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Synthesis and antibacterial activity of 1β-methyl-2-[5-(pyrrolidine or piperidine-2-N-substituted carbamoyl) pyrrolidin-3-ylthio]carbapenem derivatives

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A series of new 1β-methylcarbapenems having a substituted pyrrolidine or piperidine-2-N-substituted carbamoyl pyrrolidine moiety were synthesized. Their *in vitro* antibacterial activities against both Gram-positive and Gram-negative bacteria were tested and the effect of substituent on the carbamoyl pyrrolidine was investigated. Of these new carbapenems, **7e** and **7f** showed the most potent antibacterial activity and are worth further studying.

Key words: synthesis; antibacterial activity; 1β-Methylcarbapenem.

INTRODUCTION

Carbapenem antibiotics, which were developed in the late 1970s, are some of the most potent types of antibacterial agents and are among those used as last resort against infections in the clinical field, due to their broad antibacterial spectra and potent bactericidal effects [1]. They play an important role in the treatment of severe infections in hospitals. In particular, carbapenems bearing a 1β-methyl substituent, exemplified by meropenem [2], biapenem [3], ertapenem [4], doripenem [5], tebipenem [6] have excellent antibacterial activities and good resistance to renal dehydropeptidase I (DHP-I). However, they are limited in their use, as their activity against resistant Gram-positive bacteria such as methicillin-resistant *Staphylococcus aureus* (MRSA) and Gram-negative pathogens is relatively weak [7]. There is an urgent need to find new antibiotics with stable properties, long $t_{1/2}$, less side effects and more potent activities.

During the past decade, a large number of carbapenem derivatives have been synthesized and investigated. These include a (3S)-pyrrolidin-3-ylthio group introduced as the C-2 side chain of the carbapenem nucleus. As a result, some carbapenem derivatives with potent *in vitro* antibacterial activity have been identified [8-12].

Previously, we reported that 1β-methyl carbapenem compounds containing 5′-pyrrolidine or piperidine derivatives substituted pyrrolidin-3′-ylthio group as C-2 side chain have improved antibacterial activity [13].

In this study, we describe the synthesis and antibacterial activity of new lβ-methyl-carbapenems having 5′-(pyrrolidine or piperidine-2-N-substituted carbamoyl) pyrrolidin -3-ylthio as C-2 side chain and discuss our approach to improve the antibacterial activity of the carbapenems.

INVESTIGATIONS AND RESULTS

The general synthetic route leading to new carbapenems involved the preparation of appropriately protected thiols group at the 3-position containing substituted pyrrolidine ring as a side chain. The intermediates thus prepared were then coupled with carbapenem diphenylphosphates, followed by deprotection of the protected carbapenems in a usual manner.

Synthesis of the intermediates (**4a-f**) was conducted as shown in Scheme 1. The starting material (2*S*, 4*R*)-4-acetylthio-1- (allyloxy carbonyl) pyrrolidine-2-carboxylic acid (**1**) was prepared according to ref. [14]. The compounds (**2a-f**) were prepared according to ref. [15]. The preparation of compounds (**4a-f**) was achieved as follows: compound (**1**) was activated with ethyl chloroformate followed by reaction with compounds (**2a-f**) to afford compounds (**3a-f**). Then the compounds (**3a-f**) were readily hydrolyzed with an aqueous/methanolic solution of 4N NaOH to give mercaptan compounds (**4a-f**), which were used in the subsequent reaction without purification.

Finally, the reaction of 1β-methyl-carbapenem nucleus allyl (1*R*, 5*S*, 6*S*)-2- (diphenyl-phosphoryloxy)-6-[(*R*)-1-hydroxyethyl]-1-methylca rbapen-2-em-3-carb-oxylate (**5**) was prepared according to ref. [16] and mercaptan compounds

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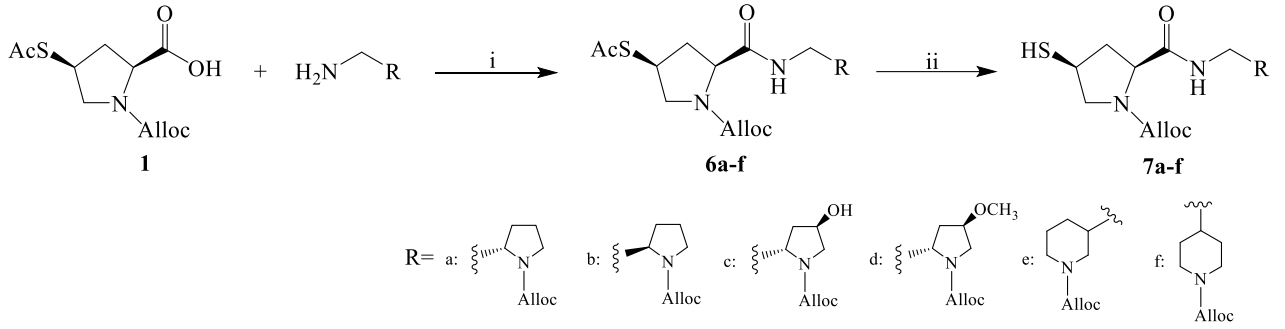
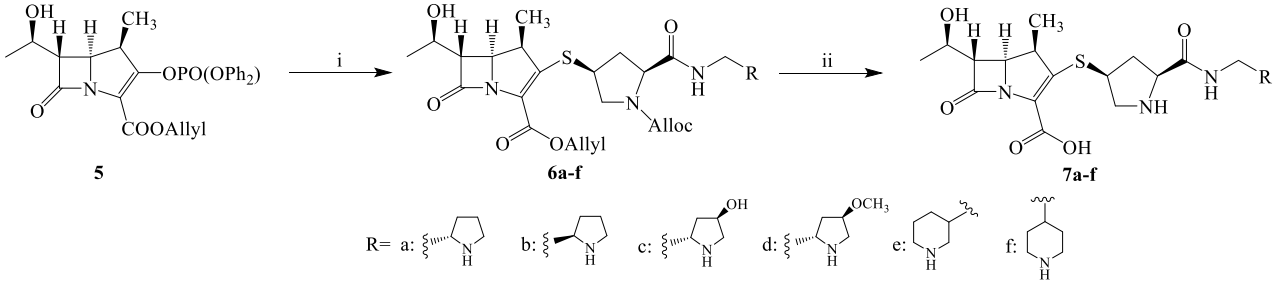
(**4a-f**) in the presence of diisopropylethylamine gave the corresponding protected carbapenem esters (**6a-f**). Deprotection of these compounds by treating with 1,3-dimethyl-barbituric acid (NDMBA), tetrakis-(triphenylphosphine)-palladium(0) ($Pd(PPh_3)_4$) and triphenylphosphine (Ph_3P) gave the corresponding carbapenems (**7a-f**) [17], as shown in Scheme 2.

Measurement of *in vitro* antibacterial activity: The MIC of a compound was defined as the lowest concentration that visibly inhibited growth. The MIC was determined by the standard agar dilution method using test agar. The *in vitro* antibacterial activities of the new carbapenems (**7a-f**) against Gram-positive and Gram-negative bacteria are listed in Table 1. For comparison, the MIC value of meropenem as positive control is also listed.



Scheme 1. Scheme of synthesis of intermediate compounds **4a-f** (i) ethyl chloroformate/Et_3N/THF/-5℃/5h; (ii) 4N NaOH/MeOH/0-5℃/3h.



Scheme 2. Scheme of synthesis of 1β-methylcarbapenem compounds **7a-f** (i) **4a-f**, DIPEA/DCM/-5℃/5h; (ii) NDMBA/Ph_3P/$Pd(PPh_3)_4$/THF/-5-0℃/6h.

Table 1. *In vitro* antibacterial activity (MIC, μg/ml) of the carbapenem derivatives

	7a	**7b**	**7c**	**7d**	**7e**	**7f**	**MPM**
Staphylococcus aureus 26003	0.39	0.39	0.78	0.39	0.098	0.195	0.39
Pneumococcal pneumonia 31002	0.195	0.195	0.098	0.098	0.098	0.098	0.098
Staphylococcus albus 26101	0.39	0.39	0.78	0.39	0.098	0.78	0.39
Enterococcus 32220	0.39	0.39	0.78	0.39	0.098	0.195	6.25
Gamma streptococcus 32206	6.25	6.25	6.25	6.25	12.5	12.5	6.25
Staphylococcus epidermidis 26069	0.39	0.39	0.78	0.39	0.098	0.39	0.195
Shigella boydii 51313	0.195	0.195	1.56	0.78	0.098	0.098	<0.049
Proteus mirabilis 49005	0.78	0.39	1.56	1.56	0.098	0.39	<0.049
Proteus vulgaris 49085	0.78	0.39	1.56	0.78	0.098	0.195	<0.049
Morgan proteus 49086	0.195	0.195	1.56	0.195	0.098	0.195	<0.049
Pseudomonas aeruginosa 10124	12.5	12.5	>25	>25	>25	>25	0.78
Pneumobacillus 46101	0.78	0.78	3.13	1.56	3.13	0.78	<0.049
Salmonella enteritidis 50041	0.39	0.39	1.56	0.39	0.098	0.195	<0.049
Salmonella typhi 50097	0.39	0.39	0.78	0.195	0.098	0.098	<0.049
Citrobacter 48017	0.39	0.195	3.13	1.56	3.13	0.195	<0.049
Aerobacter aerogenes 45102	0.39	0.39	3.13	0.78	3.13	0.39	<0.049
Serratia marcescens 41002	0.78	0.39	3.13	0.78	3.13	0.39	<0.049
Shigella sonnei 51081	0.195	0.39	1.56	1.56	0.098	0.098	<0.049
Shigella flexneri 51573	0.195	0.195	0.78	0.39	0.098	0.098	<0.049
Escherichia Coli 44102	0.39	0.39	1.56	0.098	0.098	0.195	<0.049

The compounds exhibited excellent antibacterial activity. Among these compounds, **7e** and **7f** showed superior or similar antibacterial activity against Gram-positive bacteria compared to MPM except *Gamma streptococcus*. Slightly lower activity was displayed on Gram-negative by **7a-f**, especially against *Pseudomonas aeruginosa* compared to MPM. It was also shown that the larger the size of the 5-substituents, the more difficult was to penetrate the cell membrane of Gram-negative bacteria and the lower was the activity against Gram-negative bacteria. The effects of substituent on the carbamoyl pyrrolidine were investigated. Results showed that the compounds of substituted pyrrolidine displayed slightly lower activity than piperidine against Gram-positive bacteria except *Gamma streptococcus*.

EXPERIMENTAL

All solvents and chemicals used were of analytical grade, purchased from Sinopharm Chemical Reagent Co., Ltd (SCRC) (China) and Aladdin Reagent, were used without further purification. The ^{1}H-NMR spectra (400 MHz) were measured on a DRX-400 spectrometer using DMSO-*d*6 or $CDCl_3$ or D_2O as solvent and TMS as an internal standard. Chemical shifts were expressed in ppm units. Multiplicities were recorded as s (singlet), brs (broad singlet), d (doublet), t (triplet), q (quartet), m (multiplet). Mass spectra were obtained on a LC-MSD 1100 spectrometer with ESI.

Spectral data of compounds **7a-f**: these are new compounds and their structures were fully confirmed by ^{1}H-NMR and ESI-MS. **7a**: Yield: 28.6%; ^{1}H-NMR (400Hz, D_2O): δ1.06 (d, 3H, *J*=7.2Hz, β-CH_3), 1.12 (d, 3H, *J*=6.0Hz, $\underline{CH_3}$CHOH), 1.82~2.01 (m, 4H), 2.45~2.58 (m, 3H), 3.02~3.13 (m, 3H), 3.62~3.69 (m, 3H), 3.87~3.96 (m, 2H), 4.02~4.07 (bs, 1H), 4.12 (bs, 1H) 4.52~4.56 (m, 2H); ESI-MS: *m/z* = 439.23 $[M+H]^+$. **7b**: Yield: 30.3%; ^{1}H-NMR (400Hz, D_2O): δ1.05 (d, 3H, *J*=7.2Hz, β-CH_3), 1.13 (d, 3H, *J*=6.4Hz, $\underline{CH_3}$CHOH), 1.76~1.98 (m, 4H), 2.43~2.55 (m, 3H), 3.00~3.11 (m, 3H), 3.61~3.70 (m, 3H), 3.86~3.98 (m, 2H), 4.05 (bs, 1H), 4.13 (bs, 1H), 4.50~4.54 (m, 2H); ESI-MS: *m/z* = 439.23 $[M+H]^+$. **7c**: Yield: 30.7%; ^{1}H-NMR (400Hz, D_2O): δ1.02 (d, 3H, *J*=7.2Hz, β-CH_3), 1.13 (d, 3H, *J*=6.0Hz, $\underline{CH_3}$CHOH), 1.53~1.64 (m, 2H), 2.55~2.70 (m, 3H), 3.07~3.19 (m, 3H), 3.49~3.59 (m, 4H), 3.95~4.00 (m, 3H), 4.13 (bs, 1H), 4.51~4.55 (m, 2H); ESI-MS: *m/z* = 455.25 $[M+H]^+$. **7d**: Yield: 32.4%; ^{1}H-NMR (400Hz, D_2O): δ1.01 (d, 3H, *J*=7.2Hz, β-CH_3), 1.14 (d, 3H, *J*=6.0Hz, $\underline{CH_3}$CHOH), 1.51~1.63 (m, 2H), 2.51~2.65 (m, 3H), 2.79 (s, 3H), 3.03~3.16 (m, 3H), 3.53~3.66 (m, 4H), 3.94~3.98 (m, 3H), 4.14 (bs, 1H), 4.50~4.54 (m, 2H); ESI-MS: *m/z* = 469.20 $[M+H]^+$. **7e**: Yield: 32.7%; ^{1}H-NMR (400Hz, D_2O): δ1.05 (d, 3H, *J*=7.2Hz, β-CH_3), 1.15 (d, 3H, *J*=6.0Hz, $\underline{CH_3}$CHOH), 1.50~1.63 (m, 4H), 2.50~2.62 (m, 1H), 2.76~2.87 (m, 3H), 2.97~3.05 (m, 3H), 3.50~3.55 (m, 2H), 3.61~3.65 (m, 2H), 3.95~4.06 (m, 3H), 4.11~4.15 (m, 1H), 4.50~4.54 (m, 2H); ESI-MS: *m/z*=453.21 $[M+H]^+$. **7f**: Yield: 31.2%; ^{1}H-NMR (400Hz, D_2O): δ1.03 (d, 3H, *J*=7.2Hz, β-CH_3), 1.11 (d, 3H, *J*=6.0Hz, $\underline{CH_3}$CHOH), 1.60~1.67 (m, 4H), 2.65~2.75 (m, 4H), 2.96~3.09 (m, 3H), 3.56~3.66 (m, 2H), 3.61~3.65 (m, 2H), 3.94~3.98 (m, 3H), 4.02~4.11 (m, 1H), 4.50~4.54 (m, 2H); ESI-MS: *m/z* =453.26 $[M+H]^+$.

CONCLUSIONS

We have designed and synthesized a novel series of new 1β-methyl-2-[5-(pyrrolidine or piperidine-2-N-substituted carbamoyl) pyrrolidin-3-ylthio] carbapenem derivatives. These compounds were prepared from **1** in the reaction with the pyrrolidine or piperidine group containing derivatives (**2a-f**). The antibacterial activity of the obtained carbapenem derivatives was determined by the standard agar dilution method. Then the MIC values were calculated and compared with positive control (MPM). We have found that **7e** and **7f** showed superior or similar antibacterial activity against Gram-positive bacteria compared to MPM except *Gamma streptococcus*, and were worth further studying. Due to the increased size of the 5-substituents, their penetration into the cell membrane of Gram-negative bacteria is hampered and the derivatives display slightly lower antibacterial activity than MPM in most cases.

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СИНТЕЗА И АНТИБАКТЕРИАЛНА АКТИВНОСТ НА ПРОИЗВОДНИ НА 1β-МЕТИЛ-2-[5-(ПИРОЛИДИН ИЛИ ПИПЕРИДИН-2-N-ЗАМЕСТЕНИ КАРБАМОИЛ) ПИРОЛИДИН-3-ИЛТИО] КАРБАПЕНЕМ

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(Резюме)

Синтезирана е серия от нови производни на 1β-метил-2-[5-(пиролидин или пиперидин-2-n-заместени карбамоил) пиролидин-3-илтио]карбапенем. Изпитана е *in vitro*тяхната антибактериална активност спрямо Грам-положителни и Грам-отрицателни бактерии, като е изследван ефекта на заместителите на карбаноил-пиролидина. Най-висока антибактериална активност проявяват 7e и 7f – производните на карбапенема и заслужават следващи изследвания.