SYNTHESES AND BIOACTIVITIES OF ACETOPHTHALIDIN AND ITS DERIVATIVES

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Abstract —Acetophthalidin is a potent cell cycle inhibitor which was isolated from a fungal strain. Both enantiomers of acetophthalidin and its derivatives were synthesized, and their bioactivities were examined.

INTRODUCTION

In 1996, Osada *et al.* isolated acetophthalidin (**1**, Figure 1) from a culture broth of a fungal strain BM923.[1] This simple phenolic lactone shows a potent inhibitory activity to the cell cycle progression of the mouse tsFT210 cells in the M phase. Due to the instability of **1**, however, it has isomerized readily to inactive trihydroxymellein (**2**) during the isolation and was obtained only as a racemate by heating the isolated **2** under acidic conditions. This nature as well as its strong activity made us to synthesize both enantiomers of **1** to clarify the difference of their activities.[2,3]

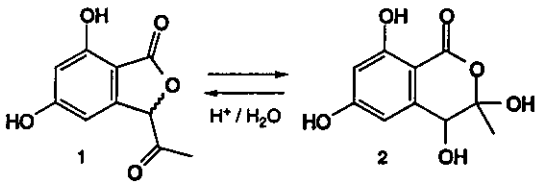


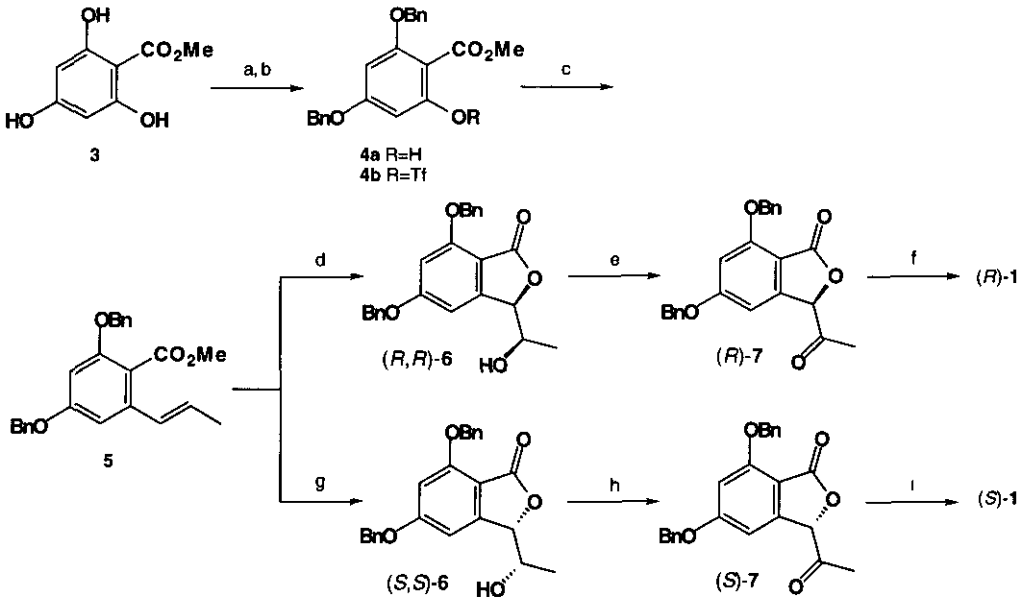
Figure 1.

RESULTS AND DISCUSSION

We started from methyl phloroglucinolcarboxylate[4] (**3**, Scheme 1). Benzylation of **3** using 2.1 eq. of benzyl bromide gave its 2,4-dibenzyl ether (**4a**) as a major product (47%). The dibenzyl ether (**4a**) was treated with trifluoromethanesulfonic anhydride to give **4b** as colorless needles (77%). Coupling of **4b** with excess tri-*n*-butyl-1-propenyltin (1:1 *E*/*Z* mixture)[5,6] under Stille's conditions[7] afforded a mixture

of (*E*)- and (*Z*)-olefins (**5**) (3:1 by ^{1}H NMR), which on recrystallization from hexane / ethyl acetate gave almost pure (*E*)-isomer (50:1, 59%). Asymmetric dihydroxylation of **5** with AD-mix-β[8] gave optically active hydroxylactone (*R*, *R*)-**6** of 99.7% e.e. (by HPLC, see Experimental), which was recrystallized to give pure (*R*, *R*)-**6** with ~100% e.e.

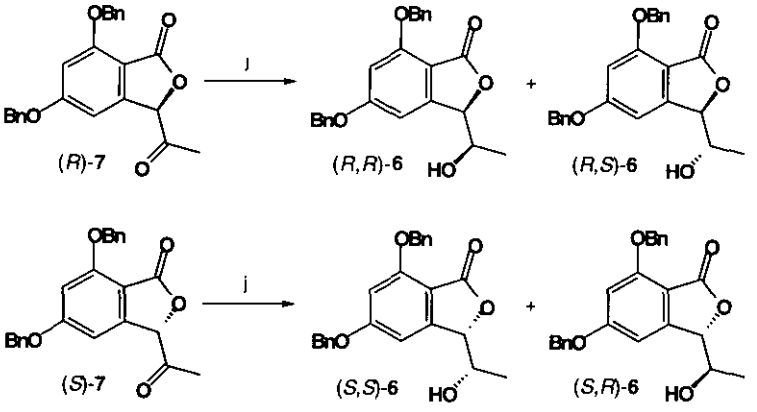
The next oxidation step was critical because we had to optimize the procedure to avoid racemization and/or isomerization to trihydroxymellein derivative. Thus, we selected Dess-Martin oxidation[9,10] or two-phase Jones oxidation[11] to oxidize the *sec*-hydroxyl group of (*R*, *R*)-**6** because the reaction was fast and (*R*)-**7** was obtained as a single product. When PCC or PDC was used, the reaction became slower and undesired isomerization to trihydroxymellein derivative occurred. It was unsuccessful to determine its enantiomeric purity directly by HPLC with several columns with optically active stationary phase. We then tried the following method (Scheme 2). Reduction of (*R*)-**7** with lithium tri-*t*-butoxyaluminohydride in THF afforded a mixture of (*R*, *R*)-**6** and (*R*, *S*)-**6**, and the enantiomeric purity of (*R*, *R*)-**6** was determined by HPLC (see Experimental). It seemed partial racemization occurred during the oxidation (or the reduction), that is, two-phase Jones oxidation gave (*R*)-**7** with 87.0% e.e., while Dess-Martin oxidation gave that with 89.7% e.e.



Scheme 1. a) BnBr (2.1 eq.), K_2CO_3, NaI, DMF, rt, 12 h, 47%. b) Tf_2O, pyridine, CH_2Cl_2, –40 °C, 0.5 h; 0 °C, 3.5 h, 77%. c) MeCH=CHSnBu$^n_3$ (*EZ* mixture, 1.7 eq.), Pd(0)[PPh_3]$_4$, LiCl, THF, 90 °C, 7 d; recryst'n, 59%. d) AD-mix-β, $MeSO_2NH_2$, Bu^{t}OH, H_2O, 4 °C, 12 d, recryst'n, 75%. e) Dess-Martin reagent, CH_2Cl_2, rt, 1 h, 91%. f) H_2, 10% $Pd(OH)_2$-C, AcOEt, rt, 15 min, 71%. g) AD-mix-α, $MeSO_2NH_2$, Bu^{t}OH, H_2O, 4 °C, 3 d; recryst'n, 79%. h) Dess-Martin reagent, CH_2Cl_2, rt, 1 h, 99.5%. i) H_2, 10% $Pd(OH)_2$-C, AcOEt, rt, 15 min, 87%.

The crude (*R*)-**7** obtained by Dess-Martin oxidation was immediately recrystallized from hexane / ethyl acetate without heating to give pure (*R*)-**7** as a colorless fine needles (91% yield). Finally, benzyl groups were hydrogenolyzed using large amount of 10% $Pd(OH)_2$-C in ethyl acetate in less than 15 min

to give (*R*)-**1** which was immediately recrystallized as a colorless powder (71%). The mp was 189.0~201.0 °C (decomp) and the specific rotation was $[\alpha]^{20}_{D}$ +23° (*c* 0.89, AcOEt). ^{1}H NMR and IR spectra were identical with those in the literature.[1]



Scheme 2. j) $Li(Bu^tO)_3AlH$, THF, −30°C, 1 h.

In the same manner, (*S*)-**1** was synthesized. Asymmetric dihydroxylation of **5** with AD-mix-α[8] gave (*S*, *S*)-**6** of 99.3% e.e. (by HPLC), which was recrystallized to give (*S*, *S*)-**6** with ~100% e.e. (79% yield). Dess-Martin oxidation of (*S*, *S*)-**6** afforded (*S*)-**7** which was recrystallized at low temperature to give pure (*S*)-**7** in 99.5% yield. Enantiomeric purity of (*S*)-**7** determined in the same procedure (Scheme 2) was 92.7% e.e. Then it was hydrogenated similarly to give (*S*)-**1** which was immediately reprecipitated as a colorless powder (87%). Its mp was 190.0~201.0 °C (decomp) and its specific rotation was $[\alpha]^{20}_{D}$ −27° (*c* 0.72, AcOEt). ^{1}H-NMR spectrum and IR spectrum data were identical with those of (*R*)-**1**.

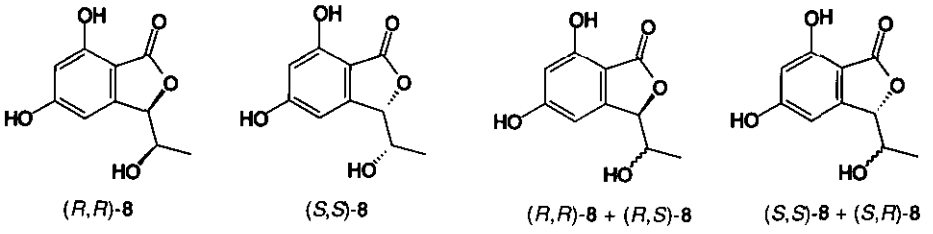
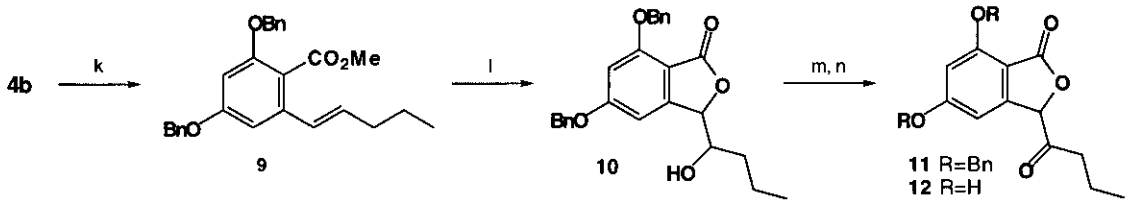


Figure 2.

Several derivatives were also synthesized as below. The hydroxy analogs of Figure 2 were derived from their dibenzyl ethers, respectively (see Experimental). On the other hand, the racemic butyryl analogue (**12**) was also synthesized in the same manner with (*R*)- and (*S*)-**1** using 1-pentenyl tri-*n*-butyltin as a coupling partner with **3b** and osmium tetroxide instead of AD-mix (Scheme 3). The bioactivity of these products was examined with a mouse mammary carcinoma cell line FM3A. The results were



Scheme 3. k) $Me(CH_2)_2CH=CHSnBu^n_3$, $Pd(PPh_3)_4$, LiCl, THF, reflux, overnight, 88%; l) OsO_4, NMO, Bu'OH, H_20, rt, overnight, 82%; m) Dess-Martin reagent, CH_2Cl_2, rt, 30 min, 93%; n) H_2, 10% $Pd(OH)_2$-C, AcOEt, rt, 9 min, 75%.

shown in Figure 3. The racemic **1** from natural source, (*R*)-**1** and (*S*)-**1** accumulated cells in G2/M phase, and there was no difference of cell cycle inhibition. This result suggests that the racemization may occur faster than the appearance of the activity. As the hydroxy analogs were all inactive, the carbonyl group must be necessary to show the activity. Interestingly, racemic **12** inhibited the cell growth completely, but did not accumulate the cells in G2/M phase. Acetophthalidin (**1**) might inhibit the specific molecule(s) necessary for the M phase progression, however, racemic **12** might easily affect other molecules required for the cell viability.

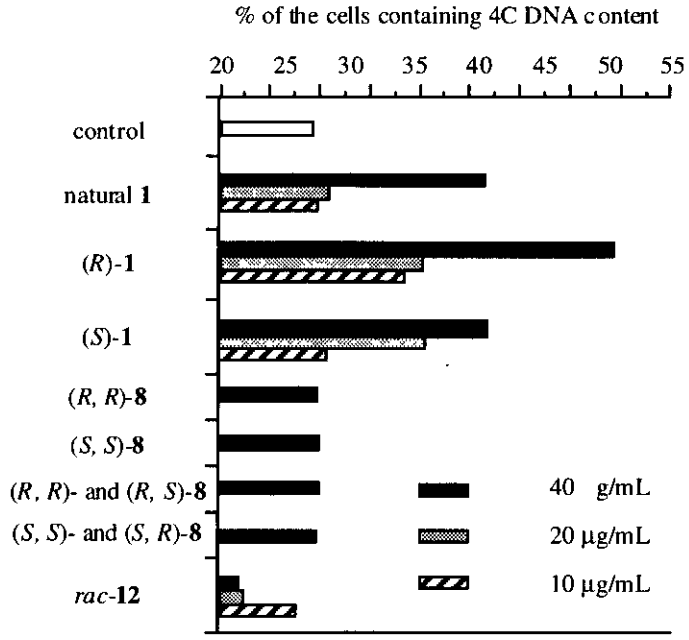


Figure 3. The cell cycle population in the G2/M phase was determined by flow cytometry.

In conclusion, we synthesized the both enantiomers of acetophthalidin and its derivatives. The overall yield was 10% for (*R*)-**1** and 15% for (*S*)-**1**. As described in the literature, the synthetic **1** was also very unstable ($T_{1/2}$ of $[\alpha]_D$ was 33 min in MeOH at 22°C), and against our expectation, both the enantiomers showed no difference in respect of the cell cycle inhibition. From the bioassay of other derivatives, it

was clarified that the carbonyl group must be necessary to appear the activity and the length of side chain might have some important roll for M phase specific cell cycle inhibition.

EXPERIMENTAL

IR spectra: Jasco FT / IR 230 spectrophotometer. ^{1}H-NMR spectra: JEOL JNM EX-90 (90 MHz) spectrometer, Bruker AC-300 spectrometer (300 MHz) or JEOL JNM GSX-500 spectrometer (500 MHz). ^{13}C-NMR spectra: JEOL JNM GSX-500 spectrometer (125 MHz). Specific rotations: Jasco DIP-371 polarimeter. Refractive indexes: Atago 1T refractometer. HPLC: Shodex DS-4 and SSC UV detector 3000B (254 nm). HRMS spectra: JEOL JMS-SX102 / SX102. Column chromatography: Merck Kieselgel 60 (Art. Nr. 7734). Preparative silica gel TLC: Merck Kieselgel F-254. Melting points: Yanako micro-melting point apparatus. Boiling point and melting points are uncorrected.

Methyl 2,4-dibenzyloxy-6-hydroxybenzoate (**4a**).
Benzyl bromide (4.10 mL, 34.5 mmol) was added to a suspension of **3** (3.10 g, 16.8 mmol), potassium carbonate (4.72 g, 68.4 mmol) and sodium iodide (0.806 g, 5.38 mmol) in dimethylformamide (30 mL) at 0°C. The reaction mixture was stirred at rt for 12 h. It was poured into ice-water and acidified with concentrated hydrochloric acid to adjust pH 5. The mixture was extracted with ether and dichloromethane. The combined organic layer was successively washed with 15% aqueous sodium thiosulfate solution, water and brine, dried with anhydrous sodium sulfate and concentrated *in vacuo*. The residue was chromatographed over silica gel (250 g) and elution with hexane / ethyl acetate (15:1-5:1) afforded a mixture of **4a** and tribenzyl ether (9.50 g) as a slightly pink solid. It was recrystallized from hexane / ethyl acetate (3:1) to give pure **4a** (2.89 g, 47%) as colorless needles; mp 117.0°C; IR (KBr): ν = 2950, 1640, 1620, 1580, 1500, 1440, 1430, 1400, 1380, 1320, 1300, 1260, 1215, 1170, 1100, 1045, 1030, 800, 730 cm^{-1}; ^{1}H-NMR (90 MHz in $CDCl_3$): δ = 3.91 (3H, s, -CO_2CH_3), 5.04 (2H, s, -OCH_2Ph), 5.07 (2H, s, -OCH_2Ph), 6.13 (1H, d, *J* = 2.2 Hz, 3- or 5-H), 6.22 (1H, d, *J* = 2.2 Hz, 3- or 5-H), 7.25-7.60 (10H, m, -$OCH_2C_6H_5$), 12.01 (1H, s, 6-OH); *Anal.* Calcd for $C_{22}H_{20}O_5$: C, 72.51; H, 5.53. Found: C, 72.37; H, 5.55.

Methyl 2,4-dibenzyloxy-6-trifluoromethanesulfonyloxybenzoate (**4b**).
Trifluoromethanesulfonic anhydride (0.86 mL, 7.14 mmol) was added to a solution of **4a** (2.00 g, 5.49 mmol) and pyridine (1.33 mL, 16.5 mmol) in dry dichloromethane (20 mL) at –40°C. The mixture was stirred at –40°C for 30 min and then at 0°C for 3.5 h. It was poured into ice-water and extracted with ether. The organic layer was washed with brine, dried with anhydrous magnesium sulfate and concentrated *in vacuo*. The residure was chromatographed over silica gel (100 g) and elution with hexane / ethyl acetate (15:1-0:1) afforded 5.49 g of **4b** as a slightly pink solid. This was recrystallized from hexane / ethyl acetate (3:1) to give pure **4b** (2.10 g, 77%) as colorless needles; mp 95.0-95.5°C; IR (KBr): ν = 3040, 2950, 1720, 1620, 1570, 1440, 1420, 1400, 1330, 1300, 1280, 1240, 1220, 1210, 1190,

1170, 1140, 1110, 1060, 1035, 840, 730, 770 cm^{-1}; ^{1}H-NMR (90 MHz in $CDCl_3$): δ = 3.90 (3H, s, $-CO_2CH_3$), 5.03 (2H, s, $-OCH_2Ph$), 5.11 (2H, s, $-OCH_2Ph$), 6.02 (1H, d, *J* = 2.2 Hz, 3- or 5-H), 6.08 (1H, d, *J* = 2.2 Hz, 3- or 5-H), 7.30-7.46 (10H, m, $-OCH_2C_6H_5$); *Anal.* Calcd for $C_{23}H_{19}O_7F_3S$: C, 55.64; H, 3.86. Found: C, 56.07; H, 3.92.

Methyl 2,4-dibenzyloxy-6-(1-propenyl)benzoate (**5**).

A mixture of the triflate (**4b**) (15.0 g, 30.2 mmol), dry lithium chloride (3.10 g, 73.1 mmol), 1-propenyl-tri-*n*-butyltin[5,6] (*E* / *Z* = 1:1, 18.6 g, 51.8 mmol), tetrakis(triphenylphosphin)palladium (0) (715 mg, 0.619 mmol) in dry tetrahydrofuran (90 mL) was refluxed for 7 d under argon. The reaction mixture was cooled, poured into 10% aqueous NH_3 solution and extracted with ether. The organic layer was successively washed with 10% aqueous NH_3 solution, saturated aqueous sodium bicarbonate solution, water and brine, dried with anhydrous magnesium sulfate and concentrated *in vacuo*. The residue was chromatographed over silica gel (300 g) and elution with hexane / ethyl acetate (10:1-3:1) gave crude **5** (13.7 g) as a colorless solid (*E* / *Z* = 3:1). It was recrystallized twice from hexane / ethyl acetate (2:1, 5:1) to give almost pure (*E*)-**5** (*E* / *Z* = 50:1) as a colorless needles; mp 101.0-102.0°C; IR (KBr): ν = 3030, 2940, 1720, 1600, 1580, 1450, 1440, 1380, 1355, 1290, 1260, 1240, 1220, 1170, 1100, 1040, 970, 830, 730, 700 cm^{-1}; ^{1}H-NMR (500 MHz in $CDCl_3$) for (*E*)-iomer: δ = 1.89 (3H, dd, *J* = 1.7 Hz, 6.5 Hz, $=CHCH_3$), 3.89 (3H, s, $-CO_2CH_3$), 5.05 (2H, s, $-OCH_2Ph$), 5.06 (2H, s, $-OCH_2Ph$), 6.20 (1H, dq, *J* = 15.5 Hz, 6.5 Hz, $-CH=CHCH_3$), 6.41 (1H, dq, *J* = 15.5 Hz, 1.7 Hz, $-CH=CHCH_3$), 6.47 (1H, d, *J* = 2.0 Hz, 3- or 5-H), 6.71 (1H, d, *J* = 2.0 Hz, 3- or 5-H), 7.28-7.43 (10H, m, $-OCH_2C_6H_5$); for (*Z*)-isomer (partial): δ = 1.71 (3H, dd, *J* = 7.0 Hz, 1.8 Hz, $=CHCH_3$), 3.86 (3H, s, $-CO_2CH_3$), 5.09 (2H, s, $-OCH_2Ph$), 5.84 (1H, dq, *J* = 11.4 Hz, 7.0 Hz, $-CH=CHCH_3$), 6.43 (1H, m, $-CH=CHCH_3$), 6.51 (1H, d, *J* = 2.0 Hz, 3- or 5-H), 6.53 (1H, d, *J* = 2.0 Hz, 3- or 5-H); *Anal.* Calcd for $C_{25}H_{24}O_4$: C, 77.30; H, 6.23. Found: C, 77.27; H, 6.22.

5,7-Dibenzyloxy-3-(1-hydroxyethyl)phthalide (**6**).

(a) *(R, R)-isomer:* Methanesulfonamide (1.60 g, 16.8 mmol) was added to a two-phase stirred mixture of AD-mix-β (23.2 g), water (80 mL) and *t*-butyl alcohol (80 mL) at rt, and the reaction mixture was then ice-cooled. The olefin (**5**) (3.25 g, 8.38 mmol) was added to the resulting orange suspension and the reaction mixture was vigorously stirred at 4°C for 5 d. After sodium sulfite (12.4 g) was added, the mixture was warmed to rt and stirred for 1 h. It was diluted with water and extracted with ethyl acetate. The organic layer was washed with water and brine, dried with anhydrous magnesium sulfate and concentrated *in vacuo*. The residue was chromatographed over silica gel (150 g) and elution with hexane / ethyl acetate (2:1-1:2) gave (*R, R*)-**6** (2.82 g) as a colorless solid. Recrystallization from hexane / ethyl acetate (3:2) afforded pure (*R, R*)-**6** (2.44 g, 75%) as colorless powder; $[\alpha]_D^{19}$ –40.5° (*c* 1.17, MeOH); mp 122.0-123.5°C; IR (KBr): ν = 3500, 3420, 1730, 1610, 1500, 1450, 1380, 1350, 1340, 1210, 1170, 1070, 825, 730, 700 cm^{-1}; ^{1}H-NMR (500 MHz in $CDCl_3$): δ = 1.34 (3H, d, *J* = 6.3 Hz, $-CH(OH)CH_3$), 1.90 (1H, br dd, *J* = 3.7 Hz, 6.0 Hz, -OH,), 4.10 (1H, ddq, *J* = 4.1 Hz, 6.0 Hz, 6.3 Hz, $-CH(OH)CH_3$), 5.06 (2H, s, $-OCH_2Ph$), 5.17 (1H, br d, *J* = 4.1 Hz, 3-H), 5.25 (2H, s, $-OCH_2Ph$), 6.54 (1H,

d, J = 1.6 Hz, 4- or 6-H), 6.60 (1H, m, 4- or 6-H), 7.28-7.48 (10H, m, -$OCH_2C_6H_5$); *Anal.* Calcd for $C_{24}H_{22}O_5$: C, 73.83; H, 5.68. Found: C, 73.67; H, 5.68.

(b) *(S, S)-isomer:* In the same manner as described above, the olefin **5** (3.25 g, 8.38 mmol) was treated with AD-mix-α (192 mg), methanesulfonamide (15.0 mg), water (1 mL) and *t*-butyl alcohol (2 mL) to give (*S*, *S*)-**6** (24.8 mg, 79%) as colorless powder; $[\alpha]_D^{21}$ +40.9° (*c* 1.36, MeOH); mp 121.5-122.0°C; IR spectrum was identical with that of (*R*, *R*)-**6**; ^{1}H-NMR (500 MHz in $CDCl_3$): δ = 1.34 (3H, d, J = 6.3 Hz, -CH(OH)CH_3), 1.95 (1H, br d, J = 6.0 Hz, -OH,), 4.10 (1H, ddq, J = 4.1 Hz, 6.0 Hz, 6.3 Hz, -CH(OH)CH_3), 5.06 (2H, s, -OCH_2Ph), 5.17 (1H, br d, J = 4.1 Hz, 3-H), 5.25 (2H, s, -OCH_2Ph), 6.54 (1H, d, J = 1.6 Hz, 4- or 6-H), 6.60 (1H, m, 4- or 6-H), 7.28-7.48 (10H, m, -$OCH_2C_6H_5$); *Anal.* Calcd for $C_{24}H_{22}O_5$: C, 73.83; H, 5.68. Found: C, 73.60; H, 5.67.

Determination of Enantiomeric Purity of ***6****.*

This was determined by HPLC (Ceramospher Chiral RU-1, 4.6 mm ϕ × 250 mm (SHISEIDO); eluent, MeOH; flow rate, 0.3 mL/min). (*R*, *R*)-**6**: (before recrystallization) t_R = 36.8 min (99.86%), 44.2 min (0.14%); (after recrystallization) t_R = 36.8 min (single peak). These figures proved the enantiomeric purity of crude (*R*, *R*)-**6** to be 99.7% e.e. and that of purified (*R*, *R*)-**6** to be about 100% e.e. (*S*, *S*)-**6**: (before recrystallization) t_R = 37.1 min (0.33%), 44.9 min (99.67%); (after recrystallization) t_R = 36.3 min (0.01%), 42.9 min (99.92%). These figures proved the enantiomeric purity of crude (*S* ,*S*)-**6** to be 99.3% e.e. and that of purified (*S*, *S*)-**6** to be about 100% e.e.

5,7-Dibenzyloxy-3-(1-acetyl)phthalide (**7**).

(a) *(R)-isomer:* Dess-Martin periodinane (870 mg, 2.05 mmol) was added to the alcohol ((*R*, *R*)-**6**) (295 mg, 0.756 mmol) in dry dichloromethane (15 mL) at rt and the reaction mixture was stirred for 1 h. It was diluted with ether, quenched with saturated aqueous sodium bicarbonate solution and sodium sulfite, and extracted with ethyl acetate. The organic layer was successively washed with saturated aqueous sodium bicarbonate solution, water and brine, dried with anhydrous magnesium sulfate and concentrated *in vacuo* to *ca.* 5 mL of solution. It was cooled to 4°C to afford crystalline solid, which was filtered to give pure (*R*)-**7** (266 mg, 91%) as colorless fine needles; $[\alpha]_D^{22}$ +171° (*c* 0.95, $CHCl_3$); mp 135.0-137.0°C; IR (KBr): ν = 3450, 1760, 1720, 1610, 1500, 1380, 1355, 1335, 1240, 1200, 1160, 1020, 840, 740, 700 cm^{-1}; ^{1}H-NMR (500 MHz in $CDCl_3$): δ = 2.21 (3H, s, -C(=O)CH_3), 5.06 (2H, s, -OCH_2Ph), 5.25 (2H, s, -OCH_2Ph), 5.51 (1H, br s, 3-H), 6.57 (1H, d, J = 1.8 Hz, 4- or 6-H), 6.71 (1H, m, 4- or 6-H), 7.30-7.50 (10H, m, -$OCH_2C_6H_5$); *Anal.* Calcd for $C_{24}H_{20}O_5$: C, 74.21; H, 5.19. Found: C, 73.73; H, 5.18.

(b) *(S)-isomer:* In the same manner as described above, the alcohol ((*S*, *S*)-**6**) (225 mg, 0.576 mmol) was treated with Dess-Martin periodinane (627 mg, 1.48 mmol) in dry dichloromethane (10 mL) to give (*S*)-**7** (222 mg, 99.5%) as colorless fine needles; $[\alpha]_D^{19}$ –166° (*c* 1.24, $CHCl_3$); mp 133.0-135.0°C; IR and ^{1}H-NMR spectra were identical with those of (*R*)-**7**; *Anal.* Calcd for $C_{24}H_{20}O_5$: C, 74.21; H, 5.19. Found: C, 73.82; H, 5.12.

Reduction of ***7*** *with lithium tri-t-butoxyaluminohydride.*

(a) *(R)-isomer:* A solution of (*R*)-**7** (6.2 mg, 0.0160 mmol) in tetrahydrofuran (2.5 mL) was added to a mixture of lithium tri-*t*-butoxyaluminohydride (1.0 M solution in tetrahydrofuran (60 μL, 0.060 mmol) in tetrahydrofuran (1 mL) at –45°C. After stirring the reaction mixture at –45°C for 3 h, it was poured into saturated aqueous ammonium chloride solution and extracted with ether. The organic layer was successively washed with saturated aqueous sodium bicarbonate solution, water and brine, dried with magnesium sulfate and concentrate *in vacuo* to give (*R*, *R*)- and (*R*, *S*)-**6** (6.2 mg, quant.); IR (KBr): ν = 3460, 2980, 1760, 1610, 1500, 1460, 1380, 1330, 1240, 1210, 1160, 1060, 1020, 840, 740, 700 cm^{-1}; ^{1}H-NMR (500 MHz in $CDCl_3$) for (*R*, *R*)-**6**: δ = 1.34 (3H, d, *J* = 6.3 Hz, -CH(OH)CH_3), 1.93 (1H, d, *J* = 6.0 Hz, -OH), 4.10 (1H, ddq, *J* = 4.2 Hz, 6.0 Hz, 6.3 Hz, -C*H*(OH)CH_3), 5.06 (2H, s, -OCH_2Ph), 5.17 (1H, d, *J* = 4.1 Hz, 3-H), 5.25 (2H, s, -OCH_2Ph), 6.53 (1H, d, *J* = 1.6 Hz, 4- or 6-H), 6.60 (1H, dd, *J* = 1.0 Hz, 1.6 Hz, 4- or 6-H), 7.28-7.49 (10H, m, -$OCH_2C_6H_5$); for (*R*, *S*)-**6**: δ = 1.24 (3H, d, *J* = 6.3 Hz, -CH(OH)CH_3), 1.99 (1H, d, *J* = 7.1 Hz, -OH), 4.04 (1H, ddq, *J* = 4.7 Hz, 7.1 Hz, 6.3 Hz, -C*H*(OH)CH_3), 5.06 (2H, s, -OCH_2Ph), 5.22 (1H, d, *J* = 4.7 Hz, 3-H), 5.25 (2H, s, -OCH_2Ph), 6.53 (1H, d, *J* = 1.6 Hz, 4- or 6-H), 6.63 (1H, dd, *J* = 1.0 Hz, 1.6 Hz, 4- or 6-H), 7.28-7.49 (10H, m, -$OCH_2C_6H_5$).

(b) *(S)-isomer:* In the same manner as described above, (*S*)-**7** (10.2 mg, 0.0263 mmol) was treated with lithium tri-*t*-butoxyaluminohydride (1.0 M solution in tetrahydrofuran, 60 μL, 0.060 mmol) and tetrahydrofuran (3.5 mL) to give (*S*, *S*)- and (*S*, *R*)-**6** (10.3 mg, quant.) as colorless crystalline solid; IR and ^{1}H-NMR spectra was identical with those of (*R*, *R*)- and (*R*, *S*)-**6**.

Determination of Enantiomeric Purity of regenerated **6**.

This was determined by HPLC (Ceramospher Chiral RU-1, 4.6 mm ϕ × 250 mm (SHISEIDO); eluent, MeOH; flow rate, 0.3 mL/min). (*R*, *R*)-**6** from (*R*)-**7**: t_R = 39.5 min (94.81%), 47.8 min (5.19%). This figure proved the enantiomeric purity of regenerated (*R*, *R*)-**6** to be 89.6% e.e. (*S* ,*S*)-**6** from (*S*)-**7**: t_R = 39.5 min (3.66%), 47.6 min (96.34%). This figure proved the enantiomeric purity of regenerated (*S* ,*S*)-**6** to be 92.7% e.e.

5,7-Dihydroxy-3-(1-acetyl)phthalide (acetophthalidin, **1**).

(a) *(R)-isomer:* A mixture of (*R*)-**7** (97.8 mg, 0.252 mmol) and 20% palladium hydroxide on carbon (wet, Degussa type E101 NE/W, Aldrich, 432 mg) in ethyl acetate (17 mL) was stirred at rt for 15 min under hydrogen atomosphere. It was filtered through Celite® and the filter cake was washed with ethyl acetate. The combined filtrate and washings were concentrated *in vacuo* to to *ca.* 3 mL of solution. Hexane was added to this solution to give colorless crystalline powder, which was filtered to give pure (*R*)-**1** (37.1 mg, 71%); $[\alpha]_D^{20}$ +23.4° (*c* 0.89, AcOEt); mp 189.0-201.0°C (decomp); IR (KBr): ν = 3310, 3170, 1720, 1620, 1480, 1360, 1330, 1230, 1200, 1160, 1045, 760, 695 cm^{-1}; ^{1}H-NMR (500 MHz in DMSO-d_6): δ = 2.17 (3H, s, -C(=O)CH_3), 5.80 (1H, s, 3-H), 6.34 (1H, d, *J* = 1.8 Hz, Ar-H), 6.38 (1H, dd, *J* = 1.8 Hz, 1.0 Hz, Ar-H), 10.56 (1H, s, Ar-OH), 10.75 (1H, s, Ar-OH); ^{13}C-NMR (125 MHz in DMSO-d_6): δ = 25.9, 82.3, 100.7, 102.0, 103.2, 148.0, 158.6, 165.0, 167.4, 203.5; HRFABMS *m/z* = 208.0378 (Calcd for $C_{10}H_8O_5$: 208.0372).

(b) *(S)-isomer:* In the same manner as described above, (*S*)-**7** (97.7 mg, 0.252 mmol) was treated with hydrogen, 20% palladium hydroxide on carbon (wet, Degussa type E101 NE/W, Aldrich, 421 mg) in ethyl acetate (15 mL) to give (*S*)-**1** (45.5 mg, 87%) as colorless crystalline powder; $[\alpha]_D^{20}$ –26.7° (*c* 0.72, AcOEt); mp 190.0-201.0°C (decomp); IR, ^{1}H- and ^{13}C- NMR spectra were identical with those of (*R*)-**1**; HRFABMS *m/z* = 208.0370 (Calcd for $C_{10}H_8O_5$: 208.0372).

5,7-Dihydroxy-3-(1-hydroxyethyl)phthalide (**8**).

(a) *(R, R)-isomer:* A mixture of (*R, R*)-**6** (50.5 mg, 0.129 mmol) and 20% palladium hydroxide on carbon (wet, Degussa type E101 NE/W, Aldrich, 359 mg) in ethyl acetate (5 mL) was stirred at rt for 30 min under hydrogen atmosphere. It was filtered through Celite® and the filter cake was washed with ethyl acetate. The combined filtrate and washings were concentrated *in vacuo* to give (*R, R*)-**6** (22.9 mg, 85%) as a colorless solid. A small portion of this sample was recrystallized for analysis and bioassay to give pure (*R, R*)-**8** as colorless crystalline powder; $[\alpha]_D^{19}$ –62.0° (*c* 0.31, MeOH); mp 206.5-208.0°C; IR (KBr): ν = 3200, 1730, 1710, 1620, 1480, 1360, 1330, 1290, 1220, 1165, 1060 cm^{-1}; ^{1}H-NMR (500 MHz in DMSO-d_6): δ = 1.03 (3H, d, *J* = 6.3 Hz, -CH(OH)C*H*$_3$), 4.00 (1H, m, -C*H*(OH)CH$_3$), 4.92 (1H, d, *J* = 4.7 Hz, -OH), 5.13 (1H, d, *J* = 3.0 Hz, 3-H), 6.26 (1H, d, *J* = 1.0 Hz, Ar-H), 6.35 (1H, br s, Ar-H), 10.32 (2H, br, Ar-OH); HRFABMS *m/z* = 210.0493 (Calcd for $C_{10}H_{10}O_5$: 210.0528).

(b) *(S, S)-isomer:* In the same manner as described above, the alcohol (*S, S*)-**6** (109 mg, 0.281 mmol) was treated with hydrogen, 20% palladium hydroxide on carbon (wet, Degussa type E101 NE/W, Aldrich, 368 mg) in ethyl acetate (10 mL) to give (*S, S*)-**7** (51.6 mg, 87%) as colorless crystalline powder; $[\alpha]_D^{18}$ +60.7° (*c* 0.29, MeOH); mp 206.0-208.0°C; IR spectrum was identical with that of (*R, R*)-**8**; ^{1}H-NMR (500 MHz in DMSO-d_6): δ = 1.03 (3H, d, *J* = 6.3 Hz, -CH(OH)C*H*$_3$), 4.00 (1H, m, -C*H*(OH)CH$_3$), 4.92 (1H, d, *J* = 4.7 Hz, -OH), 5.13 (1H, d, *J* = 3.0 Hz, 3-H), 6.26 (1H, br s, Ar-H), 6.35 (1H, br s, Ar-H), 10.28 (2H, br, Ar-OH); HRFABMS *m/z* = 210.0515 (Calcd for $C_{10}H_{10}O_5$: 210.0528).

(c) *(R, R)- + (R, S)-isomer:* In the same manner as described above, a mixture of alcohols (*R, R*)- and (*R, S*)-**6** (26.6 mg, 0.0682 mmol) was treated with hydrogen, 20% palladium hydroxide on carbon (wet, Degussa type E101 NE/W, Aldrich, 245 mg) in ethyl acetate (5 mL) to give a mixture of (*R, R*)- and (*R, S*)-**8** (8.9 mg, 62%) as colorless crystalline powder; IR (KBr): ν = 3320, 3160, 2980, 2940, 1690, 1610, 1480, 1360, 1220, 1160, 1075, 1060, 1040, 980, 840, 780 cm^{-1}; ^{1}H-NMR (500 MHz in DMSO-d_6) for (*R, R*)-isomer: δ = 1.03 (3H, d, *J* = 6.3 Hz, -CH(OH)C*H*$_3$), 4.00 (1H, m, -C*H*(OH)CH$_3$), 4.92 (1H, br d, *J* = 4.7 Hz, -OH), 5.13 (1H, d, *J* = 3.0 Hz, 3-H), 6.26 (1H, d, *J* = 1.8 Hz, Ar-H), 6.35 (1H, br, Ar-H), 10.38 (2H, br, Ar-OH); for (*R, S*)-isomer: δ = 1.00 (3H, d, *J* = 6.3 Hz, -CH(OH)C*H*$_3$), 3.80 (1H, m, -C*H*(OH)CH$_3$), 5.08 (1H, d, *J* = 4.8 Hz, 3-H), 5.17 (1H, br d, *J* = 5.0 Hz, -OH), 6.28 (1H, d, *J* = 1.8 Hz, Ar-H), 6.38 (1H, br, Ar-H), 10.38 (2H, br, Ar-OH).

(d) *(S, S)- + (S, R)-isomer:* In the same manner as described above, a mixture of alcohols (*S, S*)- and (*S, R*)-**6** (31.6 mg, 0.0810 mmol) was treated with hydrogen, 20% palladium hydroxide on carbon (wet, Degussa type E101 NE/W, Aldrich, 256 mg) in ethyl acetate (5 mL) to give a mixture of (*S, S*)- and (*S, R*)-**8** (8.9 mg 52%) as colorless crystalline solid; IR and ^{1}H-NMR spectra were identical with those of (*R, R*)- and (*R, S*)-**8**.

1-Pentenyl-tri-n-butyltin.

A mixture of 1-pentyne (13.0 mL, 132 mmol), tri-*n*-butyltin hydride (26.5 mL, 98.5mmol) and AIBN (0.180 g, 1.10 mmol) was stirred at 130°C for 3.5 h under argon. After cooling, a brown precipitate was excluded by decantation and the residue was distilled to give 1-pentenyl-tri-*n*-butyltin (*E* / *Z* = 7:1, 32.3 g, 68 %) as a colorless oil; n_D^{23} 1.4795; IR (KBr): ν = 2960, 2940, 2875, 2850, 1600, 1460, 1420, 1380, 1340, 1070, 990, 960, 870, 860 cm^{-1}; ^{1}H-NMR (500 MHz in $CDCl_3$) for (*E*)-iomer: δ = 0.82-0.95 (9H, m, -Sn$CH_2$$CH_2$- and 5-H), 0.89 (9H, t, *J* = 7.3 Hz, -Sn$(CH_2)_2CH_2CH_3$), 1.31 (6H, m, -Sn$(CH_2)_2CH_2CH_3$), 1.36-1.58 (8H, m, -Sn$CH_2CH_2CH_2CH_3$ and 4-H), 2.11 (2H, br dt, *J* = 6.8 Hz, 6.8 Hz, 3-H), 5.87 (1H, br d, *J* = 19.0 Hz, 1-H), 5.94 (1H, dt, *J* = 5.9 Hz, 19.0 Hz, 2-H); for (*Z*)-isomer (partial): δ = 2.00 (2H, br dt, *J* = 7.4 Hz, 7.4 Hz, 3-H), 5.79 (1H, br d, *J* = 12.4 Hz, 1-H), 5.51 (1H, dt, *J* = 12.4 Hz, 6.9 Hz, 2-H); *Anal.* Calcd for $C_{17}H_{36}Sn$: C, 56.85; H, 10.10. Found: C, 56.92; H, 10.04.

Methyl 2,4-dibenzyloxy-6-(1-pentenyl)benzoate (**9**).

A mixture of the triflate (**4b**) (3.01 g, 6.07 mmol), dry lithium chloride (0.810 g, 19.1 mmol), 1-pentenyl-tri-*n*-butyltin (*E* / *Z* = 7:1, 2.63 g, 7.33 mmol), tetrakis(triphenylphosphin)palladium (0) (166 mg, 0.138 mmol) in dry tetrahydrofuran (20 mL) was refluxed for overnight under argon. The reaction mixture was cooled, poured into 10% aqueous NH_3 solution and extracted with ether. The organic layer was successively washed with 10% aqueous NH_3 solution, saturated aqueous sodium bicarbonate solution, water and brine, dried with anhydrous magnesium sulfate and concentrated *in vacuo*. The residue was chromatographed over silica gel (140 g) and elution with hexane / ethyl acetate (10:1-3:1) gave crude **9** (2.22 g, 88%) as a yellow oil (*E* / *Z* = 7:1); n_D^{23} = 1.5710; IR (KBr): ν = 2960, 2940, 1730, 1650, 1600, 1580, 1500, 1455, 1430, 1380, 1320, 1290, 1260, 1160, 1100, 1040, 820, 740, 700 cm^{-1}; ^{1}H-NMR (500 MHz in $CDCl_3$) for (*E*)-iomer: δ = 0.94 (3H, t, *J* = 7.4 Hz, -CH_2CH_3), 1.48 (2H, tq, *J* = 7.4 Hz, 7.4 Hz, -$CH_2CH_2CH_3$), 2.17 (2H, ddt, *J* = 1.5 Hz, 7.2 Hz, 7.4 Hz, =$CHCH_2CH_2$-), 3.87 (3H, s, -CO_2CH_3), 5.04 (2H, s, -OCH_2Ph), 5.05 (2H, s, -OCH_2Ph), 6.16 (1H, dt, *J* = 15.5 Hz, 7.2 Hz, -CH=$CHCH_2$-), 6.37 (1H, dt, *J* = 15.5 Hz, 1.5 Hz, -CH=$CHCH_2$-), 6.45 (1H, d, *J* = 2.0 Hz, 3- or 5-H), 6.71 (1H, d, *J* = 2.0 Hz, 3- or 5-H), 7.26-7.42 (10H, m, -$OCH_2C_6H_5$); for (*Z*)-isomer: δ = 0.86 (3H, t, *J* = 7.4 Hz, -CH_2CH_3), 1.38 (2H, tq, *J* = 7.4 Hz, 7.4 Hz, -$CH_2CH_2CH_3$), 2.07 (2H, ddt, *J* = 1.8 Hz, 7.2 Hz, 7.4 Hz, =$CHCH_2CH_2$-), 3.83 (3H, s, -CO_2CH_3), 5.04 (2H, s, -OCH_2Ph), 5.07 (2H, s, -OCH_2Ph), 5.69 (1H, dt, *J* = 11.5 Hz, 7.2 Hz, -CH=$CHCH_2$-), 6.42 (1H, dt, *J* = 11.5 Hz, 1.8 Hz, -CH=$CHCH_2$-), 6.45 (1H, d, *J* = 2.0 Hz, 3- or 5-H), 6.50 (1H, d, *J* = 2.0 Hz, 3- or 5-H), 7.26-7.42 (10H, m, -$OCH_2C_6H_5$); *Anal.* Calcd for $C_{27}H_{28}O_4$: C, 77.86; H, 6.78. Found: C, 77.67; H, 6.79.

5,7-Dibenzyloxy-3-(1-hydroxybutyl)phthalide (**10**).

A mixture of **9** (140 mg, 0.335 mmol), osmium tetroxide (*ca.* 0.02 M in Bu^{t}OH, 2 mL) and NMO (50% in water, 1 mL) was stirred at rt for overnight. After excess sodium sulfite was added, the mixture was diluted with water and extracted with ethyl acetate. The organic layer was successively washed with 1N hydrochloric acid, water and brine, dried with anhydrous magnesium sulfate and concentrated *in vacuo*. The residue was chromatographed over silica gel (5 g) and elution with hexane / ethyl acetate (3:1) gave

10 (115 mg, 82%) as a colorless foam; IR (film): ν = 3450, 2960, 2920, 1750, 1600, 1500, 1460, 1380, 1330, 1230, 1210, 1160, 1060, 1025, 840, 740, 700 cm^{-1}; ^{1}H-NMR (500 MHz in $CDCl_3$) for *syn*-alcohol: δ = 0.96 (3H, d, *J* = 7.2 Hz, -CH_2CH_3), 1.20-1.70 (4H, m, -$CH_2CH_2CH_3$), 1.75 (1H, br, -OH), 3.95 (1H, m, -CH(OH)CH_2-), 5.06 (2H, s, -OCH_2Ph), 5.23 (1H, d, *J* = 3.2 Hz, 3-H), 5.24 (2H, s, -OCH_2Ph), 6.52 (1H, d, *J* = 1.8 Hz, 4- or 6-H), 6.59 (1H, m, 4- or 6-H), 7.26-7.48 (10H, m, -$OCH_2C_6H_5$); for *anti*-alcohol (partial): δ = 0.92 (3H, d, *J* = 7.2 Hz, -CH_2CH_3), 1.94 (1H, br, -OH), 3.85 (1H, m, -CH(OH)CH_2-), 6.63 (1H, m, 4- or 6-H); HRFABMS *m/z* = 418.1787 (Calcd for $C_{26}H_{26}O_5$: 418.1780).

5,7-Dibenzyloxy-3-(1-butyryl)phthalide (**11**).

Dess-Martin periodinane (235 mg, 0.555 mmol) was added to the alcohol (**10**) (89.3 mg, 0.214 mmol) in dry dichloromethane (5 mL) at rt and the reaction mixture was stirred for 30 min. It was diluted with ether, quenched with saturated aqueous sodium bicarbonate solution and sodium sulfite, and extracted with ether. The organic layer was successively washed with saturated aqueous sodium bicarbonate solution, water and brine, dried with anhydrous magnesium sulfate and concentrated *in vacuo* to *ca.* 1 mL of solution. This was cooled to 4°C to afford crystalline solid, which was filtered to give pure **11** (83.1 mg, 93%); mp 72.0-74.0°C; IR (KBr): ν = 2960, 1780, 1760, 1720, 1600, 1500, 1450, 1380, 1360, 1325, 1200, 1170, 1030, 1010, 830, 740, 700 cm^{-1}; ^{1}H-NMR (500 MHz in $CDCl_3$): δ = 0.84 (3H, t, *J* = 7.4 Hz, -CH_2CH_3), 1.45-1.64 (2H, m, -CH_2CH_3), 2.38 (1H, ddd, *J* = 6.3 Hz, 7.2 Hz, 18.5 Hz, -$CH_2CH_2CH_3$), 2.68 (1H, ddd, J = 6.0 Hz, 8.2 Hz, 18.5 Hz, -$CH_2CH_2CH_3$), 5.06 (2H, s, -OCH_2Ph), 5.25 (2H, s, -OCH_2Ph), 5.52 (1H, br, 3-H), 6.56 (1H, d, *J* = 1.8 Hz, 4- or 6-H), 6.71 (1H, dd, *J* = 1.8 Hz, 1.0 Hz 4- or 6-H), 7.28-7.52 (10H, m, -$OCH_2C_6H_5$); *Anal.* Calcd for $C_{26}H_{24}O_5$: C; 74.98; H, 5.81. Found: C, 74.50; H, 5.73.

5,7-Dihydroxy-3-(1- butyryl)phthalide (**12**).

A mixture of **11** (82.5 mg, 0.198 mmol) and 20% palladium hydroxide on carbon (wet, Degussa type E101 NE/W, Aldrich, 689 mg) in ethyl acetate (12 mL) was stirred at rt for 9 min under hydrogen atmosphere. It was filtered through Celite® and the filter cake was washed with ethyl acetate. The combined filtrate and washings were concentrated *in vacuo* to *ca.* 2 mL of solution. Hexane was added to this solution to give colorless crystalline solid, which was filtered to give pure **12** (35.0 mg, 75%); mp 170.0-172.0°C; IR (KBr): ν = 3130, 2960, 1730, 1700, 1630, 1600, 1480, 1360, 1330, 1200, 1160, 1070, 1040, 850 cm^{-1}; ^{1}H-NMR (300 MHz in DMSO-d_6): δ = 0.79 (3H, t, *J* = 7.4 Hz, -CH_2CH_3), 1.43 (2H, m, -CH_2CH_3), 2.44 (1H, ddd, *J* = 6.8 Hz, 6.8 Hz, 18.0 Hz, -$CH_2CH_2CH_3$), 2.61 (1H, ddd, *J* = 6.8 Hz, 7.4 Hz, 18.0 Hz, -$CH_2CH_2CH_3$), 5.80 (1H, s, 3-H), 6.34 (1H, d, *J* = 1.5 Hz, Ar-H), 6.37 (1H, br, Ar-H), 10.53 (1H, s, Ar-OH), 10.70 (1H, s, Ar-OH); HRFABMS *m/z* = 236.0713 (Calcd for $C_{12}H_{12}O_5$: 236.0684).

Bioassay of the synthesized compounds.

The bioassay for the compounds was carried out as previously reported[12] with slight modifications. Briefly, mouse mammary carcinoma FM3A cells were cultured in a humidified atmosphere of 5% CO_2, 95% air at 37°C in RPMI1 640 medium supplemented with 5% calf serum. Exponentially growing FM3A cells were seeded at 2 x 105 cells /ml and treated with acetophthalidin derivatives for 24 h.

These cells were fixed with cold 70% EtOH and stained with propidium iodide (Sigma Chemical Co.). The cell population in G2/M phase was determined by flow cytometry (Profile II, Coulter Co., Healeah, FL). Cytotoxicity was checked by microscopic observation.

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