SYNTHESIS AND PHARMACOLOGICAL ACTIVITY OF METABOLITES OF VASOPRESSIN V_1 RECEPTOR ANTAGONIST, OPC-21268

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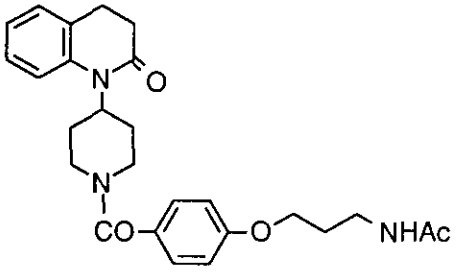
Abstract - The metabolites of 1-[1-[4-(3-acetylaminopropoxy)benzoyl]-4-piperid-yl]-3,4-dihydro-2(1*H*)-quinolinone (OPC-21268, **1**), vasopressin V_1 receptor antagonist were synthesized to confirm the proposed structures and to examine their vasopressin V_1 receptor antagonistic activity. The structures 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**.

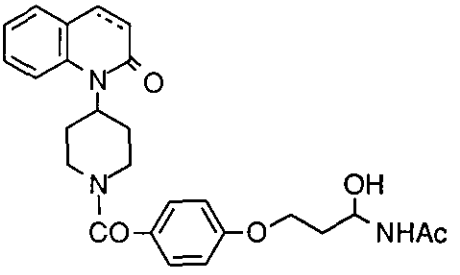
A new vasopressin V_1 receptor antagonist, OPC-21268 (**1**) (1-[1-[4-(3-acetylaminopropoxy)benzoyl]-4-piperidyl]-3,4-dihydro-2(1*H*)-quinolinone) was synthesized by Ogawa *et al.*[1,2] and is now under clinical trial. This compound is an orally effective, nonpeptide antagonist for the arginine vasopressin (AVP) receptor and turned out to be a selective V_1 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(1*H*)-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,[3] 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.

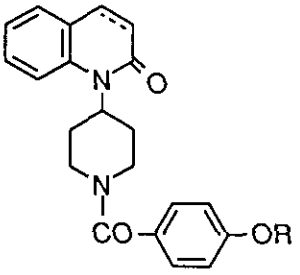
Figure 1



1(OPC-21268)

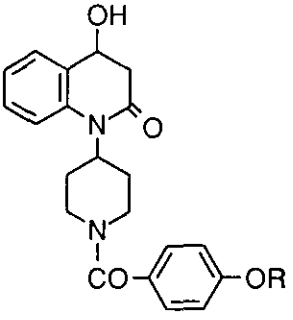


2a: Bond of C(3) and C(4),single
2b: Bond of C(3) and C(4),double



3: R= NHAc, Bond of C(3) and C(4), double

4a: R=H, Bond of C(3) and C(4), single



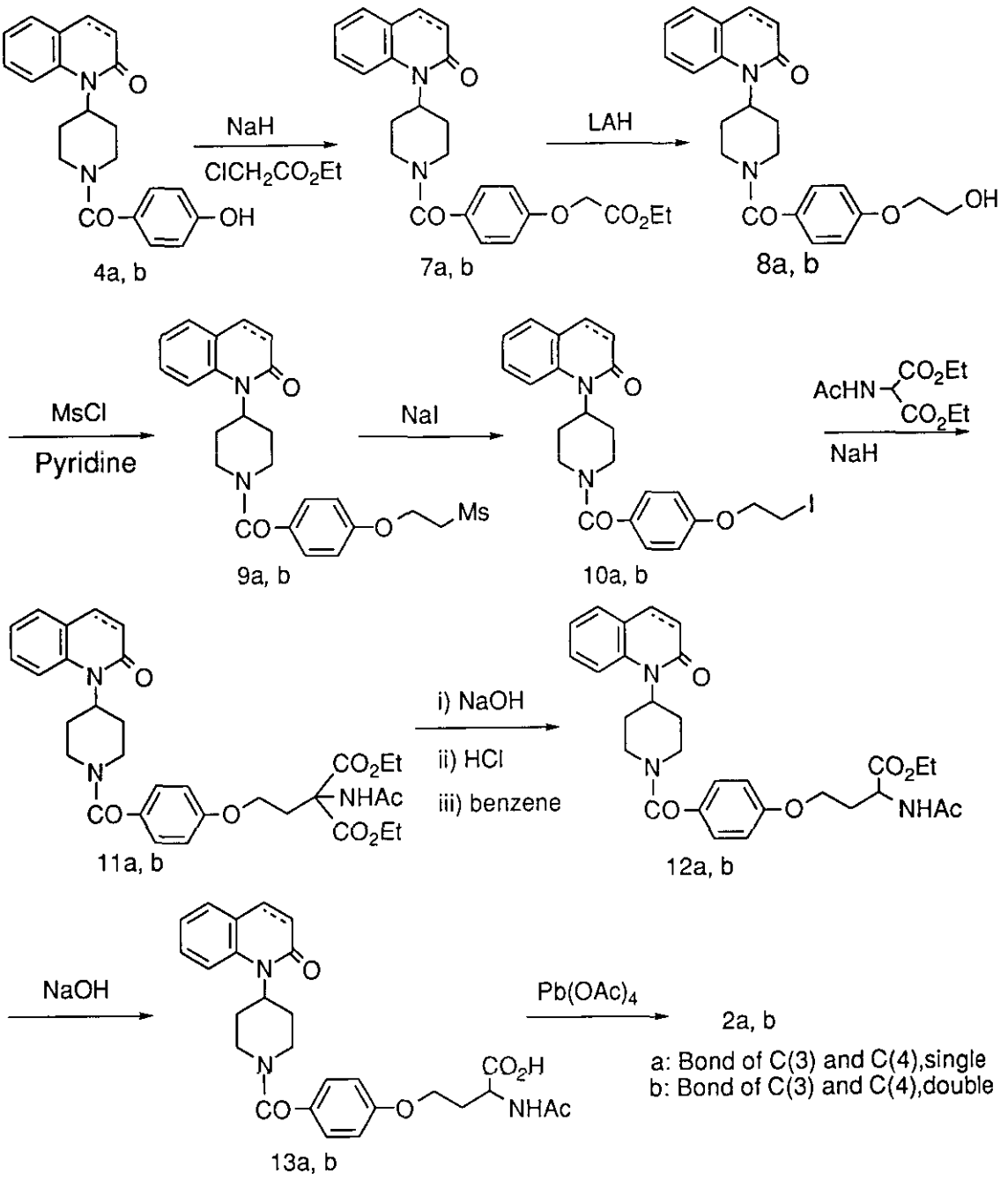
5: R=H
6: R= NHAc

SYNTHESIS

Initially, *N*-acetylhemiaminal metabolites (**2a** and **b**) were synthesized by the pathway shown in Scheme 1. Alkylation of phenol derivative (**4a**)[2] 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

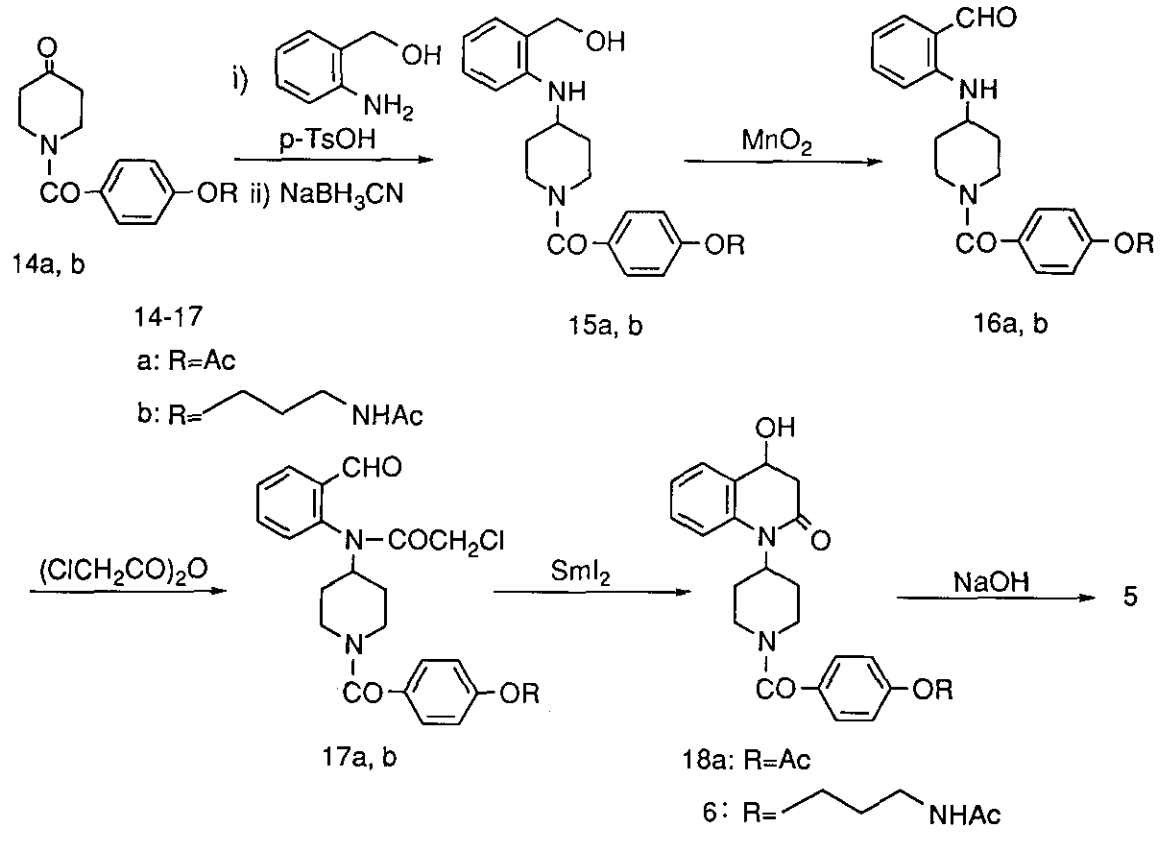
Scheme 1



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**),[5] *via* **12b**, which was hydrolyzed with NaOH, followed by oxidative decarboxylation with lead tetraacetate.

Next, we planed the synthesis of 3,4-dihydro-4-hydroxy-2(1*H*)-quinolinone metabolites (**5** and **6**). The 3,4-dihydro-4-hydroxy-2(1*H*)-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(1*H*)-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 $NaBH_3CN$ 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 SmI_2 gave the 4-hydroxy-2(1*H*)-quinolinone derivative (**18a**) in 18 % yield. Hydrolysis of **18a** with NaOH gave 4-hydroxy-2(1*H*)-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(1*H*)-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 V_1 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 V_1 receptor binding affinity

Compd.No.	**1**	**2a**	**2b**	**3**	**4a**	**5**	**6**
Receptor affinity[c] IC_{50} (μM)	0.44	0.6	3.3	1.2	1.3	>100	>100

[c] Compounds were tested for their ability to displace [^{3}H]AVP from its specific binding sites in rat liver (V_1 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 $CDCl_3$ on a Bruker AC-200 spectrometer with tetramethylsilane as an internal standard.

Ethyl 2-[[4-[4-[3,4-Dihydro-2-oxo-1*H*-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(1*H*)-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 ℃ for 1 h. The mixture was poured into water and extracted with ethyl acetate. The combined organic phases were dried over $MgSO_4$ 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(1*H*)-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 ℃ for 2 h and at room temperature for 1 h. Water was carefully added to destroy the excess $LiAlH_4$. The mixture was poured into water and extracted with CH_2Cl_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_2Cl_2 : 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(1*H*)-quinolinone (9a)

To a stirred solution of **8a** (4.5 g, 11.4 mmol) and pyridine (5.4 g, 68.4 mmol) in $CHCl_3$ (50 ml) was added $MeSO_2Cl$ (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 CH_2Cl_2. The CH_2Cl_2 layers were dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by column chromatography (silica gel, eluent; CH_2Cl_2 : 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(1*H*)-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 $CHCl_3$. The $CHCl_3$ solution was washed with water and saturated NaCl solution, dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified

by column chromatography (silica gel, eluent; CH_2Cl_2 : MeOH = 50 : 1) to give **10a** (18.3 g, 81 %) as white powder (from EtOH), mp 157 - 159 ℃. 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 $C_{23}H_{25}N_2O_3I$: 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-1*H*-quinolin-1-yl)-1-piperidyl]carbonyl]phenoxy]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 ℃ for 1.5 h. After removal of the solvent, the residue was poured into water and extracted with $CHCl_3$. The $CHCl_3$ layer was dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by column chromatography (silica gel, eluent; CH_2Cl_2 : 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 $C_{32}H_{39}N_3O_8 \cdot 1/2\ H_2O$: 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-1*H*-quinolin-1-yl]-1-piperidyl]-carbonyl]phenoxy]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 $CHCl_3$. The $CHCl_3$ solution was dried over $MgSO_4$ 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_2Cl_2 : MeOH = 50 : 1). Recrystallization from AcOEt-hexane gave **12a** (1.0 g, 50 %) as white granules, mp 158 - 160 ℃. 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 $C_{29}H_{35}N_3O_6$: 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-1*H*-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_2Cl_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_2Cl_2 : MeOH = 30 : 1 to 10 : 1) and recrystallized from Et_2O - AcOEt to give **13a** (2.8 g, 90 %) as white granules, mp 114 - 116 ℃. 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 $C_{27}H_{31}N_3O_6 \cdot 1/2\ H_2O$: 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(1*H*)-

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 $NaHCO_3$ solution (9 ml) was added to the reaction mixture and the solution was extracted with AcOEt. The combined organic phases were dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by column chromatography and triturated in Et_2O - 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 (2 H, d, *J* = 8.6 Hz). Fab-ms (pos.) m/z : 466 $[M-H]^+$. *Anal.* Calcd for $C_{26}H_{31}N_3O_5 \cdot H_2O$: 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 CH_2Cl_2 - hexane), mp 137 - 139 ℃, 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 $C_{27}H_{29}N_3O_6 \cdot 1/2\ H_2O$: 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 $C_{26}H_{29}N_3O_5 \cdot 1.25\ H_2O$: 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 $SOCl_2$ (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 K_2CO_3 (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 CH_2Cl_2. The combined organic phases were dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by column chromatography (CH_2Cl_2 : MeOH = 100 : 1) and recrystallized from AcOEt - hexane to give **14a** (63.5 g, 87 %) as white powder, mp 102 - 103 ℃. 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 $C_{14}H_{15}NO_4$: C,

64.36; H, 5.79; N, 5.36. Found : C, 64.19; H, 5.88; N, 5.05.

1-[(4-Acetoxy)benzoyl]-4-[(2-hydroxymethyl)anilino]piperidine (15a)

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 CH_2Cl_2. The combined organic phases were dried over $MgSO_4$ 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 $CHCl_3$ (500 ml) was refluxed for 20 h. To the reaction mixture was added water and MnO_2 was removed by filtration. The $CHCl_3$ layers was separated, dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by column chromatography (silica gel, eluent; CH_2Cl_2 : 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), 3.22 - 3.32 (2 H, m), 3.60 - 4.00 (2 H, m), 4.10 - 4.60 (1 H, br s), 6.70 (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; CH_2Cl_2 : 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-Acetoxy)benzoyl]-4-piperidyl]-3,4-dihydro-4-hydroxy-2(1*H*)-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 CH_2Cl_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_2Cl_2 : 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(1*H*)-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 CH_2Cl_2. The CH_2Cl_2 layer was dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by column chromatography (silica gel, eluent; CH_2Cl_2 : MeOH = 20 : 1) and preparative thin layer chromatography (silica gel, solvent; CH_2Cl_2 : MeOH = 15 : 1, 3 times). Recrystallization from Et_2O-hexane gave **5** (20 mg, 7.5 %) as white granules, mp 142 - 144 ℃. Nmr (DMSO-d_6) 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 $C_{21}H_{22}N_2O_4 \cdot 3/2\ H_2O$: 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. x 150 mm; solvent, acetonitrile : 20 mM KH_2PO_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_2CO_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_2Cl_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_2Cl_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_2Cl_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 $C_{26}H_{31}N_3O_5 \cdot H_2O$: C, 64.58; H, 6.88; N, 8.69. Found: C, 64.23; H, 6.95; N, 8.41.

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