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Synthesis and *In Vitro* Cytotoxicity of Cinnamaldehydes to Human Solid Tumor Cells

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Cinnamaldehydes and related compounds were synthesized from various cinnamic acids based on the 2'-hydroxycinnamaldehyde isolated from the bark of *Cinnamomum cassia* Blume. The cytotoxicity to human solid tumor cells such as A549, SK-OV-3, SK-MEL-2, XF498 and HCT15 were measured. Cinnamic acid, cinnamates and cinnamyl alcohols did not show any cytotoxicity against the human tumor cells. Cinnamaldehydes and realted compounds were resistant to A549 cell line up to 15 μg/ml. In contrast, HCT15 and SK-MEL-2 cells were much sensitive to these cinnamaldehyde analogues which showed ED_{50} values 0.63~8.1 μg/ml. Cytotoxicity of the saturated aldehydes was much weak compared to their unsaturated aldehydes. From these studies, it was found that the key functional group of the cinnamaldehyde-related compounds in the antitumor activity is the propenal group.

Key words : Cinnamaldehyde, Human tumor cell, Cytotoxicity, *Cinnamomum cassia*

INTRODUCTION

A large number of aliphatic and aromatic aldehydes and their derivatives showed cytotoxicity against tumor cell lines (Piantadosi, *et al.*, 1964). Several of these aldehydes and their derivatives were also used clinically as antitumor drugs (Goldin, *et al.*, 1966). Cinnamaldehydes, one of aromatic aldehydes, are widely distributed in nature. It was reported that cinnamaldehydes have an antimutagenic effect in the cells mutagenized with 4-nitroquinoline-N-oxide (4NQO) (Feron, *et al.*, 1991) and aromatic aldehydes including cinnamaldehyde protect cells from inactivating effect of cis-diamminedichloroplatinum (II) (cis-DDP) (Dornish, *et al.*, 1989). These previous studies showed the feasibility of a clinical application of cinnamaldehydes to enhance the therapeutic effect of *cis*-DDP.

Billman and Tonnis (1971) reported substituted aralkyl aldehydes and described an antitumor activity. However, they could not find an antitumor effect of the cinnamaldehydes because the aralkyl aldehydes were only screened against leukemia L-1210. Kwon, *et al.*, 1996 and 1997 reported the farnesyl transferase inhibitory and antiangiogenic activity of 2'-hydroxycinnamaldehyde and related compounds.

In this paper, we report cytotoxic activity studies of naturally occurring compounds and synthetic derivatives against human solid tumor cell lines such as A 549, SK-OV-3, SK-MEL-2, XF498 and HCT15 *in vitro*. And we also discussed the relationship between the propenal group of cinnamaldehydes and its biological activities.

MATERIALS AND METHODS

Reagents

Chemical reagents were purchased from Aldrich Chemical Co. (St. Louis, MO, USA) unless otherwise mentioned.

2'-Hydroxycinnamaldehyde (**4a**) was isolated from stem bark of *Cinnamomum cassia* according to the reported procedure (Kwon *et al.*, 1996).

Instruments

UV and FT-IR spectra were recorded on a Shimadzu UV-265 and Bio-Rad Digible Division FTS-80 spectrophotometer, respectively. NMR spectra were measured in $CDCl_3$ or acetone-d_6 (Aldrich) on a Brucker AM 500 and Varian 300 MHz spectrometer. Exact mass

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of the unknown compounds were measured on a VG ZAB-7070 by the University of California at Riverside Mass Spectrometry Facility using the electron impact (EI) or the chemical ionization (CI) mode with NH_3.

Methyl 3-(2'-hydroxyphenyl)-2-propenate (2)

To a solution of 2'-hydroxycinnamic acid (**1**, 10 g) dissolved in 100 mL of CH_3OH, diazomethane (CH_2N_2), which was prepared from Diazal, was slowly added at 0°C. The reaction was followed by TLC (Hexane: EtOAc=4:6, v/v) until the starting material was completely disappeared, then the addition of CH_2N_2 was stopped. After concentration *in vacuo,* the residue was chromatographed to give 9.2g of **2** as a white solid.

3-(Substituted phenyl)-2-propenol (3)

Methyl 2'-hydroxycinnamate (**2**, 5 g) dissolved in the solution of THF was placed in a 500-mL three-necked round-bottomed flask equipped with a magnetic stirrer and a thermometer in dry-ice-acetonitrile (-40°C), and then Diisobutyl-aluminium hydride (DIBAL-H) was slowly added via cannula into the solution until the starting material (**2**) was completely disappeared on TLC. After the addition was completed, the reaction mixture was quenched with saturated ammonium chloride (NH_4Cl) solution. The reaction mixture was extracted with EtOAc (2×200 mL) and the organic layers were collected, then it was dried with sodium sulfate (Na_2SO_4). After concentration *in vacuo,* the residue was chromatographed on silica gel to give 4.8 g of **3a** as a colorless solid.

3-(2'-Hydroxyphenyl)-2-propenol(3a): HRMS: $[MH]^+$; 150.0673 ($C_9H_{11}O_2$), Calcd. 150.0680. mp: 83~88°C. IR (KBr pellet, cm^{-1}): 3400 cm^{-1} (H-bonded OH), 3460 cm^{-1} (free OH), 1630, 1490 cm^{-1} (ar, C=C), 1045, cm^{-1} (C-O bond). ^1H NMR (300 MHz, acetone-d_6) δ 7.40 (1H, dd, *J*=1.5, 7.5 Hz), 7.05 (1H, td, *J*=1.2, 8.1 Hz), 6.89 (1H, d, *J*=16.2 Hz, C_6H_4-CH=CH-), 6.85 (1H, dd, *J*=1.1, 8.1 Hz), 6.79 (1H, td, *J*=1.1, 7.5 Hz), 6.39 (1H, dt, *J*=5.4, 15.9 Hz, C_6H_4-CH=CH-CH_2OH), 4.22 (2H, d, *J*=4.8 Hz, -CH_2OH).

3-(3'-Hydroxyphenyl)-2-propenol(3b): HRMS: $[MH]^+$; 150.0674 ($C_9H_{11}O_2$), Calcd. 150.0680. mp: 87~88°C. IR (KBr pellet, cm^{-1}): 3100~3400 cm^{-1} (H-bonded OH), 3500 cm^{-1} (free OH), 1630, 1490 cm^{-1} (ar, C=C), 980 cm^{-1} (C-O bond). ^1H NMR (300 MHz, acetone-d_6): δ= 7.12 (1H, t, *J*=8.1 Hz), 6.89 (1H, d, *J*=1.5 Hz), 6.87 (1H, s), 6.70 (1H, dt, *J*=1.8, 8.1 Hz), 6.53 (1H, d, *J*=7.5 Hz, C_6H_4-CH=CH-), 6.33 (1H, dt, *J*=7.5, 15.9 Hz, C_6H_4-CH=CH-CHOH), 4.21 (2H, d, *J*=3.9 Hz, CH_2OH).

3-(2'-Chlorophenyl)-2-propenol(3c): ^1H NMR (300 MHz, acetone-d_6) δ 7.62 (1H, dd, *J*=7.2, 1.8 Hz), 7.37 (1H, dd, *J*=1.2, 7.8 Hz), 7.27 (1H, td, *J*=7.2, 1.5 Hz), 7.21 (1H, dt, *J*=8.7, 1.8 Hz), 7.00 (1H, d, *J*=15.9 Hz, C_6H_4-CH=CH-), 6.43 (1H, dt, *J*=15.9, 4.8 Hz, C_6H_4-CH=CH-CHOH), 4.15 (2H, d, *J*=4.8 Hz, CH_2OH).

3-(Substituted phenyl)-2-propenal (4): Compound **4a** was isolated from the bark of *Cinnamomum cassia* Blume and synthesized from 2-hydroxycinnamyl alcohol (Kwon *et al.*, 1996). 3-(2-Hydroxyphenyl)-2-propenol (**3a**, 1 g) dissolved in 100 mL of methylene chloride was placed in a round-bottomed flask equipped with a magnetic stirring bar and a reflux condenser, and then manganese oxide (MnO_2, 2×2 g) was added into the reaction vessel (25°C). The reaction mixture was refluxed in sand bath for 4 hours. When **3a** was completely disappeared on the TLC, manganese oxide was removed with a filter paper and washed with acetone (2×50 mL). After concentration *in vacuo*, the residue was chromatographed on silica gel to give **4a** (320 mg, 32%) as a yellowish soild.

Compounds **4b** and **4c** were also prepared by the same way as that of the compound **4a** from **3b** and **3c**, respectively.

3-(2'-Hydroxyphenyl)-2-propenal (2'-Hydroxycinnamaldehyde, 4a): HRMS: $[MH]^+$; 149.0594 ($C_9H_9O_2$), calcd. 149.0605. mp: 121~125°C. IR (KBr pellet, cm^{-1}): 3000~3500 cm^{-1} (H-bonded OH), 1658 cm^{-1} (C=O, aldehyde), 1612, 1600, 1450 cm^{-1} (ar, C≃C) and 1145 cm^{-1} (C-O bond). ^1H NMR (300 MHz, acetone-d_6) δ 9.68 (1H, d, *J*=7.8 Hz, -CHO), 7.89 (1H, d, *J*=16.2 Hz, C_6H_4-CH=CH-), 7.63 (1H, dd, *J*=7.7, 1.5 Hz, ar), 7.30 (1H, td, *J*=7.7, 1.5 Hz), 7.00 (1H, d, *J*=7.8 Hz, ar), 6.92 (1H, td, *J*=7.5 Hz, 1.1 Hz, ar), 6.84 (1H, dd, *J*=7.8 Hz, 16.2 Hz, C_6H_4-C*H*=CH-CHO).

3-(3'-Hydroxyphenyl)-2-propenal (4b): HRMS: $[MH]^+$; 149.0599 ($C_9H_9O_2$), calcd. 149.0605. mp: 52~54°C. ^1H NMR (300 MHz, acetone-d_6) δ 9.79 (1H, d, *J*=7.5 Hz, -CHO), 8.01 (1H, d, *J*=15.9 Hz, C_6H_4-CH=CH-), 7.91 (1H, t, *J*=7.2 Hz), 7.54 (1H, d, *J*=7.5 Hz), 7.48 (1H, dt, *J*=7.2, 1.8 Hz), 7.42 (1H, td, *J*=7.2, 1.5 Hz), 6.80 (1H, dd, *J*=7.5, 15.9 Hz, C_6H_4- CH=CH-CHO).

3-(2'-Chlorophenyl)-2-propenal (4c): HRMS: $[MH]^+$; 167.0263 ($C_9H_{10}ClO_2$), calcd. 167.0263. mp: 118~120°C. IR (KBr pellet, cm^{-1}): 3000~3500 cm^{-1} (H-bonded OH), 1680 cm^{-1} (C=O, aldehyde), 1600, 1470 cm^{-1} (ar, C=C) and 1170 cm^{-1} (C-O bond). ^1H NMR (300 MHz, acetone-d_6) δ 9.69 (1H, d, *J*=7.8 Hz, -CHO), 7.60 (1H, d, *J*=15.9 Hz, C_6H_4-CH=CH-), 7.29 (1H, t, *J*=7.8), 7.20 (1H, s), 7.16 (1H, d, *J*=2.4 Hz), 6.96 (1H, dd, *J*= 8.25, 2.4 Hz), 6.69 (1H, dd, *J*=7.8, 15.9 Hz, C_6H_4-CH =CH-CHO).

3-(2'-Methoxyphenyl)-2-propenal (2'-Methoxycinnamaldehyde 5a): Compound **5a** was synthesized from methyl 2'-methoxycinnamate, which was prepared with the excess addition of diazomethane to 2-hydroxycinnamic acid in CH_3OH following the procedure described in synthesis of **4a**. 2'-Methoxycinnaldehyde is also commercially available from Aldrich Chemical Co.

Mp: 47~50°C. ^1H NMR (300 MHz, $CDCl_3$) δ 9.68 (1H, d, *J*=7.8 Hz, -CHO), 7.83 (1H, d, *J*=15.9 Hz, C_6H_4-

CH=CH-), 7.53 (1H, dd, J=1.8, 1.8 Hz, ar, 6H), 7.40 (1H , td, J=1.8, 1.5 Hz, ar, 4H), 7.00 (1H, d, J=7.5 Hz, ar, 3H), 6.95 (1H, td, J=3.3, 8.1 Hz, ar, 5H), 6.78 (1H, dt, J=7.8, 16.2 Hz, C_6H_4-CH=CH-CHO), 3.9 (3H, s, OCH_3).

3-(2'-O-Acetylphenyl)-2-propenal (5b): Mp: 82~83°C. R_f=0.65 (Hexane:EtOAc=3:7, v/v). ^{1}H-NMR (300 MHz, acetone-d_6) δ 9.71 (1H, d, J=7.5 Hz, -CHO), 7.88 (1H, dd, J=1.5, 8.1 Hz), 7.78 (1H, d, J=16.0 Hz, C_6H_4-CH=CH-), 7.52 (1H, dt, J=1.5, 7.8 Hz), 7.35 (1H, t, J=1.2, 7.8 Hz), 7.23 (1H, dd, J=1.2, 8.1 Hz), 6.77 (1H, dd, J=7.5, 16.0 Hz, C_6H_4-CH=CH-CHO).

3-(2'-O-Benzoylphenyl)-2-propenal (5c): HRMS: $[M]^+$; 253.0867 ($C_{16}OH_{12}O_3$), calcd. 253.0864. mp: 78~80 °C. R_f=0.70 (Hexane:EtOAc=3:7, v/v). ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.64 (1H, d, J=7.8 Hz, -CHO), 8.27 (1H, d, J=3.6 Hz), 7.74 (1H, d, 16.1 Hz, C_6H_4-CH=CH-), 7.69 (1H, d, J=8.4, 2.1 Hz), 7.59 (2H, m), 7.55 (1H, m), 7.52 (1H, m), 7.38 (1H, m), 7.28 (1H, m), 6.55 (1H, dd, J=7.8, 16.1 Hz, C_6H_4-CH=CH-CHO).

3-(2'-O-Isopropionylphenyl)-2-propenal (5d): HRMS: $[MH]^+$; 219.1018 ($C_{13}H_{15}O_3$), calcd. 219.1021. mp: 75~77°C. R_f=0.71 (Hexane:EtOAc=3:7, v/v). ^{1}H-NMR (300 MHz, acetone-d_6) δ 9.70 (1H, d, J=7.5 Hz, -CHO), 7.88 (1H, dd, J=1.7, 7.8 Hz), 7.71 (1H, d, 16.1 Hz, C_6H_4-CH=CH-), 7.51 (1H, m), 7.35 (1H, m), 7.21 (1H, m), 6.78 (1H, dd, J=7.5, 16.1 Hz, C_6H_4-CH=CH-CHO), 2.96 (1H, m), 1.34 (6H, d, 4.5 Hz).

3-(2'-methylcarboxyphenyl)-2-propenal (5e): HRMS: $[MH]^+$; 191.0911 ($C_{11}H_{11}O_3$), calcd. 191.0901. mp: 38~41°C. ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.68 (1H, d, -CHO), 8.45 (1H, d, C_6H_4-CH=CH-), 8.1 (1H, dd, ar), 7.65 (1H, d, ar), 7.6 (1H, td, ar), 7.5 (1H, td, ar), 6.65 (1H, dt, 16.2 Hz, C_6H_4-CH=CH-CHO), 4.0 (3H, s, $COOCH_3$).

3-[2'-O-Benzylphenyl]-2-propenal (5f): Compound **5f** was synthesized from methyl 2'-hydroxycinnamate (**2**) and benzyl bromide as starting materials.

3-[2'-O-Benzylphenyl]-2-propenal (5f): HRMS: $[M]^+$; 204.1153 ($C_{16}H_{14}O_2$), calcd. 204.1150. mp: 53~55°C. R_f=0.76 (Hexane:EtOAc=3:7, v/v). ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.66 (1H, d, J=7.6 Hz, -CHO), 7.9 (1H, d, J=15.9 Hz, C_6H_4-CH=CH-), 7.8 (1H, dd, J=8.4, 2.1 Hz, ar, 6H), 7.34~7.46 (6H, m), 7.00 (2H, m), 6.78 (1H, dd, J=7.6, 15.9 Hz, C_6H_4-CH=CH-CHO), 5.16 (2H, s, -O-CH_2-).

3-[2'-O-(4-Methoxybenzyl)-phenyl]-2-propenal (5g): HRMS: $[MH]^+$; 269.1190 ($C_{17}H_{17}O_3$), calcd. 269.1178. mp: 84~87°C. R_f=0.82 (Hexane:EtOAc=3:7, v/v). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.7 (1H, d, J=7.6 Hz, -CHO), 7.9 (1H, C_6H_4-CH=CH-), 7.6 (1H, d, J=9.4 Hz), 7.4 (3H, m), 7.1 (2H, m), 6.9 (2H, m), 6.8 (1H, dd, J=7.6, 16.0 Hz, C_6H_4-CH=CH-CHO), 5.2 (2H, s, -O-CH_2-), 3.8 (3H, s, $-OCH_3$).

3-[2'-O-(4-Methylbenzyl)-phenyl]-2-propenal (5h): HRMS: $[MH]^+$; 253.1232 ($C_{17}H_{17}O_2$), calcd. 253.1228. mp: 76~79°C. R_f=0.81 (Hexane:EtOAc=3:7, v/v). ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.7 (1H, d, J=7.5 Hz, -CHO), 7.9 (1H, d, J=16.1 Hz, C_6H_4-CH=CH-), 7.6 (1H, d, J=9.4 Hz), 7.5 (1H, m), 7.4 (2H, m), 7.3 (2H, m), 7.1 (2H, m) 6.8 (1H, dd, J=7.5, 16.1 Hz, C_6H_4-CH=CH-CHO), 5.2 (2H, s, -O-CH_2-.), 2.4 (3H, s, $-CH_3$).

3-[2'-O-(4-Chlorobenzyl)-phenyl]-2-propenal (5i): HRMS: $[MH]^+$; 273.0675 ($C_{17}H_{14}ClO_2$), calcd. 273.0682. mp: 94~95°C. R_f=0.82 (Hexane:EtOAc=3:7, v/v). ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.69 (1H, d, J=7.5 Hz, -CHO), 7.89 (1H, d, J=16.1 Hz, C_6H_4-CH=CH-), 7.59 (1H, dd, J=1.5, 1.5 Hz), 7.32~7.42 (1H, m), 7.00 (2H, m), 6.8 (1H, dd, J=7.5, 16.1 Hz, C_6H_4-CH=CH-CHO), 5.14 (2H, s, -O-CH_2-).

3-[2'-O-(4-Nitrobenzyl)-phenyl]-2-propenal (5j): HRMS: $[MH]^+$; 284.0917 ($C_{17}H_{14}NO_4$), calcd. 284.0922. mp: 122~124°C. R_f=0.60 (Hexane:EtOAc=3:7, v/v). ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.7 (1H, d, J=7.8 Hz, -CHO), 8.29 (2H, dd, J=2.1, 1.8 Hz), 7.9 (1H, d, J=16.1 Hz, C_6H_4-CH=CH-), 7.62 (2H, m), 7.60 (1H, m), 7.4 (1H, m), 7.03 (2H, m), 6.92 (1H, m) 6.8 (1H, dd, J=7.8, 16.1 Hz, C_6H_4-CH=CH-CHO), 5.3 (2H, s, -O-CH_2-).

3-[2'-O-Phenylsulfonylphenyl]-2-propenal (5k): 5k was synthesized by the reaction of **4a** with phenylsulfonyl chloride in the presence of triethyl amine.

HRMS: $[MNH_4]^+$; 306.0799 ($C_{15}H_{16}NO_4S$), calcd. 306.0800. mp: 75~80°C. R_f=0.51 (Hexane:EtOAc=3:7, v/v). ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.40 (1H, d, J=7.5 Hz, -CHO), 7.81 (2H, m), 7.67 (1H, m), 7.62 (2H, m), 7.52 (4H, m), 7.42 (1H, m), 7.32 (4H, m), 6.45 (1H, dd, J=7.5, 16.0 Hz, C_6H_4-CH=CH-CHO).

3-[2'-Methoxyphenyl]-2-propanal (6): To a solution of methyl 3-[2'-methoxyphenyl]-2-propenate (1 g) in 100 mL of CH_3OH was added a catalytic amount of 5% Pd-charcoal and the mixture was stirred under gentle stream of H_2 at room temperature for 3 hours. After filtration of the reaction mixture, the filtrate was concentrated and chromatographed to give 3-(2'-methoxyphenyl)-2-propanol **6**.

To a solution containing 0.1 mL of oxalyl chloride in 2 mL of CH_2Cl_2 was added a solution containing 0.15 mL of dimethyl sulfoxide in 2 mL of CH_2Cl_2 at -78°C. The resulting mixture was stirred for 15 min at -78°C, and a solution containing 0.2 g of 3-(2'-methoxyphenyl)-2-propanol in 2 mL of CH_2Cl_2 was added, and the mixture was allowed to warm to room temperature during a period of 15 min. The reaction mixture was quenched with water and extracted with ethyl acetate. The combined organic layers were washed with small amount of 1 N HCl solution and dried over anhydrous $MgSO_4$. The solvent was removed under reduced pressure, and the residue was purified by silica gel chromatography to give 0.14g of **7** as a colorless oil.

3-[2'-Methoxyphenyl]-2-propanal (6): HRMS: $[MH]^+$;

165.0908 ($C_{10}H_{13}O_2$), calcd. 165.0915. R_f=0.70 (Hexane: EtOAc=3:7, v/v). ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.80 (1H, t, *J*=1.5 Hz, -CHO), 7.23 (2H, m), 6.95 (2H, m), 3.82 (s, OCH_3), 2.96 (2H, t, *J*=7.8 Hz), 2.72 (2H, dt, *J* =1.5, 7.8 Hz).

3-[2'-O-Benzoylphenyl]-2-propanal (9): Compound **9** was also prepared from methyl 3-[2'-O-benzoylphenyl]-2-propenate by the above method for compound **6**.

HRMS: $[MNH_4]^+$; 272.1300 ($C_{16}H_{18}NO_3$), calcd. 272.1286. R_f=0.65 (Hexane:EtOAc=3:7, v/v). ^{1}H NMR (300 MHz, $CDCl_3$) δ 9.89 (1H, s, -CHO), 8.36 (2H, m), 7.88 (1H, m), 7.71 (2H, m), 7.45 (4H, m), 3.05 (2H, m), 2.92 (2H, m).

Cells

Human tumor cells used in the experiment were A 549 (non small lung cell), SK-OV-3 (ovary), SK-MEL-2 (skin), XF498 (central nerve system) and HCT15 (colon). Stock cell cultures were grown in T-25 (Falcon) flasks containing 10 ml of RPMI 1640 medium with glutamine, sodium bicarbonate, gentamycin, amphotericin and 5% of fetal bovine serum. The cells were dissociated with 0.25% trypsin and 2 mM 1,2-cyclohexanediaminetetraacetic acid (CDTA) in PBS in case of transferring or dispensing before experiment. The cells were maintained in the incubator at 37°C in a humidified atomosphere of 5% CO_2 in air continuously except when adding drugs.

Cytotoxicity assay *in vitro*

All experimental procedures were followed up the NCI(USA)'s protocol based on the Sulforhodamine B (SRB) method as described previously (Ryu *et al.*, 1992; Skehan *et al.*, 1990). Briefly, tumor cells were inoculated over a series of standard 96-well flat bottom microtiter plates on day 0. These cells were then preincubated on the microtiter plate for 24 hours. The compounds were added to the wells in six 2-fold dilutions starting from the highest concentrations. The actual assay involved an incubation of the compounds for 48 hours with the cancer cells. At the termination of the incubation, the culture medium in each well was removed, and the cells were fixed with cold 10% trichloroacetic acid (TCA). The microplates were washed and dried after incubation at 4°C for 1 hour with TCA. And then, 0.4% SRB solution was added and incubated for 30 minutes at room temperature. The cells were washed again, and the bound stain was solubilized with 10 mM unbuffered Tris base solution (pH 10.5), and the absorbances were measured spectrophotometrically at 520 nm and 690 nm in a microtiter plate reader. The absorbance measured at 690 nm was subtracted from the absorbance at 520 nm so as to eliminate the effects of non-specific absorbance.

The data were transferred and transformed into a Lotus-123 format and survival fractions were calculated by comparing the drug-treated with controls. All data represented the average values for a minimum of three wells.

RESULTS AND DISCUSSION

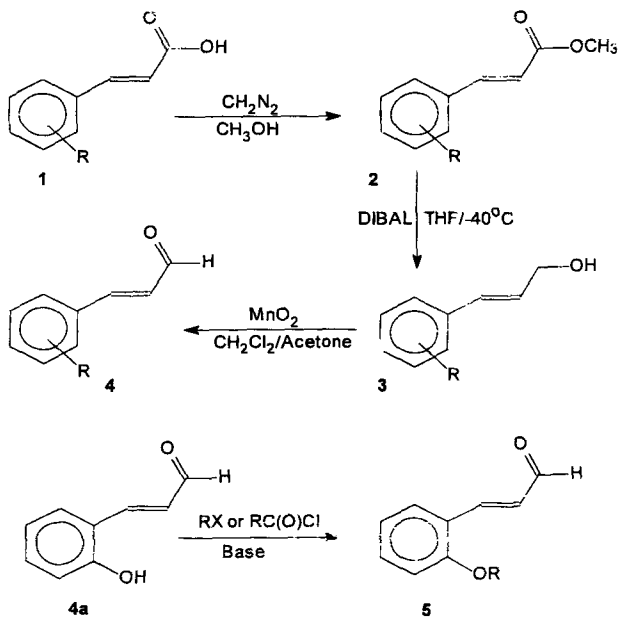
Chemistry

The preparation of desired compounds is detailed in Scheme 1. The commercially available cinnamic acids (**1**) were converted to methyl cinnamates (**2**) by the methylation with diazomethane, which was prepared from Diazal. The methyl esters were reduced by DIBAL to lead the corresponding alcohols (**3**) and followed by oxidation with MnO_2 to give cinnamaldehydes (**4**).

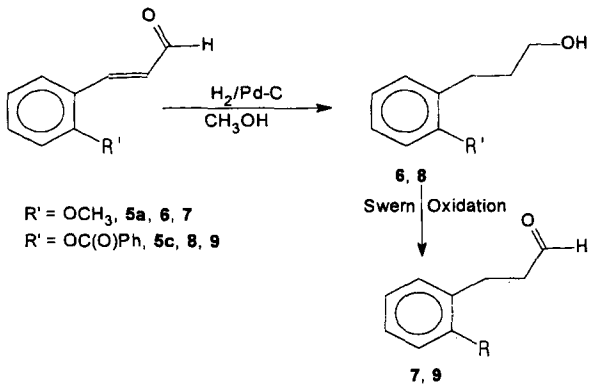
Based on finding obtained using the natural compounds **4a** and **5b** isolated from *Cinnamomum cassia* Blume, the propenal and free phenolic hydroxy groups were identified as targeting sites to study the structure-activity relationship.

Compounds **5a-j** were synthesized by alkylation or acylation of 2'-hydroxycinnamaldehyde **4a** with the corresponding reagents in good yields. 3'-Hydroxy- **4b** and 2'-chloro-cinnamaldehyde **4c** were also prepared for the study of structure-activity relationship from the corresponding cinnamic acids.

The saturated aryl aldehydes **7** and **9** were prepared from **5a** and **5c** by reduction with H_2/Pd-C under atmosphere to see the relationship between double bond in side chain and the biological activity. In the case of compounds **7** and **9**, a different approach



Scheme 1. Preparation of cinnamaldehydes.



Scheme 2. Preparation of saturated aryl aldehydes.

was necessitated by the low reactivity of the alcohols, and the saturated alcohols **6** and **8** were oxidized by Swern oxidation (DMSO/oxalyl chloride) as shown in Scheme 2.

New cinnamaldehydes and their derivatives were characterized by NMR and exact mass spectrometry.

Biological activities

In vitro cytotoxicity of ninteen substituted aryl aldehydes and related compounds in this work is shown in Table I. Standard serial dilution techniques were used for ED_{50} determinations against the human solid tumor cells. Our first goal was to determine the key functional groups in order to narrow the possible range of derivatives for further variation. A549 cell are resistant to all of the synthesized compounds and SK-OV-3 and XF498 cell lines are moderately sensitive to cinnamaldehydes. Cinnamic acid, corresponding alcohols and saturated aryl aldehydes (**7**) did not show any cytotoxicity up to 15 μg/ml except XF498 (ED_{50} value 9.24 μg/ml against compound **7**) in the human tumor cells. However, one of saturated aldehydes 9 is moderately sensitive against SK-MEL-2 with ED_{50} value 5.06 μg/ml), XF498 with ED_{50} value 5.11 μg/ml and HCT15 with ED_{50} value 3.34 μg/ml. Comparison of the substituted positions **4a** with **4b** indicates that the activity of 2'-hydroxy **4a** is about 2-4 times stronger than 3'-hydroxy **4b**, however, the cytotoxicity of **4b** and 2'-chloro **4c** is not greatly variable. The most potent activity against HCT15 was displayed by **4a**, whilst the most potent activity against SK-MEL-2 was displayed by **5b**. In case of a alkyl-substituted cinnamaldehyde **5a**, the cytotoxicity against tumor cells is about 10 times lower than the 2'-OH **4a**. Changing the alkyl substituent R from methyl to polar groups, such as methoxy, chloro and nitro, was examined with the hope of improving activity in comparison to the parent 2'-O-benzyl **5f**. As shown in Table I, the activity had slightly changed with the same trend. We also prepared the compounds **5b**, **5c**, **5d** and **5e** containing acyl or benzoyl group to improve the activity, however, the only slightly improved activity of **5b** and **5e** against SK-Mel-2 and HCT15 was found.

These results suggested that the propenal group of the synthesized cinnamic analogs seems to play a critical role in the antitumor activity of the compounds and the free hydroxy or acyl-substituted groups are also considered to improve the activity of the cinnamaldehydes.

Recently, it was reported that cinnamaldehydes and

Table I. Cytotoxicity of cinnamaldehydes and related compounds against human tumor cells

	Compound	ED_{50} (μg/ml)				
	R	A549	SK-OV-3	SK-MEL-2	XF498	HCT15
1	2'-OH	>20	>20	>20	>20	>20
2	2'-OH	>20	>20	>20	>20	>20
3	2'-OH	>20	>20	>20	>20	>20
4a	2'-OH	>20	1.82	1.63	3.03	0.63
4b	3'-OH	>20	9.17	3.28	6.11	3.40
4c	2'-Cl	>20	4.85	3.12	3.97	3.03
5a	2'-OCH_3	>20	14.17	7.83	5.41	8.06
5b	2'-$OC(O)CH_3$	>20	3.28	0.93	3.27	1.31
5c	2'-OC(O)Ph	>20	3.12	1.49	3.54	1.44
5d	2'-$OC(O)CH(CH_3)_2$	>20	6.27	2.58	13.97	13.08
5e	2'-$C(O)OCH_3$	>20	3.28	0.93	3.27	1.44
5f	2'-OCH_2Ph	>20	8.02	4.72	14.29	6.05
5g	2'-OCH_2Ph-4-OCH_3	17.20	5.77	2.62	8.84	2.43
5h	2'-OCH_2Ph-4-CH_3	16.64	4.42	2.35	11.21	3.16
5i	2'-OCH_2Ph-4-Cl	>20	5.43	4.50	12.86	4.17
5j	2'-OCH_2Ph-4-NO_2	>20	8.70	4.86	14.92	5.62
5k	2'-$OS(O)_2Ph$	15.20	4.06	1.67	1.71	1.09
7	2'-OCH_3	>20	>20	>20	9.24	>20
9	2'-OC(O)Ph	>20	>20	5.06	5.11	3.34
	Cisplatin	1.25	0.83	0.79	0.88	2.13

related compounds inhibited farnesyl transferase which is a critical enzyme for the activation of *ras oncogene* (Kwon, *et al.*, 1996) and antiangiogenic activity of the cinnamaldehydes was also reported (Kwon, *et al.*, 1997). Therefore, the synthesized compounds will be useful for the combination therapeutic agents with clinically used antitumor drugs.

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REFERENCES CITED

Billman, J. H. and Tonnis, J. A., Substituted aralkyl aldehydes: preparation and antitumor evaluation. *J. Pharm. Sci.*, 60, 1188-1192 (1971).

Dornish, J. M., Petterson, E. O. and Oftebro, R., Modifying effect of cinnamaldehyde and cinnamaldehyde derivatives on cell inactivation and cellular uptake of cis-diamminedichloroplatinum (II) in human NHIK 3025 cells. *Cancer Res.*, 49, 3917-3921 (1989).

Goldin, A., Serpick, A. A., Mantel, N., Experimental screening procedures and clinical predictability value. *Cancer Chemoth. Rep.*, 50, 190-231(1966).

Kwon, B. M., Cho, Y. K., Lee, S. H., Nam, J. Y., Bok, S. H., Chun, S. K., Kim, J. A. and Lee, I. R., 2'-Hydroxycinnamaldehyde from stem bark of *cinnamomum cassia*. *Planta Med.* 62, 193-184 (1996).

Kwon, B. M., Lee, S. H., Cho, Y. K., Bok, S. H., So, S. H., Youn, M. R. and Chang, S. I., Synthesis and biological activity of cinnamaldehydes as angiogenesis inhibitors. *Bioorg. Med. Chem. Letters*, 7, 2473-2476 (1997).

Rutten, B. and Gocke, E., The antimutagenic of cinnamaldehyde is due to a transient growth inhibition. *Mutation Res.* 201, 97-105 (1988).

Piantadosi, C., Skulason, V. G., Irvin, J. L., Powell, J. M. and Hall, L., Potential antitumor agents. Schiff-bases and hydrazone derivatives of pyrimidine-4-carboxaldehydes. *J. Med. Chem.*, 7, 337-348 (1964).

Ryu, S. Y., Choi, S. U., Lee, C. O. and Zee, O. P., Anticancer activity of psoralea corylifolia. *Arch. Pharm. Res.*, 15, 356-359 (1992).

Skehan, P. Streng, R. Scudiero, D., Monks, A., McMahon, J., Vistica, D., Warrenm J. T., Bokesch, H., Kenney, S. and Boyd, M. R. New colorimetric cytotoxicity assay for anticancer-drug screening. *J. Natl. Cancer Inst.* 82, 1107-1112 (1990)