Studies on 2(1H)-Quinolinone Derivatives as Gastric Antiulcer Active Agents. Synthesis and Antiulcer Activity of the Metabolites of 2-(4-Chlorobenzoylamino)-3-[2(1H)-quinolinon-4-yl]propionic Acid

MINORU UCHIDA, FUJIO TABUSA,* MAKOTO KOMATSU, SEIJI MORITA, TOSHIMI KANBE and KAZUYUKI NAKAGAWA

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

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The metabolites of 2-(4-chlorobenzoylamino)-3-[2(1H)-quinolinon-4-yl]propionic acid (OPC-12759) (1), which has a potent antiulcer activity towards acetic acid-induced gastric ulcer, were synthesized to confirm their structures and to examine their antiulcer activity. The structures of the major metabolites (2-4) in the rat were identified by means of comparisons with the synthetic compounds. The antiulcer activity of the metabolites (2-4) was found to be lower than that of 1.

Keywords—metabolite; 2-(4-chlorobenzoylamino)-3-[2(1H)-quinolinon-4-yl]propionic acid; antiulcer activity; 2-(4-chlorobenzoylamino)-3-[6-hydroxy-2(1H)-quinolinon-4-yl]propionic acid; 2-(4-chlorobenzoylamino)-3-[8-hydroxy-2(1H)-quinolinon-4-yl]propionic acid; 2-amino-3-[2(1H)-quinolinon-4-yl]propionic acid

In the previous paper,1 we described the synthesis and the antiulcer activity towards acetic acid-induced gastric ulcer, which is a model of chronic ulcer,2 of 2(1H)-quinolinone derivatives having an ω-amino acid moiety. After examination of the pharmacological properties of these compounds, 2-(4-chlorobenzoylamino)-3-[2(1H)-quinolinon-4-yl]propionic acid (OPC-12759) (1) was selected as the most promising compound, and is now under clinical trial. In metabolic studies,3 three major metabolites, OPC-12759 analogues (2 and 3) hydroxylated on the 2(1H)-quinolinone ring and 2-amino-3-[2(1H)-quinolinon-4-yl]propionic acid (4), were isolated from biological fluids of rats. In order to confirm the

![Fig. 1](image-url)
structures, three metabolites (2–4) were synthesized as described below, and tested for antulcer activity towards the acetic acid-induced gastric ulcer.

**Synthesis**

2-(4-Chlorobenzoylamino)-3-[8-hydroxy-2(1H)-quinolinon-4-yl]propionic acid (2) and 2-(4-chlorobenzoylamino)-3-[6-hydroxy-2(1H)-quinolinon-4-yl]propionic acid (3) were synthesized in the same manner. First, N-(o-bromoacetooctetyl)-2 or 4-methoxyaniline (5a or 5b) was cyclized using polyphosphoric acid (PPA) to 4-bromomethyl-(6 or 8)-methoxy-2(1H)-quinolinone (6a or 6b). In this cyclization, the use of conc. sulfuric acid was found to lower the yield of 6a or 6b. The 4-bromomethyl derivative (6a or 6b) was condensed with diethyl acetylamidomalate in the presence of sodium ethoxide to give the z-aminomalonic acid ester derivative (7a or 7b). Next, 7a or 7b was treated with 47% HBr, and 4-chlorobenzoated in EtOH to afford crude 2 or 3. The crude material thus obtained was esterified with thionyl chloride in EtOH, and purified by silica gel column chromatography. Finally, pure 2 or 3 was obtained by hydrolysis of the ester derivative with 5% KOH methanolic solution. The synthesis of 2-amino-3-[2(1H)-quinolinon-4-yl]-propionic acid (4) has already been reported by us.

The metabolites (2–4) from the biological fluids were identical with the corresponding synthetic compounds on the basis of mass spectra (MS) and high-performance liquid chromatography (HPLC) comparisons.

**Biological Results**

The antulcer activities of synthetic metabolites prepared above towards acetic acid-induced gastric ulcer in the rat were tested by the same method as described in a previous paper, and the results are summarized in Table I. All metabolites were found to have lower potency than 1. These results would suggest that the antulcer activity of OPC-12759 (1) in vivo arises from OPC-12759 itself, and not from the metabolites.
TABLE I. Antiulcer Activity of OPC-12759 and Their Metabolites

<table>
<thead>
<tr>
<th>Compd. No.</th>
<th>Activity (healing ratio, %)</th>
</tr>
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<tbody>
<tr>
<td>1</td>
<td>+ +  (38.5)</td>
</tr>
<tr>
<td>2</td>
<td>-   (6.4)</td>
</tr>
<tr>
<td>3</td>
<td>-   (6.1)</td>
</tr>
<tr>
<td>4</td>
<td>-   (-7.7)</td>
</tr>
</tbody>
</table>

a) Evaluation and healing ratio are defined in the previous report.\textsuperscript{11}

Experimental

Melting points were determined with a Yamato MP-21 apparatus and are uncorrected. Infrared (IR) spectra were recorded on a JASCO IR-810 spectrometer. Nuclear magnetic resonance (NMR) spectra were recorded on a Varian EM-390, JEOL JMN FX-200 or Brucker WH-400 spectrometer in CDCl$_3$ with tetramethylsilane as an internal standard or in d$_6$-DMSO with 3-(trimethylsilyl)propionic acid-d$_4$ as an internal standard. MS were obtained on a Varian MAT-312 instrument. 5a\textsuperscript{19} and 5b\textsuperscript{20} were prepared by reference to the known method.\textsuperscript{21}

Preparation of 4-Bromomethyl-8-methoxy-2(1H)-quinolinone (6a) — Compound 5a (13.6 g) was added to PPA prepared from phosphorus pentoxide (40 g) and phosphoric acid (40 ml) and the mixture was heated at 70–80 °C for 3 h. The reaction mixture was poured into ice-water, and the precipitate was collected by filtration and washed sufficiently with 5% NaHCO$_3$ and water. Recrystallization from EtOH–H$_2$O gave yellow needles (9.5 g, 75%), mp 223–224°C. NMR (CDCl$_3$) $\delta$: 3.92 (3H, s), 4.88 (2H, s), 6.77 (1H, s), 7.10–7.53 (3H, m), 8.67 (1H, brs); IR (KBr): 1645, 1605 cm$^{-1}$. Anal. Calcd for C$_{11}$H$_{10}$BrNO$_2$: C, 49.28; H, 3.76; N, 5.22. Found: C, 49.46; H, 3.68; N, 5.03.

Preparation of 4-Bromomethyl-6-methoxy-2(1H)-quinolinone (6b) — Compound 5b (6 g) was added to PPA prepared from phosphorus pentoxide (18 g) and phosphoric acid (18 ml), and the mixture was heated at 110–120 °C for 3 h. The reaction mixture was poured into ice-water, and the resulting precipitate was collected by filtration and washed sufficiently with 5% NaHCO$_3$ and water. Recrystallization from EtOH–H$_2$O gave pale yellow needles (2.7 g, 48%), mp 248–250.5°C (dec.). NMR (CDCl$_3$) $\delta$: 4.39 (3H, s), 5.49 (2H, s), 7.30 (1H, s), 7.57–7.86 (3H, m), 12.40 (1H, brs). IR (KBr): 1670, 1620 cm$^{-1}$. Anal. Calcd for C$_{11}$H$_{10}$BrNO$_2$: C, 49.28; H, 3.76; N, 5.22. Found: C, 49.31; H, 3.74; N, 5.28.

Preparation of Ethyl 2-Acetylamino-2-ethoxy-carbonyl-3-[8-methoxy-2(1H)-quinolinone-4-yl]propanoic acid (7a) — Sodium metal (0.7 g) was dissolved in EtOH (70 ml), and diethyl acetylamidomalonate (6.8 g) was added to the solution. Stirring was continued for 1 h, then 6a (7 g) was added, and the whole was refluxed for 3 h. The solvent was evaporated off, and the residue was dissolved in CH$_2$Cl$_2$. The CH$_2$Cl$_2$ layer was washed with water and sat. NaCl, and dried over Na$_2$SO$_4$. After evaporation of the solvent, the residue was chromatographed on a silica gel column (eluant; CH$_2$Cl$_2$ : MeOH = 50:1). Recrystallization from AcOEt–hexane gave a white powder (10.1 g, 96%), mp 101–105°C. NMR (CDCl$_3$) $\delta$: 1.30 (6H, t, $J = 7$ Hz), 1.94 (3H, s), 3.94 (2H, s), 4.18–4.35 (4H, m), 6.36 (1H, s), 6.76 (1H, s), 6.96 (1H, d, $J = 7.5$ Hz), 7.10 (1H, t, $J = 7.5$ Hz), 7.28 (1H, d, $J = 7.5$ Hz), 9.18 (1H, brs). IR (KBr): 1740, 1660, 1650 cm$^{-1}$. Anal. Calcd for C$_{28}$H$_{26}$N$_{2}$O$_{6}$: C, 55.68; H, 6.31; N, 6.49. Found: C, 55.89; H, 6.06; N, 6.56.

Preparation of Ethyl 2-Acetylamino-2-ethoxy-carbonyl-3-[6-methoxy-2(1H)-quinolinone-4-yl]propanoic acid (7b) — Compound 7b (2.65 g, 70%) was prepared by a synthetic procedure similar used for 7a with 60% NaH (0.4 g), diethyl acetylamidomalonate (2.2 g) and 6b (2.5 g). Colorless prisms from EtOH, mp 207–208.5°C. NMR (CDCl$_3$) $\delta$: 1.16 (6H, t, $J = 7$ Hz), 1.71 (3H, s), 3.57 (2H, s), 3.67 (3H, s), 4.05 (4H, q, $J = 7$ Hz), 6.00 (1H, s), 6.86–7.23 (3H, m), 8.13 (1H, s), 11.46 (1H, brs). IR (KBr): 1735, 1660, 1650 cm$^{-1}$. Anal. Calcd for C$_{28}$H$_{26}$N$_{2}$O$_{6}$: C, 59.40; H, 5.98; N, 6.93. Found: C, 58.92; H, 5.92; N, 6.86.

Preparation of 2-(4-Chlorobenzoyl)-3-[8-hydroxy-2(1H)-quinolinone-4-yl]propanoic acid (2) — Compound 7a (9 g) was added to 47% HBr (80 ml) and the mixture was refluxed for 10 h, then the solvent was evaporated off. The residue and K$_2$CO$_3$ (15.4 g) were dissolved in H$_2$O (180 ml) and EtOH (120 ml), and an acetone (25 ml) solution of 4-chlorobenzoyl chloride (11.7 g) was added to the above mixture under ice-water cooling. After being stirred for 2 h the reaction mixture was poured into water, and acidified with 10% HCl. The resulting precipitate was collected by filtration, washed with water and dried. This crude 2 was suspended in EtOH (30 ml) and thionyl chloride (1.5 ml) was added dropwise to the suspension. The mixture was refluxed for 2 h, then the solvent was evaporated off, and the residue was dissolved in CH$_2$Cl$_2$. The solution was washed with water and dried over Na$_2$SO$_4$. After evaporation of the solvent, the residue was chromatographed on a silica gel column (eluant; CH$_2$Cl$_2$ : MeOH = 50:1). Evaporation of the solvent gave the ester derivative of 2 (4.5 g, 49%). This ester derivative (2 g) was dissolved in 5% KOH methanolic solution (20 ml) and refluxed for 2 h. After evaporation of the MeOH, the residue was dissolved in water and acidified with 10% HCl. The precipitate was collected by filtration, dissolved in dil. NaOH and decolorized with active
charcoal. After removal of the active charcoal by filtration, the filtrate was acidified with 10% HCl. The precipitate was collected by filtration and washed with water. Recrystallization from dimethylformamide (DMF)-H₂O gave 2 (1.65 g, 89%) as a white powder, mp 328—329 °C. NMR (d₂-DMSO) δ: 3.12—3.57 (3H, m), 4.70—4.77 (1H, m), 6.45 (1H, s), 6.94—7.32 (3H, m), 7.55 (2H, d, J = 8.5 Hz), 7.82 (2H, d, J = 8.5 Hz), 8.91 (1H, d, J = 8.5 Hz), 10.35 (1H, br s), 13.06 (1H, br s). IR (KBr): 1715, 1660, 1650 cm⁻¹. Anal. Calcd for C₁₉H₁₅ClN₂O₂: C, 59.00; H, 3.91; N, 7.24. Found: C, 59.04; H, 3.66; N, 7.03.

Preparation of 2-(4-Chlorobenzoylamino)-3-[6-hydroxy-2(1H)-quinolin-4-yl]propionic Acid (3)—Compound 3 (0.9 g, 19%) was prepared by a synthetic procedure similar to that used for 2 with 7b (4 g), 47% HBr (50 ml), K₂CO₃ (4.8 g) and 4-chlorobenzoyl chloride (2.7 g). A pale brown powder from EtOH—H₂O, mp 315.5—318 °C (dec.). NMR (d₆-DMSO) δ: 3.00—3.50 (2H, m), 4.53—4.87 (1H, m), 6.37 (1H, s), 6.85—7.23 (3H, m), 7.44 (2H, d, J = 8.5 Hz), 7.77 (2H, d, J = 8.5 Hz), 8.83 (1H, br d, J = 8 Hz), 11.33 (1H, br s). IR (KBr): 1720, 1660 cm⁻¹. Anal. Calcd for C₁₉H₁₅ClN₂O₂·1/2 H₂O: C, 57.66; H, 4.07; N, 7.08. Found: C, 57.68; H, 3.95; N, 6.99.

Biological Method—Antiulcer activity was measured by the reported method,¹¹ against acetic acid-induced gastric ulcer in rats.

References