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Synthesis and anticancer activity of lavendustin A derivatives containing arylethenylchromone substituents

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Abstract

2-Styrylchromones, which are relatively scarce in nature, have been reported to possess potent cytotoxicities on KB cell line. Lavendustin A, a metabolite of *Streptomyces griseolavendus*, has been shown to inhibit a growth of A431 cell line. Accordingly, a series of compounds **3a–g** having structural features of styrylchromones and lavendustin A were synthesized and evaluated for cytotoxicity using SRB assay on four tumor cell lines. Compounds **3a–g** were synthesized by the condensation of 2-methylchromone derivative **7** with several aromatic aldehydes. Among synthesized, compound **3e** showed the significant cytotoxic activity on HCT-15 cell line with IC_{50} values of 7.17 μg/ml indicating that lavendustin A derivatives containing 2-arylethenylchromone ring have a potential in anti-tumor application.

Keywords: Styrylchromone; Arylethenylchromone; Lavendustin A; Cytotoxicity; HCT-15

1. Introduction

2-Arylchromones, also known as flavones, are among the most ubiquitous classes of natural products occurring in the plant kingdom. On the other hand, 2-styrylchromones are relatively scarce in nature and only a few including hormothamnione (**1**) have been identified from the blue-green algae species [1,2]. Consequently, syntheses of **1** have reported due to its unique structure and cytotoxic effects [3]. Regardless of isolation of **1**, the carbon skeleton of 2-styrylchromones has already been known; however, the synthesis of its derivatives for structure–activity relationship study has not been accomplished [4].

Lavendustin A (**2**), a metabolite of *Streptomyces griseolavendus*, has been shown to inhibit protein-tyrosine kinase in A431 cell line [5]. Some stilbene and benzylaniline derivatives, which can be considered as the fragments of lavendustin A have been synthesized to reduce polarity or simplify the structure of **2**, and evaluated for cytotoxicity in cancer cell cultures [6] (Fig. 1).

Recently, hybridization of natural products has been proposed as a promising strategy in the development of new leads for medicinal application. The biological activities of several new hybrids have been found to exceed those of the parent compounds [7].

In this study, we synthesized a new structure of compounds by combining structures of styrylchromone and lavendustin A to form hybrid **3** and evaluated them for cytotoxicities on tumor cell lines.

2. Results and discussion

2.1. Synthesis

To investigate the influence of styrylchromone rings on cytotoxicity, we designed a series of compounds **3**, where *p*-methoxybenzyl and phenethyl substituents on nitrogen are fixed, while aromatic substituents on styrylchromone ring were varied. Compound **4** was selected as a starting material since amine substituents and arylethenyl group can readily be introduced on C-8 and C-2 positions of chromone ring, respectively, through reductive-amination and aldol-type condensation reactions (Scheme 1).

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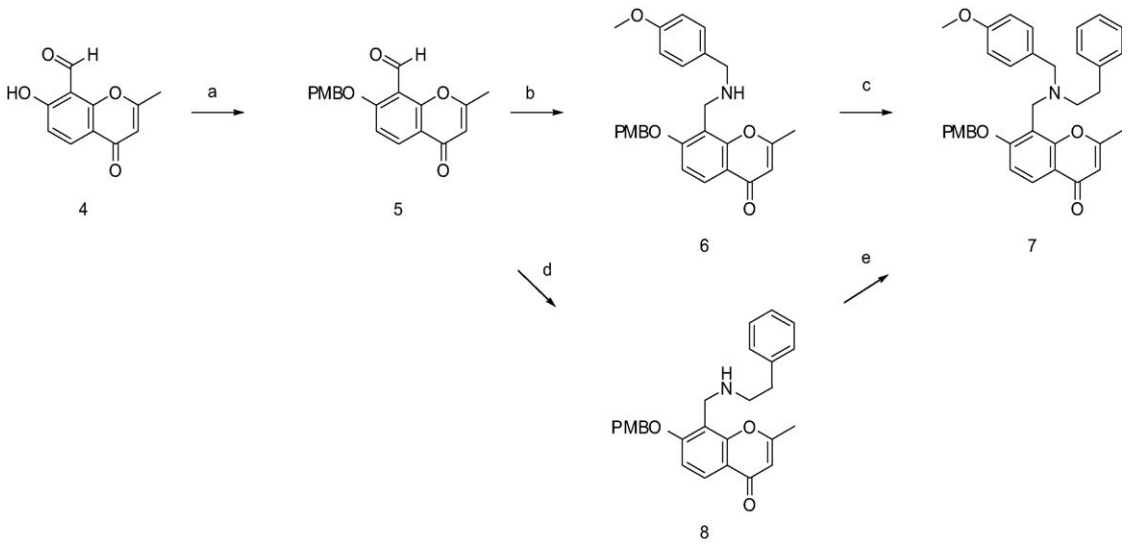


Scheme 1. Reagents and conditions. a) PMB-Cl, NaI, K_2CO_3, DMF; b) *p*-methoxybenzylamine, $NaBH_4$, MeOH; c) phenethyl bromide, NaI, NaH, DMF; d) phenethylamine, $NaBH_4$, MeOH; e) PMB-chloride, NaI, NaH, DMF.

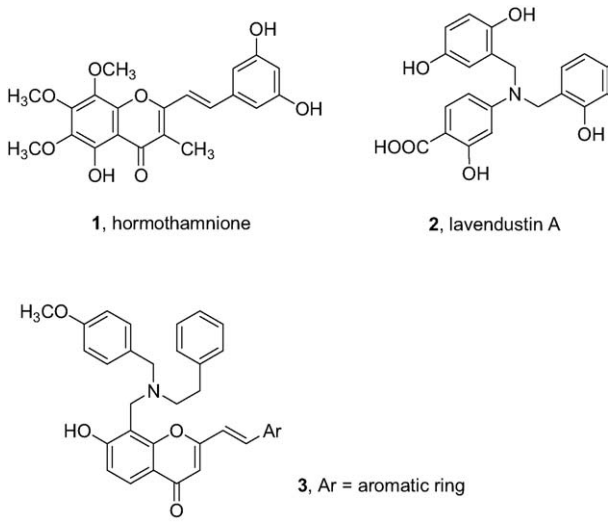


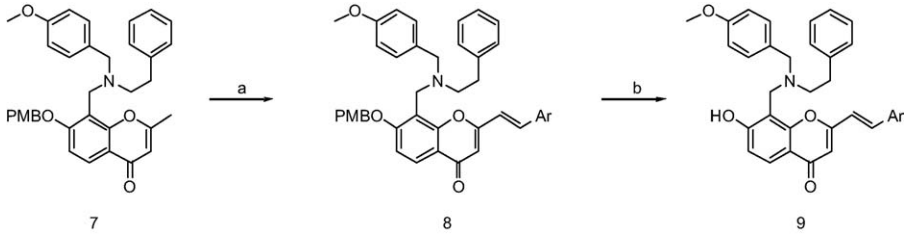
Fig. 1.

C-7 hydroxyl group of **4** [4,8] was protected with the reaction of *p*-methoxybenzyl chloride (PMB-Cl) to afford **5** in 90% yield. To introduce amine group in chromone ring using reductive-amination reaction, compound **5** was reacted with *p*-methoxybenzylamine in methanol followed by treatment of $NaBH_4$ to provide **6** in 76% yield [9]. Aminoalkylation reaction of **6** with phenethyl bromide using NaH as a base in DMF afforded **7** only in 12% yield. Although this reaction was carried out using weak base, DBU in DMSO, the yield was not improved resulting in 11% yield of **7**. As the low yield was thought to be due to the decomposition of phenethyl bromide to styrene through the elimination of HBr by the base, we decided to introduce phenethyl group firstly through reductive-amination with phenethylamine and then *p*-methoxybenzyl group. Thus, compound **5** was again treated with phenethylamine and $NaBH_4$ in MeOH to afford **8** in 93% yield. As expected, the aminoalkylation of **8** with PMB-Cl and NaH in the presence of catalytic amount of NaI provided **7** in improved 57% yield.

Next, for the synthesis of target compounds **3**, arylethenyl group was introduced through aldol-type condensation of **7** with aromatic aldehydes as depicted in Scheme 2. In order to examine the effects of aromatic substituents on cytotoxicity, various aromatic and heteroaromatic aldehydes were used. Compound **7** was treated with aldehydes using KOH in EtOH to afford **9a–g** in 37–92% yields [10]. Finally, PMB-protected compounds **9a–g** were subjected to hydrolysis condition by treatment of trifluoroacetic acid (TFA) in CH_2Cl_2 to provide **3a–g** in 36–86% yield.

2.2. Biological activity

The synthesized compounds **3a–g** were evaluated for their cytotoxic activities against four tumor cell lines, including hu-



Scheme 2. Reagents and conditions. a) Ar-CHO, KOH, EtOH; b) TFA, CH_2Cl_2.

man lung carcinoma (A-549), human colon cancer cells (HCT-15), human epidermoid carcinoma (KB), and malignant melanoma (SK-MEL-2) using SRB assay [11]. The results are shown as IC_{50} values in Table 1, which are mean values from three independent experiments.

Most of compounds synthesized exhibited weak or no cytotoxicity under the concentration of 100 μg/ml on KB and SK-MEL-2 cell lines. However, they showed significant cytotoxicity on A-549 and HCT-15 cell lines in the range of 7.17–83.7 μg/ml of IC_{50} values. Especially, they showed marked structure–activity relationships on HCT-15 cell. Compounds **3b** and **3c**, which have electron releasing methoxy groups on arylethenylchromone ring showed decreased cytotoxicity when compared to that of **3a**. On the other hand, compounds **3d**, **3e**, and **3g**, which have electron withdrawing bromide or heteroaromatic ring exhibited potent cytotoxicity with IC_{50} values in the range of 7.17–11.98 μg/ml on HCT-15 cell. Regarding the cytotoxicity on A-549 cell line, noticeable structure–activity relationships were not found. Among the tested compounds, compounds **3a** and **3e** showed the most potent cytotoxicity.

3. Experimental section

3.1. Chemistry

^1H and ^{13}C NMR spectra were recorded on a Gemini Varian-300 (300 and 75 MHz, respectively). Analytical thin layer chromatographies (TLC) were carried out by precoated silica gel (E. Merck Kiesegel $60F_{254}$ layer thickness 0.25 mm). Flash column chromatographies were performed with Merck Kiesegel 60 Art 9385 (230–400 mesh). All solvents used were purified according to standard procedures.

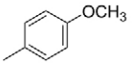
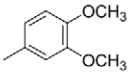
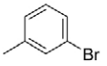
3.1.1. 7-(4-Methoxybenzyloxy)-2-methyl-8-formylchromone (5)

To a stirred solution of 7-hydroxy-8-formyl-2-methylchromone (**4**, 100 mg, 0.49 mmol) in DMF (5 ml) was added successively K_2CO_3 (135 mg, 1.21 mmol) and *p*-methoxybenzyl chloride (PMB-Cl, 0.13 ml, 0.94 mmol), and catalytic amount of NaI. The reaction mixture was warmed up to 55 °C and further stirred for 1 h. The mixture was diluted with EtOAc and washed with brine. The organic layer was dried over $MgSO_4$, concentrated, and purified by flash column chromatography (CH_2Cl_2/CH_3OH = 98:2) to afford **5** (145 mg, 91%) as a yellow solid. ^1H NMR (300 MHz, $CDCl_3$) δ 10.60 (1H, s), 8.32 (1H, d, J = 9.0 Hz), 7.36 (2H, d, J = 8.5 Hz), 7.12 (1H, d, J = 9.0 Hz), 6.93 (2H, d, J = 8.5 Hz), 6.14 (1H, s), 5.22 (2H, s), 3.81 (3H, s), 2.40 (3H, s); ^{13}C NMR (75 MHz, $CDCl_3$) δ 187.18, 176.77, 166.63, 164.91, 159.10, 156.48, 133.30, 133.02, 129.15, 128.61, 126.99, 117.58, 114.24, 113.87, 113.21, 110.78, 110.61, 71.34, 64.91, 55.32, 29.69, 20.50.

3.1.2. (7-(4-Methoxybenzyloxy)-8-[(4-metoxybenzylamino) methyl]-2-methylchromone(6)

To a stirred solution of **5** (480 mg, 1.47 mmol) and 4-methoxybenzylamine (0.39 ml, 2.94 mmol) in MeOH (10 ml) was added portion-wise $NaBH_4$ (111 mg, 2.93 mmol) at 0 °C. After 5 min, the mixture was warmed up to room temperature and further stirred for 1 h. The reaction mixture was poured into water and extracted with EtOAc. The organic layer was dried over $MgSO_4$, concentrated, and purified by flash column chromatography (CH_2Cl_2/CH_3OH = 98:2), and further purified by

Table 1
The chemical yields of **9** and **3** and cytotoxicity of **3** on cancer cell lines in vitro
[a] Yield of **9** from **7**. [b] Yield of **3** from **9**. [c] IC_{50} value of compound against each cancer cell line, which was defined as a concentration (μg/ml) that caused 50% inhibition of cell growth in vitro.

entry	Ar	Yields of 9[a] (%)	Yields of 3[b] (%)	Cytotoxicity of 3 (IC_{50}, μg/mL)[c]			
				A549	HCT-15	KB	SK-MEL-2
a		51	62	17.9 ± 2.9	49.37 ± 1.1	34.98 ± 6.6	>100
b		76	36	63.0 ± 18.2	81.41 ± 5.4	>100	>100
c		63	86	83.7 ± 13.6	74.53 ± 5.3	>100	>100
d		42	48	63.8 ± 1.0	9.22 ± 1.8	>100	>100
e		38	65	23.1 ± 7.4	7.17 ± 2.3	38.56 ± 3.0	62.53 ± 15.3
f		92	37	57.7 ± 10.7	29.52 ± 8.6	82.32 ± 4.1	>100
g		76	80	32.2 ± 8.3	12.98 ± 4.2	61.13 ± 19.1	>100
	cisplatin			0.56 ± 0.12	0.63 ± 0.2	0.90 ± 0.29	1.65 ± 0.12

recrystallization with EtOAc and hexane to afford **6** (500 mg, 76%) as a yellow solid. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.06 (1H, d, J = 9.2 Hz), 7.30 (2H, d, J = 8.4 Hz), 7.15 (2H, d, J = 8.4 Hz), 7.03 (1H, d, J = 9.2 Hz), 6.89 (2H, d, J = 8.8 Hz), 6.78 (2H, d, J = 8.8 Hz), 6.05 (1H, s), 5.09 (2H, s), 4.02 (2H, s), 3.79 (3H, s) 3.75 (3H, s), 3.69 (2H, s), 2.27 (3H, s); ^{13}C NMR (75 MHz, $CDCl_3$) δ 118.00, 165.77, 160.89, 159.67, 158.57, 155.60, 132.17, 129.24, 127.97, 125.90, 117.60, 116.19, 114.11, 113.67, 109.92, 70.62, 55.27, 55.22, 52.52, 40.72, 20.48.

*3.1.3. 7-(4-Methoxybenzyloxy)-8-[(phenethylamino)methyl]-2-methylchromone (**8**)*

Compound **8** was obtained from **5** with phenethylamine by using the similar procedure for **6**. Yield: 93%; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.07 (1H, d, J = 9.0 Hz), 7.30–7.08 (7H, m), 7.03 (1H, d, J = 9.0 Hz), 6.92–6.88 (2H, m), 6.07 (1H, s), 5.06 (2H, s), 4.07 (2H, s), 3.82 (3H, s), 2.87–2.74 (4H, m), 2.28 (3H, s); ^{13}C NMR (75 MHz, $CDCl_3$) 178.26, 166.01, 161.05, 159.90, 155.90, 140.05, 129.37, 128.86, 128.68, 128.22, 126.43, 126.23, 117.79, 116.07, 114.35, 110.17, 70.82, 55.56, 50.21, 41.42, 36.31, 20.77.

3.1.4. 7-(4-Methoxybenzyloxy)-8-{[phenethyl-(4-methoxybenzyl)amino]methyl}-2-methylchromone (7)

To a solution of **8** (103 mg, 0.22 mmol) in DMF (1.3 ml) was added NaH (60% in oil, 9.6 mg, 0.24 mmol) at 0 °C and stirred for 15 min. The mixture was treated with a solution of PMB-Cl (0.03 ml, 0.24 mmol) and catalytic amount of NaI in DMF (1.0 ml) and warmed up to room temperature. After further stirring for 2 h, the reaction mixture was poured into water and extracted with EtOAc. The organic layer was dried over $MgSO_4$, concentrated, and purified by flash column chromatography (CH_2Cl_2/CH_3OH = 98:2) to afford **7** (75 mg, 57%). ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.06 (1H, d, J = 8.3 Hz), 7.30 (2H, d, J = 8.7 Hz), 7.17–7.08 (7H, m), 7.01(1H, d, J = 8.3 Hz), 6.92 (2H, d, J = 9.0 Hz), 6.75 (2H, d, J = 8.7 Hz), 6.06 (1H, s), 5.08 (2H, s), 3.90 (2H, s), 3.78 (3H, s), 3.75 (3H, s), 3.63 (2H, s), 2.74–2.68 (4H, m), 2.22 (3H, s); ^{13}C NMR (75 MHz, $CDCl_3$) δ 178.46, 166.09, 161.56, 159.88, 158.73, 156.72, 141.10, 132.26, 130.10, 129.54, 128.83, 128.47, 128.34, 126.23, 125.94, 117.88, 115.89, 114.32, 113.67, 110.25, 70.93, 58.62, 55.53, 55.21, 46.35, 33.70, 20.82.

*3.1.5. General procedure for **9a–g** by the condensation reaction of **9** with aromatic aldehydes*

To a solution of **9** (0.2 mmol, 1 eq.) in EtOH was added powdered KOH (10 eq.) and stirred for 5 min. The mixture was treated with aromatic aldehydes (3 eq.) and warmed up to 55 °C. After stirring for 1–2 h, the reaction mixture was cooled to 0 °C diluted with water, and neutralized with 3 N HCl. The mixture was extracted with EtOAc and the organic layer was dried over $MgSO_4$, concentrated, and purified by flash column chromatography (CH_2Cl_2/CH_3OH = 98:2) to afford **9a–g** as yellow solids

*3.1.6. (E)-7-(4-methoxybenzyloxy)-8-{[phenethyl-(4-methoxybenzyl)amino]methyl}-vinylchromone (**9a**)*

Yield: 51%; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.11 (1H, d, J = 8.9 Hz), 7.59 (1H, d, J = 16.0 Hz), 7.49–7.46 (2H, m), 7.40–7.35 (5H, m), 7.18–7.10 (5H, m) 7.04 (1H, d, J = 8.9 Hz), 6.97 (2H, m), 6.91 (2H, d, J = 8.6 Hz), 6.77 (1H, d, J = 16.0 Hz) 6.72 (2H, d, J = 8.6 Hz), 6.29 (1H, s), 5.14 (2H, s), 4.13 (2H, s), 3.82 (3H, s), 3.76 (2H, s), 3.72 (3H, s), 2.84–2.78 (4H, m); ^{13}C NMR (75 MHz, $CDCl_3$) δ 178.30, 161.66, 161.51, 159.62, 158.43, 155.87, 140.72, 136.68, 135.07, 131.55, 129.66, 129.27, 128.98, 128.60, 128.17, 127.67, 126.11, 125.71, 120.71, 118.16, 115.80, 114.07, 113.42, 110.07, 70.69, 57.91, 55.30, 55.20, 47.18, 33.10.

*3.1.7. (E)-7-(4-methoxybenzyloxy)-8-{[phenethyl-(4-methoxybenzyl)amino]methyl}-2-(4-methoxybenzyl) vinylchromone (**9b**)*

Yield: 31%; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.09 (1H, d, J = 9.0 Hz), 7.53 (1H, d, J = 15.9 Hz), 7.38 (2H, d, J = 8.7 Hz), 7.34 (2H, d, J = 8.4 Hz), 7.16–7.11 (6H, m), 7.01 (1H, d, J = 9.0 Hz), 6.95–6.87 (5H, m), 6.70 (2H, d, J = 8.7 Hz), 6.60 (1H, d, J = 15.9 Hz), 6.25 (1H, s), 5.20 (2H, s), 4.10 (2H, s), 3.82 (3H, s), 3.79 (3H, s), 3.73 (2H, s), 3.70 (3H, s), 2.79–2.77 (4H, m); ^{13}C NMR (75 MHz, $CDCl_3$) δ 178.54, 162.27, 161.82, 161.23, 159.86, 158.68, 156.12, 140.99, 136.62, 131.86, 129.91, 129.50, 128.84, 128.54, 128.41, 128.11, 126.31, 125.94, 118.55, 118.42, 116.01, 114.68, 114.31, 113.67, 110.23, 109.58, 70.94, 58.17, 55.53, 47.44, 33.35, 29.98.

*3.1.8. (E)-7-(4-methoxybenzyloxy)-8-{[phenethyl-(4-methoxybenzyl)amino]methyl}-2-(3′,4′-dimethoxybenzyl) vinylchromone (**9c**)*

Yield: 76%; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.13 (1H, d, J = 9.0 Hz), 7.59 (1H, d, J = 15.9 Hz), 7.40–6.66 (24H, m), 6.29 (1H, s), 5.21–5.17 (2H, m), 4.17–4.14 (2H, m), 3.95–3.74 (17H, m), 2.82–2.80 (4H, m); ^{13}C NMR (75 MHz, $CDCl_3$) δ 178.54, 162.17, 161.81, 159.87, 158.69, 156.17, 150.94, 149.55, 140.92, 136.91, 131.86, 129.87, 129.50, 128.84, 128.43, 126.34, 125.97, 122.03, 118.76, 118.45, 115.94, 114.32, 113.68, 111.44, 110.23, 109.68, 70.96, 58.11, 56.25, 56.04, 55.53, 55.43, 47.41, 33.19, 24.99, 24.85.

*3.1.9. (E)-7-(4-methoxybenzyloxy)-8-{[phenethyl-(4-methoxybenzyl)amino]methyl}-2-bromovinylchromone (**9d**)*

Yield: 42%; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.06 (1H, d, J = 8.7 Hz), 8.01 (1H, d, J = 15.9 Hz), 7.64 (1H, d, J = 7.5 Hz), 7.59 (1H, d, J = 8.4 Hz), 7.34 (2H, d, J = 8.1 Hz), 7.20 (1H, d, J = 7.5 Hz), 7.17–7.00 (7H, m), 6.68–6.51 (3H, m), 6.28 (1H, s), 5.12 (2H, s), 4.10 (2H, br s), 3.78 (3H, s), 3.74 (2H, s), 3.70 (3H, s), 2.80–2.72 (4H, m); ^{13}C NMR (75 MHz, $CDCl_3$) δ 178.55, 162.20, 161.11, 159.86, 158.61, 155.92, 141.08, 135.22, 133.71, 132.00, 130.99, 129.97, 129.53, 128.87, 128.53, 128.35, 128.11, 127.52, 126.33, 125.88, 125.61, 123.46, 118.19, 116.26, 114.30, 113.57, 110.97, 110.54, 70.9, 58.31, 55.72, 55.53, 55.45, 47.32, 33.42.

*3.1.10. (E)-7-(4-methoxybenzyloxy)-8-{[phenethyl-(4-methoxybenzyl)amino]methyl}-2-(pyrdin-2-yl)vinylchromone **(9e)***

Yield: 38%; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.65 (1H, d, J = 4.2 Hz), 8.09 (1H, d, J = 9.3 Hz), 7.67–6.88 (19H, m), 6.72–6.68 (2H, m), 6.36 (1H, s), 5.13 (2H, s), 4.12 (2H, s), 3.80 (3H, s), 3.75 (2H, s), 3.70 (3H, s), 2.79–2.76 (4H, m); ^{13}C NMR (75 MHz, $CDCl_3$) δ 177.86, 164.20, 159.94, 159.30, 159.09, 138.64, 136.82, 134.50, 131.01, 128.59, 128.55, 127.05, 126.51, 126.13, 124.80, 123.73, 123.55, 116.85, 115.32, 114.03, 111.77, 107.92, 57.09, 55.12, 54.75, 49.58, 33.12, 29.65, 24.52, 24.46, 24.41.

*3.1.11. (E)-7-(4-methoxybenzyloxy)-8-{[phenethyl-(4-methoxybenzyl)amino]methyl}-2-(furan-3yl)vinylchromone **(9f)***

Yield: 92%; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.12 (1H, d, J = 8.7 Hz), 7.50–7.45 (3H, m), 7.38 (2H, d, J = 8.4 Hz), 7.21–7.14 (6H, m), 7.06 (1H, d, J = 9.0 Hz), 6.99–6.92 (5H, m), 6.76 (2H, d, J = 8.7 Hz), 6.66 (2H, s), 6.51 (1H, d, J = 15.9 Hz), 6.25 (1H, s), 5.15 (2H, s), 3.83 (2H, s), 3.75 (6H, s), 2.80–2.78 (4H, m); ^{13}C NMR (75 MHz, $CDCl_3$) δ 178.54, 161.86, 159.88, 158.69, 156.11, 144.68, 144.07, 140.91, 131.81, 129.87, 129.54, 128.81, 128.44, 126.98, 126.35, 125.99, 123.51, 120.64, 118.37, 115.92, 114.32, 113.69, 110.28, 109.63, 107.49, 70.95, 60.64, 58.20, 55.53, 55.43, 47.40, 33.40, 21.29, 14.46.

*3.1.12. (E)-7-(4-methoxybenzyloxy)-8-{[phenethyl-(4-methoxybenzyl)amino]methyl}-2-(thiophen-2-yl)vinylchromone **(9g)***

Yield: 76%; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.12 (1H, d, J = 8.4 Hz), 7.72 (1H, d, J = 15.6 Hz), 7.38 (3H, d, J = 9.8 Hz), 7.19–6.99 (11H, m), 6.96 (1H, d, J = 8.4 Hz), 6.80 (1H, d, J = 8.7 Hz), 6.69 (1H, d, J = 15.6 Hz), 6.26 (1H, s), 5.16 (2H, s), 4.13 (2H, s), 3.84 (3H, s), 3.75 (6H, s), 2.81–2.79 (4H, m); ^{13}C NMR (75 MHz, $CDCl_3$) δ 178.42, 161.09, 161.46, 159.90, 158.71, 156.10, 140.96, 140.76, 131.83, 130.10, 129.90, 129.69, 129.54, 128.87, 128.44, 128.01, 126.38, 125.99, 119.91, 118.42, 115.94, 114.35, 113.71, 110.31, 110.00, 70.97, 58.18, 55.55, 55.46, 47.46, 33.42.

3.1.13. General procedure for PMB-deprotection

A solution of **9a–f** (0.2 mmol) in CH_2Cl_2 (5.0 ml) was treated with TFA (5.0 ml) and stirred at room temperature for 30 min. The reaction mixture was extracted with CH_2Cl_2 and the organic layer was dried over $MgSO_4$, concentrated, and purified by flash column chromatography (CH_2Cl_2/CH_3OH = 98:2) to afford **3a–g** as yellow solids.

*3.1.14. (E)-7-hydroxy-8-{[phenethyl-(4-methoxybenzyl)amino]methyl}-vinylchromone **(3a)***

Yield: 62%. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.05 (1H, s, J = 8.7 Hz), 7.57–7.16 (15H, m), 6.90–6.78 (4H, m), 6.28 (1H, s), 4.22 (1H, s), 3.83 (2H, s), 3.73 (3H, s), 3.04–2.93 (4H, m); ^{13}C NMR (75 MHz, $CDCl_3$) δ 177.94, 164.11. 160.48, 159.39, 154.80, 138.69, 135.95, 134.99, 131.15, 129.02, 128.68, 128.61, 127.63, 126.60, 126.21, 120.69, 116.88, 115,24, 114.08, 110.52, 107.86, 56.85, 55.15, 54.73, 49.50, 33.15.

*3.1.15. (E)-7-hydroxy-8-{[phenethyl-(4-methoxybenzyl)amino]methyl}-2-(4-methoxy-benzyl)vinylchromone **(3b)***

Yield: 30%; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.02 (1H, d, J = 8.7 Hz), 7.48 (2H, d, J = 8.7 Hz), 7.32–7.14 (12H, m), 6.95 (2H, d, J = 8.7 Hz), 6.87–6.84 (3H, m), 6.64 (1H, d, J = 15.9 Hz), 6.22 (1H, s), 4.18 (2H, s), 3.86 (3H, s), 3.79 (2H, s), 3.71 (3H, s), 2.96–2.82 (4H, m); ^{13}C NMR (75 MHz, $CDCl_3$) δ 177.94, 164.78, 163.36, 159.58, 155.58, 136.08, 131.06, 129.78, 128.87, 128.14, 127.85, 126.20, 115.14, 114.25, 110.30, 107.97, 58.16, 57.58, 55.38, 48.76, 20.49.

*3.1.16. (E)-7-hydroxy-8-{[phenethyl-(4-methoxybenzyl)amino]methyl}-2-(3′,4′-dimethoxy-benzyl)vinylchromone **(3c)***

Yield: 86%; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.04 (1H, d, J = 8.7 Hz), 7.33–7.09 (14H, m), 6.94–6.81 (5H, m), 6.65 (1H, d, J = 15.9 Hz), 6.27 (1H, s), 4.22 (2H, s), 4.00 (2H, s), 3.96 (3H, s), 3.82 (3H, s), 3.72 (3H, s), 2.99–2.96 (4H, m); ^{13}C NMR (75 MHz, $CDCl_3$) δ 212.25, 169.10, 164.30, 161.16, 159.60, 149.69, 138.97, 136.11, 131.41, 128.91, 128.84, 128.30, 127.00, 126.84, 126.39, 122.43, 188.75, 117.09, 155.39, 144.29, 111.42, 110.07, 109.43, 108.12, 89.33, 86.28, 74.15, 56.95, 56.28, 55.37, 55.02, 49.72, 33.44, 29.91, 25.00, 24.65, 21.67, 19.56.

*3.1.17. (E)-7-hydroxy-8-{[phenethyl-(4-methoxybenzyl)amino]methyl}-2-bromovinylchromone **(3d)***

Yield: 48%; ^{1}H NMR (300 MHz, $CDCl_3$) δ 7.99 (1H, d, J = 9.0 Hz), 7.90 (1H, d, J = 16.2 Hz), 7.71 (1H, dd, J = 1.5, 8.1 Hz), 7.65 (1H, dd, J = 1.2, 7.8 Hz), 7.37–7.09 (10H, m), 6.85 (2H, dd, J = 1.5, 9.0 Hz), 6.75 (1H, d, J = 16.2 Hz), 6.27 (1H, s), 4.25 (2H, s), 3.76 (2H, s), 3.73 (3H, s), 2.96–2.89 (4H, m); ^{13}C NMR (75 MHz, $CDCl_3$) δ 178.00, 159.96, 159.62, 154.91, 138.68, 134.71, 134.56, 133.60, 131.08, 130.95, 128.76, 128.69, 128.09, 127.19, 126.69, 125.49, 122.81, 114.32, 57.76, 55.29, 49.84, 32.56, 30.99.

*3.1.18. (E)-7-hydroxy-8-{[phenethyl-(4-methoxybenzyl)amino]methyl}-2-(2-pyrdin-2-yl)vinylchromone **(3e)***

Yield: 65%; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.71–8.68 (1H, d, J = 4.2 Hz), 8.04 (1H, d, J = 8.8 Hz), 7.81–7.72 (1H, m), 7.48–7.13 (11H, m), 6.91–6.86 (3H, m), 6.36 (1H, s), 4.23 (2H, s), 3.81 (2H, s), 3.74 (3H, s), 2.97–2.90 (4H, m); ^{13}C NMR (75 MHz, $CDCl_3$) δ 178.58, 161.99, 161.26, 159.87, 158.65, 156.15, 153.46, 150.35, 140.97, 137.07, 135.33, 131.87, 129.92, 129.85, 129.51, 128.84, 128.39, 126.39, 125.93, 125.00, 124.59, 123.96, 118.44, 116.09, 114.32, 113.66, 111.72, 110.41, 70.96, 58.21, 55.63, 55.54, 55.43, 47.44, 33.43.

*3.1.19. (E)-7-hydroxy-8-{[phenethyl-(4-methoxybenzyl)amino]methyl}-2-(2-furan-3-yl)vinylchromone **(3f)***

Yield: 37%; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.01 (1H, d, J = 9.0 Hz), 7.32–7.05 (10H, m), 6.88–6.78 (4H, m), 6.43 (1H, d, J = 16.2 Hz), 6.19 (1H, s), 4.10 (2H, s), 3.99 (2H, s),

3.71 (3H, s), 2.95–2.83 (4H, m); ^{13}C NMR (75 MHz, $CDCl_3$) δ 177.99, 163.89, 160.89, 159.38, 158.48, 155.39, 144.56, 142.54, 138.59, 131.19, 131.06, 129.85, 129.19, 128.68, 128.59, 126.60, 126.22, 123.26, 119.22, 118.38, 116.79, 115.12, 114.23, 114.09, 109.82, 109.30, 107.75, 107.25, 56.97, 55.17, 54.86, 49.40, 33.17, 32.76, 31.66, 29.71.

*3.1.20. (E)-7-hydroxy-8-{[phenethyl-(4-methoxybenzyl)amino] methyl}-2-(2-thiophen-2-yl)vinylchromone (**3g**)*

Yield: 80%; ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.01 (1H, d, J = 9.0 Hz), 7.45–6.83 (16H, m), 6.55 (1H, d, J = 15.6 Hz), 6.21 (1H, s), 4.16 (2H, s), 3.78 (2H, s), 3.71 (3H, s), 2.97–2.89 (4H, m); ^{13}C NMR (75 MHz, $CDCl_3$) δ 182.17, 166.92, 165.77, 164.87, 159.84, 144.34, 140.215, 136.36, 135.12, 134.65, 133.08, 132.68, 132.54, 131.41, 125.22, 122.46, 120.89, 118.69, 113.43, 108.36, 61.80, 59.30, 58.38, 53.817, 53.53, 53.25, 52.86, 52.68, 52.40, 52.11, 35.21.

3.2. Biological test

RPMI 1640 medium, fetal bovine serum (FBS), penicillin, and streptomycin were purchased from Life Technologies Inc. (Grand Island, NY, USA). Sulforhodamine B, dimethyl sulfoxide (DMSO) and cisplatin were purchased from Sigma Chemical Co. (MO, U.S.A.). Human lung cancer (A549), human epidermoid carcinoma (KB), human colon (HCT-15), and human melanoma (SK-MEL2) cancer cell limes were supplied from the Korean Cell Line Bank, Seoul National University.

Cytotoxic activity of the synthesized compounds against human cancer cell lines was investigated using the SRB assay as described previously [11]. All cell lines were grown in RPMI 1640 (Gibco BRL) supplemented with 10% (V/V) heat inactivated fetal bovine serum (FBS) and maintained at 37 °C in a humidified atmosphere with 5% CO_2. The cells (5×10^3 cells/well for KB, SK-MEL-2, HCT-15 and 2×10^3 cells/well for A-549) were seeded into 96-well plate. Various concentrations of samples were added to each well in triplicate, then incubated at 37 °C with 5% CO_2 for 72 h such that time cells are in the exponential phase of growth at the time of drug addition. After incubation, the 100 μl of formalin solution were gently added to the wells. Microplates were left for 30 min at room temperature, washed five times with tap water. The 100 μl of 0.4% SRB solution was added to each well and left at room temperature for 30 min. SRB was removed and the plates were washed five times with 1% acetic acid before air drying. Bound SRB was solubilized with 100 μl 10 mM unbuffered Tris-base solution (Sigma) and plates were left on a plate shaker for at least 10 min. The optical density was measured using a microplate reader (Versamax, Molecular Devices) with a 520 nm wavelength and the anticancer effective concentration was expressed as an IC_{50}.

4. Conclusion

A series of compounds **3a–g** having structural features of styrylchromones and lavendustin A were synthesized through reductive-amination, aminoalkylation, and aldol-type condensation reactions from **4**. Synthesized compounds were evaluated for their cytotoxicities on four tumor cell lines. Among tested, compound **3e** showed the most potent cytotoxic activity on HCT-15 cell line with IC_{50} values of 7.17 μg/ml indicating that lavendustin A derivatives containing 2-arylethenylchromone ring have a potential in anti-tumor application.

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