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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

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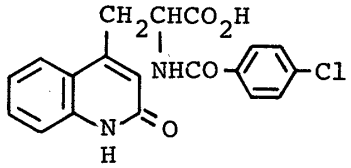
The metabolites of 2-(4-chlorobenzoylamino)-3-[2(1*H*)-quinolinon-4-yl]propionic acid (OPC-12759) (**1**), which has a potent antiulcer activity towards acetic acid-induced gastric ulcer, were synthesized to confirm their structures and to examine their antiulcer activity. The structures of the major 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



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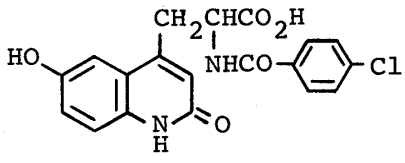
OPC-12759



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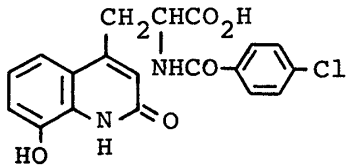

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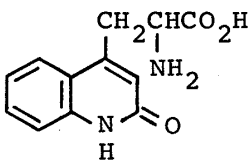

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Fig. 1

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*-(ω-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 acid[4] was found to lower the yield of **6a** or **6b**. The 4-bromomethyl derivative (**6a** or **6b**) was condenced with diethyl acetamidomalonate in the presence of sodium ethoxide to give the α-aminomalonic acid 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.

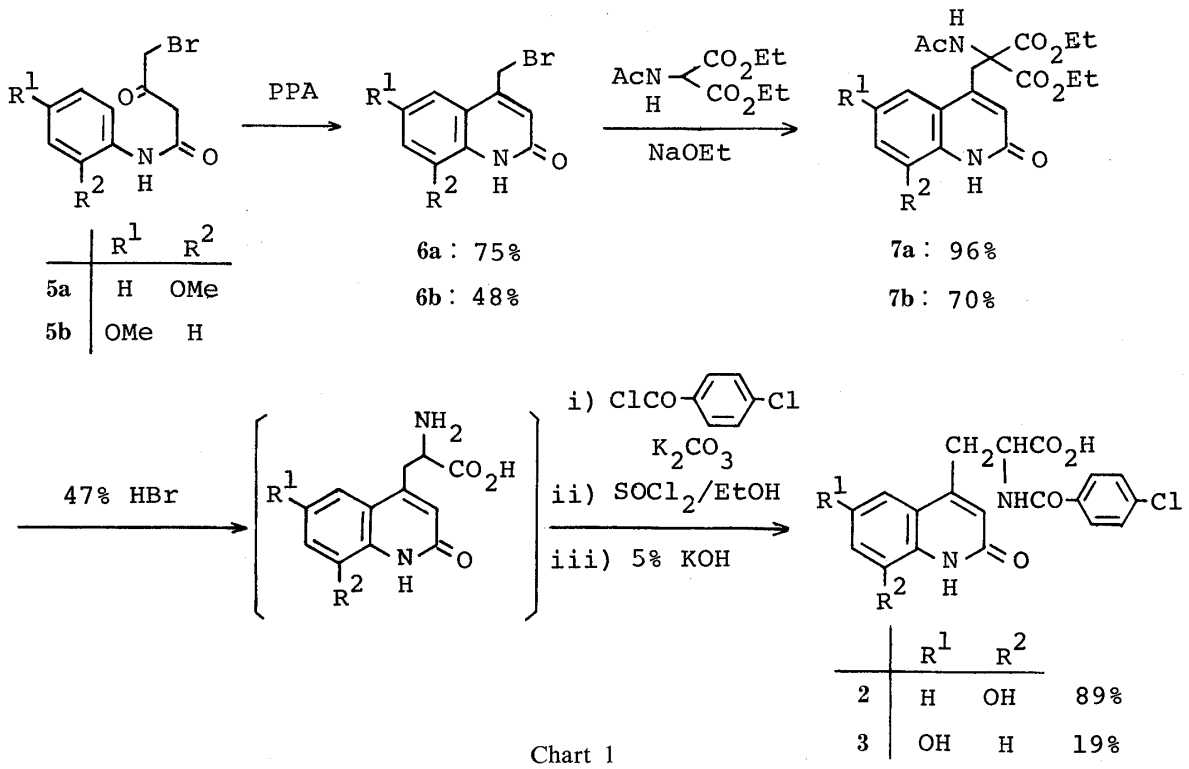

Chart 1

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 Brucker WH-400 spectrometer in $CDCl_3$ 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% $NaHCO_3$ and water. Recrystallization from EtOH–H_2O gave yellow needles (9.5 g, 75%), mp 223—224 °C. NMR ($CDCl_3$) 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 $C_{11}H_{10}BrNO_2$: 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(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% $NaHCO_3$ and water. Recrystallization from EtOH–H_2O gave pale yellow needles (2.7 g, 48%), mp 248—250.5 °C (dec.). NMR ($CDCl_3$) 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 $C_{11}H_{10}BrNO_2$: 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-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 CH_2Cl_2. The CH_2Cl_2 layer was washed with water and sat. NaCl, and dried over Na_2SO_4. After evaporation of the solvent, the residue was chromatographed on a silica gel column (eluant; CH_2Cl_2 : MeOH = 50 : 1). Recrystallization from AcOEt–hexane gave a white powder (10.1 g, 96%), mp 101—105 °C. NMR ($CDCl_3$) 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 $C_{20}H_{24}N_2O_7 \cdot 3/2\ H_2O$: 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-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 ($CDCl_3$) 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 $C_{20}H_{24}N_2O_7$: 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 K_2CO_3 (15.4 g) were dissolved in H_2O (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 CH_2Cl_2. The solution was washed with water and dried over Na_2SO_4. After evaporation of the solvent, the residue was chromatographed on a silica gel column (eluant; CH_2Cl_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)–H_2O 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 $C_{19}H_{15}ClN_2O_5$: 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), K_2CO_3 (4.8 g) and 4-chlorobenzoyl chloride (2.7 g). A pale brown powder from EtOH–H_2O, 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 $C_{19}H_{15}ClN_2O_5 \cdot 1/2\ H_2O$: 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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