THE SYNTHESIS AND BIOLOGICAL CHARACTERISTICS OF NEW ORALLY ACTIVE CEPHEMS

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The chemotherapeutic significance of oral antibacterial agents is evidenced by their ability to generate annual sales of several billion dollars. Although many useful agents are currently available, the search for new, more potent compounds with broader spectra of activity continues. Our own efforts in this area have been influenced by the activity and pharmacokinetic properties of FK-482 (I).

FK-482 is characterized by a (Z)-2-(2-aminothiazol-4-yl)-2-hydroxyiminoacetamido group at C-7 of a 3-vinyl cephalosporin nucleus. The 3-vinyl nucleus has previously been shown to support oral bioavailability with other side chains attached to the C-7 nitrogen. Thus, we chose to append the (Z)-2-(2-aminothiazol-4-yl)-2-hydroxyiminoacetamido group to the 3-chlorocephalosporin and 3-chloro-l-carba-1-dethiacephalosporin nuclei since they serve as the foundation for the well known oral agent Ceclor (cefaclor) and the recently introduced Lorabid (loracarbef).

Experimental

All reactions described herein were performed under an inert atmosphere of dry nitrogen in flame-dried glassware unless otherwise noted. All reagents were used as supplied unless stated otherwise. Melting points were recorded on a Thomas-Hoover apparatus and are uncorrected. NMR spectra were recorded at 300 MHz with a General Electric QE-300 instrument, at 270 MHz with a Bruker W-M instrument and at 90 MHz with a JEOL FX-90 instrument. Chemical shifts are recorded in parts per million (δ) relative to tetramethylsilane. IR spectra were recorded on a Nicolet MX-1 FT-IR, optical rotations were measured on a Perkin-Elmer 241 spectrometer, and UV spectra were obtained on a Cary 219. The mass spectral data were obtained on either a CEC-21-140 or a Varian MAT-731 spectrometer. All MPLC separations were conducted on Merck Lobar columns (Lichrorep RP-18) with the help of a Fluid Metering Inc. pump. Analytical HPLC separations were performed on a Varian chromatographic system utilizing a MicroPak MCH-5 N-cap 15 cm x 4 mm column and a variable wavelength UV detector set to record at 254 nm.

Preparation of (Z)-2-(2-aminothiazol-4-yl)-2-hydroxyiminoacetamido-3-chloro-3-cephem-4-carboxylic Acid (5a)

To a solution of 2 (1.65 g, 2.46 mmol) in CH₂Cl₂ (25 ml) was added N-methylmorpholine (0.5 ml, 4.93 mmol) and POCl₃ (0.23 ml, 2.46 mmol) at 0°C and stirred for 15 minutes. To the resulting solution N-methylmorpholine (0.5 ml, 4.93 mmol) and 3a (1.0 g, 2.46 mmol) was added and the reaction stirred for 1 hour at room temperature (RT). The reaction was diluted with EtOAc, washed with 1N HCl, saturated NaHCO₃ solution and brine, dried over MgSO₄, filtered and concentrated. The crude product was purified by flash chromatography on silica gel (2% EtOAc-CH₂Cl₂) to give 1.47 g of 4a as a light yellow foam: mp >105°C (dec); IR (KBr) cm⁻¹ 3379, 3051, 1791, 1738, 1688, 1523, 1492, 1448, 1347, 1217; ¹H NMR (300MHz, DMSO-d₆) δ 9.95 (d, J=12Hz, 1H), 8.75 (s, 1H), 8.14 (d, J=9Hz, 2H), 7.71 (d, J=9Hz, 2H), 7.29-7.08 (m, 30H), 6.56 (s, 1H), 5.96~5.87 (m, 1H), 5.42~5.24 (m, 3H), 3.89 (ABq, J=18Hz, 2H): UV (EtOH) 248 nm (ε = 29,800).

Preparation of (Z)-2-(2-aminothiazol-4-yl)-2-hydroxyiminoacetamido-3-chloro-3-cephem-4-carboxylic Acid (5a)

To a solution of 4a (1.45 g, 1.42 mmol) in CH₃CN
(140 ml) and H₂O (45 ml) at 40°C was added NaHCO₃ (14.28 g, 170 mmol). The suspension was stirred for 1 minute, then Na₂S₂O₄ (9.86 g, 56.7 mmol) was added as a solid over 2 minutes with gas evolution. After 6 minutes stirring, the reaction was poured into H₂O - CH₂Cl₂ and the pH was lowered to 3 with concentrated HCl. The aqueous layer was extracted with CH₂Cl₂, the organic layer was washed with brine, dried over Na₂SO₄, filtered and concentrated. The crude product was purified by flash chromatography on silica gel (2.5% AcOH - EtOAc) to yield 740 mg of 5a (53%) as a yellow solid: ¹H NMR (300 MHz, DMSO-<d>) δ 9.56 (d, J=9 Hz, 1H), 7.78 (s, 1H), 7.38-7.05 (m, 30H), 6.57 (s, 1H), 5.92-5.84 (m, 1H), 5.28 (d, J=6 Hz, 1H), 3.80 (ABq, J= 15 Hz, 2H).

Preparation of (7S,6R)-7-[[2-(Amino-4-thiazolyl)hydroximinoacetyl]amino]-3-chloro-3-cephem-4-carboxylic Acid (6a)

To a solution of 5a (740 mg, 0.75 mmol) in THF (6 ml) was added 75% formic acid (6 ml) and the reaction was stirred at 45°C for 2.5 hours. The reaction was diluted with CH₃CN and concentrated. The crude product was purified by C₁₈ reverse phase medium pressure liquid chromatography (3% CH₃CN-H₂O) to yield 85 mg (27%) of 6a as an off-white solid: mp >205°C; IR (KBr) cm⁻¹ 1764, 1615, 1532, 1537; ¹H NMR (300 MHz, DMSO-d₆) δ 9.44 (d, J=8.7 Hz, 1H), 7.11 (s, 2H), 6.61 (s, 1H), 5.62-5.56 (m, 1H), 5.07 (d, J=6.7 Hz, 1H), 3.54 (ABq, J=13.5 Hz, 2H); FAB-MS m/z 426 (M⁺), 428 (M⁺2).

Preparation of p-Nitrobenzyl (7S,6R)-7-[[2-[(Tritylamino)-4-thiazolyl]trityloximinoacetyl]amino]-3-chloro-1-carba-l-dethia-3-cephem-4-carboxylate (4b)

To a solution of 2 (672 mg, 1.0 mmol) in CH₂Cl₂ (20 ml) was added N-methylmorpholine (0.22 ml, 2.0 mmol) and POCl₃ (0.09 ml, 1.0 mmol) at 0°C and stirred for 10 minutes. To the resulting solution N-methylmorpholine (0.22 ml, 2.0 mmol) and 3b (388 mg, 1 mmol) was added and the reaction stirred for 4 hours at RT. The reaction was diluted with EtOAc, washed with 1N HCl and brine, dried over MgSO₄, filtered and concentrated. The crude product was purified by flash chromatography on silica gel (2×: 40% EtOAc - hexanes followed by 4% EtOAc - CH₂Cl₂) to give 384 mg (38%) of 4b: mp >140°C (dec); IR (KBr) cm⁻¹ 3486, 1780, 1737, 1682, 1523, 1448, 1347, 1206; ¹H NMR (300 MHz, DMSO-d₆) δ 9.43 (d, J=9 Hz, 1H), 8.73 (s, 1H), 8.20 (d, J=9 Hz, 2H), 7.68 (d, J=9 Hz, 2H), 7.27-7.07 (m, 30H), 6.57 (s, 1H), 5.58~5.54 (m, 1H), 5.40 (ABq, J=13.5 Hz, 2H), 3.96~3.88 (m, 1H), 2.58~2.40 (m, 2H), 1.78~1.64 (m, 1H), 1.30~1.19 (m, 1H); FD-MS m/z 1,004 (M⁺), 1,006 (M⁺²); UV (EtOH) 250 nm (ε=31,000).

Preparation of (7S,6R)-7-[[2-[(Tritylamino)-4-thiazolyl]trityloximinoacetyl]amino]-3-chloro-1-carba-1-dethia-3-cephem-4-carboxylic Acid (5b)

To a solution of 4b (310 mg, 0.31 mmol) in CH₃CN (25 ml) and H₂O (10 ml) at 45°C was added NaHCO₃ (3.12 g, 37.0 mmol). The suspension was stirred for 1 minute, then Na₂S₂O₄ (2.15 g, 12.3 mmol) was added as a solid over 2 minutes with gas evolution. After 5 minutes stirring, the reaction was poured into H₂O - CH₂Cl₂ and the pH was lowered to 4 with concentrated HCl. The aqueous layer was extracted with CH₂Cl₂ (2×) and the combined organics were washed with brine, dried over Na₂SO₄, filtered and concentrated. The crude product was purified by flash chromatography on silica gel (2×: 40% EtOAc - CH₂Cl₂) to yield 194 mg of 5b (62%) as a white solid: ¹H NMR (300 MHz, DMSO-<d>) δ 9.43 (d, J=9 Hz, 1H), 8.77 (s, 1H), 7.36~7.03 (m, 30H), 6.59 (s, 1H), 5.54~5.46 (m, 1H), 3.90~3.82 (m, 1H), 2.57~2.23 (m, 2H), 1.76~1.62 (m, 1H), 1.24~1.15 (m, 1H).

Preparation of (7S,6R)-7-[[2-(Amino-4-thiazolyl)hydroximinoacetyl]amino]-3-chloro-1-carba-1-dethia-3-cephem-4-carboxylic Acid (6b)

To a solution of 5b (410 mg, 0.47 mmol) in THF (3 ml) was added 50% formic acid (3 ml) and the reaction was stirred at 45°C for 4 hours. The reaction was diluted with CH₃CN and concentrated. The crude product was purified by C₁₈ reverse phase medium pressure liquid chromatography (4% CH₃CN-H₂O) to yield 73 mg (38%) of 6b as an off-white solid: mp >200°C; IR (KBr) cm⁻¹ 1751, 1617, 1532, 1537; ¹H NMR (300 MHz, DMSO-d₆) δ 9.12 (d, J=9 Hz, 1H), 7.10 (s, 2H), 6.65 (s, 1H), 5.29~5.22 (m, 1H), 3.73~3.64 (m, 1H), 2.49~2.21 (m, 2H), 1.86~1.67 (m, 2H); FAB-MS m/z 408 (M⁺), 410 (M⁺²).

**Pharmacology**

Male Sprague-Dawley rats were dosed intravenously with test compounds at 20 mg/kg in 0.9% saline. Dosing and blood sampling were carried out through an indwelling jugular vein cannula, thus permitting serial sampling from individual rats. Plasma levels were determined from samples col-
lected over a 6-hour time course. Male CD-1 mice were dosed both orally and intravenously with test compound at 20 mg/kg in 0.9% saline. The 0~4 hours cumulative urinary recovery was collected in 0.1 M sodium citrate buffer, pH 6.5, from animals placed in metabolism cages. Plasma and urine samples were stored at -70°C prior to analysis.

Oral bioavailability was calculated as the oral/intravenous ratio of antibacterial activity recovered in the urine following a 20 mg/kg dose.

Antibiotic concentrations were determined with an agar well diffusion assay (bioassay) employing Escherichia coli (ATCC4157) as the bacterial test strain. Standard curves were generated from rat plasma spiked with the compound under study. Urine samples were analyzed by comparison to a standard curve prepared in 0.1 M sodium citrate buffer, pH 6.5. Urine samples were diluted with citrate buffer so that the drug concentration would fall into the range of the standard curve.

**Results and Discussion**

Preparation of the two compounds was accomplished as shown in Scheme 1. Thus, acylation of the appropriate nucleus followed by removal of the protecting groups provided the desired products. Both 6a and 6b exhibited potent Gram-positive and Gram-negative antibacterial activity as determined by an agar dilution method and summarized in Table 1. Unfortunately, the compounds were inactive against Pseudomonas.

**Scheme 1.**

![Scheme 1](image)

Table 1. Antibacterial activity against selected organisms.

<table>
<thead>
<tr>
<th>Test organisms</th>
<th>MIC μg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1 (FK-482)</td>
</tr>
<tr>
<td>------------------------</td>
<td>------------</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em> XI.1</td>
<td>0.5</td>
</tr>
<tr>
<td><em>S. epidermidis</em> 222</td>
<td>0.5</td>
</tr>
<tr>
<td><em>Streptococcus pneumoniae</em> PARK</td>
<td>0.06</td>
</tr>
<tr>
<td><em>Haemophilus influenzae</em> RES 76</td>
<td>0.25</td>
</tr>
<tr>
<td><em>Escherichia coli</em> EC14</td>
<td>0.125</td>
</tr>
<tr>
<td><em>Klebsiella pneumoniae</em> X26</td>
<td>0.06</td>
</tr>
<tr>
<td><em>Enterobacter aerogenes</em> C32</td>
<td>0.05</td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em> X239</td>
<td>&gt;128</td>
</tr>
</tbody>
</table>

Tr = Triphenylmethyl
Reagents: (a) POCl₃, N-methylmorpholine, CH₂Cl₂, (b) NaHCO₃, Na₂S₂O₄, H₂O/CH₃CN, (c) 75% formic acid/THF.
Table 2. Urinary recovery of antibacterial activity in CD-I mice.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Urinary recovery (% dose)</th>
<th>Oral bioavailabilitya (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (FK-482)</td>
<td>58.9</td>
<td>15.0</td>
</tr>
<tr>
<td>6a (LY215891)</td>
<td>47.2</td>
<td>&lt;7.1</td>
</tr>
<tr>
<td>6b (LY215890)</td>
<td>49.1</td>
<td>16.2</td>
</tr>
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</table>

a Oral bioavailability = urinary recovery (po)/urinary recovery (iv)

The pharmacokinetic profiles of the 3-chloro derivatives 6a and 6b were evaluated in male Sprague-Dawley rats following intravenous administration. The drug concentration at 30 minutes was 11.5 μg/ml for 6a and 14.7 μg/ml for 6b. The limited sensitivity of the plasma assays precluded half-life determination, however, the results suggest that these compounds were rapidly cleared.

The oral absorption of compounds 1, 6a and 6b was tested experimentally in CD-I mice. Oral bioavailability was calculated as the po/iv ratio of antibacterial activity recovered in the urine following a 20 mg/kg dose. The results, shown in Table 2, demonstrate that the oral bioavailability of the 1-carbacephalosporin analogue 6b, while relatively low, is greater than that of 6a and 1.

This observation combined with the well documented stability of the 1-carbacephalosporins relative to their 1-sulfur counterparts has prompted us to select 6b for more detailed studies. In addition, esters of these types of compounds have been demonstrated to exhibit enhanced oral bioavailability. Thus, our intention is to prepare such ester prodrugs of 6b in the hope of obtaining well-absorbed antimicrobial agents for clinical studies. The results of these continuing studies will be reported in due course.

Acknowledgments

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References


