NEW ORALLY ACTIVE CEPHALOSPORIN ESTERS

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In our search for new orally active cephalosporins, we found that 7β-[2-(2-amino-4-thiazolyl)-(Z)-2-(methoxyimino)acetamido]-3-[3-methyl-1,2,4-thiadiazol-5-yl]thiomethyl]-3-cephem-4-carboxylic acid (6e) possessed potent antibacterial activities and that the esters of 6e, in particular the compound 9e, were well absorbed by oral administration to rodents.

This paper describes the synthesis and the biological properties of esters of 6e and related compounds compared with cefixime (CFIX) and ceferam pivoxil (CFTM-PI).

The compounds tested were prepared as shown in Schemes 1 and 2. Pivaloyloxymethyl esters, 9a and 9b, were prepared as reported in the ref. The other pivaloyloxymethyl esters, 9c, 9d and 9e, were prepared via another procedure, which is shown in Scheme 2. Other esters 11p~11w were prepared in the manner similar to that of 9e from the sodium salt of 5e and the corresponding halides. The latter halides were prepared from chloromethyl chlorosulfate (or chloromethyl iodide) and the corresponding carboxylic acids.

The in vitro antibacterial activity of the compounds 6c~6e and the reference compounds was determined by the serial 2-fold agar dilution method and the results are given as MIC (µg/ml) in Table 1. The activity of compounds 6c, 6d and 6e was as potent as that of parenteral cephalosporins (cefotaxime: CTX and cefmenoxime: CMX). The activity of these compounds against Staphylococcus aureus was even superior to that of CTX and CMX.
Scheme 2.

5 (Na)\[\text{ICH}_2\text{OOC}(\text{CH}_3)_3\] → 7

\[
\begin{align*}
\text{HCONH} & \quad \text{COOCH}_2\text{OOC}(\text{CH}_3)_3 \\
\text{N} & \quad \text{N} \\
\text{O} & \quad \text{CH}_3 \\
\end{align*}
\]

8 → 9

\[
\begin{align*}
\text{H}_2\text{N} & \quad \text{COOCH}_2\text{OOC}(\text{CH}_3)_3 \\
\text{N} & \quad \text{CH}_3 \\
\text{O} & \quad \text{N} \\
\end{align*}
\]

R = a, b, c, d, e

5e (Na)\[\text{NaI}\] → 10

\[
\begin{align*}
\text{HCONH} & \quad \text{COOCH}_2\text{OOCOR}' \quad \text{COOCH}_2\text{OOCOR}' \\
\text{N} & \quad \text{N} \\
\text{O} & \quad \text{CH}_3 \\
\end{align*}
\]

10 → 11

\[
\begin{align*}
\text{H}_2\text{N} & \quad \text{COOCH}_2\text{OOCOR}' \\
\text{N} & \quad \text{CH}_3 \\
\text{O} & \quad \text{N} \\
\end{align*}
\]

R' = p, q, r, s, t, u, v, w
Table 1. In vitro antibacterial activity of the cephalosporins (MIC: μg/ml).

<table>
<thead>
<tr>
<th></th>
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<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>CTX (6a)</td>
<td>CH2OCOCH3</td>
<td>0.78</td>
<td>0.78</td>
<td>&lt;0.013</td>
<td>&lt;0.013</td>
<td>&lt;0.013</td>
<td>&lt;0.013</td>
<td>6.25</td>
<td>0.10</td>
</tr>
<tr>
<td>CMX (6b)</td>
<td>CH2S^N'</td>
<td>1.56</td>
<td>0.78</td>
<td>0.10</td>
<td>&lt;0.013</td>
<td>0.05</td>
<td>&lt;0.013</td>
<td>12.5</td>
<td>0.20</td>
</tr>
<tr>
<td>6c</td>
<td>CH2S^N'CH3</td>
<td>0.05</td>
<td>0.20</td>
<td>&lt;0.013</td>
<td>&lt;0.013</td>
<td>&lt;0.013</td>
<td>&lt;0.013</td>
<td>12.5</td>
<td>0.20</td>
</tr>
<tr>
<td>6d</td>
<td>CH2S^N'CH3</td>
<td>0.20</td>
<td>0.39</td>
<td>0.05</td>
<td>&lt;0.013</td>
<td>0.05</td>
<td>&lt;0.013</td>
<td>12.5</td>
<td>0.20</td>
</tr>
<tr>
<td>6e</td>
<td>CH2S^N'CH3</td>
<td>0.10</td>
<td>0.39</td>
<td>0.05</td>
<td>&lt;0.013</td>
<td>0.10</td>
<td>&lt;0.013</td>
<td>12.5</td>
<td>0.39</td>
</tr>
<tr>
<td>CFTM</td>
<td>CH2N=NCH3</td>
<td>1.56</td>
<td>1.56</td>
<td>0.20</td>
<td>&lt;0.013</td>
<td>0.20</td>
<td>&lt;0.013</td>
<td>100</td>
<td>1.56</td>
</tr>
</tbody>
</table>

MICs were determined by the serial 2-fold agar dilution method.

Test organisms and abbreviations: S.a., Staphylococcus aureus FDA 209P; S.e., Staphylococcus epidermidis IAM 1296; E.c., Escherichia coli NIHJ JC-2; K.p., Klebsiella pneumoniae ATCC 10031; P.m., Proteus mirabilis GN 2425; P.v., Proteus vulgaris OX-19; P.a., Pseudomonas aeruginosa IFO 3451; S.m., Serratia marcescens X-100.

While the oral cephalosporins CFIX and cefeteram (CFTM) were highly active against Gram-negative bacteria, they showed only weak activities against Gram-positive bacteria. Cefuroxime (CXM), cefotiam (CTM) and cefaclor (CCL) showed less potent activity than 6c, 6d and 6e against both Gram-positive and Gram-negative bacteria.

The pivaloyloxymethyl esters of these compounds (6a ~ 6e) were prepared to estimate the oral absorption in rodents. The pivaloyloxymethyl esters are generally accepted as useful prodrug esters in oral administration of β-lactam antibiotics. The results are shown in Table 2.

It is interesting to note that 9e, the methyl substituted analogue of 9d, exhibited higher oral absorption than 9d in both rats and mice. The oral absorption bioavailability of 9e was higher than those of CFTM-PI and cefuroxime axetil (CXM-AX) in both rats and mice.

As the pivaloyloxymethyl ester of 6e gave good urinary recovery and oral absorption, several kinds of esters other than pivaloyloxymethyl ester of 6e were prepared and evaluated. The results are shown in Table 3. No better ester than pivaloyloxymethyl were found. The 1-methylpyrrolylcarbonyloxymethyl ester (11p) was moderately well absorbed orally.

A comparison test of oral absorption between 9e and CFTM-PI was conducted. In Figs. 1 and 2, the serum levels of 6e and CFTM after oral administration of 9e and CFTM-PI in mice and rats are shown. The serum level of 6e was equal to that of CFTM in mice and about two times as high as that of CFTM in rats. Moreover, T1/2 of 6e in serum was about two times as long as that of CFTM in both mice and rats. In consideration of these pharmacokinetic data together with the antibacterial activities of 6e, compound 9e was evaluated as a good oral cephalosporin.

Experimental

IR spectra were recorded on a Hitachi model EPI-G3 spectrophotometer. NMR spectra were recorded on Nihon Denshi a FX-270 (270 MHz) spectrometer using TMS as an internal standard.
Table 2. Urinary recovery and relative bioavailability of the cephalosporins after oral administration.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Dosing route</th>
<th>Urinary recovery (%)</th>
<th>Relative bioavailability (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Rats</td>
<td>Mice</td>
</tr>
<tr>
<td>9a</td>
<td>po</td>
<td>4.1 ± 0.5</td>
<td>NT</td>
</tr>
<tr>
<td>6a</td>
<td>iv</td>
<td>40.0 ± 2.0</td>
<td>NT</td>
</tr>
<tr>
<td>9b</td>
<td>po</td>
<td>9.0 ± 0.8</td>
<td>NT</td>
</tr>
<tr>
<td>6b</td>
<td>iv</td>
<td>68.2 ± 12.1</td>
<td>NT</td>
</tr>
<tr>
<td>9c</td>
<td>po</td>
<td>6.0 ± 0.5</td>
<td>12.9</td>
</tr>
<tr>
<td>6c</td>
<td>iv</td>
<td>36.2 ± 5.4</td>
<td>38.0</td>
</tr>
<tr>
<td>9d</td>
<td>po</td>
<td>5.3 ± 0.3</td>
<td>25.9</td>
</tr>
<tr>
<td>6d</td>
<td>iv</td>
<td>50.1 ± 0.8</td>
<td>58.0</td>
</tr>
<tr>
<td>9e</td>
<td>po</td>
<td>18.8 ± 5.9</td>
<td>26.6</td>
</tr>
<tr>
<td>6e</td>
<td>iv</td>
<td>48.7 ± 5.4</td>
<td>40.5</td>
</tr>
<tr>
<td>CFTM-PI</td>
<td>po</td>
<td>14.3 ± 5.7</td>
<td>21.0</td>
</tr>
<tr>
<td>CFTM</td>
<td>iv</td>
<td>49.8 ± 9.0</td>
<td>65.5</td>
</tr>
<tr>
<td>CXM-AX</td>
<td>po</td>
<td>17.5 ± 0.4</td>
<td>38.8</td>
</tr>
<tr>
<td>CXM</td>
<td>iv</td>
<td>58.0 ± 4.3</td>
<td>83.0</td>
</tr>
</tbody>
</table>

- Urinary recovery of 9 (po) = \( \frac{\text{Urinary recovery of 6 (iv)}}{100} \).
- Mean ± SD (n = 3), male JCL-SD rats.
- A group of eight male SLC-ICR mice was housed in each cage.
- As suspension in a 0.5%-methyl cellulose solution.
- NT: Not tested.

Table 3. Cmax and AUC of the cephalosporins (11p~11w) after oral administration in mice (20 mg/kg).

<table>
<thead>
<tr>
<th>Compound</th>
<th>Cmax ((\mu g/ml))</th>
<th>Tmax (minutes)</th>
<th>AUC (0~2 hours) ((\mu g \cdot \text{minute/ml}))</th>
<th>Relative bioavailability (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>9e</td>
<td>44.1</td>
<td>15</td>
<td>4,350</td>
<td>84.6</td>
</tr>
<tr>
<td>11p</td>
<td>26.1</td>
<td>30</td>
<td>2,874</td>
<td>56.0</td>
</tr>
<tr>
<td>11q</td>
<td>22.3</td>
<td>60</td>
<td>2,390</td>
<td>46.5</td>
</tr>
<tr>
<td>11r</td>
<td>12.9</td>
<td>30</td>
<td>1,400</td>
<td>27.3</td>
</tr>
<tr>
<td>11s</td>
<td>8.6</td>
<td>60</td>
<td>784</td>
<td>15.0</td>
</tr>
<tr>
<td>11t</td>
<td>16.6</td>
<td>60</td>
<td>1,710</td>
<td>33.0</td>
</tr>
<tr>
<td>11u</td>
<td>7.8</td>
<td>60</td>
<td>821</td>
<td>16.0</td>
</tr>
<tr>
<td>11v</td>
<td>1.6</td>
<td>60</td>
<td>144</td>
<td>3.0</td>
</tr>
<tr>
<td>11w</td>
<td>2.7</td>
<td>120</td>
<td>209</td>
<td>4.1</td>
</tr>
</tbody>
</table>

- Relative bioavailability (%) = \(100 \times \frac{\text{AUC 11p~11w, po}}{\text{AUC 6e, iv}}\).

7β-[2-(2-Amino-4-thiazolyl)-(Z)-2-(methoxyimino)acetamido]-3-(3-methyl-1,2,4-thiadiazol-5-yl)thiomethyl-3-cephem-4-carboxylic Acid (6e)

To a suspension of 25 (3.05 g) and 4e (3.00 g) in DMF (19 ml) was added triethylamine (2.64 g). The mixture was stirred at room temperature for 2 hours. To the reaction mixture was added water (135 ml) and the insoluble material was filtered off with the aid of Celite. The filtrate was adjusted to pH 2.2 by the addition of 2 N HCl. The precipitate was collected by filtration, washed with cold water and dried in vacuo to give a crude product (2.36 g). The filtrate was further extracted by EtOAc several times. The combined extracts were washed with saturated
Mice: 20 mg/kg. 6e: T1/2, 390 minutes; AUC (0–4 hours), 5,780 μg-minute/ml. CFTM: T1/2, 43 minutes; AUC (0–4 hours), 2,700 μg-minute/ml.

Rats: 25 mg/kg. 6e: T1/2, 140 minutes; AUC (0–4 hours), 5,010 μg-minute/ml. CFTM: T1/2, 48 minutes; AUC (0–4 hours), 2,270 μg-minute/ml.

aqueous NaCl and dried over MgSO4. Concentration of dried extracts gave also the crude product (2.69 g); a total yield of crude product was 5.05 g. The crude products were combined and purified by the preparative liquid chromatography on a reverse phase column, LiChroprep RP-8, with a mobile phase consisting of 0.01 M phosphate buffer (pH 6.8), MeCN, and THF (80:10:10). The fractions containing the product were concentrated in vacuo to a small volume and adjusted to pH 2 by the addition of 2 N HCl at 0–5°C. The precipitate was collected, washed on a filter with cold water and dried in vacuo over phosphorus pentoxide.

\[ ^1H \text{ NMR (270 MHz, DMSO-}d_6) \delta 2.52 (3H, s, thiadiazole-CH}_3, 3.55 \] and 3.76 (2H, ABq, J = 18 Hz, 2-H, 2-H, 3.83 (3H, s, =NOCH}_3), 4.23 and 4.63 (2H, ABq, J = 13 Hz, 3-CH}_2, 5.14 (1H, d, J = 4.6 Hz, 6-H), 5.77 (1H, dd, J = 4.6 and 8 Hz), 6.73 (1H, s, thiazole-5H), 7.24 (2H, br, NH}_2), 9.60 (1H, d, J = 8 Hz, CONH).

\[ 7β-[2-(2-Formylamino-4-thiazolyl)-(Z)-2-(methoxyimino)acetamido]-3-(3-methyl-1,2,4-thiadiazol-5-yl)thiomethyl-3-cephem-4-carboxylic Acid (5e) \]

To an ice-cold solution of DMF (1.67 g) in EtOAc (27 ml) was added POCl3 (2.17 g) and the mixture was stirred at –5~0°C for 2 hours. To the resulting mixture was added 2-(2-formylamino-4-thiazolyl)-(Z)-2-(methoxyimino)acetic acid (6e) (3.27 g) and the mixture was stirred at –5~0°C for 2 hours. The reaction mixture was added to an ice-cold solution of a silyl derivative of 7-amino-3-(3-methyl-1,2,4-thiadiazol-5-yl)thiomethyl-3-cephem-4-carboxylic acid (4e), prepared by adding N,O-bis(trimethylsilyl)acetamide (7.92 g) to a suspension of 4e (4.34 g) in EtOAc (86 ml), and the mixture was stirred at –5~0°C for 1.5 hours. To the reaction mixture was added a solution of water (26 ml) and MeOH (5.5 ml) and the mixture was stirred for 30 minutes. The resulting crystals were collected by filtration, washed with EtOAc and dried in vacuo to give the product (3.49 g).

IR (KBr) cm\(^{-1}\) 1780, 1680; \(^1H \text{ NMR (270 MHz, DMSO-}d_6) \delta 2.52 (3H, s, CH}_3, 3.54 \] and 3.76 (2H, ABq, J = 18 Hz, 2-H, 2-H, 3.89 (3H, s, =NOCH}_3), 4.23 and 4.62 (2H, ABq, J = 13.5 Hz, 3-CH}_2, 5.15 (1H, d, J = 5 Hz, 6-H), 5.81 (1H, dd, J = 5 and 8 Hz, 7-H), 7.40 (1H, s, thiazole-5H), 8.50 (1H, s, HCONH), 9.69 (1H, d, J = 8 Hz, CONH), 12.62 (1H, s, HCONH).

Sodium Salt of 5e

Compound 5e (590 mg) was dissolved in a solution of NaHCO3 (94 mg) and H2O (17 ml), and the solution was lyophilized to give the sodium salt of 5e.

Pivaloyloxymethyl 7β-[2-(2-Formylamino-4-thiazolyl)-(Z)-2-(methoxyimino)acetamido]-3-(3-methyl-1,2,4-thiadiazol-5-yl)thiomethyl-3-cephem-4-carboxylate (8e)

To an ice-cold solution of 5e (Na salt, 0.61 g) in DMF (3 ml) was added iodomethyl pivalate (0.41 g) and the mixture was stirred at 5°C for 1 hour. The mixture was poured into a mixture of EtOAc (30 ml) and water (10 ml) with stirring, and the organic layer
was separated and washed with water (10 m x 2) and saturated brine (10 ml), then dried over MgSO₄. The solvent was evaporated in vacuo and the residue was triturated with Et₂O and n-hexane. The solid was collected by filtration and dried in vacuo to give 8e (0.4 g).

IR (KBr) cm⁻¹ 1790, 1755, 1690; ¹H NMR (270 MHz, DMSO-d₆) δ 1.14 (9H, s, C(CH₃)₃), 2.52 (3H, s, CH₃), 3.61 and 3.81 (2H, ABq, J=18Hz, 2-H₂), 3.88 (3H, s, -NOCH₃), 4.23 and 4.59 (2H, ABq, J=13.5Hz, 3-CH₂), 5.20 (1H, d, J=5Hz, 6-H), 5.85~5.98 (3H, m, 7-H, COOCH₂), 7.42 (1H, s, thiazole-5H), 8.51 (1H, s, a-HCO), 9.70 (1H, d, J=8Hz, CONH).

Pivaloyloxymethyl 7β-[2-(2-Amino-4-thiazolyl)-(Z)-2-(methoxyimino)acetamido]-3-(3-methyl-1,2,4-thiadiazol-5-yl)thiomethyl-3-cephem-4-carboxylate (9e)

To an ice-cold solution of 8e (1.14g) in a mixture of MeOH (5ml) and THF (5ml) was added cone HC1 (1.5ml) and the mixture was stirred at 0~5°C for 8 hours. To the reaction mixture was added water (30ml) and the mixture was adjusted to pH2.5 by the addition of saturated aqueous NaHCO₃. The organic solvent was removed in vacuo and the resulting crystals were collected by filtration, washed with water and dried in vacuo to give 9e (1.01 g).

¹H NMR (270 MHz, CDCl₃) δ 1.22 (9H, s, (CH₃)₃), 2.60 (3H, s, CH₃), 3.62 and 3.75 (2H, ABq, J=18 Hz, 2-H₂), 4.10 (3H, s, -NOCH₃), 4.20 and 4.68 (2H, ABq, J=13Hz, 3-CH₂), 5.07 (1H, d, J=5Hz, 6-H), 5.85~5.99 (3H, m, 7-H, COOCH₂), 6.6~7.1 (2H, br, NH₂), 7.00 (1H, s, thiazole-5H), 7.80 (1H, d, J=9 Hz, CONH).

Chloromethyl L-1-Benzylloxy carbonyl-2-pyrrolidinecarboxylate (7u)

To the mixture of L-1-benzyloxycarbonyl-2-pyrrolidinecarboxylic acid (6.0 g), NaHCO₃ (5.04 g) and tetrabutylammonium hydrogensulfate (0.90 g) in a mixture of CH₂Cl₂ (60 ml) and H₂O (60 ml) was added a solution of chloromethyl chlorosulfate reacted (4.75 g) in CH₂Cl₂ (30 ml) with stirring. The mixture was stirred at room temperature for 42 hours. Organic layer was separated and the aqueous layer was extracted with CHCl₃ (100 ml). Extracts were combined and dried over MgSO₄. The solvent was removed in vacuo to give the product (8.35 g).

¹H NMR (270 MHz, CDCl₃) δ 1.88~2.32 (4H, m, pyrrolidine-3,4-H₄), 3.44~3.69 (2H, m, pyrrolidine-5H₂), 4.35~4.45 (1H, m, pyrrolidine-2H), 5.04~5.20 (2H, m, benzyl-CH₂), 5.55~5.86 (2H, m, CICH₂OCO), 7.28~7.37 (5H, m, phenyl).

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