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Synthesis and Biological Evaluation of Novel 1β-Methylcarbapenems with Isothiazoloethenyl Side Chains

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Abstract—The synthesis of novel 1β-methylcarbapenems **1a**,**b** bearing isothiazoloethenyl moieties at C-5 position of pyrrolidine ring and their biological evaluation are described. Both compounds showed potent and well-balanced antibacterial activity as well as high stability to DHP-I. Especially, 5-isothiazole derivative **1a** exhibited excellent DHP-I stability and advanced pharmacokinetics profiles, compared to 5-isoxazole derivative **2**, imipenem, and meropenem.

1β-Methylcarbapenems such as meropenem[1] and ertapenem[2,3] have been extensively studied recently because of their chemical and metabolic stabilities as well as potent antimicrobial activities.[4]

In the preceding paper,[5] we reported that a series of novel 1β-methylcarbapenems containing isoxazoloethenyl substituents at C-5 position of pyrrolidine ring showed excellent antibacterial activity and high DHP-I stability. In this series, 5-isoxazole derivative **2** exhibited the best combination of antibacterial activity and stability to DHP-I (Fig. 1).

As a continuation of these studies, we carried out introduction of isothiazole instead of isoxazole into the pyrrolidine ring in order to improve the activity against Gram-positive strains by the greater lipophilicity of sulfur relative to oxygen.[6,7] As expected, 5-isothiazole derivative **1a**, which is an isostere of our earlier 5-isoxazole derivative **2**, showed slightly improved activity against Gram-positive bacteria without the severe loss of activity against Gram-negative bacteria compared to **2**. In particular, 1a exhibited markedly enhanced DHP-I stability and advanced pharmacokinetic profiles than those of **2**, imipenem, and meropenem.

Herein, we wish to describe the synthesis of novel 1β-methylcarbapenems **1a**,**b** with isothiazoloethenyl side chains and biological evaluation including pharmacokinetics and in vivo efficacy for the selected **1a**.

Chemistry

5-Substituted isothiazole **10a**, Wittig agent for the synthesis of isothiazoloethenyl moiety, was prepared by linear route as shown in Scheme 1.

Propagyl alcohol (**3**) was oxidized with CrO_3 under reduced pressure to give propynal (**4**),[8] which was treated with sodium thiosulfate to afford aldehyde dithionite **5**.[9] Cyclization of **5** in liquid NH_3 followed by formylation of the resulting isothiazole (**6**)[10] in the presence of *n*-BuLi provided 5-formylisothiazole (**7**).[11] Subsequent reduction of **7** with $NaBH_4$ in THF gave the alcohol **8**, which was brominated with *N*-bromosuccinimide in the presence of AIBN to give 5-bromomethylisothiazole (**9**). Triphenylphosphonium bromide **10a** was obtained by treatment of **9** with triphenylphosphine in CH_3CN.

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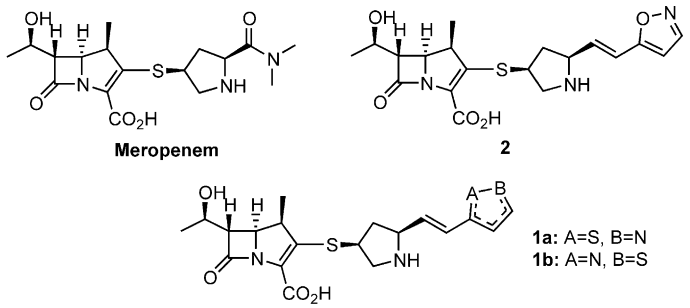
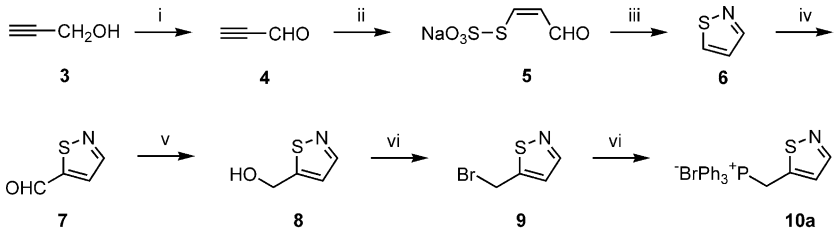


Figure 1.



Scheme 1. Reagents and reaction conditions: (i) CrO_3, H_2SO_4–H_2O, 30 mmHg, 2–10 °C, 3 h (35%); (ii) $Na_2S_2O_3$, AcOH, acetone–H_2O, −5 °C, 30 mim (65%); (iii) liquid NH_3, −60 °C to rt, 2 h (60%); (iv) *n*-BuLi, DMF, THF, −78 °C, 1 h (73%); (v) $NaBH_4$, THF (83%); (vi) PPh_3, CBr_4, CH_2Cl_2, −20 °C, 30 mim (80%); (vii) PPh_3, CH_3CN, reflux, 3 h (63%).

3-Substituted isothiazole **10b** was prepared by using procedures analogous to those described above (Scheme 2).

3-Methylisothiazole (**14**) was successfully synthesized from 3-butyn-2-ol (**1**) as a starting material via oxidation of alcohol with CrO_3, addition of sodium thiosulfate, and construction of isothiazole ring in liquid NH_3. Monobromination of **14** with *N*-bromosuccinimde in the presence of AIBN gave 3-bromomethylisothiazole (**15**) and subsequent treatment of **15** with triphenylphosphine in CH_3CN afforded the desired phosphonium salt **10b**.

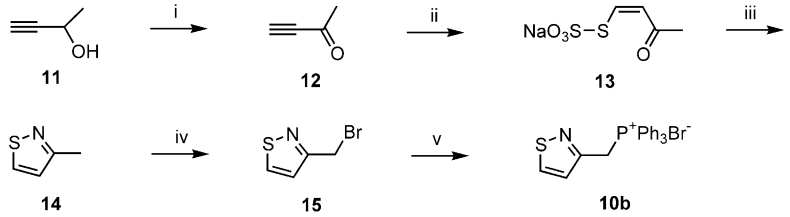
Introduction of ethenylpyrrolidine group was achieved by Wittig methodology of formylpyrrolidine **16** with phosphonium salts **10a**,**b** in THF in the presence of sodium bis(trimethylsilyl)amide as a base to give the mesyl protected isothiazoloethenylpyrrolidines **17a**,**b** (Scheme 3). The formylpyrrolidine **16** was prepared from hydroxymethylpyrrolidine by the known method.[12] In this reaction, the *E*-isomers were obtained exclusively whereas the *Z*-isomers did not detect as meaningful amounts.

After the conversion of mesyl group of **17a**,**b** with potassium thioacetate, the resulting thioacetates with inverted configuration were hydrolyzed to afford the thiols **18a**,**b**. Treatment of enolphosphate **19**[4] with freshly prepared the thiols **18a**,**b** in the presence of diisopropyl-ethylamine afforded the protected 1β-methylcarbapenems **20a**,**b**. Deprotection of **20a**,**b** with Bu_3SnH in the presence of catalytic amount of $Pd(PPh_3)_4$ provided the corresponding 1β-methylcarbapenems **1a**,**b**[13] as an amorphous solid by lyophilization, after purification by column chromatography on Diaion HP-20, respectively.

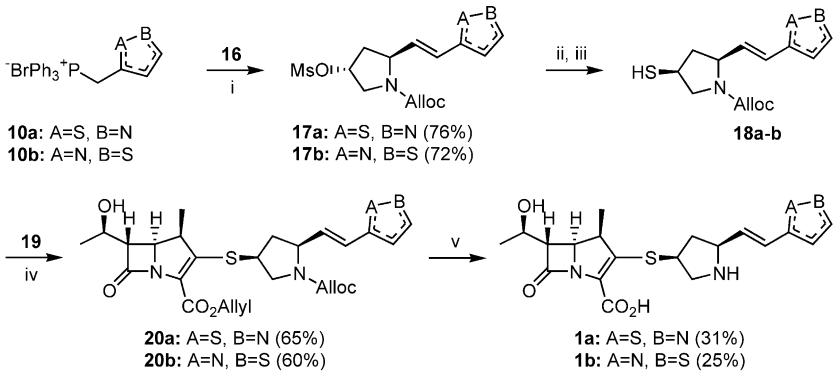
Biological Properties

The in vitro antibacterial activity and stability to porcine renal DHP-I of novel 1β-methylcarbapenems prepared above are listed in Table 1, together with those of **2**, imipenem, and meropenem as reference compounds.

With the exception of slightly reduced antipseudomonal activities, both compounds **1a**,**b** exhibited potent antibacterial activities against a wide range of Gram-positive and Gram-negative organisms and high stability to DHP-I superior to those of imipenem and meropenem. In this class of analogues, 5-isothiazole derivative **1a** showed more potent antibacterial activity and higher DHP-I stability than 3-isothiazole derivative **1b**. Carbapenem **1a** exhibited potent and well-balanced anti-



Scheme 2. Reagents and reaction conditions: (i) CrO_3, H_2SO_4–H_2O, 30 mmHg, 2–10 °C, 3 h (38%); (ii) $Na_2S_2O_3$, AcOH, acetone–H_2O, −5 °C, 30 mim (60%); (iii) liquid NH_3, −60 °C to rt, 2 h (52%); (iv) NBS, AIBN, CCI4, reflux, 15 h (45%); (v) PPh_3, CH_3CN, reflux, 3 h (63%).



Scheme 3. Reagents and reaction conditions: (i) (2*S*,4*R*)-4-Methanesulfonyloxy-2-formyl-1-allyloxy-carbonylpyrrolidine (**16**), NaHMDS, THF, −78 °C to rt, 1.5 h; (ii) AcSK, CH_3CN, reflux, 5 h; (iii) 2 N NaOH, MeOH, 0 °C to rt, 30 min; (iv) Alloc (1*R*,5*S*,6*S*)-2-(diphenylphosphoryloxy)-6-[(1*R*)-1-hydroxyethyl]-1-methylcarbapen-2-em-3-carboxylate (**19**), DIEA, CH_3CN, 0 °C, 1.5 h; (v) $Pd(PPh_3)_4$, Bu_3SnH, CH_2Cl_2, 0 °C, 1 h.

Table 1. In vitro antibacterial activity and DHP-I stability of **1a,b**

Organism	MIC (μg/mL)[a]				
	1a	**1b**	**2**	IPM[b]	MPM[c]
Streptococcus pyogenes 308A	0.004	0.007	0.004	0.007	0.013
Staphylococcus aureus SG 511	0.013	0.025	0.025	0.025	0.195
Staphylococcus aureus 285	0.025	0.025	0.049	0.025	0.195
Staphylococcus aureus 503	0.013	0.013	0.013	0.013	0.098
Escherichia coli 078	0.025	0.049	0.025	0.098	0.025
Escherichia coli 1507E	0.025	0.049	0.025	0.195	0.025
Psuedomonas aeruginosa 9027	0.391	1.563	0.195	0.781	0.195
Psuedomonas aeruginosa 1592E	0.391	1.563	0.195	1.563	0.195
Psuedomonas aeruginosa 1771M	0.195	0.391	0.098	0.195	0.049
Salmonella typhimurium	0.049	0.049	0.049	0.781	0.049
Klebsiella aerogenes 1522E	0.049	0.098	0.049	0.391	0.049
Enterobacter cloacae 1321E	0.049	0.025	0.025	0.195	0.025
DHP-I stability[d]	3.36	1.42	1.95	0.19	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.

Table 2. Pharmacokinetic parameters[a] of **1a**

	1a	**2**	IPM	MPM
$T_{1/2}$ (min)	23.5±6.8	16.3±1.5	3.46±0.1	3.99±4.1
AUC (μg/min/mL)	1801±486	1132±93	330±23	383±36
CL (mL/mim/kg)	11.6±2.7	17.8±1.5	61.5±3.7	54.2±3.7

[a]At a single intravenous administration of 20 mg/kg in rat.

Table 3. In vitro protective effects[a,b] of **1a** and Meropenem

	1a	MPM
S. pyogens 77A	0.32 (0.16–0.64)	4.72 (2.59–8.61)
S. aureus Y-80-1953	1.27 (0.6–2.54)	4.49 (2.74–8.02)
E. coli 078	0.32 (0.16–0.61)	1.11 (0.65–1.90)
P. aeruginosa 1771M	6.17 (3.39–11.24)	5.43 (3.02–9.75)

[a]At a single subcutaneous administration in mice.
[b]PD_{50} (mg/kg), parenthesis: 95% confidence limits.

bacterial activity against both Gram-positive and Gram-negative bacteria including *P. aeruginosa* isolates. Especially, **1a** displayed excellent DHP-I stability compared to 5-isoxazole derivative **2**, imipenem, and meropenem. And also, **1a** possessed highly effective in vitro potency against respiratory tract pathogens, especially such as *S. pyogens*, *S. aureus*, *S. pneumoniae*, *K. pneumoniae*, and *Morxella catarrhalis*.[14] Based on a favorable combination of in vitro antibacterial activity and DHP-I stability, 5-isothiazole derivative **1a** was selected for further evaluation.

The selected carbapenem **1a** was evaluated for pharmacokinetics and in vivo therapeutic efficacy in systematic infections in mice. **1a** possessed excellent pharmacokinetics profiles in rat compared to those of **2**, imipenem, and meropenem, and the results were listed in Table 2.

It showed that the half-life of **1a** was 6–7-fold longer than those of imipenem and meropenem. Furthermore, **1a** displayed 4–6 times higher value in AUC and approximately 5 times lower value in clearance than imipenem and meropenem.

In vivo activities of **1a** together with the data of meropenem as a reference were shown in Table 3. As a result, **1a** showed excellent in vivo therapeutic efficacy in systemic infections caused by *S. pyogenes* 77A, *S. aureus* Y-80-1953, and *E coli* 078 in mice. **1a** displayed similar value to meropenem against *P. aeruginosa* 1771M.

In summary, the title 1β-methylcarbapenems exhibited potent antibacterial activity against the target organisms, including *P. aeruginosa* isolates. Introduction of isothiazole instead of isoxazole into the pyrrolidine ring resulted in slightly improved activity against Gram-positive bacteria, especially remarkably enhanced DHP-I stability and pharmacokinetics. Taking the overall biological and physical properties into account, **1a** was selected as a good candidate.

Acknowledgements

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13. Spectral data: **1a**: ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.07 (d, 3H, $J=7.2$ Hz), 1.16 (d, 3H, $J=6.0$ Hz), 1.57 (m, 1H), 2.65 (m, 1H), 3.05 (dd, 1H, $J=3.8$, 3.8 Hz), 3.28–3.36 (m, 3H), 3.78 (m, 1H), 3.95 (m, 1H), 4.05–4.12 (m, 2H), 6.32 (dd, 1H, $J=7.7$, 7.7 Hz), 6.77 (d, 1H, $J=18.5$ Hz), 7.19 (s, 1H), 8.27 (s, 1H); FABHRMS *m*/*z* calcd for $C_{19}H_{23}N_3O_4S_2Na$ $(M+Na)^+$ 444.1028, found 444.1029. **1b:** ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.14 (d, 3H, $J=7.3$ Hz), 1.20 (d, 3H, $J=6.3$ Hz), 1.86 (m, 1H), 2.76 (m, 1H), 3.25–3.40 (m, 3H), 3.68 (m, 1H), 3.82–4.07 (m, 1H), 4.16 (m, 2H), 4.37 (m, 1H), 6.55 (dd, 1H, $J=8.2$, 8.2 Hz), 6.88 (d, 1H, $J=15.8$ Hz), 7.49 (d, 1H, $J=4.8$ Hz), 8.80 (d, 1H, $J=4.1$ Hz); FABHRMS *m*/*z* calcd for $C_{19}H_{23}N_3O_4S_2Na$ $(M+Na)^+$ 444.1028, found 444.1014.
14. MIC (μg/mL) data. *S. pyogens* 308A: 0.04 (**1a**), 0.007 (IPM), 0.013 (MPM); *S. pyogens* 77A: 0.002 (**1a**), 0.004 (IPM), 0.002 (MPM); *S. aureus* 6538P: 0.031 (**1a**), 0.016 (IPM), 0.13 (MPM); *S. aureus* giogio: 0.016 (**1a**), 0.008 (IPM), 0.13 (MPM); *S. aureus* 77: 0.25 (**1a**), 0.13 (IPM), 2 (MPM); *S. aureus* 241: 8 (**1a**), 8 (IPM), 32 (MPM); *S. pneumoniae* PG-R PN01: 0.25 (**1a**), 0.25 (IPM), 0.25 (MPM); *K. pneumoniae* 2011E: 0.063 (**1a**), 0.13 (IPM), 0.031 (MPM); *M. catarrhalis* 25240: $\leq$0.008 (**1a**), $\leq$0.008 (IPM), $\leq$0.008 (MPM).