0.05	<0.013	0.05	0.10
K.p. ATCC 10031		0.025	0.05	<0.013	0.05	0.025
P.m. GN 2425		0.05	0.20	0.05	0.20	0.10
P.a. IFO 3451		0.78	0.20	0.20	0.39	0.39
S.m. X 100		0.05	0.10	0.05	0.20	0.05
E.c. ML 1410 RP4[a]		0.05	0.20	0.025	0.20	0.05
P.v. GN 7919[a]		0.10	0.39	0.10	0.39	0.20
S.m. GN 6473[a]		0.10	0.10	0.10	0.20	0.20
DHP-I[b] $T_{1/2}$ (minute)		44	160	150	140	ne

Organism	Compound No.	MIC (μg/ml) 2e	2f	3	4a	4b
S.a. FDA 209P		0.39	0.10	0.05	0.10	0.025
S.p. Cook		0.025	<0.013	<0.013	0.025	<0.013
E.c. NIHJ JC-2		0.025	0.10	0.20	0.10	0.10
K.p. ATCC 10031		0.025	0.025	0.10	0.05	0.20
P.m. GN 2425		0.10	0.10	0.78	0.39	0.10
P.a. IFO 3451		0.78	0.39	1.56	1.56	1.56
S.m. X 100		0.05	0.10	0.39	0.20	0.20
E.c. ML 1410 RP4[a]		0.025	0.10	0.78	0.20	0.39
P.v. GN 7919[a]		0.20	0.20	1.56	0.39	0.78
S.m. GN 6473[a]		0.20	ne	0.39	0.39	0.39
DHP-I[b] $T_{1/2}$ (minute)		320	130	390	340	480

[a] β-Lactamase producing strain.

[b] Partially purified renal DHP-I of swine[18].

Abbreviations: *S.a.*, *Staphylococcus aureus*; *S.p.*, *Staphylococcus 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*; ne, Not examined.

that of the parent compound (**1**). The quaternization drastically improved the stabilities to DHP-I as reported by other groups[6~13], especially in the case of compound **2e**. This increase of the DHP-I stability could not be completely explained by the mere presence of the positive charge, since compound **1** should also exist as the ammonium form by protonation in neutral media (pH *ca.* 7). It is

Table 2. Pharmacokinetic parameters[c] of carbapenem compounds **1** and **2a** following co-administration with cilastatin (20 mg/kg each, i.v.) to rats.

Compound No.	n[d]	$T_{1/2}$ (minute)	CL_{tot}[e] (ml/minute/kg)	CL_s[f] (ml/minute/kg)	V_d (ml/kg)	C_{60}[g]
1	3	10.8±0.6	15.8±1.6	3.7	245±16	1.7±0.4
1+PBC[h]	3	13.9±0.3	12.1±0.7		244±19	4.3±0.2
2b	3	13.4±1.5	13.9±1.1	−0.4	269±47	3.3±0.7
2b+PBC[h]	3	14.0±0.2	14.3±1.6		288±36	3.5±0.3

[c] Analysis by the one-compartment model.
[d] Number of animals tested.
[e] Total body clearance (Dose/AUC).
[f] Tubular secretion clearance (ΔCL_{tot}).
[g] Concentration of the carbapenem in plasma after 60 minutes.
[h] Probenecid: dose = 40 mg/kg.

suggested that the bulkiness around the quaternary nitrogen atom might be another important factor. Concerning the further increase of resistance to DHP-I in the case of compound **2e**, the presence of a carboxyl group, which exists as a carboxylate anion, might decrease the affinity to the enzyme by ionic repulsion. Other derivatives, that have an alkylene spacer between the pyrrolidine ring and the quaternary heterocycle (compounds **3** and **4**), showed less antipseudomonal activity, whereas they exhibited greater stability to DHP-I. The latter observation coincides with the previous studies[2,12].

Compound **2b** showed the most excellent and well-balanced antimicrobial activity among the synthesized compounds. We investigated the pharmacokinetics of compound **2b** in rats to compare it with the parent compound (**1**). Unlike humans, in the case of rats, DHP-I is also distributed in their lung with high activity as well as the kidney, and the pulmonary DHP-I affects the elimination rate from systemic circulation of carbapenem[15]. To estimate the clearance of carbapenem without metabolism by DHP-I, each compound was co-administered with cilastatin at a dose (20 mg/kg each, iv) which inhibits DHP-I activity sufficiently, but dose not inhibit renal tubular secretion of a typical β-lactam compound, such as penicillin G (data not shown). Plasma concentrations after intravenous administration to rats were determined by the bioassay method described for the analysis of meropenem[16] by using *Bacillus subtilis* ATCC 6633 as the test organism. The pharmacokinetic parameters of compounds **1** and **2b** were calculated by one-compartment model analysis[17] (Table 2). **2b** exhibited longer plasma half life ($T_{1/2}$) than **1**. Although the $T_{1/2}$ of **1** was prolonged by the co-administration of probenecid, which prohibited the tubular secretion in the kidney, such prolongation of $T_{1/2}$ was not observed in the case of **2b**. It indicated that **2b** was disposed mainly by glomerular filtration in the kidney and this might be due to the presence of the quaternary ammonium group. As long-time action might be expected from the increased $T_{1/2}$, it could be assumed that **2b** may have greater efficacy *in vivo*.

In conclusion, the title compounds show the expected improvements in pharmacokinetic properties. Further evaluation of **2a**~**2c** including *in vivo* studies is in progress.

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