Arch Pharm Res Vol 25, No 5, 590-599, 2002

Archives of Pharmacal Research
http://apr.psk.or.kr

Synthesis and Cytotoxicity of Some Rigid Derivatives of Methyl 2,5-Dihydroxycinnamate

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(Received May 21, 2002)

Eight rigid compounds designed as esterase-stable analogues of methyl 2,5-dihydroxycinnamate (**1**) were synthesized. These derivatives include 2-(2',5'-dihydroxybenzylidene)cyclopentenone (**3a**), 2-(2',5'-dihydroxybenzylidene)cyclohexanone (**3b**), 2,6-bis(2',5'-dihydroxybenzy-lidene)cyclohexanone (**4b**), 2,6-bis(2',5'-dihydroxybenzylidene)cyclopentenone (**4a**), (*E*)-3-(2',5'-dihydroxybenzylidene)pyrrolidin-2-one (**5**), (*E*)-5-(2',5'-dihydroxybenzylidene)-1,2-isothiazolidine-1,1-dioxide (**6**), 4-(2',5'-dihydroxyphenyl)-5H-furan-2-one (**7**), and 3-(2',5'-dihydroxyphenyl)cyclopent-2-ene-1-one (**8**). Among the eight compounds, the furanone **7** and cyclopentenone **8** showed the most potent cytotoxicity with IC_{50} values of 0.39-0.98 μg/mL. Compound **8** was further brominated, phenylated and methylated at the α position to give three corresponding analogues, including 2-bromo-3-(2',5'-dihydroxyphenyl)cyclopent-2-ene-1-one (**24**), 3-(2',5'-dihydroxyphenyl)-2-phenylcyclopent-2-ene-1-one (**27**), and 3-(2',5'-dihydroxyphenyl)-2-methylcyclopent-2-ene-1-one (**28**). Among the three, the most enhanced activity was observed with the phenylated compound **27**.

Key words: Structure-activity relationship, Cytotoxicity, Cyclopentenone

INTRODUCTION

As a result of our effort to find antitumor agents from Vietnamese medicinal plants we have previously identified methyl caffeic ester (**MCE**, Fig. 1) as a moderate cytotoxic metabolite of *Notopterygium incisum* (Nam *et al.*, 2000). Modification taken on this molecule led to the finding of methyl 2,5-dihydroxycinnamate (**1**) with very potent cytotoxicity in a panel of human tumor cell lines (Nam *et al.*, 2001a). However, **1** showed only marginal antitumor activity in mice. This was attributed to the lability of the compound towards esterase hydrolysis *in vivo*. Our preceding efforts have been made to replace an esterase-labile methoxy group in **1** by various phenyl rings, resulting in chalcones **2** with significant bioactivity (Nam *et al.*, 2002a). Continued in vein of our further investigation in order to find more potent cytotoxic agents with improved kinetic profiles (e.g. more stable to esterase-mediated hydrolysis), a number of **1** analogues, compounds **3~8** (Fig. 2), were designed.

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Compounds **3a** and **3b** were synthesized based on the observation that a simple *E*-2-benzylidenecyclohexanone (BZH) showed a marked cytotoxicity towards an epidermoid carcinoma of the nasopharynx (IC_{50} of 1.94 μg/mL) (Dimmock *et al.*, 1976).

It has been mentioned in the previous parts that reaction of α,β-unsaturated ketones with thiol could be one plausible mechanism mediating the cytotoxicity of this type of compounds. Dimmock *et al.* (1993) hypothesized in a recent publication that successive chemical attacks of cellular constituents may be highly deleterious to malignant cells. Thus, the preparation for bioevaluation of bis alkylators **4a** and **4b** was considered. In fact, several studies have shown that various neoplasms are more vulnerable to multiple chemical insults than the corresponding normal cells (Tsutsui *et al.*, 1986; Michell *et al.*, 1987) and hence compounds with selective toxicity for neoplastic tissues may evolve using this approach.

Further, the incorporation of the nitrogen into **3a**, resulting in compound **5**, was rationalized from the previous report that the presence of such functional group proved to increase the affinity of the related compounds for thiols but not for amino or hydroxy groups found in nucleic acids; therefore potentiation of their cytotoxicity

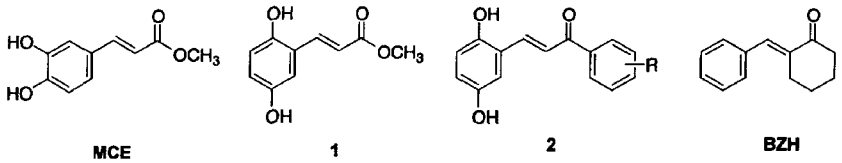


Fig. 1 Structure of methyl caffeic ester (**MCE**), methyl 2,5-dihydroxycinnamate (**1**), 2,5-dihydroxychalcones (**2**), and *E*-2-benzylidenecyclohexanone (**BZH**)

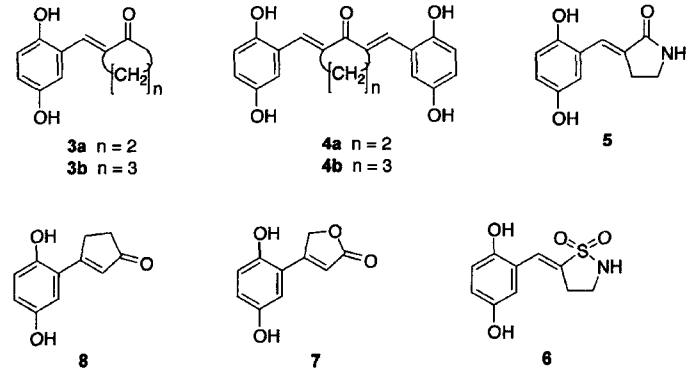
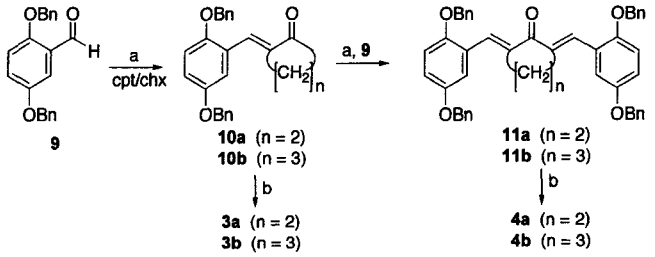


Fig. 2 Structures of some analogues of **1** planned to be investigated.



Scheme 1. *Reagents and Conditions*: (a) $Ba(OH)_2$, MeOH, 44-47% for **10a, b** and 35-66% for **11a, b**, 40 °C, 12 h; (b) HCl/AcOH, 36-75% for **3a, b** and 44-47% for **4a, b**, rfx, 2 h.

could be expected while mutagenic and carcinogenic side effects should be absent (Dimmock *et al.*, 1993, Benvenuto *et al.*, 1983).

Next, compound **6** in which a sulfonamide was present instead of an amide functionality in compound **5** was designed in consideration of potential roles played by the sulfonyl group as a bioisostere of the carbonyl functionality. Actually, such practice has been documented to be fruitful for a number of recently reported anticancer agents (Huang *et al.*, 2001, Supuran *et al.*, 2000a, Supuran *et al.*, 2000b).

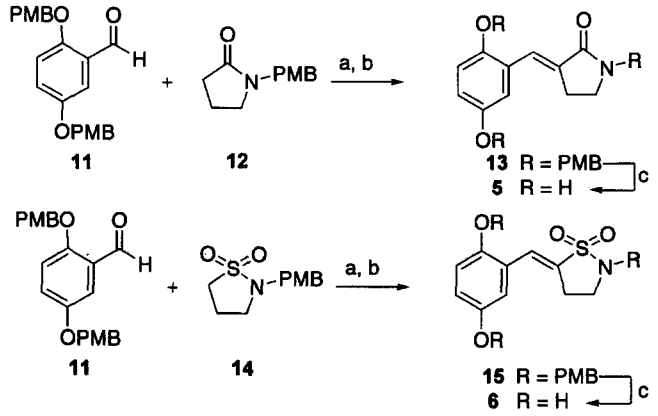
The syntheses of compounds **7** and its closely analogous one compound **8**, were attempted in view of the prevalence of the furanone moiety in antitumor agents, such as podophyllotoxins and camptothecins.

Initial screening for cytotoxicity of compounds **3~8** revealed that **7** and **8** were most potent with IC_{50} values ranged 0.35~0.48 μg/mL in several cancer cell lines. Compound **8** was chosen for further development. Three analogues **24, 27** and **28** were further synthesized. In this paper, details on the syntheses and cytotoxicity of the target compounds including **3~8, 24, 27** and **28** are described and discussed.

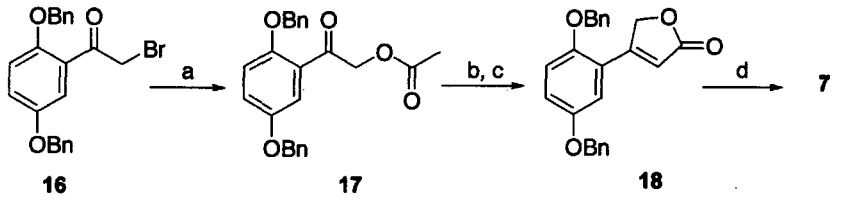
RESULTS AND DISCUSSION

Chemistry

A Claisen-Schmidt condensation between 2,5-dibenzyloxybenzaldehyde **9**, which was prepared from 2,5-dihydroxybenzaldehyde using a procedure described previously (Nam *et al.*, 2001b), and the stoichiometric amount of cyclopentanone (cpt) or cyclohexanone (chx) led to intermediates **10a, 10b** (Scheme 1). Removal of the benzyl groups by refluxing these intermediates in HCl/AcOH furnished **3a** and **3b** in moderate overall yields. A similar reaction of **10a** or **10b** with a stoichiometric amount of **9** gave the corresponding intermediates **11a** and **11b** which upon removal of the benzyl groups afforded **4a** and **4b**, respectively. The evidence from thin-layer chromatography (TLC) and ^{1}H-NMR spectroscopy indicated that the compounds **3a, b~4a, b** were iso-



Scheme 2. *Reagents and Conditions*: (a) LDA, THF, 55% for **13** and 52% for **15**, -78°C, 3 h; (b) cat. *p*-TsOH, MeOH, 36% for **13**, 37% for **15**, rt, 3 h; (c) TFA, rfx, 3 h. 37% for **5** and 42% for **6**.



Scheme 3. *Reagents and Conditions*: (a) AcOH, TEA, acetonitrile, rt, 30 min; (b) DBU, benzene, rfx, 18 h; (c) cat. *p*-TsOH, MeOH, 75%, rt, 3 h; (d) H_2, Pd/C, MeOH, 90%, 1 atm, rt, 90 min.

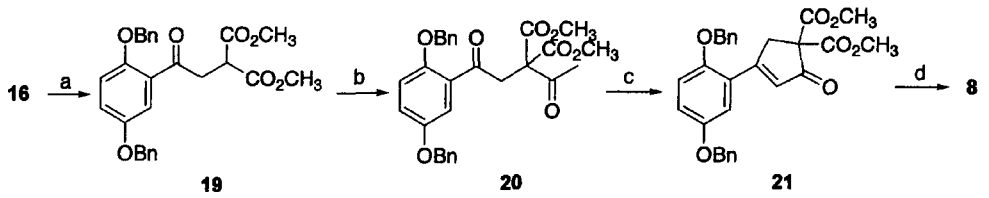
merically pure. The absorptions of the olefinic protons in the ^{1}H-NMR spectra were located at 7.49~7.53 ppm, which is indicative of compounds possessing the *E* configuration (Dimmock *et al.*, 1999); for the corresponding Z isomers, these protons would be predicted to be located at higher field, in a range of 6.22~6.31 ppm (Smith *et al.*, 1973, Hassner *et al.*, 1964).

Compounds **5** was synthesized as shown in Scheme 2 by an aldol-like reaction between 2,5-di(*p*-methoxybenzy-loxy) benzaldehyde (**11**) and *N*-(*p*-methoxybenzyl)pyrollidin-2-one (**12**). The reaction was mediated by lithium diisopropylamine (LDA) in THF to give a condensed product which was dehydrated using a catalytic amount of *p*-toluenesulfonic acid (*p*-TsOH) to give **13**. **11** was prepared from 2,5-dihydroxybenzaldehyde as described in our previous report (Nam *et al.*, 2001b) and *N*-(*p*-methoxybenzyl)pyrollidin-2-one (**12**) was prepared from 4-chlorobutanoyl chloride as described in literature (Li *et al.*, 1995). Compound **13** was obtained as a major product and determined to adopt *E*-configuration because the chemical shift of the olefinic proton appeared at 7.51 ppm. The chemical shift of the olefinic proton in the corresponding *Z*-isomer should appear at around 6.26 ppm (Smith *et al.*, 1973, Hassner *et al.*, 1964). Subsequent deprotection of **13** afforded **5** in 37% yield.

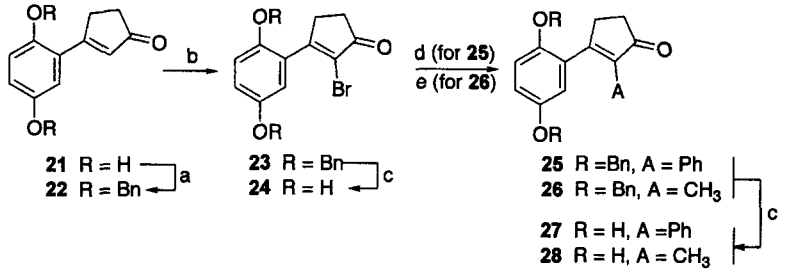
Compound **6** was obtained by coupling of ben-zaldehyde **11** with *N*-(*p*-methoxybenzyl)-γ-sultam **14** (Scheme 2). The intermediate **14** was prepared in a good yield (90%) from 3-chloropropanesulfonyl chloride by a method described previously (Puig *et al.*, 2000). The aldol-like reaction of **11** with **14**, followed by dehydration leading to **15** (*E*-configured) as a major product was much the same as described for **13**. Deprotection of **15** gave **6** in a moderate yield (42%).

Compound **7** was constructed as shown in Scheme 3. Nucleophilic substitution of **16**, which was prepared by bromination of **11** as detailed in our preceding report (Nam *et al.*, 2001b), with anhydrous acetic acid led the intermediate **17**. Cyclization of the intermediate **17** was effected by DBU to give a diastereoisomeric mixture of alcohol products, which was then dehydrated directly using a catalytic amount of *p*-TsOH to give **18**. Subsequent hydrogenation at 1 atm and room temperature removed the benzyl protecting groups and furnished **7** in a moderate yield. Fortunately, under these mild conditions, no hydrogenation of the olefin was detected.

The synthesis of compound **8** was based on sequential malonate alkylation-acylation followed by ring-closure and decarboxylation (Scheme 4). The synthesis began with the alkylation of dimethyl malonate with **16** to give **19**. The alkylation was found most effective by slow addition of a solution of **16** in acetone to a mixture of dimethyl malonate and K_2CO_3 in anhydrous acetone at 45°C. Acetylation of **19** to give **20** was mediated by $MgBr_2.Et_2O$ using pyridine as base. The optimal procedure for this



Scheme 4. *Reagents and Conditions*: (a) K_2CO_3, dimethyl malonate, 85%, 40°C, 5 h; (b) i) $MgBr_2.Et_2O$, pyridine, 0°C, 3 h; ii) CH_3COCl, 81%, -25°C, 2 h then 1N HCl; (c) TEA, acetonitrile, 100%, 30 min; (d) 3*M* H_2SO_4/AcOH, 37%, rfx, 3 h.



Scheme 5. *Reagents and Conditions*: (a) BnCl, K_2CO_3, NaI, acetone, 98%, rfx, 4 h; (b) Br_2, CH_3Cl, 75%, rt, 3 h; (c) 3N HCl/AcOH, 41% for 24, 59% for 27 and 49% for 28, reflux, 2 h; (d) $C_6H_5B(OH)_2$, 5 mol % $Pd(PPh_3)_4$, 2M Na_2CO_3, ethanol, toluene, 71%, rfx, 12 h; (e) Me_4Sn, $Pd(OAc)_2$, P(*o*-tolyl)$_3$, TEA, 73%, DMF.

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 **20** in good yield. Cyclization of **20** was readily achieved using TEA as base. Decarboxylation of **21** was effected by refluxing in 3*M* H_2SO_4-AcOH at 90°C for 10 h. Conveniently, under this condition both benzyl groups used to protect the phenol hydroxyls were removed to afford **8** in one step

Some analogues of **8** were prepared as shown in Scheme 5. First, the dihydroxy functionality was protected as benzyloxy groups using a benzylation procedure (Nam *et al.* 2001b). Bromination of the protected **22** using Br_2/MC gave **23** in good yield (75%). Suzuki coupling reaction of **23** with phenylboronic acid mediated by tetrakis(triphenylphosphine)palladinium(0) ($Pd(PPh_3)_4$) furnished the 2-phenyl substituted derivative **25** while Stille coupling reaction of **23** with tetramethyltin catalyzed by palladinium acetate ($Pd(OAc)_2$) and tri(*o*-tolyl)phosphine (P(*o*-tolyl)$_3$) afforded the 2-methyl substituted derivative (**26**). Subsequent deprotection of the benzyl groups was initially carried out by hydrogenation over Pd/C (5%) at the atmospheric pressure and room temperature for 90 min, as described for **7**. However, concomitant reduction of the olefinic moiety was also observed. Later, **24, 27** and **28** were obtained in moderate yields (41~57%) by refluxing in 3*N* HCl/AcOH for 2 h.

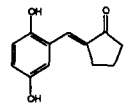
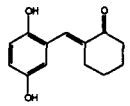
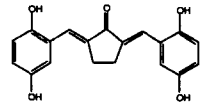
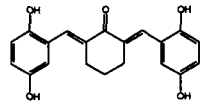
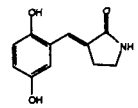
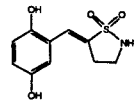
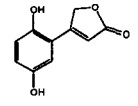
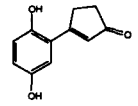
Cytotoxicity in tumor cell lines

The synthesized compounds **3~8, 24** and **27, 28** were evaluated for cytotoxic activity in three tumor cell lines including B16 (murine melanoma), HCT116 (human colon tumor) and A431 (human epidermoid carcinoma). For comparison purpose, BZH was included in all assays. The results are summarized in Table 1. As shown in this table, all compounds were found to be active in all three cell lines; the IC_{50} values were ≤ 4 μg/mL. To get an idea of relative potency of compounds in this series, the average potencies (AP) (averages of the IC_{50} values) are used and the data are included in Table 1.

The figures presented therein show that a cyclohexanone derivative **4b** was roughly 2.3-fold as potent as *E*-2-benzylidenecyclohexanone (BZH), suggesting an important role played by the 2,5-dihydroxy moiety toward the bioactivity of this molecular type. Between **3a** and **3b**, again **3b** was found to display a nearly 65% increase in cytotoxicity compared to **3a**. Conformationally the cyclohexanone derivative **3b** is more rigid than the cyclopentenone **3a** and thus, this result was similar to a recent study with cytotoxic 2-cyclobenzoalkanones wherein compounds having rigid or semirigid conformations were reported to exhibit greater activity (Dimmock *et al.*, 1999).

Next, the two compounds **4a** and **4b** were originally designed as bisalkylators based on **3a** and **3b** which possessed α,β-unsaturated ketone moieties. The design was rationalized from a hypothesis stated by Dimmock *et al.* (1993) that successive chemical attacks of cellular proponents may be highly deleterious to malignant cells. Thus, compounds **4a** and **4b** having two electrophilic sites

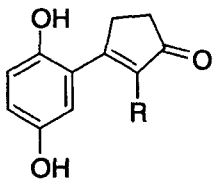
Table 1. Cytotoxicity of compounds 44~51 against some tumor cell lines[1]

No	Compound	Cytotoxicity (IC_{50}[2], μg/mL)			
		B16	HCT116	A431	AP[3]
3a		1.51	1.65	2.00	1.72
3b		1.01	1.23	1.12	1.12
4a		1.37	1.46	3.21	2.01
4b		2.41	1.99	2.00	1.80
5		1.98	2.03	1.95	1.98
6		1.65	3.09	>10	4.91
7		0.39	0.75	0.35	0.49
8		0.47	0.98	0.59	0.68
BZH		2.74	3.01	1.97	2.57
Adriamycin		0.21	0.78	1.01	0.67

[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]Average potency.

were expected to show a greater cytotoxicity than **3a** and **3b**. Actually however, the IC_{50} of **4a** and **4b** were found to be larger than those of **3a** and **3b** (2.01 μg/mL of **4a** vs. 1.72 μg/mL of **3a** and 1.80 μg/mL of **4b** vs. 1.12 μg/mL of **3b**). Thus, in actuality **3a** was slightly more cytotoxic than **4a** while **3b** was found to be 1.6-fold as potent as **4b**. These results suggest that the hypothesis proposed by Dimmock *et al.* referenced above was not viable for this cluster of compounds. One possible explanation for this could be that the second alkylation of GSH was unlikely

Table 2. Cytotoxicities of 24, 27 and 28 in some tumor cell lines[1]



Cpd	R	Cytotoxicity (IC_{50}[2], μg/mL)		
		B16	HCT116	A431
24	-Br	7.31	6.58	5.39
27	$-C_6H_5$	0.55	0.41	0.21
28	$-CH_3$	2.41	1.23	0.99
8	H	0.47	0.98	0.59
ADR[3]		0.21	0.78	1.01

[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.

ensued after the first one, partly due to the bulkiness of GSH and decrease of electrophilicity at the second β-methine carbon caused by the first GSH incorporation on the molecule.

The sulfonamide **6** proved to be less active, indicating that a bulky sulfonamide group was not favorable for interaction of the compound with receptors at the binding site. However, an amide **5** retained much activity of **3a**, signaling the incorporation of the nitrogen atom in this manner was tolerable. Further development of this type of compounds may be profitable avenue to pursue stemming from a view that the nitrogen atom is prevalent in many of the currently used drugs.

The most interesting results from this series were observed with the two compounds **7** and **8** which were most active in this group. The IC_{50} values of these two derivatives were found in the range of 0.35~0.98 μg/mL in the three cell lines tested. A notable feature that should be addressed is the likelihood of the difference in mechanism of action of these two compounds compared to the previously discussed ones. Since the β-methine carbons of the α,β-unsaturated ketone moieties in the furanone **7** and cyclopentenone **8** are sterically hindered, alkylation of GSH is unlikely a mechanism of the cytotoxicity of these two compounds. Furthermore, the *para*-dihydroxy functionality could not account for the whole bioactivity of **7** and **8**, taking into consideration the fact that this moiety was present in all of the compounds in this series. Thus, a mechanism others than GSH alkylation or formation of quinonoid metabolites may come into play.

Compound **8** was selected for a further modification since the presence of the cyclopentenone moiety has been

very rarely documented in literature with anticancer agents, while a furanone moiety is common and has a disadvantage of being too labile towards esterases *in vivo*.

The α position in **8** was chosen as a site for the first line of modifications due to the easiness of substitution of the active α-proton by different groups. In concert to discerning the electronic effect as well as the shape and size of the substituents at this position, three groups, namely bromine, methyl, and phenyl were first attempted. Correspondingly, three synthesized compounds **24**, **27** and **28** were evaluated for cytotoxicity in three cell lines B16, HCT116 and A431. The results summarized in Table 2 clearly indicated that the introduction of the bromine group at this α position in **8** greatly diminished its activity in all of the three cell lines. Whether the actual culprit was solely due to the electronic effect was hardly perceivable since another reason, e.g. instability of this compound might also be a cause. The methyl group was found to have a similar effect; compound **28** was two- to five-fold less cytotoxic than **8**. Interestingly, the phenyl moiety was proved to be worthwhile for further investigation. Though compound **27** was slightly less active than **8** in B16 cells, it showed a more than 2 times the cytotoxicity of **8** in the rest two cell lines. Thus, the phenyl group seemed to be favorable for the bioactivity of this compound, probably due to the enhanced interaction of the compounds newly introduced phenyl ring with a complementary aryl moiety at a binding site through a van der Waals bonding, as is the case for combretastatin derivatives (lit). Further studies on the structure-activity relationships of compound **27** have been carried out and the results will be reported in our next communication (Nam *et al.*, 2002c).

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.

2-(2',5'-Dibenzyloxybenzylidene)cyclopentenone (10a)

This compound was prepared from **9** and 1 mol equivalent of cyclopentanone by the same procedure described for the synthesis of chalcones in our previous publication (Nam *et al.*, 2002a). Yield 47%; IR (KBr) 1660 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 90 MHz) δ 1.44-1.53 (2H, m), 2.01-2.25 (2H, m), 2.95-3.11 (2H, m), 5.27 (4H, overlapped), 6.54-6.87 (2H, m), 6.89 (1H, s), 7.12-7.46 (m, 10H), 7.52 (1H, s).

2-(2',5'-Dibenzyloxybenzylidene)cyclohexanone (10b)

This compound was prepared from **9** and 1 mol equivalence of cyclohexanone by the same procedure described for the synthesis of **10a**. Yield 44%; IR (KBr) 3010, 1670, 1280, 1250, 1150, 1130 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 90 MHz) δ 1.64-1.86 (4H, m), 2.18-2.35 (2H, m), 2.97-3.15 (2H, m), 5.27 (4H, overlapped), 6.54-6.67 (2H, m), 6.75 (1H, s), 7.12-7.46 (m, 10H), 7.52 (1H, s).

2-(2',5'-Dihydroxybenzylidene)cyclopentenone (3a)

Compound **10a** (1.05 g, 3 mmol) was dissolved in AcOH (15 mL). To the resulting solution was added 3 mL of 1*N* HCl. The mixture was refluxed at 100°C for 3 h and solvents were removed at diminished pressure. The residue was redissolved in water (50 mL) and extracted with EA (50 mL). The EA layer was dried, concentrated and purified over a silica gel column eluting with gradient EA in hexane (1:3→1:1) to give the expected compound **3a** (0.48 g). Yield 75%; m.p. 135-137°C; IR (KBr) 3350, 1670, 1250, 1180 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 90 MHz) δ 1.47-1.55 (2H, m), 1.96-2.20 (2H, m), 2.93-3.07 (2H, m), 6.56-6.68 (2H, m), 6.86 (1H, s), 7.51 (1H, s).

2-(2',5'-Dihydroxybenzylidene)cyclohexanone (3b)

This compound was obtained from **10b** by the same procedure described for **3a**. Yield 36%; m.p. 116-118 °C; IR (KBr) 3450, 1680, 1250, 1180, 1120 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 90 MHz) δ 1.61-1.83 (4H, m), 2.15-2.31 (2H, m), 2.93-3.11 (2H, m), 6.57-6.71 (2H, m), 6.83 (1H, s), 7.49 (1H, s).

2,6-bis(2',5'-Dibenzyloxybenzylidene)cyclopentenone (11a)

This compound was prepared by subjecting compound **10a** to a second condensation with 1 mol equivalent of **9**, using the same procedure for the synthesis of **10a**. Yield 35%; ^{1}H-NMR ($CDCl_3$, 90 MHz) δ 1.93-2.08 (4H, s), 5.26 (8H, s), 6.55-6.75 (4H, m), 6.89 (2H, m), 7.22-7.61 (22H, m).

2,6-bis(2',5'-Dibenzyloxybenzylidene)cyclohexanone (11b)

This compound was prepared by subjecting compound **10b** to a second condensation with 1 mol equivalent of **9**, using the same procedure for the synthesis of **10a**. Yield 66%; IR (KBr) 3020, 1670, 1240, 1210, 1180, 1120 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 90 MHz) δ 1.60-1.82 (2H, m), 2.15-2.33

(4H, m), 5.20-5.29 (8H, overlapped), 6.55-6.78 (4H, m), 6.99-7.12 (2H, m), 7.15-7.55 (22H, m).

2,6-bis(2',5'-Dihydroxybenzylidene)cyclopentenone (4a)

This compound was prepared from compound **11a** using the same procedure for the synthesis of **3a**. Yield 47%; m.p. 124-126°C; IR (KBr) 3450, 1680, 1250, 1150 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 90 MHz) δ 1.96-2.15 (4H, s), 6.54-6.75 (4H, m), 6.88 (2H, s), 7.53 (2H, s).

2,6-bis(2',5'-Dihydroxybenzylidene)cyclohexanone (4b)

This compound was prepared from compound **11b** using the same procedure for the synthesis of **3a**. Yield 44%; m.p. 121-124°C; IR (KBr) 3350, 1660, 1250, 1170 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 90 MHz) δ 1.57-1.80 (2H, m), 2.11-2.30 (4H, m), 6.57-6.76 (4H, m), 6.86 (2H, s), 7.52 (2H, s).

(E)-3-[2',5'-bis(4-methoxybenzyloxy)benzylidene)-N-(4'-methoxybenzyl)pyrrolidin-2-one (13)

To a stirred solution of diisopropylamine (15.5 mL, 111 mmol), BuLi in hexane (1.60 M, 69.5 mL, 111 mmol) was added dropwise under ice-cooling over a period of 20 min. After completion of the addition, stirring was continued for another 15 min and the reaction mixture was cooled to -78°C, followed by the addition of THF (100 mL). To this mixture were added **12** (19.0 g, 101 mmol), 2,5-bis(4-methoxybenzyloxy)benzaldehyde (**11**) (32.4 g, 90.5 mmol), and hexamethylphosphamide (HMPA, 30 mL) in THF (70 mL) dropwise over a period of 20 min. The reaction mixture was stirred for 30 min at the same temperature and then warmed to room temperature. The mixture was poured into 2*N* HCl (100 mL) under ice-cooling and extracted with EA (250 mL). The organic layer was washed with saturated $NaHCO_3$ (300 mL) and brine (300 mL), then dried, and evaporated. The residue was purified by chromatography on silica gel eluting with hexane-EA (4:1 to 1:1) to give 27.3 g (55% based on benzaldehyde) of a colorless solid. To a solution of this solid (10.9 g, 19.9 mmol) in toluene (150 mL) was added *p*-TsOH (2.49 g, 13 mmol), and the mixture was refluxed for 30 min. After cooling to room temperature, the mixture was poured into saturated $NaHCO_3$ (150 mL) and extracted with EA (150 mL). The organic layer was washed with water (150 mL) and brine (150 mL) and then dried and evaporated. The residue was subjected to column chromatography on silica gel, eluting with hexane-EA (3:1) to give compound **13** ((E)-3-[2,5-bis(4-methoxybenzyloxy)benzylidene)-N-(4-methoxybenzyl) pyrrolidin-2-one) as a major product (3.9 g, 36%). m.p. 135-137°C; IR (KBr) 3010, 2910, 1660, 1580, 1430, 1250, 1180, 1150, 1140 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 90 MHz) δ 2.12-2.31 (2H, m), 3.11-3.28 (2H, m), 3.75 (3H, s), 3.88 (6H, overlapped), 4.41 (2H, s), 5.27 (4H, overlapped), 6.51-6.73 (2H, m), 6.87-7.02 (5H, m), 7.09-7.35 (8H, m), 7.48 (1H, s).

(E)-3-(2',5'-Dihydroxybenzylidene)pyrrolidin-2-one (5)

Compound **13** (2.48 g, 5 mmol) was dissolved in TFA (30 mL). The reaction mixture was refluxed for 18 h. TFA was removed and the residue was dissolved in water (50 mL), extracted with EA (50 mL). The EA layer was concentrated by an evaporator and the residue was purified on a silica gel column eluting with gradient EA in hexane to provide 0.46 g of **5**. Yield 37%; m.p. 102-104°C; IR (KBr) 3540, 3020, 2915, 1640, 1600, 1580, 1250 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 90 MHz) δ 2.10-2.30 (2H, m), 3.12-3.28 (2H, m), 6.55-6.77 (2H, m), 6.87 (1H, s), 7.51 (1H, s).

(E)-2-(4'-Methoxybenzyl)-5-[2',5'-bis(4'-methoxybenzyloxy)benzylidene)-1,2-isothiazolidin-e-1,1-dioxide (15)

15 was obtained from **14** by the same procedure described for **13**. Yield 52% and 37% for each step, ^{1}H-NMR ($CDCl_3$, 90 MHz) δ 3.07-3.19 (4H, m), 3.76 (3H, s), 3.81 (2H, s), 5.22 (4H, overlapped), 6.54-6.67 (2H, m), 6.88-7.08 (5H, m), 7.11-7.43 (8H, m), 7.49 (1H, s).

(E)-5-(2',5'-Dihydroxybenzylidene)-1,2-isothiazolidine-1,1-dioxide (6)

This compound was obtained from **15** by the same procedure described for **5**. Yield 41%; m.p. 201-204°C; IR (KBr) 3450, 3020, 2910, 1620, 1580, 1460, 1250 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 90 MHz) δ 3.02-3.13 (2H, m), 3.22-3.34 (2H, m), 6.57-6.68 (2H, m), 6.88 (1H, s), 7.49 (1H, s).

2-(2',5'-Dibenzyloxyphenyl)-2-oxoethyl acetate (17)

2-Bromo-(2,5-dibenzyloxy)acetophenone (33, 4.11 g, 10 mmol) was dissolved in 30 mL of dry acetonitrile. To the resulting solution was added acetic acid (0.62 g, 10 mmol) and triethylamine (1.01 g, 1.29 mL, 10 mmol). The reaction mixture was stirred at room temperature for 30 min and solvent was removed. Work-up included dissolving the resulting syrup in water then extracted with MC. The MC layer was concentrated to produce an oil which solidified upon standing overnight. This intermediate was used directly for the next reaction without any further purification.

4-(2',5'-Dibenzyloxyphenyl)-5H-furan-2-one (18)

Compound **17** obtained from the previous reaction was dried and dissolved in benzene (30 mL). To this solution was added DBU (1.8 mL, 12 mmol) and the resulting mixture was refluxed for 18 h. The mixture was passed through a pad of silica gel and the concentrated *in vacuo* to give an oil. This oil was directly dissolved in 30 mL of MeOH and *p*-TsOH (500 mg) was added. The mixture was stirred at room temperature for 3 h. MeOH was

removed, followed by water addition, MC extraction and evaporation. The residue obtained was purified over a silica gel column eluting with gradient EA in hexane to give 3.08 g of **18**. Yield 75% in two steps; m.p. 101-104°C; IR (KBr) 3020, 2915, 1680, 1620, 1580, 1250 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 90 MHz) δ 5.15 (2H, s), 5.22 (4H, overlapped), 6.31 (1H, s), 6.55-6.68 (2H, m), 6.91 (1H, s), 7.02-7.38 (10H, m).

4-(2' 5'-Dihydroxyphenyl)-5H-furan-2-one (7)

Compound **18** (2.5 g, 6.7 mmol) was dissolved in MeOH and Pd/C (200 mg) was added. The reaction mixture was stirred at room temperature under an umbrella of 1 atm hydrogen gas for 90 min. The mixture was filtered through a pad of silica gel and the filtrate was crystallized from MeOH/Et_2O to afford 1.15 g of **7**. Yield 90% m.p. 114-116°C; IR (KBr) 3540, 3460, 3010, 2920, 1675, 1620, 1580, 1250 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 90 MHz) δ 5.23 (2H, s), 6.45 (1H, s), 6.57-6.70 (2H, m), 6.88 (1H, s).

Dimethyl 2-[2'-(2',5'-dibenzyloxyphenyl)-2'-oxoethyl] propane-1,3-dioate (19)

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 (**19,** 18.75 g, 84%) as a yellowish oil which was used for the next step without any further purification.

Dimethyl 2-[2'-(2',5'-dibenzyloxyphenyl)-2'-oxoetyl]-2-acetyl)propane-1,3-dioate (20)

The oil **19** (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 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, acetyl chloride (1.84 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 (**20,** 9.28 g, 81%). ^{1}H-NMR ($CDCl_3$, 90 MHz) δ 2.15 (3H, s), 3.87 (6H, s), 3.92 (2H, s), 5.26 (4H, overlapped), 6.61-6.73 (2H, m), 6.89 (1H, s), 7.03-7.42 (10H, m).

Methyl-3-(2',5'-dibenxyloxyphenyl)-1-methoxycarbonyl-2-oxocyclopent-3-enecarboxylate (21)

The oil **20** (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 (**73,** 8.35 g, 100%). ^{1}H-NMR ($CDCl_3$, 90 MHz) δ 2.62-2.79 (2H, m), 3.11-3.27 (2H, m), 5.25 (4H, overlapped), 6.27 (1H, s), 6.66-6.79 (2H, m), 6.93 (1H, s), 7.00-7.39 (10H, m).

3-(2',5'-Dihydroxyphenyl)cyclopent-2-ene-1-one (8)

The residue **21** 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 **8** (1.71 g, 37%). IR (KBr) 3450, 3340, 3010, 2920, 1680 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 300 MHz) δ 2.65-2.79 (2H, m), 3.15-3.31 (2H, m), 6.27 (1H, s), 6.68 (1H, d, J = 8.8 Hz), 6.73 (1H, dd, J = 2.1, 8.8 Hz), 6.92 (1H, d, J = 2.1 Hz).

3-(2',5'-Dibenzyloxyphenyl)cyclopent-2-ene-1-one (22)

This compound was obtained from **8** by the same procedure described for **9, 11**. Yield 98%; IR (KBr) 3010, 2920, 1680 cm^{-1}.

2-Bromo-3-(2',5'-dibenzyloxyphenyl)cyclopent-2-ene-1-one (23)

This compound was obtained from **22** by the same procedure described for **16**. Yield 75%; IR (KBr) 3010, 2920, 1680 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 90 MHz) δ 2.66-2.81 (2H, m), 3.17-3.33 (2H, m), 5.27 (4H, overlapped), 6.68-6.75 (2H, m), 6.89 (1H, m), 6.99-7.35 (10H, m).

2-Bromo-3-(2',5'-dihydroxyphenyl)cyclopent-2-ene-1-one (24)

This compound was obtained from **23** by the same procedure described for **3a**. Yield 41%; IR (KBr) 3430, 3400, 3010, 2920, 1670 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 90 MHz) δ 2.78-2.86 (2H, m), 3.20-3.35 (2H, m), 6.65-6.77 (2H, m), 6.86 (1H, m).

3-(2',5'-Dibenzyloxyphenyl)-2-phenylcyclopent-2-ene-1-one (25)

Under a blanket of nitrogen, to a stirred solution of **23** (5.74 g, 12.8 mmol) and 1.89 g of phenylboronic acid (15.4 mmol) in a mixed solvent of 35 mL of toluene, 35 mL of ethanol, and 35 mL of 2 M Na_2CO_3 was added 1 g (0.86 mmol) of $Pd(PPh_3)_4$. After refluxing with vigorous stirring for 3 h, the solvent was removed *in vacuo*. The residue was dissolved in EA, washed with water and brine, and dried over Na_2SO_4. Purification by silica gel chromatography with gradient EA in hexane gave 3.99 g of the title compound **79**. Yield 71%; IR (KBr) 3020, 2915, 1680, 1620, 1580, 1250 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 90 MHz) δ

2.61-2.75 (2H, m), 2.87-3.05 (2H, m), 5.24 (4H, overlapped), 6.52-6.73 (2H, m), 6.83 (1H, m), 7.06-7.35 (13H, m), 7.41-7.52 (2H, m).

3-(2',5'-Dihydroxyphenyl)-2-phenylcyclopent-2-ene-1-one (27)

This compound was obtained from **25** by the same procedure described for **3a**. Yield 59%; IR (KBr) 3450, 3360, 3015, 2920, 1675, 1615, 1590, 1250 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 90 MHz) δ 2.67-2.81 (2H, m), 2.92-3.14 (2H, m), 6.57-6.81 (2H, m), 6.92 (1H, m), 7.11-7.32 (3H, m), 7.46-7.57 (2H, m).

3-(2',5'-Dibenzyloxyphenyl)-2-methylcyclopent-2-ene-1-one (26)

To a solution of **23** (7.99 g, 17.8 mmol) in 45 mL of anhydrous DMF were added 6.36 g (35.6 mmol) of tetramethyltin, 0.07 g (0.33 mmol) of palladium (II) acetate, 0.43 g (1.42 mmol) of tri-*o*-tolylphosphine, and 2.46 mL (1.79 g, 17.8 mmol) of Et_3N. The mixture was heated at 100°C overnight. After being cooled, the mixture was diluted with 45 mL of dioxane and filtered through Celite. The filtrate was partitioned between EA and water. The organic layer was washed, dried, and concentrated. The residue was purified by column chromatography, eluting with EA/hexane (1:2) to give 4.85 g of the title compound **26**. Yield 73%; IR (KBr) 3010, 2920, 1660 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 90 MHz) δ 1.99 (3H, s), 2.61-2.75 (2H, m), 3.11-3.28 (2H, m), 5.22 (4H, overlapped), 6.71-6.89 (2H, m), 7.05 (1H, m), 7.12-7.44 (10H, m).

3-(2',5'-Dihydroxyphenyl)-2-methylcyclopent-2-ene-1-one (28)

This compound was obtained from **26** by the same procedure described for **3a**. Yield 49%; IR (KBr) 3440, 3400, 3020, 2910, 1670 cm^{-1}; ^{1}H-NMR ($CDCl_3$, 90 MHz) δ 2.01 (3H, s) 2.72-2.84 (2H, m), 3.21-3.33 (2H, m), 6.71-6.88 (2H, m), 7.04 (1H, m).

Cytotoxicity assays.

Tumor cells were maintained in plastic dishes in RPMI-1640 supplemented with 10% fetal bovine serum. On day 0, 180 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.*, 1993; Nam *et al.* 2002b). Compounds were examined in three independent assays, and the values shown for these compounds are averages of three determinations.

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