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5-Arylidene-2(5*H*)-Furanone Derivatives: Synthesis and Structure-Activity Relationship for Cytotoxicity

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Thirty-eight 5-arylidene-2(5*H*)-furanone derivatives possessing halo-, methoxy-, oxo-, dioxo-, and thiophenyl groups as well as anthraquinone and naphthquinone moieties were synthesized, and their cytotoxicity was evaluated against various cancer cell lines. The introduction of halogen atoms or nitro group at aromatic ring of 5-arylidene-2(5*H*)-furanone was shown to increase the cytotoxicity with 5-(3-nitrobenzylidene)-2(5*H*)-furanone (**21**) being the most potent. Among anthracenyl or naphthalenyl derivatives, (*E*)-5-[2-(1,4-dimethoxy-9,10-dioxo) anthracenyl]-2(5*H*)-furanone (**34**) showed the most potent cytotoxic activity.

Key words: 5-Aryline-2(5*H*)-furnaone, Synthesis, Cytotoxicity

INTRODUCTION

The root of *Pulsatilla koreana* Nakai (Ranunculaceae) has been used for the treatment of amoebic dysentery and malaria in Korean traditional medicine. The *Pulsatilla* genus is known to contain ranunculin, anemonin, protoanemonin, triterpenes, and saponins. Among them, ranunculin (**RAN**, **1**, Fig. 1) exhibited a remarkable cytotoxic activity against KB and Bel-7402 cells with ED_{50} values of 0.21 and 0.35 μM, respectively. Its deglucosylated product, protoanemonin (**PA**, **2**), also had a cytotoxic activity (Li, R. Z. *et al.*, 1993).

PA readily dimerizes into noncytototoxic anemonin (**AN**, **3**) under light or physiological conditions. In plant cells, **RAN** is hydrolysed to anemonol, γ-hydroxymethyl butenolide, which is easily dehydrated to **PA**, and then, **PA**, in turn, dimerizes to the more stable **AN** (R. Hill *et al.*, 1951).

Based on this chemistry, we assumed that **RAN** can be metabolized *via* the same route used in cancer cells to produce **PA**, a metabolite, which could directly damage cells. Moreover, it was reported that γ-alkylidene butenolide moiety, as well as **PA**, was included as a common structure in a number of drugs with diverse biological activities, such as antifungal, antibacterial, and anti-inflammatory effects (Ei-ichi Negishi *et al.*,1997).

Therefore, we envisioned that the introduction of relatively stable arylidene group instead of methylene group at C-5 can prevent the dienone of **PA** from dimerizing to the **AN**- form, thereby retaining the cytotoxic dienone moiety in the structure (Fig. 2).

On this rationale, we synthesized thirty-eight 5-arylidene-2(5*H*)-furanone derivatives and evaluated their cytotoxicity against several human cancer cell lines.

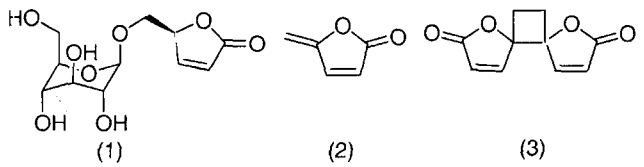

Fig. 1. The structures of ranunculin (**RAN**, **1**), protoanemonin (**PA**, **2**), and anemonin (**AN**, **3**)

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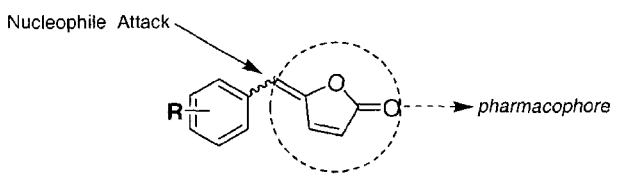


Fig. 2. Protoanemonin moiety as pharmacophore.

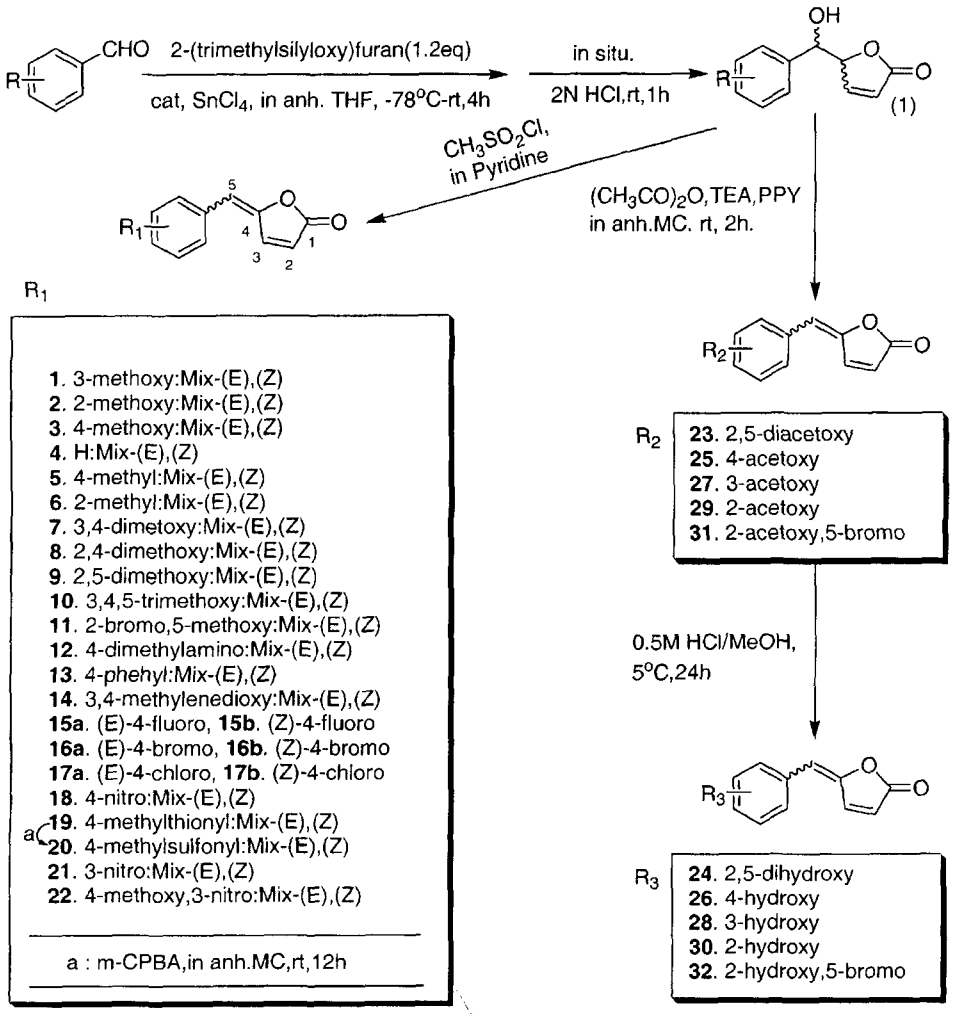
CHEMISTRY

Recently, 2-trimethylsiloxyfuran has emerged as a keystone for the synthesis of a wide variety of 5-substitued-2(5*H*)-furanones (Leo A. Paquette, p.5297-5300). The synthesis was readily achieved by the reaction with an electrophile followed by desilylation. The facile condensation of 2-trialkylsilyloxyfurans with carbonyl compounds and their derivatives has spawned many synthetic applications. Therefore, each aldehyde was reacted with 2-trimethylsiloxyfuran, which were then followed by desilylation and dehydration reactions to provide each 5-arylidene-2(5*H*)-furanone derivative (**1-38**).

An intermediate of 5-arylidene-2(5*H*)-furanone, 5-(1'-hydroxyaryl)-2(5*H*)-furanone, can be prepared through the reaction of aldehydes with 2-(trimethylsilyloxy)furan, in the presence of a catalytic amount of $SnCl_4$ as an activator in dry tetrahydrofuran (THF) at -78 °C under N_2 for 2 h. The mixture, while being stirred, was slowly warmed to room temperature for 2 h, and then, *in situ*, the reaction mixture was reacted with 2N HCl for an additional 1 h (desilylation reation). In the final step, dehydration of 5-(1'-hydroxyaryl)-2(5*H*)-furanone was achieved using either methansulfonyl chloride (CH_3SO_2Cl) in dry pyridine or acetic anhydride and 4-pyrrolidinopyrine (PPY) in the mixed solution of triethylamine (TEA) and anhydrous methylene chloride (MC) as shown in Scheme 1 (Manat *et al.*, 1998; Casiraghi *et al.*, 1995).

5-Arylidene-2(5*H*)-furanones **1-22** were prepared by employing the method mentioned above, and analogs **23-38** were synthesized through the use of the same dehydration reaction. The deacetylation of **23**, **25**, **27**, **29**, and **31** was achieved by reacting with 0.5 M HCl in MeOH at 5 °C for 24 h to give phenolic compounds of **24**, **26**, **28**, **30**, and **32**, respectively (Jozef A. J. M. Vekemans *et al.*, 1987).

The diasteromers of the haloaryls **15-17** and the oxyaryls **23-32** were completely separated by using the silica gel



Scheme 1. Synthetic pathway of 5-arylidene-2(5*H*)-furanone derivatives **1-32**

column eluted with ethyl acetate (EA)/cyclohexane (Cy.Hx) (increasing EA). However, the diasteromeric resolution of other compounds was unsuccessful.

5-Anthracenyl-2(5*H*)-furanone derivates **33-35** and 5-naphthalenyl-2(5*H*)-furanone derivates **36-38** were synthesized by the reaction between 2-formyl-1,4,9,10-tetramehtoxyanthracene and 2-formyl-1,4,9,10-tetramethoxy naphthalene as shown in Schemes 2 and 3 (Jin, G. Z *et al.*, 1998; Song, G. Y. *et al.*, 1999). The oxidative demethylation of **33** and **36** with cerium diammonium nitrate (CAN) afforded **34**, **37**, and **38** in moderate yields (38-87%). In the anthracenyl or naphthalenyl derivatives, only (*E*)-form compounds were synthesized. The reason for this result was fully explained in previous reports, which was briefly mentioned above.

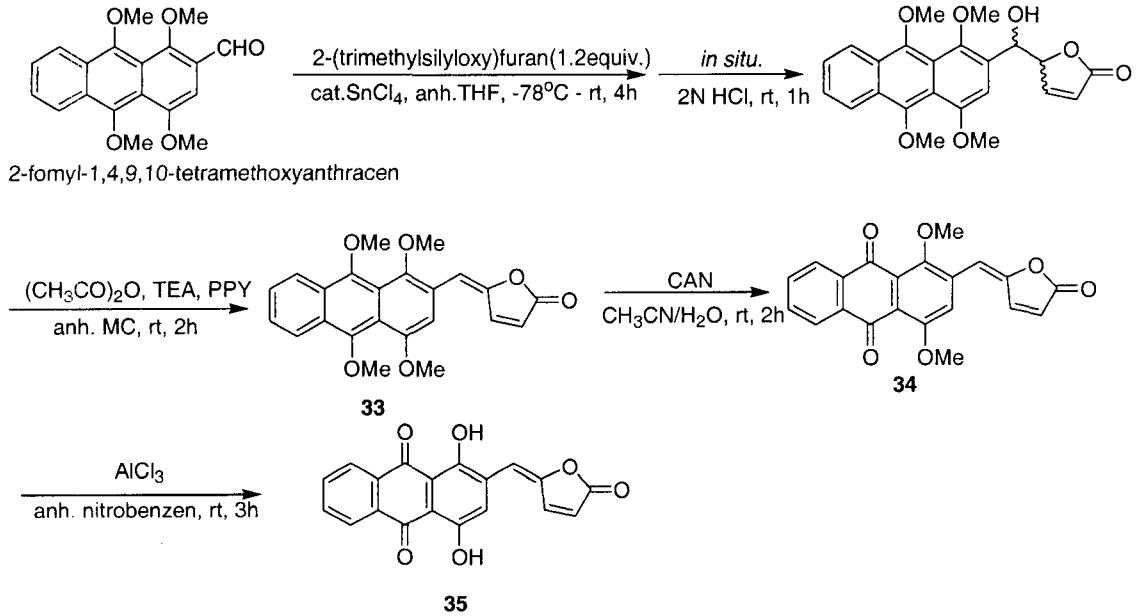
MATERIALS AND METHODS

All reactions were carried out in an atmosphere of N_2 unless otherwise noted. Chemical reagents were purchased from Aldrich Chemical Company. The solvents were of extra pure grade and were obtained from local suppliers. THF was distilled from sodium metal immediately prior to use. Organic extracts or filtrates were washed with brine, dried over anhydrous Na_2SO_4, and concentrated *in vacuo*. Thin layer chromatography (TLC) was carried out using E. Merck Silica Gel 60 precoated plates. Column chromatography was performed with Merck-EM Type 60 (70~230 mesh). Melting points were determined by the capillary method on Electrothermal IA9200 digital melting point apparatus and were uncorrected. Infrared spectra were recorded as thin films on KBr disks on the JASCO, IR Report-100 Spectrophotometer. 1H-NMR spectra were obtained using the Jeol EX-90 (90 MHz) Spectrometer. Chemical shift were reported in ppm's (δ) relative to Tetramethyl Silane (TMS) as an internal standard.

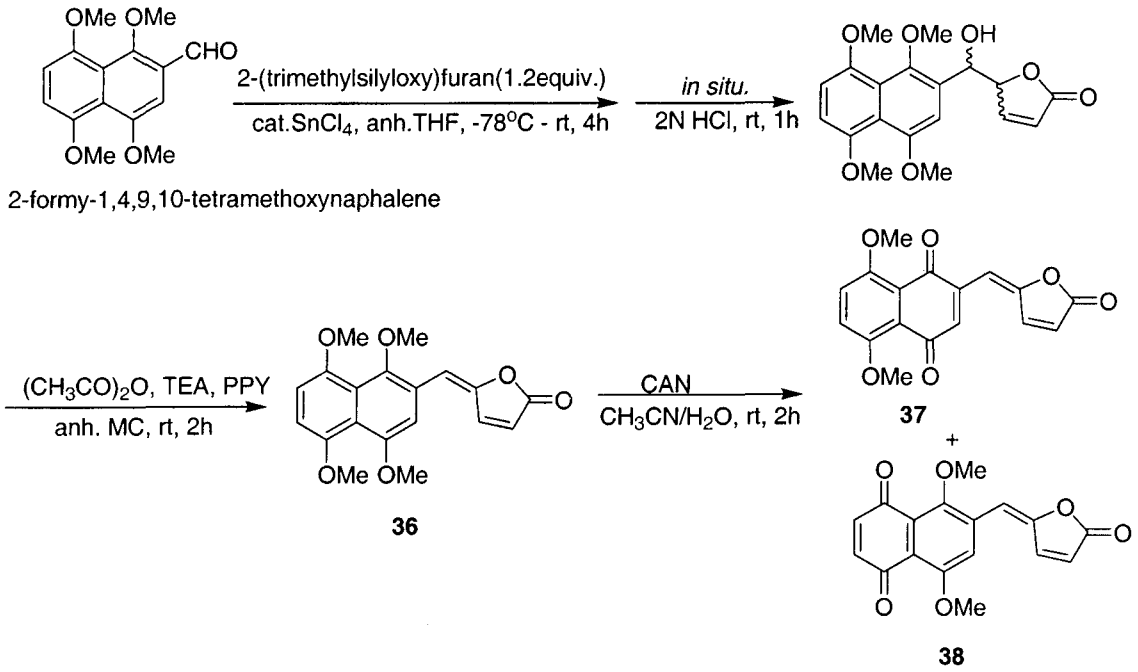
In vitro cytotoxic assay

Cytotoxic assay was performed by using A-549, NIH, and SK-OV-3 human tumor cell lines, which were supplied by KRIBB (Korea Research Institute of Biosciences and Biotechnology). The cells were grown at 37 °C in RPMI 1640 medium supplemented with 10% FBS, and they were separated using PBS containing 0.25% trypsin and 3 mM EDTA. Cell suspensions (5×10^3-2×10^4 cells/mL) were added to each well of a 96-well plate and were incubated at 37 °C for 24 h. Each compound was dissolved in DMSO and was diluted with this medium to five different concentrations of 0.3, 1, 3, 10 and 30 μg/mL. The concentration of DMSO was adjusted to be below 0.2%. After removing the well medium through aspiration, each of the sample solutions (20 μL) was added to the above well plates, which were placed in a 5% CO_2 incubator for 48 hrs. The protein stain assay was performed according to the SRB method (Skehan *et al.*, 1990).

(*E*), (*Z*)-5-(3-Methoxybenzylidene)-2(5*H*)-furanone (1) : To a solution of 3-methoxy benzaldehyde (500 mg, 3.67 mmol) in dry THF (25 mL) under N_2, a solution of 2-(trimethylsilyloxy)furan (1.2 eq, 740 mL) in dry THF (20 mL) at -78 °C was added dropwise for 1 h, and then, $SnCl_4$ was added as a catalyst to the reaction mixture,



Scheme 2. Synthetic pathway of 5-anthracenyl-2(5*H*)-furanone derivatives **33-35**



Scheme 3. Synthetic pathway of 5-naphthalenyl-2(5*H*)-furanone derivatives **36-38**

which was stirred for an additional 1 h. The mixture, while being stirred, was slowly warmed to room temperature for 2 h, and then, *in situ*, 10 mL of 2N HCl was added to the reaction mixture for an additional 1 h to break the siloxane complex. The reaction mixture was quenched with water (50 mL) and was extracted with MC (200 mL×2), and the organic layer was combined, washed with brine, dried over anhydrous Na_2SO_4, and concentrated *in vacuo* to give a mixture of syn/anti-isomer of 4-[1-hydroxy-1-(3-methoxyphenyl)methyl]-2-buten-4-olide as an intermediate (638 mg). The intermediate (300 mg) was stirred with anhydrous pyridine (5 mL) in the presence of CH_3SO_2Cl (2 eq.), which was added dropwise for 1 h under N_2 for 12 h. The reaction mixture was quenched with water (50 mL) and was extracted with MC (100mL). The combined organic extracts was washed with brine, dried over anhydrous Na_2SO_4, and evaporated *in vacuo*, which was purified by column chromatography with gradient solvent condition (Cy.Hx : EA = 10 : 1 → 5 : 1 → 1 : 1), to obtain a mixture of **(*E*)** and **(*Z*)-5-(3-methoxybenzylidene)-2(5*H*)-furanone (1)** (the mixing ratio = 42% : 58% in ^{1}H-NMR spectra); Yield : 47%; mp : 102~104 °C; **(*E*)-5-(3-methoxybezylidene)-2(5*H*)-furanone (1a)** : ^{1}H-NMR (90 MHz, in $CDCl_3$) : δ 7.48 (d, *J* = 5.4 Hz, 1H), 7.38-7.23 (m, 3H), 7.09-7.05 (m, 1H), 6.59 (s , 1H), 6.21 (dd, *J* = 5.4, *J* = 1.7 Hz, 1H), 3.87 (s, 3H); **(*Z*)-5-(3-methoxybezylidene)-2(5*H*)-furanone (1b)** : ^{1}H-NMR (90 MHz, in $CDCl_3$) : δ 7.48 (d, *J* = 5.4 Hz, 1H), 7.38-7.23 (m, 3H), 7.09-7.05 (m, 1H), 6.05 (dd, *J* = 5.4, *J'* = 0.72 Hz, 1H), 6.08 (s, 1H), 3.88 (s, 3H)

Compounds of **2** - **22** was prepared with the same method as **1**.

The mixing ratio of **(*E*)-** to **(*Z*)-5-(2-methoxybenzylidene)-2(5*H*)-furanone (2)** was 46% to 54%; Yield : 52%; mp : 102~104 °C; IR ν_{max} cm^{-1} (KBr) : 2900, 2850, 1785, 1750, 1500, 1230, 1050, 1020, 880, 800; **(*E*)-5-(2-methoxybenzylidene)-2(5*H*)-furanone (2a)** : ^{1}H-NMR (90 MHz, in $CDCl_3$) : δ 7.81-7.75 (m, 1H), 7.47 (d, *J* = 5.3 Hz, 1H), 6.85-6.87 (m, 3H), 6.94 (s, broad, 1H), 6.25 (dd, *J* = 5.3, *J'* = 1.98 Hz, 1H), 3.82 (s, 3H); **(*Z*)-5-(2-methoxybenzylidene)-2(5*H*)-furanone (2b)** : ^{1}H-NMR (90 MHz, in $CDCl_3$) : δ 7.81-7.75 (m, 1H), 7.47 (d, *J* = 5.3 Hz, 1H), 6.85-6.87 (m, 3H), 6.55 (s,1H), 6.15 (dd, *J* = 5.3, *J'* = 0.72 Hz, 1H), 3.82 (s, 3H)

The mixing ratio of **(*E*)-** to **(*Z*)-5-(4-methoxybenzylidene)-2(5*H*)-furanone (3)** was 39% to 61%; Yield : 43.1%; mp : 106~108 °C; IR ν_{max} cm^{-1} (KBr) : 2930, 2850, 1785, 1750, 1600, 1500, 1300, 1250, 1170, 1120, 1020, 930, 890, 810; **(*E*)-5-(4-methoxybezylidene)-2(5*H*)-furanone (3a)** : ^{1}H-NMR (90 MHz, in $CDCl_3$) : δ 7.71 (d, *J* = 9.1 Hz, 2H), 7.44 (d, *J* = 5.4 Hz, 1H), 6.87 (d, *J* = 9.1 Hz, 2H), 6.74 (s, broad, 1H), 6.27 (dd, *J* = 5.4, *J'* = 1.89 Hz, 1H), 3.80 (s, 3H); **(*Z*)-5-(4-methoxybezylidene)-2(5*H*)-furanone (3b)** : ^{1}H-NMR (90 MHz, in $CDCl_3$) : δ 7.71 (d, *J* = 9.1 Hz, 2H), 7.44 (d, *J* = 5.4 Hz, 1H), 6.87 (d, *J* = 9.1 Hz, 2H), 6.12 (dd, *J* = 5.4, *J'* = 0.8 Hz, 1H), 5.99 (s ,1H), 3.80 (s, 3H)

The mixing ratio of **(*E*)-** to **(*Z*)-5-(benzylidene)-2(5*H*)-furanone (4)** was 43% to 57%; Yield : 48%; mp : 86~88 °C; IR ν_{max} cm^{-1} (KBr) : 3010, 2900, 1750, 1640, 1602, 1542, 1501, 1065, 940; **(*E*)-5-(bezylidene)-2(5*H*)-furanone (4a)** : ^{1}H-NMR (90 MHz, in $CDCl_3$) : δ 7.80 (d, *J*

= 7.2 Hz, 2H), 7.50 (d, *J* = 5.3 Hz, 1H), 7.47-7.30 (m, 3H), 6.80 (s, 1H), 6.34 (d, *J* = 5.3 Hz, 1H); **(*Z*)-5-(bezylidene)-2(5*H*)-furanone (4b)** : ^{1}H-NMR (90 MHz, in $CDCl_3$) : δ 7.80 (d, *J* = 7.2 Hz, 2H), 7.50 (d, *J* = 5.3 Hz, 1H), 7.47-7.30 (m, 3H), 6.22 (d, *J* = 5.3 Hz, 1H), 6.04 (s, 1H)

The mixing ratio of **(*E*)-** to **(*Z*)-5-(4-methylbenzylidene)-2(5*H*)-furanone (5)** was 48% to 52%. Yield : 55.6%; mp : 59~60 °C; IR ν_{max} cm^{-1} (KBr) : 3100, 2900, 1750, 1640, 1602, 1550, 1501, 1100, 1070, 930, 880, 820; **(*E*)-5-(4-methylbenzylidene)-2(5*H*)-furanone (5a)** : ^{1}H-NMR (90 MHz, in $CDCl_3$) : δ 7.65 (d, *J* = 8.4 Hz, 2H), 7.45 (d, *J* = 5.4 Hz, 1H), 7.16 (d, *J* = 8.3 Hz, 1H), 6.76 (s, broad, 1H), 6.27 (dd, *J* = 5.4, *J* = 1.98 Hz, 1H), 2.38 (s, 3H); **(*Z*)-5-(4-methylbenzylidene)-2(5*H*)-furanone (5b)** : ^{1}H-NMR (90 MHz, in $CDCl_3$) : δ 7.65 (d, *J* = 8.4 Hz, 2H), 7.45 (d, *J* = 5.4 Hz, 1H), 7.16 (d, *J* = 8.3 Hz, 1H), 6.15 (d, *J* = 5.4 Hz, 1H), 6.01 (s, 1H), 2.38 (s, 3H)

The mixing ratio of **(*E*)-** to **(*Z*)-5-(2-methylbenzylidene)-2(5*H*)-furanone (6)** was 27% to 73%; Yield : 57%; IR ν_{max} cm^{-1} (KBr) : 3100, 2900, 1750, 1640, 1602, 1540, 1501, 1100, 1070, 930, 760; **(*E*)-5-(2-methylbenzylidene)-2(5*H*)-furanone (6a)** : ^{1}H-NMR (90 MHz, in $CDCl_3$) : δ 8.14-8.10 (m, 1H), 7.50 (d, *J* = 5.4 Hz, 1H), 7.32-7.19 (m, 3H), 6.48 (s, 1H), 6.25 (d, *J* = 5.4 Hz, 1H) 2.35 (s, 3H); **(*Z*)-5-(2-methylbenzylidene)-2(5*H*)-furanone (6b)** : ^{1}H-NMR (90 MHz, in $CDCl_3$) : δ 8.14-8.10 (m, 1H), 7.50 (d, *J* = 5.4 Hz, 1H), 7.32-7.19 (m, 3H), 6.20 (d, *J* = 5.4 Hz, 1H), 6.19 (s, 1H), 2.35 (s, 3H).

The mixing ratio of **(*E*)-** to **(*Z*)-5-(3,4-dimethoxybenzylidene)-2(5H)-furanone (7)** was 36% to 66%; Yield : 58%; mp : 114~117 °C; IR ν_{max} cm^{-1} (KBr) : 3080, 2900, 1760, 1650, 1620, 1540, 1500, 1280, 1080, 1070, 830, 810; **(*E*)-5-(2,3-dimethoxybenzylidene)-2(5*H*)-furanone (7a)** : ^{1}H-NMR (90 MHz, in $CDCl_3$) : δ 7.45 (d, *J* = 1.98 Hz,1H), 7.44 (d, *J* = 5.4 Hz, 1H), 6,92 (s, 1H), 6.83 (s ,1H), 6.73 (s, broad, 1H), 6.28 (dd, *J* = 5.6, *J* = 1.7 Hz,1H), 3.95 (s, 3H), 3.93 (s, 3H); **(*Z*)-5-(2,3-dimethoxybenzylidene)-2(5*H*)-furanone (7b)** : ^{1}H-NMR (90 MHz, in $CDCl_3$) : δ 7.45 (d, *J* = 1.98 Hz,1H), 7.44 (d, *J* = 5.4 Hz, 1H), 6,92 (s, 1H), 6.83 (s, 1H), 6.12 (dd, *J* = 5.3, *J'* = 0.9 Hz, 1H), 5.98 (s, 1H), 3.95 (s,3H), 3.93 (s, 3H)

The mixing ratio of **(*E*)-** to **(*Z*)-5-(2,4-dimethoxybenzylidene)-2(5*H*)-furanone (8)** was 40% to 60%; Yield : 53%; mp : 185~190 °C; IR ν_{max} cm^{-1} (KBr) : 3040, 2900, 1750, 1650, 1620, 1540, 1500, 1475, 1280, 1090, 840; **(*E*)-5-(2,4-dimethoxybenzylidene)-2(5*H*)-furanone (8a)** : ^{1}H-NMR (90 MHz, in $CDCl_3$) : δ 8.15 (d, *J* = 8.64 Hz, 1H), 7.44 (d, *J* = 5.4 Hz,1H), 6.93 (s, broad, 1H), 6.56-6.42 (m, 2H), 6.21 (dd, *J* = 5.67, *J'* = 1.71 Hz, 1H), 3.85 (s, 6H); **(*Z*)-5-(2,4-dimethoxybenzylidene)-2(5*H*)-furanone (8b)** : ^{1}H-NMR (90 MHz, in $CDCl_3$) : δ 8.15 (d, *J* = 8.64 Hz, 1H), 7.44 (d, *J* = 5.4 Hz,1H), 6.56-6.63 (m ,2H), 6.45 (s, 1H), 6.08 (d, *J* = 5.4 Hz,1H), 3.85 (s, 6H)

The mixing ratio of **(*E*)-** to **(*Z*)-5-(2,5-dimethoxybenzylidene)-2(5*H*)-furanone (9)** was 45% to 55%; Yield : 56%; mp : 76~80 °C; IR ν_{max} cm^{-1} (KBr) : 3100, 2950, 2850, 1790, 1740, 1590, 1540, 1490, 1230, 1100, 1050, 1020, 960, 880; **(*E*)-5-(2,5-dimethoxybenzylidene)-2(5*H*)-furanone (9a)** : ^{1}H-NMR (90 MHz, in $CDCl_3$) : δ 7.73 (dd, *J* = 5.58, *J'* = 0.81 Hz, 1H), 7.46 (d, *J* = 5.4 Hz, 1H), 6.92 (s, broad, 1H), 6.85-6.83 (m, 2H), 6.23 (dd, *J* = 5.54, *J'* = 1.7 Hz, 1H), 3.80 (s, 3H), 3.78 (s, 3H); **(*Z*)-5-(2,5-dimethoxybenzylidene)-2(5*H*)-furanone (9b)** : ^{1}H-NMR (90 MHz, in $CDCl_3$) : δ 7.73 (dd, *J* = 5.58, *J'* = 0.81 Hz, 1H), 7.46 (d, *J* = 5.4 Hz, 1H), 6.85-6.83 (m, 2H), 6.53 (s ,1H), 6.13 (d, *J* = 5.4 Hz, 1H), 3.80 (s, 3H), 3.78 (s, 3H)

The mixing ratio of **(*E*)-** to **(*Z*)-5-(3,4,5-trimethoxybenzylidene)-2(5*H*)-furanone (10)** was 54% to 46%; Yield : 55.7%; mp : 115~118 °C; IR ν_{max}cm^{-1} (KBr) : 3100, 2900, 1760, 1590, 1500, 1450, 1320, 1250, 1120, 1000, 920, 880, 820; **(*E*)-5-(3,4,5-trimethoxybenzylidene)-2(5*H*)-furanone (10a)** : ^{1}H-NMR (90 MHz, in $CDCl_3$) : δ 7.78 (d, *J* = 5.4 Hz, 1H) 6.74 (s, 1H), 6.57 (s, 2H), 6.30 (d, *J* = 5.4, *J* = 1.8 Hz, 1H), 3.89 (s, 9H); **(*Z*)-5-(3,4,5-trimethoxybenzylidene)-2(5*H*)-furanone (10b)** : ^{1}H-NMR (90 MHz, in $CDCl_3$) : δ 7.78 (d, *J* = 5.4 Hz, 1H), 6.57 (s, 2H), 6.15 (dd, *J* = 5.4, *J'* = 0.8 Hz, 1H), 5.95 (s , 1H), 5.98 (s, 1H), 3.90 (s, 9H)

The mixing ratio of **(*E*)-** to **(*Z*)-5-(2-bromo, 5-methoxy)-2(5*H*)-fuarnone (11)** was 10% to 90%; Yield : 54.7%; mp : 158~159 °C; IR ν_{max} cm^{-1} (KBr) : 2950, 2850, 1780, 1760, 1750, 1540, 1480, 1280, 1260, 1120, 1020, 810; **(*E*)-5-(2-bromo,5-methoxy)-2(5*H*)-furanone (11a)** : ^{1}H-NMR (90 MHz, in $CDCl_3$) : δ 8.27 (d, *J* = 3.78 Hz, 1H), 7.46 (d, *J* = 5.4 Hz, 1H), 7.34 (d, *J* = 8.8 Hz, 1H), 6.85 (s, 1H), 6.71 (d, *J* = 8.8 Hz, 1H), 6.30 (dd, *J* = 5.4, *J* = 1.89 Hz, 1H), 3.86 (s, 3H); **(*Z*)-5-(2-bromo,5-methoxy)-2(5*H*)-furanone (11b)** : ^{1}H-NMR (90 MHz, in $CDCl_3$) : δ 8.27 (d, *J* = 3.78 Hz, 1H), 7.46 (d, *J* = 5.4 Hz, 1H), 7.34 (d, *J* = 8.8 Hz, 1H), 6.71 (d, *J* = 8.8 Hz, 1H), 6.47 (s, 1H), 6.18 (dd, *J* = 5.4, *J* = 0.72 Hz, 1H), 3.86 (s, 3H).

The mixing ratio of **(*E*)-** to **(*Z*)-5-(4-N,N-dimethylaminobenzylidene)-2(5*H*)-furanone (12)** was 80% to 20%; Yield : 49%; (E),(Z)-mixture (E : Z = 80 : 20); mp : 191~193 °C; IR n_{max} cm^{-1} (KBr) : 3150, 2925, 1790, 1760, 1590, 1500, 1110, 940, 900, 820, 720; **(*E*)-5-(4-N,N-dimethylaminobenzylidene)-2(5*H*)-furanone (12a)** : ^{1}H-NMR (90 MHz, in $CDCl_3$) : δ 8.21 (d, *J* = 8.8 Hz, 2H), 7.73 (d, *J* = 5.58 Hz, 1H), 7.45 (d, *J* = 8.8 Hz, 2H), 6.78 (s, 1H), 6.40 (dd, *J* = 5.58, *J'* = 1.98 Hz, 1H), 2.98 (s, 6H); **(*Z*)-5-(4-N,N-dimethylaminobenzylidene)-2(5*H*)-furanone (12b)** : ^{1}H-NMR (90 MHz, in $CDCl_3$) : δ 8.21 (d, *J* = 8.8 Hz, 2H), 7.73 (d, *J* = 5.58 Hz, 1H), 7.45 (d, *J* = 8.8 Hz, 2H), 6.30 (dd, *J* = 5.58, *J* = 0.72 Hz, 1H), 6.05 (s, 1H), 2.98 (s, 6H)

The mixing ratio of **(*E*)-** to **(*Z*)-5-(4-phenylbenzylidene)-2(5*H*)-furanone (13)** was 75% to 25%; Yield : 55%; mp :

165~167 °C; IR ν_{max} cm^{-1} (KBr) : 2930, 2850, 1790, 1750, 1710, 1550, 1300, 1100, 940, 900, 800, 770, 700, 680; **(*E*)-5-(4-phenylbenzylidene)-2(5*H*)-furanone (13a) :** ^{1}H-NMR (90 MHz, in $CDCl_3$) : δ 7.87 (d, *J* = 5.4 Hz, 1H), 7.68-7.60 (m, 4H), 7.54-7.45 (m, 4H), 7.41-7.37 (m, 1H), 6.83 (s, 1H), 6.36 (dd, *J* = 5.4, *J'* = 1.7 Hz, 1H); **(*Z*)-5-(4-phenylbenzylidene)-2(5*H*)-furanone (13b) :** ^{1}H-NMR (90 MHz, in $CDCl_3$) : δ 7.87 (d, *J* = 5.4 Hz, 1H), 7.68-7.60 (m, 4H), 7.54-7.45 (m, 4H), 7.41-7.37 (m ,1H), 6.23 (d, *J* = 5.4 Hz, 1H), 6.08 (s, 1H)

The mixing ratio of **(*E*)-** to **(*Z*)-5-(3,4-methylenedioxyl)-2(5*H*)-furanone (14)** was 47% to 53%; Yield : 58%; mp : 158~160 °C; IR ν_{max} cm^{-1} (KBr) : 3100, 2925, 2850, 1770, 1740, 1540, 1500, 1490, 1440, 1260, 1120, 1040, 940, 880, 800; **(*E*)-5-(3,4-methylenedioxyl)-2(5*H*)-furanone (14a) :** ^{1}H-NMR (90 MHz, in $CDCl_3$) : δ 7.47-7.45 (m, 2H), 7.18 (dd, *J* = 8, *J'* = 1.9 Hz, 1H), 6.83 (d, *J* = 8 Hz, 1H), 6.70 (s, 1H), 6.17 (d , *J* = 5.0 Hz, 1H), 6.02 (s, 2H); **(*Z*)-5-(3,4-methylenedioxyl)-2(5*H*)-furanone (14b) :** ^{1}H-NMR (90 MHz, in $CDCl_3$) : δ 7.47-7.45 (m, 2H), 7.18 (dd, *J* = 8, *J* = 1.92 Hz, 1H), 6.83 (d, *J* = 8 Hz, 1H), 6.17 (d , *J* = 5.0 Hz, 1H), 6.01 (s, 2H), 5.98 (s, 1H)

(*E*)-5-(4-Fluorobenzylidene)-2(5*H*)-furanone (15a) : Yield : 23%; mp : 106~110 °C; IR ν_{max} cm^{-1} (KBr) : 3100, 2900, 1790, 1750, 1600, 1540, 1500, 1230, 1120, 1070, 940, 900, 820; ^{1}H-NMR (90 MHz, in $CDCl_3$) : δ 7.73 (dd, *J* = 5.67, *J* = 0.72 Hz, 1H), 7.47-7.01 (m, 4H), 6.75 (s, 1H), 6.32 (dd, *J* = 5.58, *J'* = 1.98 Hz, 1H), **(*Z*)-5-(4-fluorobenzylidene)-2(5*H*)-furanone (15b) :** Yield : 31%; mp : 131~133 °C; IR ν_{max} cm^{-1} (KBr) : 3100, 2900, 1780, 1740, 1600, 1540, 1500, 1240, 1120, 1070, 930, 890, 820; ^{1}H-NMR (90 MHz, in $CDCl_3$) : δ 7.87-7.71 (m, 2H), 7.46 (d, *J* = 5.4 Hz, 1H), 7.27-6.99 (m, 2H), 6.19 (d, *J* = 5.13 Hz, 1H), 6.00 (s, 1H).

(*E*)-5-(4-Bromobenzylidene)-2(5*H*)-furanone (16a) : Yield : 21%; mp : 83~85 °C; IR ν_{max} cm^{-1} (KBr) : 3100, 2950, 1785, 1760, 1540, 1480, 1110, 1070, 940, 898, 820, 720; ^{1}H-NMR (90 MHz, in $CDCl_3$) : δ 7.75 (d, *J* = 5.2 Hz, 1H), 7.54 (d, *J* = 8.8 Hz, 2H), 7.22 (d, *J* = 8.8 Hz, 2H), 6.72 (s, 1H), 6.37 (dd, *J* = 5.6, *J'* = 2.0 Hz, 1H), **(*Z*)-5-(4-bromobenzylidene)-2(5*H*)-furanone (16b) :** Yield : 36%; mp : 102~105 °C; IR ν_{max} cm^{-1} (KBr) : 3100, 2900, 1780, 1750, 1580, 1540, 1480, 1100, 1070, 940, 880, 820; ^{1}H-NMR (90 MHz, in $CDCl_3$) : δ 7.71-7.46 (m, 5H), 6.22 (d, *J* = 5.4 Hz, 1H), 5.97 (s, 1H).

(*E*)-5-(4-Chlorobenzylidene)-2(5*H*)-furanone (17a) : Yield : 23%; mp : 105~106 °C; IR ν_{max} cm^{-1} (KBr) : 3100, 2900, 1790, 1750, 1540, 1490, 1110, 940, 900, 820; ^{1}H-NMR (90 MHz, in $CDCl_3$) : δ 7.73 (dd, *J* = 5.67, *J* = 0.72 Hz, 1H), 7.46-7.33 (m, 4H), 6.73 (s, 1H), 6.33 (dd, *J* = 5.63, *J* = 1.98 Hz, 1H). **(*Z*)-5-(4-chlorobenzylidene)-2(5*H*)-furanone (17b) :** Yield : 36%; mp : 121~123 °C; IR ν_{max} cm^{-1} (KBr) : 3100, 2900, 1780, 1740, 1600, 1540, 1490, 1120, 1090, 1070, 1020, 940, 880, 820, 770. ^{1}H-NMR (90 MHz, in $CDCl_3$) : δ 7.77-7.67 (m, 2H), 7.52-7.26 (m, 3H), 6.22 (dd, *J* = 5.4, *J*= 0.99 Hz, 1H), 5.98 (s, 1H).

The mixing ratio of **(*E*)-** to **(*Z*)-5-(4-nitrobenzylidene)-2(5*H*)-furanone (18)** was 1% to 99%; Yield : 24%; mp : 183~185 °C; IR ν_{max} cm^{-1} (KBr) : 3100, 2900, 1790, 1750, 1590, 1510, 1340, 1300, 1100, 940, 890, 880, 820, 800, 750, 700; **(*Z*)-5-(4-nitrobenzylidene)-2(5*H*)-furanone (18b) :** ^{1}H-NMR (90 MHz, in $CDCl_3$) : δ 8.23 (d, *J* = 6.8 Hz, 1H), 7.88 (d, *J* = 6.8 Hz, 1H), 7.52 (d, *J* = 5.67 Hz, 1H), 6.32 (dd, *J* = 5.5, *J*= 0.8 Hz, 1H), 6.08 (s, 1H)

The mixing ratio of **(*E*)-** to **(*Z*)-5-(4-methylthionyl-benzylidene)-2(5*H*)-furanone (19)** was 1% to 99%; Yield : 56.6%; mp : 118~120 °C; IR ν_{max} cm^{-1} (KBr) : 3100, 2920, 2850, 1780, 1740, 1540, 1500, 1090, 1070, 940, 890, 800; **(*Z*)-5-(4-methylthionylbenzylidene)-2(5*H*)-furanone (19b) :** ^{1}H-NMR (90 MHz, in $CDCl_3$) : δ 7.67 (d, *J* = 8.55 Hz, 2H), 7.44 (d, *J* = 5.67 Hz, 1H), 6,83 (d, *J* = 8.1 Hz, 1H), 6.16 (dd, *J* = 5.31, *J'* = 0.99 Hz, 1H), 2.51 (s, 3H).

(*Z*)-5-(4-Methansulfonylbenzylidene)-2(5*H*)-furanone (20b) : To a solution of **19b** (100 mg) that was dissolved in MC 20 mL, 250 mg of m-CPBA was added slowly at 0 °C, and stirring was continued for an additional 12 h while also being slowly warmed to room temperature. The reaction mixture was quenched with 50 mL of water and was extracted with 100 mL of MC, and the combined organic extracts was washed with brine, dried over anhydrous Na_2SO_4 and evaporated *in vacuo*, which was purified by column chromatography with the eluant (Cy.Hx : EA = 3 : 1), to obtain **20b** (white solid, 27.6 mg : Yield : 24.1%; mp : 169~171 °C; IR ν_{max} cm^{-1} (KBr) : 3400, 2930, 1800, 1760, 1590, 1560, 1400, 1290, 1140, 1100, 960, 940, 890, 870, 780, 760; ^{1}H-NMR (90 MHz, in $CDCl_3$) : δ 7.86 (s, 4H), 7.52 (d, *J* = 5.4 Hz, 1H), 6.30 (dd, *J* = 5.4, *J'* = 0.72 Hz, 1H), 6.07 (s, 1H), 3.08 (s, 3H)

The mixing ratio of **(*E*)-** to **(*Z*)-5-(3-nitrobenzylidene)-2(5*H*)-furanone (21)** was 56% to 44%; Yield : 19.8%; mp : 133~135 °C; IR ν_{max} cm^{-1} (KBr) : 3400, 2900, 1760, 1730, 1560, 1540, 1520, 1350, 1200, 1100, 940, 910, 820, 740; **(*E*)-5-(3-nitrobenzylidene)-2(5*H*)-furanone (21a) :** ^{1}H-NMR (90 MHz, in $CDCl_3$) : δ 8.27-8.22 (m, 2H), 7.76 (d, *J* = 5.67 Hz, 1H), 7.68-7.60 (m, 2H), 6.8 (s, 1H), 6.45 (dd, *J* = 5.3, *J'* = 1.72 Hz, 1H); **(*Z*)-5-(3-nitrobenzylidene)-2(5*H*)-furanone (21b) :** ^{1}H-NMR (90 MHz, in $CDCl_3$) : δ 8.27-8.22 (m, 2H), 7.76 (d, *J* = 5.67 Hz, 1H), 7.68-7.60 (m, 2H), 6.30 (dd, *J* = 5.3, *J* = 0.72 Hz, 1H), 6.08 (s, 1H).

The mixing ratio of **(*E*)-** to **(*Z*)-5-(3-methoxy,4-nitrobenzylidene)-2(5*H*)-furanone (22)** was 61% to 39%; Yield : 15%; mp : 143~145 °C; IR ν_{max} cm^{-1} (KBr) : 3400, 2900, 1790, 1750, 1540, 1490, 1100, 940, 900, 820, 800; **(*E*)-5-(4-methoxy,3-nitrobenzylidene)-2(5*H*)-furanone (22a) :** ^{1}H-NMR (90 MHz, in $CDCl_3$) : δ 7.68-7.58 (m, 1H), 7.44 (d, *J* = 5.4 Hz, 1H), 7.05 (s, 1H), 6.60-6.52 (m, 1H),

6.81 (s, 1H), 6.47 (dd, J = 5.4, J' = 1.83 Hz, 1H); **(*Z*)-5-(4-methoxy,3-nitrobenzylidene)-2(5*H*)-furanone (22b)** : ^{1}H-NMR (90 MHz, in $CDCl_3$) : δ 7.68-7.58 (m, 1H), 7.44 (d, J = 5.4 Hz, 1H), 7.05 (s, 1H), 6.60-6.52 (m, 1H), 6.17 (d, J = 5.4 Hz, 1H), 5.96 (s, 1H).

The mixing ratio of **(*E*)-** to **(*Z*)-5-(2,5-diacetoxybenzylidene)-2(5*H*)-furanone (23)** was 99% to 1%; To a solution of 4-[1-hydroxy-1-(2,5-dihydroxyphenyl)-2-buten-4-olide (286 mg, 1.29 mmol), 20 mL of MC, which was stirred with $(CH_3CO)_2O$ (3.3 eq.) and PPY as a catalyst, was added slowly to a solution of TEA in 100 mL of MC at 0 °C for 1 h, and stirring was continued for an additional 3 h. The reaction mixture was quenched with 50 mL of water and was extracted with 100 mL of MC. The combined organic extracts was washed with brine, dried over anhydrous Na_2SO_4 and evaporated *in vacuo*, which was purified by column chromatography with a gradient solvent condition (Cy.Hx : EA = 10 : 1 → 5 : 1 → 1 : 1) to obtain **23a** (pale yellow solid, 129 mg); Yield : 35%; mp : 105~110 °C; IR ν_{max} cm^{-1} (KBr) : 3100, 2900, 1760, 1750, 1580, 1560, 1440, 1380, 1200, 1160, 1120, 940, 900, 800; **(*E*)-5-(2,5-diacetoxybenzylidene)-2(5*H*)-furanone (23a)** : ^{1}H-NMR (90 MHz, in acetone) : δ 7.94 (dd, J = 5.6, J' = 0.72 Hz, 1H), 7.38-7.35 (m, 1H), 7.25-7.22 (m, 1H), 6.72 (s, 1H), 6.49 (dd, J = 5.67, J' = 1.71 Hz, 1H), 2.33 (s, 3H), 2.28 (s, 3H).

Compounds **25a, 25b, 27a, 27b, 29a, 29b, 31a, 31b,** were prepared using the same method as **23a**.

(*E*)-5-(2,5-Dihydroxybenzylidene)-2(5*H*)-furanone (24a) : **23a** (100 mg, 0.45 mmol) was reacted with 0.5 M HCl in MeOH at 5 °C for 24 h. The reaction mixture was extracted with EA (100 mL × 2), and the combined organic extracts was washed with brine, dried over anhydrous Na_2SO_4 and evaporated *in vacuo*, which was purified by column chromatography with a gradient solvent condition (Cy.Hx : EA = 3 : 1 → 1 : 1), to obtain **24a** (yellow solid, 54 mg) : Yield : 60%; mp : 185~187 °C; IR ν_{max} cm^{-1} (KBr) : 3280, 3220, 2910, 2850, 1750, 1600, 1540, 1500, 1480, 1340, 1200, 1160, 1130, 1100, 940, 810; ^{1}H-NMR (90 MHz, in acetone) : δ 8.34 (s, 1H), 8.05 (d, J = 5.58 Hz, 1H), 7.91 (s, 1H), 6.91-6.80 (m, 3H), 6.79 (s, 1H), 6.38 (dd, J = 5.54, J' = 1.98 Hz, 1H).

Compounds **26a, 26b, 28a, 28b, 30a, 30b, 32a, 32b,** were prepared with the same method as **24a**.

(*E*)-5-(4-Acetoxybenzylidene)-2(5H)-furanone (25a) : Yield : 14%; mp : 86~88 °C; IR ν_{max} cm^{-1} (KBr) : 3100, 2900, 1760, 1600, 1500, 1370, 1240, 1170, 1110, 1020, 920, 900, 820. 660; ^{1}H-NMR (90 MHz, in $CDCl_3$) : δ 7.76 (d, J = 6.1 Hz, 1H), 7.44-7.10 (m, 4H), 6.77 (s, 1H), 6.31 (dd, J = 5.54, J = 1.71 Hz, 1H), 2.32 (s, 3H), **(*Z*)-5-(4-acetoxybenzylidene)-2(5H)-furanone (25b)** : Yield : 15%; mp : 113~115 °C; IR ν_{max} cm^{-1} (KBr) : 3100, 2900, 1780, 1760, 1550, 1500, 1370, 1200, 1100, 940, 870, 810, 760; ^{1}H-NMR (90 MHz, in $CDCl_3$) : δ 7.76 (dd, J = 6.84, J' = 1.98 Hz, 2H), 7.46 (d, J = 5.4 Hz, 1H), 7.09 (dd, J = 6.84, J' = 1.98 Hz, 2H), 6.18 (dd, J = 5.4, J' = 0.72 Hz, 1H), 6.02 (s, 1H), 2.31 (s, 3H).

(*E*)-5-(4-Hydroxybenzylidene)-2(5*H*)-furanone (26a) : Yield : 52%; mp : 162~165 °C; IR ν_{max} cm^{-1} (KBr) : 3350, 2910, 1750, 1740, 1730, 1690, 1600, 1500, 1280, 1220, 1170, 1120, 1100, 900, 880, 810; ^{1}H-NMR (90 MHz, in acetone) : δ 8.80 (d, J = 6.7 Hz, 1H), 8.11 (dd, J = 5.67, J' = 0.81 Hz, 1H), 7.73 (q, 1H), 7.47-7.38 (dd, J = 6.84, J' = 1.71 Hz, 3H), 6.36 (dd, J = 5.58, J' = 1.71 Hz, 1H), 6.20 (s, 1H).

(*Z*)-5-(4-Hydroxybenzylidene)-2(5*H*)-furanone (26b) : Yield : 41%; mp : 180~182 °C; IR ν_{max} cm^{-1} (KBr) : 3350, 2900, 1730, 1720, 1580, 1540, 1370, 1280, 1210, 1120, 1080, 930, 890, 810, 790, 770, 690; ^{1}H-NMR (90 MHz, in acetone) : δ 8.89 (s, 1H), 7.83-7.38 (m, 3H), 6.87 (d, J = 8.82 Hz, 1H), 6.25-6.22 (m, 2H).

(*E*)-5-(3-Acetoxybenzylidene)-2(5*H*)-furanone (27a) : Yield : 13%; mp : 92~94 °C; IR ν_{max} cm^{-1} (KBr) : 3100, 2900, 1760, 1580, 1550, 1450, 1370, 1200, 1160, 1120, 930, 900, 800, 690; ^{1}H-NMR (90 MHz, in acetone) : δ 8.10 (dd, J = 5.90, J' = 0.72Hz, 1H), 7.47-7.42 (m, 2H), 7.36-7.31 (m, 1H), 7.21-7.16 (m, 1H), 6.86 (s, 1H), 6.48 (dd, J = 5.67, J' = 1.8 Hz, 1H), 2.30 (s, 3H), **(*Z*)-5-(3-acetoxybenzylidene)-2(5*H*)-furanone (27b)** : Yield : 17%; mp : 89~91 °C; IR ν_{max} cm^{-1} (KBr) : 3100, 2900, 1750, 1740, 1580, 1550, 1480, 1450, 1370, 1210, 1150, 1020, 890, 810, 790, 770, 690; ^{1}H-NMR (90 MHz, in acetone) : δ 7.86 (d, J = 5.4 Hz, 1H), 7.62-7.57 (m, 3H), 7.19 (m, 1H), 6.35 (dd, J = 5.4, J' = 0.99 Hz, 1H), 6.33 (s, 1H), 2.30 (s, 3H).

(*E*)-5-(3-Hydroxybenzylidene)-2(5*H*)-furanone (28a) : Yield : 46%; mp : 87~90 °C; IR ν_{max} cm^{-1} (KBr) : 3375, 2920, 1720, 1650, 1540, 1440, 1370, 1280, 1210, 1120, 930, 820, 690; ^{1}H-NMR (90 MHz, in $CDCl_3$) : δ 7.47(d, J = 5.4 Hz, 1H), 7.43-7.41(m, 1H), 7.31-7.24(m, 1H), 6.92-6.82 (m, 1H), 6.21 (d, J = 5.4 Hz, 1H), 5.01 (b, 1H), **(*Z*)-5-(3-hydroxybenzylidene)-2(5*H*)-furanone (28b)** : Yield : 47%; mp : 148~150 °C; IR ν_{max} cm^{-1} (KBr) : 3375, 2930, 1740, 1600, 1510, 1440, 1370, 1280, 1220, 1170, 1100, 930, 890, 880, 810; ^{1}H-NMR (90 MHz, in acetone) : δ 8.65 (b, 1H), 7.82 (d, J = 5.22 Hz, 1H), 7.40-7.23 (m, 3H), 6.90-6.82 (m, 1H), 6.30 (d, J = 5.22 Hz, 1H), 6.23 (s, 1H)

(*E*)-5-(2-Acetoxybenzylidene)-2(5*H*)-furanone (29a) : Yield : 14%; mp : 108~110 °C; IR ν_{max} cm^{-1} (KBr) : 3100, 2900, 1780, 1750, 1740, 1560, 1480, 1440, 1370, 1220, 1190, 1090, 940, 900, 800; ^{1}H-NMR (90 MHz, in $CDCl_3$) : δ 7.67 (d, J = 5.67 Hz, 1H), 7.35-7.12 (m, 4H), 6.71 (s, 1H), 6.30 (dd, J = 5.67, J' = 1.71 Hz, 1H), 2.31 (s, 3H), **(*Z*)-5-(2-acetoxybenzylidene)-2(5*H*)-furanone (29a)** : Yield : 12%; mp : 102~105 °C; IR ν_{max} cm^{-1} (KBr) : 3100, 2900, 1780, 1760, 1740, 1680, 1560, 1480, 1460, 1370, 1220, 1200, 1110, 940, 880, 820, 750; ^{1}H-NMR (90 MHz,

in $CDCl_3$) : δ 7.47 (d, *J* = 5.4 Hz, 1H), 7.40-7.05 (m, 4H), 6.21 (d, *J* = 5.4 Hz, 1H), 6.11 (s, 1H), 2.38 (s, 3H).

(*E*)-5-(2-Hydroxybenzylidene)-2(5*H*)-furanone (30a) : Yield : 41%; IR ν_{max} cm^{-1} (nujol) : 3350, 2950, 1740, 1640, 1460, 1260, 1120, 1100, 940, 910; ^{1}H-NMR (90 MHz, in DMSO) : δ 10.2 (b, 1H), 7.99 (d, *J* = 5.4 Hz, 1H), 7.96-7.10 (m, 4H), 6.85 (d, *J* = 1.98 Hz, 1H), 6.37 (dd, *J* = 5.54, *J'* = 1.98 Hz, 1H), **(*Z*)-5-(2-hydroxybenzylidene)-2(5*H*)-furanone (30b) :** Yield : 37%; mp : 166~169 °C; IR n_{max} cm^{-1} (KBr) : 3300, 2930, 1730, 1640, 1460, 1260, 1120, 1090, 940, 900; ^{1}H-NMR (90 MHz, in DMSO) : δ 10.19 (b, 1H), 8.02 (d, *J* = 5.49 Hz, 1H), 7.88 (d, *J* = 7.83 Hz, 1H), 7.30-7.13 (m, 1H), 6.96-6.90 (m, 1H), 6.65 (s, 1H), 6.39 (d, *J* = 5.4 Hz, 1H).

The mixing ratio of **(*E*)-** to **(*Z*)-5-(2-acetoxy, 5-bromobenzylidene)-2(5H)-furanone (31)** was 99% to 1%; Yield : 14%; mp : 113~115 °C; IR ν_{max} cm^{-1} (KBr) : 3100, 2900, 1790, 1760, 1740, 1540, 1460, 1370, 1280, 1230, 1210, 1160, 1100, 1020, 900, 880, 820; **(*E*)-5-(2-acetoxy, 5-bromobenzylidene)-2(5H)-furanone (31a) :** ^{1}H-NMR (90 MHz, in $CDCl_3$) : δ 7.96 (d, *J* = 2.97 Hz, 1H), 7.66-7.52 (m, 1H), 7.66-7.52 (m, 2H), 7.03-6.90 (q, 1H), 6.44 (s, 1H), 6.26 (dd, *J* = 5.4, *J'* = 0.81 Hz, 1H), 2.32 (s, 3H)

The mixing ratio of **(*E*)-** to **(*Z*)-5-(2-bromo, 5-hydroxybenzylidene)-2(5*H*)-furanone (32)** was 99% to 1%; Yield : 60%; mp : 156~160 °C; IR ν_{max} cm^{-1} (KBr) : 3350, 2930, 1740, 1650, 1470, 1250, 1130, 1100, 940, 910, 810, 720; **(*E*)-5-(2-bromo, 5-hydroxybenzylidene)-2(5*H*)-furanone (32a) :** ^{1}H-NMR (90 MHz, in $CDCl_3$) : δ 7.68 (d, *J* = 5.67 Hz, 1H), 7.55-7.43 (q, 1H), 6.88-6.69 (m, 2H), 6.46 (s, 1H), 6.32 (dd, *J* = 5.67, *J'* = 1.98 Hz, 1H)

(*E*)-5-[2-(1,4,9,10-Tetramethoxy)anthracenyl]-2(5*H*)-furanone (33) : 33 (pale-brown solid, 365 mg) is prepared by using the reaction of 1.5 g of 2-formyl-1,4,9,10-tetramethoxyanthracene and 2-(trimethylsilyloxy)furan under the same method as **1** : Yield : 20%; mp : 107~111 °C; IR ν_{max} cm^{-1} (KBr) : 3400, 2910, 1750, 1740, 1590, 1540, 1490, 1230, 1100, 1050, 1020, 960, 880, 800; ^{1}H-NMR (90 MHz, in $CDCl_3$) : δ 8.42-8.31 (q, 2H), 7.63-7.46 (m, 4H), 6.80 (s, 1H), 6.21 (d, *J* = 5.4 Hz, 1H), 4.14 (s, 3H), 4.01 (s, 3H), 3.99 (s, 3H), 3.88 (s, 3H).

(*E*)-5-[2-(1,4-Dimethoxy-9,10-dioxo)anthracenyl]-2(5*H*)-furanone (34) : To a solution of **33** (360 mg, 0.92 mmol) in 10 mL of MeCN, a solution of CAN (2.5 eq.) in 20 mL of water was added slowly at room temperature for 1 h, and stirring was continued for an additional 2 h. The reaction mixture was quenched with 50 mL of water and was extracted with MC (100 mL × 2). The combined organic extracts was washed with brine, dried over anhydrous Na_2SO_4 and evaporated *in vacuo*, which was purified by column chromatography with a gradient solvent condition (Cy.Hx : EA = 5 : 1 → 1 : 1), to obtain **34** (red solid, 291 mg) : Yield : 87%; mp : 116~119 °C; IR ν_{max} cm^{-1} (KBr) : 3400, 2930, 2850, 1780, 1740, 1660, 1590, 1400, 1350, 1240, 1100, 1040, 1000, 940, 880, 780; ^{1}H-NMR (90 MHz, in $CDCl_3$) : δ 8.47-8.34 (q, 2H), 7.85-7.71 (m, 3H), 7.54 (d, *J* = 5.67 Hz, 1H), 6.58 (s, 1H), 6.36 (d, *J* = 5.67 Hz, 1H), 4.01 (s, 6H).

(*E*)-5-[2-(1,4-Dihydoxy-9,10-dioxo)anthracenyl]-2(5*H*)-furanone (35) : To a solution of **34** (150 mg, 0.41 mmol) in 10 mL of anhydrous nitrobenzene, $AlCl_3$ (1 g, excess) was added slowly, and stirring was continued for an additional 2 h. The reaction mixture was quenched with 50 mL of water and was extracted with MC (100 mL × 2). The combined organic extracts was washed with brine, dried over anhydrous Na_2SO_4 and evaporated *in vacuo*, which was purified by column chromatography with a gradient solvent condition (Cy.Hx : EA = 3 : 1 → 1 : 1 → 1 : 3), to obtain **35** (red solid, 43.4 mg) : Yield : 32%; mp : 245~247 °C; IR ν_{max} cm^{-1} (KBr) : 3500, 2920, 2850, 1800, 1770, 1730, 1620, 1580, 1540, 1430, 1350, 1250, 1100, 1020, 940, 880, 780; ^{1}H-NMR (90 MHz, in $CDCl_3$) : δ 12.77 (s, 1H), 8.43-8.24 (q, 2H), 7.91-7.80 (m, 3H), 6.21 (d, *J* = 5.13 Hz, 1H), 6.66 (s, 1H), 6.33 (d, *J* = 5.4 Hz, 1H)

(*E*)-5-[2-(1,4,9,10-Tetramethoxy)napthalenyl]-2(5*H*)-furanone (36) : 36 (pale-brown solid, 334 mg) is prepared by using the reaction of 1 g of 2-formyl-1,4,9,10-tetramethoxynaphthalene and 2-(trimethylsilyloxy)furan under the same method as **1** : Yield : 27%; mp : 123~125 °C; IR ν_{max} cm^{-1} (KBr) : 3400, 2910, 1750, 1740, 1600, 1540, 1500, 1210, 1100, 1030, 950, 800; ^{1}H-NMR (90 MHz, in $CDCl_3$) : δ 7.86 (d, *J* = 5.4 Hz, 1H), 7.18 (s, 1H), 6.92-6.89 (m, 2H), 6.81 (s, 1H), 6.19 (d, *J* = 5.4 Hz, 1H), 4.02 (s, 3H), 3.96 (s, 3H), 3.91 (s, 3H), 3.79 (s, 3H).

(*E*)-5-[2-(5,8-Dimethoxy-1,4-dixo)naphthalenyl]-2(5*H*)-furanone (37) : 37 (red solid, 123 mg) is prepared by the same method as **34** : Yield : 45%; mp : 226~230 °C; IR ν_{max} cm^{-1} (KBr) : 3400, 2925, 2850, 1790, 1740, 1700, 1650, 1560, 1370, 1290, 1270, 1230, 1140, 1120, 820. ^{1}H-NMR (90 MHz, in $CDCl_3$) : δ 7.78 (d, *J* = 5.58 Hz, 1H), 7.56 (d, *J* = 5.4 Hz, 1H), 7.35-7.34 (m, 2H), 6.79 (s, 1H), 6.33 (d, *J* = 5.4 Hz, 1H), 3.97 (s, 6H).

(*E*)-5-[2-(1,4-Dimethoxy-5,8-dioxo)napthalenyl]-2(5*H*)-furanone (38) : 38 (red solid, 123 mg) is prepared using the same method as **34** : Yield : 38%; mp : 252~255 °C; IR ν_{max} cm^{-1} (KBr) : 3400, 2925, 1780, 1760, 1740, 1660, 1650, 1260, 1245, 1100, 1060, 1020, 950; ^{1}H-NMR (90 MHz, in $CDCl_3$) : δ 8.20 (s, 1H), 7.58 (d, *J* = 5.4 Hz, 1H), 6.81 (d, *J* = 1.57 Hz, 2H), 6.55 (s, 1H), 6.19 (dd, *J* = 5.4, *J'* = 0.72 Hz, 1H), 4.06 (s, 3H), 3.86 (s, 3H).

RESULTS AND DISCUSSION

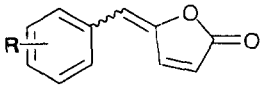
The arylidenebutenolides that were synthesized were divided into three categories according to the aromatic character of the arylidenes. Various functional groups were

introduced in the aromatic ring in order to observe their electronic and spatial effects (Scheme 1). The anthraquinone (Scheme 2) or naphthoquinone structure (Scheme 3), as a cytotoxic moiety, was linked to the furanone ring to promote a synergic effect on the cytotoxicity.

The structural determination of (*E*) or (*Z*) was based on the difference in the chemical shift of H-1, which was in agreement with the previous observation (Manat *et al.*, 1998). For example, for (*E*)-5-(3-methoxybenzylidene)-2(5*H*)-furanone (**1a**), the chemical shift of H-1 was observed at δ 6.21 as a doublet of a doublet (J = 5.4, J' = 1.7 Hz), whereas that of (*Z*)-5-(3-methoxybenzylidene)-2(5*H*)-furanone (**1b**) appeared at δ 6.05 as a doublet of a doublet (J = 5.4 Hz, J' = 0.72 Hz). In addition, the H-4 of the former appeared down field than the latter (δ 6.59, singlet vs. 6.08, singlet). On the basis of these differences, the structure of other diastereomers, as well as the *E/Z* ratio of the unresolved isomeric mixtures, can be easily determined by using the same ^{1}H-NMR spectrometric method.

The cytotoxicities of the total thirty-eight 5-arylidene-2(5*H*)-furanone derivatives were measured against human cancer cell lines (A549 : human lung cancer, NIH3T : fibroblast cancer, SK-OV-3 : human ovarian cancer). The results are demonstrated in Table I and Table II. Out of thirty-eight

Table I. The cytotoxic activity of 5-arylidene-2(5*H*)-furanone derivatives **1-32**

Compound		Cytotoxicity (ED_{50}, μg/mL)[a]		
		A-549[b]	NIH3T[c]	SK-OV-3[d]
ranunculin[e]		7.53	13.6	17.3
protoanemonin[f]		9.38	13.8	15.1
				
	R[g]			
1	3-methoxy-	>30	NT[h]	NT
2	2-methoxy-	>30	NT	NT
3	4-methoxy-	>30	NT	NT
4	H	>30	NT	NT
5	4-methyl-	>30	NT	NT
6	2-methyl-	>30	NT	NT
7	3,4-dimethoxy-	>30	NT	NT
8	2,4-dimethoxy-	>30	NT	NT
9	2,5-dimethoxy-	>30	NT	NT
10	3,4,5-trimethoxy-	>30	NT	NT
11	2-bromo, 5-methoxy-	>30	NT	NT
12	4-dimethylamino-	17.6	15.1	14.9
13	4-phenyl-	>30	NT	NT
14	3,4-methylenedioxy-	15.2	12.8	10.9
15	4-fluoro-	(*E*)[i] : 17.6, (Z)[j] : 15.8	(*E*) : 13.5, (*Z*) : 15.8	(*E*) : 18.6, (*Z*) : 15.9
16	4-bromo-	(*E*) : 14.8, (*Z*) : 12.6	(*E*) : 16.2, (*Z*) : 14.7	(*E*) : 17.4, (*Z*) : 8.0
17	4-chloro-	(*E*) : 21.0, (*Z*) : 19.6	(*E*) : 15.2, (*Z*) : 12.5	(*E*) : 6.12, (*Z*) : 17.7
18	4-nitro-	16.2	13.3	13.6
19	4-methylthiony-	>30	NT	NT
20	4-methylsulfonyl-	>30	NT	NT
21	3-nitro-	5.9	19.5	16.4
22	4-methoxy,3-nitro-	25.4	20.8	20.0
23	2,5-diacetoxy-	(*E*) : 24.7	(*E*) : 14.3	(*E*) : 20.6
24	2,5-dhydroxy-	(*E*) : 10.7	(*E*) : 17.5	(*E*) : 16.3
25	4-acetoxy-	(*E*) > 30, (*Z*) > 30	(*E*) > 30, (*Z*) > 30	(*E*) > 30, (*Z*) > 30
26	4-hydroxy-	(*E*) > 30, (*Z*) > 30	(*E*) > 30, (*Z*) > 30	(*E*) > 30, (*Z*) > 30
27	3-acetoxy-	(*E*) > 30, (*Z*) : 17.3	(*E*) > 30, (*Z*) : 20.3	(*E*) > 30, (*Z*) : 15.6
28	3-hydroxy-	(*E*) > 30, (*Z*) > 30	(*E*) > 30, (*Z*) > 30	(*E*) > 30, (*Z*) > 30
29	2-acetoxy-	(*E*) : 30.0, (*Z*) : 20.9	(*E*) : 29.9, (*Z*) : 19.8	(*E*) : 14.5, (*Z*) : 8.8
30	2-hydroxy-	(*E*) > 30, (*Z*) > 30	(*E*) > 30, (*Z*) > 30	(*E*) >30, (*Z*) > 30
31	2-acetoxy, 5-bromo-	(*E*) > 30, (*Z*) > 30	(*E*) : 16.3, (*Z*) : 21.9	(*E*) : 12.8, (*Z*) : 18.6
32	2-bromo, 5-hydroxy-	(*E*) : 19.5, (*Z*) : 20.0	(*E*) : 17.9, (*Z*) : 15.1	(*E*) : 16.8, (*Z*) : 15.2

[a]The concentration that produces a 50% reduction in cell growth, [b]Human lung cancer, [c]Fibro-blast cell, [d]Human ovarian cancer, [e-f]Ranunculin and protoanemonin were synthesized directly according to Campbells method and was used in cytotoxicity test *in vitro*, [g]The functional groups substituted in the benzene ring of 5-arylidene-2(5*H*)-furanone derivatives, [h]Not test, [i-j](*E*) is (*E*)-isomer and (*Z*) is (*Z*)-isomer in 5-arylidene-2(5*H*)-furanone derivatives.

Table II. The cytotoxic activity of 5-anthracenyl-2-(5*H*)-furanone derivatives (**33-35**) and 5-naphthalenyl-2(5*H*)-furanone derivatives (**36-38**)

Compound		Cytotoxicity (ED_{50}, μg/mL)[a]		
		A-549[b]	NIH3T[c]	SK-OV-3[d]
33	(*E*)-5-[2-(1,4,9,10-tetramethoxy)anthracenyl]-2(5H)-furanone	>30	>30	>30
34	(*E*)-5-[2-(1,4-dimethoxy-9,10-dioxo)anthracenyl]-2(5H)-furanone	16.5	9.2	10.4
35	(*E*)-5-[2-(1,4-dihydoxy-9,10-dioxo)anthracenyl]-2(5H)-furanone	30.6	23.4	29.8
36	(*E*)-5-[2-(1,4,9,10-tetramethoxy)napthalenyl]-2(5H)-furanone	>30	>30	>30
37	(*E*)-5-[2-(5,8-dimethoxy-1,4-dioxo)naphthalenyl]-2(5H)-furanone	>30	>30	>30
38	(*E*)-5-[2-(1,4-dimethoxy-5,8-dioxo)naphthalenyl]-2(5H)-furanone	>30	>30	>30

[a] The concentration that produces a 50% reduction in cell growth, [b] Human lung cancer, [c] Fibro-blast cell, [d] Human ovarian cancer

synthesized furanones, fifteen compounds showed a moderate level of cytotoxicity (ED_{50}; 30-5.9 μg/mL).

For 5-arylidene-2(5*H*)-furanone derivatives (**1-32**), the electronic effect played an important role; the compounds with an electron-withdrawing group, such as nitro, halo, and acetoxy groups, on the aromatic ring showed a stronger cytotoxicity than those with an electron-donating group, such as the methoxy, thionyl, and hydroxy groups. Among these compounds, 5'-(3-nitrobenzylidene)-2(5*H*)-furanone (**21**), 5'-(4-chlorobenzylidene)-2(5*H*)-furanone (**17**), 5'-(3-bromobenzylidene)-2(5*H*)-furanone (**16**) illustrated a significant level of cytotoxicity (ED_{50}s: 5.9-21.0 μg/mL) against all of the cancer cell-lines that were tested. Thus, it was assumed that the decrease of electron denisty in the structure enhanced the electrophilic property of the dienone in furanones, which resulted in the acceleration of the interaction with bionucleophiles.

5-Anthracenyl-2-(5*H*)-furanone derivatives (**33-35**) and 5-naphthalenyl-2(5*H*)-furanone derivatives (**36-38**) exhibited a moderate level of cytotoxicity. Thus, the synergistic effect expected from the protoanemonin and quinone moieties could not be observed. Among all the (*E*)-5-[2-(1,4-dimethoxy-9,10-dioxo)anthracenyl]-2(5*H*)-furanones, (*E*)-5-[2-(1,4-dimethoxy-9,10-dioxo)anthracenyl]-2(5*H*)-furanone (**34**) illustrated the best cytotoxicity (ED_{50}s: 9.2-16.5 μg/mL).

As shown in Table I, the configuration around the exo-double bond played no role in the cytotoxicity, which implies that that spatial influence of aryl groups on the cytotoxic furanone moiety is not a determining factor for cytotoxicity.

These findings led to the following conclusions: 1) the assumption on protoanemonin moiety as a pharmacophore was reasonable, and 2) the electron-withdrawing group on the aromatic ring in 5-arylidene-2(5*H*)-furanone derivatives potentiated the cytotoxicity, but the synergistic effect that was expected from the combination of two pharmacophores was not observed.

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