Synthesis and Antibacterial Activity of Novel 4-Pyrrolidinylthio Carbapenems II: 2-Alkyl and 2-Arylthiomethyl Derivatives

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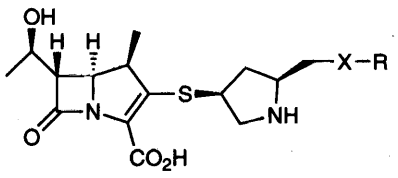
A number of carbapenem antibiotics, for example imipenem, panipenem and meropenem are currently in clinical use due to their broad antibacterial spectra and potent bactericidal effects.[1] However, a number of problems still remain with these agents, in particular, activity against resistant Gram-positive bacteria such as methicillin-resistant *Staphylococcus aureus* (MRSA) and the Gram-negative pathogen *Pseudomonas aeruginosa*, is relatively weak. Furthermore, stability to the renal enzyme dehydropeptidase-I (DHP-I) is poor, especially for the first generation examples imipenem and panipenem, but is also not exceptionally good for meropenem, the prototypical second generation carbapenem. As a part of our research for novel carbapenems possessing potent broad spectrum antibacterial efficacy and good DHP-I stability, we earlier described a novel series of 2-alkoxymethylpyrrolidin-4-ylthio derivatives **2** and reported the biological profile, including *in vivo* efficacy.[2] These compounds possessed good DHP-I stability, high urinary recovery, and good activity against *P. aeruginosa*, however activity against MRSA was not sufficient.

In order to improve activity, especially against MRSA, we have investigated the synthesis and biological activity of 2-alkylthiomethyl isosteres **1** of our earlier 2-alkoxymethyl series **2**. We expected that activity against Gram-positive bacteria, including MRSA, would improve as a result of the greater lipophilicity of sulfur relative to oxygen, whilst we postulated that activity against Gram-negative bacteria would be maintained, since under certain circumstances sulfur functions as a bioisostere[3] of oxygen. A limited number of 2-alkylthiomethylpyrrolidin-4-ylthio carbapenems were described recently,[4~6] however substituents were limited to aliphatic side chains. In this paper we wish to disclose the synthesis and biological evaluation of some novel 2-alkyl and 2-arylthiomethyl carbapenem derivatives, including examples with basic heteroaromatics and cationic quaternary ammonium salts, several examples of which displayed excellent *in vitro* antibacterial activity against MRSA.

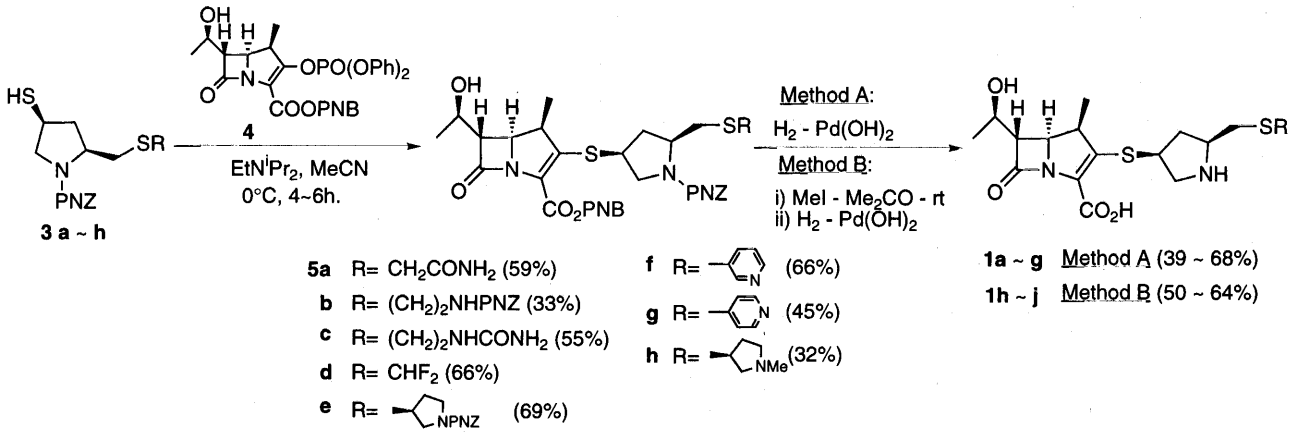
The synthetic route to prepare the novel 2-alkyl and 2-arylthiomethylpyrrolidin-4-ylthio carbapenem derivatives is shown in Scheme 1. Synthesis of carbapenems was achieved by a similar method described in our previous paper[2] from thiols (**3a~h**) which are obtained readily from hydroxyproline. Thiols **3a~h** were treated with the activated carbapenem **4** in the presence of Hünig's base to give protected carbapenem derivatives **5**, which were subsequently deprotected by hydrogenolysis to give **1a~g** (method A). In the case of cationic side chains, quaternary salt formation was achieved with methyl iodide, subsequent deprotection then afforded carbapenems **1h~j** (method B). For example, a mixture of **4** (1 equiv.) and **3f** (1.3 equiv.) in acetonitrile was treated with Hünig's base (1.4 equiv.) at 0°C for 2 hours. Standard work-up and silica-gel chromatography then gave **5f** (66%) [IR (Nujol) 1770 cm^{-1} (β-lactam C=O), 1680 cm^{-1} (PNZ C=O); ^1H NMR (200 MHz, $CDCl_3$) δ 8.61 (br s, 1H), 8.43~8.38 (m, 1H), 8.22 (d, 4H, J=8.8 Hz), 7.81 (d, 1H, J=7.9 Hz), 7.66 (d, 2H J=8.8 Hz), 7.47 (br d, 2H, J=8.5 Hz), 7.27~7.08 (m, 1H), 5.51 and 5.23 (ABq, 2H, J=13.7 Hz), 5.13 (br s, 2H), 4.29~3.14 (m, 10H), 2.70~2.55 (m, 1H), 2.17~1.95 (m, 1H), 1.36 (d, 3H, J=6.2 Hz), 1.27 (d, 3H, J=6.6 Hz)]. Deprotection of **5f** was best achieved by hydrogenolysis in a buffered THF-H_2O medium. For example, a solution of **5f** in THF-phosphate buffer (0.1 N, pH=6.3) was treated with $Pd(OH)_2$-C (20%) under a hydrogen atmosphere for 5 hours at room temperature. Filtration, concentration, purification by HP-20 column chromatography, and lyophilization gave **1f** as a white powder (60%). [IR (Nujol) 1755 cm^{-1} (β-lactam C=O), MS (FAB) 436 (MH^+), ^1H NMR (90 MHz, D_2O) δ 8.63 (s, 1H), 8.49 (d, 1H, J=5.4 Hz), 8.03 (d, 1H, J=8.6 Hz) 7.50 (dd, 1H, J=8.6, 5.4 Hz), 4.40~3.30 (m, 10H), 3.00~2.68 (m,



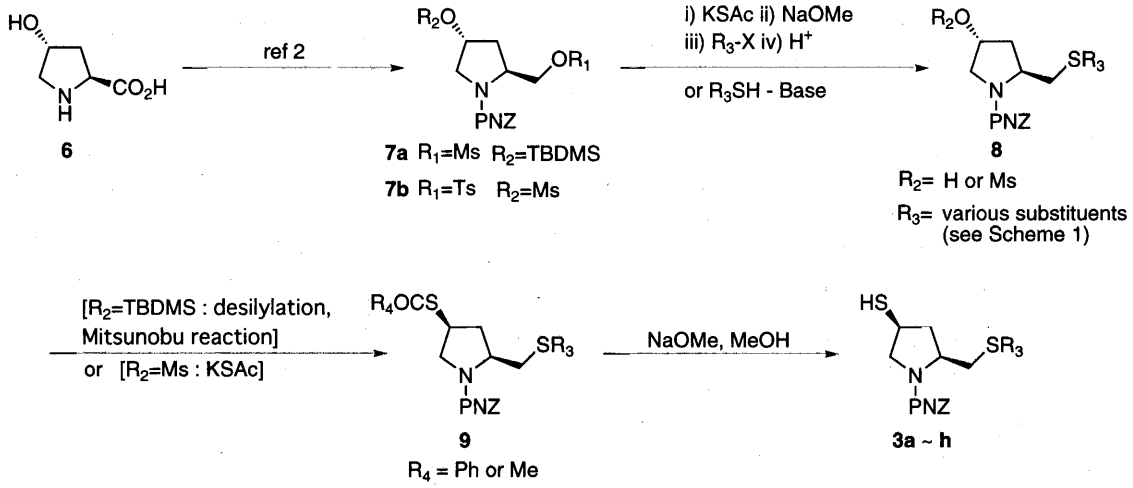
1a~j X=S

2a~c X=O

Scheme 1. Synthesis of novel alkyl (aryl) thiomethyl-substituted carbapenems.



Scheme 2. Synthesis of key pyrrolidine thiols (**3**).

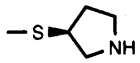
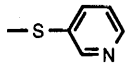
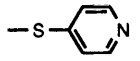
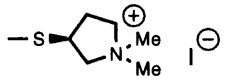
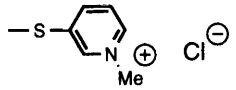
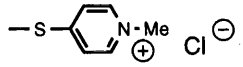


1H), 2.02~1.53 (m, 1H), 1.39 (d, 3H, J=5.4 Hz), 1.29 (d, 3H, J=8.1 Hz)]. Carbapenems containing a quaternary cationic center in the side chain were obtained by reaction of the fully protected derivative **5f~h** with methyl iodide, followed by deprotection. Ion exchange chromatography before lyophilization served to exchange the counterion to chloride.

Thiols **3a~h** were generally prepared from 4-hydroxyproline **6** as summarized in Scheme 2. **6** was converted to a mesylate or tosylate (**7**) by similar methods as described in the literature.[2,6] **7a** was then converted to the acetylthio derivative by reaction with a thioacetate salt; subsequent deprotection, *S*-alkylation and desilylation gave **8** (R_2=H). The doubly activated derivative **7b** was converted to **8** (R_2=Ms) selectively by reaction with various thiol derivatives. Conversion of **8** to the thioacetates **9** was achieved the Mitsunobu method or reaction with a thioacetate salt;[2,6] straightforward deprotection (NaOMe–CH_3CN) then gave thiols **3a~h**. Some of the derivatives **8** required additional conversions to produce the appropriate starting material. The protected amine **8** (R_2=H, R_3=SCH_2CH_2NHPNZ) and urea **8** (R_2=H, R_3=$SCH_2CH_2NHCONH_2$) were obtained from amide **8** (R_2=H, R_3=SCH_2CONH_2) by reduction (B_2H_6) and subsequent protection or urea formation by KOCN. Cyclic amine **8** (R_2=H, R_3=*N*-methylpyrrolidinylthio) was obtained by borane reduction of the corresponding *N*-formylpyrrolidine.

The antibacterial activities of the new carbapenems prepared in this work are shown in Table 1. MICs were obtained by the standard agar dilution method. As references, the antibacterial activity of panipenem and meropenem, as well as 2-alkoxymethyl derivatives (**2a~c**), which were described in our earlier report[2], and the

Table 1. Antibacterial activity (MIC) of novel carbapenems (**1**).

	R	S.a.	S.a.(R)	E.c.	K.p.	E.cl.	P.m.	P.v.	P.a.1	P.a.2
	Panipenem	0.025	50	0.2	0.1	0.39	1.56	1.56	0.36	0.39
	Meropenem	0.1	25	0.025	0.025	0.05	0.05	0.1	0.2	0.39
2a	OCH_2CONH_2	0.1	50	0.1	0.1	0.2	0.78	0.78	0.39	0.78
2b	$OCH_2CH_2NH_2$	0.05	12.5	0.2	0.39	0.78	3.13	3.13	1.56	1.56
2c	$OCH_2CH_2NHCONH_2$	0.1	25	0.2	0.2	0.39	0.78	0.39	0.39	0.78
1a	SCH_2CONH_2	0.1	1.56	0.2	0.05	0.1	0.2	0.2	3.13	1.56
1b	$SCH_2CH_2NH_2$	≦0.025	3.13	0.1	0.1	0.39	1.56	1.56	1.56	0.39
1c	$SCH_2CH_2NHCONH_2$	0.050	12.5	0.1	0.05	0.2	0.39	0.39	6.25	1.56
1d	$SCHF_2$	0.05	0.39	0.05	0.05	0.1	0.1	0.2	12.5	3.13
1e		≦0.025	6.25	0.2	0.1	0.2	0.78	0.39	1.56	0.78
1f		≦0.025	12.5	0.05	≦0.025	0.05	0.2	0.1	25	6.25
1g		0.05	0.39	0.1	0.05	0.39	0.2	0.2	100	6.25
1h		0.05	12.5	0.2	0.39	0.39	0.78	0.78	3.13	0.78
1i		0.05	12.5	0.39	0.39	0.78	1.56	0.78	25	3.13
1j		0.1	25	0.39	0.2	0.39	0.78	0.78	25	1.56

S.a., *S. aureus* 209P JC-1; S.a.(R), *S. aureus* 3004; E.c., *E. coli* NIHJ JC-2; K.p., *K. pneumoniae* 12; E.cl., *E. cloacae* 60; P.m., *P. mirabilis* 1; P.v., *P. vulgaris* IAM 1025; P.a.1, *P. aeruginosa* IAM 1095; P.a.2, *P. aeruginosa* FP 1457.

Table 2. Urinary recovery and DHP-I stability of selected novel carbapenems.

Compound	**1a**	**1b**	**1d**	**1e**	**Meropenem**
DHP-I Stability*1 (Human)	0.76	0.44	0.43	0.125	1.0
Urinary Recovery*2	24	21	38	39	20

*1 DHP-I Stability is given relative to meropenem
*2 Recovery (%) in Rats after s.c. administration (20 mg/kg)

carbamoylmethyl derivative **1a**, which was described by LEE *et al.*[4] are included. The effect of incorporation of a variety of heteroaryl, basic, and cationic substituents to the thiomethyl side chain was examined, as well as a number of aliphatic derivatives containing polar functionality.

At first we synthesized the aliphatic derivatives **1a**~**c** for comparison with the reference compounds **2a**~**c**. Activity against MRSA was clearly improved in **1a**~**c**; this is presumably an effect of increased lipophilicity due to the sulfur atom as postulated. In the aliphatic series (**1a**~**e**), fluoromethyl derivative **1d** was especially potent against MRSA. However, the activity of these derivatives (**1a**~**e**) against *P. aeruginosa* was relatively weak

compared to **2a**～**c**, except for amine derivatives (**1b**, **e**), which displayed well balanced activity against both MRSA and *P. aeruginosa*. Next, we introduced a pyridine moiety (**1f**, **g**) as an aromatic amine function. Amongst these compounds, 4-pyridine (**1g**) was especially potent against MRSA, although activity against *P. aeruginosa* was reduced. To improve activity against *P. aeruginosa*, formation of the quaternary salts (**1h**～**k**) was investigated, but it was not effective. As a result, our strategy to improve activity (especially against MRSA) was partially successful. We could find very potent derivatives (**1d**, **g**) against MRSA, and well balanced derivatives (**1b**, **e**) which displayed relatively potent activity against both MRSA and *P. aeruginosa*.

Urinary recovery and DHP-I stability of selected carbapenems from this novel series are shown in Table 2. Whilst DHP-I stability is superior to meropenem, urinary recovery is relatively low.

In summary, we have prepared a novel series of 2-alkyl and 2-arylthiomethylpyrrolidin-4-ylthio carbapenems *via* coupling reaction of thiols **3a**～**h** with activated carbapenem **4**. The antibacterial activity of lipophilic thio alkyl 4-pyrrolidinylthio carbapenem against MRSA was improved compared to the reference compounds, panipenem and meropenem.

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