SYNTHESIS AND β-LACTAMASE INHIBITORY ACTIVITY OF 7α-HYDROXYETHYL CEPHEM DERIVATIVES

Sir:

Recent advances in the chemistry of β-lactam antibiotics have created novel nuclei, such as carbapenems. One of the structural peculiarities of carbapenems is to have 6α-hydroxyethyl side chain as well as the highly strained ring system. On the other hand, the most of the cephalosporin antibiotics has the amide side chain at the 7β-position, and so the biological property of the corresponding cephalosporin having hydroxyethyl side chain at the 7α-position has become of interest in recent years.

Accordingly, the Merck group investigated homothienamycin 11) as a new carbacephem and reported that its antibacterial activity was quite low level. We thought that the poor activity was mainly due to the low reactivity of β-lactam ring because there was no electron-withdrawing group (EWG) at the both of the 3 and 7 positions.

In cephalosporin chemistry, it was said that the EWG at the 3 position played an important role for reactivity of β-lactam ring6); therefore, we attempted to introduce strong EWG's at the 3 position of 7α-hydroxyethyl cephem derivatives in order to find new biologically active cephem derivatives as shown in 2.

Silver salt 7 was obtained from 3-chloro-1,2-propanediol via 4 steps (Scheme 1). Azetidinone 8) was treated with 7 and sodium iodide in acetonitrile to give 9. Phosphorane 10 was obtained by the condensation of allyl glycoxylate with 9, conversion to the chloride with SOCl₂ and subsequent reaction with PPh₃.

Cephem 12 was obtained by an intramolecular Wittig reaction7) of 10 in the presence of hydroquinone8) followed by desilylation and subsequent oxidation with Collins reagent. Deprotection of 12 was effected with hydrochloric acid to give 13 (Scheme 2). Oxime 14, derived from 13 by the treatment with hydroxylamine hydrochloride, was dehydrated to give the cyano derivative 15. Deprotection of 15 was accomplished with palladium(0)-catalyzed exchange9) to form 16, sodium 7α-[(1R)-1-hydroxyethyl]-3-cyano-3-cephem-4-carboxylate. Similarly, 18, sodium 7α-[(1R)-1-hydroxyethyl]-3-[(Z)-2-cyano-

--

Fig. 1. Structures of 1 and 2.

\begin{align*}
1 & \quad X=\text{CH}_{2} \quad R=-\text{S}^{-}\text{\text{NH}}_{2} \quad R'=\text{H} \\
2 & \quad X=\text{S} \quad R=\text{EWG} \quad R'=\text{Na}
\end{align*}

Scheme 1.

\begin{align*}
3 & \quad \text{Cl} \quad \text{OH} \\
& \quad \xrightarrow{\text{a,b}} \quad \text{TrS} \quad \text{OH} \quad \text{OR} \\
4 & \quad R=\text{H} \quad \text{(yield 80\%)} \\
& \quad \xrightarrow{\text{c,d}} \quad \text{RS} \quad \text{O} \quad \text{Si} \\
5 & \quad R=-\text{OSi} \quad \text{(yield 66\%)} \\
6 & \quad R=\text{Tr} \quad \text{(yield 46\%)} \\
7 & \quad R=\text{Ag} \quad \text{(yield 98\%)}
\end{align*}

--

\text{a) \text{TrSH}, tert-BuOK - THF, b) tert-Bu(CH₃)₂SiCl, imidazole - DMF, c) (CF₃CO)₂O, DMSO - CH₂Cl₂, d) AgNO₃, pyridine, THF - MeOH.}

\text{\textsuperscript{1} IR and \textsuperscript{1}H NMR data of 13: IR (CH₃Cl) cm\textsuperscript{-1}: 3600, 1790, 1735, 1670, 1600, 1380, 1345, 1235; \textsuperscript{1}H NMR (90 MHz, CDCl₃) δ 1.31 (3H, d, J=7 Hz), 2.48 (1H, br s), 3.28 and 3.97 (2H, Abq, J=17 Hz), 3.38 (1H, dd, J=3 and 5 Hz), 4.35 (1H, m), 4.72~4.90 (3H, m), 5.70~6.20 (1H, m), 9.65 (1H, s).}

\text{\textsuperscript{11} IR and \textsuperscript{1}H NMR data of 16: IR (Nujol) cm\textsuperscript{-1}: 3500~3250, 2200, 1760, 1620, 1590, 1450, 1330; \textsuperscript{1}H NMR (90 MHz, D₂O) δ 1.28 (3H, d, J=7 Hz), 3.48 and 3.78 (2H, Abq, J=17 Hz), 3.57 (1H, dd, J=3 and 5 Hz), 4.33 (1H, m), 4.87 (1H, d, J=3 Hz).}

\text{\textsuperscript{111} IR and \textsuperscript{1}H NMR data of 18: IR (Nujol) cm\textsuperscript{-1}: 2210, 1750, 1610, 1340; \textsuperscript{1}H NMR (90 MHz, D₂O) δ 1.31 (3H, d, J=7 Hz), 3.52 (1H, dd, J=3 and 5 Hz), 3.83 and 4.10 (2H, Abq, J=17 Hz), 4.32 (1H, m), 4.86 (1H, d, J=3 Hz), 5.35 (1H, d, J=12 Hz), 7.00 (1H, d, J=12 Hz).}
The MICs of 16, 18 and 20 against Staphylococcus aureus and Escherichia coli are shown in Table 1. The cephems of this series showed only poor activity. Fig. 2 shows the binding affinities of 20, imipenem and cefazolin (CEZ) for penicillin-binding proteins in E. coli. Interestingly, the affinity pattern of 20 is similar to that of imipenem. This fact suggested that the hydroxyethyl moiety determined the affinity pattern regardless of the ring systems, and that the relative weakness of the affinities of 20 resulted in poor MIC values.

Scheme 2.

a) NaI - CH₃CN, b) CH₂=CHCH₂OOCCHO → H₂O - toluene, reflux 3 hours, c) SOCl₂, 2,6-lutidine - THF, d) PPh₃, sodium 2-ethyl hexanoate, Pd(PPh₃)₄ - EtOAc, e) hydroquinone - xylene, reflux 13 hours, f) BF₃·Et₂O - CH₃CN, g) Collins reagent - CH₂Cl₂, h) 2 N HCl - THF.

Scheme 3.

α) NH₂OH·HCl - (CH₃)₂CHOH, b) SOCl₂ - CHCl₃, reflux 1.5 hours, c) PPh₃, sodium 2-ethyl hexanoate, Pd(PPh₃)₄ - EtOAc.

Interestingly, it was found that 16, 18 and 20 had potent β-lactamase inhibitory activity. As can be seen from Table 2, the degree of activity seems to be in order of the electron-withdrawing effect of the side chain at the 3 position. In particular, 16 exhibits superior inhibitory activity against cephalosporinase to that of sulbactam and clavulanic acid. Furthermore, 16 displayed synergistic activity with cefotizoxime (CZX). The MIC data of 1:1 combination of CZX plus 16 against several representative β-lactamase producing bacteria is shown in Table 3.

In summary, 7α-hydroxyethyl cephems, which have EWG at the 3 position, have poor antibacterial activity, however exhibit potent β-lactamase inhibitory activity and synergistic activity in...
Table 1. MICs of 16, 18 and 20.

<table>
<thead>
<tr>
<th>Organism</th>
<th>16 (µg/ml)</th>
<th>18 (µg/ml)</th>
<th>20 (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Staphylococcus aureus 209P JC-1</td>
<td>50</td>
<td>100</td>
<td>25</td>
</tr>
<tr>
<td>Escherichia coli NIHJ JC-2</td>
<td>100</td>
<td>&gt;100</td>
<td>100</td>
</tr>
</tbody>
</table>

*a* Mueller-Hinton agar 10^{-3}; Stamp method; 37°C, 20 hours.

Table 2. β-Lactamase inhibitory activity.*

<table>
<thead>
<tr>
<th>β-Lactamase</th>
<th>ID_{50} (µg/ml)</th>
<th>16</th>
<th>18</th>
<th>20</th>
<th>Sulbactam</th>
<th>Clavulanic acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>TEM PCase</td>
<td></td>
<td>17</td>
<td>&gt;500</td>
<td>30</td>
<td>1.2</td>
<td>1.0</td>
</tr>
<tr>
<td>(Escherichia coli 18)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ia CSase (Enterobacter cloacae 91)</td>
<td>&lt;0.03</td>
<td>&lt;0.78</td>
<td>33</td>
<td>42</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>Ib CSase (E. coli HB101/pCF3)</td>
<td>&lt;0.5</td>
<td>14</td>
<td>450</td>
<td>14</td>
<td>7.8</td>
<td></td>
</tr>
<tr>
<td>Ic CSase (Proteus vulgaris 9)</td>
<td>0.9</td>
<td>&lt;7.8</td>
<td>&lt;0.5</td>
<td>&lt;0.5</td>
<td>0.6</td>
<td></td>
</tr>
</tbody>
</table>

*a* Serial dilutions of a β-lactamase inhibitor were incubated with enzyme solution for 10 minutes at 37°C. Residual β-lactamase activity was determined spectrophotometrically using the chromogenic substrate nitrocefin at 482 nm. ID_{50} was calculated as the concentration inhibiting 50% of activity.

Table 3. MIC data* of 1:1 combination of 16 with ceftizoxime (CZX).

<table>
<thead>
<tr>
<th>Organism</th>
<th>16 - CZX (1:1)</th>
<th>16 (µg/ml)</th>
<th>CZX (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Morganella morganii 181</td>
<td>25</td>
<td>&gt;100</td>
<td>100</td>
</tr>
<tr>
<td>Citrobacter freundii 3007</td>
<td>3.13</td>
<td>100</td>
<td>6.25</td>
</tr>
<tr>
<td>C. freundii 3014</td>
<td>12.5</td>
<td>50</td>
<td>25</td>
</tr>
<tr>
<td>Enterobacter cloacae 3011</td>
<td>12.5</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>E. cloacae 3022</td>
<td>1.56</td>
<td>100</td>
<td>25</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa FP1457</td>
<td>12.5</td>
<td>&gt;100</td>
<td>25</td>
</tr>
</tbody>
</table>

*a* Mueller-Hinton agar 10^{-3}; Stamp method; 37°C, 20 hours.
Fig. 2. Binding affinities* of 20, imipenem and cefazolin (CEZ) for penicillin-binding proteins (PBP) in Escherichia coli.

□ 20, ○ imipenem, △ CEZ.

* Concentration required to inhibit binding of $[^{14}\text{C}]$benzylpenicillin to each protein by 50%.

There are few reports of the cephalosporins which have β-lactamase inhibitory activity. Further detailed descriptions and synthesis of 1-oxacephem derivatives are underway.

Shintaro Nishimura
Nobuyoshi Yasuda
Hiroshi Sasaki
Yoshimi Matsumoto
Toshiaki Kamimura
Kazuo Sakane
Takao Takaya

Fujisawa Pharmaceutical Co., Ltd.,
New Drug Research Laboratories,
2-1-6 Kashima, Yodogawa-ku,
Osaka 532, Japan

(Received July 15, 1988)