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Synthesis, Cytotoxicity and Antitumor Activity of 2,3-Diarylcyclopent-2-ene-1-ones

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Two series of 2,3-diarylcyclopent-2-ene-1-ones including 2-aryl-3-(2,5-dihydroxyphenyl)cyclopent-2-ene-1-ones (**2a~2f**) and 3-aryl-2-(3',4',5'-trimethoxyphenyl)cyclopent-2-ene-1-one (**3a~3j**) were synthesized and evaluated for the cytotoxicity against three tumor cell lines; B16F10, HCT116 and A431. It was found that the 3,4,5-trimethoxy substituent was optimal for the bioactivity of compounds in series **2**. Meanwhile, compounds in series **3** exhibited the most potent cytotoxicity with 3-aryl ring being 4-methoxyphenyl (compound **3f**), (3-hydroxy-4-methoxy)phenyl (compound **3e**), or (3-amino-4-methoxy)phenyl (compound **3j**).

Key words: 2,3-Diarylcyclopent-2-ene-1-ones, Cytotoxicity, and Antitumor activity

INTRODUCTION

In our preceding paper we have reported compound **1a** as a potent cytotoxic agent in both murine and human tumor cell lines (Nam *et al.*, 2002a). Further manipulation carried out on this small molecule revealed that substitution of the electron-withdrawing groups like bromine into α-carbon led to compound **1a** with decreased cytotoxicity. Replacement of the a-bromide by a methyl group also decreased activity. However, when the phenyl ring was attached at this position, compound **1d** was found to have twice activity of **1a**. Thus, the phenyl group seemed to be favorable for the bioactivity of this compound. It was postulated that the phenyl moiety probably enhanced interaction of the compound with a complementary aryl moiety at a binding site on receptors through a van der Waals bonding. If that, introduction of different substituents on this phenyl ring may significantly affect the bioactivity of the compound. In this study, we have prepared a series of compound **2** and found that the 3,4,5-trimethoxy substituent deemed optimal for this formula's activity. We fixed this substituted pattern on this ring and continued to investigate various substitutes for the 2,5-dihydroxy moiety. In this paper we would like to detail the results obtained from these investigations.

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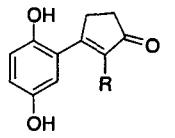

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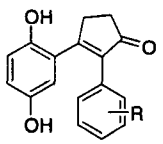

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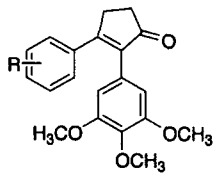
a R = H, **b** R = Br
c R = CH_3, **d** R = Ph



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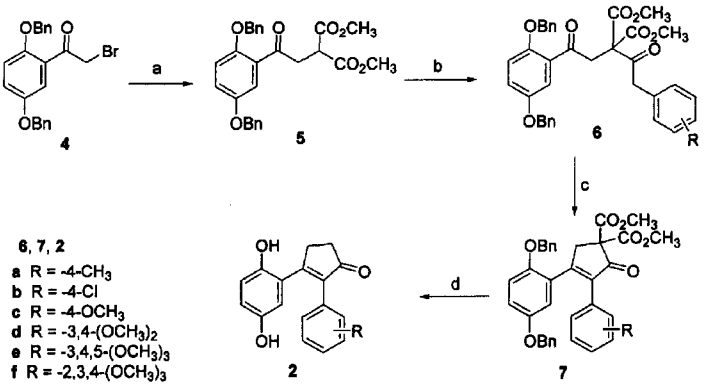
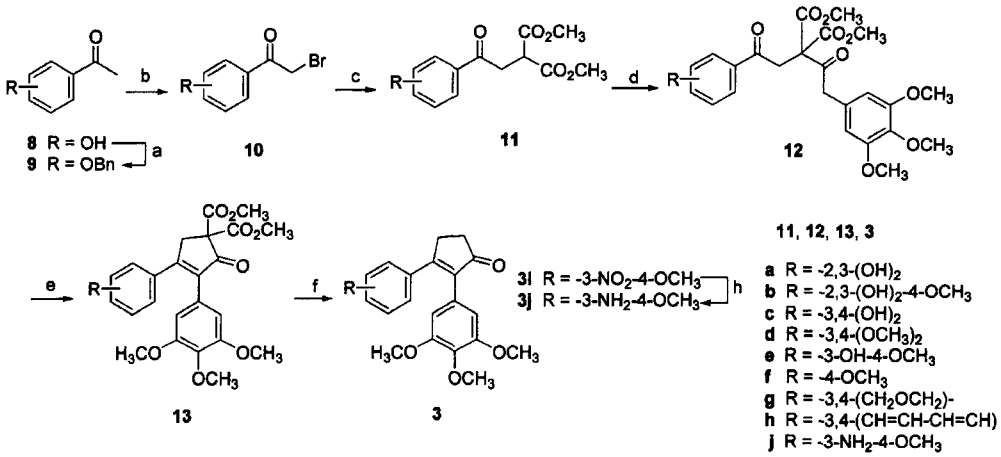
RESULTS AND DISCUSSION

Chemistry

The synthesis of a series of 3-(2,5-dihydroxyphenyl)-2-arylcyclopent-2-ene-1-one (**2a~2f**) was completed using the same method described in our previous report (Nam *et al.*, 2002a). Briefly, alkylation of dimethyl malonate with **4**, which was prepared as detailed in literature (Nam *et al.*, 2002a), gave **5**. The alkylation was found most effective by slow addition of a solution of **4** in acetone to a mixture of dimethyl malonate and K_2CO_3 in anhydrous acetone at 45°C. Acetylation of **5** to give **6** was mediated by



Scheme 1. *Reagents and Conditions*: (a) K_2CO_3, dimethyl manolate, 79-85%, 40°C, 5 h; (b) i) $MgBr_2.Et_2O$, pyridine, 0°C, 3 h; ii) $ArCH_2COCl$, 75-83%, -25°C, 2 h then 1N HCl (Ar = R-phenyl); (c) TEA, 95-100%, acetonitrile, 30 min; (d) 3*M* H_2SO_4/AcOH, 27-41%, rfx, 10 h. For R groups, refer to Table 1.



Scheme 2. *Reagents and Conditions*: (a) BnCl, K_2CO_3, 88-90%, Acetone, reflux; (b) Br_2, 90-95%, CH_3Cl, rt, 3 h; (c) K_2CO_3, 93-96%, dimethyl manolate, 40°C, 5 h; (d) i) $MgBr_2.Et_2O$, pyridine, 0°C, 3 h; ii) 3,4,5-trimethoxyphenylacetyl chloride, 86-96%, -25°C, 2 h then 1N HCl; (e) TEA, 98-100%, acetonitrile, 30 min; (f) 3*M* H_2SO_4/AcOH, 68-73%, rfx, 10 h; (h) Zn/AcOH, 68%, rt, 2 h.

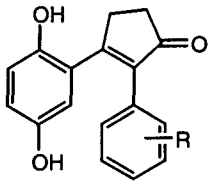
$MgBr_2.Et_2O$ using pyridine as base. The optimal procedure for this acetylation involved preparation of the Mg enolate, using $MgBr_2.Et_2O$-Pyridine in a mixture of MeCN and THF at 0°C, followed by reaction with acetyl chloride at 30°C. THF was used as a co-solvent to prevent acetonitrile from freezing. This procedure gave **6** in good yield (49-77%) Cyclization of **6** was readily achieved using TEA as base. Decarboxylation of **7** was effected by refluxing in 3*M* H_2SO_4-AcOH at 90°C for 10 h. Con-veniently, under this condition both benzyl groups used to protect the phenol hydroxyls were removed to afford **2** in one step.

A series of 3-aryl-2-(3,4,5-trimethoxyphenyl)cyclopent-2-ene-1-one (**3a~3i**) was obtained by yet the same method for **2**, starting from bromoacetophenones (**10**). Most of the bromoacetophenones **10** were commercially available. Those not purchasable were easily synthesized by bromination of the corresponding acetophenones, protected as benzyloxyacetophenone wherever the phenolic hydroxy group(s) was present (Nam *et al.*, 2001a). Alkylation of dimethyl manolate with **10** gave the intermediates **11** which were then acylated with 3,4,5-trimethoxyphenylacetyl chloride to give **12**. Subsequent cyclization of **12** led to the pentenones **13**. Decarboxylation of **13** and concomitant removal of the benzyl protecting group(s) furnished the final products **3a~3i**. Compound **3j** was obtained in moderate yield (68%) by reduction of the nitro group in **3i** using Zn/AcOH.

Cytotoxicity and antitumor activity

We reasoned that if the cytotoxicity of compound **1d** was potentiated by enhancement of the binding affinity of the compound toward the complementary hydrophobic moiety at a binding site on receptors through a van der Waals bonding, the introduction of the electron rich groups should be beneficial. Therefore we focused on these kinds of substituents. Accordingly a series of **2**'s analogues, compounds **2a~f**, having various electron releasing groups (ERGs) were prepared. For the purpose

Table 1. Cytotoxicities of Compounds 2a~2f in Some Cancer Cell Lines[1]



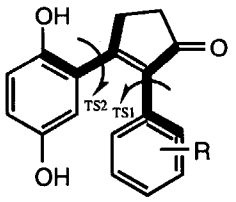
Cpd	R	Cytotoxicity (IC_{50}[2], μg/mL)			
		B16F10	HCT116	A431	AP
2a	-4-CH_3	1.21	1.75	2.01	1.66
2b	-4-Cl	2.00	3.71	1.53	2.41
2c	-4-OCH_3	0.34	0.31	0.27	0.30
2d	-3,4-$(OCH_3)_2$	0.42	0.39	0.35	0.38
2e	-3,4,5-$(OCH_3)_3$	0.11	0.23	0.15	0.16
2f	-2,3,4-$(OCH_3)_3$	1.01	1.74	0.99	1.25
1d	-H	0.55	0.41	0.21	0.39
ADR[3]		0.21	0.78	1.01	0.67

[1]Cancer cell lines: B16F10, murine melanoma; HCT116, colon cancer; A431, human epidermoid carcinoma. [2]A samples concentration produces a 50% reduction in cell growth. The values shown were the averages from a triplicate experiment. [3]Adriamycin, used as a positive control.

of comparison, one compound (**2b**), possessing chlorine as an ERG was also synthesized. The bioactivity of this series was measured in the three tumor cell lines including B16 (murine melanoma), HCT116 (human colon), and A431 (human epithelial carcinoma). The results, expressed as IC_{50} values, are shown in Table 1. The average IC_{50} values (AP) of each compound in three tumor cell lines were also calculated and the results from these calculations were included in this table.

A review of the data presented in table 1 revealed that the introduction of the EWG, e.g. chlorine, was found to raise the AP value in three tested cell lines by more than 7 times; thus, **2b** was 7-fold less active than **1d**. In five compounds having additional ERG(s), the cytotoxicity was found to be enhanced in three, or 60% of compounds, suggesting that the hypothesis proposed above is likely viable for this cluster of compounds. Compound **2a** possessing a methyl substituent did not show enhancement in bioactivity, indicating that the oxygen atom in methoxy group was important, probably for additional hydrogen binding between this molecule and a complementary hydroxy or amino moiety at the binding site of receptors. The activity was found to be optimal with the 3,4,5-trimethoxy substituent in this series where the AP value was recorded at 2.4 times lower than that of **1d**, while compounds bearing 4-methoxy or 3,4-dimethoxy groups were shown to be equipotent with the parent **1d**. A marked disparity in the cytotoxicity between

Table 2. Average Potency of 2e, 2f and Torsional Angles q_1 and q_2 of the two Aryl Rings at C_a and C_b around the plane of the Olefinic Moiety

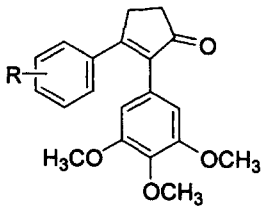


Cpd	R	AP[1]	Torsional Angle[2]	
			(TS1) θ_1	(TS2) θ_2
2e	-3,4,5-$(OCH_3)_3$	0.16	-19.5655	-24.8850
2f	-2,3,4-$(OCH_3)_3$	1.25	-25.9648	-25.8284

[1]Average IC_{50} values (mg/mL) of the compound in three cancer cell lines: B16, HCT116, and A431. carcinoma. [2]The minus value indicates the anticlockwise rotation of the phenyl ring against the olefinic plane.

2e and **2f** was noted where **2e** was nearly 8-fold as potent as **2f**. In these two compounds, the electronic nature was expected to be much similar. Hence, a cause of this discrepancy in bioactivity was mainly resulted from the position of substituted groups. The methoxy substituent at 2-position in **2f** possibly affected the conformation of this compound markedly. To glean some insights into this possible reason, the conformations of the two compounds were generated and compared. As predicted, in **2f** the presence of a methoxy group at the ortho position of the phenyl ring attached at C-α markedly forced the plane of this phenyl moiety rotating anticlockwise out of the plane of the olefinic linkage with a torsional angle (C_{ortho}-C_1-C_{α}-$C_{carbonyl}$) of 25.9648° (Table 2). The plane of the 2,5-dihydroxyphenyl ring was also rotated anticlockwise by 25.8284° out of the olefinic resonance. Whereas, in **2e** a greater degree of coplanarity between the two aryl rings and this olefinic linkage was observed. The corresponding torsional angles in **2e** were -19.5655° and -24.8850°, respectively. From this result one observation could be drawn out that, an optical torsion angles might be necessary for the bioactivity of this compound series. To examine this, several changes in the position of substituents on the β-phenyl ring in an effort to achieving a higher degree of planarity in the compound conformations were contemplated. Since, the α-3,4,5-trimethoxyphenyl moiety deemed optimal for the bioactivity, it was kept intact throughout this line of modifications. Initially, it was thought that the dihydroxy functionality was still important in mediating the cytotoxicity, effort was made to keep this moiety present and only the substituted pattern in the *b*-phenyl ring was changed. It was also perceived from viewing the conformations of **2e** and **2f** that the 5-position was not favorable for the planarity of the conformation of this compound series; accordingly the preparation of

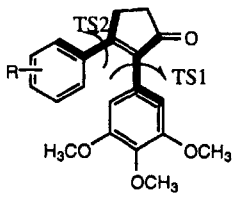
Table 3. Cytotoxicity of Compounds 3a~3j in Some Cancer Cell Lines[1]



Cpd	R	Cytotoxicity (IC_{50}[2], μg/mL)		
		B16	HCT116	A431
3a	-2,3-$(OH)_2$	0.781	0.953	0.629
3b	-2,3-$(OH)_2$-4-OCH_3	0.656	0.474	0.357
3c	-3,4-$(OH)_2$	0.090	0.092	0.120
3d	-3,4-$(OCH_3)_2$	0.073	0.067	0.091
3e	-3-OH-4-OCH_3	0.013	0.012	0.009
3f	-4-OCH_3	0.015	0.017	0.021
3g	-3,4-(CH_2OCH_2)-	0.030	0.053	0.112
3h	-3,4-(CH=CH-CH=CH)	0.029	0.031	0.034
3j	-3-NH_2-4-OCH_3	0.008	0.007	0.009
ADR		0.11-0.19	0.47-0.58	0.87-0.99

[1]Cancer cell lines: B16, murine melanoma; HCT116, colon cancer; A431 human epidermoid carcinoma. [2]A samples concentration produces a 50% reduction in cell growth. The values shown were the averages from a triplicate experiment. [3]Adriamycin, used as a positive control.

Table 4. Average potency of some compounds from series 3 and torsional angles q_1 and q_2 of the two aryl rings at C_a and C_b around the plane of the olefinic moiety



Cpd	R	AP[1]	Torsional Angle	
			(TS1) θ_1	(TS2) θ_2
2e	-2.5-$(OH)_2$	0.16	-24.8850	-19.5655
3e	-3-OH-4-OCH_3	0.011	20.1d85	18.3686
3f	-4-OCH_3	0.017	16.1287	18.91d6
3j	-3-NH_2-4-OCH_3	0.008	18.7567	19.0506

[1]Average IC_{50} values (mg/mL) in three cell lines as shown in Table 1 and 3.

compounds **3a**, **3c** was firstly suggested. Compound **3b** possessing one additional methoxy group was synthesized in an attempt to gauge the electronic effect of the substituent in this ring on bioactivity. IC_{50} values of the synthesized compounds in three cancer cell lines B16, HCT116 and A431 are shown in Table 3. The figures therein demonstrated that compound **3c** bearing a 3,4-dihydroxy moiety was more cytotoxic than **2e** which possessed a 2,5-dihydroxy functionality. It has been shown earlier that the 2,5-dihydroxy group was most favorable for bioactivity in the compounds where the formation of quinonoids was one of the predominant mechanisms of action (Nam *et al.*, 2001b). Thus, these results suggest that for the cluster of compounds in series **3**, the formation of quinones may not be important for bioactivity. This postulation was strongly espoused by compound **3d**. This compound bears a 3,4-dimethoxy substitution instead of the 3,4-dihydroxy moiety previously present in **3c**, thus the formation of quinonoid metabolites should be blocked. Actually however, **3d** was found to be more potent than **3c**.

It was noted that compound **3a** was least active in this series, further confirming that the presence of an *ortho*-substituent was not favorable for bioactivity, probably due to distorsion of the molecules conformation caused by the *ortho*-substituent as explained earlier for **2f**.

Compound **3b** bearing an additional methoxy in *para*-position was found to be slightly more potent than **3a**, thus the introduction of the methoxy group at this position seemed to be beneficial for cytotoxicity. Should this be the case, compound **3e**, resulting from **3c** by methylation of the *para*-hydroxy moiety, would be more active than **3c**. Interestingly, **3e** was revealed to be roughly 7-fold as potent as **3c** in B16 and HCT116 cells (IC_{50} values of **3e** and **3c** were 0.013 and 0.090 μg/mL in B16 and 0.012 and 0.092 μg/mL in HCT116, respectively). In A431 cell line, an even greater disparity in bioactivity between the two compounds was recorded; **3c** was found to be more than one magnitude less potent than **3e** (IC_{50} of 0.120 vs. 0.009 μg/mL). This result clearly confirmed the benefit conferred to bioativity by the *para*-methoxy group. Further, to clarify a role of the *meta*-hydroxy group, compound **3f** was prepared and evaluated. It was found that **3f** was essentially equipotent to **3e**, suggesting that the *meta*-hydroxy moiety was not important for cytotoxicity. However, in view of a highly lipophilic nature of the compound, it could be envisaged that this moiety might be important for enhancement of hydrophilicity of this compound. In addition, it could be employed as a site for a further manipulation to improve an aqueous solubility. In vein of this rationale, compound **3j** was synthesized. In this analogue an amine group, having a lower π (the hydrophobicity constant) and lower δ (the Hamlette constant) values in Craig's plot was chosen to replace the *meta*-hydroxy group in **3e**, thus it might impart **3j** with a greater hydrophilicity. As expected, **3j** showed greater water solubility compared to **3e** (Table 5), and we could further manipulate its amine moiety to improve an aqueous solubility.

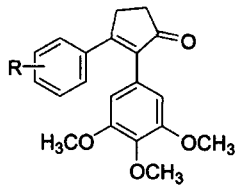
The two compounds **3g** and **3h** were prepared to gain some discernment toward whether bulkier substituents are

Table 5. Water Solubility of Some Representative Compounds from a Series 3

Cpd	Water Solubility (mg/mL)[1]	Cpd	Water Solubility (mg/mL)
3e	<0.5	3j	0.8
3f	<0.5	3j. HCl	2.1
3h	<0.5		

[1]Measured at 25°C by a method described in Experimentals

Table 6. Antitumor Activity of Some Representative Compounds from a Series 3



Cpd	R	Antitumor activity	
		Dose (mg/kg/day)	IR[1] (%)
3e	-3-OH-4-OCH_3	15	37
3f	-4-OCH_3	15	-
3h	-3,4-(CH=CH-CH=CH)	15	-
3j. HCl	-3-NH_2-4-OCH_3	40	59
ETP[2]		36	71

[1]Inhibiton rate(%)=(1-T/C) × 100; T = mean tumor volume of the same-treated group, C = mean tumor volume of the negative control group; mice were implanted s.c. with 10^7 3LL cells/mouse, and the drugs were administered i.p.; [2]Etoposide, used as a positive control, dosed i.p. at 1, 5, and 9 day.

possible at these positions. The results suggest that introduction of the methylenedioxy or even an incorporation of a second benzene ring was tolerable though not favorable for bioactivity of this compound series.

At this stage, careful analysis of the structure-activity relationship of compounds in series **3** and their structural features, we noted that the compounds in this series could be considered as analogues of combretastatin A-4 [1-(3-hydroxy-4-methoxy)phenyl-2-(3,4,5-trimethoxy)phenyl-ethene]. More details comparison in this regard could be found in our recent publication (Nam *et al.*, 2002c).

In vivo evaluation of some representative compounds was performed with **3e**, **3f**, **3h** and **3j** in BDF1 mice bearing 3LL (Lewis lung carcinoma) cells. Due to a limited solubility in aqueous system **3e**, **3f**, and **3h** were administered at 15 mg/kg/day. Compound **3j** in a form of hydrochloride salt was evaluated at three doses, i.e. 40, 20 and 10 mg/kg/day. **3e** was found to show a marginal activity in this model with inhibition rate of 37% compared to 71% of etoposide which was used as a positive control (Table 6). The two compounds **3f** and **3h** did not show any significant inhibition on the growth of 3LL cells in the experimented mice, probably due to the low bioavailability caused by their poor water solubility (Table 5). The activity displayed by **3j**, which was more water-soluble, seemed to espouse this postulation. Evaluated at a higher dose than the preceding two compounds, i.e. at 40 mg/kg/day, **3j**.HCl produced a marked reduction in tumor mass. The inhibition rate was recorded at 59%, but still lower than that of etoposide (71%), one useful drug currently used in cancer chemotherapies. It was noteworthy that mice treated with **3j**.HCl at 40 mg/kg/day did not manifest any weight loss or decrease in water and food consumption, suggesting a non-toxic nature of this agent at the evaluated dose and thus, elevation of dosage of the compound is possible given that its pharmacokinetics, e.g. water solubility, are improved. The preparation of a series of this compound's prodrugs has been initiated and the results from this investigation will be reported elsewhere (Nam *et al.*, 2002d).

MATERIALS AND METHODS

Chemistry

Common chemicals, solvents were purchased from commercial source and distilled before use. Protected amino acids were purchased from either Sigma Aldrich Co. Ltd. (USA) or Fluka Co. Ltd. (USA). All ^{1}H-NMR were recorded using either a JEOL JNM-EX 90 (90 MHz) FT spectrometer or a Varian-Gemini 300 (300 MHz) spectrometer. Chemical shifts are reported in ppm (δ) and coupling constants are reported in Hz. Infrared spectra were measured in KBr plates on a Perkin-Elmer 1600 series FTIR. Melting points were determined using an Electrothermal IA6304 open capillary melting point apparatus and are uncorrected. Syntheses of compounds and biological procedures are described as follow.

Dimethyl 2-[2'-(2',5'-dibenzyloxyphenyl)-2'-oxoethyl]propane-1,3-dioate (5a)

A solution of 2-bromo-2',5'-dibenzyloxyacetophenone (20.76 g, 50.5 mmol) in acetone (110 mL) was added to the mixture of dimethyl malonate (53 g, 401 mmol) and K_2CO_3 (14 g, 101 mmol) over 5 h at 40°C. The mixture was stirred for an additional 1 h at the same temperature and cooled to room temperature. Evaporation of the solvent gave an oil that was suspended in water and extracted with MC. The MC layer was concentrated to give a residue which was purified over a silica gel column to yield dimethyl 2-[2'-(2',5'-dibenzyloxyphenyl)-2'-oxoethyl]propane-1,3-dioate(**5**,18.75 g, 84%) as a yellowish oil which was used for the next step without any further purification.

Dimethyl2-[2'-(2',5'-dibenzyloxyphenyl)-2'-oxoethyl]-2-(4'-methylphenylacetyl)propane-1,3-dioate (6a)

The oil 5a (10.58 g, 22.8 mmol) was dissolved in THF

(30 mL) and acetonitrile (30 mL). To the resulting solution was added magnesium bromide etherate (5.90 g, 22.8 mmol) and pyridine (3.8 mL, 47.0 mmol). The mixture was stirred for 3 h at room temperature and cooled to 30°C. After 30 min, (4-methylphenyl)acetyl chloride (22.8 mmol) was dropwise added over 30 min. The mixture was aged for a further 2 h and quenched with 30 mL of aqueous 1 *N* HCl. The reaction mixture was extracted with MC. The MC layer was dried, evaporated give a yellowish oil (6a, 9.28 g, 81%). ^{1}H-NMR ($CDCl_3$, 90 MHz) δ 2.13 (3H, s), 3.85 (6H, s), 3.95 (2H, s), 4.21 (2H, s), 5.25 (4H, overlapped), 6.84-7.03 (4H, m), 7.09-7.35 (9H, m), 7.41-7.55 (4H, m).

Methyl 4-(2',5'-dibenzyloxyphenyl)-1-methoxycarbonyl-3-(4'-methylphenyl)-2-oxocyclopent-3-ene-carbox-ylate (7a)

The oil **6a** (9.28 g, 18.2 mmol) was directly dissolved in 30 mL of acetonitrile. To the resulting solution was added 0.5 mL of triethylamine and the mixture was stirred for 30 min at room temperature. After that the reaction mixture was concentrated to give a residue (**7a,** 8.35 g, 100%). ^{1}H-NMR ($CDCl_3$, 90 MHz) d 2.16 (3H, s), 3.68 (2H, s), 3.87 (6H, s), 6.79-7.05 (2H, m), 7.21-7.35 (3H, m), 7.41-7.57 (2H, m).

3-(2',5'-Dihydroxyphenyl)-2-(4'-methylphenyl)cyclopent-2-ene-1-one (2a)

The residue **7a** was treated with a mixture of concentrated sulfuric acid (3 mL) in acetic acid (100 mL) for 8 h at 90°C to provide the final product **2a** (1.71 g). Yield 35%; IR (KBr) 3420, 3020, 2910, 161d, 1620, 1582, 1250 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 90 MHz) δ 2.17 (3H, s), 2.71-2.86 (2H, m), 2.98-3.21 (2H, m), 6.57-6.71 (2H, m), 6.3-7.14 (3H, m), 7.22-7.37 (2H, m).

Other compounds 6, 7 and 2 (b, R = 4-Cl; c, R = 4-OCH_3; d, R = 3,4-$(OCH_3)_2$; e, R = 3,4,5-$(OCH_3)_3$; and f, R =2,3,4-$(OCH_3)_3$) were synthesized by the same procedures described for 5a, 6a, and 2a, respectively. Only spectral data of the final compounds 2 (b, c, d, e, f) are shown here.

2-(4-Chlorophenyl)-3-(2',5'-Dihydroxyphenyl)cyclopent-2-ene-1-one (2b)

Yield 27%; IR (KBr) 3450, 3010, 2910, 1690, 1620, 151d, 1250 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 90 MHz) δ 2.84-2.94 (2H, m), 3.02-3.27 (2H, m), 6.44-6.61 (2H, m), 6.88 (1H, s), 7.20-7.34 (2H, m), 7.41-7.55 (2H, m).

3-(2',5'-Dihydroxyphenyl)-2-(4'-methoxyphenyl) cyclopent-2-ene-1-one (2c)

Yield 29%; IR (KBr) 3400, 3020, 2910, 161d, 1620, 1575, 1250 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 90 MHz) δ 2.81-2.93 (2H, m), 2.99-3.21 (2H, m), 375 (3H, s), 6.55-6.67 (2H, m), 6.88 (1H, s), 7.05-7.27 (2H, m), 7.35-7.46 (2H, m).

3-(2',5'-Dihydroxyphenyl)-2-(3',4'-dimethoxyphenyl) cyclopent-2-ene-1-one (2d)

Yield 38%; IR (KBr) 3420, 3020, 2910, 1690, 1620, 158, 1250 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 90 MHz) δ 2.2-2.95 (2H, m), 3.00-3.23 (2H, m), 3.78 (3H, s), 3.86 (3H, s), 6.50-6.66 (2H, m), 6.68-6.99 (2H, m), 7.11-7.22 (2H, m).

3-(2',5'-Dihydroxyphenyl)-2-(3',4',5'-trimethoxyphenyl)cyclopent-2-ene-1-one (2e)

Yield 41%; IR (KBr) 3400, 3010, 2910, 1685, 1615, 151d, 1250 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 90 MHz) δ 2.85-2.97 (2H, m), 3.01-3.27 (2H, m), 3.78 (3H, s), 3.84 (6H, s), 6.41-6.62 (2H, m), 6.72 (2H, s), 6.88-6.92 (1H, m).

3-(2',5'-Dihydroxyphenyl)-2-(2',3',4'-trimethoxyphenyl)cyclopent-2-ene-1-one (2f)

Yield 40%; IR (KBr) 3430, 3010, 2910, 1690, 1620, 151d, 1250 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 90 MHz) δ 2.75-2.81 (2H, m), 2.91-3.12 (2H, m), 3.77 (3H, s), 3.82 (3H, s), 3.2 (3H, s), 6.56-6.73 (2H, m), 6.77-6.3 (2H, m), 6.91-6.99 (1H, m).

Dimethyl2-[2'-(4'-methoxyphenyl)-2'-oxoethyl]propane-1,3-dioate (11f)

A solution of 2-bromo-4'-methoxyacetophenone (11.51 g, 50.5 mmol) in acetone (110 mL) was added to the mixture of dimethyl malonate (53 g, 401 mmol) and K_2CO_3 (14 g, 101 mmol) over 5 h at 40°C. The mixture was stirred for an additional 1 h at the same temperature and cooled to room temperature. Evaporation of the solvent gave an oil that was suspended in water and extracted with MC. The MC layer was concentrated to give a residue which was purified over a silica gel column to yield dimethyl 2-[2'-(4'-methoxyphenyl)-2-oxoethyl]propane-1,3-dioate (**11f,** 12.76 g, 95%) as a yellowish oil. ^{1}H-NMR ($CDCl_3$, 90 MHz) δ 3.64 (2H, d, J = 7.00 Hz), 3.77 (3H, s), 3.81 (6H, s), 4.08 (1H, t, *J* = 7.00 Hz), 7.02 (2H, m), 7.54 (2H, m).

Dimethyl2-[2'-(4'-methoxyphenyl)-2'-oxoethyl]-2-(3',4',5'-trimethoxyphenylacetyl)propa-ne-1,3-dioate (12f)

The oil **11f** (6.38 g, 22.8 mmol) was dissolved in THF (30 mL) and acetonitrile (30 mL). To the resulting solution was added magnesium bromide etherate (5.90 g, 22.8 mmol) and pyridin (3.8 mL, 47.0 mmol). The mixture was stirred for 3 h at room temperature and cooled to 30°C. After 30 min, 3,4,5-trimethoxyphenylacetyl chloride (4.48 g, 22.8 mmol) was dropwise added over 30 min. The mixture was aged for a further 2 h and quenched with 30

mL of aqueous 1 N HCl. The reaction mixture was extracted with MC. The MC layer was dried, evaporated give a yellowish oil (**12f**, 8.90 g, 94%). ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 3.85 (3H, s), 3.86 (6H, s), 3.87 (3H, s), 3.3 (6H, s), 3.90 (2H, s), 4.21 (2H, s), 6.55 (2H, s), 7.05 (2H, d, *J* = 8.31 Hz), 7.44 (2H, d, *J* = 8.31 Hz).

Methyl 4-(4'-methoxyphenyl)-1-methoxycarbonyl-3-(3',4',5'-trimethoxyphenyl)-2-oxocycl-opent-3-ene-carboxylate (13f)

The oil **12f** (8.9 g, 18.2 mmol) was directly dissolved in 30 mL of acetonitrile. To the resulting solution was added 0.5 mL of triethylamine and the mixture was stirred for 30 min at room temperature. After that the reaction mixture was concentrated to give a residue (**13f**, 8.57 g, 100%). ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 3.72 (3H, s), 3.77 (3H, s), 3.85 (6H, s), 3.86 (6H, s), 6.62 (2H, s), 7.00 (2H, d, *J* = 8.40 Hz), 7.55 (2H, d, J = 8.40 Hz),

3-(4'-Methoxyphenyl)-2-(3',4',5'-trimethoxyphecy-clopent-2-ene-1-one (3f)

The residue **13f** was treated with a mixture of concentrated sulfuric acid (3 mL) in acetic acid (100 mL) for 8 h at 90°C to provide the final product **3f** (4.69 g, 74%). ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 2.64-2.82 (2H, m), 3.14-3.34 (2H, m), 3.73 (3H, s), 3.87 (6H, s), 3.88 (3H, s), 6.58 (2H, s), 6.88 (2H, d, *J* = 8.49 Hz), 7.67 (2H, d, *J* = 8.49 Hz).

Other compounds of the general structures **11**, **12**, **13**, and **3** (**a**, R = 2,3-$(OH)_2$; **b**, R = 2,3-$(OH)_2$-4-OCH_2; **c**, R = 3,4-$(OH)_2$; **d**, R = 3,4-$(OCH_3)_2$; **e**, R = 3-OH-4-OCH_3; **g**, R = 3,4-(CH_2OCH_2); **h**, R = 3,4-(CH=CH-CH=CH); and **i**, R = 3-NO_2-4-OCH3 (Table 3)) were synthesized by the same procedures described for **11f**, **12f**, **13f**, and **3f**, respectively. Only the spectral data for the final series **3** are shown here.

3-(2',3'-Dihydroxyphenyl)-2-(3',4',5'-trimethoxyphe-nyl)cyclopent-2-ene-1-one (3a)

This compound was synthesized from 2-bromo-2,3-dibenzyloxyacetophenone, dimethyl malonate and 3,4,5-trimethoxyphenylacetyl chloride by the same methodology as described for **3f**. Yield: 73%, ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 2.67-2.85 (2H, m), 3.12-3.24 (2H, m), 3.77 (3H, s), 3.87 (6H, s), 6.61 (2H, s), 6.66 (2H, d, *J* = 7.87 Hz), 6.70-6.84 (2H, m), 6.86 (1H, d, *J* = 7.87 Hz).

3-[(2',3'-Dihydroxy-4'-methoxy)phenyl]-2-(3',4',5'-tri-methoxyphenyl)cyclopent-2-ene-1-one (3b)

This compound was synthesized from 2-bromo-(2,3-dibenzyloxy-4-methoxy)acetophenone, dimethyl malonate and 3,4,5-trimethoxyphenylacetyl chloride by the same methodology as described for **3f**. Yield: 70%, ^{1}H-NMR ($CDCl_3$, 90 MHz) δ 2.64-2.81 (2H, m), 2.3-3.15 (2H, m), 3.81 (3H, s), 3.88 (6H, s), 3.95 (3H, s), 6.57 (2H, s), 6.67-6.85 (2H, m).

3-(3',4'-Dihydroxyphenyl)-2-(3',4',5'-trimethoxyphe-nyl)cyclopent-2-ene-1-one (3c)

This compound was synthesized from 2-bromo-3,4-dibenzyloxyacetophenone, dimethyl malonate and 3,4,5-trimethoxyphenylacetyl chloride by the same methodology as described for **3f**. Yield: 68%, ^{1}H-NMR ($CDCl_3$, 90 MHz) δ 2.64-2.75 (2H, m), 2.3-3.03 (2H, m), 3.81 (3H, s), 3.86 (6H, s), 6.58 (2H, s), 6.66-6.87 (2H, m), 6.98-7.05 (1H, m).

3-(3',4'-Dimethoxyphenyl)-2-(3',4',5'-trimethoxyphe-nyl)cyclopent-2-ene-1-one (3d)

This compound was synthesized from 2-bromo-3,4-dimethoxyacetophenone, dimethyl malonate and 3,4,5-trimethoxyphenylacetyl chloride by the same methodology as described for **3f**. Yield: 71%, ^{1}H-NMR ($CDCl_3$, 90 MHz) δ 2.74-2.88 (2H, m), 2.96-3.14 (2H, m), 3.78 (3H, s), 3.82 (3H, s), 3.86 (6H, s), 3.94 (3H, s), 6.62 (2H, s), 6.68-6.3 (2H, m), 7.05 (1H, s).

3-[(3'-Hydroxy-4'-methoxy)phenyl]-2-(3',4',5'-triethox-yphenyl)cyclopent-2-ene-1-one (3e)

This compound was synthesized from 2-bromo-(3-benzyloxy-4-methoxy)acetophenone, dimethyl malonate and 3,4,5-trimethoxyphenylacetyl chloride by the same methodology as described for **3f**. Yield: 70%, ^{1}H-NMR ($CDCl_3$, 90 MHz) δ 2.65-2.76 (2H, m), 3.02-3.13 (2H, m), 3.74 (3H, s), 3.78 (6H, s), 3.85 (3H, s), 6.47 (2H, s), 6.2-7.07 (2H, m), 7.15 (1H, s).

3-(3',4'-Methylenedioxyphenyl)-2-(3',4',5'-trimethox-yphenyl)cyclopent-2-ene-1-one (3g)

This compound was synthesized from 2-bromo-3,4-methylenedioxyacetophenone, dimethyl malonate and 3,4,5-trimethoxyphenylacetyl chloride by the same methodology as described for **3f**. Yield: 72%, ^{1}H-NMR ($CDCl_3$, 90 MHz) δ 2.72-2.84 (2H, m), 2.92-3.12 (2H, m), 3.81 (3H, s), 3.85 (6H, s), 5.95 (2H, s), 6.62 (2H, s), 6.81-7.05 (3H, m).

3-(2'-Naphthyl)-2-(3',4',5'-trimethoxyphenyl)cyclo-pent-2-ene-1-one (3h)

This compound was synthesized from 2-bromonaphthone, dimethyl malonate and 3,4,5-trimethoxyphenylacetyl chloride by the same methodology as described for **3f**. Yield: 68%, ^{1}H-NMR ($CDCl_3$, 90 MHz) δ 2.64-2.82 (2H, m), 3.14-3.34 (2H, m), 3.82 (6H, m), 3.93 (3H, m), 6.51 (2H, s), 7.25-7.67 (3H, m), 7.88-8.00 (4H, m).

3-[(3'-Nitro-4'-methoxy)phenyl]-2-(3',4',5'-trimethox-yphenyl)cyclopent-2-ene-1-one (3i)

This compound was synthesized from 2-bromo-(3-nitro-4-methoxy)acetophenone, dimethyl malonate and 3,4,5-trimethoxyphenylacetyl chloride by the same methodology as described for **3f**. Yield: 69%, ^{1}H-NMR ($CDCl_3$, 90 MHz) δ 2.58-2.72 (2H, m), 2.84-3.02 (2H, m), 3.76 (3H, s), 3.85 (3H, s), 3.88 (6H, s), 6.67 (2H, s), 7.05-7.34 (2H, m), 7.81 (1H, s).

3-[(3'-Amino-4'-methoxy)phenyl]-2-(3',4',5'-trimethoxyphenyl)cyclopent-2-ene-1-one (3j)

This compound was synthesized by reduction of the nitro group in **3i** using Zn/AcOH and procedures described in our previous publication (Nam *et al.*, 2001a). Yield: 68%; m.p. ; ^{1}H-NMR ($CDCl_3$, 90 MHz) δ 2.59-2.73 (2H, m), 2.88-3.05 (2H, m), 3.84 (3H, s), 3.87 (6H, s), 3.91 (3H, s), 6.54 (2H, s), 6.71-6.79 (3H, m).

Molecular modelling and torsional angles calculation.

The structures of compounds were built and optimized using a HyperChem molecular modelling programme, version 6.0. The molecules were minimized using the MM+ molecular mechanics module with the Polak-Ribiere algorithm. From molecules built therein torsional angles were derived. Atomic charges were calculated using a SNDO semi-emperical force field module.

Measurement of water solubility.

Each compound (0.5 mg for **3e**, **g**, **h** and 3 mg/mL for **3j** and **3j**.HCl) was mixed in 1 mL of distilled water and the resulting mixture was stirred at room temperature for 24 h. The insolubles were filtered off and the filtrates were analyzed for dissolved concentration. The complete dissolution was not observed with for **3e**, **g**, **h**, hence the water solubility of these compounds was recorded as < 0.5 mg/mL without further HPLC evaluation. The filtrates of **3j** and **3j**.HCl were calibrated with the standard solution (1 mg/mL of **3j** in MeOH). No decomposition of compounds was observed.

Cytotoxicity assays.

Tumor cells were maintained in plastic dishes in RPMI-1640 supplemented with 10% fetal bovine serum. On day 0, 100 μL of a tumor cell suspension (3×10^4 cells/mL in culture medium) were seeded in each well of 96 well plates. The plates were incubated in a 5% CO_2 incuabator at 37°C for 24 h then samples in 20 μL culture medium were added at various concentrations. The plates were incubated for another 48 h. Cytotoxicity was measured by SRB's method as described in literature (Skehan *et al.*, 1990; Nam *et al.*, 2002b). Compounds were examined in three independent assays, and the values shown for these compounds are averages of three determinations.

Antitumor experiments.

Antitumor experiments were carried as described in our previous reports (Nam *et al.*, 2002b; Kim *et al.*, 2002). Briefly, 3LL cells were inoculated s.c. into BDF1 mice on day 0 (1×10^7 cells/mouse/0.2 mL PBS). Test compounds were dissolved in a medium comprising of 5% DMSO and 20% Cremophor®. Each compound was dosed with six injections from day 1 and every two days after. Body weights were tracked every day and tumor sizes were measured with calipers from day 9. Tumor volumes were calculated by the following equation: tumor volume (mm^3) = [length (mm) x width $(mm)^2$]/2. The inhibition ratio was evaluated as (1-T/C) x 100% (where T is the mean tumor volume of the treated group, C is the mean tumor volume of the control group). Each group consisted of 10 mice. Etoposide was administered at day 1, 5, and 9 at 36 mg/kg/day. No death was recorded within the experimented period.

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