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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Structure–activity relationship study of growth inhibitory 2-styrylchromones against carcinoma cells

Chen Lin · Pei-Jung Lu · Chia-Ning Yang · Christopher Hulme · Arthur Y. Shaw

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Abstract The structure–activity relationship study of 2-styrylchromones against carcinoma cell growth is discussed in the present report. Taking advantage of 2-styrylchromone as a molecular template, a series of structural modifications was carried out and examined on several carcinoma cell lines. Interestingly, AGS cells exhibited more sensitivity in response to methoxy-bearing compounds, of which compound **23** (3,4,5-trimethoxy group on ring B) showed the most potent activity with a GI_{50} value of 1.3 μM. Surprisingly, as methoxy groups in **12** and **24**–**27** were demethylated to generate their hydroxyl counterparts **28**–**32**, none of them displayed appreciable activity against all carcinoma cells. We further confirmed the pivotal role of rigidity for growth inhibitory activity between the rigid **12** and its flexible counterpart **33**. Taken together, in the present report, we have clearly demonstrated the structure–activity relationship study of 2-styrylchromones targeting carcinoma cell growth.

Keywords Structure–activity relationship study · 2-Styrylchromone · Carcinoma cells · Growth inhibition

C. Lin
Department of Chemistry, Tamkang University, New Taipei City 251, Taiwan

P.-J. Lu
Institute of Clinical Medicine, National Cheng Kung University, Tainan 701, Taiwan

C.-N. Yang
Institute of Biotechnology, National University of Kaohsiung, Kaohsiung 811, Taiwan

C. Hulme · A. Y. Shaw (✉)
BIO5 Oro Valley, University of Arizona, 1580 E. Hanley Blvd., Oro Valley, AZ 85737, USA
e-mail: shaw@pharmacy.arizona.edu

C. Hulme · A. Y. Shaw
Department of Pharmacology and Toxicology, College of Pharmacy, The University of Arizona, Tucson, AZ 85721, USA

Introduction

Regardless of the availability of computer-assisted strategies toward drug discovery programs, drug-like core structures stemmed from natural products are still highly demanded due to their structural novelty and biologic relevance. It can be agreed that abundant clinically used drugs either mimic naturally occurring molecules or bear scaffolds that are fully or partially employed from natural products (Balunas and Kinghorn, 2005). For instance, the phase 2 clinical trial candidate flavopiridol (Kelland, 2000; Senderowicz and Sausville, 2000), bearing a chromone (**1**) core structure, is a cyclin-dependent kinase (CDK) inhibitor derived from the indigenous Indian plant *Dysoxylum binectariferum* (Fig. 1). This chromone (**1**) core structure is found in flavone (**2**), isoflavone (**3**), and 2-styrylchromone (**4**), of which are ubiquitously distributed in several species of higher plants (Fig. 1). It is noteworthy that 2-styrylchromones (**4**) are a small group of chromone derivatives specifically recognized by a styryl group appended at 2-position of the chromone (**1**) core structure.

Naturally occurring homothamnione, 6-desmethoxyhomothamnione, and 5-hydroxy-2-styrylchromone have been characterized for antitumor and neuroprotective activities (Gerwick *et al.*, 1986; Gerwick, 1989). Interestingly, several 2-styrylchromone derivatives have been synthesized before the isolation of first natural compounds. 2-Styrylchromones possess a broad spectrum of biologic activities, including

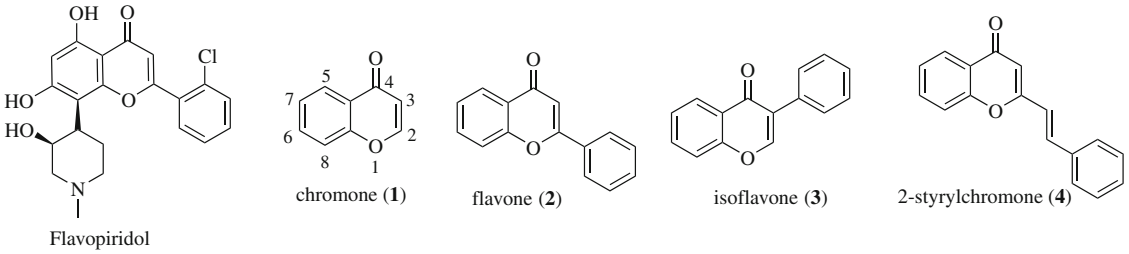


Fig. 1 Schematic representation of flavopiridol, chromones (**1**), flavones (**2**), isoflavones (**3**), and 2-styrylchromones (**4**)

anti-allergic (Doria *et al.*, 1979), antitumor (Momoi *et al.*, 2005; Marinho *et al.*, 2008), antiviral (Desideri *et al.*, 2000; Desideri *et al.*, 2003; Conti *et al.*, 2005), antioxidant (Filipe *et al.*, 2004; Gomes *et al.*, 2007), anti-inflammatory (Gomes *et al.*, 2009), and to serve as antagonists of A_3 adenosine receptor (Karton *et al.*, 1996), inhibitors of xanthine oxidase (Fernandes *et al.*, 2002). Recently, we embarked on the synthesis of 2-styrylchromones with aim to examine their antiproliferative effect on human carcinoma cell lines. Our results indicated that the exposure of HeLa cells to 2-styrylchromones lead to significant G1 phase cell cycle arrest, DNA fragmentation, and programed cell death (Shaw *et al.*, 2009). To continue our effort on the elucidation of carcinoma cells in response to 2-styrylchromones, herein, we report the synthesis of some newly synthesized 2-styrylchromones aimed at examining the structure–activity relationship of growth inhibition of carcinoma cell lines.

Results and discussion

Chemistry

As shown in Fig. 2, a series of 2-styrylchromones and their modifications was carried out in a systematic manner. In Route A, we incorporated various substituents on the ring B to give **6**–**22** (Fig. 3, Scheme 1). The structural modifications in Route B were mainly originated from the results of Route A. Accordingly, ring A modification of 2-styrylchromone scaffold was carried out in which the 3,4,5-trimethoxy groups on ring B were maintained, such that analogs **23**–**27** were synthesized (Fig. 3, Scheme 1). In Route C, methoxy groups in **23**–**27** were completely removed via boron tribromide (BBr_3)-mediated demethylation to yield the corresponding polyphenol analogs **28**–**32** (Fig. 3, Scheme 2). In Route D, we examined whether the styryl group in compound **12** is essential for the activity by which hydrogenation was carried out to generate the corresponding **33** (Fig. 3, Scheme 3).

Growth inhibition

In this study, growth inhibition of 2-styrylchromones was examined in vitro against a panel of human carcinoma cell lines, including AGS (gastric carcinoma cell), HeLa (cervical epithelioid carcinoma cell), OVCA (ovarian carcinoma cell), SKHep (hepatocellular carcinoma cell), H460 (large lung carcinoma cell), PC-3 (prostate carcinoma cell), SW620 (colorectal adenocarcinoma cell), and BT483 (mammary gland adenocarcinoma cell). We employed the MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) assay to study the growth inhibition. The compound concentration causing 50 % cell growth inhibition (GI_{50}) was determined by interpolation from dose–response curves.

As shown in Table 1, modifications of 2-styrylchromones on ring B revealed some interesting structure–activity correlations. For example, compared to their counterpart of unsubstituted **6**, upon the introduction of methoxy groups to generate compounds **7**–**12**, **9** (4-OMe) and **12** (3,4,5-tri-OMe) exhibited significant improvement in potency against AGS cells with GI_{50} values of 8 and 2 μM, respectively. Similarly, **9** (4-OMe, GI_{50}, 7 μM), **8** (3-OMe, GI_{50}, 17 μM) and **12** (3,4,5-tri-OMe, GI_{50}, 6 μM) displayed potent activity, while **7** (2-OMe, GI_{50}, >40 μM), **10** (3,5-di-OMe, GI_{50}, >40 μM) and **11** (3,4-di-OMe, GI_{50}, >40 μM) resulted in the loss of activity against HeLa cells. On the other hand, only **9** (4-OMe) and **12** (3,4,5-tri-OMe) showed appreciable improvement in potency against OVCA cells with GI_{50} values of 6 and 8 μM, respectively. Interestingly, both mono-substituted **8** (3-OMe, GI_{50}, 8 μM) and **9** (4-OMe, GI_{50}, 5 μM) exhibited more potency than that of tri-substituted **12** (3,4,5-tri-OMe, GI_{50}, 14 μM) against SKHep cells. Except for **7** and **11,** without any activity, **8**, **9**, **10,** and **12** displayed dramatically improved activity against H460 cells with GI_{50} values of 16, 7, 25, and 6 μM, respectively. Meanwhile, benzene ring was replaced with pyridine ring to generate **14** (4-pyridyl) and **15** (3-pyridyl). Surprisingly, only **14** showed more potency against a panel of five cell lines against HeLa, OVCA, and SKHep cells with GI_{50} values of 24, 10, and 15 μM, respectively. This discrepancy in activity might be attributed to the preferred interactions between molecular targets and **14** for favored hydrogen bond interaction. Compared to its counterpart **6**, **16** bearing 4-*N*, *N*-dimethylamino group on ring B exhibited more potent activity against AGS, OVCA, and H460 cells with GI_{50} values of 9, 10, and 8 μM, respectively. As the benzene ring was introduced with strong electron-withdrawing groups such as 3,4-di-fluoro group in **18**, a remarkable improvement

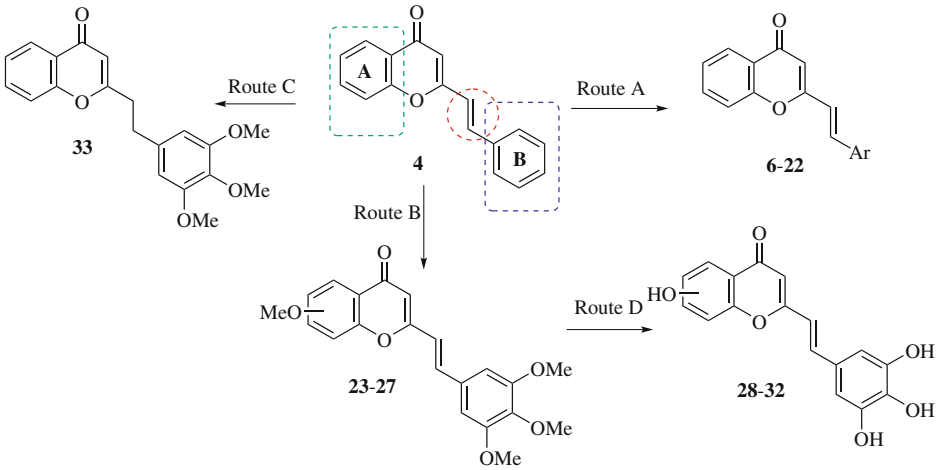
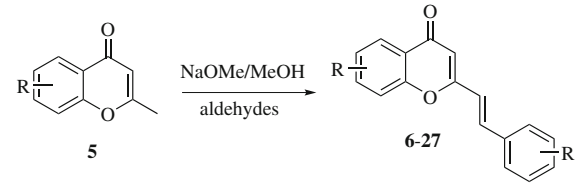
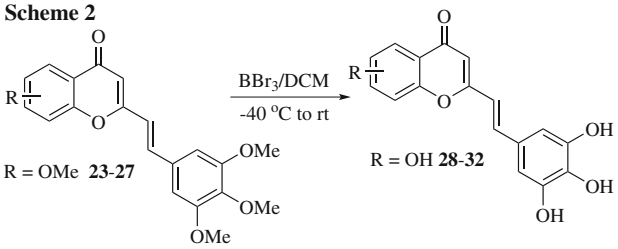


Fig. 2 Overall synthetic routes of structure–activity relationship study of 2-styrylchromones

Scheme 1



Scheme 2



Scheme 3

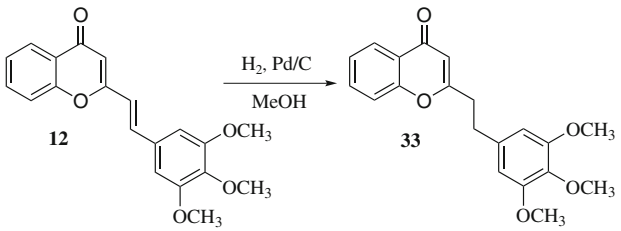


Fig. 3 Synthetic schemes employed for the structure–activity relationship of 2-styrylchromones

in activity against AGS, HeLa, SKHep, and H469 cells was found with GI_{50} values of 15, 9.9, 9, and 18 μM, respectively. According to data shown in Table 1, all five carcinoma cell lines exhibited higher sensitivity in response to electron-withdrawing substituents on ring B with moderate activity.

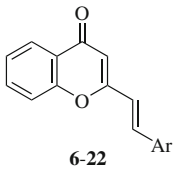
We further focused on the ring A structural modifications of 2-styrylchromone scaffold in which ring B remained 3,4,5-trimethoxy groups owing to **12** with the most potent activity against AGS cells. As a consequence, polymethoxy analogs **22–27** were synthesized and evaluated for their growth inhibition. To our surprise, except for **23** (5-OMe) which showed improved activity with a GI_{50} value of 1.3 μM against AGS cells, all analogs exhibited decreased potency with GI_{50} values between 10 and 24 μM as indicated in Table 2. Both **24** (6-OMe) and **26** (5,7-di-OMe) displayed equal potency against H460 cells with GI_{50} values of 10 μM, which is less active than that of counterpart **22** (GI_{50}, 6 μM). Moreover, as polymethoxy analogs were removed in Route D to obtain polyphenols **28–32**, the activity completely disappeared. These findings might be attributable to the alternation of physical properties, particularly loss of hydrophobicity for appropriate cell membrane penetration.

To examine whether the rigidity in 2-styrylchrome skeleton plays a role for the activity, the double bond of styryl group in analogs **12** was subjected to undergo hydrogenation which afforded the corresponding product **33**. Interestingly, the activity of **33** was dramatically decreased in comparison to its counterpart **12** as indicated in Table 3. Among seven cell lines, only BT483, OVCA, and SKHep showed moderate activity in response to **33** treatment with GI_{50} values of 21, 24, and 19 μM, respectively. This finding clearly suggested that a certain level of rigidity in the structure plays an important role for activity.

Conclusion

In conclusion, we have demonstrated that modifications of ring B exhibited certain degree of significant structure–activity relationships than that of ring A. Particularly, AGS cells showed more sensitivity to the tested compounds, in which compound **23** bearing polymethoxy group exhibited the most potent activity with a GI_{50} value of 1.3 μM. As methoxy groups in **12** and **24–27** were removed to the counterpart **28–32** with hydroxyl groups, none of them displayed appreciable activity against all carcinoma cells. Moreover, we further demonstrated that the vinyl group is

Table 1 Growth inhibition of 2-styrylchromones **6–22** against carcinoma cells



6-22

No.	Ar	GI_{50} (μM)[a]				
		AGS	HeLa	OVCA	SKHep	H460
6	Ph	>40	25 ± 2	21 ± 2	>40	>40
7	2-OMe–Ph	>40	>40	>40	>40	>40
8	3-OMe–Ph	>40	17 ± 1	21 ± 3	8 ± 1	16 ± 3
9	4-OMe–Ph	8 ± 0.4	7 ± 1	6 ± 1	5 ± 1	7 ± 2
10	3,5-Di-OMe–Ph	>40	>40	>40	21 ± 2	25 ± 4
11	3,4-Di-OMe–Ph	>40	>40	27 ± 2	>40	>40
12	3,4,5-Tri-OMe–Ph	2 ± 0.2	6 ± 1	8 ± 3	14 ± 2	6 ± 1
13	2-Benzo[*d*][1,3]dioxol-5-yl	>40	14 ± 1	>40	>40	>40
14	4-Pyridyl	>40	24 ± 2	10 ± 1	15 ± 3	>40
15	3-Pyridyl	>40	16 ± 1	>40	>40	>40
16	4-NMe_2–Ph	9 ± 1	24 ± 2	10 ± 1	>40	8 ± 2
17	4-Br–Ph	>40	24 ± 4	23 ± 1	22 ± 2	17 ± 3
18	3,4-Di-F–Ph	15 ± 1	9.9 ± 1	23 ± 2	9 ± 1	18 ± 4
19	4-Cl–Ph	>40	>40	11 ± 1	9 ± 2	22 ± 3
20	4-CF_3–Ph	25 ± 3	29 ± 6	>40	14 ± 4	19 ± 4
21	4-OCF_3–Ph	>40	16 ± 2	>40	>40	21 ± 5
22	4-NO_2–Ph	14 ± 1	8 ± 1	>40	10 ± 1	16 ± 3
Doxorubicin		0.02 ± 0.01	0.33 ± 0.04	0.95 ± 0.07	0.09 ± 0.03	0.11 ± 0.02

[a] GI_{50} values are presented as the Mean ± SEM (standard error of the mean) from four to six separated experiments

pivotal for activity since **33** with a rotatable single bond between the chromone core structure and ring B significantly decreased growth inhibitory activity. Taken together, we have successfully synthesized a series of 2-styrylchromone derivatives and examined their growth inhibition activity against a panel of carcinoma cell lines for further study.

Experimental

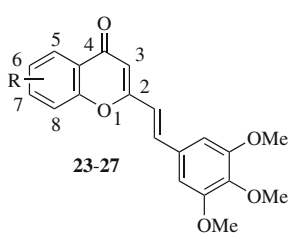
Chemistry

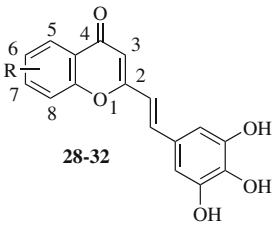
Chemical reagents and organic solvents were purchased from TCI and Alfa Aesar unless otherwise mentioned. Melting points were determined by Fargo MP-2D. Nuclear magnetic resonance spectra (^{1}H NMR) were measured on a Bruker AC-300 instrument. Chemical shifts (δ) are reported in parts per million relative to the TMS peak. Mass spectra were obtained by FAB on a Jeol JMS-700 instrument. Flash column chromatography was performed with silica gel (230–400 mesh).

(*E*)-2-Styryl-4*H*-chromen-4-one (**6**)

Sodium (0.69 g, 30.0 mmol) was gradually added to dry methanol (30 ml) and the mixture was stirred until the solution reached room temperature. **5** (0.73 g, 5.0 mmol) and benzaldehyde (0.64 g, 6.0 mmol) were added and the resulting mixture was allowed to stir at reflux for 18 h. After this period, the solution was poured into iced water and the pH was adjusted to 4 with aqueous HCl. The yellow solid was removed by filtration, taken up in DCM, and purified with silica gel chromatography (eluent, DCM: ethyl acetate = 4:1) to give **6** (0.93 g, 75 %) as a white solid. Mp 134 °C (lit. (Silva *et al.*, 1998) 131–133 °C). ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.31 (s, 1H), 6.76 (d, J = 16.0 Hz, 1H), 7.43–7.34 (m, 4H), 7.58–7.50 (m, 3H), 7.59 (d, J = 16.0 Hz, 1H), 7.66 (dd, J = 8.7, 7.2 Hz, 1H), 8.18 (d, J = 7.9 Hz, 1H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$) δ 110.8, 118.0, 120.4, 124.3, 125.1, 125.8, 127.8, 129.1, 130.0, 133.8, 135.1, 137.1, 156.2, 161.9, 178.5 ppm. HRMS $(M)^+$ calcd. for $C_{17}H_{12}O_2$ 248.0837; found 248.0834.

Table 2 Growth inhibition of 2-styrylchromones **23–32** against carcinoma cells

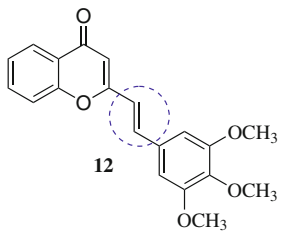


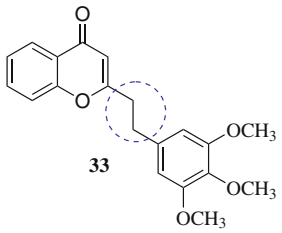


No.	R	GI_{50} (μM)[a]			
		AGS	PC-3	H460	SW620
23	5-OMe	1.3 ± 0.07	20 ± 4	>40	17 ± 3
24	6-OMe	10 ± 1	29 ± 5	10 ± 2	26 ± 3
25	7-OMe	12 ± 1	>40	>40	>40
26	5,7-Di-OMe	10 ± 0.2	>40	10 ± 2	27 ± 4
27	7,8-Di-OMe	24 ± 2	>40	>40	>40
28	H	>40	>40	>40	>40
29	6-OH	>40	>40	>40	>40
30	7-OH	>40	>40	>40	>40
31	5,7-Di-OH	>40	>40	>40	>40
32	7,8-Di-OH	>40	>40	>40	>40
Doxorubicin		0.02 ± 0.01	1.76 ± 0.71	0.11 ± 0.02	0.03 ± 0.005

[a] GI_{50} values are presented as the Mean ± SEM (standard error of the mean) from four to six separated experiments

Table 3 Comparison of **12** with its counterpart **33** in regard to their growth inhibition against carcinoma cells





No.	GI_{50} (μM)[a]						
	AGS	BT483	HeLa	OVCA	SKHep	H460	SW620
12	2 ± 0.2	10 ± 2	6 ± 1	8 ± 3	14 ± 2	6 ± 1	8 ± 0.2
33	>40	21 ± 4	>40	24 ± 4	19 ± 1	>40	>40

[a] GI_{50} values are presented as the Mean ± SEM (standard error of the mean) from four to six separated experiments

(*E*)-2-(2-Methoxystyryl)-4*H*-chromen-4-one (**7**)

*Compound **7** was synthesized from the procedure described for compound **6***

Yield 57.8 %. Mp 118.2 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.92 (s, 3H), 6.31 (s, 1H), 6.85 (d, $J = 16.1$ Hz, 1H), 6.93 (d, $J = 7.9$ Hz, 1H), 6.98 (dd, $J = 7.6$, 7.5 Hz, 1H), 7.32 (dd, $J = 7.6$, 7.9 Hz, 1H), 7.37 (dd, $J = 7.9$, 7.0 Hz, 1H), 7.53 (d, $J = 8.4$ Hz, 1H), 7.56 (d, $J = 7.5$ Hz, 1H), 7.65 (dd, $J = 7.0$, 8.4 Hz, 1H), 7.90 (d, $J = 16.1$ Hz, 1H), 8.18 (d, $J = 7.9$ Hz, 1H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$) δ 55.7, 110.4, 111.3, 118.1, 120.9, 121.0, 124.1, 124.3, 125.0, 125.7, 128.3, 131.2, 132.5, 133.6, 156.2, 158.1, 162.6, 178.6 ppm. HRMS $(M+1)^+$ calcd. for $C_{18}H_{14}O_3$ 279.0976; found 279.1019.

(*E*)-2-(3-Methoxystyryl)-4*H*-chromen-4-one (**8**)

*Compound **8** was synthesized from the procedure described for compound **6***

Yield 42.8 %. Mp 139.9 °C. ^{1}H NMR (300 MHz, $CDCl_3$)δ 3.87(s, 3 H), 6.33 (s, 1 H), 6.77 (d, $J = 16.0$ Hz, 1H), 6.95 (dd, $J = 7.9$, 7.7 Hz, 1H), 7.10 (s, 1H), 7.18 (d, $J = 7.7$ Hz, 1H), 7.32 (d, $J = 7.9$ Hz, 1H), 7.39 (dd, $J = 7.9$, 7.4 Hz, 1H), 7.53 (d, $J = 7.8$ Hz, 1H), 7.58 (d, $J = 16.0$ Hz, 1H), 7.68 (dd, $J = 7.8$, 7.4 Hz, 1H), 8.18(d, $J = 7.9$ Hz, 1H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$) δ 55.5, 110.9, 112.9, 115.8, 118.0, 120.5, 120.7, 124.3, 125.1, 125.8, 130.1, 133.9, 136.5, 137.0, 156.2, 160.2, 161.8, 178.5 ppm. HRMS $(M+1)^+$ calcd. for $C_{18}H_{14}O_3$ 279.0976; found 279.1017.

(*E*)-2-(4-Methoxystyryl)-4*H*-chromen-4-one (**9**)

*Compound **9** was synthesized from the procedure described for compound **6***

Yield 43.9 %. Mp 140.3 °C (lit. (Makrandi and Kumari, 1989) 137–139 °C). ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.86(s, 3H), 6.29 (s, 1H), 6.65 (d, $J = 16.0$ Hz, 1H), 6.94 (d, $J = 7.0$ Hz, 2H), 7.38 (dd, $J = 7.5$, 7.6 Hz, 1H), 7.52 (d, $J = 7.7$ Hz, 1H), 7.54 (d, $J = 7.0$ Hz, 1H), 7.57 (d, $J = 16.0$ Hz, 1H), 7.67 (dd, $J = 7.7$, 7.7 Hz, 1H), 8.20 (d, $J = 7.6$ Hz, 1H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$) δ 55.5, 110.9, 112.8, 115.7, 118.0, 120.5, 120.7,124.2, 125.1, 125.8, 130.1, 133.9, 136.5, 137.0, 156.1, 160.1, 161.7, 178.6 ppm. HRMS $(M+1)^+$ calcd. for $C_{18}H_{14}O_3$ 279.0976; found 279.1014.

(*E*)-2-(3,5-Dimethoxystyryl)-4*H*-chromen-4-one (**10**)

*Compound **10** was synthesized from the procedure described for compound **6***

Yield 45.3 %. Mp 152.9 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.83 s, 6H), 6.31 (s, 1H), 6.48 (t, J = 2.1, 2.3 Hz, 1H), 6.70 (s, 2H), 6.73 (d, J = 15.9 Hz, 1H), 7.37 (dd, J = 8.0, 7.0 Hz, 1H), 7.51 (d, J = 8.7 Hz, 1H), 7.52 (d, J = 15.9 Hz, 1H), 7.66 (dd, J = 8.7, 7.0 Hz, 1H), 8.17 (d, J = 8.0 Hz, 1H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$) δ 55.5, 102.1, 105.7, 110.8, 117.9, 120.7, 124.1, 125.1, 125.7, 133.8, 136.9, 137.0, 156.1, 161.2, 161.7, 178.5 ppm. HRMS $(M+1)^+$ calcd. for $C_{19}H_{16}O_4$ 309.1082; found 309.1124.

(*E*)-2-(3,4-Dimethoxystyryl)-4*H*-chromen-4-one (**11**)

*Compound **11** was synthesized from the procedure described for compound **6***

Yield 30.2 %. Mp 166.1 °C (lit. (Cheema *et al.*, 1932) 165 °C). ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.92 (d, J = 11.0 Hz, 6H), 6.28 (s, 1H), 6.62 (d, J = 15.9 Hz, 1H), 6.87 (d, J = 8.2 Hz, 1H), 7.08 (s, 1H), 7.14 (d, J = 8.2 Hz, 1H), 7.35 (dd, J = 7.4, 7.7 Hz, 1H), 7.49 (d, J = 8.0 Hz, 1H), 7.53 (d, J = 15.9 Hz, 1H), 7.64 (dd, J = 7.4, 8.0 Hz, 1H), 8.16 (d, J = 7.7 Hz, 1H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$) δ 56.1, 109.6, 110.1, 111.4, 117.9, 118.2, 122.2, 124.3, 125.1, 125.8, 128.2, 133.7, 137.0, 149.5, 151.1, 156.2, 162.3, 178.5 ppm. HRMS $(M+1)^+$ calcd. for $C_{19}H_{16}O_4$ 309.1082; found 309.1137.

(*E*)-2-(3,4,5-Trimethoxystyryl)-4*H*-chromen-4-one (**12**)

*Compound **12** was synthesized from the procedure described for compound **6***

Yield 70 %. Mp 211.2 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.92d, J = 11.5 Hz, 9H), 6.32 (s, 1H), 6.68 (d, J = 16.0 Hz, 1H), 6.80 (s, 2H), 7.39 (dd, J = 9.0, 7.9 Hz, 1H), 7.52 (s, J = 7.7 Hz, 1H), 7.53 (d, J = 16.0 Hz, 1H), 7.67 (dd, J = 9.0, 7.7 Hz, 1H), 8.19 (d, J = 7.9 Hz, 1H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$) δ 56.2, 56.3, 61.1, 105.1, 110.5, 117.9, 119.6, 124.2, 125.1, 125.8, 130.7, 133.8, 137.0, 140.1, 153.7, 156.1, 161.8, 178.4 ppm. HRMS $(M)^+$ calcd. for $C_{20}H_{18}O_5$ 338.1154; found 338.1151.

(*E*)-2-(2-(Benzo[*d*][1,3]dioxol-5-yl)vinyl)-4*H*-chromen-4-one (**13**)

*Compound **13** was synthesized from the procedure described for compound **6***

Yield 56.4 %. Mp 211.4 °C (lit. (Momoi *et al.*, 2005) 209–210 °C). ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.08 s, 2H), 6.37 (s, 1H), 6.98 (d, J = 8.0 Hz, 1H), 7.07 (d, J = 16.1 Hz, 2H), 7.20 (d, J = 8.0 Hz, 1H), 7.39 (d, J = 1.28 Hz, 1H), 7.45 (dd, J = 7.0, 7.8 Hz, 1H), 7.61(d, J = 16.1 Hz, 1H), 7.67(d, J = 8.5 Hz, 1H), 7.80 (dd, J = 8.5, 7.0 Hz, 1H), 7.99 (d, J = 7.8 Hz, 1H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$) δ 101.8, 106.3, 108.8, 110.3, 118.0, 118.5, 124.1, 124.3, 125.1, 125.9, 129.7, 133.8, 136.8, 148.7, 149.5, 156.2, 162.2, 178.6 ppm. HRMS $(M+1)^+$ calcd. for $C_{18}H_{12}O_4$ 293.0769; found 293.0809.

(*E*)-2-(2-(Pyridin-4-yl)vinyl)-4*H*-δchromen-4-one (**14**)

*Compound **14** was synthesized from the procedure described for compound **6***

Yield 35.7 %. Mp 179.7 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.38 (s, 1H), 6.95 (d, J = 16.0 Hz, 1H), 7.37–7.43 (m, 3H), 7.51 (d, J = 16.0 Hz, 1H), 7.53 (d, J = 8.6 Hz, 1H), 7.69 (dd, J = 8.6, 7.0 Hz, 1H), 8.18 (d, J = 7.9 Hz, 1H), 8.66 (d, J = 6.0 Hz, 2H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$) δ 112.2, 117.9, 118.1, 121.6, 124.2, 124.9, 125.4, 125.9, 133.8, 134.1, 142.3, 150.7, 156.1, 160.5, 178.5 ppm. HRMS $(M+1)^+$ calcd. for $C_{16}H_{11}NO_2$ 250.0823; found 250.0867.

(*E*)-2-(2-(Pyridin-3-yl)vinyl)-4*H*-chromen-4-one(**15**)

*Compound **15** was synthesized from the procedure described for compound **6***

Yield 32.2 %. Mp 175.9 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.35 (s, 1H), 6.85 (d, J = 16.1 Hz, 1H), 7.33–7.37 (m, 1H), 7.39 (dd, J = 6.9, 7.9 Hz, 1H), 7.54 (d, J = 8.6 Hz, 1H), 7.59 (d, J = 16.1 Hz, 1H), 7.69 (dd, J = 8.6, 6.9 Hz, 1H), 7.91 (d, J = 8.0 Hz, 1H), 8.18 (d, J = 7.9 Hz, 1H), 8.59 (d, J = 4.8 Hz, 1H), 8.79 (d, J = 2.0 Hz, 1H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$) δ 111.4, 117.9, 122.4, 123.9, 124.1, 125.2, 125.7, 130.8, 133.1, 133.6, 134.1, 149.6, 150.5, 156.0, 160.9, 178.5 ppm. HRMS $(M+1)^+$ calcd. for $C_{16}H_{11}NO_2$ 250.0823; found 250.0866.

(*E*)-2-(4-(Dimethylamino)styryl)-4*H*-chromen-4-one (**16**)

*Compound **16** was synthesized from the procedure described for compound **6***

fYield 48.3 %. Mp 120.2 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.02 (s, 6H), 6.25 (s, 1H), 6.56 (d, J = 15.9 Hz, 1H), 6.70 (d, J = 8.9 Hz, 2H), 7.36 (dd, J = 8.0, 6.8 Hz, 1H), 7.48 (d, J = 8.0 Hz, 1H), 7.50 (d, J = 8.9 Hz, 1H), 7.55 (d, J = 15.9 Hz, 1H), 7.65 (dd, J = 8.0, 6.8 Hz, 1H), 8.18 (d, J = 8.0 Hz, 1H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$) δ 40.3, 109.0, 112.1, 114.9, 117.8, 123.1, 124.3, 124.8, 125.7, 129.5, 133.5, 137.7, 151.6, 156.2, 163.2, 178.5 ppm.

HRMS $(M)^+$ calcd. for $C_{19}H_{17}NO_2$ 291.1259; found 291.1260.

(*E*)-2-(4-Bromostyryl)-4*H*-chromen-4-one (**17**)

*Compound **17** was synthesized from the procedure described for compound **6***

Yield 65.1 %. Mp 225.5 °C. ^{1}H NMR(300 MHz,$CDCl_3$) δ 6.31 (s, 1H), 6.75 (d, $J = 16.0$ Hz, 1H), 7.35–7.44 (m, 3H), 7.46 (d, $J = 16.0$ Hz, 1H), 7.49–7.54 (m, 3H), 7.67 (dd, $J = 7.0, 8.0$ Hz, 1H), 8.17(d, $J = 8.0$ Hz, 1H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$) δ 111.1, 118.0, 121.1, 124.1, 124.2, 125.2, 125.8, 129.2, 132.3, 133.9, 134.0, 135.6, 156.1, 161.4, 178.5 ppm. HRMS $(M)^+$ calcd. for $C_{17}H_{11}BrO_2$ 325.9942; found 325.9941.

(*E*)-2-(3,4-Difluorostyryl)-4*H*-chromen-4-one (**18**)

*Compound **18** was synthesized from the procedure described for compound **6***

Yield 30 %. Mp 191.3 °C. ^{1}H NMR (300 MHz,$CDCl_3$) δ 6.31 (s, 1H), 6.85 (d, $J = 16.0$ Hz, 1H), 7.18 (m, 1H), 7.30 (br, 1H), 7.37 (dd, $J = 8.6$, 7.9 Hz, 1H), 7.40 (m, 1H), 7.48 (d, $J = 16.0$ Hz, 1H), 7.49 (d, $J = 7.0$ Hz, 1H), 7.67 (dd, $J = 8.6$, 7.0 Hz, 1H), 8.16 (d, $J = 7.9$ Hz, 1H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$) δ 111.3, 116.0, 116.2, 118.0, 118.2, 121.5, 124.4, 125.3, 125.9, 132.4, 134.0, 134.6, 156.1, 161.1, 178.5 ppm. HRMS $(M+1)^+$ calcd. for $C_{17}H_{10}F_2O_2$ 285.0682; found 285.0734.

(*E*)-2-(4-Chlorostyryl)-4*H*-chromen-4-one (**19**)

*Compound **19** was synthesized from the procedure described for compound **6***

Yield 67.7 %. Mp 232.4 °C (lit. (Silva *et al.*, 1998) 181–183 °C). ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.32 (s, 1H), 6.74 (d, $J = 16.0$ Hz, 1H), 7.36–7.41 (m, 3H), 7.48–7.52 (m, 3H), 7.54 (d, $J = 16.0$ Hz, 1H), 7.66 (dd, $J = 8.3, 8.0$ Hz, 1H), 8.18 (d, $J = 8.0$ Hz, 1H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$) δ 111.1, 118.0, 121.1, 124.3, 125.2, 125.9, 129.0, 129.4, 133.7, 133.9, 135.6, 135.9, 156.1, 161.5, 178.5 ppm. HRMS $(M)^+$ calcd. for $C_{17}H_{11}ClO_2$ 282.0448; found 282.0441.

(*E*)-2-(4-(Trifluoromethyl)styryl)-4*H*-chromen-4-one (**20**)

*Compound **20** was synthesized from the procedure described for compound **6***

Yield 53.5 %. Mp 175 °C. ^{1}H NMR(300 MHz,$CDCl_3$) δ 6.36 (s, 1H), 6.85 (d, $J = 16.0$ Hz, 1H), 7.39 (dd, $J = 7.9$,7.0 Hz, 1H), 7.52 (d, $J = 8.2$ Hz, 1H), 7.61 (d, $J = 16.0$ Hz, 1H), 7.67 (s, 4H), 7.68 (dd, $J = 8.2$, 7.0 Hz, 1H), 8.18 (d, $J = 7.9$ Hz, 1H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$) δ 111.7, 118.1, 122.9, 124.3, 125.3, 125.9, 126.1, 127.9, 134.1, 135.2, 138.5, 156.2, 161.1, 178.5 ppm. HRMS $(M+1)^+$ calcd. for $C_{18}H_{11}F_3O_2$ 317.0745; found 317.0784.

(*E*)-2-(4-(Trifluoromethoxy)styryl)-4H-chromen-4-one (**21**)

*Compound **21** was synthesized from the procedure described for compound **6***

Yield 56.1 %. Mp 170.2 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.34 (s, 1H), 6.75 (d, $J = 16.1$ Hz, 1H), 7.25 (d, $J = 7.4$ Hz, 2H), 7.37 (m, 1H), 7.52 (d, $J = 7.4$ Hz, 2H), 7.55 (d, $J = 16.1$ Hz, 1H), 7.61 (m, 2H), 8.18 (d, $J = 7.9$ Hz, 1H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$) δ 110.9, 115.2, 117.7, 118.6, 119.3, 122.0, 124.1, 125.0, 128.9, 133.6, 133.7, 135.0, 149.9, 155.9, 161.1, 178.3 ppm. HRMS $(M)^+$ calcd. for $C_{18}H_{11}F_3O_3$ 332.0660; found 332.0667.

(*E*)-2-(4-Nitrostyryl)-4*H*-chromen-4-one (**22**)

*Compound **22** was synthesized from the procedure described for compound **6***

Yield 61.2 %. Mp 288.7 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.5 5 s, 1H), 7.46 (d, $J = 16.0$ Hz, 1H), 7.48 (dd, $J = 8.0$, 6.7 Hz, 1H), 7.71 (d, $J = 8.2$ Hz, 1H), 7.81 (d, $J = 16.0$ Hz, 1H), 7.83 (dd, $J = 8.2$, 6.7 Hz, 1H), 7.98 (d, $J = 8.9$ Hz, 2H), 8.02 (d, $J = 8.0$ Hz, 1H), 8.29 (d, $J = 8.7$ Hz, 2H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$) δ 29.9, 112.4, 118.1, 124.4, 124.5, 124.8, 125.5, 126.1, 128.4, 134.2, 134.3, 141.4, 148.3, 156.2, 160.5, 178.5 ppm. HRMS $(M+1)^+$ calcd. for $C_{17}H_{11}NO_4$ 294.0722; found 294.0766.

(*E*)-5-Methoxy-2-(3,4,5-trimethoxystyryl)-4*H*-chromen-4-one(**23**)

*Compound **23** was synthesized from the procedure described for compound **6***

Yield 34.7 %. Mp 201.3 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.90d, $J = 10.72$ Hz, 9H), 3.99 (s, 3H), 6.22 (s, 1H), 6.60 (d, $J = 15.9$ Hz, 1H), 6.77 (s, 2H), 6.78 (d, $J = 8.5$ Hz, 1H), 7.07 (d, $J = 8.2$ Hz, 1H), 7.44 (d, $J = 15.9$ Hz, 1H), 7.54 (dd, $J = 8.5$, 8.2 Hz, 1H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$) δ 56.4, 56.6, 61.1, 104.9, 106.5, 110.1, 112.2, 114.9, 119.4, 130.8, 133.8, 136.4, 139.9, 153.7, 158.2, 159.7, 160.0, 178.4 ppm. HRMS $(M+1)^+$ calcd. for $C_{21}H_{20}O_6$ 369.1293; found 369.1298.

(*E*)-6-Methoxy-2-(3,4,5-trimethoxystyryl)-4*H*-chromen-4-one(**24**)

*Compound **24** was synthesized from the procedure described for compound **6***

Yield 36.1 %. Mp 194–195 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.92 m, 12H), 6.31 (s, 1H), 6.68 (d, J = 15.9 Hz, 1H), 6.80 (s, 2H), 7.28 (d, J = 3.1 Hz, 1H), 7.46 (d, J = 9.1 Hz, 1H), 7.51 (d, J = 15.9 Hz, 1H), 7.56 (d, J = 3.1 Hz, 1H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$) δ 56.0, 56.4, 61.1, 105.0, 105.1, 109.8, 119.3, 119.7, 123.7, 124.8, 130.7, 136.8, 140.0, 150.9, 153.7, 157.0, 161.6, 178.3 ppm. HRMS $(M+1)^+$ calcd. for $C_{21}H_{20}O_6$ 369.1293; found 369.1308.

(*E*)-7-Methoxy-2-(3,4,5-trimethoxystyryl)-4*H*-chromen-4-one(**25**)

*Compound **25** was synthesized from the procedure described for compound **6***

Yield 75 %. Mp 203.9 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.91(m, 12H), 6.24 (s, 1H), 6.64 (d, J = 15.9 Hz, 1H), 6.78 (s, 2H), 6.93 (d, J = 8.7 Hz, 1H), 6.95 (d, J = 2.3 Hz, 1H), 7.46 (d, J = 15.9 Hz, 1H), 8.07 (d, J = 8.7 Hz, 1H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$) δ 29.87, 55.9, 56.4, 61.1, 100.5, 105.0, 110.6, 114.1, 118.2, 119.7, 127.2, 130.8, 136.4, 140.0, 153.7, 157.8, 161.4, 164.3, 177.9 ppm. HRMS $(M+1)^+$ calcd. for $C_{21}H_{20}O_6$ 369.1293; found 369.1306.

(*E*)-5,7-Dimethoxy-2-(3,4,5-trimethoxystyryl)-4*H*-chromen-4-one(**26**)

*Compound **26** was synthesized from the procedure described for compound **6***

Yield 66.3 %. Mp 204.2 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.91 (m, 15H), 6.17 (s, 1H), 6.35 (d, J = 2.3 Hz, 1H), 6.53 (d, J = 2.3 Hz, 1H), 6.59 (d, J = 15.9 Hz, 1H), 6.77 (s, 1H), 7.41 (d, J = 15.9 Hz, 1H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$) δ 55.8, 56.3, 56.4, 61.1, 92.9, 96.0, 104.8, 109.5, 112.1, 119.3, 130.9, 135.8, 139.7, 153.6, 159.2, 159.7, 161.0, 164.2, 177.7 ppm. HRMS $(M+1)^+$ calcd. for $C_{22}H_{22}O_7$ 399.1399; found 399.1418.

(*E*)-7,8-Dimethoxy-2-(3,4,5-trimethoxystyryl)-4*H*-chromen-4-one (**27**)

*Compound **27** was synthesized from the procedure described for compound **6***

Yield 48.8 %. Mp 185.8 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 3.88–4.04 m, 15H), 6.25 (s, 1H), 6.68 (d, J = 15.9 Hz, 1H), 6.78 (s, 2H), 7.00 (d, J = 9.0 Hz, 1H), 7.51 (d, J = 15.9 Hz, 1H), 7.91 (d, J = 9.0 Hz, 1H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$) δ 56.4, 56.6, 61.1, 61.8, 105.1, 109.8, 110.0, 119.1, 119.9, 121.2, 130.8, 136.9, 140.1, 145.0, 153.7, 156.8, 161.6, 178.1 ppm. HRMS $(M+1)^+$ calcd. for $C_{22}H_{22}O_7$ 399.1399; found 399.1409.

(*E*)-2-(3,4,5-Trihydroxystyryl)-4*H*-chromen-4-one(**28**)

A solution of **12** (0.338 g, 1.0 mmol) in CH_2Cl_2 (10 ml) was cooled to −40 °C under argon atmosphere, and boron tribromide (1.98 g, 8.0 mmol) was added. The mixture was allowed to warm up to room temperature over a period of 16 h and was then cooled to −40 °C. Methanol (10 ml) was added to the reaction mixture, the solution was evaporated *in vacuo,* and purified with silica gel chromatography (ethyl acetate/hexane = 1:4) to afford **28** (0.159 g, 54 %). Mp 170–180 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.42 (s, 1H), 6.65 (s, 2H), 6.77 (d, J = 16.0 Hz, 1H), 7.44 (dd, J = 6.0, 7.5 Hz, 1H), 7.45 (d, J = 16.0 Hz, 1H), 7.70 (d, J = 8.2 Hz, 1H), 7.78 (dd, J = 8.2, 6.0 Hz, 1H), 7.99 (d, J = 7.5 Hz, 1H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$) δ 107.5, 109.1, 116.9, 118.3, 123.7, 124.9, 125.3, 125.7, 134.2, 136.2, 137.8, 146.4, 155.6, 162.5, 177.1 ppm. HRMS $(M)^+$ calcd. for $C_{17}H_{12}O_5$ 296.0685; found 296.0684.

(*E*)-6-Hydroxy-2-(3,4,5-trihydroxystyryl)-4*H*-chromen-4-one (**29**)

*Compound **29** was synthesized from the procedure described for compound **28***

Yield 47.2 %. Mp 173.2 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.33 (s, 1H), 6.62 (s, 2H), 6.73 (d, J = 16.0 Hz, 1H), 7.19 (d, J = 9.0 Hz, 1H), 7.26 (d, J = 2.9 Hz, 1H), 7.39 (d, J = 16.0 Hz, 1H), 7.56 (d, J = 9.0 Hz, 1H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$) δ 15.1, 102.5, 104.7, 105.6, 107.5, 108.8, 114.9, 115.1, 117.1, 125.9, 126.8, 136.1, 143.9, 146.4, 157.4, 162.9, 179.5 ppm. HRMS $(M)^+$ calcd. for $C_{17}H_{12}O_7$ 328.0583; found 328.0586.

(*E*)-7-Hydroxy-2-(3,4,5-trihydroxystyryl)-4*H*-chromen-4-one (**30**)

*Compound **30** was synthesized from the procedure described for compound **28***

Yield 34.7 %. Mp 130–140 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.27 (s, 1H), 6.63 (s, 2H), 6.70 (d, J = 16.0 Hz, 1H), 6.87 (d, J = 8.6 Hz, 1H), 6.93 (d, J = 1.9 Hz, 1H), 7.37 (d, J = 16.0 Hz, 1H), 7.81 (d, J = 8.6 Hz, 1H) ppm.^{13}C NMR (75 MHz, $CDCl_3$) δ 100.8, 104.5, 105.5, 107.9, 116.1, 117.0, 119.9, 123.8, 124.8, 133.1, 134.7, 143.9, 146.3, 157.3, 162.8,

176.2 ppm. HRMS $(M)^+$ calcd. for $C_{17}H_{12}O_6$ 312.0634; found 312.0633.

(*E*)-5,7-Hydroxy-2-(3,4,5-trihydroxystyryl)-4*H*-chromen-4-one(**31**)

*Compound **31** was synthesized from the procedure described for compound **28***

Yield 29.1 %. Mp 140–150 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.34d, $J = 16.0$ Hz, 1H), 6.45 (s, $J = 1.92$ Hz, 1H), 6.64 (d, 2H), 6.66 (d, $J = 16.0$ Hz, 1H), 6.72 (m, 1H), 7.42 (m, 1H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$) δ 56.04, 92.59, 97.83, 104.89, 107.10, 107.39, 116.13, 125.37, 136.32, 138.38, 146.23, 157.16, 161.24, 163.34, 165.17, 181.82 ppm. HRMS $(M)^+$ calcd. for $C_{17}H_{12}O_7$ 328.0583; found 328.0587.

(*E*)-7,8-Dihydroxy-2-(3,4,5-trihydroxystyryl)-4*H*-chromen-4-one (**32**)

*Compound **32** was synthesized from the procedure described for compound **28***

Yield 42.6 %. Mp 149–157 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 6.24 (s, 1H), 6.61 (s, 2H), 6.71 (d, $J = 15.9$ Hz, 1H), 6.88 (d, $J = 8.6$ Hz, 1H), 7.32 (d, $J = 8.6$ Hz, 1H), 7.60 (d, $J = 15.9$ Hz, 1H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$) δ 107.1, 108.3, 113.7, 115.1, 117.1, 125.8, 132.8, 135.7, 137.4, 146.2, 150.4, 161.6, 176.8 ppm. HRMS $(M)^+$ calcd. for $C_{17}H_{12}O_7$ 328.0583; found 328.0583.

2-(3,4,5-Trimethoxyphenethyl)-4*H*-chromen-4-one (**33**)

To a solution of **12** (0.338, 1.0 mmol) in MeOH (10 ml), Pd/C (5 mg, 10 % on charcoal) was added to the mixture. The reaction mixture was degassed and filled with H_2 balloon. The mixture was stirred at room temperature for 16 h. The solid was filtered and the filtrate was evaporated in vacuo, followed by silica gel chromatography purification to give **33** (0.208 g, 56.4 %). Mp 133.4 °C. ^{1}H NMR (300 MHz, $CDCl_3$) δ 2.93 (t, $J = 7.4$ Hz, 2H), 2.99 (t, $J = 7.4$ Hz, 2H), 3.81 (d, $J = 10.8$ Hz, 9H), 6.18 (s, 1H), 6.40 (s, 2H), 7.37 (d, $J = 7.0$ Hz, 1H), 7.42 (dd, $J = 8.0$, 9.0 Hz, 1H), 7.65 (dd, $J = 9.0$, 7.0 Hz, 1H), 8.17 (d, $J = 8.0$ Hz, 1H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$) δ 29.9, 33.7, 36.4, 56.3, 61.1, 105.5, 110.5, 117.9, 123.9, 125.2, 125.9, 133.7, 135.6, 136.9, 153.5, 156.6, 168.5, 178.4 ppm. HRMS $(M)^+$ calcd. for $C_{20}H_{20}O_5$ 341.1389; found 341.1387.

Cell culture

Cancer cells were purchased from Bioresource Collection and Research Center in Hsinchu, Taiwan. Each cell line was maintained in the standard medium and grown as a monolayer in Dulbecco's modified eagle medium (DMEM) containing 10 % fetal bovine serum, 2 mM glutamine, 100 units/ml penicillin, and 100 g/ml streptomycin. Cultures were maintained at 37 °C with 5 % CO_2 in a humidified atmosphere.

MTT assay for cell viability

Cell proliferation and viability were measured by MTT assay. Compound stock solution (10 mM in DMSO) was prepared and stored at −20 °C, and was diluted with DMSO to 0.1–1 mM range at room temperature before experiment. The final percentage of DMSO in the reaction mixture was <1 % (v/v). Cancer cells (1×10^4 cells/well) were plated in the 96-well plates and incubated in medium for 24 h, followed by the addition of serial dilutions of individual compounds. Treated cells were further incubated for 48 h. Cell survival is expressed as percentage of control cell growth. The 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT, 2 mg/ml) dye reduction assay in 96-well microplates was used. The assay is dependent on the reduction of MTT by mitochondrial dehydrogenases of viable cell to a blue formazan product, which come be measured spectrophotometrically. Tumor cells were incubated in each well with serial dilutions of the tested compounds. After 2 days of incubation (37 °C, 5 % CO_2 in a humid atmosphere) 100 µl of MTT (2 mg/ml in PBS) was added to each well and the plate was incubated for a further 2 h (37 °C). The resulting formazan was dissolved in 100 µl DMSO and read at 570 nm. The percentage of growth inhibition was calculated by the following equation: percentage growth inhibition $= (1 \times A_t/A_c) \times 100$, where A_t and A_c represent the absorbance in treated and control cultures, respectively. The drug concentration causing a 50 % cell growth inhibition (GI_{50}) was determined by interpolation from dose–response curves. All determinations were carried out in four to six separated experiments.

Statistical analysis

Data are presented as the Mean ± SEM (standard error of the mean) from four to six separated experiments. Statistical analyses were performed by Bonferroni *t* test method after ANOVA for multigroup comparison and Student's *t* test method for two-group comparison. $P = 0.05$ was considered significant. Analysis of linear regression (at least five data within 20–80 % inhibition) was used to calculate GI_{50} value.

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