Short communication

Synthesis of C6-Ethylidene Meropenem Derivative with Antimicrobial Activity

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Dedicated to Professor Branko Stanovnik on the occasion of his 70th birthday

Abstract

The development of novel β-lactamase inhibitors with activity against various clinically relevant β-lactamase producing strains is one of the most important strategies to sustain the clinical efficacy of β-lactam antibiotics. With intention to eliminate antibiotic activity of meropenem with preserved activity against β-lactamases C6-hydroxyethyl side chain of meropenem was transformed to C6-ethylidene moiety. IC50 values of C6-ethylidene derivative of meropenem were in low mM range against TEM-1, SHV-1 and AmpC enzymes and were clearly inferior to meropenem. Surprisingly, some of the antimicrobial activity of meropenem was preserved implying that C6-hydroxyethyl side chain is not essential to retain antibiotic activity of meropenem.

Keywords: Tricyclic carbapenem, antimicrobial, β-lactamase inhibitor

1. Introduction

The dramatic increase in antibacterial resistance in both community and hospital settings have resulted in limited effectiveness of current drugs and represent a real public health threat. A wide variety of pathogens have acquired antimicrobial resistances to β-lactam antibiotics by the synthesis of β-lactamases. Based on amino-acid sequence similarities, β-lactamases have been broadly grouped into four molecular classes, A, B, C and D. The β-lactamase superfamily currently has more than 700 members, many of which differ only by a single amino acid. Commercially available inhibitors potassium clavulanate, sulbactam and tazobactam, inhibit most class A and some class D β-lactamases, but activity against class C types is poor in general. NXL-104 is a non-β-lactam inhibitor currently in clinical phase I and there are several compounds under investigation as potential β-lactamase inhibitors.1,2 However, there is a great unmet medical need for broad-spectrum inhibitor of β-lactamases, that would be capable to restore diminished activity of β-lactam antibiotic in combination and extend its antimicrobial spectrum.

Recently a development of novel broad-spectrum inhibitor of β-lactamases LK-157 has been published.3,4 It has been shown that transformation of C10-hydroxyethyl side chain of sanfetrinem to C10-ethylidene moiety of LK-157 resulted in loss of antibiotic activity. The common feature of all carbapenems on the market is the C6-hydroxyethyl side chain which is believed to contribute to antibacterial activity and stability against β-lactamases. Therefore our initial assumption was to apply the same strategy also in case of meropenem to retain only β-lactamase inhibitory activity. In this paper, the synthesis of C6-ethylidene derivative of meropenem, its IC50s against TEM-1, SHV-1 and AmpC enzymes and antimicrobial activity are presented.

2. Results and Discussion

2.1. Chemistry

Commercially available meropenem (1) was chosen as the starting compound for the synthesis of C6-ethylidene meropenem derivative LK-175 (5). The synthesis of C6-ethylidene analogue has already been reported, although the final compound has never been evaluated as inhibitor of β-lactamases.5 In addition, the coupling of 3-ethylidenazetidinone fragment and 4-mercapto pyrrolidine carboxamide comprised of total 8 synthetic steps. The-
therefore, we designed a shorter and more convenient synthetic route to the LK-175 (5). Carboxylate and amine moieties of meropenem were protected as allyl ester and allyl carbamate protecting groups, respectively, followed by water elimination resulting in formation of the ethylidene moiety. The final compound was isolated upon deprotection of allyl groups (Scheme 1).

Meronem®, commercially available mixture of meropenem (1) and sodium carbonate, was first treated with allyl chloroformate in water and additional quantity of sodium carbonate to afford the corresponding N-protected allyloxycarbonyl derivative 2.6 Water was removed by liophilisation, and the mixture alkylated with allyl bromide in DMF at room temperature.7 The obtained allyl ester 3 was purified by extraction followed by column chromatography. Subsequently, water elimination under the conditions of the Mitsunobu reaction8 afforded ethylidene derivative 4, which was further purified by column chromatography. Both protecting groups were removed by tributyltin hydride in the presence of bis(triphenylphosphine)palladium(II) dichloride and water in dichloromethane.9 The stannic by-products were removed by washing the crude product with diethyl ether, followed by preparative HPLC purification and liophilization to afford LK-175 (5).

2.2 Biological Activity

The results of the inhibitory activities against class A (TEM-1 and SHV-1 from Escherichia coli) and class C (AmpC from E. cloacae) β-lactamases for LK-175, meropenem and tazobactam are presented in Table 1. IC50 values of LK-175 were in low mM range and were clearly inferior to meropenem, thus neglecting our hypothesis to prepare the equipotent inhibitor of β-lactamases by subtle structural modification.

Moreover, LK-175 exhibited antimicrobial profile against various bacterial strains as shown in Table 2. MIC values were in range of 1 to 4 μg/ml, except for MRSA-producing S. aureus ATCC 43300 with higher MIC values (8–16 μg/ml). Surprisingly, some of the antimicrobial activity of meropenem was preserved, although it is believed that C6-hydroxyethyl side chain is crucial for antibiotic activity of carbapenems and is a common feature of all carbapenem antibiotics on the market. However, our finding implies that C6-hydroxyethyl side chain is not vital to retain antibiotic activity.

3. Conclusions

There is no broad-spectrum inhibitor of β-lactamases on the market that covers also class C β-lactamases. A

| Table 1: In vitro activity of C6-ethylidene derivative of meropenem against class A (TEM-1 and SHV-1) and class C (AmpC) β-lactamases. |
|----------------|----------------|----------------|
| Compound       | TEM-1 [nM]     | SHV-1 [nM]     | AmpC [nM]    |
| Tazobactam     | 17             | 222            | 1808         |
| Meropenem      | 200            | 200            | 1            |
| LK-175         | 1000           | 2500           | 5000         |

Scheme 1. Synthetic route to LK-175 (5).

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design of meropenem derivative LK-175 with C6-hydroxyethyl moiety was based on results of broad-spectrum β-lactamase inhibitor LK-157 encouraged by finding that meropenem is also a good inactivator of AmpC. However, LK-175 exhibited only a weak β-lactamase inhibitory activity. On the other side, it is interesting to note that C6-hydroxyethyl side chain is not essential to retain antibiotic activity and various modifications could be employed in future design of novel carbapenem antibiotics.

### 4. Experimental

#### 4.1. Synthesis

##### 4.1.1. General Chemical Methods

Analytical TLC was performed on Merck silica gel 60 F254 plates (0.25 mm) and the components detected using specific spray reagents. Column chromatography was carried out on silica gel 60 (particle size 240–400 mesh). DSC spectrum was recorded on a Mettler Toledo DSC822e. IR spectrum was obtained with a Nicolet FT-IR Nexus spectrometer and optical rotation was measured on a Perkin Elmer 1241 MC polarimeter. ¹H-NMR spectra were recorded on a Varian Inova300 spectrometer in DMSO-d6 solution, with TMS as the internal standard. Mass spectra were obtained using a VG-Analytical Autospec Q mass spectrometer. LC-MS analyses were performed on an Alliance HT Waters 2795 separations module with a Waters 2996 PDA detector and an XTerra RP C18 (150 × 4.6 mm I.D., 3.5 μm particle size) analytical column and a Micro- mass Quatro micro mass spectrometer. HRMS analyses were performed on Micromass QTOF Ultima Global using ESI (negative and positive mode). HPLC analyses were carried out with Waters 2695 Separation Module with a Waters 2996 PDA detector and an XTerra RP C18 (150 × 4.6 mm I.D., 3.5 μm particle size) analytical column and a gradient elution method combining mobile phase A with 25 mM ammonium acetate (pH = 6) / acetonitrile (95/5 v/v) and mobile phase B with 25 mM ammonium acetate (pH = 6) / acetonitrile (10/90 v/v). All reported yields are yields of purified products.

**Synthesis of (4R,5S,6S)-allyl 3-[(3S,5S)-1-(allyloxy carbonyl)-5-(dimethylcarbamoyl)pyrrolidin-3-ylthio]-6-ethylidene-4-methyl-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate (4).** (4R,5S,6S)-allyl 3-[(3S,5S)-1-(allyloxy carbonyl)-5-(dimethylcarbamoyl)pyrrolidin-3-ylthio]-6-ethylidene-4-methyl-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate (4) was dissolved in dry dichloromethane (20 mL) under argon atmosphere. Triphenylphosphine (1.05 EQ, 3.31 mmol, 0.869 g) and diethyl azodicarboxylate (1.05 EQ, 3.31 mmol, 3.31 g, 0.577 g, 0.317 g) were added and the reaction mixture additionally stirred for 2–3 days at room temperature. If necessary, additional quantity of allyl bromide was added (0.05 mL + 0.1 mL). After the complete conversion was detected by HPLC, the reaction mixture was filtered and the solvent removed under reduced pressure. The crude product was dissolved in ethyl acetate (80 ml) and washed with water (3 × 80 ml) to afford 1.08 g of (4R,5S,6S)-allyl 3-[(3S,5S)-1-(allyloxy carbonyl)-5-(dimethylcarbamoyl)pyrrolidin-3-ylthio]-6-ethylidene-4-methyl-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate (3) yield: 82%, yellow oil; ¹H NMR (300 MHz, DMSO-d6) δ 1.15 (d, 6H, J = 5.4 Hz), 1.52–1.64 (m, 1H), 1.95 (s, 1H), 2.76–2.86 (m, 1H), 2.97 (s, 3H), 2.99 (s, 3H), 3.02–3.11 (m, 1H), 3.21 (dd, 1H, J = 4.5 Hz, J = 2.1 Hz), 3.52–3.57 (m, 1H), 3.71–3.84 (m, 1H), 3.91–4.02 (m, 1H), 4.04–4.10 (m, 1H), 4.17–4.21 (m, 1H), 4.42–4.50 (m, 2H), 4.57–4.59 (m, 1H), 4.66–4.74 (m, 2H), 5.05 (d, 1H, J = 3.3 Hz), 5.14–5.19 (m, 2H), 5.35–5.40 (m, 2H), 5.83–5.93 (m, 2H) [peaks for H2'alpha and H6 are under signal for H2O]. ¹³C NMR (100 MHz, DMSO-d6) δ 17.2, 21.9, 35.5, 35.6, 36.6, 43.1, 55.6, 56.2, 59.8, 60.0, 64.4, 65.0, 65.3, 116.4, 117.4, 117.9, 132.3, 133.4, 149.7, 153.2, 160.2, 170.7, 174.2. HRMS–FAB (m/z): calcd for C2₄H₂₄N₂O₅S (M+H) 508.2117; found, 508.2093. LCMS calcd for C₂₄H₂₁N₃O₅S: 507; obtd. 508 (M+H). HPLC: 93.3 area %.

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was removed under reduced pressure and the obtained oily residue purified with flash chromatography (neutral Al₂O₃). Ethylacetate: Hexane = 3 : 1 to afford 1.26 g of (4R,5S,E)-allyl 3-[(3S,5S)-1-(allyloxy carbonyl)-5-(dimethylcarbamoyl)pyrrolidin-3-ylthio]-6-ethylidene-4-methyl-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylate (4); yield 82%, yellow oil. ¹H NMR (300 MHz, DMSO-d₆) δ 1.06 (d, 3H, J = 7.2 Hz), 1.52–1.66 (m, 1H), 1.84 (d, 3H, J = 7.2 Hz), 2.79–2.86 (m, 1H), 2.83 (s, 3H), 3.02 (s, 3H), 3.08–3.18 (m, 1H), 3.58–3.72 (m, 1H), 3.74–3.88 (m, 1H), 4.42–4.52 (m, 2H), 4.70–4.81 (m, 2H), 4.92 (d, 1H, J = 9.3 Hz), 5.13–5.24 (m, 2H), 5.43 (dd, 2H, J₁ = 17.1 Hz, J₂ = 1.8 Hz), 5.88–5.98 (m, 2H), 6.47–6.53 (m, 1H), [peaks for H₂α and H₆ are under signal for H₂O]. ¹³C NMR (100 MHz, CDCl₃) δ 15.1, 17.1, 35.1, 36.0, 39.9, 40.8, 44.3, 54.1, 55.7, 61.3, 65.8, 66.2, 117.8, 118.6, 129.6, 131.4, 132.6, 138.8, 146.1, 153.9, 160.6, 168.4, 170.8. LC-MS: m/z (relative intensity): 490 (M+H, 20), 279 (100), 154 (36). HRMS–FAB (m/z): calcd for C₂₄H₃₁N₃O₆S (M+H), 490.2012; found, 490.1539. MS m/z (relative intensity): 490 (M+H), 279 (100), 154 (36). HRMS–FAB (m/z): calcd for C₂₄H₃₁N₃O₆S (M+H), 490.2012; found, 490.1539. HPLC: 92.6 area %.

**Synthesis of (4R,5S,E)-3-[(3S,5S)-5-(dimethylcarbamoyl)pyrrolidin-3-ylthio]-6-ethylidene-4-methyl-7-oxo-1-azabicyclo[3.2.0]hept-2-ene-2-carboxylic acid (5).** A solution of (4R,5S,E)-allyl 3-[(3S,5S)-1-(allyloxy carbonyl)-5-(dimethylcarbamoyl)pyrrolidin-3-ylthio]-6-ethylidene-4-methyl-7-oxo-1-aza-bicyclo[3.2.0]hept-2-ene-2-carboxylate (4) (0.083 g), Pd(PPh₃)₂Cl₂ (2.5 mg) in dry dichloromethane (5 mL) was cooled to 0 °C. Argon was bubbled into the reaction mixture followed by the addition of water (15 μL) and the addition of Bu₃SnH during stirring within 30 minutes in 5 portions (5 × 40 μL). The reaction mixture was stirred at room temperature for 15–30 min and after the complete conversion was detected by HPLC, the reaction mixture was dried with MgSO₄ and the solvent removed under reduced pressure (T bath < 40 °C). The obtained oily residue was washed with diethyl ether (3 × 20 mL). The product was purified by the preparative HPLC and lyophilized at −20 °C to afford 0.041 g of (4R,5S,E)-3-[(3S,5S)-5-(dimethylcarbamoyl)pyrrolidin-3-ylthio]-6-ethylidene-4-methyl-7-oxo-1-aza-bicyclo[3.2.0]hept-2-ene-2-carboxylic acid (5), yield 66%, yellow solid; decom at 160 °C (before mp). IR (KBr) 3436, 3296, 2967, 2931, 1752, 1701, 1653, 1608, 1508, 1381, 1244, 1137 cm⁻¹, [α]D⁰ +23.5 (H₂O, 1.04) ¹H NMR (300 MHz, DMSO-d₆) δ 1.05 (d, 3H, J = 7.2 Hz), 1.40–1.48 (m, 1H), 1.80 (d, 3H, J = 7.1), 2.52–2.60 (m, 1H), 2.80–2.86 (m, 2H), 2.83 (s, 3H), 2.96 (s, 3H), 3.05–3.08 (m, 1H), m for H₂α is under signal for water, 3.46–3.54 (m, 1H), 3.62–3.68 (m, 1H), 3.92–3.99 (m, 1H), 4.82 (d, 1H, J = 9.3 Hz), 6.42–6.45 (m, 1H). ¹³C NMR (100 MHz, CDCl₃) δ 14.7, 17.0, 35.1, 36.1, 42.8, 55.5, 57.8, 60.6, 62.8, 131.6, 139.0, 141.1, 164.1, 168.0, 171.7. HRMS–FAB (m/z): calcd for C₁₇H₂₄N₃O₄S (M+H), 366.1486; found, 366.1507. HPLC: 92.6 area %.

### 4. 2. Enzymatic Assay

All the enzyme and compound solutions were prepared in 50 mM phosphate buffer pH 7.0. Assayed enzymes were incubated in presence of various concentrations of the tested compounds for 30 minutes at 30 °C in a final volume of 500 μl. The final concentrations of enzymes were as follows: TEM-1 (5 mg/ml), SHV-1 (0.5 mg/ml) and AmpC (1 mg/ml). Than 10 μl of 5 mM nitrocefin was added to the solution and the absorbance at 482 nm was measured for 5 minutes. The initial rate was determined and IC₅₀ values were calculated from two independent experiments. The IC₅₀ was recorded as the inhibitor concentration that give a initial hydrolysis rate of nitrocefin equal to 50% of the hydrolysis rate of nitrocefin in absence of inhibitor.

### 4. 3. MIC Determination

All tested bacterial strains were purchased from the American Type Culture Collection (ATCC): E. coli ATCC 25922 (S1), E. coli ATCC 35218 (S2), S. aureus ATCC 25923 (S3), S. aureus ATCC 43300 (S4), S. aureus ATCC 29213 (S5), S. pyogenes ATCC 19615 (S6), E. faecalis ATCC 29221 (S7), H. influenzae ATCC 49247 (S8).

Bacterial susceptibility, expressed as MIC, was determined by the microbroth dilution technique as recommended by the approved standard reference recommendations of the CLSI. All tested bacterial strains were purchased from the American Type Culture Collection (ATCC): E. coli ATCC 25922 (S1), E. coli ATCC 35218 (S2), S. aureus ATCC 25923 (S3), S. aureus ATCC 43300 (S4), S. aureus ATCC 29213 (S5), S. pyogenes ATCC 19615 (S6), E. faecalis ATCC 29221 (S7), H. influenzae ATCC 49247 (S8).

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### 5. Acknowledgments

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### 6. References

1. C. Miossec, L. Poirel, D. Livermore, T. Stachurya, P. Nordmann, M. Black and P. Levasseur In Vitro Activity of the New β-Lactamase Inhibitor NXL104: Restoration of Ceftazidime...
(CAZ) Efficacy Against Carabpenem-Resistant Enterobacteriaceae Strains, 47th ICAAC, Chicago, IL, 2007, F319.

Povzetek

Razvoj novih inhibitorjev β-laktamaz z delovanjem proti klinično relevantnim sevom, ki proizvajajo β-laktamaze je eden najpomembnejših pristopov pri zagotavljanju klinične učinkovitosti β-laktamskih antibiotikov. 6-Hidroksietilno stransko verigo meropenema smo pretvorili v C6-etilidensko skupino z namenom, da ohranimo aktivnost proti β-laktamazam brez antibiotičnega delovanja. IC50 vrednosti C6-etiliden derivata proti TEM-1, SHV-1 in AmpC encimom so bile v spodnjem mM območju, kar je slabše kot pri meropenem. Ob tej strukturni spremembi se je nepričakovano ohranila protimikrobna aktivnost, kar dokazuje, da C6-hidroksietilna stranska veriga ni nujni pogoj za ohranitev antibiotične aktivnosti meropenema.