Synthesis and Antibacterial Activity of the Metabolites of 9-Fluoro-6,7-dihydro-8-(4-hydroxy-1-piperidyl)-5-methyl-1-oxo-1H,5H-benzo[i,j]quinolizine-2-carboxylic Acid (OPC-7251)

Seiji Morita, Kenji Otsubo, Minoru Uchida,* Shigekatsu Kawabata, Hisashi Tamaoka and Takefumi Shimizu

Tokushima Research Institute, Otsuka Pharmaceutical Co., Ltd., Kagauso 463-10, Kawauchi-cho, Tokushima 771-01, Japan.

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The metabolites of 9-fluoro-6,7-dihydro-8-(4-hydroxy-1-piperidyl)-5-methyl-1-oxo-1H,5H-benzo[i,j]quinolizine-2-carboxylic acid (OPC-7251) (1), which has a potent antibacterial activity against gram-positive bacteria, characteristically Propionibacterium acnes, were synthesized to confirm their structures and to examine their antibacterial activity. The structures of three major metabolites (2, 3a and 4) were identified by means of comparison with the synthetic compounds. The antibacterial activity of the metabolites (2, 3a and 4) was found to be lower than that of 1.

Keywords metabolite; 9-fluoro-6,7-dihydro-8-(4-hydroxy-1-piperidyl)-5-methyl-1-oxo-1H,5H-benzo[i,j]quinolizine-2-carboxylic acid; antibacterial activity; antibacterial agent; quinolone carboxylic acid

The quinolone carboxylic acid derivative (±)-9-fluoro-6,7-dihydro-8-(4-hydroxy-1-piperidyl)-5-methyl-1-oxo-1H,5H-benzo[i,j]quinolizine-2-carboxylic acid (OPC-7251) (1), a new antibacterial agent, was synthesized by Ishikawa et al. and is now under clinical trial. In studies on the metabolism of 1, three metabolites, OPC-7251 analogues (2 and 3a) oxidized and hydroxylated on the piperidine ring and the sulfate derivative (4) of OPC-7251, were isolated from biological fluids of rats, rabbits and dogs (Chart 1). In order to confirm the structures, the three metabolites (2, 3a and 4) were synthesized as described below. Stereoisomer (3b) of 3a was also synthesized, and these compounds were tested for antibacterial activity against gram-positive and gram-negative bacteria.

Synthesis 9-Fluoro-6,7-dihydro-5-methyl-1-oxo-8-(4-oxo-1,2,3,4-tetrahydro-1-pyridyl)-1H,5H-benzo[i,j]quinolizine-2-carboxylic acid (2) was synthesized as shown in Chart 2. Reaction of the carboxylic acid (5) with boric acid and acetic anhydride in acetic acid gave the chelate
compound (6), which was allowed to react with 4-oxo-1,2,3,4-tetrahydropyridine in the presence of sodium hydride in N,N-dimethylformamide (DMF) to give the crude tetrahydropyridine derivative, which was esterified with methanol-thionyl chloride to give methyl 9-fluoro-6,7-di-hydro-5-methyl-1-oxo-8-(4-oxo-1,2,3,4-tetrahydroy-1-pyridyl)-1H,5H-benzof[i]quinoline-2-carboxylate. Finally, the desired compound (2) was prepared by hydrolysis of the ester with sodium hydroxide.

For the formation of the structure of the metabolite (3a) two possible stereoisomers (3a, b) were synthesized by treatment of the chelate compound (6) with the protected piperidines (9a, b) in the usual manner (Chart 2). The starting trans-di-hydroxy piperidine (9b) was prepared by hydrolysis of the benzoyl derivative (8) with potassium hydroxide, and 8 was synthesized by alkylation of trans-1-benzoyl-3,4-dihydroxypiperidine (7) with methoxy-methylen chloride in the presence of sodium hydride.

The sulfate of OPC-7251 was next synthesized by the pathway shown in Chart 3. OPC-7251 was esterified with methyl iodide in the presence of potassium hydroxide in hexamethylenphosphoric triamide (HMPPA)-ethanol to give the ester (10), which was reacted with chlorosulfonic acid in pyridine to afford the pyridinum sulfate (11). Hydrolysis of 11 with potassium hydroxide gave the desired sulfate (4) (Chart 3).

The structures of the metabolites (2, 3a and 4) were identical with those of the corresponding synthetic compounds on the basis of nuclear magnetic resonance (NMR), mass spectrum (MS) and high performance liquid chromatographic comparisons.

Biological Results

The antibacterial activity against gram-positive (Staphylococcus 209p and Propionibacterium acnes ATCC 6919) and gram-negative bacteria (Escherichia coli NIHJ JC-2 and Pseudomonas aeruginosa ATCC 10145) was measured in vitro by the serial dilution method. The results are summarized in Table I. The metabolites (2, 3a) and compound (3b) exhibited potent antibacterial activity against P. acnes. The antibacterial activity of all metabolites and compound (3b) against the other bacteria tested was found to be of a lower potency than 1.

Table I. In Vitro Antibacterial Activity (Minimum Inhibitory Concentration, µg/ml)

<table>
<thead>
<tr>
<th>Compd. No.</th>
<th>S. aureus 209p</th>
<th>E. coli NIHJ JC-2</th>
<th>Ps. aeruginosa ATCC 10145</th>
<th>P. acnes ATCC 6919</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.02</td>
<td>0.39</td>
<td>3.13</td>
<td>0.10</td>
</tr>
<tr>
<td>2</td>
<td>0.20</td>
<td>0.78</td>
<td>6.25</td>
<td>0.10</td>
</tr>
<tr>
<td>3a</td>
<td>0.20</td>
<td>1.56</td>
<td>25</td>
<td>0.20</td>
</tr>
<tr>
<td>3b</td>
<td>0.20</td>
<td>3.13</td>
<td>25</td>
<td>0.20</td>
</tr>
<tr>
<td>4</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>&gt;100</td>
<td>&gt;100</td>
</tr>
</tbody>
</table>

Experimental

Melting points were determined on a Yamaoto MP-21 apparatus and are uncorrected. Infrared (IR) spectra were recorded on a JASCO IRA-2 spectrometer. NMR spectra were recorded on a Bruker AC-200 spectrometer. MS were obtained on a Varian MAT-312 and JEOL JMS-SX102 instruments.

9-Fluoro-6,7-di-hydro-5-methyl-1-oxo-1H,5H-benzof[i]quinoline-2-carboxylic Acid (BOCOCOHCOOHCl, Chelate (6) A mixture of 8,9-dihydro-5-methyl-1-oxo-1H,5H-benzof[i]quinoline-2-carboxylic acid (303 g, 1.09 mol), 46% boric acid (41.6 g, 0.6 mol) and acetic anhydride (676 ml, 6.62 mol) in acetic acid (215 ml) was stirred at 120°C for 1 h. The reaction mixture was allowed to cool (50°C) and poured into isopropyl ether. The precipitated crystals were collected by filtration. Recrystallization from acetone/nitromethane gave 6 (397 g, 90%) as a white powder, mp 203–206°C (dec.). NMR (DMSO-d6): δ 1.51 (3H, d, J = 6.76 Hz), 1.91 (6H, s), 2.20–2.40 (2H, m), 3.00–3.30 (2H, m), 5.20–5.40 (1H, m), 8.30 (1H, t, J = 8.45 Hz), 9.62 (1H, s). IR (KBr): 1715, 1470, 1275, 1060 cm⁻¹. Anal. Calcld. for C21H17BF3N5O4: C, 51.59; H, 4.12; N, 3.37. Found: C, 52.08; H, 4.08; N, 3.26.

9-Fluoro-6,7-di-hydro-5-methyl-1-oxo-8-(4-oxo-1,2,3,4-tetrahydro-1-pyridyl)-1H,5H-benzof[i]quinoline-2-carboxylic Acid (2) Sodium hydride (60% dispersion in mineral oil, 0.6 g, 14.9 mmol) was added to a stirred and ice-cooled solution of 4-oxo-1,2,3,4-tetrahydropyridine (1.3 g, 13.6 mmol) in DMF (10 ml) and the reaction mixture was stirred for 15 min. Then 6 (1.8 g, 4.55 mmol) was added and the reaction mixture was stirred at 60°C for 4 h. The mixture was poured into water and acidified with dil. HCl. The resulting precipitate was collected by filtration, washed with water and dried over MgSO₄. This crude 2 was suspended in MeOH (10 ml) and thionyl chloride (0.58 ml) was added dropwise to the suspension. The mixture was refluxed for 3 h, then the solvent was evaporated off and the residue was dissolved in CH₂Cl₂. The solution was washed with water and dried over MgSO₄. After evaporation of the solvent, the residue was purified by column chromatography (silica gel; eluent, CH₂Cl₂:MeOH = 30:1). Evaporation of the solvent gave methyl 9-fluoro-6,7-di-hydro-5-methyl-1-oxo-8-(4-oxo-1,2,3,4-tetrahydro-1-pyridyl)-1H,5H-benzof[i]quinoline-2-carboxylate (0.61 g, 64%). This ester derivative (0.61 g) was dissolved in a mixture of 30% NaOH solution (0.5 ml) and MeOH (10 ml) and refluxed for 2 h. After evaporation of the MeOH, the residue was dissolved in water and acidified with dil. HCl. The precipitate was collected by filtration and washed with water. Recrystallization from DMF (2) gave 2 (0.46 g, 29%) as yellow powder, mp >300°C. NMR (DMSO-d6): δ 1.43 (3H, d, J = 6.65 Hz), 1.90–2.30 (2H, m), 2.30–2.85 (2H, m), 3.00–3.20 (2H, m), 3.70–4.10 (2H, m), 4.85–5.00 (1H, m), 5.00–5.15 (1H, m), 7.40–7.65 (1H, m), 8.05 (1H, d, J = 10.6 Hz), 9.06 (1H, s), 14.90–15.10 (1H, brs). IR (KBr): 1715, 1660, 1590, 1460 cm⁻¹. MS m/z (%): 356 (M⁺, 19), 313 (23), 312 (100), 269 (20), 235 (12). Anal. Calcld. for C₂₅H₁₈F₂N₄O₂: C, 64.94; H, 4.81; N, 7.86. Found: C, 64.05; H, 4.84; N, 7.82.

trans-3,4-Dimethoxymethylxypiperidine (9) Sodium hydride (60%, 1.04 g, 26 mmol) was added to a stirred and ice-cooled suspension of trans-1-benzoyl-3,4-dihydroxypiperidine (7) (2.2 g, 10 mmol) and molecular sieves 4A (1.5 g) in DMF (20 ml) and the mixture was stirred for 15 min. Then a solution of methoxymethyl chloride (2 ml, 26 mmol) in water (and MeOH and ethyl alcohol was added and stirred at room temperature overnight. After removal of the molecular sieves by filtration, the filtrate was poured into MeOH, MeOH and water and extracted with toluene–AcOEt. The extract was dried over MgSO₄ and concentrated to dryness in vacuo. The residue was purified by column chromatography (silica gel; eluent, hexane/acetone = 2:1) to give 8 (2.18 g, 71%). This dimethoxymethylxypiperidine derivative (2.18 g) was dissolved in 10% KOH solution (20 ml) and the mixture was refluxed for 2.5 h, and then cooled to room temperature. The reaction mixture was extracted with CHCl₃. The extract was dried over MgSO₄ and the solvent was evaporated off in vacuo to give 9 (1.75 g, 70%) as colorless oil. NMR (CDCl₃): δ 1.40–1.60 (1H, m), 1.45–1.70 (1H, brs), 1.95–2.15 (1H, m), 2.50–2.70 (2H, m), 2.90–3.15 (1H, m), 3.20–3.35 (1H, m), 3.39 (6H, s).
Pyridinium 9-Fluoro-6,7-dihydro-8-(4-hydroxy-1-piperidyl)-2-methoxy-carbonyl-5-methyl-1-oxo-1H,5H-benzo[j]quinolizine-2-carboxylic acid (10) was obtained from the reaction mixture. The crude product was purified by column chromatography. The purified compound was characterized by NMR and mass spectrometry. The structure was confirmed by comparison with the literature. The compound was found to be stable under a variety of conditions.

References: