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REGULAR ARTICLE

Design and synthesis of (*Z*/*E*)-2-phenyl/*H*-3-styryl-2*H*-chromene derivatives as antimicrotubule agents

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Abstract. Two new series of compounds (*Z*/*E*)-2-phenyl/*H*-3-Styryl-2*H*-Chromenes **9**(**a**-**r**) and **10**(**a**-**s**) were synthesized and evaluated *in vitro* cytotoxic activities against four cancer cell lines. One compound, *(Z)-8-ethoxy-3-(4-methoxystyryl)-2-phenyl-2H-chromene* (***9g***) was found to be the most active among the tested compounds in HeLa cell lines (IC_{50}10μM). Compound **9g** arrested cells at G2/M phase, disrupted microtubule network, accumulated tubulin in the soluble fraction and manifested an increased expression of the G2/M marker, Cyclin B1.

Keywords. Combretastatin; candenatenin E; chromene; resveratrol; Wittig reaction; cytotoxicity; antimitotic activity; chromene.

1. Introduction

Cancer is a collection of different life-threatening diseases, in which a group of cells display uncontrolled growth in a body. According to the statistics, cancer is the second most common cause of death worldwide after cardiovascular diseases. The corresponding incidence and mortality statistics shows that it is growing in developing as well as developed countries.[1,2] Although the cancer research has led to a number of new and effective solutions, the medicines used as treatments have clear limitations and unfortunately, cancer is projected as the primary cause of death. Existing drugs are effective and cytotoxic and thus exhibit severe side effects, particularly on normal proliferating tissues such as the haematopoietic system. The use of novel and improved chemopreventive and chemotherapeutic agents for the prevention and treatment of cancer is on the rise. Natural products have always afforded a rich source of such agents.[3] Nowadays, there is a huge scientific and commercial interest in the discovery of new hybrid anticancer drugs,[4] with their ability to interact with more than one target, represent, in medicinal chemistry, a significant source of inspiration for the design of structural analogues with improved pharmacological profile of action acting in synergy to inhibit cancer tumor growth. Therefore, the search for potent, safe and selective hybrid anticancer drug is a crucial aspect of modern cancer research.[5] In this connection, current literature report reveals that 2*H*-chromene, a small molecule natural product and their derivatives such as KCN1,[6] S14161, BENC-511,[7] Seselin,[8] Xanthylein,[9] Lonchocarpin,[10] Acronycine,[11] and (*Z*)-(2*H*-Chromene-3-yl) methylene azolidinones[12] etc., also show potent anticancer activities. Candenatenin E[13] is a chromene derivative isolated from the heartwood of *D. candenatensis* exhibits potent cytotoxic activity against HT-29 (colon cancer), KB (human oral cancer), MCF-7 (breast adenocarcinoma), and HeLa (human cervical cancer) cell lines.[7,14] A key aspect is that the lipophilic nature of the benzopyran derivatives helps to cross the cell membrane more easily.

From the literature survey we also found that *cis* stilbene based natural product combretastatin A4, exhibited a significant role in clinical applications, particularly acting as anticancer agent.[14] Various hybrid

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combretastatin analogs have been designed and synthesized; among them, *cis* restricted heterocycles, fused heterocycles and nonsubstituted aromatic ring analogs of CA4 are most important.[15–33] Not only *cis* stilbene but also *trans*-stilbene based natural product and synthetic analogs show pronounced anticancer and antiapoptotic activities; among them, resveratrol and its analogs are most important.[14,34]

Nowadays, the preparation of hybrid compounds that possess important skeletons present in drugs/clinical agents has become an important research area for medicinal chemists.[35] This can be an effective approach to possibly avoid physicochemical/pharmacokinetic/toxicity problems that appear in the later stages of development.[36] All these observations and our interest in chromene and stilbene derivatives prompted us to explore a series of new compounds, (*Z*)-2-phenyl/H-3-styryl-2*H*-chromenes **9(a–r)** and (*E*)-2-phenyl/*H*-3-styryl-2*H*-chromenes **10(a–s)** as hybrid analogs of candenatenin E **1**, combretastatin A4(CA4) and resveratrol **2** as potential anticancer agents.

Herein we have designed the molecules on the basis of SAR studies and explored their cytotoxicity in four different cancer cell lines such as HeLa, MCF-7, A549 and DU145. Among the compounds tested, **9g** displayed the most potent antiproliferative activity against HeLa cells with IC_{50} 10.62 μM. Further, we have characterized the antiproliferative mechanism of **9g**. In addition, the structural features of the compounds along with their antiproliferative activity have been discussed.

2. Experimental

2.1 *Materials and methods*

All reactions were carried out under a positive pressure of argon and with oven-dried glassware. Melting points are uncorrected and were determined with SMP10 digital melting point apparatus using open capillary tubes. Proton magnetic resonance (^{1}H NMR) spectra were recorded using Bruker 400 spectrometers. Chemical shifts are reported in parts per million (ppm) relative to internal standards (tetramethylsilane, $\delta_H = 0.00$; $CDCl_3$, $\delta_H = 7.26$). Data are presented as follows: chemical shift (δ, ppm), multiplicity (s = singlet, d = doublet, t = triplet, q = quadruplet, m = multiplet, dd = doublet of doublet, br = broad), coupling constant (J) values are presented in Hz, integration. Carbon magnetic resonance (^{13}C NMR) spectra were recorded using Bruker 400 spectrometers. Chemical shifts are reported in parts per million (ppm) relative to internal standard (tetramethylsilane, $\delta_C = 0.00$; $CDCl_3$, $\delta_C = 77.00$). Reactions were monitored with analytical Thin Layer Chromatography (TLC) which was carried out using Merck commercial aluminium sheets coated (0.2 mm layer thickness) with Kieselgel 60 F254, with visualization by ultraviolet light and product purification by flash column chromatography. Mass spectra were determined on an Agilent Technology (HP) mass spectrometer operating at an ionization potential of 70 eV. The elemental analysis for C, H and N was carried out with an Elementary Analysen system GmbH VarioEL. All reagents and solvents used in this study were commercially available (from Sigma-Aldrich) and were used without further purification. The intermediate compounds were prepared according to the literature methods.

Table 1. Reaction time and yield of products.

Entry	R	R_1	R_2	Time(h)	Product[a]	Yield[b](%)
1	Ph	H	H	12	**5a**	89
2	Ph	OMe	H	12	**5b**	89
3	Ph	OEt	H	12	**5c**	88
4	Ph	H	Cl	16	**5d**	83
5	Ph	H	Br	18	**5e**	82
6	Ph	Cl	Cl	12	**5f**	85

2.2 *Synthesis of 2-phenyl-2H-chromene-3-carboxaldehyde **5(a–f)** (Table 1)*

Salicylaldehyde (1 equiv.) and cinnamaldehyde (1.1 equiv.) were taken in a round bottom flask and anhyd. DMSO (7 mL) was added to it followed by pyrrolidine (0.2 equiv.). Then it was stirred in an argon atmosphere at room temperature. The reaction was monitored by TLC and was found to be completed after 12 h. 50 mL of water was added to it and then extracted with ethylacetate. Organic layers were separated out and dried over Na_2SO_4, filtered and concentrated in rotavapour and purified by column chromatography on silica gel (200–330 mesh) to afford the desired product.

*2.2.1 2-Phenyl-2H-chromene-3-carboxaldehyde(**5a**)* Prepared from **3a** (Salicylaldehyde) and **4** (Cinnamaldehyde).Yellow solid, M.p. 72−74 °C. IR(KBr) cm^{-1}:3048, 2820, 2707, 1670, 1570, 1457, 1216, 1102, 996, 768, 612, 520. ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.66 (s, 1H), 7.42 (s, 1H), 7.37–7.35 (m, 2H), 7.30–7.25 (m, 5H), 6.96–6.87 (m, 2H), 6.35 (s, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 190.1, 154.9, 140.8, 139.1, 133.7, 129.5, 128.7, 126.8, 121.8, 120.0, 117.1, 74.2. Anal. Calculated for $C_6H_{12}O_2$: C, 81.34; H, 5.12%. Found: C, 81.35; H, 5.15%. ESI-HRMS $[M + Na]^+$: Calcd for $C_{16}H_{12}O_2Na$: 259.0730, Found: 259.0730.

*2.2.2 8-Methoxy-2-phenyl-2H-chromene-3-carboxaldehyde (**5b**)* Prepared from **3b** (3-Methoxy salicylaldehyde) and **4** (Cinnamaldehyde).Yellow solid, M.p. 117−119 °C. IR (KBr) cm^{-1}:3051, 2908, 2811, 2720, 1657, 1631, 1573, 1378, 1255, 1210, 1093, 964, 892, 763, 723, 691, 581, 510.^{1}H NMR (400 MHz, $CDCl_3$): δ 9.67 (s, 1H), 7.40 (s, 1H), 7.38-7.36 (m, 2H), 7.28–7.24 (m, 3H), 6.94–6.88 (m, 3H), 6.44 (s, 1H), 3.84 (s, 3H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ

190.3, 148.6, 144.2, 141.1, 139.1, 134.1, 128.7, 128.6, 126.7, 121.7, 121.4, 120.9, 116.2, 74.3, 56.4. Anal. Calculated for $C_{17}H_{14}O_3$: C, 76.68; H, 5.30%. Found: C, 76.69; H, 5.33%. ESI-HRMS $[M + Na]^+$: Calcd for $C_{17}H_{14}O_3Na$: 289.0835, Found: 289.0837.

*2.2.3 8-Ethoxy-2-phenyl-2H-chromene-3-carboxaldehyde (**5c**)* Prepared from **3c** (3-Ethoxy salicylaldehyde) and **4** (Cinnamaldehyde).Yellow solid, M.p. 98−100 °C. IR (KBr) cm^{-1}:2974, 2807, 1748, 1664, 1627, 1609, 1469, 1376, 1256, 1218, 1098, 1005, 902, 754, 689, 642, 615, 521.^{1}H NMR (400 MHz, $CDCl_3$): δ 9.70 (s, 1H), 7.41 (s, 1H), 7.40–7.37 (m, 2H), 7.30–7.26 (m, 3H), 6.96–6.88 (m, 3H), 6.46 (s, 1H), 4.08 (q, J = 8.0 Hz, 2H), 1.40 (t, J = 8.0 Hz, 3H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 190.4, 147.9, 144.7, 141.3, 139.1, 134.1, 128.6, 128.5, 126.6, 121.7, 121.5, 121.2, 118.1, 73.9, 65.1, 14.9.Anal. Calculated for $C_{18}H_{16}O_3$: C, 77.12; H, 5.75%; Found: C, 77.15; H, 5.77%. ESI-HRMS $[M + Na]^+$: Calcd for $C_{18}H_{16}O_3Na$: 303.0992, Found: 303.0985.

*2.2.4 6-Chloro-2-phenyl-2H-chromene-3-carboxaldehyde (**5d**)* Prepared from **3d** (5-Chloro salicylaldehyde) and **4** (Cinnamaldehyde). Faint yellow solid, M.p. 129−131 °C. IR (KBr) cm^{-1}:3058, 2934, 2824, 2714, 1891, 1819, 1677, 1631, 1586, 1560, 1482, 1411, 1384, 1307, 1203, 1158, 1132, 1067, 957, 814, 691, 626, 522. ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.67 (s, 1H), 7.36 (s, 1H), 7.34–7.22 (m, 7H), 6.82 (d, J = 8.0 Hz, 1H), 6.34 (s, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 189.8, 153.3, 139.3, 138.5, 134.6, 133.1, 128.9, 128.7, 128.5, 126.8, 126.7, 121.2, 118.6, 74.5. Anal.Calcd for $C_{16}H_{11}ClO_2$: C, 70.99; H, 4.10%. Found: C, 71.02; H, 4.13%.ESI-HRMS $[M + Na]^+$: Calcd for $C_{16}H_{11}ClO_2Na$: 293.0340, Found: 293.0338.

*2.2.5 6-Bromo-2-phenyl-2H-chromene-3-carboxaldehyde (**5e**)* Prepared from **3e** (5-Bromo salicylaldehyde) and **4** (Cinnamaldehyde). Yellow solid, M.p. 137−139 °C. IR (KBr) cm^{-1}: 3058, 2934, 2824, 2714, 1891, 1819, 1677, 1631, 1586, 1560, 1482, 1411, 1384, 1307, 1203, 1158, 1132, 1067, 957, 814, 691, 626, 522. ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.66 (s, 1H), 7.39–7.27 (m, 8H), 6.78 (d, J = 8.0 Hz, 1H), 6.34 (s, 1H).^{13}C NMR ($CDCl_3$, 100 MHz): δ 189.9, 153.9, 139.2, 138.6, 136.2, 134.7, 131.6, 129.1, 128.8, 126.9, 121.9, 119.2, 113.8, 74.6. Anal. Calcd for $C_{16}H_{11}BrO_2$: C, 60.98; H, 3.52%. Found: C, 61.01; H, 3.54%.ESI-HRMS $[M + Na]^+$: Calcd for $C_{16}H_{11}BrO_2Na$: 336.9835, Found: 336.9833.

*2.2.6 6, 8-Dichloro-2-phenyl-2H-chromene-3-carboxaldehyde (**5f**)* Prepared from **3f** (3,5-dichloro salicylaldehyde) and **4** (Cinnamaldehyde).Yellow solid, M.p. 136−138 °C. IR(KBr) cm^{-1}: 3071, 2811, 1683, 1631, 1462, 1398, 1333, 1242, 1171, 1086, 88, 883, 723, 652, 555, 451. ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.73 (s, 1H), 7.35–7.27 (m, 7H), 7.17 (s, 1H), 6.48 (s, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 189.8, 149.3, 138.7, 138.0, 135.4, 133.0, 129.1, 128.8, 127.1, 126.6, 123.3, 122.4, 74.8. Anal. Calcd for $C_{16}H_{10}Cl_2O_2$: C, 62.97; H, 3.30%. Found: C, 62.99; H, 3.28%.ESI-HRMS $[M + Na]^+$: Calcd for $C_{16}H_{10}Cl_2O_2Na$: 326.9950, Found: 326.9947.

Table 2. Reaction time and yield of products.

Entry	R	R_1	R_2	Time(h)	Product[a]	Yield[b](%)
1	H	OMe	H	12	**5′b**	89
2	H	OEt	H	12	**5′c**	87

2.3 *Synthesis of 2H-chromene-3-carbaldehydes **5′(b–c)** (Table 2)*

3-methoxysalicylaldehyde (1 equiv.) in dioxane (10 mL) was taken to it K_2CO_3(0.2 equiv.) and acrolein (2.2 equiv.) was added and refluxed. The reaction was monitored by TLC. After 2 h, the reaction mixture was poured into water (100 mL). The solution was extracted with ethylacetate (30 mL × 3). The combined organic layers were washed with NaOH (30 mL) and water (30 mL) successively. Then the organic layers were dried over anhydrous Na_2SO_4. The solvent was evaporated under vacuum; the crude residue was purified by column chromatography on silica gel (100–200 mesh) to afford the desired product.

*2.3.1 8-methoxy-2H-chromene-3-carbaldehyde (**5′b**)* Yellow solid, M.p. 82−84 °C. IR(KBr) cm^{-1}: 2974, 2883, 2818, 2733, 2364, 2325, 1663, 1560, 1475, 1339, 1216, 1093, 1002, 886, 782, 723, 704, 594, 490. ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.59 (s, 1H), 7.26 (s, 1H), 6.97–6.92 (m, 2H), 6.86 (dd, J = 8.0 Hz, 4.0 Hz, 1H), 5.11 (s, 2H), 3.90 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 189.9, 148.1, 145.0, 141.3, 131.8, 121.7, 121.3, 121.1, 115.5, 63.7, 56.2. Anal.Calcd for $C_{11}H_{10}O_3$: C, 69.46; H, 5.30%. Found: C, 69.49; H, 5.32%.GCMS m/z: Calcd for $C_{11}H_{10}O_3$: 190.0, Found: 190.2.

*2.3.2 8-Ethoxy-2H-chromene-3-carbaldehyde (**5′c**)* Yellow solid, M.p. 89−91 °C. IR (KBr) cm^{-1}: 2974, 2883, 2818, 2733, 2364, 2325, 1663, 1560, 1475, 1339, 1216, 1093, 1002, 886, 782, 723, 704, 594, 490. ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.60 (s, 1H), 7.26 (s, 1H), 6.96 (dd, J = 8.0Hz, 4.0 Hz, 1H), 6.91 (t, J = 8.0 Hz, 1H), 6.85 (dd, J = 4.0 Hz, 4.8Hz, 1H), 5.11 (s, 2H), 4.12 (q, J = 4.0 Hz, 2H), 1.47 (t, J = 4.8 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 189.9, 147.4, 145.4, 141.5, 131.7, 121.6, 121.3, 117.0, 64.7, 63.6, 14.8. Anal. Calcd for $C_{12}H_{12}O_3$: C, 70.57; H, 5.92%. Found: C, 70.59; H, 5.95%. GCMS m/z: Calcd for $C_{12}H_{12}O_3$: 204.0, Found: 204.1.

2.4 *Synthesis of (Z/E)-2-phenyl/H-3-Styryl-2H-chromenes **9(a–r)** and **10(a–s)** (Table 3)*

Benzyltriphenylphosphonium bromide (3 equiv.) was taken in anhydrous THF (10 mL) to it BuLi (1.6 M in Hexane) (3 equiv.) was added slowly at −78 °C and stirred in argon atmosphere for 1h. Substituted 2-phenyl-2*H*-chromene aldehyde (1 equiv.) in THF was added and stirred at −78 °C. Reaction was monitored by TLC and observed to

Table 3. Reaction time and yield of products.

Entry	R	R_1	R_2	R_3	R_4	R_5	Time(h)	Product[a]	Yield[b](%)
1	Ph	H	H	H	OMe	H	6	**9a**	38
2	Ph	H	H	H	H	H	6	**9b**	35
3	Ph	OMe	H	H	H	H	6	**9c**	39
4	Ph	OMe	H	H	OMe	H	6	**9d**	38
5	Ph	H	Br	H	OMe	H	6	**9e**	35
6	Ph	H	Cl	H	OMe	H	6	**9f**	36
7	Ph	OEt	H	H	OMe	H	6	**9g**	39
8	Ph	OEt	H	H	H	H	6	**9h**	40
9	Ph	OMe	H	OMe	OMe	OMe	6	**9i**	41
10	Ph	OEt	H	OMe	OMe	OMe	6	**9j**	40
11	Ph	OEt	H	H	OMe	OMe	6	**9k**	39
12	Ph	OMe	H	H	OMe	OMe	6	**9l**	40
13	H	OEt	H	OMe	OMe	OMe	6	**9m**	41
14	H	OMe	H	H	OMe	H	6	**9n**	40
15	H	OMe	H	OMe	OMe	OMe	6	**9o**	39
16	H	OMe	H	H	OMe	OMe	6	**9p**	39
17	H	OEt	H	H	OMe	H	6	**9q**	35
18	H	OEt	H	H	OMe	OMe	6	**9r**	35
19	Ph	H	H	H	OMe	H	6	**10a**	32
20	Ph	H	H	H	H	H	6	**10b**	34
21	Ph	Cl	Cl	H	H	H	6	**10c**	33
22	Ph	Cl	Cl	H	OMe	H	6	**10d**	33
23	Ph	OMe	H	H	H	H	6	**10e**	31
24	Ph	H	Br	H	OMe	H	6	**10f**	35
25	Ph	H	Cl	H	OMe	H	6	**10g**	34
26	Ph	OEt	H	H	OMe	H	6	**10h**	31
27	Ph	OEt	H	H	H	H	6	**10i**	30
28	Ph	OMe	H	OMe	OMe	OMe	6	**10j**	32
29	Ph	OEt	H	OMe	OMe	OMe	6	**10k**	32
30	Ph	OEt	H	H	OMe	OMe	6	**10l**	32
31	Ph	OMe	H	H	OMe	OMe	6	**10m**	30
32	H	OEt	H	OMe	OMe	OMe	6	**10n**	32
33	H	OMe	H	H	OMe	H	6	**10o**	30
34	H	OMe	H	OMe	OMe	OMe	6	**10p**	32
35	H	OMe	H	H	OMe	OMe	6	**10q**	31
36	H	OEt	H	H	OMe	H	6	**10r**	35
37	H	OEt	H	H	OMe	OMe	6	**10s**	31

be completed in 6 h. Reaction mixture was quenched by saturated NH_4Cl solution and stirred in room temperature for 1–2 h. Reaction mixture was extracted thrice with ethyl acetate and separated out. The combined organic layers were dried over anhydrous sodium sulphate (Na_2SO_4), filtered and concentrated. The crude reaction mixture was purified by flash column chromatography on silica gel (230–330 mesh) to afford the desired product.

*2.4.1 (Z)-3-(4-methoxystyryl)-2-phenyl-2H-chromene (**9a**)* Prepared from **5a** and **6b**. Yellow liquid,^{1}H NMR (400 MHz, $CDCl_3$): δ 7.46 (d, J = 8.0 Hz, 1H), 7.31–7.18 (m, 10H), 7.07–7.03 (m, 1H), 6.95–6.93 (m, 1H), 6.63 (s, 1H), 6.39 (d, J = 12.0 Hz,1H), 5.91 (d, J = 12.0 Hz, 1H), 5.89 (s, 1H), 3.80 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 159.4, 151.8, 138.6, 132.6, 129.7, 129.1, 128.8, 128.5, 128.4, 127.7, 127.6, 127.6, 126.5, 125.2, 123.5, 123.0, 121.3, 116.4, 114.1, 77.2, 55.3.Anal.Calcd for $C_{24}H_{20}O_2$: C, 84.68; H, 5.92%. Found: C, 84.70; H, 5.95%.

*2.4.2 (Z)-2-phenyl-3-styryl-2H-chromene (**9b**)* Prepared from **5a** and **6a**. White solid, M.p. 83 – 85 °C.^{1}H NMR (400 MHz, $CDCl_3$): δ 7.46–7.44 (m, 2H), 7.35 (d, J = 8.0 Hz, 2H), 7.30–7.24 (m, 6H), 7.09–7.07 (m, 1H), 7.05–7.04 (m, 1H), 6.93 (d, J = 12.0 Hz, 1H), 6.87–6.83 (m, 1H), 6.78 (s, 1H), 6.75 (d, J = 4.0 Hz, 1H), 6.38 (d, J = 12.0 Hz, 1H), 6.23 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 152.0, 139.4, 137.3, 132.2, 131.5, 129.4, 128.6, 128.3, 127.4, 126.7, 124.0, 122.1, 121.2, 116.0, 78.1. Anal.Calcd for $C_{23}H_{18}O$: C, 89.00; H, 5.85%. Found: C, 89.04; H, 5.89%.

*2.4.3 (Z)-8-methoxy-2-phenyl-3-styryl-2H-chromene (**9c**)* Prepared from **5b** and **6a**. Yellow liquid,^{1}H NMR (400 MHz, $CDCl_3$): δ 7.31–7.20 (m, 10H), 6.76 (d, J = 8.0 Hz, 1H), 6.72–6.70 (m, 1H), 6.61 (s, 1H), 6.60–6.58 (m, 1H), 6.49 (d,

J = 12.0 Hz, 1H), 6.08 (d, J = 12 Hz, 1H), 5.97 (s, 1H), 3.75 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 147.9, 141.0, 139.2, 137.3, 132.5, 131.5, 128.5, 128.4, 128.2, 127.6, 127.4, 127.3, 124.1, 122.9, 120.9, 119.2, 112.5, 77.7, 56.1. Anal.Calcd for $C_{24}H_{20}O_2$: C, 84.68; H, 5.92%. Found: C, 84.64; H, 5.89%.

*2.4.4 (Z)-8-methoxy-3-(4-methoxystyryl)-2-phenyl-2H-chromene (**9d**)* Prepared from **5b** and **6b**. Yellow solid, M.p. 129−131 °C.^{1}H NMR (400 MHz, $CDCl_3$): δ 7.29–7.21 (m, 8H), 6.81 (d, J = 8.0 Hz, 2H), 6.75 (d, J = 8.0 Hz, 1H), 6.70 (d, J = 8.0 Hz, 1H), 6.62–6.59 (m, 2H), 6.43 (d, J = 12.0 Hz, 1H), 5.99 (s, 1H), 3.80 (s, 3H), 3.77 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 158.9, 147.9, 140.9, 139.4, 132.9, 131.2, 129.9, 129.7, 128.2, 127.2, 126.1, 123.5, 123.4, 120.9, 119.1, 113.8, 112.4, 77.7, 56.1, 55.2. Anal.Calcd for $C_{25}H_{22}O_3$: C, 81.06; H, 5.99%. Found: C, 81.08; H, 5.97%. ESI-HRMS $[M + Na]^+$: Calcd for $C_{25}H_{22}O_3Na$: 393.14612, Found: 393.14671.

*2.4.5 (Z)-6-bromo-3-(4-methoxystyryl)-2-phenyl-2H-chromene (**9e**)* Prepared from **5e** and **6b**. Yellow liquid,^{1}H NMR (400 MHz, $CDCl_3$): δ 7.28–7.24 (m, 8H), 7.20 (d, J = 8.0Hz, 2H), 7.14–7.11 (m, 1H), 7.04 (d, J = 4.0 Hz, 1H), 6.60 (d, J = 8.0 Hz, 1H), 6.56 (s, 1H), 5.91 (d, J = 8.0Hz, 1H), 5.87 (s, 1H), 3.82 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 159.1, 158.5, 138.9, 133.8, 132.0, 131.6, 130.0, 129.9, 128.9, 128.5, 128.4, 128.3, 127.3, 125.4, 122.1, 117.8, 113.8, 113.6, 78.3, 55.2. Anal.Calcd for $C_{24}H_{19}BrO_2$: C, 68.75; H, 4.57%. Found: C, 68.78; H, 4.55%.

*2.4.6 (Z)-6-chloro-3-(4-methoxystyryl)-2-phenyl-2H-chromene (**9f**)* Prepared from **5d** and **6b**. Yellow liquid,^{1}H NMR (400 MHz, $CDCl_3$): δ 7.29–7.25 (m, 7H), 6.99 (d, J = 8.0 Hz, 1H), 6.91 (d, J = 4.0 Hz, 1H), 6.83 (d, J = 8.0 Hz, 2H), 6.63 (d, J = 8.0 Hz, 1H), 6.57 (s, 1H), 6.45 (d, J = 12.0 Hz, 1H), 5.92 (d, J = 12.0 Hz, 1H), 5.87 (s, 1H), 3.82 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 159.1, 150.5, 138.9, 133.8, 132.0, 129.9, 129.5, 128.7, 128.5, 128.4, 127.3, 126.0, 125.4, 123.6, 122.3, 117.3, 113.8, 78.3, 55.3.Anal. Calcd for $C_{24}H_{19}ClO_2$: C, 76.90; H, 5.11%. Found: C, 76.88; H, 5.15%.

*2.4.7 (Z)-8-ethoxy-3-(4-methoxystyryl)-2-phenyl-2H-chromene (**9g**)* Prepared from **5c** and **6b**. White solid, M.p. 113 − 115 °C.^{1}H NMR (400 MHz,$CDCl_3$): δ 7.31–7.20 (m, 7H), 6.82–6.79 (m, 2H), 6.75–6.73 (m, 2H), 6.63 (s, 1H), 6.61–6.58 (m, 1H), 6.43 (d, J = 12.0 Hz, 1H), 6.03 (d, J = 12.0 Hz, 1H), 5.98 (s, 1H), 3.98 (q, J = 8.0 Hz, 2H), 3.80 (s, 3H), 1.30 (t, J = 8.0 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 158.9, 147.1, 141.5, 139.4, 132.9, 131.1, 129.9, 129.7, 128.1, 127.2, 126.3, 123.7, 123.4, 120.8, 119.3, 114.7, 113.8, 77.5, 64.9, 55.2, 14.8. Anal. Calcd for $C_{26}H_{24}O_3$: C, 81.22; H, 6.29%. Found: C, 81.25; H, 6.27%.

*2.4.8 (Z)-8-ethoxy-2-phenyl-3-styryl-2H-chromene (**9h**)* Prepared from **5c** and **6a**. Pale yellow solid, M.p. 127−129°C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.31–7.20 (m, 8H), 6.76–6.70 (m, 2H), 6.62 (s, 1H), 6.59–6.57 (m, 1H), 6.50 (d, J = 12.0 Hz, 1H), 6.10 (d, J = 12.0 Hz, 1H), 5.95 (s, 1H), 3.96 (q, J = 8.0 Hz, 2H), 1.28 (t, J = 8.0 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 147.1, 141.6, 139.2, 137.3, 132.5, 131.4, 128.6, 128.3, 128.1, 127.7, 127.4, 127.3, 124.3, 123.3, 120.8, 119.3, 114.8, 77.5, 64.9, 14.8. Anal.Calcd for $C_{25}H_{20}O_2$: C, 84.72; H, 6.26%. Found: C, 84.75; H, 6.27%.

*2.4.9 (Z)-8-methoxy-2-phenyl-3-(3,4,5-trimethoxystyryl)-2H-chromene (**9i**)* Prepared from **5b** and **6d**. Deep yellow solid, M.p. 129−131 °C.^{1}H NMR (400 MHz, $CDCl_3$): δ 7.51 (d, J = 4.0 Hz, 2H), 7.30–7.25 (m, 3H), 6.90 (d, J = 12.0 Hz, 1H), 6.85–6.82 (m, 1H), 6.81–6.79 (m, 1H), 6.73 (s, 1H), 6.71 (s, 1H), 6.60 (s, 2H), 6.35 (d, J = 12.0 Hz, 1H), 6.31 (s, 1H), 3.87 (s, 6H), 3.83 (s, 3H), 3.79 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 152.9, 148.0, 140.9, 139.2, 137.2, 132.7, 132.5, 131.3, 128.2, 127.6, 127.2, 124.5, 123.0, 121.0, 119.1, 112.6, 105.4, 103.5, 77.3, 60.9, 56.1, 55.8. Anal. Calcd for $C_{27}H_{26}O_5$: C, 75.33; H, 6.09%. Found: C, 75.35; H, 6.07%. ESI-HRMS $[M + Na]^+$: Calcd for $C_{27}H_{26}O_5Na$: 453.16725, found: 453.16680.

*2.4.10 (Z)-8-ethoxy-2-phenyl-3-(3,4,5-trimethoxystyryl)-2H-chromene (**9j**)* Prepared from **5c** and **6d**. Pale yellow solid, M.p. 120−122 °C.^{1}H NMR (400 MHz, $CDCl_3$): δ 7.32–7.21 (m, 5H), 6.75–6.71 (m, 2H), 6.68 (s, 1H), 6.62–6.60 (m, 1H), 6.49 (s, 2H), 6.45 (d, J = 12.0 Hz, 1H), 6.15 (d, J = 12.0 Hz, 1H), 5.98 (s, 1H), 3.97 (q, J = 8.0 Hz, 2H), 3.85 (s, 3H), 3.65 (s, 6H), 1.29 (t, J = 8.0 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 152.8, 147.1, 141.6, 139.3, 137.2, 132.7, 132.5, 131.2, 128.1, 127.8, 127.1, 124.7, 123.4, 121.0, 119.2, 114.8, 105.5, 77.2, 64.9, 60.9, 55.8, 14.8. Anal. Calcd for $C_{28}H_{28}O_5$: C, 75.65; H, 6.35%. Found: C, 75.62; H, 6.37%. ESI-HRMS $[M + Na]^+$: Calcd for $C_{28}H_{28}O_5Na$: 467.18290, found: 467.18315.

*2.4.11 (Z)-3-(3,4-dimethoxystyryl)-8-ethoxy-2-phenyl-2H-chromene (**9k**)* Prepared from **5c** and **6c**. White solid, M.p. 105−107 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.33–7.31 (m, 2H), 7.23–7.21 (m, 3H), 6.90–6.87 (m, 2H), 6.79–6.73 (m, 3H), 6.65 (s, 1H), 6.60–6.58 (m, 1H), 6.45 (d, J = 8.0 Hz, 1H), 6.06 (d, J = 8.0 Hz, 1H), 5.99 (s, 1H), 3.98 (q, J = 8.0 Hz, 2H), 3.88 (s, 3H), 3.64 (s, 3H), 1.31 (t, J = 8.0 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 148.4, 147.1, 141.6, 139.5, 132.8, 131.3, 129.9, 128.2, 128.1, 127.0, 126.6, 123.9, 123.3, 121.6, 120.9, 119.2, 114.7, 111.2, 110.9, 77.4, 64.9, 55.8, 55.6, 14.8. Anal.Calcd for $C_{27}H_{26}O_4$: C, 78.24; H, 6.32%. Found: C, 78.26; H, 6.35%. ESI-HRMS$[M + Na]^+$: Calcd for $C_{27}H_{26}O_4Na$: 437.17233, found: 437.17231.

*2.4.12 (Z)-3-(3,4-dimethoxystyryl)-8-methoxy-2-phenyl-2H-chromene (**9l**)* Prepared from **5b** and **6c**. Yellow liquid,^{1}H NMR (400 MHz, $CDCl_3$): δ 7.53–7.50 (m, 2H), 7.28–7.26 (m, 3H), 6.93–6.91 (m, 3H), 6.85–6.80 (m, 2H), 6.78 (d, J = 12.0 Hz, 1H), 6.72 (d, J = 4.0 Hz, 1H), 6.39 (d, J = 12.0 Hz, 1H), 6.31 (s, 1H), 3.90 (s, 3H), 3.87 (s, 3H), 3.79 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 148.5, 147.9, 140.9, 139.5, 132.8, 131.4, 129.9, 128.2, 127.1, 126.4, 123.6, 123.0, 121.6, 120.9, 119.1, 112.5, 111.2, 110.9, 77.6, 56.2, 55.8, 55.6. Anal. Calcd for $C_{26}H_{24}O_4$: C, 77.98; H, 6.04%. Found: C, 77.96; H, 6.05%. ESI-HRMS $[M + Na]^+$: Calcd for $C_{26}H_{24}O_4Na$: 423.15668, found: 423.15675.

*2.4.13 (Z)-8-ethoxy-3-(3,4,5-trimethoxystyryl)-2H-chromene (**9m**)* Prepared from **5'c** and **6d**. Yellow solid, M.p. 75−77 °C. ^{1}H NMR (400 MHZ, $CDCl_3$): δ 6.81 (d, J = 8.0 Hz, 1H), 6.77–6.75 (m, 1H), 6.67 (d, J = 8.0 Hz, 1H), 6.55–6.52 (m, 2H), 6.46 (s, 2H), 6.21 (d, J = 12.0 Hz, 1H), 4.58 (s, 2H), 4.05 (q, J = 8.0 Hz, 2H), 3.87 (s, 3H), 3.82 (s, 6H), 1.41 (t, J = 8.0 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 152.9, 146.9, 142.9, 137.5, 133.7, 131.2, 130.8, 127.9, 125.7, 124.1, 121.2, 119.1, 113.5, 105.7, 67.1, 64.5, 61.0, 56.1, 14.9. Anal.Calcd for $C_{22}H_{24}O_5$: C, 71.72; H, 6.57%. Found: C, 71.76; H, 6.54%. ESI-HRMS $[M + Na]^+$: Calcd for $C_{22}H_{24}O_5Na$: 391.15160, found: 391.15185.

*2.4.14 (Z)-8-methoxy-3-(4-methoxystyryl)-2H-chromene (**9n**)* Prepared from **5'b** and **6b**. Yellow solid, M.p. 106−108 °C.^{1}H NMR (400 MHz, $CDCl_3$): δ 7.16 (d, J = 8.0 Hz, 2H), 6.82 (d, J = 8.0 Hz, 3H), 6.76–6.74 (m, 1H), 6.67–6.65 (m, 1H), 6.56 (d, J = 12.0 Hz, 1H), 6.48 (s, 1H), 6.17 (d, J = 12.0 Hz, 1H), 4.52 (s, 2H), 3.83 (s, 3H), 3.80 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 159.0, 147.6, 142.4, 131.9, 130.9, 130.5, 129.8, 127.1, 124.8, 124.1, 121.1, 119.0, 113.6, 111.7, 67.3, 56.0, 55.3. Anal. Calcd for $C_{19}H_{18}O_3$: C, 77.53; H, 6.16%. Found: C, 77.56; H, 6.14%. ESI-HRMS $[M + Na]^+$: Calcd for $C_{19}H_{18}O_3Na$: 317.11482, found: 317.11456.

*2.4.15 (Z)-8-methoxy-3-(3,4,5-trimethoxystyryl)-2H-chromene (**9o**)* Prepared from **5'b** and **6d**. Yellow liquid, ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.89–6.82 (m, 4H), 6.68–6.65 (m, 1H), 6.65–6.64 (m, 2H), 6.62 (d, J = 12.0 Hz, 2H), 6.48–6.39 (m, 3H), 6.34 (s, 2H), 5.01 (s, 2H), 3.77 (s, 12 H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 153.4, 147.6, 142.3, 138.2, 132.6, 130.6, 128.0, 126.0, 123.7, 123.4, 121.2, 119.3, 112.0, 103.5, 65.9, 60.9, 56.1, 56.1, 56.0. Anal.Calcd for $C_{21}H_{22}O_5$: C, 71.17; H, 6.26%. Found: C, 71.16; H, 6.24%. ESI-HRMS: Calcd for $C_{21}H_{22}O_5$: 353.13835, found: 353.13886.

*2.4.16 (Z)-3-(3,4-dimethoxystyryl)-8-methoxy-2H-chromene (**9p**)* Prepared from **5'b** and **6c**. Yellow solid, M.p. 97−99 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.83–6.79 (m, 4H), 6.76–6.74 (m, 1H), 6.67–6.65 (m, 1H), 6.55 (d, J = 8.0 Hz, 1H), 6.50 (s, 1H), 6.18 (d, J = 8.0 Hz, 1H), 4.55 (s, 2H), 3.88 (s, 3H), 3.83 (s, 6H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 148.6, 148.5, 147.6, 142.4, 131.7, 131.0, 130.8, 127.1, 125.0, 124.0, 121.4, 121.2, 119.0, 111.8, 111.5, 110.8, 67.2, 56.0, 55.9, 55.8. Anal. Calcd for $C_{20}H_{20}O_4$: C, 74.06; H, 6.21%. Found: C, 74.08; H, 6.24%.ESI-HRMS: Calcd for $C_{20}H_{24}O_4N$: 342.16998, found: 342.17097.

*2.4.17 (Z)-8-ethoxy-3-(4-methoxystyryl)-2H-chromene (**9q**)* Prepared from **5'c** and **6b**. Yellow liquid, ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.15 (d, J = 8.0 Hz, 2H), 6.83–6.80 (m, 3H), 6.75–6.73 (m, 1H), 6.65–6.63 (m, 1H), 6.55 (d, J = 12.0 Hz, 1H), 6.48 (s, 1H), 6.18 (d, J = 12.0 Hz, 1H), 4.52 (s, 2H), 4.05 (q, J = 8.0 Hz, 2H), 3.81 (s, 3H), 1.40 (t, J = 8.0 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 159.5, 146.8, 142.7, 130.9, 129.7, 127.7, 124.5, 123.8, 122.9, 121.0, 119.2, 114.2, 113.5, 65.8, 64.6, 55.3, 14.9.Anal. Calcd for $C_{20}H_{20}O_3$: C, 77.90; H, 6.54%. Found: C, 77.88; H, 6.56%.

*2.4.18 (Z)-3-(3,4-dimethoxystyryl)-8-ethoxy-2H-chromene (**9r**)* Prepared from **5'c** and **6c**. Yellow solid, M.p. 109−111°C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.82–6.77 (m, 4H), 6.76–6.74 (m, 1H), 6.66–6.64 (m, 1H), 6.55 (d, J = 8.0 Hz, 1H), 6.50 (s, 1H), 6.18 (d, J = 8.0 Hz, 1H), 4.55 (s, 2H), 4.05 (q, J = 8.0 Hz, 2H), 3.88 (s, 3H), 3.83 (s, 3H), 1.40 (t, J = 4.0 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 148.6, 148.5, 146.9, 142.9, 131.6, 130.9, 127.2, 125.2, 124.2, 121.4, 121.1, 119.1, 113.5, 111.5, 110.8, 67.2, 64.5, 55.9, 55.8, 14.9. Anal. Calcd for $C_{21}H_{22}O_4$: C, 74.54; H, 6.55%. Found: C, 77.58; H, 6.56%. ESI-HRMS $[M + Na]^+$: Calcd for $C_{21}H_{22}O_4Na$: 361.14103, found: 361.14111.

*2.4.19 (E)-3-(4-methoxystyryl)-2-phenyl-2H-chromene (**10a**)* Prepared from **5a** and **6b**. White solid, M.p. 135–137 °C; ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.47–7.44 (m, 2H), 7.31–7.25 (m, 5H), 7.08–7.01 (m, 2H), 6.93–6.84 (m, 4H), 6.83–6.80 (m, 1H), 6.78–6.74 (m, 1H), 6.35(d, J = 24.0 Hz,1H), 6.22 (s, 1H), 3.79 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 159.4, 151.8, 138.6, 132.6, 129.7, 129.1, 128.8, 128.5, 128.4, 127.7, 127.6, 126.5, 125.2, 123.5, 123.0, 121.3, 116.4, 114.1, 77. 2, 55.3.Anal. Calcd for $C_{24}H_{20}O_2$: C, 84.68; H, 5.92%. Found: C, 84.71; H, 5.94%.

*2.4.20 (E)-2-phenyl-3-styryl-2H-chromene (**10b**)* Prepared from **5a** and **6a**. Yellow solid, M.p. 131−133 °C.^{1}H NMR (400 MHz, $CDCl_3$): δ 7.34–7.23 (m, 10H), 7.05–7.02 (m, 1H), 6.93 (dd, J = 4.0 Hz, J = 8.0 Hz, 1H), 6.84–6.81 (m, 1H), 6.72 (d, J = 8.0 Hz, 1H), 6.62 (s, 1H), 6.47 (d, J = 20.0 Hz, 1H), 6.02 (d, J = 20.0 Hz, 1H), 5.86 (s, 1H).^{13}C NMR (100 MHz, $CDCl_3$): δ 152.0, 138.5, 136.9, 132.4, 129.5, 129.3, 128.6, 128.5, 127.8, 127.7, 127.2, 126.8, 126.4, 124.6, 122.8, 121.4, 116.5, 76.5.Anal. Calcd for $C_{23}H_{18}O$: C, 89.00; H, 5.85%. Found: C, 89.02; H, 5.87%.

*2.4.21 (E)-6,8-dichloro-2-phenyl-3-styryl-2H-chromene (**10c**)* Prepared from **5f** and **6a**. Yellow solid, M.p. 140−142°C.^{1}H NMR (400 MHz, $CDCl_3$): δ 7.48–7.46 (m, 2H), 7.33–7.25 (m, 5H), 7.07 (d, J = 4.0 Hz, 1H), 6.93 (d, J = 4.0 Hz, 1H), 6.86–6.82 (m, 3H), 6.63 (s, 2H), 6.42 (d, J = 16.0 Hz, 1H), 6.35 (s, 1H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 159.9, 146.3, 137.6, 134.9, 130.7, 129.2, 128.8, 128.5, 128.0, 127.5, 126.1, 125.6, 124.4, 122.2, 121.7, 114.2, 76.7.Anal. Calcd for $C_{23}H_{15}Cl_2O$: C, 72.83; H, 4.25%. Found: C, 72.82; H, 4.27%.

*2.4.22 (E)-6,8-dichloro-3-(4-methoxystyryl)-2-phenyl-2H-chromene (**10d**)* Prepared from **5f** and **6b**. Yellow solid, M.p. 172−174 °C.^{1}H NMR (400 MHz, $CDCl_3$): δ 7.48–7.46 (m, 2H), 7.33–7.25 (m, 5H), 7.07 (d, J = 4.0 Hz, 1H), 6.93 (s, 1H), 6.86-6.82 (m, 3H), 6.63 (s, 1H), 6.43 (d, J = 16.0 Hz, 1H), 6.35 (s, 1H), 3.79 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 159.9, 146.3, 137.6, 134.9, 130.7, 129.2, 128.8, 128.5, 128.0, 127.5, 126.1, 125.6, 124.4, 122.2, 121.7, 114.2, 76.7, 55.3.Anal. Calcd for $C_{24}H_{18}Cl_2O_2$: C, 70.43; H, 4.43%. Found: C, 70.42; H, 4.45%.

*2.4.23 (E)-8-methoxy-2-phenyl-3-styryl-2H-chromene (**10e**)* Prepared from **5b** and **6a**. Yellow solid, M.p. 140 − 142 °C.^{1}H NMR (400 MHz, $CDCl_3$): δ 7.52–7.50 (m, 2H), 7.38–7.20 (m,

8H), 6.95 (d, J = 20.0 Hz, 1H), 6.83–6.69 (m, 4H), 6.42 (d, J = 20.0 Hz, 1H), 6.32 (s, 1H), 3.77 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 148.3, 141.0, 138.5, 136.9, 132.7, 129.4, 128.6, 128.4, 127.8, 127.6, 127.3, 126.4, 124.5, 123.6, 121.1, 119.2, 112.8, 76.3, 56.3.Anal. Calcd for $C_{24}H_{20}O_2$: C, 84.68; H, 5.92%. Found: C, 84.65; H, 5.93%.

*2.4.24 (E)-6-bromo-3-(4-methoxystyryl)-2-phenyl-2H-chromene (**10f**)* Prepared from **5e** and **6b**. Yellow solid, M.p. 153−155 °C.^{1}H NMR (400 MHz, $CDCl_3$): δ 7.43–7.41 (m, 2H), 7.30–7.25 (m, 5H), 7.17 (d, J = 4.0 Hz,1H), 7.11–7.09 (m, 1H), 6.82 (d, J = 4.0 Hz, 2H), 6.78 (d, J = 16.0 Hz, 1H), 6.65 (s, 1H), 6.62 (d, J = 8.0 Hz, 1H), 6.36 (d, J = 16.0 Hz, 1H), 6.21 (s, 1H), 3.78 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 159.7, 159.0, 138.1, 133.8, 131.5, 129.9, 128.8, 128.7, 128.6, 127.8, 127.7, 125.0, 124.7, 122.1, 118.2, 114.2, 113.8, 113.4, 76.6, 55.3.Anal. Calcd for $C_{24}H_{19}BrO_2$: C, 68.75; H, 4.57%. Found: C, 68.76; H, 4.59%.

*2.4.25 (E)-6-chloro-3-(4-methoxystyryl)-2-phenyl-2H-chromene (**10g**)* Prepared from **5d** and **6b**. Yellow solid, M.p. 189−191 °C.^{1}H NMR (400 MHz, $CDCl_3$): δ 7.42 (d, J = 8.0 Hz, 2H), 7.30–7.25 (m, 5H), 7.03 (d, J = 4.0 Hz, 1H), 6.98–6.95 (m, 1H), 6.83 (d, J = 8.0 Hz, 2H), 6.78 (d, J = 16.0 Hz, 1H), 6.68-6.66 (m, 2H), 6.37 (d, J = 16.0 Hz, 1H), 6.21 (s, 1H), 3.79 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 159.7, 150.3, 138.1, 133.8, 129.9, 129.4, 128.7, 128.6, 127.8, 127.7, 126.1, 125.9, 124.7, 124.4, 122.3, 117.7, 114.2, 76.6, 55.3.Anal. Calcd for $C_{24}H_{19}ClO_2$: C, 76.90; H, 5.11%. Found: C, 76.89; H, 5.13%.

*2.4.26 (E)-8-ethoxy-3-(4-methoxystyryl)-2-phenyl-2H-chromene (**10h**)* Prepared from **5c** and **6b**. White solid, M.p. 132−134 °C.^{1}H NMR (400 MHz, $CDCl_3$): δ 7.52–7.49 (m, 2H), 7.32 (d, J = 8.0 Hz, 2H), 7.29–7.23 (m, 3H), 6.88–6.83 (m, 3H), 6.79–6.70 (m, 4H), 6.38 (d, J = 16.0 Hz, 1H), 6.31 (s, 1H), 3.98 (q, J = 4.0 Hz, 2H), 3.79 (s, 3H), 1.33 (t, J = 4.0 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 156.9, 144.9, 138.9, 136.1, 130.4, 127.2, 126.3, 125.8, 125.2, 125.1, 122.9, 121.6, 121.1, 118.5, 116.7, 112.2, 111.6, 73.5, 62.5, 52.8, 12.3.Anal. Calcd for $C_{26}H_{24}O_3$: C, 81.22; H, 6.29%. Found: C, 81.24; H, 6.32%.

*2.4.27 (E)-8-ethoxy-2-phenyl-3-styryl-2H-chromene (**10i**)* Prepared from **5c** and **6a**. Yellow liquid. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.50(d, J = 4.0 Hz, 2H), 7.37 (d, J = 8.0 Hz, 2H), 7.31–7.19 (m, 6H), 6.98 (d, J = 16.0 Hz, 1H), 6.79–6.76 (m, 2H), 6.73–6.71 (m, 2H), 6.43 (d, J = 16.0 Hz, 1H), 6.32 (s, 1H), 3.98 (q, J = 4.0 Hz, 2H), 1.33 (t, J = 4.0 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 147.5, 141.6, 138.5, 136.9, 132.7, 129.3, 128.6, 128.4, 127.8, 127.6, 126.4, 124.7, 124.0, 121.1, 119.4, 115.0, 76.0, 65. 0, 14.9.Anal. Calcd for $C_{25}H_{22}O_2$: C, 84.72; H, 6.26%. Found: C, 84.73; H, 6.29%.

*2.4.28 (E)-8-Methoxy-2-phenyl-3-(3,4,5-trimethoxystyryl)-2H-chromene (**10j**)* Prepared from **5b** and **6d**. Pale yellow solid, M.p. 104−106 °C.^{1}H NMR (400 MHz, $CDCl_3$): δ 7.31–7.28 (m, 2H), 7.23–7.21 (m, 3H), 6.77 (d, J = 8.0 Hz, 1H), 6.73–6.71 (m, 1H), 6.68 (s,1H), 6.62 (d, J = 8.0 Hz, 1H), 6.48 (s, 2H), 6.45 (d, J = 16.0 Hz, 1H), 6.15 (d, J = 16.0 Hz, 1H), 6.00 (s, 1H), 3.85 (s, 3H), 3.76 (s, 3H), 3.66 (s, 6H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 153.3, 148.3, 140.9, 138.4, 138.1, 132.6, 132.5, 129.2, 128.4, 127.6, 126.9, 124.5, 123.7, 121.1, 119.2, 112.7, 106.0, 103.5, 76.1, 60.9, 56.2, 56.1, 56.0. Anal. Calcd for $C_{27}H_{26}O_5$: C, 75.33; H, 6.09%. Found: C, 75.36; H, 6.12%. ESI-HRMS $[M + Na]^+$: Calcd for $C_{27}H_{26}O_5Na$: 453.16725, found: 453.16680.

*2.4.29 (E)-8-ethoxy-2-phenyl-3-(3,4,5-trimethoxystyryl)-2H-chromene (**10k**)* Prepared from **5c** and **6d**. Pale yellow solid, M.p. 118 − 120 °C.^{1}H NMR (400 MHz, $CDCl_3$): δ 7.54–7.53 (m, 1H), 7.52–7.51 (m, 1H), 7.28–7.26 (m, 3H), 6.92 (d, J = 16.0 Hz, 1H), 6.79–6.76 (m, 2H), 6.74–6.70 (m, 2H), 6.61 (s, 2H), 6.35 (d, J = 16.0 Hz, 1H), 6.32 (s, 1H), 4.00 (q, J = 8.0 Hz, 2H), 3.87 (s, 6H), 3.84 (s, 3H), 1.35 (t, J = 8.0 Hz, 3H).^{13}C NMR (100 MHz, $CDCl_3$): δ 153.4, 147.5, 141.5, 138.4, 138.1, 132.6, 132.6, 129.1, 128.4, 127.6, 127.1, 124.7, 124.0, 121.2, 119.3, 114.8, 103.5, 75.8, 65.0, 61.0, 56.1, 14.9. Anal. Calcd for $C_{28}H_{28}O_5$: C, 75.65; H, 6.35%. Found: C, 75.67; H, 6.38%. ESI-HRMS$[M + Na]^+$: Calcd for $C_{28}H_{28}O_5Na$: 467.18290, found: 467.18315.

*2.4.30 (E)-3-(3, 4-dimethoxystyryl)-8-ethoxy-2-phenyl-2H-chromene (**10l**)* Prepared from **5c** and **6c**. Yellow solid, M.p. 102−104 °C.^{1}H NMR (400 MHz, $CDCl_3$): δ 7.52 (d, J = 4.0 Hz, 2H), 7.29–7.23 (m, 3H), 6.93–6.84 (m, 3H), 6.79 (d, J = 4.0 Hz, 1H), 6.77–6.70 (m, 4H), 6.37 (d, J = 16.0 Hz, 1H), 6.31 (s, 1H), 3.99 (q, J = 8.0 Hz, 2H), 3.90 (s, 3H), 3.86 (s, 3H), 1.34 (t, J = 8.0 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 149.1, 147.5, 141.4, 138.5, 132.8, 130.0, 129.0, 128.3, 127.6, 125.7, 124.2, 123.9, 121.8, 121.1, 119.9, 119.3, 114.7, 111.2, 110.9, 108.7, 75.9, 65.0, 55.9, 55.8, 14.9. Anal. Calcd for $C_{27}H_{26}O_4$: C, 78.24; H, 6.32%. Found: C, 78.25; H, 6.35%. ESI-HRMS $[M + Na]^+$: Calcd for $C_{27}H_{26}O_4Na$: 437.17233, found: 437.17231.

*2.4.31 (E)-3-(3,4-dimethoxystyryl)-8-methoxy-2-phenyl-2H-chromene (**10m**)* Prepared from **5b** and **6c**. Yellow liquid,^{1}H NMR (400 MHz, $CDCl_3$): δ 7.32–7.21 (m, 5H), 6.91–6.88 (m, 2H), 6.81–6.71 (m, 3H), 6.65 (s, 1H), 6.62–6.59 (m, 1H), 6.45 (d, J = 16.0 Hz, 1H), 6.05 (d, J = 16.0 Hz, 1H), 6.01 (s, 1H), 3.88 (s, 3H), 3.78 (s, 3H), 3.65 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 149.1, 148.3, 140.8, 138.5, 132.8, 130.0, 129.1, 128.4, 127.6, 126.6, 125.6, 123.8, 123.7, 121.1, 119.9, 119.5, 119.1, 112.5, 111.2, 108.7, 76.3, 56.3, 55.9, 55.8. Anal.Calcd for $C_{26}H_{24}O_4$: C, 77.98; H, 6.04%. Found: C, 77.97; H, 6.09%. ESI-HRMS $[M + Na]^+$: Calcd for $C_{26}H_{24}O_4Na$: 423.15668, found: 423.15675.

*2.4.32 (E)-8-ethoxy-3-(3,4,5-trimethoxystyryl)-2H-chromene (**10n**)* Prepared from **5'c** and **6d**. Yellow liquid, ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.84–6.81 (m, 3H), 6.80–6.75 (m, 1H), 6.69–6.67 (m, 2H), 6.51 (s, 1H), 6.37 (d, J = 16.0 Hz, 1H), 5.14 (s, 2H), 4.13 (q, J = 8.0 Hz, 2H), 3.91 (s, 6H), 3.86 (s, 3H), 1.47 (t, J = 8.0 Hz, 3H). ^{13}C NMR (100 MHz,

$CDCl_3$): δ 152.3, 145.8, 141.7, 137.1, 131.6, 129.5, 126.9, 125.0, 122.8, 122.5, 120.1, 118.3, 112.6, 102.4, 64.7, 63.5, 59.9, 55.1, 13.9. Anal. Calcd for $C_{22}H_{24}O_5$: C, 71.72; H, 6.57%. Found: C, 71.74; H, 6.59%.ESI-HRMS $[M + Na]^+$: Calcd for $C_{22}H_{24}O_5Na$: 391.15160, found: 391.15185.

*2.4.33 (E)-8-methoxy-3-(4-methoxystyryl)-2H-chromene (**10o**)* Prepared from **5'b** and **6b**. Yellow solid, M.p. 124–126 °C.^{1}H NMR (400 MHz, $CDCl_3$): δ 7.38 (d, J = 8.0 Hz, 2H), 6.87 (d, J = 4.0 Hz, 2H), 6.82 (d, J = 4.0 Hz, 1H), 6.78–6.76 (m, 1H), 6.73 (d, J = 16.0 Hz, 1H), 6.69–6.67 (m, 1H), 6.45 (s, 1H), 6.40 (d, J = 16.0 Hz, 1H), 5.13 (s, 2H), 3.88 (s, 3H), 3.82 (s,3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 159.5, 147.5, 142.3, 131.0, 129.7, 127.7, 127.1, 124.5, 123.6, 122.7, 121.1, 119.2, 114.2, 111.8, 65.9, 56.0, 55.3. Anal. Calcd for $C_{19}H_{18}O_3$: C, 77.53; H, 6.16%. Found: C, 77.57; H, 6.15%. ESI-HRMS $[M + Na]^+$: Calcd for $C_{19}H_{18}O_3Na$: 317.11482, found: 317.11456.

*2.4.34 (E)-8-methoxy-3-(3,4,5-trimethoxystyryl)-2H-chromene (**10p**)* Prepared from **5'b** and **6d**. Yellow liquid, ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.84 (d, J = 8.0 Hz, 1H), 6.80–6.70 (m, 3H), 6.67 (s, 2H), 6.52 (s, 1H), 6.38 (d, J = 24.0 Hz, 1H), 5.15 (s, 2H), 3.91 (s, 9H), 3.87 (s, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 153.1, 152.2, 147.6, 142.3, 136.8, 132.0, 130.8, 127.6, 126.0, 124.9, 123.7, 121.2, 119.3, 105.1, 65.8, 61.1, 60.8, 56.1, 56.0. Anal. Calcd for $C_{21}H_{22}O_5$: C, 71.17; H, 6.26%. Found: C, 71.18; H, 6.28%.ESI-HRMS: Calcd for $C_{21}H_{22}O_5$: 353.13835, found: 353.13886.

*2.4.35 (E)-3-(3,4-dimethoxystyryl)-8-methoxy-2H-chromene (**10q**)* Prepared from **5'b** and **6c**. Yellow solid, M.p. 124–126 °C.^{1}H NMR (400 MHz, $CDCl_3$): δ 7.02–6.99 (m, 2H), 6.86–6.82 (m, 2H), 6.79–6.76 (m, 2H), 6.69–6.67 (m, 1H), 6.48 (s, 1H), 6.40 (d, J = 16.0 Hz, 1H), 5.15 (s, 2H), 3.93 (s, 3H), 3.90 (s, 6H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 149.1, 147.5, 142.2, 130.9, 130.0, 127.9, 126.6, 124.7, 123.6, 122.9, 121.1, 119.8, 119.2, 111.8, 111.2, 108.7, 65.9, 56.0, 55.9, 55.8. Anal. Calcd for $C_{20}H_{20}O_4$: C, 74.06; H, 6.21%. Found: C, 74.09; H, 6.23%. ESI-HRMS: Calcd for $C_{20}H_{24}O_4N$: 342.16998, found: 342.17097.

*2.4.36 (E)-8-ethoxy-3-(4-methoxystyryl)-2H-chromene (**10r**)* Prepared from **5'c** and **6b**. White solid, M.p. 110−112 °C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.38 (d, J = 8.0 Hz, 2H), 6.87 (d, J = 4.0 Hz, 2H), 6.80 (d, J = 8.0 Hz, 1H), 6.77–6.75 (m, 1H), 6.72 (d, J = 16.0 Hz, 1H), 6.68–6.66 (m, 1H), 6.45 (s, 1H), 6.40 (d, J = 16.0 Hz, 1H), 5.13 (s, 2H), 4.12 (q, J = 8.0 Hz, 2H), 3.82 (s, 3H), 1.46 (t, J = 8.0 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 159.5, 146.8, 142.7, 130.9, 129.7, 127.7, 127.6, 124.5, 123.8, 122.9, 121.0, 119.2, 114.2, 113.5, 65.8, 64.6, 55.3, 14.9.Anal. Calcd for $C_{20}H_{20}O_3$: C, 77.90; H, 6.54%. Found: C, 77.93; H, 6.57%.

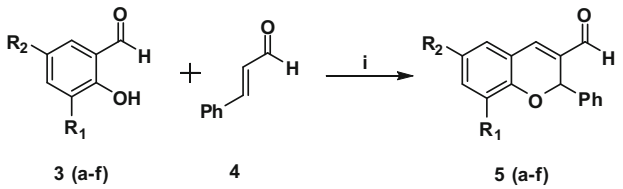
*2.4.37 (E)-3-(3,4-dimethoxystyryl)-8-ethoxy-2H-chromene (**10s**)* Prepared from **5'c** and **6c**. Yellow solid, M.p. 130–132 °C.^{1}H NMR (400 MHz, $CDCl_3$): δ 7.01–6.99 (m, 2H), 6.82 (d, J = 8.0 Hz, 1H), 6.80 (d, J = 4.0 Hz, 1H), 6.78–6.76 (m, 1H), 6.74 (d, J = 16.0 Hz, 1H), 6.67 (d, J = 8.0 Hz, 1H), 6.47 (s, 1H), 6.38 (d, J = 16.0 Hz, 1H), 5.13 (s, 2H), 4.11 (q, J = 8.0 Hz, 2H), 3.93 (s, 3H), 3.89 (s, 3H), 1.46 (t, J = 8.0 Hz, 3H). ^{13}C NMR (100 MHz, $CDCl_3$): δ 149.1, 146.8, 142.7, 130.8, 130.0, 128.7, 127.8, 124.7, 123.7, 123.0, 121.1, 119.9, 119.2, 113.5, 111.2, 108.7, 65.8, 64.6, 55.9, 14.9. Anal. Calcd for $C_{21}H_{22}O_4$: C, 74.54; H, 6.55%. Found: C, 77.56; H, 6.57%. ESI-HRMS $[M + Na]^+$: Calcd for $C_{21}H_{22}O_4Na$: 361.14103, found: 361.14111.

3. Results and Discussion

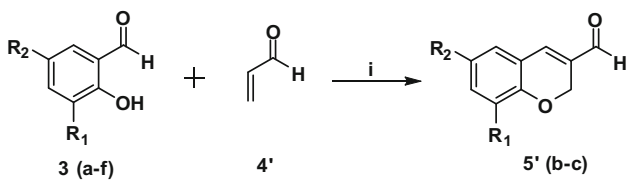
3.1 *Chemistry*

Compounds (*Z*)-2-phenyl/*H*-3-styryl-2*H*-chromenes **9**(**a**–**r**) and (*E*)-2-phenyl/*H*-3-styryl-2*H*-chromenes **10**(**a**–**s**) derivatives involves two steps. The first step involves the formation of 2*H*-chromene-3-carbaldehydes **5**(**a**–**f**) and **5'**(**b**–**c**) and the second step involves the reaction of chromene aldehyde **5**(**a**–**f**) and **5'**(**b**–**c**) with the phosphonium ylide to form the target molecules (*Z*)-2-phenyl/H-3-styryl-2*H*-chromenes **9**(**a**–**r**) and (*E*)-2-phenyl/*H*-3-styryl-2*H*-chromenes **10**(**a**–**s**). The synthesis of 2*H*-chromene-3-carbaldehyde **5**(**a**–**f**) and **5'**(**b**–**c**) started from *o*-hydroxybenzaldehyde **3**(**a**-**f**). The *o*-hydroxybenzaldehyde **3**(**a**–**f**) reacted with pyrrolidine **4** in DMSO at room temperature for 12 h to provide 2-phenyl-2*H*-chromene-3-carbaldehyde **5**(**a**–**f**) in 82–89% yield. Similarly, the *o*-hydroxybenzaldehyde **3**(**b**–**c**) was allowed to react with acrolein **4'** using K_2CO_3 in dioxane under reflux condition for 2 h to afford 2*H*-chromene-3-carbaldehyde **5'**(**b**-**c**) in 87–89% yield (Schemes 1 and 2).[37,38]

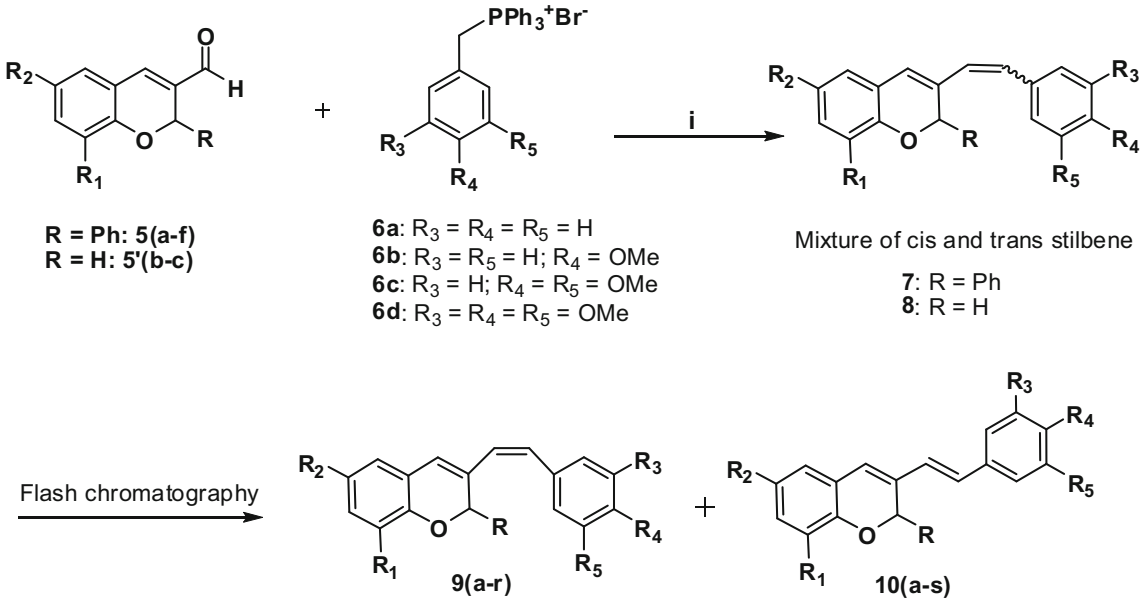
All the synthesized chromene aldehyde molecules **5**(**a**-**f**) and **5'**(**b**-**c**) were characterised by ^{1}H, ^{13}C NMR, IR and Mass spectroscopy. Melting points of the solid compounds were also taken.



Scheme 1. Synthesis of compounds **5**(**a**–**f**). Reagents and conditions: i) pyrrolidine, DMSO, rt, 12 h.



Scheme 2. Synthesis of compounds **5'**(**b**–**c**). Reagents and conditions: i) K_2CO_3, dioxane, reflux, 2 h.



Scheme 3. Synthesis of compound **9**(**a**–**r**) and **10** (**a**–**s**). Reagents and conditions: i) BuLi, THF, −78 °C, 6–8 h.

After successful synthesis of chromene aldehydes **5**(**a**–**f**) and **5'**(**b**–**c**), they are then allowed to react with the appropriate phosphonium ylide **6**(**a**-**d**) using BuLi in THF at −78 °C for 6–8 h to afford the cis and trans mixture of stilbene in good yield. Flash column chromatography purification provided the desired (*Z*)-2-phenyl/H-3-styryl-2*H*-chromenes **9**(**a**–**r**) and (*E*)-2-phenyl/*H*-3-styryl-2*H*-chromenes **10**(**a**–**s**) in 35–41% and 30–35% yields, respectively (Scheme 3). The synthesized compounds were characterized by the use of different spectroscopic techniques viz., ^{1}H, ^{13}C NMR and Mass spectroscopy. The diastereomers are well differentiated in ^{1}H NMR by the value of their coupling constant, the vicinal alkenyl protons in **9(a–r)** ranges from 8.0 to 12.0 Hz whereas for **10(a–s)** the range was from 16.0 to 24.0 Hz (Scheme 3).

3.2 *Biology*

3.2.1 In vitro anti-proliferative activity To evaluate the anti-proliferative activities of the synthesized chromene-stilbene compounds, four human cancer cell lines, MCF-7 (Breast adenocarcinoma), A549 (Lung carcinoma), DU145 (prostrate carcinoma) and HeLa (Cervical carcinoma) were tested using MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay with **CA-4**, a known anticancer drug, was used as positive control. The concentrations for antiproliferative activities (growth percentage of cells) of the synthesized compounds are shown in Table 4 in the supplementary information.

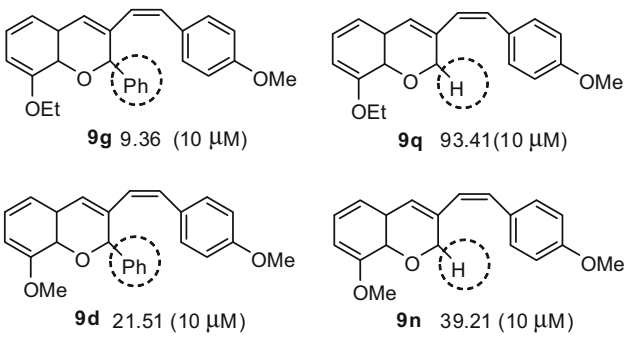


Figure 1. Comparison of antiproliferative activity of **9g/9q** and **9d/9n**.

The SAR study demonstrated that the presence of electron donating group (OEt) at the eighth position of the benzopyran ring and phenyl group in the second position of pyran ring improves anti-proliferative activity against tested cell line. But compound **9q** though quite similar to **9g** except phenyl substituent at the second position of pyran ring did not show any antiproliferative activity. This indicates that phenyl group at the second position of pyran ring has a key role in determining activity (Figure 1). Compound **9d**, **9e** and **9f** also showed good activity in HeLa cell line (Table S4).

From Table S4 (Supplementary Information) we found that among the series only the *Z*-olefinic compounds **9d**, **9e**, **9f**, **9g**, **9m** and **9n** exhibited good cytotoxicity against the HeLa cell line. Particularly compound **9g** decreased the viability of HeLa cells to 9.36% at **10**μM concentration whereas CA-4 (1 μM) exhibited similar values in parallel experiments. Then

Table 4. *In vitro* IC_{50} values of *cis* combretastatin analogs **9(d–g)** and **9(m–n)** on HeLa, MCF-7, A549 and DU145 cancer cells.

Comps.	HeLa	MCF7	A549	DU145
9d	20.73 ± 0.02	54.75 ± 0.15	66.66 ± 0.44	56.02 ± 0.09
9g	10.62 ± 0.01	75.43 ± 0.06	60.56 ± 0.26	64.37 ± 0.10
9e	22.63 ± 0.02	50.91 ± 0.11	72.76 ± 0.12	71.94 ± 0.16
9f	28.54 ± 0.11	58.38 ± 0.04	78.65 ± 0.11	73.21 ± 0.11
9m	61.45 ± 0.12	62.27 ± 0.05	58.37 ± 0.20	64.69 ± 0.07
9n	62.84 ± 0.06	86.02 ± 0.05	56.12 ± 0.12	75.40 ± 0.02
CA-4	1.32 ± 0.01	1.03 ± 0.06	1.48 ± 0.07	1.70 ± 0.03

we planned to study the half maximal inhibitory concentrations (IC_{50}) of these selective compounds (**9d, 9e, 9f, 9g, 9m** and **9n**) in the same cell lines (HeLa, MCF-7, A549 and DU145). The IC_{50} values are shown below in Table 4.

3.2.2 Antimitotic studies of compound 9g and combretastatin (CA-4) in HeLa cell lines We further studied the anti mitotic activity of most potent compound **9g** in HeLa cell lines. To examine the antimitotic effects, cell cycle analysis of most active compound **9g** (10 μM) and combretastatin(CA-4) (1 μM) standard, flow cytometry was carried out. Cell cycle analysis in HeLa cell lines revealed that treatment with **9g** and combretastatin (CA-4) exhibited 87.39% and 82.13% respectively. Accumulation of cells in G2/M phase with a concomitant decrease in the percentage of cells at G0/G1 phase was observed (Figure 2).

3.2.3 Effects of compound 9g on cellular microtubules network and nuclear morphology To examine the anti-tubulin effects of compounds 9g and CA-4 at cellular level, we treated HeLa cells at 10μM and 1μM concentrations for 18 h and analyzed the cellular microtubule network by immunofluorescence followed by nuclear staining with DAPI. Results demonstrate that cells treated with **9g** and CA-4 exhibited disrupted microtubule organization, as compared to the DMSO control (Figure 3).

HeLa cells were treated with **9g** at 10 μM and CA-4 at 1 μM concentrations for 18 h. Following the termination of the experiment, cells were fixed and stained for tubulin. DAPI was used as counterstain. The merged images of cells stained for tubulin and DAPI are represented. The photographs were taken using an Olympus confocal microscope equipped with FITC and DAPI filter settings. Data is the representative of five different fields of view.

3.2.4 Analysis of soluble versus polymerized tubulin in cells A dynamic equilibrium exists between the intracellular pool of α,β-tubulin heterodimers and the microtubule polymer. Microtubule disrupting agents target this dynamic equilibrium tubulin depolymerization agents (CA-4) inhibit polymerization. To further analyze whether the block in cell cycle at G2/M by **9g** can be reflected even in the cellular levels of soluble and polymerized tubulin (microtubules), we treated HeLa cells with **9g** at 10 μM concentration for 18 h and CA-4 (1 μM) was employed as a positive control. Following treatments, intracellular levels of soluble (free tubulin) and polymerized (tubulin from microtubules) fractions of tubulin were analyzed by immunoblotting. Results indicate that DMSO treated cells (controls) exhibited nearly equal distribution of tubulin in soluble and insoluble fraction, cells treated with **9g** and CA-4 demonstrated complete shift into soluble fraction (Figure 4A).

3.2.5 Cell cycle related expression of cyclin B1 Cyclin B1 regulates the progression of cell cycle at G2/M phase. Maximum expression of cyclin B1 expression will be seen during metaphase. Immunoblot analysis of cyclin B1 following treatment of cells with **9g** (10 μM) and CA-4 (1 μM) for 18 h resulted in an increased expression of cyclin B1 as compared to DMSO treated controls. Therefore, the increased protein levels of cyclin B1 by **9g** confirm that these compounds acts as anti-tubulin agents and block the cells at mitotic phase (Figure 4B).

3.2.6 Materials and Methods: Cell Cultures, Maintenance and Anti-proliferative Evaluation All cell lines used in this study were purchased from the American Type Culture Collection (ATCC, United States). HeLa, A549 and DU145 were grown in Dulbecco's modified Eagle's medium (containing 10% FBS in a humidified atmosphere of 5% CO_2 at 37 °C). MCF-7 cells were cultured in Eagle's minimal essential medium (MEM) containing non-essential amino acids, 1 mM sodium pyruvate, 10 mg/mL bovine insulin, and 10% FBS. The synthesized test compounds were evaluated for their in vitro antiproliferative activity in four different human cancer cell lines. MTT cell proliferation

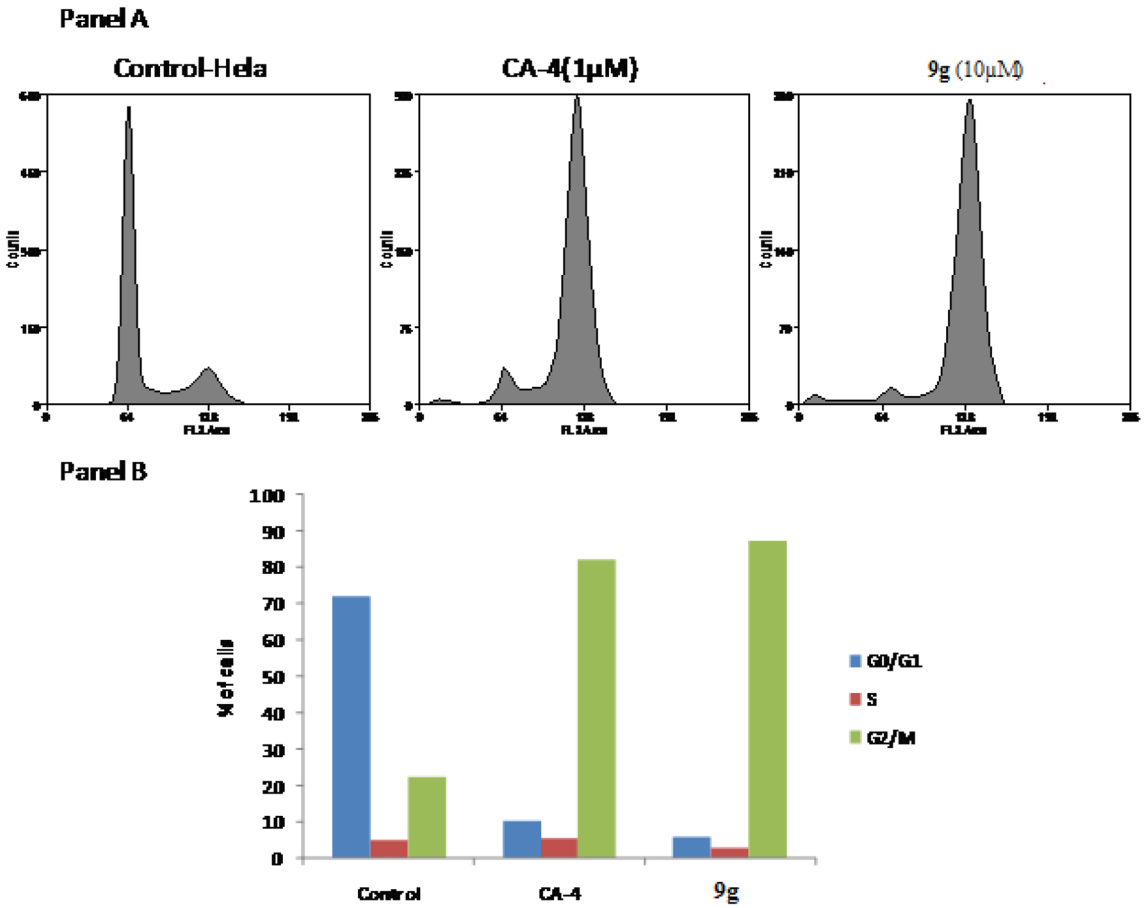


Figure 2. Anti-mitotic effects of compound **9g**. Panel A. HeLa cells were harvested after treatment with compound **9g** (10μM) and CA-4 (1μM) for 18 h. Untreated and CA-4treated cells served as control and standard. The percentage of cells in each phase of the cell cycle was quantified by flow cytometry. Panel B: Distribution of cells at G0/G1, S and G2/M phase of cell cycle following treatment with compound **9g** and CA-4 in HeLa cells.

assay was used to estimate cell viability or growth. Cell lines were grown in their respective media containing 10% fetal bovine serum. Cells were trypsinized when sub-confluent from T25 flasks/60 mm dishes and were seeded into 48-well microtiter plates in 500μL aliquots at plating densities depending on the doubling time of individual cell lines. The microtiter plates were incubated at 37 °C, 5% CO_2, 95% air, and 100% relative humidity for 24 h prior to addition of experimental drugs. Aliquots of 1μL of the test compounds were added to the wells already containing 500μL of cells, resulting in the required final drug concentrations and each compound was tested in duplicate wells. Plates were incubated further for 48 h, and the assay was terminated by the addition of 50 μL of 5% MTT and incubated for 60 min at 37 °C. Later, the plates were air-dried. The bound stain was subsequently eluted with 250μL of DMSO. Per cent growth was calculated on a plate by plate basis for test wells relative to control wells.

Compounds with good inhibition were further screened for IC50 in four concentrations (1, 10, 25 and 50 μM) of test compounds (2 μL) were evaluated in triplicates, resulting in the required final drug concentrations. Plates were incubated further for 48 h, and the assay was terminated by the addition of 10μL of 5% MTT and incubated for 60 min at 37 °C. Later, the plates were air-dried. The bound stain was subsequently eluted with 100μL of DMSO and the absorbance was read on a multimode plate reader (Perkin Elmer) at a wavelength of 565 nm. The sensitivity of the cancer cells to the test compound was expressed as IC50 values which are indicated as mean ±SD of three independent experiments.

3.2.7 Analysis of Cell Cycle HeLa cells grown in 60 mm dishes were treated with compound 9g (10 μM) and CA-4(1 μM) for 18 h. Cells were harvested with Trypsin-EDTA, fixed with ice-cold 70% ethanol at 4 °C for 30 min, ethanol was removed by centrifugation and cells were stained with 1 mL of DNA staining solution

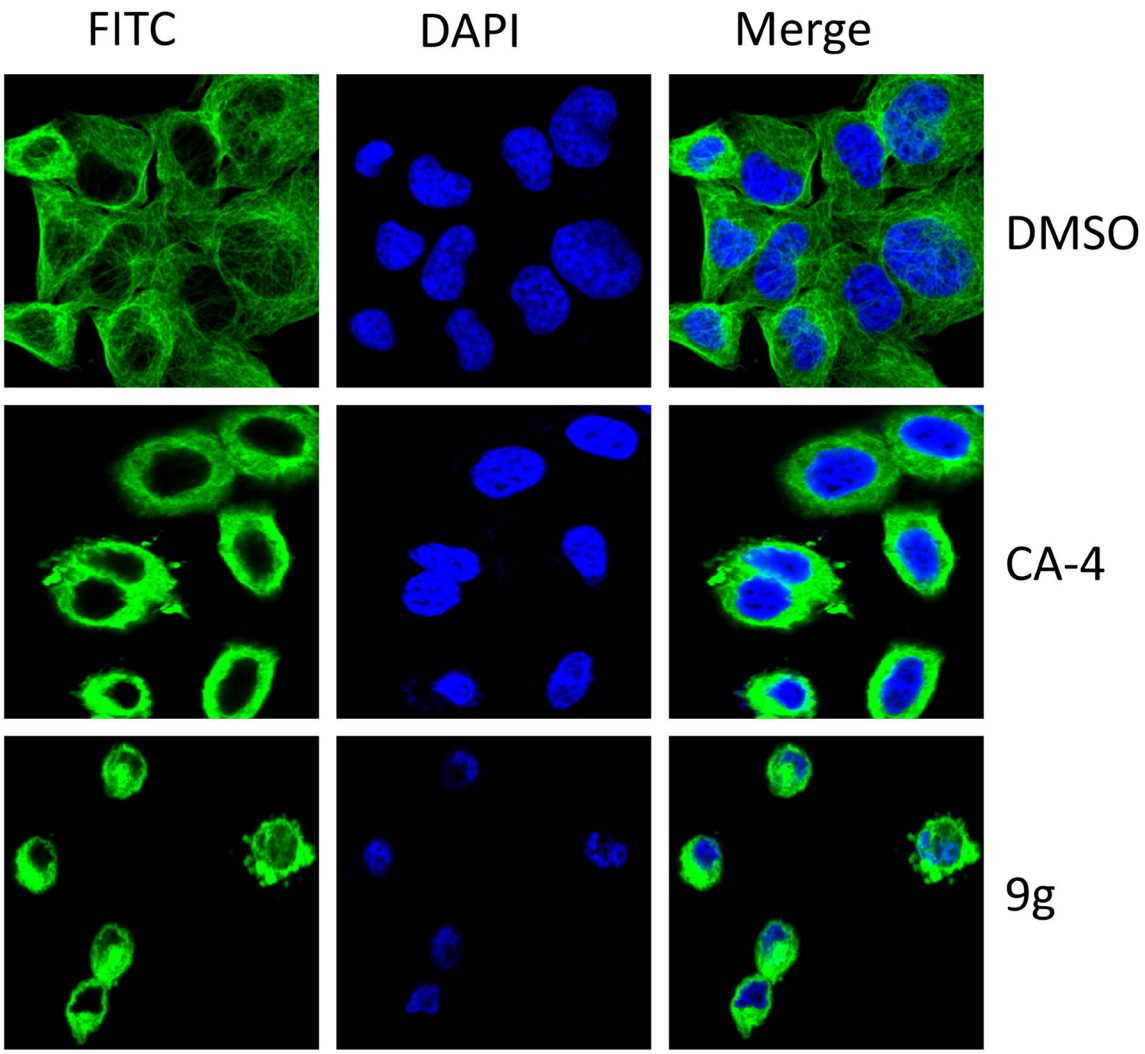


Figure 3. Effect of compound **9g** on microtubules and nuclear condensation.

[containing 50μg of Propidium Iodide (PI), and 0.1 mg RNase A] for 30 min. The DNA contents of 20,000 events were measured by flow Cytometer (MoFlow).

3.2.8 Immunofluorescence HeLa cells were seeded on glass coverslips, treated for 18 h in the presence or absence of test compounds **9g** (10 μM) and CA-4(1 μM). Following treatments, cells were fixed in 3.5% formaldehyde in phosphate-buffered saline (PBS) pH 7.4 for 10 min at room temperature. Cells were permeabilized for 6 min in PBS containing 0.5% Triton X-100 (Merck) and 0.05% Tween-20 (Merck).The permeabilized cells were blocked with 2% BSA (Merck) in PBS for 1 h. Later, the cells were incubated with the primary anti-α-tubulin antibody (Merck) (1:200) diluted in blocking solution for 4 h at room temperature. Subsequently, cells were washed thrice with PBS and then incubated with FITC labelled anti-mouse secondary antibody (Merck) (1:500) for 1 h at room temperature. Finally, cells were washed thrice with PBS and mounted in medium containing DAPI. Images were captured using the Olympus confocal microscope and analyzed with Provision software.

3.2.9 Western blot analysis Cells were seeded in 6-well plates at 2×10^5 cells per well in complete growth medium. Following treatment with compound **9g** (10 μM) and CA-4(1 μM) for 18 h, cells were washed with PBS and subsequently soluble and insoluble tubulin fractions were collected. To collect the soluble tubulin fractions, cells were permeabilized with 200 μL of pre-warmed lysis buffer [80 mM Pipes-KOH (pH 6.8), 1 mM MgCl2, 1 mM EGTA, 0.2% Triton X-100, 10% glycerol, 0.1% protease inhibitor cocktail (Sigma Aldrich)] and incubated for 3 min at 30 °C. Lysis buffer was gently removed, and mixed with 100 μL of 3x Laemmli's sample buffer (180 mMTris HCl pH 6.8, 6% SDS, 15% glycerol, 7.5% b-mercaptoethanol and 0.01% bromophenol blue). Samples were immediately heated to 95 °C for 3 min. To collect the insoluble

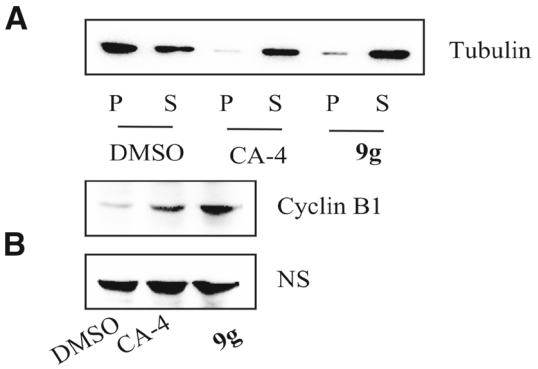


Figure 4. Effect of compound **9g** on tubulin polymerization and Cyclin-B1. **A**: HeLa cells were treated with 10 μM of **9g** and CA-4 at 1 μM concentration for 18 h. The fractions containing soluble and polymerized tubulin were collected; tubulin was detected by immunoblot analysis. **B**: HeLa cells were treated with 10 μM of **9g** and CA-4 at 1 μM concentration for 18 h. Subsequently, whole lysates were prepared and analyzed for cyclin-B1. A non-specific (NS) band at 90 kDa was used as loading control.

tubulin fraction, 300 mL of 1xLaemmli's sample buffer was added to the remaining pellet in each tube and the samples were heated to 95 °C for 3 min. Equal volumes of samples were run on an SDS-10% polyacrylamide gel and were transferred to a nitrocellulose membrane by semidry transfer at 120 mA for 1 h. Blots were probed with mouse anti-α-tubulin antibody (Merck) and further incubated with horseradish peroxidise-conjugated rabbit anti-mouse secondary antibody(Merck) at room temperature for 1 h. Bands were visualized using an enhanced chemiluminescence method (Merck).

4. Conclusion

In summary, various (*Z*)-2-phenyl/*H*-3-styryl-2*H*-Chromenes **9**(**a**–**r**) and (*E*)-2-phenyl/*H*-3-styryl-2*H*-Chromenes **10**(**a**–**s**) derivatives were synthesized and evaluated their anticancer activity against four different cancer cell line. Compounds **9d**, **9e**, **9f**, **9g**, **9m** and **9n** showed moderate to good anticancer activity, particularly in HeLa cell line. The most active molecule, **9g**, exhibited an IC_{50} value of 10.62 μM whereas CA-4 exhibited an IC_{50} value of 1.32 μM in parallel experiments. The SAR study demonstrated that the presence of electron donating groups at the 8th position of the benzopyran ring and phenyl substituent at the 2nd position of pyran ring improves anti-cancer activity against tested cell line. Further biological assessments including cell cycle analysis revealed that treatment of **9g** and combretastatin (CA-4) exhibited 87.39% and 82.13% respectively. Accumulation of cells in G2/M phase with a concomitant decrease in the percentage of cells at G0/G1 phase was observed. The result of the effect of Compound **9g**, was most evident, on cellular microtubule networks (immunofluorescence study). Compound **9g** increased the amount of tubulin in the soluble fraction, a feature of microtubule depolymerizing agents. Increased expression of cyclin B1 in cells treated with compound **9g** was found. Thus, these results suggest that (*Z*)-2-phenyl/*H*-3-styryl-2*H*-Chromenes **9**(**a**–**r**) and (*E*)-2-phenyl/*H*-3-styryl-2*H*-Chromenes **10**(**a**–**s**) derivatives particularly compound **9g** have potential to develop as a new class of tubulin polymerization inhibitors. The compounds of these series are further amenable for structural modifications and will be useful as templates for the design of new anticancer agents.

Supplementary Information (SI)

The characterization of the compounds **5**(**a**–**f**), **5'**(**b**–**c**), **9**(**a**–**r**) and **10**(**a**–**s**) using ^{1}H, ^{13}C NMR, IR and Mass data (Figures S1–S125) are given in the supplementary information. Supplementary Information is available at www.ias.ac.in/chemsci.

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