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Synthesis and Biological Activity of 1β-Methyl-2-[5′-isoxazoloethenylpyrrolidin-3′-ylthio]carbapenems

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Abstract—A new series of 1β-methylcarbapenems **1a**–**i** bearing isoxazoloethenyl groups on the pyrrolidine ring has been prepared and evaluated for in vitro antibacterial activity and stability to DHP-I. Most compounds showed excellent antibacterial activity and high stability to DHP-I superior to that of meropenem. Of these new carbapenems, **1a**,**b**,**h** exhibited the best combination of antibacterial activity and DHP-I stability.

Recently, 1β-methylcarbapenems have received much attention because of their excellent biological and chemical behavior.[1] Meropenem,[2] which has potent antibacterial activity and high stability to dehydropeptidase-I (DHP-I), has been launched on the market and several compounds are currently under clinical or preclinical evaluation.[3–6]

In a recent paper[7] we described the synthesis, antibacterial activity, and stability to DHP-I of a series of novel 1β-methylcarbapenems containing the isoxazolopyrrolidine moiety. These carbapenems exhibited potent and well-balanced antibacterial activity including *P. aeruginosa* as well as high stability to DHP-I comparable to that of meropenem. Also, Banyu scientists recently reported carbapenems possessing potent anti-MRSA activity, and demonstrated that the increased lipophilicity of the C-2 side chain of carbapenem enhanced anti-MRSA activity.[8–10] Taking these results into consideration, we investigated the incorporation of ethenyl group as a hydrophobic spacer between pyrrolidine ring and isoxazole moiety in order to enhance the anti-Gram-positive activity. The resulting new carbapenems were found to exhibit excellent antibacterial activity against both Gram-positive and Gram-negative bacteria and possess high stability to DHP-I. Especially, these carbapenems showed excellent activity against Gram-positive bacteria compared to parent carbapenems[7] containing the isoxazolopyrrolidine moiety.

In this paper, we wish to disclose the synthesis and biological evaluation of new 1β-methylcarbapenems **1a**–**i** having a 5′-isoxazoloethenylpyrrolidin-3′-ylthio group as a C-2 side chain (Fig. 1).

Chemistry

Each isoxazoloethenylthiol **12**–**17** was prepared by the sequence of reactions shown in Scheme 1. Triphenylphosphonium bromides **3a**–**f** containing the isoxazole moiety were obtained from bromomethylisoxazoles **2a**–**f** with triphenylphosphine in CH_3CN in 90–95% yields. The starting materials **2a**–**f** were prepared by known methods.[11] Introduction of the ethenyl group was performed by Wittig methodology of aldehyde **4** with the corresponding **3a**–**f** in the presence of sodium bis(trimethylsilyl)amide to yield the isoxazoloethenylpyrrolidines **5a**–**f** as a mixture of *E*- and *Z*-isomers. These geometric isomers could be separated by silica gel column chromatography. In general, *E*-isomers were more stable than the corresponding *Z*-isomers, except for 4-isoxazole **5f**. The aldehyde **4** was prepared by oxidation of hydroxymethylpyrrolidine with pyridine sulfur trioxide complex in the presence of triethyl amine.[12] The mesylates **5a**–**f** were treated with potassium thioacetate in DMF–acetone mixture to give the thioacetates **6**–**11** with inversion of the C-4 configuration, which were converted into the corresponding thiols **12**–**17**, respectively.

The thiol derivatives **18a**,**b** were prepared, starting from the ester **5b** as a mixture of geometric isomers, by the sequence outlined in Scheme 2. Reduction of the ester

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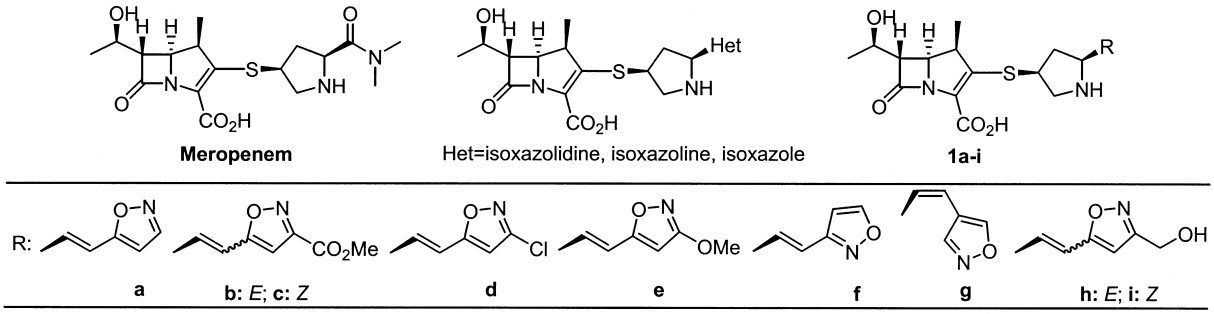
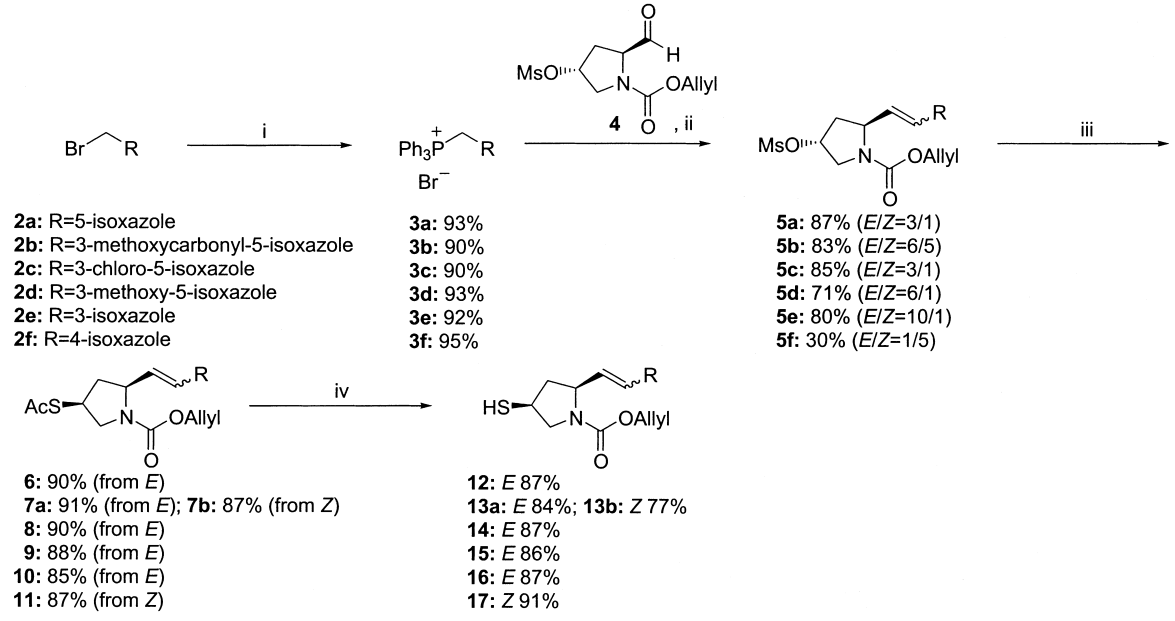
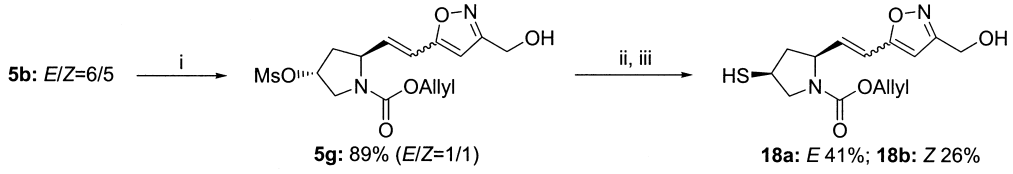


Figure 1.



Scheme 1. Reagents and reaction conditions: (i) PPh_3, CH_3CN, reflux, 4 h; (ii) NaHMDS, THF, −78 °C to rt, 5 h; (iii) AcS^-K^+, DMF–acetone, reflux, 5 h; (iv) 2 N NaOH, MeOH, 0 °C.



Scheme 2. Reagents and reaction conditions: (i) $NaBH_4$, THF–EtOH (2:3), rt, 7 h; (ii) AcS^-K^+, DMF–acetone, reflux, 5 h; (iii) 2 N NaOH, MeOH, 0 °C.

group of **5b** with sodium borohydride in THF:EtOH (2:3) mixture gave the alcohol **5g**. After the conversion of the methanesulfonyl group of **5g** with potassium thioacetate, the resulting thioacetate was hydrolyzed to afford the desired thiol mixture **18**. These geometric isomers **18a** and **18b** were separated by silica gel column chromatography.

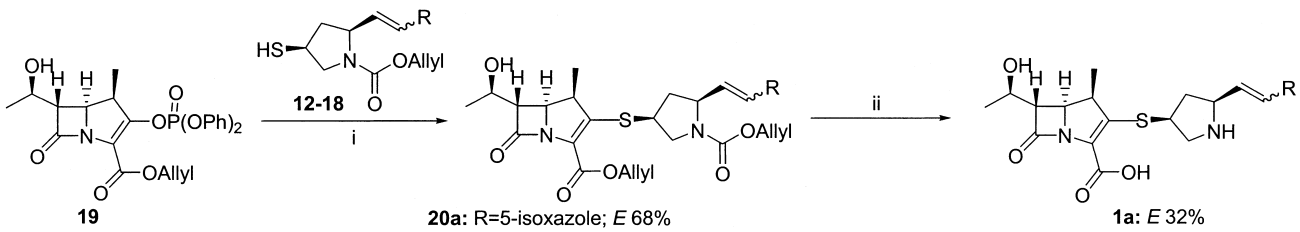
Oxazoloethenylpyrrolidinethiols **12**–**18**, freshly prepared as above, were treated with carbapenem enolphosphate **19**[1a] in the presence of DIPEA to provide the protected 1β-methylcarbapenems **20a**–**i**. Deprotection of **20a**–**i** was achieved without double bond reduction using tributyltin hydride in the presence of tetrakis(triphenylphosphine)palladium(0) to afford the title carbapenems **1a**–**i**[13] (Scheme 3).

In the case of **1a**, we were able to obtain a good crystal in distilled water, and the single crystal X-ray analysis confirmed the *E*-isomer with monohydrate, as depicted in Figure 2.

Biological Properties

Table 1 shows the in vitro antibacterial activity and stability to porcine renal DHP-I of the novel carbapenems prepared above, together with those of imipenem and meropenem as reference compounds.

With the exception of **1e**–**g**,**i**, all the compounds prepared showed comparable or superior activity to those of reference compounds against both Gram-positive



Scheme 3. Reagents and reaction conditions: (i) *i*-Pr_2NEt, CH_3CN, 0 °C; (ii) Bu_3SnH, cat. $Pd(PPh_3)_4$, 0 °C to rt, 2 h.

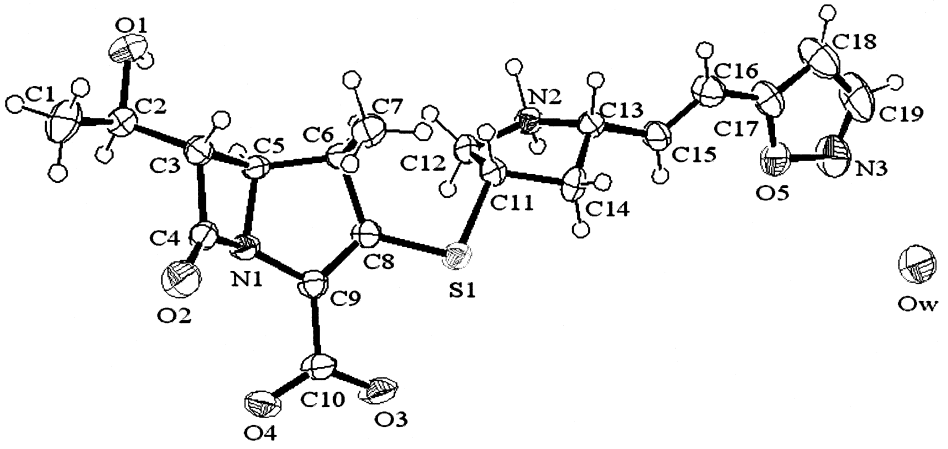


Figure 2. Crystal structure of **1a**.

Table 1. In vitro antibacterial activity and DHP-I stability of carbapenem compounds **1a**–**i**[a]

	MIC (μg/mL)[b]										
Organism	**1a**	**1b**	**1c**	**1d**	**1e**	**1f**	**1g**	**1h**	**1i**	**IPM**[c]	**MPM**[d]
S. p. 308A	0.004	0.007	0.007	0.004	0.007	0.025	< 0.002	0.007	0.013	0.007	0.013
S. a. SG 511	0.025	0.049	0.049	0.013	0.049	0.195	0.013	0.025	0.049	0.025	0.195
S. a. 285	0.025	0.049	0.049	0.025	0.025	0.195	0.025	0.025	0.049	0.025	0.195
S. a. 503	0.013	0.025	0.025	0.013	0.049	0.098	0.013	0.025	0.049	0.013	0.098
E. c. 055	0.025	0.025	0.025	0.025	0.098	0.098	0.391	0.025	0.049	0.098	0.025
E. c. 1507E	0.025	0.025	0.025	0.025	0.391	0.098	1.563	0.025	0.049	0.195	0.025
P. a. 9027	0.195	0.098	0.098	0.781	12.5	3.125	25.0	0.195	3.125	0.781	0.195
P. a. 1592E	0.195	0.195	0.195	0.781	12.5	3.125	25.0	0.195	3.125	1.563	0.195
P. a. 1771M	0.195	0.098	0.195	0.195	6.25	0.781	3.125	0.098	1.563	0.195	0.049
S.t.	0.049	0.025	0.025	0.025	0.195	0.098	0.391	0.049	0.098	0.781	0.049
K. a. 1522E	0.049	0.049	0.049	0.025	0.391	0.098	3.125	0.049	0.098	0.391	0.049
E. c. 1321E	0.025	0.025	0.025	0.013	0.195	0.049	1.563	0.025	0.049	0.195	0.025
DHP-I stability[e]	1.95	2.68	1.35	1.33	2.14	2.33	1.84	2.01	1.59	0.19	1.00

[a]Abbreviations *S. p.*, *S. pyogenes*; *S. a.*, *S. aureus*; *E. c.*, *E. coli*; *P. a.*, *P. aeruginosa*; *S. t.*, *S. typhymurium*; *K. a.*, *K. aerogenes*; *E. c.*, *E. cloacae*.
[b]MIC was determined by agar dilution method using Mueller–Hinton.
[c]IPM = imipenem.
[d]MPM = meropenem.
[e]Relative $t_{1/2}$ of hydrolosis to meropenem by partially purified porcine renal DHP-I.

and Gram-negative bacteria. Furthermore, these compounds exhibited high stability to DHP-I superior to meropenem. Carbapenems **1a**,**d**,**g**,**h** were as active as or more active than imipenem and much more active than meropenem against Gram-positive strains. Focusing on Gram-negative including *P. aeruginosa*, **1a**–**c**,**h** were better than imipenem and equivalent to meropenem. In general, 5-isoxazoles **1a**–**e**,**h**,**i** possessed higher in vitro potency than 3- and 4-isoxazoles **1h**,**g**. Among the 5-isoxazoles, **1a**,**b**,**h** showed the most potent and well-

balanced activity, but **1e**, having a methoxy group, displayed lower activity, especially against *P. aeruginosa*. As for the configuration of ethenyl group at the C-5 position of the pyrrolidine ring, geometric isomers **1b** and **1c** were almost equivalent against all tested strains, while *E*-isomer **1h** possessed several fold better activity than *Z*-isomer **1i** against Gram-negative bacteria.

Compared to the previous report,[7] introduction of the ethenyl group into the pyrrolidine ring significantly improved potency and DHP-I stability. All carbapenems were notably more stable to DHP-I than reference compounds. Carbapenems **1a**,**b**,**e**,**f**,**h** showed 2-fold better DHP-I stability than meropenem. The *E*-isomers **1b**,**h** have been found to possess greater stability to DHP-I than the *Z*-isomers **1c**,**i**, respectively.

In these series, **1a**,**b**,**h** exhibited excellent and well balanced antibacterial activity as well as high stability to DHP-I.

Acknowledgements

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13. Selected data: **1a**: ^{1}H NMR (D_2O) δ 1.10 (d, 3H, $J=7.1$ Hz), 1.16 (d, 3H, $J=6.3$ Hz), 1.83 (m, 1H), 2.71 (m, 1H), 3.22–3.33 (m, 2H), 3.34 (m, 1H), 3.58 (m, 1H), 3.96 (m, 1H), 4.09–4.14 (m, 2H), 4.26 (m, 1H), 6.41 (s, 1H), 6.47 (dd, 1H, $J=7.8$, 16.1 Hz), 6.71 (d, 1H, $J=16.1$ Hz), 8.28 (s, 1H); FABHRMS *m*/*z* calcd for $C_{19}H_{24}N_3O_5S$ $(M+H)^+$ 406.1437, found 406.1450. **1b**: ^{1}H NMR (D_2O) δ 1.12 (d, 3H, $J=7.1$ Hz), 1.18 (d, 3H, $J=6.3$ Hz), 1.77 (m, 1H), 2.72 (m, 1H), 3.20–3.34 (m, 2H), 3.36 (m, 1H), 3.55 (m, 1H), 3.87 (s, 3H), 3.93 (m, 1H), 4.10–4.16 (m, 2H), 4.25 (m, 1H), 6.58 (dd, 1H, $J=7.3$, 16.1 Hz), 6.70 (d, 1H, $J=16.1$ Hz), 6.79 (s, 1H). **1d**: ^{1}H NMR (D_2O) δ 1.24 (d, 3H, $J=7.1$ Hz), 1.31 (d, 3H, $J=6.2$ Hz), 1.91 (m, 1H), 2.87 (m, 1H), 3.31–3.50 (m, 2H), 3.48 (m, 1H), 3.76 (m, 1H), 4.07 (m, 1H), 4.19–4.32 (m, 2H), 4.48 (m, 1H), 6.65 (s, 1H), 6.69 (dd, 1H, $J=7.9$, 16.5 Hz), 6.81 (d, 1H, $J=16.5$ Hz); FABHRMS *m*/*z* calcd for $C_{19}H_{23}ClN_3O_5S$ $(M+H)^+$ 440.1048, found 440.1041. **1e**: ^{1}H NMR (D_2O) δ 1.21 (d, 3H, $J=7.1$ Hz), 1.32 (d, 3H, $J=6.3$ Hz), 1.90 (m, 1H), 2.89 (m, 1H), 3.32–3.56 (m, 2H), 3.49 (m, 1H), 3.80 (m, 1H), 3.98 (s, 3H), 4.09–4.33 (m, 3H), 4.49 (m, 1H), 6.25 (s, 1H), 6.63 (dd, 1H, $J=7.8$, 16.3 Hz), 6.90 (d, 1H, $J=16.3$ Hz); FABHRMS *m*/*z* calcd for $C_{20}H_{26}N_3O_6S$ $(M+H)^+$ 436.1543, found 436.1547. **1h**: ^{1}H NMR (D_2O) δ 1.24 (d, 3H, $J=6.9$ Hz), 1.32 (d, 3H, $J=6.3$ Hz), 1.95 (m, 1H), 2.86 (m, 1H), 3.36–3.50 (m, 3H), 3.73 (m, 1H), 4.07 (m, 1H), 4.21–4.38 (m, 2H), 4.42 (m, 1H), 4.71 (s, 2H), 6.59 (s, 1H), 6.68 (dd, 1H, $J=7.8$, 15.9 Hz), 6.83 (d, 1H, $J=15.9$ Hz). **1i**: ^{1}H NMR (D_2O) δ 1.22 (d, 3H, $J=6.9$ Hz), 1.33 (d, 3H, $J=6.3$ Hz), 1.59 (m, 1H), 2.77 (m, 1H), 3.31–3.60 (m, 4H), 3.89 (m, 1H), 4.15–4.30 (m, 2H), 4.56 (m, 1H), 4.73 (s, 2H), 6.14 (dd, 1H, $J=9.6$, 12.2 Hz), 6.51 (s, 1H), 6.53 (d, 1H, $J=12.2$ Hz); FABHRMS *m*/*z* calcd for $C_{20}H_{26}N_3O_6S$ $(M+H)^+$ 436.1543, found 436.1553.