Combretastatin-Chalcone Hybrids: Synthesis and Cytotoxicity

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Abstract: A series of all-trans-1-aryl-4-aryl-5-aryl-2,4-pentanediene-1-one (**3**), a hybridized form of chalcone and combretastatin, was synthesized and evaluated against a panel of cancer cell lines, including B16, murine melanoma; HCT116, colon cancer; A431, human epidermoid carcinoma; and human umbilical venous endothelial cells (HUVEC). Structure-activity relationships analysis of this series revealed that a 2,5-dihydroxyphenyl at position 1 of the 2,4-pentanediene-1-one was essential for cytotoxicity. all-trans-1-(2,5-Dihydroxyphenyl)-5-(4-methoxyphenyl)-4-(3,4,5-trimethoxyphenyl)-2,4-pentanediene-1-one (**3a**) was the most potent compound from this series.

Key Words: Combretastatin, chalcone, synthesis, cytotoxicity.

INTRODUCTION

In our research program to develop anticancer and antiangiogenic agents from medicinal plants, we have isolated a simple phenol, methyl caffeic acid ester [1]. This compound shows a very potent cytotoxicity against several murine and human cancer cell lines [1]. Intrigued by its activity we have synthesized a series of 3-aryl-2-propenoates [2] and rigid 2,5-dihydroxycinnamates [3]. From these studies we found methyl 2,5-dihydroxycinnamate a very potent cytotoxic agent, with IC_{50} values in NCI (US National Cancer Institute) 60 cancer cell panel at a low microgram/mL range. In continuity, we prepared a series of 2',5'-dihydroxychalcones (**1**, Fig. **1**) [4]. Most of the synthesized chalcones exhibited significant cytotoxicity against both murine and human cancer cells, with IC_{50} values at a low microgram/mL range and some chalcones also exhibited considerable *in vivo* antitumor activity, with tumor mass inhibition rates recorded at 47-61% when tested in BDF1 mice bearing B16 murine melanoma cells [4]. The results further spurred our interest in this class of compounds.

Structurally, **1** possesses two distinguished features; a hydroquinone and an α,β-unsaturated carbonyl. The hydroquinone has been known to be easily autoxidised *in vivo* to quinone which elicits cytotoxicity *per se* or through a cascade of redox cycling [5]. The α,β-unsaturated carbonyl can be considered as a Michael acceptor, an active moiety often employed in the design of anticancer drugs. A number of α,β-unsaturated ketones have demonstrated preferential activity toward thiols [6]. Alkylation with cellular nucleophiles, such as thiol in glutathione (G-SH), may also occur with chalcones like **1**. Hence, α,β-unsaturated carbonyl-containing compounds may be free from problems of mutagenicity and carcinogenicity that are associated with many alkylating agents used in cancer chemotherapy [7], while displaying potential antitumor activity. From structural consideration, introduction of an additional conjugated double bond may further enhance alkylating capability, thus may increase cytotoxicity. Based on this rationale and structural features of combretastatin A-4 (**2**, Fig. **1**), a known antimitotic anticancer agent, we have designed and synthesized a series of combretastatin-chalcone hybrids (**3**, Fig. **1**) with expectations that **3** would elicit biological activity through multiple mechanisms. In this paper, the results of synthesis and cytotoxic evaluation of these compounds are described.

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MATERIALS AND METHODS

Generals

All products were homogenous, as examined by thin-layer chromatography (TLC), performed on Whatman® 250 μm Silica Gel GF Uniplates and visualized under UV light at 254 nm. Melting points were determined with an Electrothermal Melting Point apparatus and are uncorrected. Chromatographic purification was done by the open flash silica gel column chromatography using Merck silica gel 60 (240 to 400 mesh). Nuclear magnetic resonance spectra (^{1}H NMR) were recorded using tetramethylsilane as an internal standard on a Bruker 400 MHz spectrometer with $CDCl_3$ as solvent unless otherwise indicated. Chemical shifts are reported in parts per million (ppm) downfield from tetramethylsilane as internal standard. Electrospray ionization (ESI) and high-resolution mass spectra were obtained using PE Biosystems API 2000 and Mariner® mass spectrometers, respectively. Reagents and solvents were purchased from Aldrich or Fluka Chemical Corp. (Milwaukee, WI, USA) unless noted otherwise. Solvents were distilled and dried before use.

Synthesis of Compounds

The synthesis of compounds is illustrated in Scheme **1**. Details are described as follows.

(E)-3-(4-Methoxyphenyl)-2-(3,4,5-trimethoxyphenyl)acrylic Acid (4a)

A mixture of 3,4,5-trimethoxyphenylacetic acid (30.0 g, 0.13 mol), 4-methoxy benzaldehyde (18.9 g, 0.13 mol), and triethylamine (20 mL) in acetic anhydride (Ac_2O, 100 mL) was heated at 140 °C for 12 h. After cooling, the mixture was

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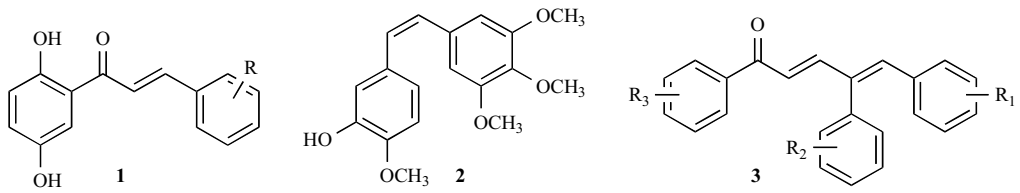


Fig. (1). Structures of 2',5'-dihydroxychalcones (**1**), combretastatin A-4 (**2**) and the designed hybrids of chalcone and combretastatin (**3**).

evaporated to dryness. The residue was diluted with aqueous NaOH for saponification. Then, the solution was acidified with acid acetic (AcOH) and extracted with methylene chloride (MC). The extract was dried over Na_2SO_4 and evaporated under *vacuum* at below 40 °C to dryness. The residue was crystallized from a mixture of ethyl acetate (EA)-hexane to give product **4a** as a white solid (16.5 g, 36%): ^{1}H-NMR (DMSO-d_6, 300 MHz) δ 7.87 (1H, s), 7.06 (2H, d, J = 8.41), 6.73 (2H, d, J = 8.41), 6.47 (2H, s), 3.90 (3H, s), 3.78 (6H, s), 3.77 (3H, s).

(E)-Methyl 3-(4-Methoxyphenyl)-2-(3,4,5-trimethoxyphenyl) acrylate (5a)

To a solution of carboxylic acid **4a** (6.5 g, 18.2 mmol) in dimethyl formamide (DMF, 60 mL) were added K_2CO_3 (6.5 g, 47.1 mmol) and methyl iodide (1.35 mL, 21.8 mmol). The reaction mixture was stirred at room temperature for 3 h and filtered, and the filtrate was evaporated under *vacuum* at below 40 °C to dryness. The residue was dissolved in EA, washed with the brine, and then dried over Na_2SO_4. After concentration, the residue was used for the next reaction without further purification.

(E)-3-(4-Methoxyphenyl)-2-(3,4,5-trimethoxyphenyl)prop-2-en-1-ol (6a)

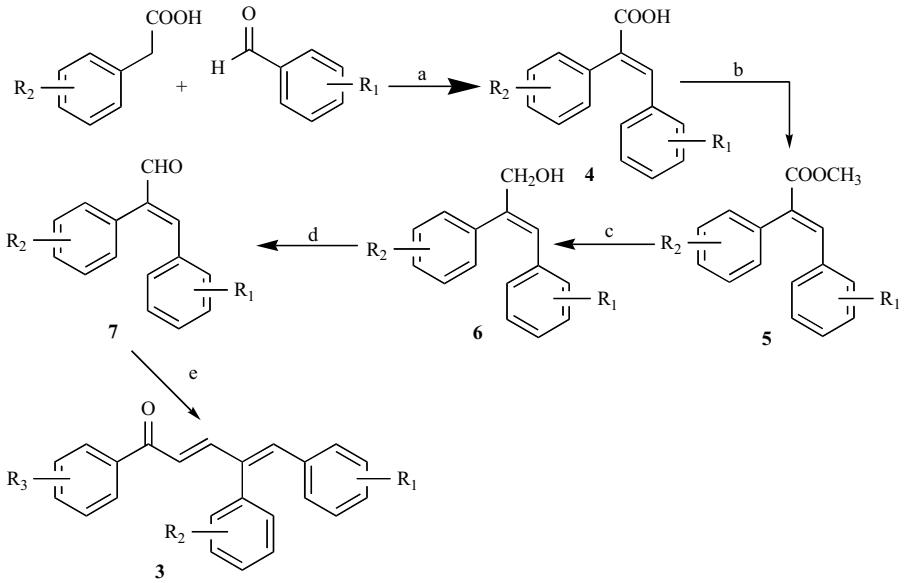
To a solution of methyl ester **5a** (10.5 g, 29.3 mmol) in anhydrous tetrahydrofuran (THF, 60 mL) was added $LiAlH_4$ (30 mL, 30 mmol, solution in THF). The solution was stirred at 0 °C for 1 h and poured into the cold brine. The solution was extracted with MC and dried over Na_2SO_4. After concentration, the residue was purified by silica gel column chromatography (33% EA/hexane) to give pure product **6a** as an oil (6.0 g, 18.2 mmol, 62%): ^{1}H-NMR (DMSO-d_6, 300 MHz) δ 6.98 (2H, d, J = 9.00), 6.69 (2H, d, J = 9.00), 6.59 (1H, s), 6.46 (2H, s), 4.43 (2H, s), 3.87 (3H, s), 3.75 (9H, s).

(E)-3-(4-Methoxyphenyl)-2-(3,4,5-trimethoxyphenyl)propenal (7a)

To a solution of **6a** (2.5 g, 7.6 mmol) in MC (25 mL) was added MnO_2 (20 g). The reaction mixture was stirred vigorously at room temperature for 8 h and filtered over Celite, and the filtrate was evaporated under *vacuum* at below 40 °C to dryness. The residue was purified by silica gel column chromatography (33% EA/hexane) to give product **7a** (1.71 g, 5.2 mmol, 68%) as white crystals: mp 109-110 °C; ^{1}H-NMR (DMSO-d_6, 300 MHz) δ 9.71 (1H, s), 7.31 (1H, s), 7.20 (2H, d, J = 7.81), 6.78 (2H, d, J = 7.81), 6.41 (2H, s), 3.91 (3H, s), 3.79 (9H, s).

All-trans-1-(2,5-Dihydroxyphenyl)-5-(4-methoxyphenyl)-4-(3,4,5-trimethoxyphenyl)-2,4-pentanediene-1-one (3a)

Condensation of **7a** with 2',5'-bis(tetrahydropyran-2-yloxy)acetophenone followed by deprotection of the pyranyl



Scheme (1). Reagents and conditions**:** (a) $(Ac)_2O$, TEA, reflux, 12 h; (b) MeI, K_2CO_3, DMF, rt, 8 h; (c) $LiAlH_4$, THF, 0 °C, 3 h. (d) MnO_2, THF, rt, 8 h; (e) $ArCOCH_3$, $Ba(OH)_2.8H_2O$, MeOH, 40 °C, 12 h then p-toluene sulfonic acid, MeOH, 5 h (for **3a**~**3l**).

groups using the same procedures described for the synthesis of **1** [1-3] gave **3a** as red crystals; mp 132-133 °C; Yield 71%; IR (KBr) 3340, 1640 cm^{-1}; ^{1}H-NMR (DMSO-d_6, 300 MHz) δ 11.76 1H, s), 9.19 (1H, s), 7.81 (1H, d, *J* = 14.91 Hz), 7.24 (1H, s), 6.96 (2H, dd, *J* = 8.85, 2.12 Hz), 6.95-6.89 (3H, m), 6.83 (2H, dd, *J* = 8.86, 2.12 Hz), 6.64 (1H, d, *J* = 14.91 Hz), 6.49 (2H, s), 3.82 (3H, s), 3.81 (3H, s), 3.73 (6H, s). Anal. calcd for $C_{27}H_{26}O_7$: C, 70.12; H, 5.67. Found C, 70.48; H, 5.59.

The following compounds were synthesized by a similar pathway described for all-trans-1-(2,5-dihydroxyphenyl)-5-(4-methoxyphenyl)-4-(3,4,5-trimethoxyphenyl)-2,4-pentanediene-1-ones (**3a**) above unless otherwise described. Only physical and spectral data of the final compounds are shown.

All-trans-1-(2,5-Dihydroxyphenyl)-4-(4-methoxyphenyl)-5-(3,4,5-trimethoxyphenyl)-2,4-pentanediene-1-one (3b)

Red crystals; mp 145-147 °C; IR (KBr) 3360, 1660 cm^{-1}; ^{1}H-NMR (DMSO-d_6, 300 MHz) δ 11.51 1H, s), 9.19 (1H, s), 7.82 (1H, d, *J* = 14.85 Hz), 7.22 (1H, s), 7.19 (2H, dd, *J* = 8.88, 2.15 Hz), 6.96 (1H, dd, *J* = 8.15, 2.04 Hz), 6.87 (1H, d, *J* = 2.04 Hz), 6.86 (1H, d, *J* = 8.15 Hz), 6.79 (2H, dd, *J* = 8.88, 2.15 Hz), 6.63 (1H, d, *J* = 14.85 Hz), 6.35 (2H, s), 3.82 (3H, s), 3.62 (3H, s), 3.49 (6H, s). HRMS *m/z* 462.1682 (M^+). Calcd for $C_{27}H_{26}O_7$: 462.1679.

All-trans-1-(2,5-Dihydroxyphenyl)-4-(3,4-dimethoxyphenyl)-5-(4-methoxyphenyl)-2,4-pentanediene-1-one (3c)

Red crystals; mp 139-140 °C; IR (KBr) 3340, 1640 cm^{-1}; ^{1}H-NMR (DMSO-d_6, 90 MHz) δ 11.68 (1H, s), 9.37 (1H, s), 7.81 (1H, d, *J* = 15.21 Hz), 7.35-7.21 (3H, m), 7.11-6.89 (4H, m), 6.61 (1H, s), 3.84 (3H, s), 3.82 (3H, s), 3.79 (3H, s). HRMS *m/z* 432.1571 (M^+). Calcd for $C_{26}H_{24}O_6$: 432.1573.

All-trans-1-(2,5-Dihydroxyphenyl)-4-(4-methoxyphenyl)-5-(4-methoxyphenyl)-2,4-pentanediene-1-one (3d)

Red crystals; mp 112-113 °C; IR (KBr) 3360, 1660 cm^{-1}; ^{1}H-NMR (DMSO-d_6, 90 MHz) δ 11.87 (1H, s), 9.21 (1H, s), 7.78 (1H, d, *J* = 15.49 Hz), 7.35-7.21 (3H, m), 7.14 (1H, s), 6.87-6.71 (4H, m), 6.57 (1H, s), 3.87 (3H, s), 3.81 (3H, s). HRMS *m/z* 402.1469 (M^+). Calcd for $C_{25}H_{22}O_5$: 402.1470.

All-trans-1-(2,5-Dihydroxyphenyl)-5-(4-methoxyphenyl)-4-phenyl-2,4-pentanediene-1-one (3e)

Red crystals; mp 141-142 °C; IR (KBr) 3350, 1660 cm^{-1}; ^{1}H-NMR (DMSO-d_6, 90 MHz) δ 12.01 (1H, s), 9.07 (1H, s), 7.82 (1H, d, *J* = 15.78 Hz), 7.47-7.23 (3H, m), 7.12 (1H, d, *J* = 2.10 Hz), 6.89-6.73 (4H, m), 6.62 (1H, s), 3.83 (3H, s). Anal. calcd for $C_{24}H_{20}O_4$: C, 77.40; H, 5.41. Found C, 77.45; H, 5.61.

All-trans-1-(2,5-Dihydroxyphenyl)-5-phenyl-4-(3,4,5-trimethoxyphenyl)-2,4-pentanediene-1-one (3f)

Red crystals; mp 155-156 °C; IR (KBr) 3360, 1660 cm^{-1}; ^{1}H-NMR (DMSO-d_6, 90 MHz) δ 11.98 (1H, s), 9.31 (1H, s), 7.92 (1H, d, *J* = 14.45 Hz), 7.51-7.21 (6H, m0), 7.07 (1H, s), 6.89-6.73 (2H, m), 6.62 (1H, s), 6.52 (2H, s), 3.83 (6H, s), 3.79 (3H, s). HRMS *m/z* 432.1566 (M^+). Calcd for $C_{26}H_{24}O_6$: 432.1573.

All-trans-1-(2,5-Dihydroxyphenyl)-4-phenyl-5-phenyl-2,4-pentanediene-1-one (3g)

Red crystals; mp 179-180 °C; IR (KBr) 3340, 1650 cm^{-1}; ^{1}H-NMR (DMSO-d_6, 90 MHz) δ 11.72 (1H, s), 9.24 (1H, s), 7.81 (1H, d, *J* = 15.59 Hz), 7.47-7.19 (11H, m), 7.11 (1H, s), 6.88-6.75 (2H, m), 6.58 (1H, s). HRMS *m/z* 342.1341 (M^+). Calcd for $C_{26}H_{24}O_6$: 342.1256.

All-trans-1-(2,5-Dihydroxyphenyl)-5-(4-ethoxyphenyl)-4-(3,4,5-trimethoxyphenyl)-2,4-pent-anediene-1-one (3h)

Red crystals; mp 166-168 °C; IR (KBr) 3340, 1640 cm^{-1}; ^{1}H-NMR (DMSO-d_6, 90 MHz) δ 12.07 (1H, s), 9.21 (1H, s), 7.91 (1H, d, *J* = 14.58 Hz), 7.56-7.21 (5H, m), 7.09 (1H, d, *J* = 1.89 Hz), 6.94-6.81 (2H, m), 6.67 (1h, s), 6.51 (2H, s), 3.99 (2H, d, *J* = 7.73 Hz), 3.88 (6H, s), 3.80 (3H, s), 1.28 (3H, t, *J* = 7.75 Hz). Anal. calcd for $C_{28}H_{28}O_7$: C, 70.57; H, 5.92. Found C, 70.46; H, 5.72.

All-trans-1-(2,5-Dihydroxyphenyl)-5-(4-chlorophenyl)-4-(3,4,5-trimethoxyphenyl)-2,4-pentanediene-1-one (3i)

Red crystals; mp 171-172 °C; IR (KBr) 3340, 1650 cm^{-1}; ^{1}H-NMR (DMSO-d_6, 90 MHz) δ 12.33 (1H, s), 9.21 (1H, s), 7.98 (1H, d, *J* = 15.51 Hz), 7.71-7.17 (6H, m), 7.05-6.61 (4H, m), 6.38 (2H, s), 3.88 (6H, s), 3.81 (3H, s). Anal. calcd for $C_{26}H_{23}ClO_6$: C, 66.88; H, 4.97. Found C, 66.57; H, 4.70.

All-trans-1-(2,5-Dihydroxyphenyl)-5-(4-methylphenyl)-4-(3,4,5-trimethoxyphenyl)-2,4-pen-tanediene-1-one (3j)

Red crystals; mp 134-135 °C; IR (KBr) 3340, 1650 cm^{-1}; ^{1}H-NMR (DMSO-d_6, 90 MHz) δ 12.21 (1H, s), 9.33 (1H, s), 7.88 (1H, d, *J* = 14.49 Hz), 7.76-7.12 (5H, m), 7.07-6.68 (5H, m), 6.41 (2H, s), 3.84 (6H, s), 3.83 (3H, s), 2.43 (3H, s). Anal. calcd for $C_{27}H_{26}O_6$: C, 72.63; H, 5.87. Found C, 72.52; H, 5.79.

All-trans-1-(2,5-Dihydroxyphenyl)-5-[(3-nitro-4-methoxy)phenyl]-4-(3,4,5-trimethoxyphenyl)-2,4-pentanediene-1-ones (3k)

Red crystals; mp 180-181 °C; IR (KBr) 3340, 1650 cm^{-1}; ^{1}H-NMR (DMSO-d_6, 90 MHz) δ 12.41 (1H, s), 9.31 (1H, s), 7.81 (1H, d, *J* = 15.89 Hz), 7.71-7.05 (4H, m), 7.00-6.62 (5H, m), 6.52 (2H, s), 3.86 (6H, s), 3.85 (3H, s), 3.79 (3H, s). Anal. calcd for $C_{27}H_{25}NO_9$: C, 63.90; H, 4.97; N, 2,76. Found C, 64.12; H, 4.73; N, 2,71.

All-trans-5-[(3-Amino-4-methoxy)phenyl]-1-(2,5-dihydroxy-phenyl)-4-(3,4,5-trimethoxyphenyl)-2,4-pentanediene-1-one (3l)

Compound **3k** (0.99 g, 2 mmol) was dissolved in AcOH (50 mL). Zn (0.65 g) was added in one portion and the mixture was stirred at room temperature for 2 h. Zn was filtered off over a pad of Celite and the filtrate was concentrated *in vacuo*. The residue was purified over a silica gel column eluting with 30% EtOAc in hexane to furnish **3l** as red crystals**.** Yield 65%; *red* crystals; mp 155-157 °C; IR (KBr) 3340, 1650 cm^{-1}; ^{1}H-NMR (DMSO-d_6, 90 MHz) δ 12.38 (1H, s), 9.21 (1H, s), 7.80 (1H, d, *J* = 15.43 Hz), 7.74-7.12 (4H, m), 6.99-6.64 (5H, m), 6.49 (2H, s), 3.87 (6H, s), 3.84 (3H, s), 3.81 (3H, s). Anal. calcd for $C_{27}H_{27}NO_7$: C, 67.91; H, 5.70; N, 2,93. Found C, 67.64; H, 5.78; N, 2,82.

All-trans-1-(4-Chlorophenyl)-5-[(3-nitro-4-methoxy)phenyl]-4-(3,4,5-trimethoxyphenyl)-2,4-pentanediene-1-one (3m)

Red crystals; mp 169-170 °C; IR (KBr) 3340, 1650 cm^{-1}; ^{1}H-NMR (DMSO-d_6, 90 MHz) δ 8.01 (1H, s), 7.81 (1H, d, *J* = 15.22 Hz), 7.75-7.56 (3H, m), 7.47-7.22 (3H, m), 7.07 (1H, d, *J* = 7.78 Hz), 6.71 (1H, s), 3.94 (3H, s), 3.88 (6H, s), 3.81 (3H, s). Anal. calcd for $C_{27}H_{24}ClNO_7$: C, 63.59; H, 4.74; N, 2,75. Found C, 63.88; H, 4.72; N, 2,91.

All-trans-5-[(3-Amino-4-methoxy)phenyl]-1-(4-chlorophenyl)-4-(3,4,5-trimethoxyphenyl)-2,4-pentanedien-1-one (3n)

This compound was synthesized from **3m** by the same procedure described for **3l**. Red crystals; mp 145-146 °C; IR (KBr) 3340, 1650 cm^{-1}; ^{1}H-NMR (DMSO-d_6, 90 MHz) δ 7.87 (1H, d, *J* = 15.49 Hz), 7.72 (2H, d, *J* = 7.84 Hz), 7.07 (1H, d, *J* = 7.84 Hz), 7.25 (1H, d, *J* = 15.49 Hz), 6.72-6.56 (4H, m), 6.52 (2H, s), 3.91 (3H, s), 3.87 (6H, s), 3.83 (3H, s). Anal. calcd for $C_{27}H_{26}ClNO_5$: C, 67.57; H, 5.46; N, 2,92. Found C, 67.43; H, 5.27; N, 2,97.

All-trans-5-[(3-Nitro-4-methoxy)phenyl]-1-(4-methylphenyl)-4-(3,4,5-trimethoxyphenyl)-2,4-pentanediene-1-one (3o)

Red crystals; mp 154-155 °C; IR (KBr) 3340, 1650 cm^{-1}; ^{1}H-NMR (DMSO-d_6, 90 MHz) δ 8.00 (1H, s), 7.88 (1H, d, *J* = 15.12 Hz), 7.78-7.70 (3H, m), 7.37-7.25 (3H, m), 7.03 (1H, d, *J* = 7.12 Hz), 6.70 (1H, s), 6.53 (2H, s), 3.95 (3H, s), 3.84 (3H, s), 3.80 (3H, s), 2.43 (3H, s). Anal. calcd for $C_{28}H_{27}NO_7$: C, 68.70; H, 5.56; N, 2,86. Found C, 68.75; H, 5.62; N, 2,63.

All-trans-5-[(3-Amino-4-methoxy)phenyl]-1-(4-methylphenyl)-4-(3,4,5-trimethoxyphenyl)-2,4-pentanediene-1-one (3p)

This compound was synthesized from **3o** by the same procedure described for **3l**. Red crystals; mp 149-152 °C; IR (KBr) 3340, 1650 cm^{-1}; ^{1}H-NMR (DMSO-d_6, 90 MHz) δ

Table 1. Cytotoxicity of Some Extended Chalcones in Cancer Cell Lines[1] and HUVECs

Cp	R_1	R_2	R_3	Cytotoxicity (IC_{50}[2], μg/mL)			
				B16	HCT116	A431	HUVEC
3a	4-OCH_3	3,4,5-$(OCH_3)_3$	2,5-$(OH)_2$	0.79	0.84	0.49	0.51
3b	3,4,5-$(OCH_3)_3$	4-OCH_3	2,5-$(OH)_2$	1.05	1.00	0.94	0.79
3c	4-OCH_3	3,4-$(OCH_3)_2$	2,5-$(OH)_2$	>10	>10	>10	>10
3d	4-OCH_3	4-OCH_3	2,5-$(OH)_2$	1.81	1.74	1.43	1.11
3e	4-OCH_3	H	2,5-$(OH)_2$	7.96	7.44	6.16	6.00
3f	H	3,4,5-$(OCH_3)_3$	2,5-$(OH)_2$	8.44	7.79	5.44	5.99
3g	H	H	2,5-$(OH)_2$	2.41	2.51	1.89	2.21
3h	4-OCH_2 CH_3	3,4,5-$(OCH_3)_3$	2,5-$(OH)_2$	1.11	1.19	1.12	1.00
3i	4-Cl	3,4,5-$(OCH_3)_3$	2,5-$(OH)_2$	3.05	3.28	3.00	2.47
3j	4-CH_3	3,4,5-$(OCH_3)_3$	2,5-$(OH)_2$	5.07	4.98	4.36	3.72
3k	4-OCH_3-3-NO_2	3,4,5-$(OCH_3)_3$	2,5-$(OH)_2$	2.47	2.54	1.99	2.01
3l	4-OCH_3-3-NH_2	3,4,5-$(OCH_3)_3$	2,5-$(OH)_2$	1.99	2.01	1.49	1.81
3m	4-OCH_3-3-NO_2	3,4,5-$(OCH_3)_3$	4-Cl	>10	>10	>10	6.42
3n	4-OCH_3-3-NH_2	3,4,5-$(OCH_3)_3$	4-Cl	>10	>10	>10	>10
3o	4-OCH_3-3-NO_2	3,4,5-$(OCH_3)_3$	4- CH_3	>10	>10	>10	>10
3p	4-OCH_3-3-NH_2	3,4,5-$(OCH_3)_3$	4- CH_3	>10	>10	>10	>10
3r	4-OCH_3-3-NO_2	3,4,5-$(OCH_3)_3$	4-OCH_3	>10	>10	>10	9.47
3q	4-OCH_3-3-NH_2	3,4,5-$(OCH_3)_3$	4-OCH_3	>10	>10	>10	>10
1 (R = H, 2',5'-dihydroxychalcone)[3]				2.25	3.29	2.44	1.89
2 (CA-4)				0.06	0.07	#[4]	#
ADR[5]				0.13	0.99	0.84	0.27

[1]Cancer cell lines: B16, murine melanoma; HCT116, colon cancer; A431, human epidermoid carcinoma. [2]A sample's concentration produces a 50% reduction in cell growth. The values shown were the averages from a triplicate experiment. [3]Data from ref. 5. [4]Not tested. [5]Adriamycin, used as a positive control.

7.88 (1H, d, *J* = 15.99 Hz), 7.76 (2H, d, *J* = 7.78 Hz), 7.52 (2H, d, *J* = 7.78 Hz), 7.31 (1H, d, *J* = 15.99 Hz), 6.67-6.52 (6H, m), 3.85 (3H, s), 3.84 (6H, s), 3.81 (3H, s), 2.43 (3H, s). Anal. calcd for $C_{28}H_{29}NO_5$: C, 72.18; H, 6.36; N, 3,05. Found C, 72.37; H, 6.47; N, 3,25.

All-trans-5-[(3-Nitro-4-methoxy)phenyl]-1-(4-methoxyphenyl)-4-(3,4,5-trimethoxyphenyl)-2,4-pentanediene-1-one (3q)

Red crystals; mp 136-138 °C; IR (KBr) 3340, 1650 cm^{-1}; ^{1}H-NMR (DMSO-d_6, 90 MHz) δ 8.11 (1H, s), 7.92 (1H, d, *J* = 14.49 Hz), 7.74 (2H, d, *J* = 8.02 Hz), 7.70 (1H, d, *J* = 7.71 Hz), 7.35-6.94 (4H, m), 6.68 (1H, s), 6.52 (2H, s), 3.90 (3H, s), 3.88 (3H, s), 3.81 (3H, s), 3.79 (3H, s). Anal. calcd for $C_{28}H_{27}NO_8$: C, 66.53; H, 5.38; N, 2,77. Found C, 66.47 H, 5.45; N, 2,68.

All-trans-5-[(3-Amino-4-methoxy)phenyl]-1-(4-methoxyphenyl)-4-(3,4,5-trimethoxyphenyl)-2,4-pentanediene-1-one (3r)

This compound was synthesized from **3q** by the same procedure described for **3l**. Red crystals; mp 173-174 °C; IR (KBr) 3340, 1650 cm^{-1}; ^{1}H-NMR (DMSO-d_6, 90 MHz) δ 7.82 (1H, d, *J* = 15.47 Hz), 7.70 (2H, d, *J* = 7.84 Hz), 7.56 (2H, d, J = 7.84 Hz), 7.39 (1H, d, *J* = 15.47 Hz), 6.72-6.58 (6H, m), 3.86 (3H, s), 3.83 (6H, s), 3.82 (3H, s), 3.78 (3H, s). Anal. calcd for $C_{28}H_{29}NO_6$: C, 70.72; H, 6.15; N, 2,95. Found C, 70.41 H, 6.32; N, 2,88.

***In vitro* Cytotoxic Assay**

The synthesized compounds were evaluated in a panel of cancer cell lines and HUVEC by SRB method with some modifications as described in detail previously [4].

RESULTS AND DISCUSSION

Chemistry

A series of combretastatin-chalcone hybrids **3** was obtained *via* a five-reaction pathway illustrated in Scheme **1**, starting from commercially available phenylacetic acids and benzaldehydes. Based-catalyzed condensation of R_1-substituted benzaldehydes with R_2-substituted phenylacetic acids in the presence of TEA and acetic anhydride at 140 °C gave the carboxylic acids **4**. In this reaction, only the *E*-form was formed. Reaction of **4** with MeI at room temperature gave methyl esters **5**. Compounds **5** were then reacted with $LiAlH_4$ to give alcohol **6** which was oxidized by MnO_2 to afford propenals **7**. A Claisen-Schmidt condensation of propenals **7** with 2',5'-bis(tetrahydropyran-2-yloxy)acetophenone in an usual way followed by deprotection of tetrahydropyranyl (THP) group as described previously [5-7] furnished the target combretastatin-chalcone hybrids **3**. These combretastatin-chalcones all adopted (*E*)-configuration as evidenced by ^{1}H-NMR spectra (coupling constants of the two newly formed olefinic protons were around 16 Hz).

Cytotoxicity

A series of the so-called "extended chalcones" or combretastatin-chalcone hybrids **3** (Table **1**) was prepared based on the earlier observation that the chalcone **1** offered considerable cytotoxic and antitumor activity and the previous study showing a series of *cis*-propenals and combretastatins with potentials as antitumor agents [1-4, 8-9]. Since 2,5-dihydroxy moiety has been previously shown to be optimal for bioactivity [4], initially 2,5-dihydroxy substitution was kept on ring C and manipulation was mainly involved the introduction of multiple methoxy substituents as R1 and R2 on ring A and B. The results presented in Table **1** demonstrate that the tetramethoxy substitution as in the two compounds **3a** and **3b** was important for cytotoxicity. Both **3a** and **3b** showed strong cytotoxic activity againts all cell lines tested, however **3a** was found to be significantly more potent than **3b**. When a 4-methoxy substituent was kept on ring B and the 3,4,5-trimethoxy substituent on ring A was replaced by different groups, all resulted compounds (**3c-3e**) were much less active than **3a**. When a 3,4,5-trimethoxy substituent on ring A was kept and the 4-methoxy group on ring B was replaced by other substituents, all compounds obtained (**3f**, **3h-l**) were also less potent than **3a**. Thus, 3,4,5-trimethoxy substituent on ring A and 4-methoxy on ring B seem to be optimal for bioactivity. Next, the 2,5-dihydroxy on ring C was replaced by different functionalities. All of the resulted compounds (**3m-3q**) were inactive. These results demonstrate that the 2,5-dihydroxy moiety on ring C was essential. From this series, **3a** turns out to be the most potential candidate for further investigation.

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