SYNTHESIS AND PHARMACOLOGICAL ACTIVITY OF METABOLITES OF VASOPRESSIN V<sub>1</sub> RECEPTOR ANTAGONIST, OPC-21268

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Abstract - The metabolites of 1-[1-[4-(3-acetylaminopropoxy)benzoyl]-4-piperid-yl]-3,4-dihydro-2(1H)-quinolinone (OPC-21268, 1), vasopressin V<sub>1</sub> receptor antagonist were synthesized to confirm the proposed structures and to examine their vasopressin V<sub>1</sub> receptor antagonistic activity. The structures of metabolites (2a - 6) were identified by means of comparison with synthetic compounds. The activity of the metabolites was found to be lower than that of 1.

A new vasopressin V<sub>1</sub> receptor antagonist, OPC-21268 (1) (1-[1-[4-(3-acetylaminopropoxy)benzoyl]-4-piperidyl]-3,4-dihydro-2(1H)-quinolinone) was synthesized by Ogawa et al.<sup>1,2</sup> and is now under clinical trial. This compound is an orally effective, nonpeptide antagonist for the arginine vasopressin (AVP) receptor and turned out to be a selective V<sub>1</sub> antagonist. Metabolism studies are an integral part of all
programs of new drug development. In metabolic studies of 1, six metabolites were isolated from dog urine (Figure 1). The metabolites (2a and 2b) were proposed on the basis of ms and nmr spectral analysis to be unstable N-acetylhemiaminals. The structure of the metabolites (3 and 4a) were indicated to be the degradation product and the dehydro OPC-21268, respectively. The structure of the metabolites (5 and 6) were proposed to be the compounds hydroxylated at the 4-position on the 2(1H)-quinolinone ring. Among these metabolites, the products having newly chiral carbon formed by the hydroxylation of 1 were included. The selective or specific production of chiral metabolites from prochiral xenobiotics is of interest in study of the metabolism. In a communication, we reported the synthesis of N-acetylhemiaminal metabolites (2a and b). In this paper we describe the synthesis and pharmacological activity of OPC-21268 metabolites.

Figure 1

SYNTHESIS

Initially, N-acetylhemiaminal metabolites (2a and b) were synthesized by the pathway shown in Scheme 1.

Alkylation of phenol derivative (4a) with ethyl chloroacetate in the presence of NaH afforded the ester (7a)
in quantitative yield. Reduction of 7a with LAH gave the alcohol (8a) in 88% yield. The alcohol (8a) was treated with MsCl in pyridine to give the mesylate (9a), which was converted to the iodide (10a) by replacement with NaI in 65% yield. Condensation of the iodide (10a) with diethyl acetamidomalonate in the

Scheme 1

\[ \text{4a, b} \xrightarrow{\text{NaH, } C\text{ICH}_2\text{CO}_2\text{Et}} \text{7a, b} \xrightarrow{\text{LAH}} \text{8a, b} \]

\[ \xrightarrow{\text{MsCl, Pyridine}} \text{9a, b} \xrightarrow{\text{NaI}} \text{10a, b} \]

\[ \xrightarrow{\text{i) NaOH, ii) HCl, iii) benzene}} \text{11a, b} \xrightarrow{\text{NaOH}} \text{12a, b} \]

\[ \xrightarrow{\text{NaOH}} \text{13a, b} \xrightarrow{\text{Pb(OAc)}_4} \text{2a, b} \]

\( \text{a: Bond of C(3) and C(4), single} \)
\( \text{b: Bond of C(3) and C(4), double} \)
presence of NaH afforded the amido derivative (11a) in 97% yield, which was hydrolyzed with NaOH, followed by decarboxylation in benzene to give the amino acid ethyl ester (12a) in 76% yield. Hydrolysis of the ester (12a) with NaOH gave the N-acetylated amino acid (13a) in 55% yield. Target compound (2a, 18%) was obtained by treatment of 13a with lead tetraacetate\(^4\) in dry DMF. Another N-acetylhemiaminal metabolite (2b) was obtained in 14% yield similarly from phenol derivative (4b),\(^4\) via 12b, which was hydrolyzed with NaOH, followed by oxidative decarboxylation with lead tetraacetate.

Next, we planned the synthesis of 3,4-dihydro-4-hydroxy-2(1H)-quinolinone metabolites (5 and 6). The 3,4-dihydro-4-hydroxy-2(1H)-quinolinones have usually been prepared by reduction of ethyl 3-(2-nitrophenyl)-3-hydroxypropionate with ferrous sulfate in 28% ammonia solution - EtOH.\(^6\) However, this method can not be applied to the metabolites (5 and 6) which have the substituent at 1-position on 2(1H)-quinolinone ring. So we attempted to cyclize 17a, b as shown in Scheme 2. Reduction of the imine from Scheme 2
4-piperidone (14a) and 2-aminobenzyl alcohol, using NaBH₃CN gave the aniline (15a), which was converted to the formyl compound (16a) by oxidation with manganese oxide in 18% yield. Treatment of 16a with chloroacetic anhydride afforded the chloroacetyl compound (17a) in 73% yield. Cyclization of 17a with SmI₂ gave the 4-hydroxy-2(1H)-quinolinone derivative (18a) in 18% yield. Hydrolysis of 18a with NaOH gave 4-hydroxy-2(1H)-quinolinone metabolite (5) in 75% yield. Another metabolite (6) was synthesized by a similar manner as described for 5. Compounds (3 and 4a) were prepared according to the reported methods.² ⁵

DISCUSSION

The structures of the metabolites (2a, 2b, 3, 4a, 5 and 6) were identical with the corresponding synthetic compounds. It is now well understood that the processes of absorption, distribution, metabolism and excretion of xenobiotics may all exhibit stereo-selectivity or specificity, and this is especially so with enzymic metabolic transformations. Most such instances involve the selective or specific production of chiral metabolites from prochiral xenobiotics. In previous paper,³ we reported that OPC-21268 (1) was not preferentially metabolized to one of the possible enantiomers of the hemiaminal metabolite (2a) in beagle dogs. The synthetic 4-hydroxy-2(1H)-quinolinone metabolite (5) was analyzed by hplc using a chiral stationary phase column. The chromatogram showed two peaks and the retention times were 10.4 min and 12.5 min. After OPC-21268 (1) was administered orally to beagle dogs, the isolated urinary metabolite (5) was subjected to hplc analysis under the same condition. It was found that the urinary metabolite showed two peaks with almost the same area intensity as the synthetic sample did. The reason for these non-stereoselective biological hydroxylation of 1 to give racemic 2a and 5 is unclear.

PHARMACOLOGICAL ACTIVITY

Vasopressin V₁ receptor antagonistic activity of metabolites was tested by the same method as described in a previous paper.¹ ² The results showed that 2a was a little less active and 2b, 3 and 4a were less active than
the mother compound (1). As regards the quinolone skeleton, introduction of a 4-hydroxy group (5 and 6) caused a marked decrease in activity.

Table 1  Effects of metabolites on AVP V1 receptor binding affinity

<table>
<thead>
<tr>
<th>Compd.No.</th>
<th>1</th>
<th>2a</th>
<th>2b</th>
<th>3</th>
<th>4a</th>
<th>5</th>
<th>6</th>
</tr>
</thead>
<tbody>
<tr>
<td>Receptor affinity&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.44</td>
<td>0.6</td>
<td>3.3</td>
<td>1.2</td>
<td>1.3</td>
<td>&gt;100</td>
<td>&gt;100</td>
</tr>
<tr>
<td>IC50 (µM)</td>
<td></td>
<td></td>
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</tbody>
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<sup>c</sup> Compounds were tested for their ability to displace [3H]AVP from its specific binding sites in rat liver (V1 receptor) plasma membrane preparations (see ref. 1).

EXPERIMENTAL

Melting points were determined with a Yamato mp-21 apparatus and are uncorrected. Nuclear magnetic resonance (nmr) spectra were recorded in CDCl3 on a Bruker AC-200 spectrometer with tetramethylsilane as an internal standard.

**Ethyl 2-[[4-[4-[3,4-Dihydro-2-oxo-1H-quinolin-1-yl]-1-piperidyl]carbonyl]phenoxy]acetate (7a)**

Sodium hydride (60 % in oil, 1.35 g, 33.8 mmol) was added to a stirred and ice-cooled solution of 3,4-dihydro-1-[1-(4-hydroxybenzoyl)-4-piperidyl]-2(1H)-quinolinone (4a, 10 g, 28.5 mmol) in DMF (100 ml). After the mixture was stirred for 10 min, ethyl 2-chloroacetate (4.14 g, 33.8 mmol) was added to the solution, and the reaction mixture was stirred at 60 °C for 1 h. The mixture was poured into water and extracted with ethyl acetate. The combined organic phases were dried over MgSO4 and concentrated in vacuo to give 7a (13 g, quant). The residue was used in the next step without purification. Nmr δ: 1.25 (3 H, t, J = 7.2 Hz), 1.84 (2 H, m), 2.04 (3 H, s), 2.53 - 2.83 (5 H, m), 2.90 - 3.10 (3 H, m), 4.28 (2H, t, J = 7.2 Hz), 4.37 (3 H, m), 4.64 (2 H, s), 6.92 (2 H, d, J = 8.6 Hz), 6.99 - 7.27 (4 H, m), 7.44 (2 H, d, J = 8.6 Hz).
3,4-Dihydro-1-[1-[4-[2-(hydroxy)ethoxy]benzoyl]-4-piperidyl]-2(1H)-quinolinone (8a)
Lithium aluminum hydride (2.16 g, 57 mmol) was added to a stirred and ice-cooled solution of 7a (12.4 g, 28.5 mmol) in THF (100 ml). The reaction mixture was stirred at 0 - 10 °C for 2 h and at room temperature for 1 h. Water was carefully added to destroy the excess LiAlH₄. The mixture was poured into water and extracted with CH₂Cl₂. The combined organic phases were dried over MgSO₄ and concentrated in vacuo. The residue was purified by column chromatography (silica gel, eluent; CH₂Cl₂ : MeOH = 4:1) to give 8a (4.6 g, 41 %) as colorless oil. Nmr δ: 1.70 - 2.00 (2 H, m), 2.53 - 2.86 (4 H, m), 2.80 - 3.30 (4 H, m), 3.96 (2 H, t, J = 4.8 Hz), 4.07 (2 H, t, J = 4.8 Hz), 4.37 (3 H, m), 6.92 (2 H, d, J = 8.6 Hz), 6.99 - 7.28 (4 H, m), 7.43 (2 H, d, J = 8.6 Hz).

3,4-Dihydro-1-[1-[4-[2-(methanesulfonyloxy)ethoxy]benzoyl]-4-piperidyl]-2(1H)-quinolinone (9a)
To a stirred solution of 8a (4.5 g, 11.4 mmol) and pyridine (5.4 g, 68.4 mmol) in CHCl₃ (50 ml) was added MeSO₂Cl (3.52 ml, 45.6 mmol) and the mixture was stirred for 25 h at room temperature. The reaction mixture was poured into 10 % HCl and extracted with CH₂Cl₂. The CH₂Cl₂ layers were dried over MgSO₄ and concentrated in vacuo. The residue was purified by column chromatography (silica gel, eluent; CH₂Cl₂ : MeOH = 30:1) to give 9a (5.26 g, 97 %) as colorless oil. Nmr δ: 1.70 - 2.10 (2 H, m), 2.53 - 2.87 (6 H, m), 2.90-3.20 (2 H, m), 3.09 (3 H, s), 4.27 (2 H, dd, J = 4.6, 6.2 Hz), 4.36 (3 H, m) 4.58 (2 H, dd, J = 4.6, 6.2 Hz), 6.93 (2 H, d, J = 8.8 Hz), 6.99 - 7.28 (4 H, m), 7.45 (2 H, d, J = 8.8 Hz).

3,4-Dihydro-1-[1-[4-[2-(iodo)ethoxy]benzoyl]-4-piperidyl]-2(1H)-quinolinone (10a)
A mixture of 9a (21.2 g, 44.9 mmol) and NaI (13.5 g, 89.8 mmol) in acetone (250 ml) was refluxed for 10 h. After evaporation of acetone, the residue was dissolved in CHCl₃. The CHCl₃ solution was washed with water and saturated NaCl solution, dried over MgSO₄ and concentrated in vacuo. The residue was purified
by column chromatography (silica gel, eluent; CH₂Cl₂ : MeOH = 50 : 1) to give 10a (18.3 g, 81 %) as white powder (from EtOH), mp 157 - 159 °C. Nmr δ : 1.60 - 2.00 (2 H, m), 2.54 - 2.87 (6 H, m), 2.90 - 3.10 (2 H, m), 3.42 (2 H, t, J = 6.6 Hz), 4.28 (2 H, t, J = 6.6 Hz), 4.38 (3 H, m), 6.92 (2 H, d, J = 8.8 Hz), 6.99 - 7.27 (4 H, m), 7.44 (2 H, d, J = 8.8 Hz). Anal. Calcd for C₂₃H₂₅N₂O₄I : C, 54.77; H, 5.00; N, 5.55. Found : C, 54.96; H, 5.03; N, 5.40.

Ethyl 2-Acetylamino-2-ethoxycarbonyl-4-[4-[4-(3,4-dihydro-2-oxo-1H-quinolin-1-yl)-1-piperidylcarbonyl]phenoxyl]butyrate (11a)

Sodium hydride (60 % in oil, 1.1 g, 27.4 mmol) was added to a solution of diethyl acetamidomalonate (4.96 g, 22.8 mmol) in DMF (200 ml) and the mixture was stirred at room temperature for 20 min. Then, 10a (9.6 g, 19 mmol) was added at room temperature and the reaction mixture was stirred at 60 - 70 °C for 1.5 h. After removal of the solvent, the residue was poured into water and extracted with CHCl₃. The CHCl₃ layer was dried over MgSO₄ and concentrated in vacuo. The residue was purified by column chromatography (silica gel, eluent; CH₂Cl₂ : MeOH = 50 : 1) to give 11a (7.7 g, 68 %) as amorphous. Nmr δ: 1.26 (6 H, t, J = 7.2 Hz), 1.70 - 1.90 (2 H, br s), 2.04 (3 H, s), 2.54 - 2.93 (8 H, m), 4.01 (2 H, t, J = 5.4 Hz), 4.14 - 4.90 (6 H, m), 6.80 (2 H, d, J = 8.6 Hz), 6.92 - 7.27 (4 H, m), 7.41 (2 H, d, J = 8.6 Hz). Ms m/z (%): 593 (M⁺, 2), 244 (85), 82 (100). Anal. Calcd for C₃₂H₃₅N₃O₄·1/2 H₂O : C, 63.77; H, 6.69; N, 6.97. Found : C, 63.79; H, 7.01; N, 6.79.

Ethyl 2-Acetylamino-4-[4-[4-(3,4-dihydro-2-oxo-1H-quinolin-1-yl)-1-piperidyl]-carbonyl]phenoxyl]butyrate (12a)

To a stirred solution of 11a (2.3 g, 3.9 mmol) in EtOH (30 ml) was added 1N NaOH solution (15.7 ml, 15.5 mmol) and the mixture was stirred at room temperature for 2 h. Then, the reaction mixture was adjusted with 1N HCl until pH 3 - 4. After removal of the solvent, the residue was poured into water and extracted with CHCl₃. The CHCl₃ solution was dried over MgSO₄ and concentrated in vacuo. The residue
was dissolved in benzene (80 ml) and the reaction mixture was refluxed for 2 h. After evaporation of benzene, the residue was purified by column chromatography (silica gel, eluent; CH$_2$Cl$_2$ : MeOH = 50 : 1). Recrystallization from AcOEt-hexane gave 12a (1.0 g, 50 %) as white granules, mp 158 - 160 °C. Nmr δ : 1.27 (3 H, t, J = 7.2 Hz), 1.80 - 2.00 (2 H, m), 2.03 (3 H, s), 2.28 - 2.50 (2 H, m), 2.54 - 2.87 (4 H, m), 2.90 - 3.20 (4 H, m), 4.06 (2 H, t, J = 5.8 Hz), 4.21 (2 H, m), 4.38 (3 H, m), 4.75 (1 H, q, J = 7.2 Hz), 6.33 (1 H, d, J = 7.4 Hz), 6.86 (2 H, d, J = 8.8 Hz), 6.99 - 7.28 (4 H, m), 7.43 (2 H, d, J = 8.8 Hz). Ms m/z (%) : 521 (M+, 5), 172 (65), 121 (25), 82 (100). Anal. Calcd for C$_2$H$_3$N$_3$O$_6$ : C, 66.78; H, 6.76; N, 8.06. Found : C, 66.68; H, 6.80; N, 7.97.

2-Acetylamino-4-[[4-[4-(3,4-dihydro-2-oxo-1H-quinolin-1-yl)-1-piperidyl]carbonyl]-phenoxy]butyric Acid (13a)

To a stirred solution of 12a (3.3 g, 6.3 mmol) in EtOH (75 ml) was added 1 N NaOH solution (13.5 ml, 12.7 mmol) at room temperature and the mixture was stirred for 2 h. The reaction mixture was poured into water, adjusted to pH 3 with 1 N HCl, and extracted with CH$_2$Cl$_2$. The combined organic phases were dried over MgSO$_4$ and concentrated in vacuo. The residue was dissolved in benzene (80 ml) and the mixture was refluxed for 2 h. After evaporation of benzene, the residue was purified by column chromatography (silica gel, eluent; CH$_2$Cl$_2$ : MeOH = 30 : 1 to 10 : 1) and recrystallized from Et$_2$O - AcOEt to give 13a (2.8 g, 90 %) as white granules, mp 114 - 116 °C. Nmr δ : 1.82 (2 H, m), 2.02 (3 H, s), 2.32 (2 H, m), 2.57 (2 H, m), 2.60 - 3.20 (6 H, m), 4.02 (2 H, m), 4.33 (3 H, m), 4.66 (1 H, m), 6.83 (2 H, d, J = 8.7 Hz), 7.00 - 7.30 (5 H, m), 7.40 (2 H, d, J = 8.7 Hz). Ms m/z (%) : 493 (M+, 1), 350 (10), 203 (10), 121 (43), 82 (100). Anal. Calcd for C$_2$H$_3$N$_3$O$_6$: 1/2 H$_2$O : C, 64.53; H, 6.42; N, 8.36. Found : C, 64.40; H, 6.42; N, 8.35.

1-[1-[4-[3-(Acetylamino-3-hydroxy)propoxy]benzoyl]-4-piperidyl]-3,4-dihydro-2(1H)-
quinolinone (2a)

A solution of lead tetraacetate (0.41 g, 0.93 mmol) in DMF (3 ml) was added dropwise to a stirred and ice-cooled solution of 13a (0.4 g, 0.81 mmol) in DMF (3 ml). The mixture was stirred at the same temperature for 30 min and at room temperature for 1.5 h. Saturated NaHCO₃ solution (9 ml) was added to the reaction mixture and the solution was extracted with AcOEt. The combined organic phases were dried over MgSO₄ and concentrated in vacuo. The residue was purified by column chromatography and triturated in Et₂O - hexane to give 2a (15 mg, 4%) as white amorphous. Nmr δ : 1.65 - 1.90 (2 H, br s), 2.00 (3 H, s), 2.12 (2 H, q, J = 5.8 Hz), 2.50 - 3.10 (8 H, m), 4.00 - 5.00 (5 H, m), 5.56 (1 H, q, J = 6.4 Hz), 6.80 (1 H, d, J = 8.6 Hz), 6.91 (2 H, d, J = 8.6 Hz), 7.03 - 7.30 (4 H, m), 7.44 (2H, d, J = 8.6 Hz). Fab-ms (pos.) m/z : 466 [M-H]⁺. Anal. Calcd for C₂₆H₂₃N₃O₃·H₂O: C, 64.58; H, 6.88; N, 8.69. Found : C, 64.34; H, 6.61; N, 8.17.

Compounds (7b - 13b and 2b) were obtained by the same procedure as described for 7a - 13a and 2a.

8b : Yield 81%, colorless oil, nmr δ : 1.70 - 2.00 (2 H, m), 2.15 (1 H, m), 2.80 - 3.40 (4 H, m), 4.00 - 4.80 (3 H, m), 3.99 (2 H, t, J = 4.2 Hz), 4.12 (2 H, t, J = 4.2 Hz), 6.65 (1 H, d, J = 9.4 Hz), 6.95 (2 H, d, J = 8.8 Hz), 7.23 (1 H, t, J = 6.0 Hz), 7.46 - 7.66 (6 H, m).

9b : Yield 91%, colorless oil, nmr δ : 1.80 - 2.00 (2 H, m), 2.70 - 3.30 (4 H, m), 3.10 (3 H, s), 4.00 - 5.00 (3 H, m), 4.28 (2 H, dd, J = 2.6, 4.4 Hz), 4.58 (2 H, dd, J = 2.6, 4.4 Hz), 6.65 (1 H, d, J = 9.4 Hz), 6.94 (2 H, d, J = 8.8 Hz), 7.23 (1 H, t, J = 8.6 Hz), 7.47 - 7.66 (6 H, m).

10b : Yield 98%, colorless oil, nmr δ : 1.70 - 2.00 (2 H, m), 2.70 - 3.30 (4 H, m), 3.43 (2 H, t, J = 6.8 Hz), 4.00 - 5.30 (3 H, m), 4.28 (2 H, t, J = 6.8 Hz), 6.65 (1 H, d, J = 9.4 Hz), 6.94 (2 H, d, J = 8.8 Hz), 7.23 (1 H, t, J = 8.2 Hz), 7.46 - 7.66 (6 H, m).

11b : Yield 58%, pale yellow oil, nmr δ : 1.26 (6 H, t, J = 7.0 Hz), 1.70 - 1.95 (2 H, br s), 2.04 (3 H, s), 2.55 - 3.30 (6 H, m), 4.02 (2 H, t, J = 5.4 Hz), 4.14 - 5.30 (7 H, m), 6.65 (1 H, d, J = 9.4 Hz), 6.82 (2 H, d, J = 8.6 Hz), 6.91 (1 H, s), 7.23 (1 H, t, J = 8.4 Hz), 7.45 (2 H, d, J = 8.6 Hz), 7.54 - 7.66 (4 H, m).
**12b**: Yield 57 %, colorless oil, nmr δ: 1.27 (3 H, t, J = 7.2 Hz), 1.70 - 1.95 (2 H, br s), 2.03 (3 H, s), 2.20 - 2.50 (2 H, m), 2.65 - 3.30 (4 H, br s), 4.07 (2 H, t, J = 5.8 Hz), 4.16 - 4.28 (2 H, m), 4.29 - 5.20 (4 H, m), 6.31 (1 H, d, J = 7.2 Hz), 6.65 (1 H, d, J = 9.4 Hz), 6.88 (2 H, d, J = 8.6 Hz), 7.23 (1 H, t, J = 8.6 Hz), 7.46 (2 H, d, J = 8.6 Hz), 7.54 - 7.66 (4 H, m).

**13b**: Yield quant., pale yellow powder (from CH₂Cl₂ - hexane), mp 137 - 139 °C, nmr δ: 1.70 - 1.95 (2 H, m), 2.02 (3 H, s), 2.29 - 2.42 (2 H, m), 2.70 - 3.30 (4 H, m), 4.04 - 4.16 (2 H, m), 4.20 - 4.60 (3 H, m), 4.71 (1 H, q, J = 6.9 Hz), 6.67 (1 H, d, J = 9.4 Hz), 6.77 (1 H, d, J = 7.1 Hz), 6.85 (2 H, d, J = 8.6 Hz), 7.24 (1 H, t, J = 7.7 Hz), 7.43 (1 H, d, J = 8.6 Hz), 7.56 - 7.67 (4 H, m).

Anal. Calcd for C₂₇H₃₉N₃O₆·1/₂H₂O: C, 64.79; H, 6.04; N, 8.39. Found: C, 64.64; H, 6.01; N, 8.11.

**2b**: Yield 14 %, white amorphous, nmr δ: 1.70 - 1.90 (2 H, br s), 2.01 (3 H, s), 2.13 (2 H, q, J = 5.6 Hz), 2.30 - 3.70 (6 H, m), 3.90 (1 H, br s), 4.10 - 4.40 (2 H, br s), 4.40 - 5.20 (3 H, m), 5.57 (1 H, m), 6.64 (1 H, d, J = 9.4 Hz), 6.75 (1 H, br s), 6.93 (2 H, d, J = 8.8 Hz), 7.23 (1 H, m), 7.45 - 7.70 (6 H, m). Anal. Calcd for C₂₆H₂₉N₃O₅·1.25 H₂O: C, 64.25; H, 6.53; N, 8.65. Found: C, 64.24; H, 6.17; N, 8.57.

**1-[(4-Acetoxy)benzoyl]-4-piperidone (14a)**

To a suspension of 4-acetoxybenzoic acid (50 g, 277.5 mmol) in 1,2-dichloroethane (500 ml) was added SOCl₂ (40.5 ml, 555 mmol) and the mixture was dissolved in acetone (250 ml). The acetone solution was added dropwise to a stirred and ice-cooled suspension of 4-piperidone hydrochloride (38.4 g, 250 mmol) and K₂CO₃ (121 g, 875.5 mmol) in acetone (250 ml) and the mixture was stirred at the same temperature for 1.5 h. The reaction mixture was adjusted to pH 2 with 10 % HCl and extracted with CH₂Cl₂. The combined organic phases were dried over MgSO₄ and concentrated in vacuo. The residue was purified by column chromatography (CH₂Cl₂ : MeOH = 100 : 1) and recrystallized from AcOEt - hexane to give 14a (63.5 g, 87 %) as white powder, mp 102 - 103 °C. Nmr δ: 2.32 (3 H, s), 2.40 - 2.70 (4 H, m), 3.70 - 4.10 (4 H, m), 7.18 (2 H, d, J = 8.6 Hz), 7.51 (2 H, d, J = 8.6 Hz). Anal. Calcd for C₁₄H₁₃NO₄: C,
A mixture of 14a (58.3 g, 223 mmol), o-aminobenzyl alcohol (27.5 g, 223 mmol) and p-toluenesulfonic acid (5.0 g, 26.3 mmol) in toluene (1.5 l) was refluxed on Dean-Stark for 5 h. After removal of toluene, the residue was dissolved in MeOH (800 ml) and AcOH (100 ml). To the stirred and ice-cooled solution was added sodium cyanoborohydride (28.2 g, 449 mmol) and the reaction mixture was stirred at room temperature for 20 h. After removal of MeOH, the residue was poured into water and extracted with CH₂Cl₂. The combined organic phases were dried over MgSO₄ and concentrated in vacuo to give 15a (40.5 g, 49%) as pale brown oil. The residue was used in the next step without purification. Nmr δ: 1.40 - 1.70 (2 H, m), 2.00 - 2.25 (2 H, m), 2.31 (3 H, s), 3.10 - 3.30 (2 H, m), 3.50 - 4.20 (2 H, m), 4.30 - 4.60 (1 H, br s), 4.62 (2 H, s), 6.65 (2 H, t, J= 8.0 Hz), 7.02 - 7.26 (4 H, m), 7.42 (2 H, d, J= 8.6 Hz).

1-[(4-Acetoxy)benzoyl]-4-[(2-formyl)anilino]piperidine (16a)

A mixture of 15a (39.0 g, 106 mmol) and manganese oxide (47 g, 539 mmol) in CHCl₃ (500 ml) was refluxed for 20 h. To the reaction mixture was added water and MnO₂ was removed by filtration. The CHCl₃ layers was separated, dried over MgSO₄ and concentrated in vacuo. The residue was purified by column chromatography (silica gel, eluent; CH₂Cl₂ : AcOEt = 30 : 1) to give 16a (14.0 g, 36%) as pale brown oil. Nmr δ: 1.50 - 1.80 (2 H, m), 2.00 - 2.20 (2 H, m), 2.30 (3 H, s), 3.10 - 3.30 (2 H, m), 3.50 - 4.20 (2 H, m), 4.30 - 4.60 (1 H, br s), 4.62 (2 H, s), 6.65 (2 H, t, J= 7.8 Hz), 7.14 (2 H, d, J= 8.5 Hz), 7.29 - 7.48 (4 H, m), 8.47 (1 H, d, J= 7.5 Hz), 9.80 (1 H, s). Ms m/z (%): 366 (M⁺, 11), 163 (27), 160 (16), 121 (100).

1-[(4-Acetoxy)benzoyl]-4-[N-(chloroacetyl)-2-formylanilino]piperidine (17a)

A solution of 16a (10.8 g, 29.5 mmol) and chloroacetic anhydride (7.5 g, 44.2 mmol) in acetone (10 ml)
was refluxed for 10 h and the mixture was stirred at room temperature for 15 h. After removal of the solvent, the residue was purified by column chromatography (silica gel, eluent; CH₂Cl₂ : AcOEt = 7 : 1) to give 17a (9.5 g, 73 %) as brown oil. Nmr δ: 0.90 - 1.20 (1 H, m), 1.30 - 1.60 (1 H, m), 1.70 - 2.20 (2 H, m), 2.30 (3 H, s), 2.80 - 3.30 (2 H, m), 3.68 (2 H, d, J = 1.6 Hz), 3.75 - 4.00 (1 H, m), 4.75 - 5.00 (2 H, m), 7.07 - 7.35 (5 H, m), 7.72 - 7.80 (2 H, m), 8.03 (1 H, dd, J = 7.2, 1.8 Hz), 10.16 (1 H, s). Ms m/z (%): 443 (M⁺, 9), 245 (46), 163 (35), 121 (100).

1-(1-[(4-Acetoxv)benzoyl]-4-piperidyl]-3,4-dihydro-4-hydroxy-2(1H)-quinolinone (18a)

Samarium iodide (0.1 M solution in THF, 120 ml, 12 mmol) was added dropwise to a ice-cooled solution of 17a (2.4 g, 5.4 mmol) in THF (20 ml) and the mixture was stirred at room temperature for 15 h. Water was added to the reaction mixture and THF was evaporated. The residue was extracted with CH₂Cl₂. The combined organic phases were dried over MgSO₄ and concentrated in vacuo. The residue was purified by column chromatography (silica gel, eluent; CH₂Cl₂ : MeOH = 30 : 1) to give 18a (0.4 g, 18 %) as pale yellow oil. Nmr δ: 1.50 - 2.00 (2 H, m), 2.31 (3 H, s), 2.57 - 2.73 (2 H, m), 2.79 (2 H, d, J = 5.0 Hz), 2.90 - 3.20 (1 H, m), 3.80 - 4.20 (1 H, br s), 4.30 - 4.50 (1 H, m), 4.82 (1 H, t, J = 5.0 Hz), 7.08 - 7.15 (4 H, m), 7.31 - 7.38 (2 H, m), 7.48 (2 H, d, J = 8.5 Hz).

3,4-Dihydro-4-hydroxy-1-[1-[(4-hydroxy)benzoyl]-4-piperidyl]-2(1H)-quinolinone (5)

To a stirred and ice-cooled solution of 18a (0.3 g, 0.73 mmol) in MeOH (5 ml) was added 1 N NaOH (1 ml, 1.0 mmol) and the mixture was stirred at the same temperature for 4 h. The reaction mixture was adjusted to pH 4 with 1 N HCl. After evaporation of MeOH, the residue was extracted with CH₂Cl₂. The CH₂Cl₂ layer was dried over MgSO₄ and concentrated in vacuo. The residue was purified by column chromatography (silica gel, eluent; CH₂Cl₂ : MeOH = 20 : 1) and preparative thin layer chromatography (silica gel, solvent; CH₂Cl₂ : MeOH = 15 : 1, 3 times). Recrystallization from Et₂O-hexane gave 5 (20 mg, 7.5 %) as white granules, mp 142 - 144 °C. Nmr (DMSO-d₆) δ: 1.60 - 2.00 (2 H, m), 2.50 - 2.70 (2 H, m), 2.90 - 3.20 (2 H, m), 3.80 - 4.50 (3 H, m), 4.64 (1 H, br s), 5.47 (1 H, br s), 6.79 (2 H, d, J = 8.2
Hz), 7.08 (1 H, m), 7.08 - 7.40 (5 H, m). Anal. Calcd for C_{21}H_{22}N_{2}O_{4}·3/2 H_{2}O: C, 64.11; H, 6.40; N, 7.12. Found: C, 64.04; H, 6.00; N, 7.04.

**Determination of Optical Purity of Metabolite (5)**

After OPC-21268 (1) was administered orally to beagle dogs, the separated urinary metabolite and synthetic 5 were subjected to hplc (column, ULTRON ES-OVM, 4.6 mm i.d. × 150 mm; solvent, acetonitrile : 20 mM KH_{2}PO_{4}=3 : 97; detector, uv 254 nm). Synthetic 5 : tR 10.4 min (48.9 %), tR 12.5 min (51.1 %); 5 obtained as the metabolite : tR 10.2 min (48.6 %), tR 12.2 min (51.4 %).

**1-[(4-(3-Acetylamino)propoxy)benzoyl]-4-piperidone (14b)**

A mixture 4-[(3-phthalimido)propoxy]benzoic acid (20 g, 61.5 mmol) and SOCl_{2} (9 ml, 123 mmol) in 1,2-dichloroethane (200 ml) was refluxed for 4 h. After removal of solvent, the residue was dissolved in acetone (55 ml). This solution was added dropwise to a stirred and ice-cooled solution of 4-piperidone hydrochloride (8.5 g, 55.3 mmol) and K_{2}CO_{3} (26.7 g, 193.2 mmol) in acetone (55 ml) and water (100 ml). The reaction mixture was stirred at room temperature for 24 h. After evaporation of acetone, the residue was extracted with CH_{2}Cl_{2}. The combined organic phases were dried over MgSO_{4} and concentrated in vacuo. A mixture of the residue (24.4 g, 60 mmol) and hydrazine monohydrate (3.64 ml, 75 mmol) in EtOH (200 ml) was refluxed for 2 h. After removal of solvent, the residue was dissolved in acetic anhydride (115 ml, 2.0 mol) and pyridine (5 ml, 61.8 mmol). The reaction mixture was stirred at room temperature for 50 h. The mixture was poured into ice-water and extracted with CH_{2}Cl_{2}. The combined organic phases were dried over MgSO_{4} and concentrated in vacuo. The residue was purified by column chromatography (silica gel, eluent; CH_{2}Cl_{2} : MeOH = 100 : 1 to 20 : 1) to give 14b (10 g, 52 %) as pale yellow oil. Nmr δ : 1.98 (3 H, s), 2.06 (2 H, t, J = 5.8 Hz), 2.5 1 (4 H, m), 3.40 - 4.00 (6 H, m), 4.05 (2 H, t, J = 5.8 Hz), 6.18 (1 H, br s), 6.93 (2 H, d, J = 8.8 Hz), 7.44 (2 H, d, J = 8.8 Hz).

Compounds (15b - 17b and 6) were obtained by the same procedure as described for 15a - 17a and 5.
15b: yield 10%, pale yellow oil, nmr δ: 1.40 - 1.60 (2 H, m), 1.95 (3 H, s), 1.99 - 2.00 (4 H, m), 3.20 (2 H, t, J = 10.6 Hz), 3.42 (2 H, dt, J = 6.6, 6.0 Hz), 3.61 (1 H, m), 3.70 - 4.50 (4 H, m), 4.02 (2 H, t, J = 5.8 Hz), 4.63 (2 H, s), 6.09 (1 H, s), 6.65 (2 H, t, J = 7.4 Hz), 6.87 (2 H, d, J = 8.8 Hz), 7.05 (1 H, d, J = 5.8 Hz), 7.19 (1 H, t, J = 7.4 Hz), 7.36 (2 H, d, J = 8.8 Hz).

16b: yield 88%, pale yellow oil, nmr δ: 1.50 - 1.70 (2 H, m), 1.97 (3 H, s), 1.99 - 2.20 (4 H, m), 3.22 - 3.33 (2 H, m), 3.42 (2 H, dt, J = 6.0, 6.3 Hz), 3.74 (1 H, m), 3.90 - 4.50 (2 H, m), 4.03 (2 H, t, J = 6.0 Hz), 6.19 (1 H, s), 6.71 (2 H, t, J = 7.7 Hz), 6.89 (2 H, d, J = 8.7 Hz), 7.40 (3 H, m), 7.48 (1 H, d, J = 7.7 Hz), 8.47 (1 H, d, J = 7.5 Hz), 9.81 (1 H, s).

17b: yield 43%, pale yellow oil, nmr δ: 1.30 - 1.60 (1 H, m), 1.60 - 1.80 (1 H, m), 1.97 (3 H, s), 1.99 - 2.30 (4 H, m), 3.00 (2 H, m), 3.42 (2 H, dt, J = 6.8, 6.2 Hz), 3.68 (2 H, d, J = 1.2 Hz), 3.90 - 4.50 (2 H, m), 4.01 (2 H, t, J = 6.0 Hz), 4.85 (1 H, m), 5.94 (1 H, s), 6.84 (2 H, d, J = 8.6 Hz), 7.27 (3 H, m), 7.72 (2 H, m), 8.03 (1 H, dd, J = 7.2, 1.8 Hz), 10.16 (1 H, s).

6: yield 10%, white amorphous, nmr δ: 1.80 - 1.90 (2 H, m), 1.93 (3 H, s), 1.97 - 2.20 (2 H, m), 2.33 (2 H, m), 2.64 (2 H, m), 2.73 (2 H, d, J = 5.3 Hz), 2.80 - 3.00 (2 H, m), 3.39 (2 H, dt, J = 7.7, 5.7 Hz), 4.01 (2 H, t, J = 6.0 Hz), 4.35 (1 H, m), 4.64 (1 H, m), 4.77 (1 H, t, J = 5.3 Hz), 6.87 (2 H, d, J = 8.7 Hz), 7.00 - 7.13 (2 H, m), 7.21 - 7.45 (4 H, m). Anal. Calcd for C29H31N3O5·H2O: C, 64.58; H, 6.88; N, 8.69. Found: C, 64.23; H, 6.95; N, 8.41.

REFERENCES


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