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Der Pharmacia Sinica, 2014, 5(4):40-46

Der Pharmacia Sinica
ISSN: 0976-8688
CODEN (USA): PSHIBD

Synthesis of 2-styrylchromones and cytotoxicity evaluation

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ABSTRACT

*A series of 2-styrylchromones **5a-h** were synthesized and their cytotoxic activity was evaluated at a very low concentration against MCF-7 and MDA-237 cell lines. All compounds showed higher cytotoxic activity for both cell lines. The structures of the newly synthesized compounds were characterized by IR, ^{1}H and ^{13}C NMR, LC-MS and elemental analysis.*

Key words: 2-styrylchromones, structural studies, cancer cell lines MCF-7 and MDA-237, cytotoxic activity.

INTRODUCTION

2-Styrylchromones are a small group of naturally occurring chromones, vinylogues of flavones (2-phenylchromones). Several analogues of these compounds have been synthesized and tested in different biological systems showed different activities with potential therapeutic applications, even before the first isolation of natural 2-styrylchromones from the green alge *Chrysophaem taylori* in 1986 [1,2]. The natural compounds were demonstrated to possess cytotoxic activity against leukaemia cells [1,2] and those obtained by synthesis exhibited antiallergic [3], antiviral [4], antitumour [5] and antagonism of A3 adenosine receptor [6] properties. Recently, these compounds have also demonstrated antioxidant properties namely xanthine oxadase inhibition [7], hepatoprotection against pro-oxidant agents in cellular and non- cellular systems [8,9] and scavenging activity against reactive oxygen and reactive nitrogen species (ROS and RNS) [10].

Further more, chromones have been found to be active in a number of plant cycles, including growth regulation, indole acetic acid oxidation and dormancy inhibition as well as exhibiting cytokine in type behaviour and stimulating oxygen up takes in plant tissues [11]. Accordingly, many synthesized chromones derivatives have extensively studied for the development of novel anticancer agents. Molecular mechanisms of anticancer effects mediated by chromones such as flavonoids could be attributed to antiproliferation, stimulation of apoptosis, cell cycle arrest and promotion of differentiation, inhibition of angiogenesis and modulation of multidrug resistance [12]. Therefore, the aim of the present work was to continue searching study a series of structure related 2-styrylchromones (Scheme 1) for their potential cytotoxic activity.

MATERIALS AND METHODS

Chemistry

Melting points were determined in open capillaries on a Mel-Temp apparatus and are uncorrected. All the reactions were monitored by thin layer chromatography (TLC) on precoated silica gel 60 F254 (mesh); spots were visualized with UV light. Merck silica gel (60-120 mesh) was used for column chromatography. The IR spectra were recorded on a Perkin-Elmer BX1 FTIR Spectrophotometer as KBr pellets and the wave numbers were given in cm^{-1}. ^{1}H NMR (400 MHz), and ^{13}C NMR (100 MHz) spectra were recorded on a Bruker AMX 400 MHz NMR spectrometer in $CDCl_3$/DMSO-d_6 solution using TMS as an internal standard. The mass spectra were recorded on Agilent 1100

LC/MSD instrument with method API-ES at 70 eV. All chemical shifts are reported in δ (ppm) using TMS as an internal standard. Elemental analysis were recorded on a Thermo Finnigan Flash EA 1112 analyzer.

General procedure for the preparation of compounds (3a-h)
Compounds (**3a-h**) was prepared by the procedure given in the literature [14]

***General procedure for the preparation of compounds* (4a-h)**
Potassium hydroxide (1.4 g, 25 mmol) was added to a solution of the appropriate cinnamoyloxy acetophenone (**3a-h)** (5 mmol) in dry pyridine (15 mL). The solution was stirred at room temperature for 2 h. After that the solution was poured into ice-cold water at pH 4. The solid obtained was taken in chloroform and washed with water and the organic layer was dried over Na_2SO_4 and the solvent was evaporated to dryness in each case. The residue was crystallized from ethanol to obtain good yields (**4a-h**).

3-Hydroxy-5-(2-hydroxy-5-acetoxyphenyl)-5-oxopenta-1,3-dienyl)phenyl acetate (4a)
Yield: 84% (brownish yellow color solid); mp 198-200 ^{0}C; IR (KBr) (ν_{max}/cm^{-1}): 3250, 1682, 1621, 1547; ^{1}H NMR (400 MHz; $CDCl_3$): δ_H 2.32 (s, 3H, OAc-5''), 2.34 (s, 3H, OAc-4'), 6.24 (s, 1H, H-2), 6.54 (d, J_{HH} = 16.0 Hz, 1H, H-4), 7.00 (d, J_{HH} = 8.5 Hz, 1H, H-3''), 7.15 (d, J_{HH} = 8.5 Hz, 2H, H-3',5'), 7.19 (dd, J_{HH} = 8.5, 2.5 Hz, 1H, H-4''), 7.46 (d, J_{HH} = 2.5 Hz, 1H, H-6''), 7.60 (d, J_{HH} = 8.5 Hz, 2H, H-2',6'), 7.82 (d, J_{HH} = 16.0 Hz, 1H, H-5), 12.20 (s, 1H, Ar-OH), 14.60 (s, 1H, enolic-OH); ^{13}C NMR (100 MHz; $CDCl_3$): δ_C 21.1, 22.1, 97.0, 114.2, 116.0, 119.5, 120.6, 122.2, 129.1, 130.3, 132.7, 139.2, 142.3, 146.8, 160.2, 174.7, 195.7; LC-MS (ESI) m/z = 381 $(M–H)^-$; Anal. Calcd. for. $C_{21}H_{18}O_7$: C, 65.96; H, 4.71; Found: C, 65.90; H, 5.02 %.

3-Hydroxy-5-(2-hydroxy-5-methoxyphenyl)-5-oxopenta-1,3-dienyl)phenyl acetate (4b)
Yield: 80% (brownish yellow color solid); mp 211-213 ^{0}C; IR (KBr) (ν_{max}/cm^{-1}): 3341, 1676, 1618, 1536; ^{1}H NMR (400 MHz; $CDCl_3$): δ_H 2.23 (s, 3H, OAc-4'), 3.80 (s, 3H, Ar-OMe), 6.24 (s, 1H, H-2), 6.62 (d, J_{HH} = 16.0 Hz, 1H, H-4), 6.88 (d, J_{HH} = 8.5 Hz, 1H, H-3''), 7.12 (dd, J_{HH} = 8.5, 2.5 Hz, 1H, H-4''), 7.18 (d, J_{HH} = 8.5 Hz, 2H, H-3',5'), 7.35 (d, J_{HH} = 2.5 Hz, 1H, H-6''), 7.64 (d, J_{HH} = 8.5 Hz, 2H, H-2',6'), 7.86 (d, J_{HH} = 16.0 Hz, 1H, H-5), 11.80 (s, 1H, Ar-OH), 14.70 (s, 1H, enolic-OH); ^{13}C NMR (100 MHz; $CDCl_3$): δ_C 21.1, 97.1, 114.3, 116.0, 119.2, 122.3, 124.7, 129.6, 132.0, 133.3, 139.2, 141.8, 152.5, 160.2, 175.3, 195.3; LC-MS (ESI) m/z = 353 $(M–H)^-$; Anal. Calcd. for. $C_{20}H_{18}O_6$: C, 67.79; H, 5.08; Found: C, 67.57; H, 5.20 %.

3-Hydroxy-5-(2-hydroxy-4-acetoxyphenyl)-5-oxopenta-1,3-dienyl)-2-methoxyphenyl acetate (4c)
Yield: 84% (brownish yellow color solid); mp 157-158 ^{0}C; IR (KBr) (ν_{max}/cm^{-1}): 3332, 1663, 1624, 1519; ^{1}H NMR (400 MHz; $CDCl_3$): δ_H 2.24 (s, 3H, OAc-4'), 2.30 (s, 3H, OAc-4''), 3.84 (s, 3H, Ar-OMe), 6.16 (s, 1H, H-2), 6.54 (d, J_{HH} = 3.4 Hz, 1H, H-3''), 6.56 (d, J_{HH} = 16.0 Hz, 1H, H-4), 6.62 (dd, J_{HH} = 7.0, 2.4 Hz, 1H, H-5''), 6.98 (d, J_{HH} = 8.3 Hz, 1H, H-5'), 7.16 (dd, J_{HH} = 8.3, 1.8 Hz, 1H, H-6'), 7.18 (d, J_{HH} = 1.8 Hz, 1H, H-2'), 7.52 (d, J_{HH} = 16.0 Hz, 1H, H-5), 7.62 (d, J_{HH} = 7.0, Hz, 1H, H-6''), 12.80 (s, 1H, Ar-OH), 14.60 (s, 1H, enolic-OH); ^{13}C NMR (100 MHz; $CDCl_3$): δ_C 20.7, 21.1, 56.0, 97.1, 109.6, 112.0, 114.3, 119.3, 120.8, 121.6, 123.4, 131.9, 133.1, 133.8, 141.9, 146.9, 151.5, 164.3, 164.5, 165.1, 174.5, 195.6; LC-MS (ESI) m/z = 413 $(M+H)^+$; Anal. Calcd. for. $C_{22}H_{20}O_8$: C, 64.07; H, 4.85; Found: C, 64.10; H, 4.83 %.

3-Hydroxy-5-(2-hydroxy-5-acetoxyphenyl)-5-oxopenta-1,3-dienyl)-2-methoxyphenyl acetate (4d)
Yield: 76% (greenish yellow color solid); mp 151-153 ^{0}C; IR (KBr) (ν_{max}/cm^{-1}): 3254, 1683, 1633, 1514; ^{1}H NMR (400 MHz; $CDCl_3$): δ_H 2.25 (s, 3H, OAc-5''), 2.28 (s, 3H, OAc-4'), 3.94 (s, 3H, Ar-OMe), 6.22 (s, 1H, H-2), 6.46 (d, J_{HH} = 16.0 Hz, 1H, H-4), 7.00 (d, J_{HH} = 8.3 Hz, 1H, H-3''), 7.05-7.25 (m, 4H, H-4'',2',5',6'), 7.43 (d, J_{HH} = 2.5 Hz, 1H, H-6''), 7.62 (d, J_{HH} = 16.0 Hz, 1H, H-5), 12.10 (s, 1H, Ar-OH), 14.55 (s, 1H, enolic-OH); ^{13}C NMR (100 MHz; $CDCl_3$): δ_C 20.6, 21.0, 56.0, 97.2, 116.1, 118.7, 120.3, 120.8, 126.5, 127.4, 127.7, 133.8, 137.6, 139.3, 141.6, 146.0, 158.4, 159.9, 161.0, 163.6, 174.8, 195.3; LC-MS (ESI) m/z = 413 $(M+H)^+$; Anal. Calcd. for. $C_{22}H_{20}O_8$: C, 64.07; H, 4.85; Found: C, 64.04; H, 5.00 %.

3-Hydroxy-5-(2-hydroxy-5-methoxyphenyl)-5-oxopenta-1,3-dienyl)-2-methoxyphenyl acetate (4e)
Yield: 78% (yellowish brown color solid); mp 132-134 ^{0}C; IR (KBr) (ν_{max}/cm^{-1}): 3290, 1641, 1627, 1552; ^{1}H NMR (400 MHz; $CDCl_3$): δ_H 2.30 (s, 3H, OAc-4'), 3.80 (s, 3H, Ar-OMe), 3.90 (s, 3H, Ar-OMe), 6.24 (s, 1H, H-2), 6.52 (d, J_{HH} = 16.0 Hz, 1H, H-4), 7.03 (d, J_{HH} = 8.3 Hz, 1H, H-3''), 6.90-7.40 (m, 4H, H-4'',2',5',6'), 7.41 (d, J_{HH} = 2.5 Hz, 1H, H-6''), 7.60 (d, J_{HH} = 16.0 Hz, 1H, H-5), 11.80 (s, 1H, Ar-OH), 14.80 (s, 1H, enolic-OH); ^{13}C NMR (100 MHz; $CDCl_3$): δ_C 20.6, 56.0, 57.2, 97.1, 114.5, 116.9, 117.6, 119.6, 121.0, 122.9, 123.6, 124.7, 137.2, 139.2, 146.5, 158.8, 159.7, 160.2, 162.8, 175.7, 195.4; LC-MS (ESI) m/z = 483 $(M–H)^-$; Anal. Calcd. for. $C_{21}H_{20}O_7$: C, 65.62; H, 5.20; Found: C, 65.64; H, 5.22 %.

3-Hydroxy-1-(2-hydroxy-4-acetoxyphenyl)-5-(3,4,5-trimethoxyphenyl)penta-2,4-dien-1-one (4f)
Yield: 70% (dark yellow color solid); mp 138-140 ^{0}C; IR (KBr) (ν_{max}/cm^{-1}): 3315, 1669, 1626, 1523; ^{1}H NMR (400 MHz; $CDCl_3$): δ_H 2.30 (s, 3H, OAc-4''), 3.76 (s, 3H, Ar-OMe-4'), 3.90 (s, 6H, Ar-OMe-3',5'), 6.20 (s, 1H, H-2), 6.54 (d, J_{HH} = 2.3 Hz, 1H, H-3''), 6.58 (dd, J_{HH} = 8.3, 2.3 Hz, 1H, H-5''), 6.60 (d, J_{HH} = 16.0 Hz, 1H, H-4), 7.50 (s, 2H, H-2', 6'), 7.60 (d, J_{HH} = 8.3 Hz, 1H, H-6''), 7.62 (d, J_{HH} = 16.0 Hz, 1H, H-5), 12.80 (s, 1H, Ar-OH), 14.58 (s, 1H, enolic-OH); ^{13}C NMR (100 MHz; $CDCl_3$): δ_C 21.3, 56.2, 61.0, 97.0, 105.6, 112.6, 115.6, 118.6, 121.4, 132.9, 136.2, 136.7, 143.4, 155.8, 157.3, 162.6, 164.9, 176.2, 195.6; LC-MS (ESI) m/z = 415 $(M+H)^+$; Anal. Calcd. for. $C_{22}H_{22}O_8$: C, 63.76; H, 5.31; Found: C, 63.77; H, 5.34 %.

3-Hydroxy-1-(2-hydroxy-5-acetoxyphenyl)-5-(3,4,5-trimethoxyphenyl)penta-2,4-dien-1-one (4g)
Yield: 74% (yellow color solid); mp 143-145 ^{0}C; IR (KBr) (ν_{max}/cm^{-1}): 3347, 1684, 1635, 1542; ^{1}H NMR (400 MHz; $CDCl_3$): δ_H 2.30 (s, 3H, OAc-5''), 3.91 (s, 3H, Ar-OMe-4'), 3.93 (s, 6H, Ar-OMe-3',5'), 6.23 (s, 1H, H-2), 6.48 (d, J_{HH} = 16.0 Hz, 1H, H-4), 6.78 (s, 2H, H-2', 6'), 6.84 (d, J_{HH} = 8.3 Hz, 1H, H-3''), 7.04 (dd, J_{HH} = 8.3, 2.2 Hz, 1H, H-4''), 7.14 (d, J_{HH} = 2.2 Hz, 1H, H-6''), 7.56 (d, J_{HH} = 16.0 Hz, 1H, H-5), 11.80 (s, 1H, Ar-OH), 14.72 (s, 1H, enolic-OH); ^{13}C NMR (100 MHz; $CDCl_3$): δ_C 20.9, 56.2, 61.0, 96.9, 105.7, 119.5, 120.6, 121.4, 124.3, 129.2, 129.5, 130.6, 140.2, 147.0, 153.5, 156.7, 165.7, 174.7, 195.3; LC-MS (ESI) m/z = 415 $(M+H)^+$; Anal. Calcd. for. $C_{22}H_{22}O_8$: C, 63.76; H, 5.31; Found: C, 63.75; H, 5.35 %.

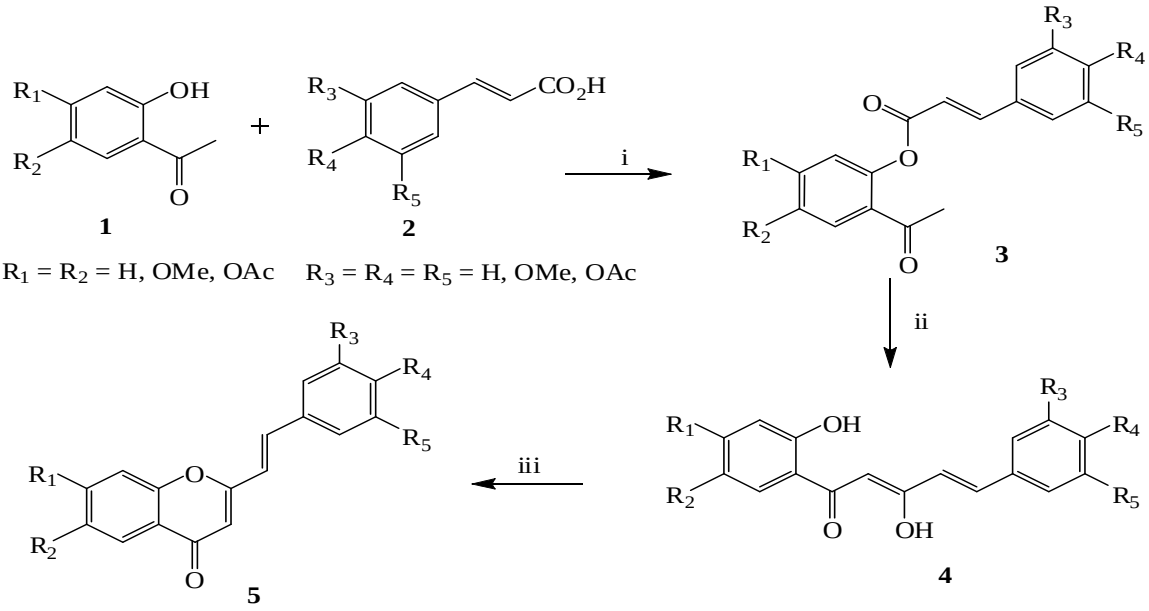
3-Hydroxy-1-(2-hydroxy-5-methoxyphenyl)-5-(3,4,5-trimethoxyphenyl)penta-2,4-dien-1-one (4h)
Yield: 75% (dark yellow color solid); mp 130-132 ^{0}C; IR (KBr) (ν_{max}/cm^{-1}): 3295, 1672, 1624, 1535; ^{1}H NMR (400 MHz; $CDCl_3$): δ_H 3.82 (s, 3H, OMe-5''), 3.91 (s, 3H, Ar-OMe-4'), 3.94 (s, 6H, Ar-OMe-3',5'), 6.28 (s, 1H, H-2), 6.52 (d, J_{HH} = 16.0 Hz, 1H, H-4), 6.80 (s, 2H, H-2', 6'), 6.94 (d, J_{HH} = 8.5 Hz, 1H, H-3''), 7.10 (dd, J_{HH} = 8.5, 2.5 Hz, 1H, H-4''), 7.14 (d, J_{HH} = 2.5 Hz, 1H, H-6''), 7.58 (d, J_{HH} = 16.0 Hz, 1H, H-5), 11.80 (s, 1H, Ar-OH), 14.70 (s, 1H, enolic-OH); ^{13}C NMR (100 MHz; $CDCl_3$): δ_C 56.2, 56.6, 61.0, 97.0, 105.4, 111.6, 118.7, 119.5, 121.4, 123.5, 130.5, 135.4, 140.0, 152.0, 153.6, 157.0, 174.7, 195.4; LC-MS (ESI) m/z = 385 $(M–H)^–$; Anal. Calcd. for. $C_{21}H_{22}O_7$: C, 65.28; H, 5.70; Found: C, 65.25; H, 5.66 %.

General procedure for the preparation of compounds (5a-h)
Concentrated H_2SO_4 (0.75 mL) was added to a solution of the appropriate 3-hydroxy-1-(2-hydroxyphenyl)-5-phenyl-2,4-pentadien-1-one (**4a-h**) (10 mmol) in glacial acetic acid (20 mL) and the reaction mixture was heated at 90^{O}C for 3 h with stirring. After cooling, the reaction mixture was poured into ice cold water and stirred for 10min. The obtained solid was collected by filtration, washed with water and dried. The residue was purified by silica gel chromatography, using $CHCl_3$: *n*-hexane (7:3) as the eluent, to affording the expected hydroxy-2-styrylchromones in good yields (**5a-h**).

2-(4-Hydroxystyryl)-6-hydroxy-4H-chromen-4-one (5a)
Yield: 96% (brick red color solid); mp 270-272 ^{0}C; IR (KBr) (ν_{max}/cm^{-1}): 3355, 1630, 1612, 1570, 1473; ^{1}H NMR (400 MHz; DMSO-d_6): δ_H 6.29 (s, 1H, H-3), 6.80 (d, J_{HH} = 8.3 Hz, 2H, H-3',5'), 6.93 (d, J_{HH} = 16.0 Hz, 1H, H-α), 7.20 (dd, J_{HH} = 8.8, 2.7 Hz, 1H, H-7), 7.27 (d, J_{HH} = 2.7 Hz, 1H, H-5), 7.54 (d, J_{HH} = 8.3 Hz, 2H, H-2',6'), 7.55 (d, J_{HH} = 16.0 Hz, 1H, H-β) 7.56 (d, J_{HH} = 8.8 Hz, 1H, H-8), 9.95 (brs, 2H, 2×Ar-OH); ^{13}C NMR (100 MHz; DMSO-d_6): δ_C 108.0, 115.9, 117.0, 119.3, 122.7, 124.4, 126.1, 129.6, 131.2, 136.3, 149.1, 154.6, 159.3, 162.0, 176.8; LC-MS (ESI) m/z = 279 $(M–H)^–$; Anal. Calcd. for. $C_{17}H_{12}O_4$: C, 72.86; H, 4.29; Found: C, 72.83; H, 4.32 %.

2-(4-Hydroxystyryl)-6-methoxy-4H-chromen-4-one (5b)
Yield: 95% (yellow color solid); mp 259-261 ^{0}C; IR (KBr) (ν_{max}/cm^{-1}): 3389, 2922, 1639, 1607, 1565, 1480; ^{1}H NMR (400 MHz; DMSO-d_6): δ_H 3.84 (s, 3H, Ar-OCH_3), 6.36 (s, 1H, H-3), 6.82 (d, J_{HH} = 8.2 Hz, 2H, H-3',5'), 6.96 (d, J_{HH} = 16.0 Hz, 1H, H-α), 7.33-7.38 (m, 2H, H-5,7), 7.55 (d, J_{HH} = 8.2 Hz, 2H, H-2',6'), 7.58 (d, J_{HH} = 16.0 Hz, 1H, H-β) 7.64 (d, J_{HH} = 9.0 Hz, 1H, H-8), 9.98 (brs, 1H, Ar-OH); ^{13}C NMR (100 MHz; DMSO-d_6): δ_C 55.6, 108.2, 115.9, 116.9, 119.6, 122.8, 124.4, 126.1, 129.6, 131.2, 136.5, 150.1, 156.3, 159.3, 162.1, 176.5; LC-MS (ESI) m/z = 293 $(M–H)^–$; Anal. Calcd. for. $C_{18}H_{14}O_4$: C, 73.46; H, 4.76; Found: C, 73.42; H, 4.77 %.

2-(4-Hydroxy-3-methoxystyryl)-7-hydroxy-4H-chromen-4-one (5c)
Yield: 85% (orange yellow color solid); mp 260-262 ^{0}C; IR (KBr) (ν_{max}/cm^{-1}): 3369, 2918, 1636, 1618, 1514, 1461; ^{1}H NMR (400 MHz; DMSO-d_6): δ_H 3.83 (s, 3H, Ar-OCH_3), 6.22 (s, 1H, H-3), 6.81 (d, J_{HH} = 8.2 Hz, 1H, H-5'), 6.87 (dd, J_{HH} = 8.6, 2.2 Hz, 1H, H-6), 6.89 (d, J_{HH} = 2.2 Hz, 1H, H-8), 6.97 (d, J_{HH} = 16.0 Hz, 1H, H-α), 7.00 (dd, J_{HH} = 8.2, 2.0 Hz, 1H, H-6'), 7.13 (d, J_{HH} = 2.0 Hz, 2H, H-2'), 7.53 (d, J_{HH} = 16.0 Hz, 1H, H-β) 7.82 (d, J_{HH} = 8.6 Hz, 1H, H-5), 8.87 (brs, 1H, Ar-OH), 9.62 (brs, 1H, Ar-OH); ^{13}C NMR (100 MHz; DMSO-d_6): δ_C 55.6, 102.2, 108.6, 114.6, 114.9, 115.6, 116.2, 117.2, 122.3, 126.4, 126.7, 136.3, 147.9, 148.7, 157.2, 161.7, 162.8, 176.2; LC-MS (ESI) m/z = 309 $(M–H)^–$; Anal. Calcd. for. $C_{18}H_{14}O_5$: C, 69.68; H, 4.5; Found: C, 69.63; H, 4.8 %.



$R_1 = R_2 =$ H, OMe, OAc $R_3 = R_4 = R_5 =$ H, OMe, OAc

For compounds 5

a) $R_1 = R_3 = R_5 =$ H; $R_2 = R_4 =$ OH
b) $R_1 = R_3 = R_5 =$ H; $R_2 =$ OMe; $R_4 =$ OH
c) $R_1 = R_4 =$ OH; $R_2 = R_5 =$ H; $R_3 =$ OMe
d) $R_1 = R_5 =$ H; $R_2 = R_4 =$ OH; $R_3 =$ OMe
e) $R_1 = R_5 =$ H; $R_2 = R_3 =$ OMe; $R_4 =$ OH
f) $R_1 =$ OH; $R_2 =$ H; $R_3 = R_4 = R_5 =$ OMe
g) $R_1 =$ H; $R_2 =$ OH; $R_3 = R_4 = R_5 =$ OMe
h) $R_1 =$ H; $R_2 =$ OMe; $R_3 = R_4 = R_5 =$ OMe

For compounds 3 and 4

a) $R_1 = R_3 = R_5 =$ H; $R_2 = R_4 =$ OAc
b) $R_1 = R_3 = R_5 =$ H; $R_2 =$ OMe; $R_4 =$ OAc
c) $R_1 = R_4 =$ OAc; $R_2 = R_5 =$ H; $R_3 =$ OMe
d) $R_1 = R_5 =$ H; $R_2 = R_4 =$ OAc; $R_3 =$ OMe
e) $R_1 = R_5 =$ H; $R_2 = R_3 =$ OMe; $R_4 =$ OAc
f) $R_1 =$ OAc; $R_2 =$ H; $R_3 = R_4 = R_5 =$ OMe
g) $R_1 =$ H; $R_2 =$ OAc; $R_3 = R_4 = R_5 =$ OMe
h) $R_1 =$ H; $R_2 =$ OMe; $R_3 = R_4 = R_5 =$ OMe

***Reagents and Conditions**: (i) pyridine, $POCl_3$, rt. 4-5 h (ii) Dry pyridine, KOH, rt. 1-2 h and (iii) AcOH, aq.H_2SO_4, 90–95 ^0C, 2-3 h.*

Scheme 1. Synthesis of 2-styrylchromones 5a-h

2-(4-Hydroxy-3-methoxystyryl)-6-hydroxy-4H-chromen-4-one (5d)

Yield: 88% (yellow color solid); mp 268-270 ^0C; IR (KBr) (ν_{max}/cm^{-1}): 3456, 2968, 1638, 1589, 1525, 1461; ^1H NMR (400 MHz; DMSO-d_6): δ_H 3.83 (s, 3H, Ar-OCH_3), 6.28 (s, 1H, H-3), 6.81 (d, J_{HH} = 8.1 Hz, 1H, H-5'), 7.00 (d, J_{HH} = 16.0 Hz, 1H, H-α), 7.12 (dd, J_{HH} = 8.1, 1.7 Hz, 1H, H-6'), 7.21 (dd, J_{HH} = 8.9, 2.8 Hz, 1H, H-7), 7.27 (d, J_{HH} = 2.8 Hz, 1H, H-5), 7.29 (d, J_{HH} = 16.0 Hz, 1H, H-β), 7.52 (d, J_{HH} = 1.7 Hz, 2H, H-2'), 7.56 (d, J_{HH} = 8.9 Hz, 1H, H-8), 9.57 (brs, 1H, Ar-OH), 9.94 (brs, 1H, Ar-OH); ^{13}C NMR (100 MHz; DMSO-d_6): δ_C 55.7, 107.7, 108.0, 110.8, 115.7, 117.3, 119.3, 122.3, 122.7, 124.4, 126.6, 136.7, 148.0, 148.8, 149.1, 154.6, 162.0, 176.7; LC-MS (ESI) m/z = 309 $(M–H)^-$; Anal. Calcd. for. $C_{18}H_{14}O_5$: C, 69.68; H, 4.5; Found: C, 68.66; H, 4.7 %.

2-(4-Hydroxy-3-methoxystyryl)-6-methoxy-4H-chromen-4-one (5e)

Yield: 88% (grey color solid); mp 230-232 ^0C; IR (KBr) (ν_{max}/cm^{-1}): 3228, 1643, 1607, 1529, 1382; ^1H NMR (400 MHz; DMSO-d_6): δ_H 3.84 (s, 6H, Ar-OCH_3), 6.34 (s, 1H, H-3), 6.82 (d, J_{HH} = 8.1 Hz, 1H, H-5'), 7.02 (d, J_{HH} = 16.0 Hz, 1H, H-α), 7.13 (dd, J_{HH} = 8.1, 2.2 Hz, 1H, H-6'), 7.39- 7.33 (m, 2H, H-5,7), 7.53 (d, J_{HH} = 2.2 Hz, 2H, H-2'), 7.58 (d, J_{HH} = 16.0 Hz, 1H, H-β), 7.64 (d, J_{HH} = 9.0 Hz, 1H, H-8), 9.55 (brs, 1H, Ar-OH); ^{13}C NMR (100 MHz; DMSO-d_6): δ_C 55.7, 105.0, 108.2, 110.9, 115.7, 117.1, 119.6, 122.4, 122.8, 124.2, 126.6, 136.9, 148.0, 148.9, 150.1, 156.4, 162.2, 176.6; LC-MS (ESI) m/z = 323 $(M–H)^-$; Anal. Calcd. for. $C_{19}H_{16}O_5$: C, 70.37; H, 4.94; Found: C, 70.33; H, 5.00 %.

2-(3,4,5-Trimethoxystyryl)-7-hydroxy-4H-chromen-4-one (5f)

Yield: 80% (suff color solid); mp 250-252 ^0C; IR (KBr) (ν_{max}/cm^{-1}): 3460, 2931, 2850, 1635, 1616, 1593; ^1H NMR (400 MHz; DMSO-d_6): δ_H 3.69 (s, 3H, Ar-OCH_3), 3.84 (s, 6H, Ar-OCH_3), 6.26 (s, 1H, H-3), 6.84 (dd, J_{HH} = 8.8, 2.1 Hz, 1H, H-6), 6.96 (d, J_{HH} = 9.0 Hz, 1H, H-8), 7.06 (s, 2H, H-2',6'), 7.13 (d, J_{HH} = 16.1 Hz, 1H, H-α), 7.56 (d, J_{HH} = 16.1 Hz, 1H, H-β), 7.58 (d, J_{HH} = 8.8 Hz, H-5), 9.64 (brs, 1H, Ar-OH); ^{13}C NMR (100 MHz; DMSO-d_6): δ_C 55.9, 60.1, 105.5, 107.4, 109.2, 114.7, 116.4, 120.1, 130.6, 136.2, 136.7, 139.0, 153.1, 157.3, 161.4, 162.7, 176.4; LC-MS (ESI) m/z = 355 $(M+H)^+$; Anal. Calcd. for. $C_{20}H_{18}O_6$: C, 67.80; H, 5.08; Found: C, 67.75; H, 5.12 %.

2-(3,4,5-Trimethoxystyryl)-6-hydroxy-4H-chromen-4-one (5g)
Yield: 80% (orange yellow color solid); mp 249-251 ^{0}C; IR (KBr) (ν_{max}/cm^{-1}): 3368, 2926, 1639, 1613, 1575; ^{1}H NMR (400 MHz; DMSO-d_6): δ_H 3.69 (s, 3H, Ar-OCH$_3$), 3.84 (s, 6H, Ar-OCH$_3$), 6.32 (s, 1H, H-3), 7.06 (s, 2H, H-2',6'), 7.17 (d, J_{HH} = 16.1 Hz, 1H, H-α), 7.23 (dd, J_{HH} = 8.9, 2.8 Hz, 1H, H-7), 7.28 (d, J_{HH} = 2.8 Hz, H-5), 7.54 (d, J_{HH} = 8.9 Hz, 1H, H-8), 7.59 (d, J_{HH} = 16.1 Hz, 1H, H-β), 9.98 (brs, 1H, Ar-OH); ^{13}C NMR (100 MHz; DMSO-d_6): δ_C 55.9, 60.1, 105.3, 107.6, 108.8, 119.3, 120.0, 122.9, 124.4, 130.6, 136.3, 138.9, 149.1, 153.1, 154.7, 161.5, 176.8; LC-MS (ESI) m/z = 355 (M+H)$^{+;}$ Anal. Calcd. for. $C_{20}H_{18}O_6$: C, 67.80; H, 5.08; Found: C, 67.76; H, 5.10 %.

2-(3,4,5-Trimethoxystyryl)-6-methoxy-4H-chromen-4-one (5h)
Yield: 92% (greenish yellow color solid); mp 174-176 ^{0}C; IR (KBr) (ν_{max}/cm^{-1}): 2932, 2833, 1650, 1605, 1580; ^{1}H NMR (400 MHz; DMSO-d_6): δ_H 3.69 (s, 3H, Ar-OCH$_3$), 3.84 (s, 9H, Ar-OCH$_3$), 6.38 (s, 1H, H-3), 7.06 (s, 2H, H-2',6'), 7.18 (d, J_{HH} = 16.1 Hz, 1H, H-α), 7.32-7.40 (m, 2H, H-5,7), 7.61(d, J_{HH} = 16.1 Hz, 1H, H-β), 7.64 (d, J_{HH} = 9.0 Hz, 1H, H-8); ^{13}C NMR (100 MHz; DMSO-d_6): δ_C 55.6, 55.9, 60.1, 105.3, 108.9, 110.6, 117.6, 119.6, 123.0, 124.2, 130.6, 136.4, 139.0, 150.1, 153.1, 156.3, 161.6, 176.6; LC-MS (ESI) m/z = 369 (M+H)$^{+;}$ Anal. Calcd. for. $C_{21}H_{20}O_6$: C, 68.48; H, 5.43; Found: C, 68.42; H, 5.45 %.

Cytotoxic activity
The compounds **5a-h** was subjected to MTT assay to determine cytotoxic capability.

Cell lines and culture
Human breast cancer MCF-7 and MDA-237 cells were grown in DMEM (Dulbecco's Modified Eagle's Medium) media containing 10% FBS, 1 mM nonessential amino acids, 1 mM sodium pyruvate and 1% penicillin/streptomycin. The cells were plated in T-25 tissue culture flask and incubated at 37 ^{0}C in a 5% CO_2 atmosphere with 90% humidity.

MTT Cytotoxicity assay
The effects of the compounds on human cell growth, presented as the percentage of viable cells were evaluated by the MTT method [15-19]. Cells were plated on a 96-well plate at 3×10^3 cells/well and exposed to the test compounds (0.1- 50 µM) for 72 h. Cultures were also treated with DMSO as the medium control. After 72 h of treatment 10 mL of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) solution (20 µL of 5 mg/mL) was added to each well and the plates were incubated for 2-4 h at 37 ^{0}C. The supernatant was then removed from formazan crystals and 100 mL of DMSO was added to each well. The absorbance at 570 nm was read using an Eliza Plate Reader. The cell viability was calculated by the average of three (n = 3) independent experiments of compound-containing wells by that of DMSO-control wells. Three separate experiments were accomplished to determine the IC_{50} values.

RESULTS AND DISCUSSION

New 2-styrylchromones (**5a**-**h**) were prepared in good yields 80-96%. The synthesis was carried out according to the three-step sequence in Scheme 1 and based on the Baker-Venkataraman rearrangement [13]. This involved in the formation of the desired 2'-cinnamoyloxyacetophenones from substituted *ortho*-hydroxy acetophenones and cinnamic acid derivatives in pyridine using $POCl_3$ as a condensing agent. A strong base such as potassium hydroxide abstracts a proton from the methyl ketone and the resultant carbanion attacks the ester carbonyl resulting in the conversion of the cinnamoyloxyacetophenones to the keto enols. A ring chain tautomerism then occurs in this intermediate which is subsequently protonated by the strong catalyst sulphuric acid and attack by the 2'-hydroxy group ultimately results in the more stable 2-styrylchromone products by elimination. The cinnamic acid (**2)** was prepared by an aldol condensation and elimination reaction from the corresponding benzaldehydes and malonic acid before reacted with the corresponding acetophenones. The structures of the prepared compounds were elucidated using ^{1}H and ^{13}C NMR spectroscopies along with mass spectrometry and IR spectroscopy.

In fact, the cinnamoyloxyacetophenone (**4a**) was established by the IR spectra present only one carbonyl absorption shifted to 1621 cm^{-1} by the strong intra molecular hydrogen bonding. The ^{1}H NMR spectra shows two signals at 14.60 and 12.20 ppm, due to the protons of the enolic and the phenolic hydroxyl groups, respectively. Moreover, the diene structure is confirmed by the presence of three signals, each for one olefinic proton, a singlet at about 6.24 ppm for H–2 and a pair of doublets at 6.54 and 7.82 ppm for H–4 and H–5, respectively. The high value of the corresponding coupling constant (J about 16.0 Hz) establishes the *trans* configuration of this double bond and the structure was confirmed by the detection of the molecular ion at *m*/*z* 381 in the LC-MS. The other intermediates (**4a**-**h**) had similar NMR data and the structures were elucidated in the same manner as 31. The cyclodehydration of 2,4-pentadien-1-ones (**4a**-**h**) by refluxing in sulphuric acid gave the corresponding 2-styrylchromones (**5a**-**h**) was confirmed by the IR spectrum of these compounds exhibited a band in the region 1630–1650 cm^{-1} for α, β-

unsaturated carbonyl group and strong absorption band at 3228–3460 cm^{-1} showed presence of OH groups. In the ^1H NMR spectra, the characteristic signals for the H-3 was observed around δ 6.22–6.38 ppm. The resonance signal to β–H (δ 7.29–7.61 ppm) was appeared at higher frequency values than that of α–H (δ 6.93–7.18 ppm) due to the mesomeric deshielding effect of chromone ring and also further confirmed by LC-MS spectra of all the 2-styrylchromones showed intense molecular ion peak in negative and positive ion mode at respective molecular weights.

Cytotoxicity

The synthesized compounds **5a-h** were subjected to MTT assay to determine growth inhibitory/cytotoxic capability against two cancer cell lines MCF-7 and MDA-237 at concentration ranging from 0.1 µM to 50 µM for 72 h are presented in Figs. 1 and 2. The clinically applied anticancer agent, doxorubicin, was used as positive control in this study. Most of the compounds showed remarkable activity against MCF-7 and MDA-237 cells. 2-Styrylchromones influenced the cell growth in a concentration-dependent manner and caused 70% growth inhibition at the highest applied concentration (10 µM). The compound **5f** nearly inactive at all tested concentrations (0.1, 1 and 10 µM), while the compound **5c** was more active in all concentrations (0.1, 1 and 10 µM) against both MCF-7 and MDA-237 cells.

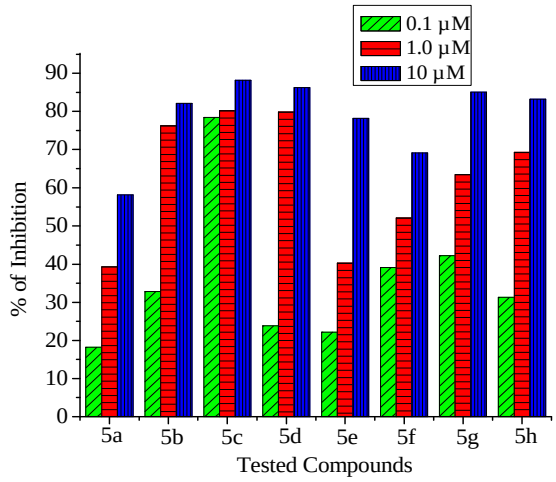


Fig 1. Effect of the 2-styrylchromones on the growth of inhibition of MCF-7 breast cancer cell line

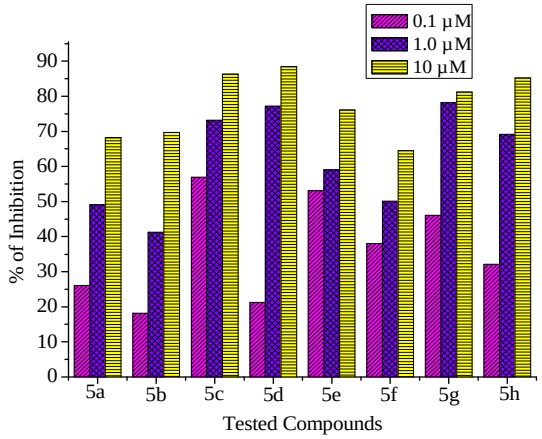


Fig 2. Effect of the 2-styrylchromones on the growth of inhibition of MDA-237 breast cancer cell line

CONCLUSION

Evaluation of the cytotoxicity results shows that the presence of methoxy substituted 2-styrylchromones **5c** and **5d** results in a significant increase of cytotoxic activity when compared to the activity of the **5a** and **5b** compounds. Moreover, the cytotoxicity of all the above synthesized compounds was higher in all the cell lines that were tested in comparison to that of reference anticancer agent doxorubicin.

Acknowledgment
One of the authors K.Vasu is thankful to the University of Grant Commission (UGC), New Delhi, for financial assistance under research project UGC Lr. No. F.36-237/2008 and Sujen Life Sciences, Tirupati for cytotoxic activity testing are gratefully acknowledged.

REFERENCES

[1] Gerwick WH, Lopez A, Van Dyune GD, Clardy J, Ortiz W, Baez A, *Tetrahedron Lett*, **1986**, 27, 1979.
[2] Gerwick WH, *J. Nat. Prod*, **1989**, 52, 252.
[3] Doria G, Romeo C, Forgione A, Sberze P, Tibolla N, Corno ML, Cruzzola G, Cadelli G, *Eur. J. Med. Chem*,**1979**, 14, 347.
[4] Desideri N, Conti C, Mastromarino P, Mastropaolo F, *Antiviral Chem. Chemother*, **2000**, 11, 373.
[5] Brion D, Le Baut G, Zammatio F, Pierre A, Atassi G, Belachm L, *Chem. Abstr*, **1991**, 116, 106092k.
[6] Karton Y, Jiang JL, Ji XD, Melman N, Olah ME, Stiles GL, Jacobson KA, *J. Med. Chem*, **1996**, 39, 2293.
[7] Fernandes E, Carvalho F, Silva AMS, Santos CMM, Pinto DCGA, Cavaleiro JAS, Bastos MLJ, *Enzyme Inhib. Med. Chem*, **2002**, 17, 45.
[8] Fernandes E, Carvalho M, Carvalho F, Silva AMS, Santos CMM, Pinto DCGA, Cavaleiro JAS, Bastos ML, *Arch. Toxicol*, **2003**, 77, 500.
[9] Filipe P, Silva AM, Morliere P, Brito CM, Patterson LK, Hug GL, Silva JN, Cavaleiro JA, Maziere JC, Freitas JP, Santus R, *Biochem. Pharmacol*, **2004**, 67, 2207.
[10] Gomes A, Fernandes E. Silva AM, Santos CM, Pinto DC, Cavaleiro JA, Lima JL, *Bioorg. Med. Chem*, **2007**, 15, 6027.
[11] Detty MR, *Organometallics*, **1988**, 7, 2188.
[12] Ren W, Qiao Z, Wang H, Zhu L, Zhang L, *Med. Res. Re,*. **2003**, 23, 519.
[13] Pinto DCGA, Silva AMS, Cavaleiro JAS, *New Journal of Chemistry*, **2000**, 24, 85.
[14] Ana G, Ondrej N, Marisa F, Diana C, Daniela R, Andrea GPRF, Artur MSS, Raquel SGRS, Diana CGAP, Augusto CT, Jose ASC, Eduarda F, Jose LFCL, *Bioorg. Med. Chem*, **2009**, 17, 7218.
[15] Li M, Zhang Z, Hill DL, Chen X, Wang H, Zhang R, *Cancer Res*, **2005**, 65, 8200.
[16] Wang W, Zhang X, Qin JJ, Voruganti S, Nag SA, Wang MH, Wang H, Zhang R, *PLoS One*, **2012,** 7, e41586.
[17] Wahi AK, Singh A, *Der Chemica Sinica*, **2011**, 2, 11.
[18] Baviskar BA, Khadabadia SS, Deore SL, Shiradkar MR, *Der PharmacianSinica*, **2012**, 3, 24.
[19] Rathinam P, Dhana SSS, Kothapalli BCS, Somasundaram J, *European Journal of Experimental Biology*, **2012**, 2, 882.