Synthesis of Metabolites of the Cerebroprotecting Agent 7-Hydroxy-1-[[[4-(3-methoxyphenyl)-1-piperazinyl]acetyl]amino]-2,2,4,6-tetramethylindan (OPC-14117)

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A hydroxylated metabolite (5a) of 7-hydroxy-1-[[[4-(3-methoxyphenyl)-1-piperazinyl]acetyl]amino]-2,2,4,6-tetramethylindan (OPC-14117), which is a novel cerebroprotective agent with central nervous system-stimulating activity, was synthesized by use of a model cytochrome P-450 system. In order to confirm the structure of this compound it was prepared by another route. The structures of several metabolites (5a, 7 and 8) were identified by means of comparisons with the synthetic compounds.

Key words metabolite; cytochrome P-450; OPC-14117; cerebroprotective agent; indan ring; chemical P-450 model

A new cerebroprotective agent, OPC-14117 (7-hydroxy-1-[[[4-(3-methoxyphenyl)-1-piperazinyl]acetyl]amino]-2,2,4,6-tetramethylindan) (**1**), was synthesized by Oshiro *et al.*[1] and is now under clinical trial. OPC-14117 shows cerebroprotective and central nervous system (CNS) stimulating activities.[1] Its antilipid peroxidative activity is more potent than that of vitamin E.[2] The metabolism of **1** has been studied in the rat, dog and human. Demethylated (**2**), demethylated and hydroxylated (**3**), and hydroxylated derivatives (**4**), hydroxylated derivatives (**5a** and **6**) of the methyl group on the indan ring, further oxidized analogues (**7** and **8**) of the alcohol, and two glucuronides (**9** and **10**), in addition to the degradation product (**11**) have been proposed as metabolites.

Cytochrome P-450 enzymes play a very important role in drug metabolism, and in recent years, chemical models for P-450 have been developed and applied to drug metabolism studies.[3] Such methods can afford relatively large amounts of products with few synthetic steps. Here, we wish to report the application of chemical P-450 model systems to the study of OPC-14117 metabolism.

Results and Discussion

Fenton's reagent is a well known chemical model for cytochrome P-450.[4] Initially, the Fenton reaction of OPC-14117 (**1**) gave the hydroxylated compound **5a** in 2.8% yield. We investigated the effect of chelators[5] upon the Fenton oxidation of **1**. Acetylacetone gave a good result (**5a**, 3.3% yield), but others failed to give the target product. In the Fenton reaction with acetylacetone, the other products were the unreacted starting material and three unknown compounds. Next, we tried Groves model[6] using *meso*-tetraphenylporphinatoiron (III) chloride (Fe(III)TPPCl). Oxidation of **1** with Fe(III)TPPCl and iodosobenzene (PhIO) in dichloromethane–methanol–water (4 : 0.9 : 0.1) gave the methoxy compound **5b** in 3.6% yield. The methyl group presumably came from the solvent (MeOH). So, metabolite **5a** (2.6% yield) was prepared by similar oxidation of **1** in benzene. In Groves reaction the other products were the unreacted starting material and two unknown compounds. Another metabolite **7** was obtained by further oxidation of **5a** with pyridinium chlorochromate (PCC) in 60% yield.

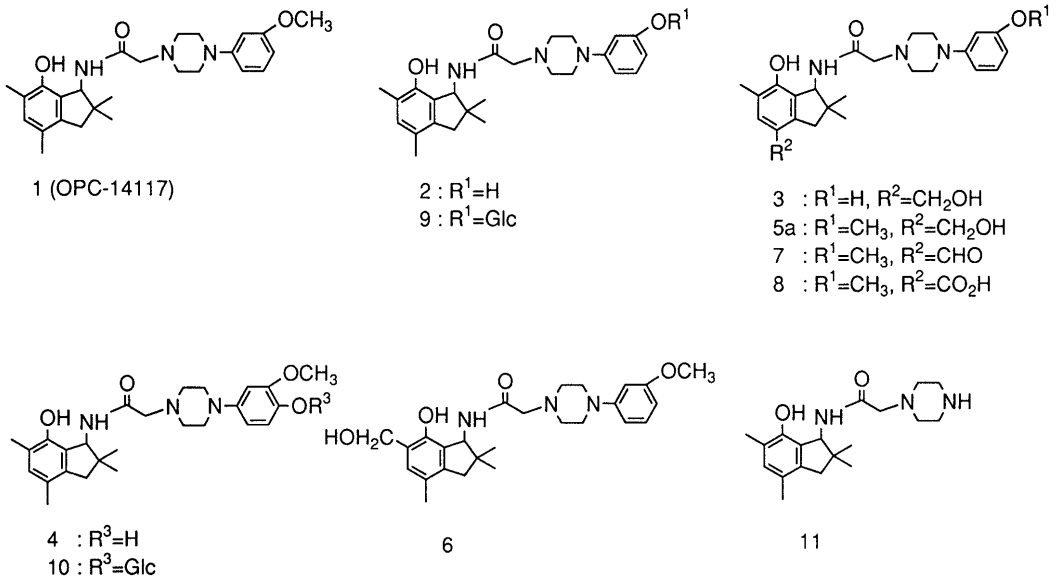

Chart 1

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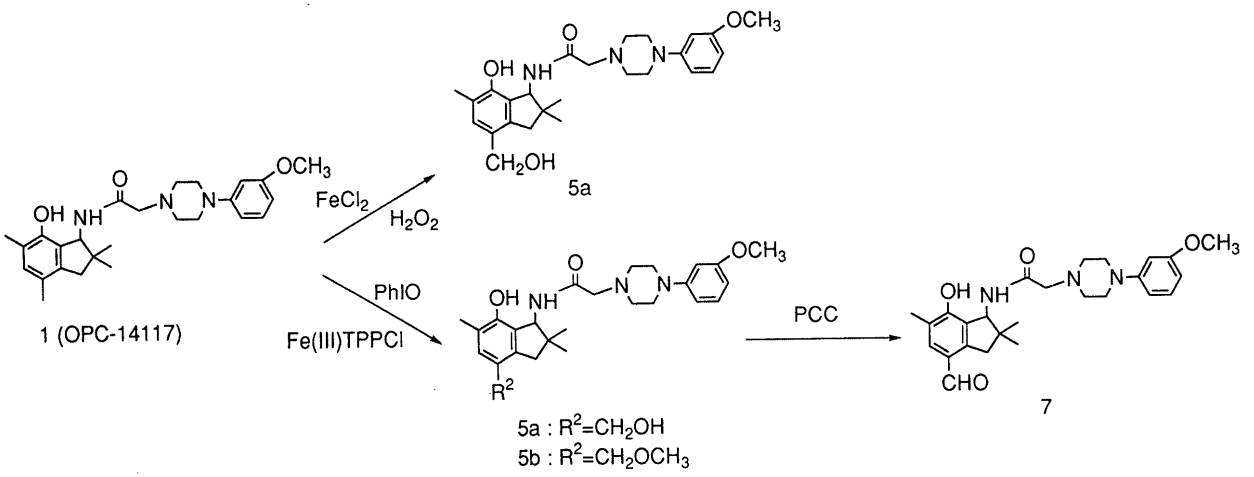

Chart 2

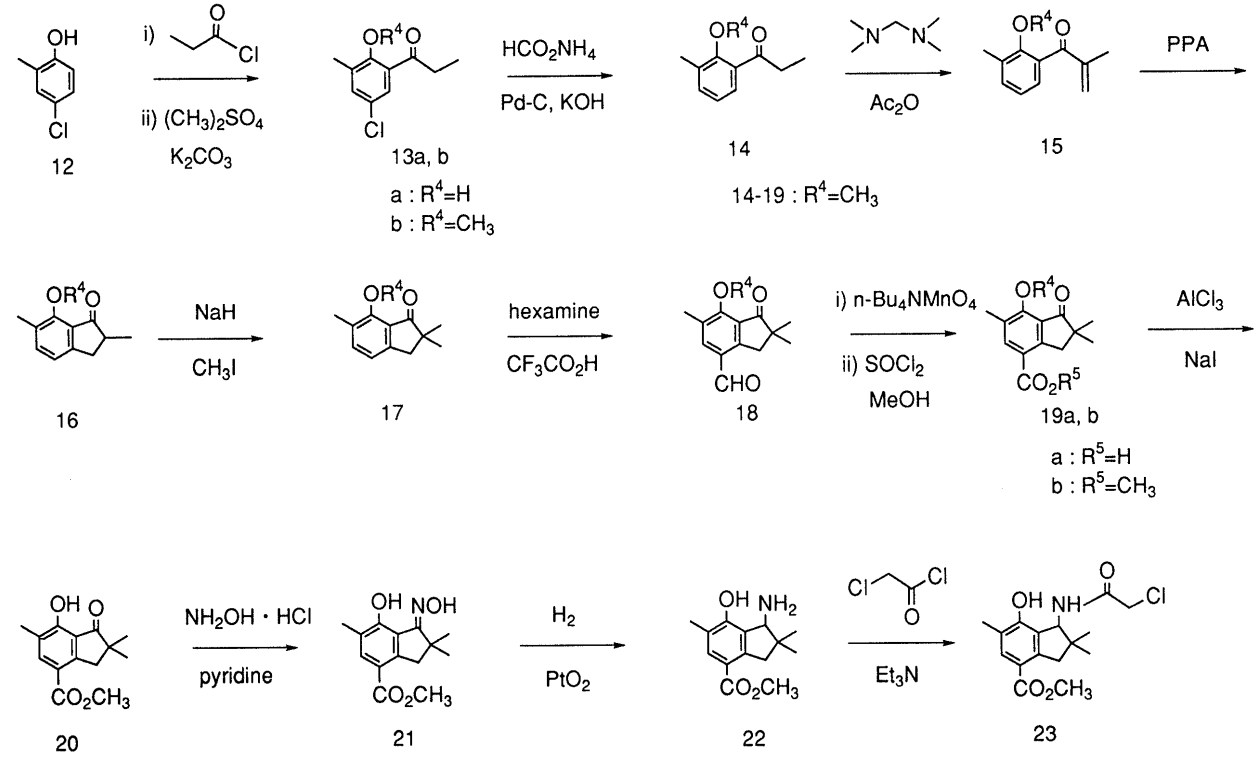

Chart 3

In order to confirm the structure, the metabolite **5a** was synthesized as shown in Charts 3 and 4. Friedel–Crafts acylation of 4-chloro-2-methylphenol (**12**) with propionyl chloride in the presence of aluminum chloride gave the propanone **13a**, which was methylated with dimethyl sulfate and potassium carbonate to give the methyl ether **13b**, followed by reduction with ammonium formate, palladium carbon (Pd–C) and potassium hydroxide to afford 1-(2-methoxy-3-methylphenyl)-1-propanone (**14**) in 61% yield. Treatment of **14** with *N*,*N*,*N*′,*N*′-tetramethyldiaminomethane and acetic anhydride gave the methylpropenone **15**, which reacted with PPA to give the indanone **16** in 43% yield. Methylation of 7-methoxy-1-indanone (**16**) with methyl iodide in the presence of sodium hydride (NaH) gave the 7-methoxy-2,2-dimethyl derivative **17**, which was formylated with hexamethylenetetramine in trifluoroacetic acid to afford the formyl compound **18** in 84% yield. Oxidation of **18** with tetrabutylammonium permanganate gave the carboxylic acid **19a**, which was converted to the ester **19b** by esterification with methanol–thionyl chloride. Treatment of **19b** with aluminum chloride-sodium iodide afforded the hydroxy derivative **20** in 71% yield. Treatment of **20** with hydroxylamine hydrochloride in pyridine gave the oxime **21**. Hydrogenation of **21** with platinum oxide afforded 1-aminoindan (**22**), which was converted to the amide **23** by treatment with 2-chloroacetyl chloride in the presence of triethylamine in 96% yield. Condensation of **23** with 1-(3-methoxyphenyl)piperazine gave the 1-(piperazinyl)acetamido derivative **24** in 62% yield. The alcohol **5a** was synthesized by reduction of the ester **24** with lithium aluminum hydride (LAH) in 54% yield. Hydrolysis of **24**

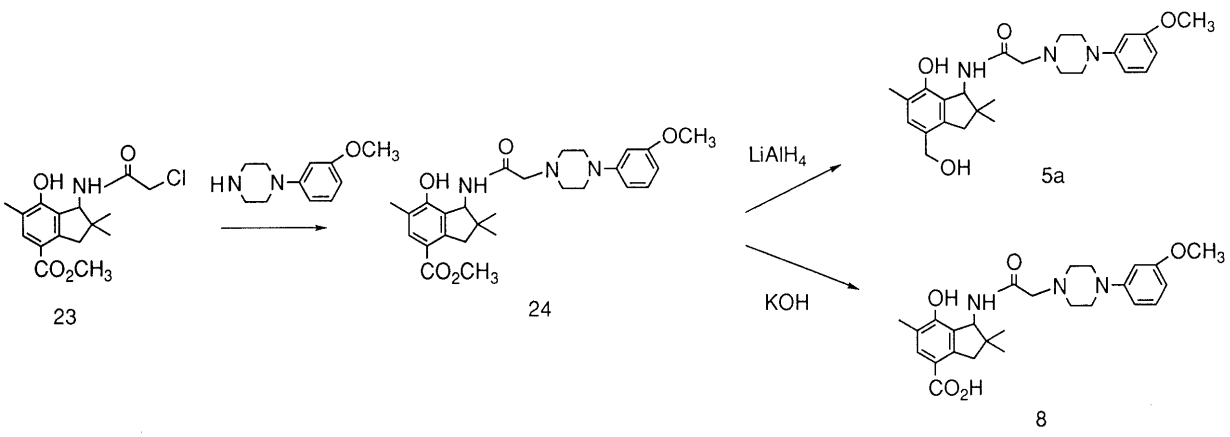

Chart 4

with potassium hydroxide afforded the carboxylic acid **8** in 87% yield.

Conclusion

The metabolite **5a** derived from the chemical model systems was identical with the corresponding synthetic compound based on NMR and MS comparisons, and high-performance liquid chromatographic (HPLC) behavior. Total yield of the metabolite **5a** was 5.03% *via* the stepwise procedure from 4-chloro-2-methylphenol (15 steps). Thus, the model cytochrome P-450 systems offer an alternative, facile preparative method. The synthetic metabolite **5a** was identical with that formed *in vivo*. The metabolites **7** and **8** also were identical with the corresponding synthetic compounds. The mother compound **1** is mainly metabolized at the 4-position of the indan ring to afford the carboxylic acid **8** in animals and human, and this pathway was thus duplicated in the model cytochrome P-450 system. The antioxidant activities of the synthetic metabolites (**5a**, **7** and **8**) were found to be lower than that of **1**.

Experimental

Melting points were determined with a Yamato MP-21 apparatus and are uncorrected. NMR spectra were recorded in $CDCl_3$ on Bruker AC-200 and AVANCE DPX 250 spectrometers with tetramethylsilane as an internal standard. IR spectra were recorded on a JASCO IR-810 spectrometer. MS were obtained on Thermoquest GCQ and JEOL JMS-SX 102A mass spectrometers. HPLC was performed with a Shimadzu LC-6A liquid chromatograph and an SPD-6A ultraviolet spectrophotometric detector.

Fenton Reaction of 1 A 7 N HCl–EtOH (1 ml) solution was added to a suspension of **1** (440 mg, 1.0 mmol) in EtOH (10 ml). After the removal of EtOH, the residue was dissolved in CH_3CN (27 ml) and EtOH (4 ml). To this solution, 70% perchloric acid (810 μl, 5.6 mmol) and ferrous chloride (1.49 g, 7.5 mmol) were added, and the mixture was stirred for 10 min. Then, a solution of 30% hydrogen peroxide (570 μl, 5.0 mmol) in water (7.5 ml) was added dropwise at room temperature and the reaction mixture was stirred for 1.5 h at the same temperature. A 10% sodium hydrogen sulfite aqueous solution (15 ml) and a 28% ammonia solution (3 ml) were added at 0—10 °C. After removal of the resulting precipitates by filtration, the filtrate was extracted with CH_2Cl_2. The extract was dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by column chromatography (silica gel; eluent, hexane: AcOEt = 1 : 1 to CH_2Cl_2 : MeOH = 30 : 1 to 15 : 1) and preparative thin layer chromatography (silica gel; solvent, hexane : AcOEt = 1 : 1, 2 developments) to give **5a** (13 mg, 2.8%) as a brown oil. NMR δ: 1.13 (3H, s), 1.26 (3H, s), 2.22 (3H, s), 2.63—2.95 (6H, m), 3.03—3.17 (7H, m), 3.79 (3H, s), 4.52 (2H, d, J = 5.5 Hz), 4.70 (1H, d, J = 7.8 Hz), 6.46—6.54 (3H, m), 7.02 (1H, s), 7.18 (1H, t, J = 8.7 Hz), 8.06 (1H, d, J = 7.8 Hz), 9.19 (1H, s). MS m/z (%): 453 (M^+, 11), 438 (8), 205 (100), 191 (27), 162 (29).

Fenton Reaction of 1 (with Chelator) A solution of **1** (440 mg, 1.0 mmol), ferrous chloride (200 mg, 1.0 mmol), acetylacetone (100 mg, 1.0 mmol) and 30% hydrogen peroxide (0.4 ml, 3.3 mmol) in CH_3CN (6 ml) and H_2O (3 ml) was stirred for 6 h at room temperature. Compound **5a** (15 mg, 3.3%) was obtained by the same procedure as described above for the Fenton reaction.

Groves Model Reaction of 1 (in CH_2Cl_2–MeOH–H_2O) A solution of **1** (440 mg, 1.0 mmol) was prepared in CH_2Cl_2–MeOH–H_2O (8 ml : 1.8 ml : 0.2 ml), then PhIO (220 mg, 1.0 mmol) and Fe(TPP)Cl (70 mg, 0.1 mmol) were added, and the mixture was stirred for 4 h at room temperature. After removal of the solvent, the residue was purified by column chromatography (silica gel; eluent, hexane : AcOEt = 2 : 1) to give the methoxy compound **5b** (15 mg, 3.6%) as a brown oil. NMR δ: 1.12 (3H, s), 1.24 (3H, s), 2.21 (3H, s), 2.65—2.93 (6H, m), 3.00—3.16 (6H, m), 3.34 (3H, s), 3.78 (3H, s), 4.27 (2H, s), 4.69 (1H, d, J = 7.7 Hz), 6.45 (2H, s), 6.52 (1H, d, J = 8.4 Hz), 6.98 (1H, s), 7.17 (1H, t, J = 8.4 Hz), 8.05 (1H, d, J = 7.4 Hz), 9.14 (1H, s). MS m/z (%): 467 (M^+, 12), 452 (8), 205 (100), 191 (40), 70 (94).

Groves Model Reaction of 1 (in Benzene) A solution of **1** (440 mg, 1.0 mmol) was prepared in benzene (60 ml), then PhIO (220 mg, 1.0 mmol) and Fe(TPP)Cl (70 mg, 0.1 mmol) were added and the mixture was stirred for 48 h at room temperature. After evaporation of the benzene, the residue was purified by column chromatography (silica gel; eluent, hexane : AcOEt = 1 : 1) and thin layer chromatography (silica gel; solvent, hexane : AcOEt = 1 : 1, 2 developments) to give **5a** (12 mg, 2.6%) as a brown oil. Its NMR spectrum was identical with that of **5a** obtained by means of the Fenton reaction.

4-Formyl-7-hydroxy-1-[4-(3-methoxyphenyl)-1-piperazinylacetyl-amino]-2,2,6-trimethylindan (7) Compound **5a** (55 mg, 0.12 mmol) was added to a mixture of pyridinium chlorochromate (40 mg, 0.18 mmol) and Celite (40 mg) in CH_2Cl_2 (1 ml) at room temperature, and the mixture was stirred for 1.5 h. After removal of the solvent, the residue was purified by column chromatography (silica gel; eluent, CH_2Cl_2 : MeOH = 50 : 1) and recrystallized from AcOEt–hexane to give **7** (32 mg, 60%) as a white powder, mp 162—164 °C. NMR δ: 1.12 (3H, s), 1.30 (3H, s), 2.27 (3H, s), 2.58—2.81 (4H, m), 3.04—3.30 (8H, m), 3.79 (3H, s), 4.65 (1H, d, J = 7.4 Hz), 6.44 (1H, d, J = 1.4 Hz), 6.46 (1H, s), 6.53 (1H, dd, J = 1.4, 8.4 Hz), 7.18 (1H, t, J = 8.4 Hz), 7.51 (1H, s), 8.15 (1H, d, J = 7.0 Hz), 9.88 (1H, s), 10.22 (1H, s). IR (KBr): 3348, 2810, 1678, 1638, 1602, 1525, 1273, 1207, 971 cm^{-1}. MS m/z (%): 451 (M^+, 15), 205 (72), 162 (32), 70 (100). *Anal.* Calcd for $C_{26}H_{33}N_3O_4$: C, 69.15; H, 7.37; N, 9.31. Found: C, 68.93; H, 7.27; N, 9.21.

HPLC Analysis Chromatographic conditions were as follows: column, TSK-80TM (4.6 × 150 mm); mobile phase, acetonitrile–20 mM sodium sulfate aqueous solution–acetic acid (35 : 65 : 1); flow rate, 1.0 ml/min; detection, UV at 254 nm; retention time, 13.1 min (99.6%).

1-(5-Chloro-2-hydroxy-3-methylphenyl)-1-propanone (13a) A stirred solution of 4-chloro-2-methylphenol (50 g, 0.35 mol) in propionyl chloride (35 ml, 0.4 mol) was heated at 80—90 °C for 2 h. To this mixture was added aluminum chloride (67 g, 0.5 mol), and the whole was stirred at the same temperature for 1 h, then poured into ice-water and extracted

with CH_2Cl_2. The extract was dried over $MgSO_4$ and concentrated *in vacuo* to give **13a** (71.3 g, quant.) as a pale orange oil. NMR δ: 1.23 (3H, t, J=7.4 Hz), 2.24 (3H, s), 2.97 (2H, q, J=7.4 Hz). IR (KBr): 2983, 1642, 1425, 1266, 1052, 755 cm^{-1}. MS m/z (%): 198 (M^+, 22), 171 (33), 169 (100), 113 (13), 77 (45). HRMS (FAB) Calcd for $C_{10}H_{11}ClO_2$: 198.0448 (M^+). Found: 198.0465.

1-(5-Chloro-2-methoxy-3-methylphenyl)-1-propanone (13b) A solution of **13a** (570 mg, 2.9 mmol) and NaOH (190 mg, 4.8 mmol) in EtOH (10 ml) was treated with methyl sulfate (0.35 ml, 3.7 ml). The reaction mixture was refluxed for 2 h, then diluted with water and extracted with CH_2Cl_2. The extract was dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by column chromatography (silica gel; eluent, hexane : AcOEt = 20 : 1) to give **13b** (450 mg, 74%) as a colorless oil. NMR δ: 1.17 (3H, t, J=7.2 Hz), 2.29 (3H, s), 2.95 (2H, q, J=7.2 Hz), 3.72 (3H, s), 7.26 (1H, d, J=2.6 Hz), 7.31 (1H, d, J=2.6 Hz). IR (neat): 2940, 1681, 1469, 1233, 1005, 877 cm^{-1}. MS m/z (%): 214 (2), 213 (2), 212 (M^+, 5), 185 (33), 180 (100), 125 (20). HRMS (FAB) Calcd for $C_{11}H_{14}ClO_2$: 213.0682 (MH^+). Found: 213.0659.

1-(2-Methoxy-3-methylphenyl)-1-propanone (14) A suspension of 10% Pd–C (80 mg) in 2.5 N KOH aqueous solution (2.3 ml) was prepared, then **13b** (1.0 g, 4.8 mmol) in EtOH (10 ml) and ammonium formate (1.2 g, 19 mmol) were added. The reaction mixture was heated at 60 °C for 20 min. After removal of the catalyst by filtration, the filtrate was evaporated *in vacuo*. The residue was poured into water and extracted with AcOEt. The extract was dried over $MgSO_4$ and concentrated *in vacuo* to give **14** (690 mg, 82%) as a colorless oil. NMR δ: 1.18 (3H, t, J=7.3 Hz), 2.32 (3H, s), 2.97 (2H, q, J=7.3 Hz), 3.74 (3H, s), 7.05 (1H, t, J=7.3 Hz), 7.30 (1H, dd, J=7.5, 1.6 Hz), 7.34 (1H, dd, J=7.5, 1.6 Hz). IR (neat): 2939, 1688, 1465, 1221, 1006, 777 cm^{-1}. MS m/z (%): 179 (M^++1, 50), 163 (21), 149 (100), 91 (22). HRMS (FAB) Calcd for $C_{11}H_{15}O_2$: 179.1072 (MH^+). Found: 179.1069.

1-(2-Methoxy-3-methylphenyl)-2-methyl-2-propen-1-one (15) *N,N,N′,N′*-Tetramethyldiaminomethane (48 ml) was added to **14** (15 g, 84 mmol) at 0 °C with stirring, and then acetic anhydride (48 ml) was added. The reaction mixture was heated at 90 °C for 3 h. The mixture was poured into water and extracted with CH_2Cl_2. The extract was dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by column chromatography (silica gel; eluent, hexane : AcOEt = 50 : 1) to give **15** (13.3 g, 83%) as a colorless oil. This compound was used in the next step without further purification. NMR δ: 2.05 (3H, t, J=1.0 Hz), 2.30 (3H, s), 3.69 (3H, s), 5.63 (1H, t, J=0.8 Hz), 5.96 (1H, t, J=1.2 Hz), 7.00 (1H, t, J=7.5 Hz), 7.07 (1H, dd, J=7.6, 2.4 Hz), 7.25 (1H, dd, J=7.5, 2.4 Hz). MS m/z (%): 191 (28), 190 (M^+, 19), 175 (17), 149 (100), 135 (23), 91 (36).

7-Methoxy-2,6-dimethyl-1-indanone (16) A mixture of phosphoric acid (80 g) and phosphorus pentoxide (80 g) was heated at 110—120 °C for 15 min, then **15** (12 g, 63 mmol) was added, and the reaction mixture was stirred at the same temperature for 20 min. The mixture was poured into ice-water and concentrated *in vacuo*. The residue was purified by column chromatography (silica gel; eluent, hexane : AcOEt = 100 : 1) to give **16** (6.2 g, 52%) as a brown oil. NMR δ: 1.29 (3H, d, J=7.3 Hz), 2.27 (3H, s), 2.61—2.76 (2H, m), 3.26—3.37 (1H, m), 3.95 (3H, s), 7.04 (1H, d, J=7.5 Hz), 7.38 (1H, d, J=7.5 Hz). IR (neat): 2929, 1710, 1601, 1483, 1257, 1051, 829 cm^{-1}. MS m/z (%): 191 (100), 190 (M^+, 42), 161 (25), 115 (21), 91 (29). HRMS (FAB) Calcd for $C_{12}H_{15}O_2$: 191.1072 (MH^+). Found: 191.1071.

7-Methoxy-2,2,6-trimethyl-1-indanone (17) Sodium hydride (60%, 1.4 g, 35 mmol) was added to a stirred and ice-cooled solution of **16** (5.5 g, 34 mmol) in *N,N*-dimethylformamide (DMF) (60 ml) and the reaction mixture was stirred for 10 min. Then, methyl iodide (2.2 ml, 35 mmol) was added dropwise at 0—10 °C. The whole was stirred at 50 °C for 1 h, poured into water and extracted with AcOEt. The extract was dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by column chromatography (silica gel; eluent, hexane : AcOEt = 100 : 1 and 50 : 1) to give **17** (5.2 g, 88%) as a yellow oil. NMR δ: 1.22 (6H, s), 2.28 (3H, s), 2.92 (3H, s), 3.96 (3H, s), 7.02 (1H, d, J=7.5 Hz), 7.40 (1H, d, J=7.5 Hz). IR (neat): 2961, 1711, 1601, 1483, 1220, 1054, 799 cm^{-1}. MS m/z (%): 204 (M^+, 69), 189 (100), 175 (93), 149 (52), 115 (63). HRMS (FAB) Calcd for $C_{13}H_{17}O_2$: 205.1229 (MH^+). Found: 205.1238.

4-Formyl-7-methoxy-2,2,6-trimethyl-1-indanone (18) A solution of **17** (3.4 g, 16.6 mmol) and hexamine (3.5 g, 24.9 mmol) in trifluoroacetic acid (50 ml) was refluxed for 6 h. After removal of the solvent, the residue was poured into water and the solution was extracted with AcOEt. The extract was washed with water and saturated $NaHCO_3$ solution, dried over $MgSO_4$ and concentrated *in vacuo* to give **18** (3.7 g, 96%) as a pale yellow oil. NMR δ: 1.26 (6H, s), 2.34 (3H, s), 3.32 (2H, s), 4.10 (3H, s), 7.87 (1H, s), 10.09 (1H, s). IR (neat): 2961, 1693, 1573, 1485, 1265, 1106, 802, 588 cm^{-1}. MS m/z (%): 233 (100), 232 (M^+, 37), 217 (18), 189 (19), 115 (18), 91 (22). HRMS (FAB) Calcd for $C_{14}H_{17}O_3$: 233.1178 (MH^+). Found: 233.1180.

7-Methoxy-2,2,6-trimethyl-1-oxoindan-4-carboxylic Acid (19a) Tetrabutylammonium permanganate (4.3 g) was added to a solution of **18** (3.7 g, 16 mmol) in pyridine (50 ml) and the reaction mixture was stirred for 5 h at room temperature, then poured into 10% HCl–AcOEt (1 : 1). After removal of the insoluble material by filtration through Celite, the filtrate was extracted with AcOEt. The extract was washed with water, dried over $MgSO_4$ and concentrated *in vacuo*. The residue was recrystallized from acetone–AcOEt to give **19a** (3.7 g, 94%) as colorless needles, mp 167.5—169 °C. NMR δ: 1.25 (6H, s), 2.33 (3H, s), 3.35 (2H, s), 4.07 (3H, s), 8.19 (1H, s). IR (KBr): 2965, 1713, 1681, 1573, 1415, 1242, 1059, 731 cm^{-1}. MS m/z (%): 248 (M^+, 100), 233 (86), 219 (67), 215 (35), 187 (34), 115 (30). *Anal.* Calcd for $C_{14}H_{16}O_4$: C, 67.73; H, 6.50. Found: C, 67.79; H, 6.50.

Methyl 7-Methoxy-2,2,6-trimethyl-1-oxoindan-4-carboxylate (19b) Thionyl chloride (1.47 ml, 20.1 mmol) was added dropwise with stirring to a solution of **19a** (3.7 g, 14.9 mmol) in MeOH (50 ml) at 0—10 °C. After having been stirred at room temperature for 6 h, the reaction mixture was evaporated to dryness *in vacuo*. The residue was dissolved in AcOEt. This solution was washed with water, dried over $MgSO_4$ and concentrated *in vacuo*. The residue was recrystallized from hexane to give **19b** (3.8 g, 97%) as colorless prisms, mp 88—89 °C. NMR δ: 1.23 (6H, s), 2.31 (3H, s), 3.29 (2H, s), 3.92 (3H, s), 4.03 (3H, s), 8.10 (1H, s). IR (KBr): 2962, 1713, 1585, 1485, 1380, 1239, 1065, 1001, 778 cm^{-1}. MS m/z (%): 262 (M^+, 100), 247 (76), 233 (69), 187 (40), 159 (39), 115 (45). *Anal.* Calcd for $C_{15}H_{18}O_4$: C, 68.69; H, 6.92. Found: C, 68.77; H, 6.93.

Methyl 7-Hydroxy-2,2,6-trimethyl-1-oxoindan-4-carboxylate (20) Sodium iodide (5.0 g, 33 mmol) was added to a stirred and ice-cooled solution of **19b** (3.8 g, 14 mmol) in acetonitrile (100 ml). Then, $AlCl_3$ (4.5 g, 34 mmol) was added and the reaction mixture was heated at 60 °C for 1 h, poured into water and extracted with AcOEt. The extract was washed with water, dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by column chromatography (silica gel; eluent, hexane–AcOEt = 98 : 2) and the product was recrystallized from hexane to give **20** (2.7 g, 78%) as colorless needles, mp 130—131 °C. NMR δ: 1.26 (6H, s), 2.27 (3H, s), 3.29 (2H, s), 3.90 (3H, s), 8.06 (1H, s), 9.88 (1H, s). IR (KBr): 2964, 1716, 1680, 1428, 1298, 1200, 1070, 726 cm^{-1}. MS m/z (%): 248 (M^+, 100), 233 (73), 205 (63), 189 (88), 145 (44), 115 (45). *Anal.* Calcd for $C_{14}H_{16}O_4$: C, 67.73; H, 6.50. Found: C, 67.63; H, 6.48.

Methyl 7-Hydroxy-2,2,6-trimethyl-1-oxoindan-4-carboxylate Oxime (21) A mixture of **20** (2.65 g, 10.7 mmol), hydroxylamine hydrochloride (0.89 g, 12.8 mmol) and pyridine (1.3 ml) in MeOH (60 ml) and $CHCl_3$ (15 ml) was refluxed for 2 h. After removal of the solvent, the residue was poured into water and the solution was extracted with $CHCl_3$. The extract was washed with 10% HCl and water, dried over $MgSO_4$ and concentrated *in vacuo*. The residue was recrystallized from AcOEt–hexane to give **21** (2.85 g, quant.) as colorless prisms, mp 133—135 °C. NMR δ: 1.55 (6H, s), 2.26 (3H, s), 3.25 (2H, s), 3.87 (3H, s), 7.82 (1H, s), 9.70 (1H, s). IR (KBr): 3372, 2955, 1693, 1596, 1439, 1243, 1211, 1102, 969, 776 cm^{-1}. MS m/z (%): 263 (M^+, 52), 230 (25), 214 (29), 213 (72), 198 (100). *Anal.* Calcd for $C_{14}H_{17}NO_4$: C, 63.87; H, 6.51; N, 5.32. Found: C, 63.95; H, 6.45; N, 5.39.

Methyl 1-Amino-7-hydroxy-2,2,6-trimethylindan-4-carboxylate (22) A mixture of **21** (2.85 g, 10.8 mmol) and PtO_2 (0.28 g) in AcOH (100 ml) was hydrogenated at 60 °C and 4 kg/cm^2. The mixture was cooled to room temperature and the catalyst was removed by filtration. The filtrate was concentrated *in vacuo* and the residue was dissolved in $CHCl_3$. The $CHCl_3$ solution was washed with saturated $NaHCO_3$ solution, dried over $MgSO_4$ and concentrated *in vacuo*. The residue was recrystallized from CH_2Cl_2–hexane to give **22** (2.59 g, 96%) as pale violet needles, mp 188—191 °C. NMR δ: 0.89 (3H, s), 1.24 (3H, s), 2.21 (3H, s), 2.86 (1H, d, J=17.0 Hz), 3.20 (1H, d, J=17.0 Hz), 3.83 (3H, s), 4.17 (1H, s), 7.67 (1H, s). IR (KBr): 3378, 2952, 1693, 1591, 1433, 1311, 1212, 891, 775 cm^{-1}. MS m/z (%): 249 (M^+, 26), 232 (100), 217 (48), 200 (28), 185 (44), 173 (60). *Anal.* Calcd for $C_{14}H_{19}NO_3$: C, 67.45; H, 7.68; N, 5.62. Found: C, 67.13; H, 7.71; N, 5.49.

Methyl 1-(Chloroacetylamino)-7-hydroxy-2,2,6-trimethylindan-4-carboxylate (23) Chloroacetyl chloride (0.61 ml) was added dropwise to a stirred and ice-cooled solution of **22** (1.59 g, 6.38 mmol) and triethylamine (0.89 ml) in $CHCl_3$ (50 ml). Stirring was continued for 2 h, then the mixture was poured into 10% HCl. The $CHCl_3$ layer was separated, dried over $MgSO_4$ and concentrated *in vacuo*. The residue was recrystallized from CH_2Cl_2–hexane to give **23** (2.2 g, quant.) as colorless prisms, mp 221—222 °C. NMR δ: 1.22 (3H, s), 1.32 (3H, s), 2.33 (3H, s), 3.14 (1H, d, J=17.5 Hz), 3.23 (1H, d, J=17.5 Hz), 3.85 (3H, s), 4.11 (1H, d, J=15.8 Hz), 4.17 (1H, d, J=15.8 Hz), 4.69 (1H, d, J=7.5 Hz), 7.30 (1H, d, J=7.5 Hz), 7.75 (1H, s), 9.20 (1H, s). IR (KBr): 3303, 2955, 1667, 1537, 1271, 1211, 1023, 780 cm^{-1}. MS *m/z* (%): 325 (M^+, 19), 248 (41), 232 (100), 217 (23), 200 (35), 173 (55). *Anal.* Calcd for $C_{16}H_{20}ClNO_4$: C, 58.99; H, 6.19; N, 4.30. Found: C, 58.70; H, 6.02; N, 4.31.

Methyl 7-Hydroxy-1-[4-(3-methoxyphenyl)-1-piperazinylacetylamino]-2,2,6-trimethylindan-4-carboxylate (24) A solution of **23** (5.0 g, 15.5 mmol), 1-(3-methoxyphenyl)piperazine hydrochloride (6.2 g, 23.2 mmol) and triethylamine (11 ml, 79.4 mmol) in acetonitrile (150 ml) was refluxed for 5 h. After removal of the solvent, the residue was poured into water and extracted with AcOEt. The extract was dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by column chromatography (silica gel; eluent, hexane : AcOEt = 2 : 1) to give **24** (6.1 g, 82%) as a pale yellow amorphous powder. NMR δ: 1.10 (3H, s), 1.27 (3H, s), 2.24 (3H, s), 2.65 (2H, m), 2.74 (2H, m), 3.16 (8H, m), 3.79 (3H, s), 3.84 (3H, s), 4.66 (1H, d, J=7.5 Hz), 6.45 (2H, m), 6.53 (1H, d, J=8.5 Hz), 7.18 (1H, dd, J=8.5, 8.5 Hz), 7.78 (1H, s), 8.13 (1H, d, J=7.7 Hz), 9.92 (1H, br s). IR (KBr): 2951, 1709, 1599, 1517, 1202, 1017, 759 cm^{-1}.

7-Hydroxy-4-(hydroxymethyl)-1-[4-(3-methoxyphenyl)-1-piperazinylacetylamino]-2,2,6-trimethylindan (5a) A suspension of **24** (0.24 g, 0.5 mmol) in dry THF (4 ml) was treated with $LiAlH_4$ (41 mg, 1.1 mmol) under cooling in ice-water. The reaction mixture was stirred at room temperature for 5 h. Dilute HCl was carefully added to destroy the excess $LiAlH_4$. The mixture was extracted with CH_2Cl_2. The extract was dried over $MgSO_4$ and concentrated *in vacuo*. The residue was purified by column chromatography (silica gel; eluent, hexane : AcOEt = 3 : 2) and the product was recrystallized from AcOEt–hexane to give **5a** (0.12 g, 54%) as white granules, mp 108—110 °C. NMR δ: 1.13 (3H, s), 1.26 (3H, s), 2.22 (3H, s), 2.63—2.95 (6H, m), 3.03—3.17 (7H, m), 3.79 (3H, s), 4.52 (2H, d, J=5.5 Hz), 4.70 (1H, d, J=7.8 Hz), 6.46—6.54 (3H, m), 7.02 (1H, s), 7.18 (1H, t, J=8.7 Hz), 8.06 (1H, d, J=7.8 Hz), 9.19 (1H, s). IR (KBr): 3289, 2829, 1637, 1495, 1203, 762 cm^{-1}. MS *m/z* (%): 454 (16), 453 (M^+, 54), 205 (100), 191 (36), 70 (28). *Anal.* Calcd for $C_{25}H_{35}N_3O_4$: C, 68.85; H, 7.78; N, 9.26. Found: C, 68.77; H, 8.14; N, 9.10.

HPLC Analysis Chromatographic conditions were as follows: column, TSK-80TM (4.6 × 150 mm); mobile phase, acetonitrile–water–acetic acid (25 : 75 : 1); flow rate, 1.0 ml/min; detection, UV at 254 nm; retention time, 7.1 min (99.3%). The retention time of **5a** derived from 4-chloro-2-methylphenol was identical with that of the *in vivo* metabolite **5a** and that of **5a** obtained from cytochrome P-450 model systems.

7-Hydroxy-1-[4-(3-methoxyphenyl)-1-piperazinylacetylamino]-2,2,6-trimethylindan-4-carboxylic Acid (8) A solution of **24** (1.95 g, 4.05 mmol) in 10% KOH (20 ml) and MeOH (20 ml) was stirred at room temperature for 2 d, then acidified with 10% HCl and extracted with $CHCl_3$. The extract was washed with water, dried over $MgSO_4$ and concentrated *in vacuo*. The residue was recrystallized from AcOEt to give **8** (1.64 g, 87%) as a white powder, 210 °C (dec.). NMR δ: 1.11 (3H, s), 1.27 (3H, s), 2.24 (3H, s), 2.68—2.74 (4H, m), 3.06—3.30 (8H, m), 3.78 (3H, s), 4.67 (1H, d, J=7.4 Hz), 6.46—6.55 (3H, m), 7.18 (1H, t, J=8.4 Hz), 8.13 (1H, d, J=7.4 Hz), 9.55—10.20 (1H, br s). IR (KBr): 3300, 2961, 1698, 1600, 1208, 1169, 781 cm^{-1}. MS *m/z* (%): 467 (M^+, 18), 205 (100), 162 (27), 91 (33), 70 (80). *Anal.* Calcd for $C_{26}H_{33}N_3O_5$: C, 66.79; H, 7.11; N, 8.99. Found: C, 66.38; H, 7.08; N, 8.90.

HPLC Analysis Chromatographic conditions were as follows: column, TSK-80TM (4.6 × 150 mm); mobile phase, acetonitrile–water–acetic acid (30 : 70 : 1); flow rate, 1.0 ml/min; detection, UV at 254 nm; retention time, 6.8 min (99.1%).

References and Notes

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