SYNTHESIS AND IN VITRO ACTIVITY OF A NEW CARBAPENEM, RS-533

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The synthesis and in vitro antimicrobial activity of a new synthetic carbapenem, (5R,6S)-6-[(R)-1-hydroxyethyl]-2-[(S)-l-acetimidoylpyrrolidin-3-ylthio]-l-carbapen-2-em-3-carboxylic acid (RS-533), are described. The MIC values of related penems and carbapenems are also given for comparison with those of the new carbapenem.

The discovery of the potent broad-spectrum antibiotic thienamycin (THM)1–4) has arisen chemical and microbiological interest in carbapenems and structurally related penems. Extensive molecular modifications of THM and penems have been made in pursuit of greater stability and potency and, as a result, clinically useful β-lactams, N-formimidoylthienamycin (MK0787)5–7) and an oral penem (Sch 29482)8) have been obtained. Prior to the present work on carbapenems, we were concerned with the synthesis9,10) and bioassay of new penems, during which we discovered 6-(1-hydroxyethyl)-2-(pyrrolidin-3-ylthio)penem-3-carboxylic acids (R-1 and S-1)11,12) which were found to have potent in vitro activity comparable to THM. This finding led us to synthesize carbapenem congeners in the expectation that the carbapenems would display greater activity than either the penems or THM. As a result of extensive syntheses ranging from penems to carbapenems, we ultimately obtained a new carbapenem, RS-533; one of the most promising antibiotics in view of the potent in vitro and in vivo activity13) against a wide range of bacteria.

Chart 1.

Synthesis

In preparing the desired carbapenem compound, we utilized the 2-oxocarbapenam (9b), first synthesized by the Merck group,14) as the most reliable intermediate for carbapenem synthesis. Although 9a and 9b have been synthesized by way of the β-keto esters (4a and 4b) by several routes,15–18) we prepared 4a and 4b starting from the acetoxyazetidinone (2) and the 3-pyrrolidinocrotonic acid esters as shown in Chart 2.
In the first attempt methyl 3-pyrrolidinocrotonate was, after lithiation with $n$-butyllithium,\(^{19}\) allowed to react with 2 to furnish 5 in very poor yield. The $\beta$-keto ester (5) was, however, produced in reasonable yield when diethylaluminum chloride was added to the lithiated enamino ester prior to reaction with 2. In this process, the initially formed enamino ester (3b) was treated with silica gel containing a small amount of water to give the $\beta$-keto ester (5). The enamino ester (3a) similarly prepared was treated with aqueous HCl - MeOH followed by hydrolysis using silica gel - H$_2$O to give 4a in 33% yield from 2, while $p$-nitrobenzyl 3-pyrrolidinocrotonate failed to yield the corresponding $\beta$-keto ester (4b). The $\beta$-keto ester (7) could be obtained by the hydrolysis of the $\beta$-keto methyl ester (5) with pig liver esterase to the $\beta$-keto acid (6) followed by esterification with $p$-nitrobenzyl iodide. The enzymatic hydrolysis has advantage over the acid or alkaline hydrolysis of $\beta$-keto esters to $\beta$-keto carboxylic acids which accompanies decarboxylation. Removal of the tert-butyldimethylsilyl group of 7 was performed by treatment with aqueous HCl in methanol. The $\beta$-keto esters (4a and 4b) were converted into 9a and 9b, respectively, via diazo compounds (8a and 8b) according to the established method.\(^{14}\) The 2-oxocarbapenams (9a and 9b) were treated with diphenylphosphoryl chloride in the presence of diisopropylethylamine\(^ {14}\) and then with (S)-1-$p$-nitrobenzoylcarbonyl-3-mercaptopyrrolidine* to furnish the carbapenem derivatives (10a and 10b). Deprotection of the $p$-nitrobenzyl and $p$-nitrobenzoxycarbonyl groups of 10b was performed by hydrogenolysis over 10% Pd-C yielding 11 in good yield, while similar treatment of 10a gave 11 in poor yield. The pyrrolidinylthiocarbapenem (11) was crystallized from water to give the semihydrate of 11 as colorless fine prisms. Treatment of 11 with ethyl acetimidate afforded RS-533. The two carbapenems, 11 and RS-533, may be represented as zwitter ions. The NMR spectrum of RS-533 indicates that it exists in water as two interconvertible rotamers (approximately 1:1) possibly responsible for a double bond character of the acetimidoylpyrrolidinyl linkage. Details will be given in the near future.

\* (S)-1-$p$-Nitrobenzoxycarbonyl-3-mercaptopyrrolidine was prepared in the following four steps starting from (R)-3-hydroxy-L-proline: 1) $p$-NO$_2$C$_6$H$_4$CH$_2$OCOCl, Et$_3$N/cyclohexanol, 0°C; 2) MeSO$_2$Cl, Et$_3$N/CH$_2$Cl$_2$, 0°C; 3) Ac$_2$Na/DMF, 60°C; 4) MeONa/MeOH, 0°C. (R)-3-Hydroxy-L-proline was prepared from trans-4-hydroxy-L-proline according to the known method.\(^{20}\)
Antimicrobial Activity

The in vitro antimicrobial activities of RS-533 and 11 were tested by the serial agar dilution method. The minimal inhibitory concentrations (MIC) against a variety of Gram-positive and Gram-negative bacteria are listed in Table 1 and compared with those of THM, R-1 and S-1. The carbapenem 11 is 4~5 times more active than THM against Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, etc. Although RS-533 is slightly less active than 11 as far as the in vitro activity concerned, the former proved to be better in mice infected with a variety of bacteria.

Table 1. Antimicrobial activities of RS-533, 11, S-1, R-1, and THM.

<table>
<thead>
<tr>
<th>Organism</th>
<th>MIC (µg/ml)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>RS-533</td>
</tr>
<tr>
<td>Bacillus subtilis PCI-219</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Staphylococcus aureus 209P</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Staphylococcus aureus 56*</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Escherichia coli NIHJ</td>
<td>0.05</td>
</tr>
<tr>
<td>Escherichia coli 609**</td>
<td>0.05</td>
</tr>
<tr>
<td>Salmonella enteritidis Gaertner</td>
<td>0.05</td>
</tr>
<tr>
<td>Shigella flexneri 2a Komagome</td>
<td>0.02</td>
</tr>
<tr>
<td>Klebsiella pneumoniae 806</td>
<td>0.05</td>
</tr>
<tr>
<td>Enterobacter cloacae 963</td>
<td>0.4</td>
</tr>
<tr>
<td>Serratia marcescens 1850</td>
<td>0.1</td>
</tr>
<tr>
<td>Proteus vulgaris 1420</td>
<td>1.5</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa 1001</td>
<td>6.2</td>
</tr>
</tbody>
</table>

* Penicillinase producer.
** Cephalosporinase producer.
Nutrient agar: Inocula were diluted 100-fold after overnight culture. Final inoculum size was one-loopful of 10^7 cfu/ml.

** Experimental **

IR spectra were recorded on a Jasco A-2 spectrometer and UV spectra were obtained on a Cary 14 CM-50 (Serial 1258) spectrometer. Nuclear magnetic resonance spectra were recorded on a Jeol
JNM GX-400 or a Varian EM 360L spectrometer. Chemical shifts are reported in parts per million (δ) using, unless otherwise specified, tetramethylsilane (TMS) as an internal standard. Rotations were determined on a Perkin-Elmer 241 polarimeter.

**Benzyl 4-[(3S,4R)-3-[(R)-1-Hydroxyethyl]-2-oxoazetidin-4-yl]-3-oxobutyrate (4a)**

A solution of benzyl 3-pyrrolidinocrotonate (5.81 g, 20 mmol) in THF (70 ml) was treated with a solution of n-BuLi (20 mmol) of hexane (12.3 ml, 1.63 mmol/ml) according to the procedure given in ref 19. To the resulting solution was added a solution of Et₂AlCl (20 mmol) in hexane (23.0 ml, 0.871 mmol/ml) at -60°C with stirring. The mixture was stirred for 45 minutes at the same temperature and a solution of 2 (1.15 g, 4 mmol) in THF (10 ml) was added dropwise. After being stirred at -60°C for 0.5 hour and at 0°C for 45 minutes, the mixture was poured into ice-water and extracted with EtOAc. After removal of the insoluble material by filtration, the extract was washed with water, dried over MgSO₄ and evaporated in vacuo to leave an oily residue. The oil was dissolved in a mixture of conc. HCl (10 ml) and MeOH (50 ml) and the solution was stirred at 0°C for 1 hour. The mixture was neutralized with 5%aq. NaHCO₃ and extracted with EtOAc. The extract was washed withaq. NaCl and dried over MgSO₄, and the residue obtained by removal of the solvent was treated with silica gel (30 g) in benzene (50 ml) - H₂O (4.5 ml) at room temperature for 1 hour. The mixture was loaded on silica gel and chromatographed eluting with EtOAc - MeOH (30:1) to give 4a (402 mg, 33% yield) as an oil. NMR (CDCl₃) δ 1.22 (3H, d, J=6.0 Hz), 2.6-3.0 (3H, m), 3.50 (2H, s), 3.3-4.3 (3H, m), 5.11 (2H, s), 6.77 (1H, br.s), 7.31 (5H, s). IR (CHCl₃) 3430, 1755, 1710 cm⁻¹.

**Methyl 4-[(3S,4R)-3-[(R)-1-tert-Butyldimethylsilyloxyethyl]-2-oxoazetidin-4-yl]-3-oxobutyrate (5)**

Methyl 3-pyrrolidinocrotonate¹⁹ (10.2 g, 60.3 mmol) was treated successively with n-BuLi (37.0 ml, 60.3 mmol), Et₂AlCl (69.3 ml, 0.870 mmol/ml) and 2 (3.47 g, 12.1 mmol) as described for 4a. Without HCl treatment the crude product was subjected to hydrolysis with silica gel - H₂O and then chromatographed on silica gel with cyclohexane - EtOAc (1:4) to give 5 (1.25 g, 30% yield) as an oil. NMR (CDCl₃) δ 0.08 (6H, s), 0.88 (9H, s), 1.22 (3H, d, J=6.5 Hz), 2.4-3.1 (3H, m), 3.48 (2H, s), 3.75 (3H, s), 3.8-4.4 (2H, m), 6.15 (1H, br.s). IR (CHCl₃) 3430, 1755, 1720 cm⁻¹.

**p-Nitrobenzyl 4-[(3S,4R)-3-[(R)-1-Hydroxyethyl]-2-[(S)-1-p-nitrobenzyloxycarbonylpyrrolidin-3-yl-thio]-1-carbapen-2-em-3-carboxylate (10b)**

Diphenylphosphoryl chloride (1.76 ml, 8.62 mmol) and iso-Pr₂EtN (1.50 ml, 8.62 mmol) were added to an ice-cooled solution of 9b (2.50 g, 7.18 mmol) in anhydrous CH₂CN (100 ml) and the mixture was stirred for 0.5 hour. Then iso-Pr₂EtN (1.50 ml, 8.62 mmol) and a solution of 3-(S)-mercapto-1-p-nitrobenzoyloxy carbonylpyrrolidine (2.43 g, 8.62 mmol) in CH₂CN (7 ml) were added and the mixture was stirred at 0°C for 1 hour. The reaction mixture was diluted with EtOAc, washed successively with H₂O, aq. 5% NaHCO₃ and aq. NaCl, and dried over MgSO₄. The solvent was evaporated in vacuo to leave a residue which was treated with a small amount of EtOAc to give 10b (2.79 g) as a powder. The filtrate was chromatographed on silica gel with EtOAc to give additional amount of 10b (1.00 g)
as a powder. Yield 86%. NMR (CDCl₃) δ 1.34 (3H, d, J=6.0 Hz), 1.9 ~ 3.0 (3H, m), 3.1 ~ 4.5 (10H, m), 5.24 (2H, s), 5.22, 5.53 (2H, AB-q, J=14.0 Hz), 7.52, 8.21 (4H, A₂B₂, J=9.0 Hz), 7.66, 8.21 (4H, A₂B₂, J=9.0 Hz). IR (KBr) 3560, 1780, 1705, 1350 cm⁻¹.

Benzyl (5R,6S)-6-[(R)-1-Hydroxyethyl]-2-[(S)-1-p-nitrobenzoyloxycarbonylpyrrolidin-3-ylthio]-1-carbapen-2-em-3-carboxylate (10a)

The benzyl ester 10a (303 mg) was obtained from 9a (280 mg, 0.92 mmol) as described for 10b. NMR (DMF-d₇) δ 1.23 (3H, d, J=6.0 Hz), 1.7-2.7 (2H, m), 3.1-4.5 (10H, m), 5.06, 5.33 (2H, AB-q, J=16.5 Hz), 5.31 (2H, s), 7.2-7.7 (5H, m), 7.73, 8.26 (4H, A₂B₂, J=8.5 Hz). IR (Nujol) 3400, 1770, 1708, 1695, 1350 cm⁻¹.

(5R,6S)-6-[(R)-1-Hydroxyethyl]-2-[(S)-pyrrolidin-3-ylthio]-1-carbapen-2-em-3-carboxylic Acid (11)

a) A mixture of 10b (5.00 g, 8.17 mmol) in a solution of THF (100 ml) and 0.1 M phosphate buffer (pH 7.0, 100 ml) was shaken with 10% Pd-C (4.0g) for 1.5 hours under a H₂ atmosphere. After removal of the catalyst by filtration through celite, the filtrate was concentrated in vacuo and filtered. The filtrate was washed with EtOAc, concentrated in vacuo to a half volume and chromatographed on a column of Diaion HP-20AG (Mitsubishi Chemical Industries, Ltd.). Fractions eluted with 5% aq. acetone were lyophilized to give 11 (1.8 g, 74% yield) as a powder which was crystallized from H₂O to give fine prisms, mp >270°C (dec.). [α]ᵣ +73° (c 0.31, H₂O). NMR (400 MHz, D₂O/TMS) δ 1.09 (3H, d, J=6.4 Hz, CH₃CH), 1.87, 1.99 (1H, m, pyrrolidine H-4), 2.06, 2.08 (1.5 H each, s, CH₂C=N), 2.10-2.35 (1H, m, pyrrolidine H-4), 3.01, 3.05 (0.5 H each, q, Jₚₖₘ=17.6 Hz, Jₚₖₑ₋ₙ=8.8, 9.8 Hz, respectively, 2×H-1), 3.16 (1H, dd, J=12.4, 4.4 Hz, pyrrolidine H-2), 3.20-3.25 (2H, m, H-6, pyrrolidine H-5), 3.31-3.37 (1H, m, pyrrolidine H-5), 3.51 (1H, dd, J=12.4, 6.6 Hz, pyrrolidine H-2), 3.80-3.85 (1H, m, SCH), 4.00-4.06 (2H, m, H-5, H-8). IR (KBr) 3400, 2800-2000, 1765, 1590 cm⁻¹. UV λmax nm (ε) 297 (8,330).

b) The benzyl ester (10a) was treated with H₂/10% Pd-C and worked up as described above to give 11 in yield of 9%. (5R,6S)-6-[(R)-1-Hydroxyethyl]-2-[(S)-1-acetimidoylpyrrolidin-3-ylthio]-1-carbapen-2-em-3-carboxylic Acid (RS-533)

A solution of 11 (3.3 g, 11.1 mmol) in 0.1 M phosphate buffer (pH 7.0, 350 ml) was adjusted to pH 8.5 with 1 N NaOH at 0°C and ethyl acetimidate hydrochloride (6.88 g, 55.5 mmol) was added in portions while adjusting to pH 8.5. After stirring for 10 minutes at pH 8.5 the reaction mixture was neutralized with aq. 5% HCl and passed through Diaion HP-20AG. Fractions eluted with aq. 5% acetone were lyophilized to give RS-533 (3.17 g, 84% yield) as a powder. NMR (400 MHz, D₂O/TMS) δ 1.09 (3H, d, J=6.3 Hz, CH₃CH). 1.87 ~ 1.99 (1H, m, pyrrolidine H-4), 2.06, 2.08 (1.5 H each, s, CH₂C=N), 2.10-2.35 (1H, m, pyrrolidine H-4), 3.01, 3.05 (0.5 H each, q, Jₚₖ₈=17.6 Hz, Jₚₖₑ₋ₙ=8.8, 9.8 Hz, respectively, H-1), 3.00, 3.06 (0.5 H each, q, Jₚₖₑ₋ₙ=17.6 Hz, Jₚₖₑ₋ₙ=8.8, 9.8 Hz, respectively, H-1), 3.23 (1H, dd, J=5.9, 2.5 Hz, H-6), 3.24-3.89 (4H, m, pyrrolidine 2- & 5-methylene protons), 3.78-3.89 (1H, m, SCH), 4.00-4.06 (2H, m, H-5, H-8). IR (KBr) 3400, 2800-2000, 1765, 1590 cm⁻¹. An analytical sample was obtained as follows. The amorphous powder was dissolved in MeOH, seeded with crystals of previously obtained authentic sample and allowed to stand in a freezer to give colorless fine prisms, mp 198 ~ 200°C (dec.), which were dried at 40°C in vacuo for 46 hours. UV λmax nm (ε) 298 (10,400).

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References


