NOTE

Synthesis and *in vitro* antibacterial activity of new meropenem analogs

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Carbapenems are one of the most potent types of antibacterial agents and are among those used as a last resort against infections in the clinical field. Recently, they have been classified into three classes according to their chemical structures. According to this classification, meropenem belongs to 1 β -methylcarbapenems containing (3*S*)-pyrrolidine-3-ylthio moiety.¹ The 1 β -methyl group slows renal hydrolysis by dehydropeptidase-I (DHP-I) and allows usage as a single agent.^{2,3} Carbapenems with (3*S*)-pyrrolidine-3-ylthio moiety are reported for their broad-spectrum and potent antibacterial activity.⁴

In the present investigation, we report the synthesis and *in vitro* antibacterial evaluation of three new meropenem analogs against 18 bacterial strains. Our target is to discover new carbapenem derivatives with improved antibacterial activity (Figure 1).

Our general synthetic route leading to new carbapenem derivatives involved the preparation of appropriately protected thiols containing pyrrolidine ring as a side chain and coupling reaction with the carbapenem diphenylphosphate, followed by deprotection of the resulting protected carbapenems in a usual manner.

The carboxylic acid **1** was treated with ethyl chloroformate and diazomethane to give the diazo compound **2**.^{5,6} Interaction of **2** with hydrobromic acid afforded the α -bromoacetyl derivative **3**.⁵ Nucleophilic substitution of the bromo group of **3** with dimethylamino moiety was carried out using dimethylammonium chloride in the presence of triethylamine to give **4**. Formation of the methoxyimino

compound **5** was successfully performed by refluxing the carbonyl compound **4** with *N*-methoxymethanamine hydrochloride in pyridine. Reduction of the carbonyl group of **4** using sodium borohydride afforded the corresponding hydroxyl compound **6**. Deprotection of the trityl group to mercaptans **7–9** was achieved by treatment of **4–6** with trifluoroacetic acid in the presence of triethylsilane (Scheme 1).

Reaction of the diphenylphosphate derivative **10**² with the thiols **7–9** in the presence of diisopropylamine yielded the corresponding 2-substituted carbapenems **11a–c**. Deprotection of these compounds by treatment with tetrakis(triphenylphosphine)palladium (0) and tributyltin hydride gave the target compounds **1a–c** (Scheme 2).

The final products were purified by column chromatography and their purities (>95%) were determined by HPLC. Their structures were confirmed by ¹H-NMR and LC-MS.

MIC values were determined by the agar dilution method using test agar. An overnight culture of bacteria in tryptose broth was diluted to about 10⁶ cells per ml with the same broth, and inoculated with an inoculation device onto agar containing serial two-fold dilutions of the test compounds. The organisms were incubated at 37 °C for 18–20 h. The MICs of the test compounds were defined as the lowest concentration that visibly inhibited growth.

The *in vitro* antibacterial activities of the new meropenem analogs **1a–c** against Gram-negative and Gram-positive bacteria are listed in Table 1. For comparison, the MIC values of imipenem and meropenem are also listed. Compounds **1a, b** displayed superior or similar activities against Gram-positive bacteria to meropenem, and against Gram-negative bacteria except *Pseudomonas aeruginosa* 5455 and imipenem-resistant *Acinetobacter baumannii* to imipenem. Compound **1b** showed higher or similar activity against Gram-positive bacteria to imipenem also. Of special interest, compound **1a** showed better or similar activity against third generation cephalosporins-resistant *Enterobacter cloacae* and *Serratia marcescens* and imipenem-resistant *Pseudomonas aeruginosa* to both imipenem and meropenem.

Figure 1 Structures of meropenem and the target compounds **1a–c**.

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Scheme 1 Reagents and conditions: (i) $\text{ClCOOC}_2\text{H}_5$, CH_2N_2 , THF, $-20 \sim -30^\circ\text{C}$ then rt, 4 h; (ii) HBr , THF, -10°C , 4 h; (iii) $(\text{CH}_3)_2\text{NH}\cdot\text{HCl}$ (4 eq.), TEA (8 eq.), CH_3CN , rt, 2 h; (iv) $\text{CH}_3\text{NHOCH}_3\cdot\text{HCl}$ (1.2 eq.), $\text{C}_5\text{H}_5\text{N}$, reflux, 8 h; (v) NaBH_4 (1 eq.), THF, 0°C , 8 h; (vi) trifluoroacetic acid, triethylsilane, dry CH_2Cl_2 , rt, 2 h.

Scheme 2 Reagents and conditions: (i) **7–9**, diisopropylethylamine, CH_3CN , 5 h; (ii) tetrakis(triphenylphosphine)palladium (0), tributyltin hydride, dry CH_2Cl_2 , 0°C , 1 h.

The hydroxyl compound **1c** showed the lowest activity compared with **1a**, **b** bearing carbonyl and methoxyimino moieties, respectively, and meropenem and imipenem. This suggests that hydroxyl group is unfavorable for antibacterial activity of these compounds.

Upon comparing the activity of the carbonyl compound **1a** with that of the methoxyimino derivative **1b**, compound **1a** was more active against Gram-negative bacteria. On the contrary, compound **1b** showed higher activity against Gram-positive bacteria. From these

Table 1 *In vitro* antibacterial activity (MIC, $\mu\text{g ml}^{-1}$) of the meropenem analogues **1a–c**

Strain	1a	1b	1c	IPM ^a	MPM ^b
<i>Escherichia coli</i> 04	0.013	0.049	0.391	0.195	0.025
<i>Klebsiella pneumonia</i> 523	0.049	0.098	0.781	0.781	0.049
<i>Citrobacter freundii</i> 323	0.025	0.049	0.391	0.391	0.025
<i>Enterobacter cloacae</i> 34R ^c	0.391	1.563	25	1.563	0.391
<i>Enterobacter cloacae</i> 34	0.049	0.049	0.391	0.781	0.025
<i>Serratia marcescens</i> 3349R ^c	3.125	25	>100	3.125	6.25
<i>Serratia marcescens</i> 3349	0.049	0.098	1.563	0.391	0.049
<i>Acinetobacter baumannii</i> 2289	12.5	6.25	>100	12.5	6.25
<i>Acinetobacter baumannii</i> 2289R ^d	25	25	>100	12.5	25
<i>Pseudomonas aeruginosa</i> 5455R ^d	12.5	50	>100	25	12.5
<i>Pseudomonas aeruginosa</i> 5455	3.125	50	>100	0.391	6.25
<i>Haemophilus influenza</i> 1210	3.125	3.125	25	6.25	3.125
<i>Safrhodococcus aureus</i> 1218	6.25	0.781	25	0.781	6.25
<i>Coagulase negative staphylococci</i>	0.195	0.049	0.391	0.025	0.098
<i>Enterococcus faecalis</i> 2347	6.25	1.563	25	1.563	12.5
<i>Streptococcus pneumonia</i> 0025	0.007	0.004	0.025	0.007	6.25
<i>Streptococcus pyogenes</i> 9889	0.007	0.004	0.025	0.004	0.007
<i>Streptococcus agalactiae</i> 32	0.025	0.007	0.098	0.013	0.049

^aImipenem.

^bMeropenem.

^cThird-generation cephalosporins resistant.

^dImipenem resistant.

results, it can be concluded that carbonyl group is favorable for activity against Gram-negative bacteria. On the other hand, methoxyimino moiety is more favorable for Gram-positive bacterial inhibition.

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