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Synthesis and Cytotoxicity of 2,5-Dihydroxychalcones and Related Compounds

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A series of 2, 5-dihydroxychalcones and related compounds were synthesized, and their cytotoxicities against tumor cell lines and human umbilical venous endothelial cells (HUVEC) evaluated. It was found that chalcones, with electron-withdrawing substituents on an A ring, exhibited significant cytotoxicities. Among the synthesized compounds, 2'-chloro-2, 5-dihydroxychalcone (9) was most potent, with an IC_{50} value as low as 0.31 μg/mL. This compound also exhibited a significant cytotoxic selectivity toward HUVEC.

Key words: Chalcone, Cytotoxicity, Structure-activity relationship

INTRODUCTION

In our search for novel antitumor agents from natural sources, a phenyl propanoid, methyl 3, 4-dihydroxycinnamate (methyl caffeate **1**, Fig. 1), was isolated from the plant *Notopterygium incisum*. This compound showed significant cytotoxicities against various tumor cell lines, and greatly inhibited the invasion of B16 melanoma cells (Nam *et al.*, 2000). By varying the aromatic moiety, methyl 2, 5-dihydroxycinnamate (**2**) was obtained, with a cytotoxicity 10-fold as potent as **1** (Nam *et al.*, 2001). Structurally, **2** possesses two distinguished features; a hydroquinone and an α,β-unsaturated carbonyl. The hydroquinone has been known to easily autoxidise, *in vivo,* to the quinone, which elicits cytotoxicity *per se* or through a cascade of redox cycling (Ollinger *et al.*, 1989). The α,β-unsaturated carbonyl can be considered as a Michael acceptor, an active moiety often employed in the design of anticancer drugs (Ahn and Sok, 1996). A number of α,β-unsaturated ketones have demonstrated preferential activity toward thiols (Lee *et al.*, 1971). Alkylation with cellular thiols, such as glutathione (GSH), may also occur with cinnamates, such as **2**, leading to adducts at the β-position. Hence, α,β-unsaturated carbonyl-containing compounds may be free from the problems of mutagenicity and carcinogenicity associated with many alkylating agents used in cancer chemotherapy (Lee *et al.*, 1977). Thus, **2** presents an interesting lead for the development of anticancer agents.

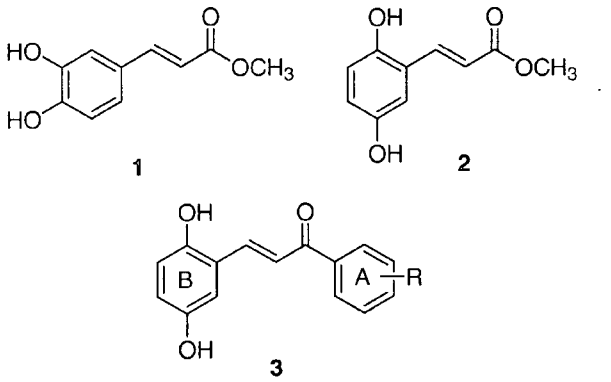

Fig. 1. Structures of cinnamates and chalcones

In a previous study, compound **2**, despite a potent cytotoxicity *in vitro*, was shown to have marginal *in vivo* antitumor activity, with a tumor mass growth inhibition rate of 45% in BDF1 mice bearing B16 murine melanoma cells. The low *in vivo* activity was attributed to the fast hydrolysis of the compound in plasma (personal unpublished results). This observation prompted us to replace the methoxy group in **2** with various substituted phenyls. The resulting 2,5-dihydroxychalcones, **3**, should be stable in term of hydrolysis, thus would be expected to

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be more active *in vivo* given that they retain cytotoxicities *in vitro*. In this paper, the synthesis and cytotoxicity of a series of such 2,5-dihydroxychalcones are described.

MATERIALS AND METHODS

Chemicals and solvents were of reagent grade and used without further purification. The tumor cell lines were obtained from the cancer cell bank at the Korea Research Institute of Bioscience and Biotechnology (KRIBB). The media, sera and other reagents used for the cell cultures were purchased from GIBCO Co. Ltd. Melting points were determined on an electrothermal melting point apparatus, and are uncorrected. The IR spectra were recorded on a Jasco Report-100 IR spectrometer. The ^{1}H-NMR spectra were recorded on a Varian-Gemini 90 MHz spectrometer, unless otherwise stated, using tetramethyl silane as the internal standard. Mass spectra were determined with a JEOL JMS-D-100 mass spectrometer. Elemental analyses were within ± 0.4% of the theoretical values, unless otherwise noted. Analytical thin layer chromatography was performed on a plastic sheet (0.2 mm) precoated with silica gel 60 F_{254} (E. Merck). Silica gel 60 (70-230 mesh, E. Merck) was used for column chromatography.

Synthesis

Most of chalcones were synthesized by a Claisen-Schmidt condensation of the appropriate acetophenones with 2, 5-dihydroxybenzaldehyde, protected as the tetrahydropyranyl ether (Scheme 1).

(*E*)-3-(2,5-Dihydroxyphenyl)-1-phenyl-2-propene-1-one (7)

2, 5-Dihydroxybenzaldehyde (3.45 g, 25 mmol) and pyridinium *p*-toluenesulfonate (0.15 g, 0.6 mmol) were stirred at room temperature, for 0.5 h, in methylene chloride (CH_2Cl_2, 100 mL), and then 3, 4-dihydro-α-pyran (13 mL) in CH_2Cl_2 (20 mL) was added drop wise. The reaction mixture was stirred at room temperature for 4 h, then washed twice with water, dried and evaporated *in vacuo*. The residue yielded crude 2, 5-bis(tetrahydropyran-2-yloxy)benzaldehyde (**5**).

Crude **5**, acetophenone (2.6 g, 25 mmol) and barium hydroxide octahydrate (8.15 g, 25 mmol) were dissolved in MeOH (100 mL). The reaction mixture was stirred for 12 h at 40 °C and then evaporated *in vacuo*. Water (100 mL) was added, the mixture neutralized with HCl (1M, 35 mL) and extracted with CH_2Cl_2. The organic layer was separated, washed with water, dried and evaporated *in vacuo*. The residue yielded crude (*E*)-3-[2, 5-bis(tetrahydropyran-2-yloxy)phenyl]-1-phenyl-2-propene-1-one (**6**).

Crude **6** and *p*-toluenesulfonic acid (0.2 g, 1.05 mmol) were dissolved in MeOH (100 mL). The mixture was stirred for 4 h at room temperature, and then evaporated *in vacuo*. Water (100 mL) was added, the resulting mixture neutralized with 5% $NaHCO_3$ (50 mL) and extracted with EtOAc. The organic layer was separated, washed with water, dried and concentrated *in vacuo*. The residue was purified on a silica gel column eluted with hexane-EtOAc (4:1) to give **7** (4.2 g, 45%) as a pale yellow solid; m.p. 187-190°C; IR (KBr) 3340, 1680, 1640, and 1580 cm^{-1}; MS (CI-MS) m/z 240 (M^+); ^{1}H-NMR (DMSO-d_6) δ 8.17 (1H, d, *J* = 16.5 Hz), 7.55~7.89 (5H, m), 7.39 (1H, d, *J* = 16.5 Hz) and 6.58~6.85 (3H, m).

The following chalcones were synthesized by the same procedure described above.

(*E*)-3-(2,5-Dihydroxyphenyl)-1-(2-fluorophenyl)-2-propene-1-one (8)

Yellow crystals; Yield 51%; m.p. 135-137°C; IR (KBr) 3350, 1680, 1630, and 1580 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ 8.24 (1H, d, *J* = 16.0 Hz), 7.51~7.97 (2H, m), 7.56 (1H, d, *J* = 16.0 Hz), 7.15~7.32 (2H, m) and 6.61~6.83 (3H, m); Anal. ($C_{15}H_{11}FO_3$) C, H.

(*E*)-1-(2-Chlorophenyl)-3-(2,5-dihydroxyphenyl)-2-propene-1-one (9)

Yellow crystals; Yield 36%; m.p. 113-115°C; IR (KBr) 3320, 1680, 1620, and 1580 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ 8.21 (1H, d, *J* = 16.0), 7.49~7.95 (2H, m), 7.43 (1H, d, *J* = 16.0 Hz), 7.21~7.37 (2H, m) and 6.55~6.78 (3H, m); Anal. ($C_{15}H_{11}ClO_3$) C, H.

(*E*)-1-(3-Chlorophenyl)-3-(2,5-dihydroxyphenyl)-2-propene-1-one (10)

Yellow crystals; Yield 47%; m.p. 125-127°C; IR (KBr) 3330, 1680, 1640, and 1580 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ 8.17 (1H, d, *J* = 16.2 Hz), 7.40~7.85 (5H, m) and 6.76~6.97 (3H, m); Anal. ($C_{15}H_{11}ClO_3$) C, H.

(*E*)-1-(4-Chlorophenyl)-3-(2,5-dihydroxyphenyl)-2-propene-1-one (11)

Yellow crystals; Yield 43%; m.p. 151-152°C; IR (KBr) 3340, 1675, 1635, and 1580 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ 8.08 (1H, d, *J* = 16.3 Hz), 7.81 (2H, d, *J* = 8.7 Hz), 7.45 (2H, d, *J* = 8.7 Hz), 7.41 (1H, d, *J* = 16.3 Hz) and 6.69~6.95 (3H, m); Anal. ($C_{15}H_{11}ClO_3$) C, H.

(*E*)-1-(2-Bromophenyl)-3-(2,5-dihydroxyphenyl)-2-propene-1-one (12)

Reddish crystals; Yield 42%; m.p. 132-133 °C; IR (KBr) 3320, 1680, 1620, and 1580 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ 8.15 (1H, d, *J* = 16.5), 7.51~7.86 (2H, m), 7.41 (1H, d, *J* = 16.5 Hz), 7.15~7.41 (2H, m) and 6.50~6.99 (3H, m); Anal. ($C_{15}H_{11}BrO_3$) C, H.

(*E*)-1-(4-Bromophenyl)-3-(2,5-dihydroxyphenyl)-2-propene-1-one (13)
Reddish crystals; Yield 45%; m.p. 143-145°C; IR (KBr) 3330, 1680, 1634, and 1580 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ 8.18 (1H, d, *J* = 16.3 Hz), 7.75 (2H, d, *J* = 9.4 Hz), 7.40 (2H, d, *J* = 9.4 Hz), 7.39 (1H, d, *J* = 16.3 Hz) and 6.73~6.99 (3H, m); Anal. ($C_{15}H_{11}BrO_3$) C, H.

(*E*)-3-(2,5-Dihydroxyphenyl)-1-(2,3,4,5,6-pentafluorophenyl)-2-propene-1-one (14)
Reddish crystals; Yield 37%; m.p. 157-159°C; IR (KBr) 3350, 1660, 1620, and 1580 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ 8.18 (1H, d, *J* = 16.3 Hz), 7.39 (1H, d, *J* = 16.3 Hz) and 6.66~6.87 (3H, m); Anal. ($C_{15}H_7F_5O_3$) C, H.

(*E*)-3-(2,5-Dihydroxyphenyl)-1-(4-nitrophenyl)-2-propene-1-one (15)
Reddish crystals; Yield 41%; m.p. 139-141°C; IR (KBr) 3350, 1680, 1630, and 1575 cm^{-1}; ^{1}H-NMR (300 MHz, DMSO-d_6) δ 8.06 (1H, d, *J* = 16.1 Hz), 7.84 (2H, d, *J* = 8.4 Hz), 7.45 (2H, d, *J* = 8.4 Hz), 7.41 (1H, d, *J* = 16.1 Hz) and 6.67~6.89 (3H, m).

(*E*)-1-(2,5-Difluorophenyl)-3-(2,5-dihydroxyphenyl)-2-propene-1-one (16)
Reddish crystals; Yield 44%; m.p. 127-129°C; IR (KBr) 3330, 1680, 1615, and 1590 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ 8.07 (1H, d, *J* = 16.0 Hz), 7.47~7.85 (2H, m), 7.41(1H, d, *J* = 16.0 Hz) and 6.65~6.89 (4H, m).

(*E*)-1-(3,5-Difluorophenyl)-3-(2,5-dihydroxyphenyl)-2-propene-1-one (17)
Reddish crystals; Yield 41%; m.p. 115-117°C; IR (KBr) 3340, 1680, 1620, and 1580 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ 8.03 (1H, d, *J* = 16.0 Hz), 7.67 (2H, d, *J* = 2.1 Hz), 7.40 (1H, d, *J* = 16.0 Hz), 7.01 (1H, d, *J* = 2.1 Hz) and 6.47~ 6.74 (3H, m).

(*E*)-1-(2,3-Dichlorophenyl)-3-(2,5-dihydroxyphenyl)-2-propene-1-one (18)
Reddish crystals; Yield 37%; m.p. 148-149°C; IR (KBr) 3345, 1680, 1610, and 1590 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ 8.17 (1H, d, *J* = 16.0 Hz), 7.25~7.56 (4H, m) and 6.51~ 6.82 (3H, m).

(*E*)-1-(3,4-Dichlorophenyl)-3-(2,5-dihydroxyphenyl)-2-propene-1-one (19)
Reddish crystals; Yield 42%; m.p. 131-132°C; IR (KBr) 3350, 1680, 1640, and 1580 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ 8.11 (1H, d, *J* = 15.8 Hz), 7.38~7.55 (3H, m), 7.14 (1H, d, *J* = 9.4 Hz) and 6.56~6.84 (3H, m).

(*E*)-1-(3,5-Dichlorophenyl)-3-(2,5-dihydroxyphenyl)-2-propene-1-one (20)
Reddish crystals; Yield 45%; m.p. 124-126°C; IR (KBr) 3340, 1680, 1630, and 1580 cm^{-1}; ^{1}H-NMR (300 MHz, DMSO-d_6) δ 8.07 (1H, d, *J* = 16.1 Hz), 7.65 (2H, d, *J* = 2.3 Hz), 7.42(1H, d, *J* = 16.1 Hz), 7.11 (1H, d, *J* = 2.3 Hz) and 6.51~6.79 (3H, m).

(*E*)-1-[(5-Bromo-2-methoxy)phenyl]-3-(2,5-dihydroxyphenyl)-2-propene-1-one (21)
Reddish crystals; Yield 64%; m.p. 160-162°C; IR (KBr) 3340, 2910, 1670, 1620, and 1580 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ 8.03 (1H, d, *J* = 15.8 Hz), 7.21~7.52 (3H, m) and 6.41~6.84 (4H, m); Anal. ($C_{16}H_{13}BrO_4$) C, H.

(*E*)-3-(2,5-Dihydroxyphenyl)-1-(2-methylphenyl)-2-propene-1-one (22)
Yellow crystals; Yield 57%; m.p. 171-173°C; IR (KBr) 3340, 2910, 1680, 1620, and 1580 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ 8.19 (1H, d, *J* = 16.0 Hz), 7.39~7.71 (5H, m) and 6.63~6.84 (3H, m).

(*E*)-3-(2,5-Dihydroxyphenyl)-1-(4-methylphenyl)-2-propene-1-one (23)
Yellow crystals; Yield 65%; m.p. 168-170°C; IR (KBr) 3320, 2915, 1680, 1620, and 1575 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ 8.01 (1H, d, *J* = 16.3 Hz), 7.71 (2H, d, *J* = 8.7 Hz), 7.43 (1H, d, *J* = 16.3 Hz), 7.29 (2H, d, *J* = 8.7 Hz) and 6.59~6.87 (3H, m).

(*E*)-3-(2,5-Dihydroxyphenyl)-1-(2-methoxyphenyl)-2-propene-1-one (24)
Reddish crystals; Yield 68%; m.p. 154-155°C; IR (KBr) 3340, 2920, 1680, 1620, and 1580 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ 8.27 (1H, d, *J* = 15.9 Hz), 7.40~7.74 (4H, m), 6.63~ 6.92 (4H, m) and 3.91 (3H, s); Anal. ($C_{17}H_{14}O_4$) C, H.

(*E*)-3-(2,5-Dihydroxyphenyl)-1-(3-methoxyphenyl)-2-propene-1-one (25)
Reddish crystals; Yield 71%; m.p. 137-139°C; IR (KBr) 3350, 2915, 1675, 1615, and 1580 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ 8.31 (1H, d, *J* = 16.5 Hz), 7.01~7.38 (4H, m), 7.41 (1H, d, *J* = 16.5 Hz), 6.47~6.71 (3H, m) and 3.89 (3H, s).

(*E*)-3-(2,5-Dihydroxyphenyl)-1-(4-methoxyphenyl)-2-propene-1-one (26)
Reddish crystals; Yield 69%; m.p. 157-159°C; IR (KBr) 3330, 2920, 1680, 1610, and 1580 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ 8.11 (1H, d, *J* = 16.2 Hz), 7.68 (2H, d, *J* = 8.5 Hz), 7.41 (1H, d, *J* = 16.2 Hz), 6.97 (2H, d, *J* = 8.5 Hz), 6.54~6.85 (3H, m) and 3.89 (3H, s).

(*E*)-3-(2,5-Dihydroxyphenyl)-1-(4-thiomethylphenyl)-2-propene-1-one (27)
Reddish crystals; Yield 60%; m.p. 119-120°C; IR (KBr)

3340, 2915, 1675, 1620, and 1580 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ 8.01 (1H, d, *J* = 15.7 Hz), 7.59 (2H, d, *J* = 8.2 Hz), 7.39 (1H, d, *J* = 15.7 Hz), 6.87 (2H, d, *J* = 8.2 Hz), 6.55~6.87 (3H, m) and 3.98 (3H, s).

(*E*)-3-(2,5-Dihydroxyphenyl)-1-(4-methylsulfonylphenyl)-2-propene-1-one (28)

Compound **22** (2 mmol, 598 mg) was dissolved in CH_2Cl_2 (20 mL). The resulting solution was cooled to 0°C, and *m*-chloroperoxybenzoic acid (CPBA, 2.2 mmol, 345 mg) added. The mixture was stirred for 2 h and water (30 mL) added. The reaction mixture was extracted with ethyl acetate (50 mL×2). The ethyl acetate extracts were combined, concentrated and crystallized from hexane/CH_2Cl_2 to furnish the expected product as reddish crystals (528 mg). Yield 80%; m.p. 145-147°C; IR (KBr) 3330, 2920, 1680, 1620, 1580, and 1380 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ 8.12 (1H, d, *J* = 16.1 Hz), 7.63 (2H, d, *J* = 7.8 Hz), 7.42 (1H, d, *J* = 16.1 Hz), 6.99 (2H, d, *J* = 7.8 Hz), 6.48~6.84 (3H, m) and 3.23 (3H, s); Anal. ($C_{17}H_{14}O_5S$) C, H.

(*E*)-3-(2,5-Dihydroxyphenyl)-1-(2,3-dimethoxyphenyl)-2-propene-1-one (29)

Yellowish crystals; Yield 58%; m.p. 114-115°C; IR (KBr) 3340, 2900, 1670, 1620, and 1580 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ 7.97 (1H, d, *J* = 15.8 Hz), 7.41 (1H, d, *J* = 15.8 Hz), 6.94~7.25 (3H, m), 6.45~6.79 (3H, m), 3.95 (3H, s) and 3.84 (3H, s).

(*E*)-3-(2,5-Dihydroxyphenyl)-1-(2,4-dimethoxyphenyl)-2-propene-1-one (30)

Yellowish crystals; Yield 67%; m.p. 158-160°C; IR (KBr) 3330, 2910, 1680, 1620, and 1580 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ 8.00 (1H, d, *J* = 16.4 Hz), 57.61 (1H, m), 7.38 (1H, d, *J* = 16.4 Hz), 6.39~6.75 (5H, m), 3.91 (3H, s) and 3.89 (3H, s).

(*E*)-3-(2,5-Dihydroxyphenyl)-1-(2,5-dimethoxyphenyl)-2-propene-1-one (31)

Yellowish crystals; Yield 71%; m.p. 138-141°C; IR (KBr) 3320, 2915, 1690, 1610, and 1590 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ 7.99 (1H, d, *J* = 16.0 Hz), 7.37 (1H, d, *J* = 16.0 Hz), 7.25 (1H, m), 6.51~6.82 (5H, m), 3.92 (3H, s) and 3.90 (3H, s).

(*E*)-3-(2,5-Dihydroxyphenyl)-1-(3,4-dimethoxyphenyl)-2-propene-1-one (32)

Yellowish crystals; Yield 74%; m.p. 155-156°C; IR (KBr) 3340, 2920, 1690, 1620, and 1580 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ 8.01 (1H, d, *J* = 16.1 Hz), 7.38 (1H, d, *J* = 16.1 Hz), 7.29 (1H, d, *J* = 2.7 Hz), 7.26 (1H, dd, *J* = 8.4, 2.7 Hz), 6.94 (1H, d, *J* = 8.4 Hz), 6.51~6.82 (3H, m), 3.93 (3H, s) and 3.92 (3H, s).

(*E*)-3-(2,5-Dihydroxyphenyl)-1-(3,5-dimethoxyphenyl)-2-propene-1-one (33)

Yellowish crystals; Yield 71%; m.p. 171-172°C; IR (KBr) 3320, 2915, 1680, 1625, and 1575 cm^{-1}; ^{1}H-NMR (300 MHz, DMSO-d_6) δ 8.00 (1H, d, *J* = 16.0 Hz), 7.45 (1H, d, *J* = 16.0 Hz), 7.28 (2H, d, *J* = 2.5 Hz), 6.84 (1H, t, *J* = 2.5 Hz), 6.53~6.82 (3H, m) and 3.91 (6H, s).

(*E*)-3-(2,5-Dihydroxyphenyl)-1-(2,3,4-trimethoxyphenyl)-2-propene-1-one (34)

Yellowish crystals; Yield 59%; m.p. 128-130°C; IR (KBr) 3340, 2910, 1670, 1620, and 1580 cm^{-1}; ^{1}H-NMR (300 MHz, DMSO-d_6) δ 7.95 (1H, d, *J* = 15.5 Hz), 7.58 (1H, d, *J* = 15.5 Hz), 7.29 (1H, d, *J* = 8.6 Hz), 6.66 (1H, dd, *J* = 7.8, 2.4 Hz), 6.51 (1H, d, *J* = 8.6 Hz), 6.44 (1H, d, *J* = 7.8 Hz), 6.41 (1H, d, *J* = 2.4 Hz), 3.95 (3H, s), 3.89 (3H, s) and 3.88 (3H, s).

(*E*)-3-(2,5-Dihydroxyphenyl)-1-(2,4,5-trimethoxyphenyl)-2-propene-1-one (35)

Yellowish crystals; Yield 77%; m.p. 149-151°C; IR (KBr) 3330, 2920, 1680, 1610, and 1580 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ 7.84 (1H, d, *J* = 16.0 Hz), 7.47 (1H, d, *J* = 16.0 Hz), 7.27 (1H, s), 6.72 (1H, d, *J* = 2.5 Hz), 6.61 (1H, dd, *J* = 8.1, 2.5 Hz), 6.51 (1H, s), 6.44 (1H, d, *J* = 8.5 Hz), 3.94 (3H, s), 3.90 (3H, s) and 3.89 (3H, s).

(*E*)-3-(2,5-Dihydroxyphenyl)-1-(2,4,6-trimethoxyphenyl)-2-propene-1-one (36)

Yellowish crystals; Yield 66%; m.p. 139-140°C; IR (KBr) 3350, 1680, 1620, and 1575 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ 7.85 (1H, d, *J* = 16.0 Hz), 7.51 (1H, d, *J* = 16.0 Hz), 6.67 (2H, s), 6.39~6.65 (3H, m), 3.87 (6H, s) and 3.85 (3H, s).

(*E*)-3-(2,5-Dihydroxyphenyl)-1-(3,4,5-trimethoxyphenyl)-2-propene-1-one (37)

Yellowish crystals; Yield 62%; m.p. 171-173°C; IR (KBr) 3350, 1680, 1620, and 1575 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ 7.85 (1H, d, *J* = 16.0 Hz), 7.51 (1H, d, *J* = 16.0 Hz), 6.67 (2H, s), 6.39~6.65 (3H, m), 3.87 (6H, s) and 3.85 (3H, s).

(*E*)-3-(2,5-Dihydroxyphenyl)-1-(2-hydroxyphenyl)-2-propene-1-one (38)

Reddish crystals; Yield 45%; m.p. 159-161°C; IR (KBr) 3360, 1680, 1625, and 1590 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ 7.84 (1H, d, *J* = 15.8 Hz), 7.44 (1H, d, *J* = 15.8 Hz), 6.84~7.42 (4H, m), 6.66 (1H, d, *J* = 2.1 Hz), 6.52 (1H, d, *J* = 8.5 Hz) and 6.43 (1H, dd, *J* = 8.5, 2.1 Hz).

(*E*)-3-(2,5-Dihydroxyphenyl)-1-(3-hydroxyphenyl)-2-propene-1-one (39)

Reddish crystals; Yield 35%; m.p. 145-147°C; IR (KBr) 3340, 1680, 1610, and 1580 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ

7.78 (1H, d, J = 16.0 Hz), 7.41 (1H, d, J = 16.0 Hz), 7.32 (1H, s), 7.00~7.38 (3H, m) and 6.45~6.75 (3H, m).

(*E*)-3-(2,5-Dihydroxyphenyl)-1-(4-hydroxyphenyl)-2-propene-1-one (40)
Reddish crystals; Yield 41%; m.p. 121-123°C; IR (KBr) 3350, 1680, 1610, and 1580 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ 7.84 (1H, d, J = 16.2 Hz), 7.55 (2H, d, J = 7.9 Hz), 7.39 (1H, d, J = 16.2 Hz), 6.94 (2H, d, J = 7.9 Hz) and 6.53~6.79 (3H, m).

(*E*)-3-(2,5-Dihydroxyphenyl)-1-[(2-hydroxy-5-methoxy)phenyl]-2-propene-1-one (41)
Yellowish crystals; Yield 41%; m.p. 119-121°C; IR (KBr) 3340, 2910, 1680, 1620, and 1580 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ 8.00 (1H, d, J = 16.0 Hz), 7.39 (1H, d, J = 16.2 Hz), 6.88~7.21 (3H, m), 6.44~6.75 (3H, m) and 3.94 (3H, m).

(*E*)-3-(2,5-Dihydroxyphenyl)-1-[(2-hydroxy-6-methoxy)phenyl]-2-propene-1-one (42)
Yellowish crystals; Yield 57%; m.p. 140-141°C; IR (KBr) 3345, 2915, 1680, 1625, and 1575 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ 8.01 (1H, d, J = 15.9 Hz), 7.42 (1H, d, J = 15.9 Hz), 6.76~7.00 (3H, m), 6.43~6.75 (3H, m) and 3.89 (3H, m).

(*E*)-3-(2,5-Dihydroxyphenyl)-1-(2,3-dihydroxyphenyl)-2-propene-1-one (43)
Yellowish crystals; Yield 29%; m.p. 149-151°C; IR (KBr) 3350, 1680, 1620, and 1580 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ 8.11 (1H, d, J = 16.0 Hz), 7.35 (1H, d, J = 16.0 Hz), 6.85~7.21 (3H, m) and 6.51~6.82 (3H, m).

(*E*)-3-(2,5-Dihydroxyphenyl)-1-(2,4-dihydroxyphenyl)-2-propene-1-one (44)
Yellowish crystals; Yield 38%; m.p. 141-142°C; IR (KBr) 3340, 1680, 1620, and 1575 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ 8.02 (1H, d, J = 16.0 Hz), 7.51 (1H, d, J = 8.2 Hz), 7.41 (1H, d, J = 16.0 Hz) and 6.39~6.82 (5H, m).

(*E*)-3-(2,5-Dihydroxyphenyl)-1-(2,5-dihydroxyphenyl)-2-propene-1-one (45)
Yellowish crystals; Yield 35%; m.p. 144-145°C; IR (KBr) 3345, 1680, 1610, and 1590 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ 7.99 (1H, d, J = 16.1 Hz), 7.41 (1H, d, J = 16.1 Hz), 6.88~7.02 (3H, m) and 6.44~6.75 (3H, m).

(*E*)-3-(2,5-Dihydroxyphenyl)-1-(3,4-dihydroxyphenyl)-2-propene-1-one (46)
Yellowish crystals; Yield 28%; m.p. 111-113°C; IR (KBr) 3350, 1680, 1615, and 1585 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ 8.10 (1H, d, J = 16.2 Hz), 7.45 (1H, d, J = 16.1 Hz), 6.88~7.11 (3H, m) and 6.51~6.87 (3H, m).

(*E*)-3-(2,5-Dihydroxyphenyl)-1-(3,5-dihydroxyphenyl)-2-propene-1-one (47)
Yellowish crystals; Yield 31%; m.p. 131-132°C; IR (KBr) 3340, 1680, 1610, and 1590 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ 8.05 (1H, d, J = 15.4 Hz), 7.40 (1H, d, J = 15.4 Hz), 6.71 (2H, s) and 6.50~6.82 (4H, m).

(*E*)-3-(2,5-Dihydroxyphenyl)-1-(3,4-methylenedioxyphenyl)-2-propene-1-one (48)
Yellowish crystals; Yield 68%; m.p. 141-153°C; IR (KBr) 3350, 1680, 1615, and 1585 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ 8.00 (1H, d, J = 16.0 Hz), 7.42 (1H, d, J = 16.0 Hz), 5.91 (2H, s), 6.84~7.10 (3H, m) and 6.51~6.83 (3H, m).

(*E*)-3-(2,5-Dihydroxyphenyl)-1-(pyridin-4-yl)-2-propene-1-one (49)
Yellowish crystals; Yield 20%; m.p. 111-112°C; IR (KBr) 3330, 1680, 1620, and 1580 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ 8.43~8.56 (2H, m), 8.01~8.12 (2H, m), 7.99 (1H, d, J = 16.0 Hz), 6.70 (1H, d, J = 16.1 Hz) and 6.51~6.71 (3H, m).

(*E*)-3-(2,5-Dihydroxyphenyl)-1-(2-naphthyl)-2-propene-1-one (50)
Yellowish crystals; Yield 65%; m.p. 147-148°C; IR (KBr) 3350, 1680, 1620, and 1580 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ 8.01 (1H, d, J = 16.0 Hz), 7.61~8.00 (5H, m), 7.51 (1H, d, J = 16.0 Hz), 7.22~7.49 (2H, m) and 6.47~6.79 (3H, m).

(*E*)-3-(2,5-Dihydroxyphenyl)-1-(4-bisphenyl)-2-propene-1-one (51)
Yellowish crystals; Yield 56%; m.p. 145-146°C; IR (KBr) 3340, 1680, 1610, and 1590 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ 7.99 (1H, d, J = 16.0 Hz), 7.42 (1H, d, J = 16.0 Hz), 6.79~7.40 (9H, m) and 6.50~6.78 (3H, m).

(*E*)-3-(2,5-Dihydroxyphenyl)-1-(indol-3-yl)-2-propene-1-one (52)
Yellowish crystals; Yield 25%; m.p. 121-123°C; IR (KBr) 3420, 3340, 1680, 1620, and 1580 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ 8.23~8.26 (1H, m), 8.00 (1H, d, J = 16.1 Hz), 7.45~7.47 (1H, m), 7.02~7.35 (2H, m), 6.72 (1H, d, J = 16.1 Hz) and 6.51~6.71 (3H, m).

(*E*)-3-(2,5-Dihydroxyphenyl)-1-(quinolin-2-yl)-2-propene-1-one (53)
Reddish crystals; Yield 27%; m.p. 118-120°C; IR (KBr) 3340, 1680, 1615, and 1575 cm^{-1}; ^{1}H-NMR (DMSO-d_6) δ 8.40~8.43 (2H, m), 7.85~8.01 (2H, m), 7.83 (1H, d, J = 16.0 Hz), 7.45~7.55 (2H, m) and 6.49~6.75 (4H, m).

Cytotoxicity assay

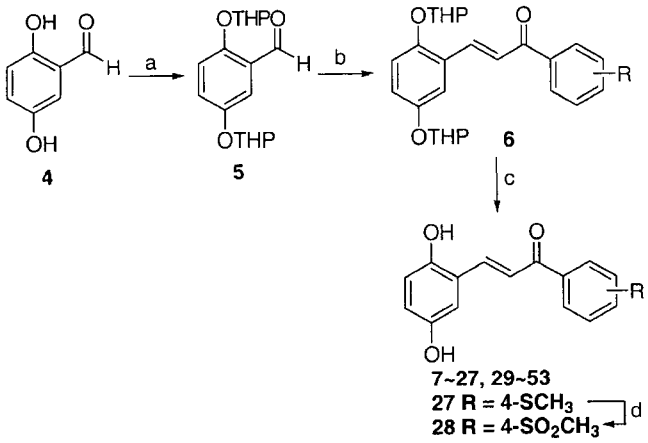
The *in vitro* cytotoxicities against tumor cell lines were

measured using the SRBs method, as described previously (Skehan *et al.*, 1990).

RESULTS AND DISCUSSION

The 2, 5-dihydroxychalcones (**7~27** and **29~53**) were synthesized, as depicted in Scheme 1, in moderate to good yields. A main step involved condensation of 2, 5-dihydroxynbenzaldehyde, (**4**), protected as the tetrahydropyranyl ether **5**, with various acetophenones, also protected as the tetrahydropyranyl ether wherever a phenol group was present, using barium hydroxide as a base. The ^{1}H-NMR spectra revealed that the olefinic bond in these chalcones adopted the *E* configuration (the coupling constants of the two olefinic protons were around 16 Hz). A chalcone **28** was obtained in a 90% yield by the oxidation of **27** with *m*-CPBA (*m*-chloroperoxybenzoic acid).

The synthesized chalcones were evaluated for their cytotoxic activities in three tumor cell lines, including B16 (murine melanoma), HCT116 (human colon cancer cells) and A431 (human epidermoid carcinoma). As is already widely known, the formation of new blood vessels from human umbilical venous endothelial cells (HUVEC), so-called angiogenesis, is prerequisite for the growth of solid tumors (Folkman, 1971). The inhibition of angiogenesis has been postulated as an attractive approach for cancer treatment (Gastl *et al.*, 1997), and a compound showing selective cytotoxicity against HUVECs may have potential for further development as an angiogenesis inhibitor. For that reason, the HUVEC line was included in the assay panel in an attempt to unveil a potential selective cytotoxicity against this cell line by the compounds tested. The results, expressed as IC_{50} values (a concentration produces 50% reduction in cell growth), are summarized in Table I.



Scheme 1. Synthesis of 2,5-dihydroxychalcones
a) pyridinium *p*-toluenesulfonate, 3, 4-dihydro-2*H*-pyran, CH_2Cl_2, rt, 5h. b) $ArCOCH_3$, $Ba(OH)_2.8H_2O$, MeOH, 40°C, 12 h. c) *p*-toluenesulfonic acid, MeOH, 5 h. and d) *m*-CPBA, CH_2Cl_2, rt, 4 h.

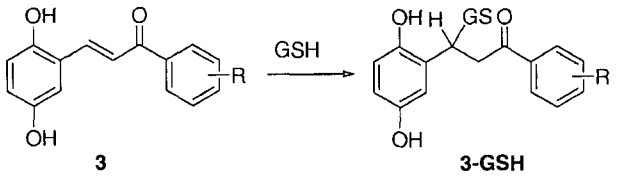
As shown in Table I, most of the synthesized compounds exhibited significant cytotoxicities. Generally, the compounds bearing electron-withdrawing group(s) on the A ring **8~20** exerted comparable or slightly enhanced cytotoxicities (IC_{50} values ranged from 0.31 to 2.49 μg/mL) compared to compound **7** bearing no substituent on the A ring (IC_{50} values, 1.97~2.77 μg/mL). A similar result was observed with compound **21**, which possesses both electron-withdrawing and electron-releasing groups. For these 2, 5-dihydroxychalcones, the formation of quinonoid metabolites from a para-hydroquinone moiety might be one mechanism that leads to their cytotoxicities (Nam *et al.*, 2001). Moreover, it has previously been shown that a number of α,β-unsaturated ketones demonstrate preferential activity toward bionucleophiles, such as thiols (Lee *et al.*, 1971). Thus, alkylation with cellular nucleophiles, such as SH, OH and NH, may also occur with chalcones **3**, leading to adducts at the β-position (Fig. 2). The substitution of the electron-withdrawing group(s) on the A ring was expected to activate the β-position, and thus promote the alkylation of bionucleophiles, resulting in enhanced cytotoxicities of the related compounds **8~20**.

For other compounds, **22~27** and **38~40**, an electron-releasing group on the A-ring was expected to retard the alkylation reaction, and thus decrease their cytotoxicities. Actually, however, they exhibited comparable bioactivities to **7** and other halogenated 2, 5-dihydroxychalcones. It has been reported that a number of chalcones show antimitotic activities (Edwards *et al.*, 1990) and protein tyrosine kinase (TK) inhibitory activities (Gazit *et al.*, 1991). Mitosis inhibition is a mechanism of action of many currently used anticancer agents, like combretastatin A-4, vincristine and vinblastine (Pratt *et al.*, 1994), and inhibition of tyrosine kinases has been an attractive approach for cancer treatment in recent years (Kolibaba and Druker, 1997). It is highly possible that the 2, 5-dihydroxychalcones synthesized might also exert antimitotic and/or TK inhibitory activities. It could be postulated that, in the compounds **22~27** and **38~40**, these activities are likely to produce predominant effects over the retardative effects of single electron-releasing groups substituted onto the A ring, resulting in cytotoxicities comparable to those of **7** and other halogenated 2, 5-dihydroxychalcones. Nevertheless, with a few exceptions, the retardative effects of the electron-releasing groups in compounds **31** and **42-43** could be realized in chalcones with multiple electron-donating substituents, as demonstrated by **32~37** and **44-45**, where a substantial decrease in the cytotoxicity compared to **7** was observed. Replacement of the phenyl ring by extended or heteroaromatic rings **49~53** produced derivatives with retained cytotoxicities. Of particular interest, it was found that the 2-chlorinated chalcone, **9**, showed a significant cytotoxic selectivity toward HUVECs. For example, its IC_{50}

Table I. Cytotoxicities of the synthesized 2,5-dihydroxychalcones in tumor cell lines[1] and HUVECs

Cpd	R[6] or A ring (for **49**, **51~53**)	Cytotoxicity (IC_{50}[2], μg/mL)			
		B16	HCT116	A431	HUVEC
7	H	1.97	2.77	2.01	1.99
8	2-F	1.96	1.99	1.87	1.45
9	2-Cl	1.51	1.67	1.24	0.31
10	3-Cl	1.26	1.55	1.37	1.12
11	4-Cl	2.00	2.35	1.96	2.01
12	2-Br	1.73	1.81	1.57	1.61
13	4-Br	1.59	1.64	1.55	1.45
14	2,3,4,5,6-F_5	2.11	2.32	1.99	2.00
15	4-NO_2	2.01	2.49	2.74	1.99
16	2,5-F_2	1.81	1.87	2.01	1.71
17	3,5-F_2	1.99	1.75	2.00	1.54
18	2,3-Cl_2	1.78	1.83	1.65	1.61
19	3,4-Cl_2	1.79	2.07	1.83	2.24
20	3,5-Cl_2	2.14	1.98	2.17	1.95
21	2-OCH_3-5-Br	1.15	2.08	1.44	1.01
22	2-CH_3	2.56	3.01	2.43	2.17
23	4-CH_3	2.61	3.00	2.41	1.97
24	2-OCH_3	1.71	2.23	1.87	1.34
25	3-OCH_3	1.91	3.01	1.71	2.01
26	4-OCH_3	3.07	4.11	3.99	3.47
27	4-SCH_3	2.47	5.99	2.61	1.79
28	4-SO_2CH_3	2.19	3.97	2.07	2.11
29	2,3-$(OCH_3)_2$	3.91	4.05	3.09	3.10
30	2,4-$(OCH_3)_2$	4.70	7.51	3.14	2.94
31	2,5-$(OCH_3)_2$	1.87	3.02	2.99	3.01
32	3,4-$(OCH_3)_2$	11.57	9.35	5.09	6.41
33	3,5-$(OCH_3)_2$	11.09	10.27	9.37	8.88
34	2,3,4-$(OCH_3)_3$	4.11	10.94	5.39	3.92
35	2,4,5-$(OCH_3)_3$	12.49	11.28	11.01	10.08
36	2,4,6-$(OCH_3)_3$	3.11	4.56	2.15	2.00
37	3,4,5-$(OCH_3)_3$	4.56	10.26	7.44	4.15
38	2-OH	1.43	3.19	2.05	1.48
39	3-OH	1.57	4.53	2.76	2.01
40	4-OH	3.58	4.03	2.99	2.51
41	2-OH-5-OCH_3	4.67	4.83	3.99	3.49
42	2-OH-6-OCH_3	2.31	2.57	2.06	1.99
43	2,3-$(OH)_2$	1.42	2.14	2.09	2.01
44	2,4-$(OH)_2$	10.67	11.38	10.41	8.75
45	2,5-$(OH)_2$	10.16	14.46	26.65	10.01
46	3,4-$(OH)_2$	2.97	11.46	5.35	2.41
47	3,5-$(OH)_2$	4.16	4.99	2.11	3.05
48	3,4-OCH_2O	9.78	10.17	7.54	11.24
49	Pyridin-4-yl	2.00	2.34	1.98	1.93
50	2-Naphtyl	1.49	2.97	1.99	1.49
51	4-Bisphenyl	1.13	3.01	2.14	1.12
52	Indol-3-yl	2.09	2.16	1.95	1.48
53	Quinolin-2-yl	1.94	2.11	2.07	1.84
	2[3]	0.12	0.17	0.15	-[4]
	ADR[5]	0.10	0.11	0.19	0.13

[1]Cancer cell lines: B16, murine melanoma; HCT116, colon cancer; A431, human epidermoid carcinoma. [2]A sample concentrations produce a 50% reduction in cell growth. The values shown are the average from triplicate experiments. [3]Methyl 2, 5-dihydroxycinnamate, synthesized as described previously (Nam *et al.*, 2001). [4]Not tested. [5]Adriamycin, used as a positive control. [6]Refer to Fig.1 and Scheme 1.



3 **3-GSH**

Fig. 2. Thiol-alkylation of chalcones, **3**, leading to adducts at the β-position

value against HUVEC was found to be 5.6-fold lower than that against HCT116. Compound **9** was also the most potent in the series, with IC_{50} values as low as 0.31 μg/mL. Unfortunately, however, the bioactivity of the whole series was found to be nearly one magnitude lower than that of compound **2**. Despite the fact the 2,5-dihydroxy-chalcones are obviously more stable than **2**, in terms of hydrolysis, their cytotoxic profiles presented here should be sufficiently interesting and encouraging for this class of compounds to be further explored as anticancer agents.

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