Synthesis and Structure-activity Relationship of 2-(5-Substituted pyrrolidin-4-ylthio)-1β-carbapenems

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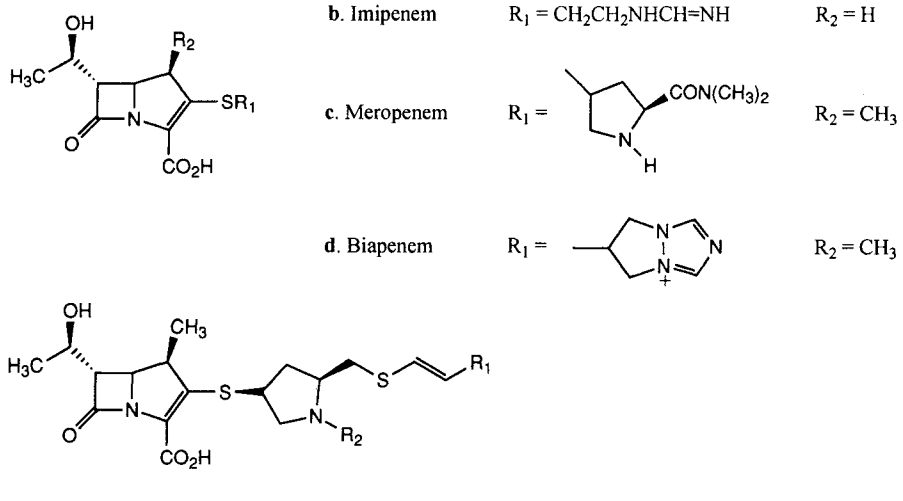
The carbapenems are important β-lactam antibiotics with a unique nuclear structure that differs from the penam and cephem nuclei of penicillins and cephalosporins, respectively. Because of their unrivaled antibacterial spectra and remarkable stabilities toward a wide range of β-lactamases as embodied in imipenem (**b**) and the fact that even imipenem has been of great deal interest to medicinal chemists in finding new and improved carbapenems[1~5]. Despite the inherent problem of chemical and metabolic instability combined with poor semi-synthetic methods for this class of compounds, considerable progress has been made. Two new carbapenems, meropenem (**c**) and biapenem (**d**) have been shown to possess better microbiological and pharmacokinetic profiles[6,7]. Meropenem is stable to renal DHP and it has recently been approved for clinical use in some countries. In recent years, a few other analogs such as DX-8739, GV-104326, BO-2727 and S-4661 have been reported to possess some advantages over the known derivatives[8~11].

Although the low molecular weight, excellent β-lactamase stability and high affinity for bacterial PBPs of carbapenems provide exceptional antibacterial properties, the nature of the C-2 substitution has also been recognized as major contributor to the antibacterial properties of carbapenems. The presence of a basic or quaternary amino moiety at C-2 considerably improves the chemical stability and extends the relative antibacterial spectrum relative to compounds with less basic side chains at the same position[5,12]. In recent years, further attempts were made to improve the antimicrobial activity against clinically resistant Gram-negative strains including *Pseudomonas* spp. by exchanging C-2 position substituents with more basic or cationic substituents. There are limited reports[13] on the effect of C-2 position substituents in carbapenems toward the Gram-positive strains including highly resistant *S. aureus*.

Recently we reported[14~16] the synthesis and biological properties of new carbapenem compounds having 2′-aromatic heterocyclic pyrrolidine and 2′-substituted carbamoyl pyrrolidine as the C-2 side chain.

As a continuation of this program to obtain better antibacterial activities against *Pseudomonas* spp. and greater stability to renal DHP-I we focused our attention on the modification of the substitution on the pyrrolidine side chain. Some carbapenem derivatives with a 2′-hydroxyalkyl or substituted carbamoyalkyl pyrrolidin-

Fig. 1. Thienamycin, imipenem and carbapenem antibiotics having a (4*S*)-pyrrolidin-4-ylthio group at C-2 position.



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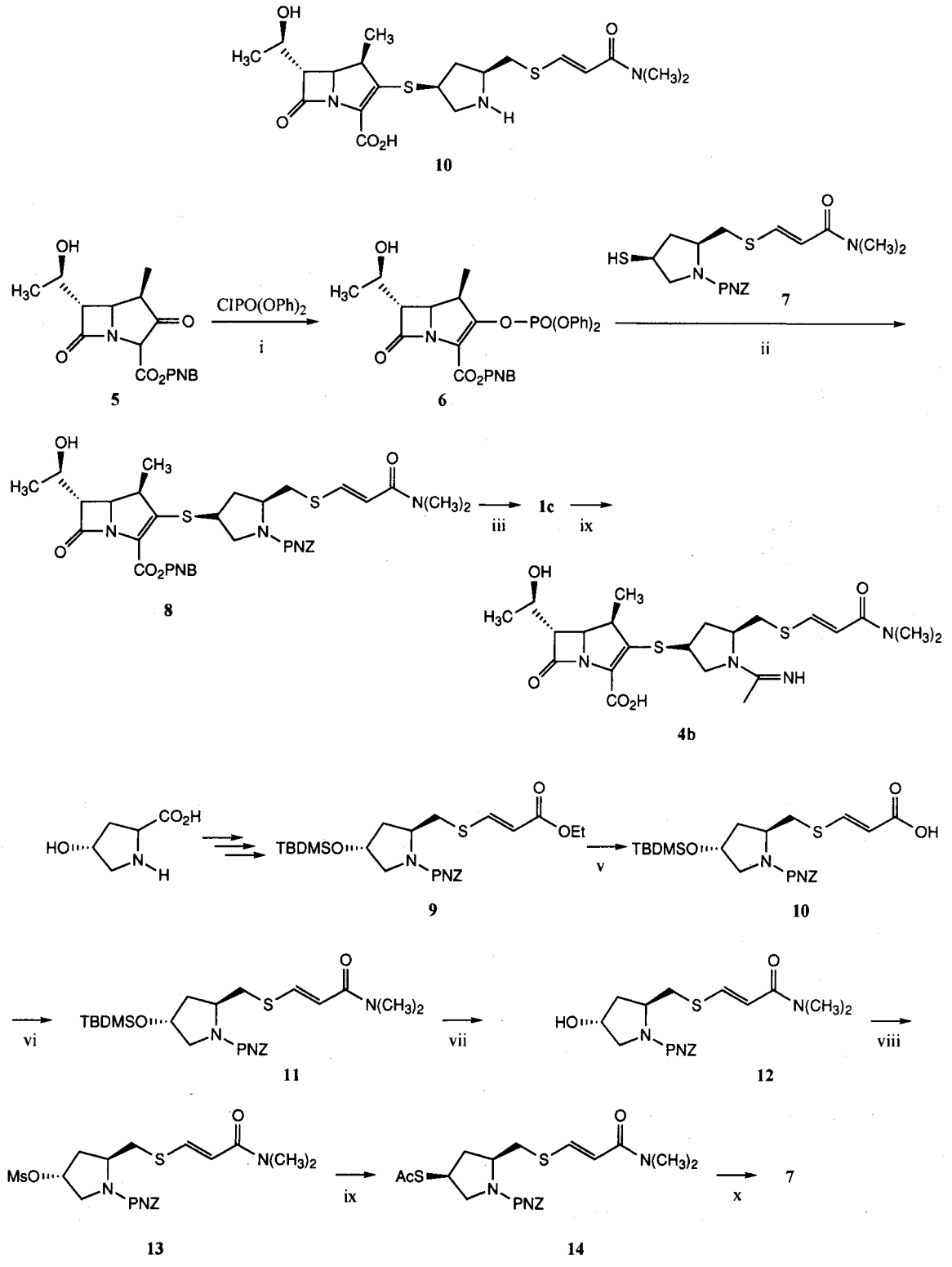
Scheme 1.



Reagents and conditions: i) *i*-Pr_2NEt, CH_3CN, 0°C, 1h; ii) *i*-Pr_2NEt, CH_3CN, -20°C, 2h; iii) 1M NH_4Cl, Fe powder, MOPS-THF, 0°C, 1h; iv) $CH_3C{=}NH(OEt){\cdot}HCl$, 10% K_2CO_3, pH = 8.5, 0°C, 2h; v) 28% NaOMe, MeOH, 0°C, 0.5h, acidify; vi) $(Im)_2CO$, CH_3CN, $HN(CH_3)_2$, rt, 4h; vii) 6N HCl, MeOH, rt, 2h; viii) MsCl, Et_3N, CH_2Cl_2, 0°C, 1h; ix) KSAc, DMF, 70°C, 2h; x) 4N NaOH, MeOH, 0°C, acidify.

4-ylthio group at the C-2 position have been reported in the literature[17,18]. Since we found that the compound (**1c**), having a carbamoylethenyl group at the C-2 position of pyrrolidine, showed good antibacterial activity, our subsequent research was focused on the biological properties of these compounds (Table 1). The results indicated that the carbamoylethenylmercaptopyrrolidine group (**1c**) was the most appropriate substituent for both good antipseudomonal activity and improved stability against DHP-I. The synthetic route chemistry employed for the synthesis of title compounds is similar to those reported in the literature[19,20] and the typical procedure is shown in Scheme 1. The bicyclic keto compound (**5**) was treated with diphenyl chlorophosphate in the presence of diisopropylethyl-amine to obtain a common intermediate enol phosphate (**6**) *in situ* which was then reacted with freshly prepared (**7**) to form carbapenem (**8**).

(**8**): Yield 52.7%, IR (nujol) cm^{-1}: 1779, 1718, 1680; ^1H NMR (400 MHz, $CDCl_3$) δ: 1.28 (3H, d, J=6.5 Hz), 1.32 (3H, d, J=6.3 Hz), 3.06 (3H, s), 3.13 (3H, s), 3.34 (1H, dd, J=3.0 and 7.0 Hz), 3.48 (1H, m), 4.16~4.38 (2H, m), 6.62 (1H, d, J=16.0 Hz), 7.69 (4H, dd, J=8.0 and 9.0 Hz), 8.15 (1H, d, J=16.0 Hz), 8.23 (4H, dd, J=7.0 and 9.0 Hz).

Deprotection of (**8**) by 1 M aqueous NH_4Cl followed by iron powder at ice temperature in the presence of 3-morpholino propanesulfonic acid (MOPS) buffer (0.1, pH=7.0) provided the target molecule. (1*R*,5*S*,6*S*)-2-[(2*S*,4*S*)-{(2-*N*,*N*-dimethyl)carbamoylethenylmercaptomethyl}pyrrolidin-4-ylthio]-6-[(1*R*)-1-hydroxyethyl]-1-mercapto-1-carbapen-2-em-3-carboxylic acid (**1c**). Column purification of the crude product on Diaion HP-20 gave the *N*,*N*-dimethylcarbamoylethenylmercaptopyrrolidine carbapenem derivative as an white amorphous solid (**1c**): yield 27.5%; UV λ_{max} (0.1 M MOPS buffer, pH=7.0): 298.5 nm; IR (KBr) cm^{-1}: 1750, 1630, 1600, 1390, ^1H NMR (400 MHz, D_2O) δ: 1.16 (3H, d, J=8.0 Hz), 1.27 (3H, d, J=6.5 Hz), 3.01 (3H, s), 3.17 (3H, s), 3.34~3.68 (4H, m), 4.14~4.30 (2H, m), 6.62 (1H, d, J=16.0 Hz), 7.84 (1H, d, J=16.5 Hz). *N*-Methylimidoylation of compound (**1c**) with methyl acetimidate hydrochloride in 10% K_2CO_3 solution provided an off-white amorphous solid (**4b**). (**4b**): yield 78.5%; IR (KBr) cm^{-1}: 1755, 1715, 1690, 1655, 1630 ^1H NMR (400 MHz, D_2O) δ: 1.21 (3H, d, J=7.0 Hz), 1.27 (3H, d, J=7.4 Hz), 1.60~1.75 (1H, m), 2.39 (3H, s), 3.25~4.45 (6H, m), 6.64 (1H, d, J=16.5 Hz), 7.85 (1H, d, J=18.0 Hz).

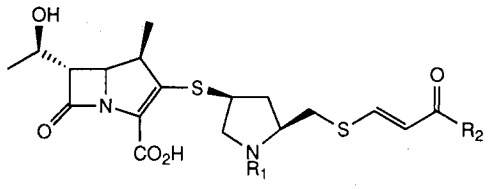
The common intermediate, the 4′-*tert*-butyldimethylsilyl-2′-*N*,*N*-dimethylcarbamoylethenylmercaptomethylpyrrolidine (**9**) was synthesized by known methods[17~21] using *trans*-4-hydroxy-L-proline as a starting material. Reaction of the compound (**9**) with 28% NaOMe in methanol afforded the carboxyethenylmercaptopyrrolidine compound (**10**). Amination of compound (**10**) was carried out with carbonyldiimidazole and dimethylamine in acetonitrile to gave dimethylcarbamoylethenylpyrrolidine compound (**11**). Desilylation of compound (**11**) carried out with 6 N HCl in methanol gave the 4′-hydroxycarbamoylethenylpyrrolidine compound (**12**). After mesylation of compound (**12**), the mesylated carbamoylethenylmercaptopyrrolidine compound (**13**) was converted into the acetylthiopyrrolidine compound (**14**) with potassium thioacetate in DMF, and the acetyl protecting group was readily hydrolyzed with 4 N NaOH solution to gave the new thiocarbamoylethenylpyrrolidine compound (**7**). Thus, the thiopyrrolidine compound (**7**) was obtained in **12** steps with the high overall yield of 21.4%.

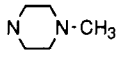
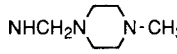
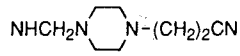
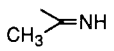
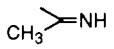
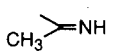
Results and Discussion

The minimum inhibitory concentrations (MICs) of the novel carbapenems for Gram-positive and Gram-negative bacteria and stability data ($T_{1/2}$) with DHP-I are listed in Table 1, along with the values for imipenem and meropenem for comparison. The nonheterocyclic pyrrolidinyl carbapenem derivatives (**1a~2b** and **4a~4c**), except for compound (**3a**), exhibited enhanced antibacterial activity against *P. aeruginosa* compared to the heterocyclic pyrrolidine carbapenems (**3b**, **3c**). These *N*-acetimidoylated pyrrolidine carbapenem derivatives (**4a~4c**) did not show high activity against *P. aeruginosa*, whereas they possessed good stability to DHP-I.

The novel carbapenem compounds (**1a**, **1c**, **1j**) exhibited enhanced or similar antibacterial activity to imipenem against *P. aeruginosa*. As the extent of alkyl group in the carbamoyl group increased, antibacterial activity generally decreased Gram-negative bacteria, as shown by compounds (**1a**, **1c**, **1j**) which exhibited higher activity against *P. aeruginosa* than compounds (**1b**, **1d~1k**). There was no significant difference between the activity of meropenem and that of compound (**1c**). Carbamoyloxyalkyl-substituted pyrrolidine compound (**1j**) exhibited higher activity than hydroxy, amino and carbamoylalkyl-substituted compounds (**1e~1i** and **1k**). Sulfamoyl-substituted carbamoyl pyrrolidine compounds (**2a**, **2b**) did not show higher activity than imipenem and meropenem against *P. aeruginosa*. Introduction of a heterocyclic

Table 1. Antibacterial activity and DHP-1 stability of carbapenem derivatives.



Comp.	R_1	R_2	MIC (μg/ml)[a]						DHP-1[c]
			S.a.[b]	*S.p.*	*E.c.*	*P.a.*	*K.a.*	*En.c.*	($T_{1/2}$) min
1a	H	NH_2	0.05	0.01	0.10	0.20	0.20	0.05	480
1b	H	$NHCH_3$	0.05	0.01	0.10	0.39	0.20	0.10	505
1c	H	$N(CH_3)_2$	0.05	0.01	0.10	0.10	0.10	0.05	516
1d	H	$NCH_3(Et)$	0.10	0.05	1.56	6.25	0.39	0.05	548
1e	H	$NH(CH_2)_2OH$	0.05	0.01	0.05	0.39	0.20	0.05	498
1f	H	$NHCH(OH)CH_3$	0.10	0.05	0.39	6.25	0.39	0.10	463
1g	H	$NHCH(OH)CH_2OH$	0.10	0.10	0.10	1.56	0.39	0.10	510
1h	H	$NHCH_2CONH_2$	0.05	0.01	0.10	0.39	0.20	0.10	423
1i	H	$NHCH_2CON(CH_3)_2$	0.39	0.05	0.78	3.13	0.39	0.05	475
1j	H	$NHCH_2OCONH_2$	0.05	0.01	0.10	0.20	0.20	0.05	405
1k	H	$NHCH_2NH_2$	0.10	0.01	0.78	0.78	0.39	0.05	478
2a	H	$NHSO_2NH_2$	0.05	0.01	0.39	0.20	0.78	0.10	387
2b	H	$NHSO_2N(CH_3)_2$	0.05	0.05	1.56	3.13	3.12	0.20	409
3a	H		0.10	0.39	0.78	0.78	0.39	0.20	548
3b	H		0.10	0.39	1.56	6.25	3.12	0.20	578
3c	H		0.39	0.78	3.12	12.50	3.12	0.20	590
4a		NH_2	0.78	0.78	0.39	1.56	0.20	0.05	610
4b		$N(CH_3)_2$	0.39	0.39	0.39	0.78	0.20	0.20	640
4c		$NHCH_2OCONH_2$	0.39	0.78	3.12	3.13	0.39	0.20	635
Imipenem			0.10	0.01	0.20	0.20	0.20	0.05	34
Meropenem			0.10	0.01	0.10	0.10	0.10	0.05	152

[a] Agar dilution method

[b] *S.a.*; *Staphylococcus aureus* SG 51, *S.p.*; *Streptococcus pyogenes* A77, *E.c.*; *Escherichia coli* O55, *P.a.*; *Pseudomonas aeruginosa* 1771M, *K.a.*; *Klebsiella aerogenes* 1522E, *En.c.*; *Enterobacter cloacae* 1321E

[c] DHP-1; Dehydropeptidase-1 (Sigma Chemical Co.: Kidney acetone powder -porcine, type II)

alkylsubstituted carbamoyl group (**3a**~**3c**) significantly lowered the antibacterial activity against *P. aeruginosa*. In general, piperazinylmethyl substituted carbamoylpyrrolidine compounds (**3a**~**3c**) shown high resistance to enzymatic stability DHP-I. Most the novel carbapenem derivatives shown enhanced or similar antibacterial activity to imipenem against Gram-positive and Gram-negative bacteria except *P. aeruginosa*. Based on the overall biological and physico-chemical properties the novel compound (**1c**) was selected for further evaluation and is presently under biological evaluation.

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