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Studies on 2(1*H*)-Quinolinone Derivatives as Gastric Antiulcer Active Agents. Synthesis and Antiulcer Activity of the Metabolites of 2-(4-Chlorobenzoylamino)-3-[2(1*H*)-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(1*H*)-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(1*H*)-quinolinon-4-yl]propionic acid; antiulcer activity; 2-(4-chlorobenzoylamino)-3-[6-hydroxy-2(1*H*)-quinolinon-4-yl]propionic acid; 2-(4-chlorobenzoylamino)-3-[8-hydroxy-2(1*H*)-quinolinon-4-yl]propionic acid; 2-amino-3-[2(1*H*)-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(1*H*)-quinolinone derivatives having an α -amino acid moiety. After examination of the pharmacological properties of these compounds, 2-(4-chlorobenzoylamino)-3-[2(1*H*)-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(1*H*)-quinolinone ring and 2-amino-3-[2(1*H*)-quinolinon-4-yl]-propionic acid (**4**), were isolated from biological fluids of rats. In order to confirm the

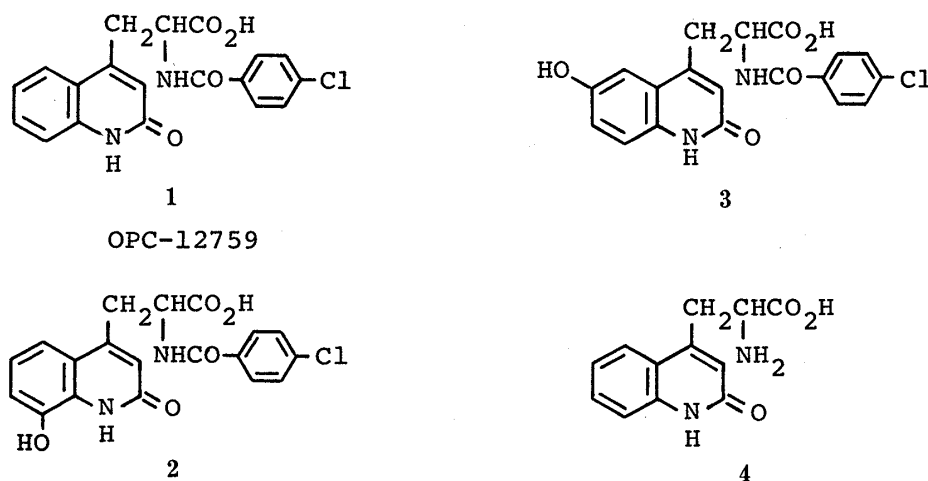


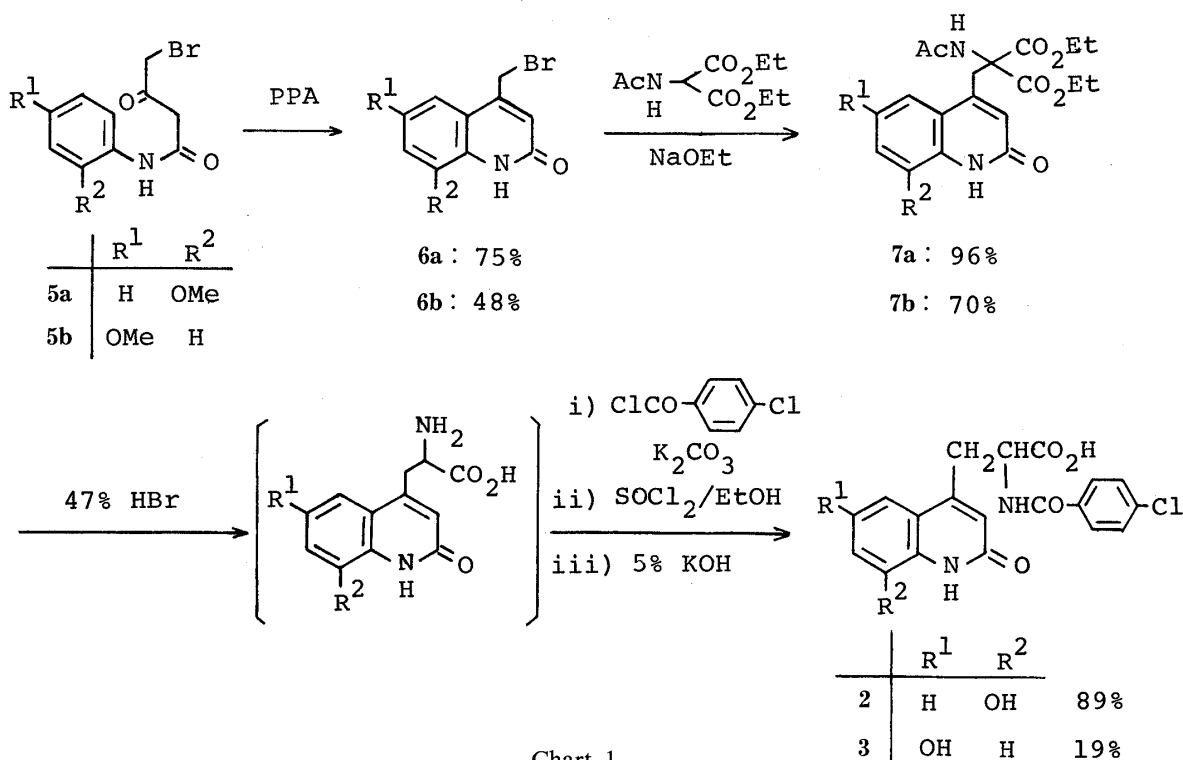
Fig. 1

structures, three metabolites (2—4) were synthesized as described below, and tested for antiulcer activity towards the acetic acid-induced gastric ulcer.

Synthesis

2-(4-Chlorobenzoylamino)-3-[8-hydroxy-2(1*H*)-quinolinon-4-yl]propionic acid (2) and 2-(4-chlorobenzoylamino)-3-[6-hydroxy-2(1*H*)-quinolinon-4-yl]propionic acid (3) were synthesized in the same manner. First, *N*-(*o*-bromoacetoacetyl)-2 or 4-methoxyaniline (5a or 5b)⁴⁾ was cyclized using polyphosphoric acid (PPA) to 4-bromomethyl-(6 or 8)-methoxy-2(1*H*)-quinolinone (6a or 6b). In this cyclization, the use of conc. sulfuric acid⁴⁾ was found to lower the yield of 6a or 6b. The 4-bromomethyl derivative (6a or 6b) was condensed with diethyl acetamidomalonate in the presence of sodium ethoxide to give the α -aminomalonate ester derivative (7a or 7b). Next, 7a or 7b was treated with 47% HBr, and 4-chlorobenzoylated 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(1*H*)-quinolinon-4-yl]-propionic acid (4) has already been reported by us.¹⁾

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 antiulcer 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,¹⁾ and the results are summarized in Table I. All metabolites were found to have lower potency than 1. These results would suggest that the antiulcer 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

Compd. No.	Activity (healing ratio, %) ^{a)}
1	++ (38.5)
2	— (6.4)
3	— (6.1)
4	— (−7.7)

a) Evaluation and healing ratio are defined in the previous report.¹⁾

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 Bruker WH-400 spectrometer in CDCl₃ with tetramethylsilane as an internal standard or in *d*₆-DMSO with 3-(trimethylsilyl)propionic acid-*d*₆ as an internal standard. MS were obtained on a Varian MAT-312 instrument. **5a**⁵⁾ and **5b**⁶⁾ were prepared by reference to the known method.⁴⁾

Preparation of 4-Bromomethyl-8-methoxy-2(1*H*)-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% NaHCO₃ and water. Recrystallization from EtOH–H₂O gave yellow needles (9.5 g, 75%), mp 223–224 °C. NMR (CDCl₃) δ: 3.92 (3H, s), 4.88 (2H, s), 6.77 (1H, s), 7.10–7.53 (3H, m), 8.67 (1H, br s); IR (KBr): 1645, 1605 cm^{−1}; *Anal.* Calcd for C₁₁H₁₀BrNO₂: 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(1*H*)-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% NaHCO₃ and water. Recrystallization from EtOH–H₂O gave pale yellow needles (2.7 g, 48%), mp 248–250.5 °C (dec.). NMR (CDCl₃) δ: 4.39 (3H, s), 5.49 (2H, s), 7.30 (1H, s), 7.57–7.86 (3H, m), 12.40 (1H, br s). IR (KBr): 1670, 1620 cm^{−1}. *Anal.* Calcd for C₁₁H₁₀BrNO₂: C, 49.28; H, 3.76; N, 5.22. Found: C, 49.31; H, 3.74; N, 5.28.

Preparation of Ethyl 2-Acetylmino-2-ethoxycarbonyl-3-[8-methoxy-2(1*H*)-quinolinon-4-yl]propionate (7a)—Sodium metal (0.7 g) was dissolved in EtOH (70 ml), and diethyl acetamidomalonate (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 CH₂Cl₂. The CH₂Cl₂ layer was washed with water and sat. NaCl, and dried over Na₂SO₄. After evaporation of the solvent, the residue was chromatographed on a silica gel column (eluant; CH₂Cl₂ : MeOH = 50 : 1). Recrystallization from AcOEt–hexane gave a white powder (10.1 g, 96%), mp 101–105 °C. NMR (CDCl₃) δ: 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, br s). IR (KBr): 1740, 1660, 1650 cm^{−1}. *Anal.* Calcd for C₂₀H₂₄N₂O₇·3/2 H₂O: C, 55.68; H, 6.31; N, 6.49. Found: C, 55.89; H, 6.06; N, 6.56.

Preparation of Ethyl 2-Acetylmino-2-ethoxycarbonyl-3-[6-methoxy-2(1*H*)-quinolinon-4-yl]propionate (7b)—Compound **7b** (2.65 g, 70%) was prepared by a synthetic procedure similar used for **7a** with 60% NaH (0.4 g), diethyl acetamidomalonate (2.2 g) and **6b** (2.5 g). Colorless prisms from EtOH, mp 207–208.5 °C. NMR (CDCl₃) δ: 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, br s). IR (KBr): 1735, 1660, 1650 cm^{−1}. *Anal.* Calcd for C₂₀H₂₄N₂O₇: C, 59.40; H, 5.98; N, 6.93. Found: C, 58.92; H, 5.92; N, 6.86.

Preparation of 2-(4-Chlorobenzoylamino)-3-[8-hydroxy-2(1*H*)-quinolinon-4-yl]propionic 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 K₂CO₃ (15.4 g) were dissolved in H₂O (180 ml) and EtOH (120 ml), and an acetone (25 ml) solution of 4-chlorobenzoyl chloride (11.7 g) was added to the above solution 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 CH₂Cl₂. The solution was washed with water and dried over Na₂SO₄. After evaporation of the solvent, the residue was chromatographed on a silica gel column (eluant; CH₂Cl₂ : MeOH = 50 : 1). Evaporation of the solvent gave the ester derivative of **2** (4.5 g, 49%). This ester derivative (**2**) 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 (*d*₆-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 C₁₉H₁₅ClN₂O₅: 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(1*H*)-quinolinon-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 (*d*₆-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 C₁₉H₁₅ClN₂O₅ · 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,¹⁾ against acetic acid-induced gastric ulcer in rats.

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