International Journal of
Molecular Sciences

MDPI

Article

Synthesis and Structure–Activity Relationship of Salvinal Derivatives as Potent Microtubule Inhibitors

Chi-I Chang [1,†], Cheng-Chih Hsieh [2,3,†], Yung-Shung Wein [4], Ching-Chuan Kuo [5,6], Chi-Yen Chang [7], Jrhau Lung [8], Jong-Yuh Cherng [9], Po-Chen Chu [10], Jang-Yang Chang [5,11,12,*] and Yueh-Hsiung Kuo [13,14,15,*]

1 Department of Biological Science and Technology, National Pingtung University of Science and Technology, Pingtung 912, Taiwan
2 Department of Pharmacy, Kaohsiung Veterans General Hospital, Kaohsiung 813, Taiwan
3 School of Pharmacy and Institute of Pharmacy, National Defense Medical Center, Taipei 114, Taiwan
4 Department of Chemistry, National Taiwan University, Taipei 114, Taiwan
5 Institute of Biotechnology and Pharmaceutical Research, National Health Research Institutes, Miaoli 350, Taiwan
6 Graduate Institute of Biomedical Sciences, China Medical University, Taichung 404, Taiwan
7 National Institute of Cancer Research, National Health Research Institutes, Miaoli 350, Taiwan
8 Department of Medical Research and Development, Chiayi Chang Gung Memorial Hospital, Chiayi Branch, Chiayi 613, Taiwan
9 Department of Chemistry and Biochemistry, National Chung Cheng University, Chiayi 613, Taiwan
10 Department of Cosmeceutics and Graduate Institute of Cosmeceutic, China Medical University, Taichung 404, Taiwan
11 Taipei Cancer Center, Taipei Medical University Hospital, Taipei 110, Taiwan
12 TMU Research Center of Cancer Translational Medicine, Taipei Medical University, Taipei 110, Taiwan
13 Department of Chinese Pharmaceutical Sciences and Chinese Medicine Resources, College of Pharmacy, China Medical University, Taichung 404, Taiwan
14 Department of Biotechnology, Asia University, Taichung 413, Taiwan
15 Chinese Medicine Research Center, China Medical University, Taichung 404, Taiwan
* Correspondence: jychang@nhri.org.tw or jychang@nhri.edu.tw (J.-Y.C.); kuoyh@mail.cmu.edu.tw (Y.-H.K.); Tel.: +886-3-724-6166 (ext. 30701) (J.-Y.C.); +886-4-2205-3366 (ext. 5701) (Y.-H.K.)
† These authors contributed equally to this work.

check for updates

Citation: Chang, C.-I.; Hsieh, C.-C.; Wein, Y.-S.; Kuo, C.-C.; Chang, C.-Y.; Lung, J.; Cherng, J.-Y.; Chu, P.-C.; Chang, J.-Y.; Kuo, Y.-H. Synthesis and Structure–Activity Relationship of Salvinal Derivatives as Potent Microtubule Inhibitors. *Int. J. Mol. Sci.* **2023**, *24*, 6386. https://doi.org/10.3390/ijms24076386

Academic Editor: Jack A. Tuszynski

Received: 17 February 2023
Revised: 22 March 2023
Accepted: 27 March 2023
Published: 28 March 2023

Abstract: Salvinal is a natural lignan isolated from the roots of *Salvia mitorrhiza* Bunge (Danshen). Previous studies have demonstrated its anti-proliferative activity in both drug-sensitive and -resistant cancer cell lines, with IC_{50} values ranging from 4–17 μM. In this study, a series of salvinal derivatives was synthesized and evaluated for the structure–activity relationship. Among the twenty-four salvinal derivatives, six compounds showed better anticancer activity than salvinal. Compound **25** displayed excellent anticancer activity, with IC_{50} values of 0.13–0.14 μM against KB, KB-Vin10 (overexpress MDR/Pgp), and KB-7D (overexpress MRP) human carcinoma cell lines. Based on our in vitro microtubule depolymerization assay, compound **25** showed depolymerization activity in a dose-dependent manner. Our findings indicate that compound **25** is a promising anticancer agent with depolymerization activity that has potential for the management of malignance.

Keywords: salvinal; lignan; *Salvia mitorrhiza*; anticancer; microtubule depolymerization

1. Introduction

The dynamic equilibrium between tubulin and microtubules refers to the balance between the assembly and disassembly of microtubules, which is critical for various cellular processes, such as cell shape maintenance, intracellular transport, and cell division [1,2]. Microtubules are long, tubular structures made up of αβ-tubulin dimers that polymerize to form a rigid yet dynamic network within the cell; this becomes a significant component of the cytoskeleton [3–5]. Microtubules play a crucial role in the cell cycle, where they form the spindle apparatus that separates the chromosomes during mitosis [3,6]. As a result,

microtubules have become a popular target for cancer therapies, as their disruption can prevent cancer cell proliferation [1,3,6]. Tubulin-binding agents that interfere with microtubule systems are commonly used in the treatment of hematological malignancies and solid tumors [4]. These agents can be classified into two categories based on their mechanism of action and the effects they have on microtubule polymerization [1,4,7]. The first category comprises drugs that inhibit polymerization, such as the vinca alkaloids [4]. These drugs bind to tubulin and prevent it from polymerizing into microtubules [1,4,7]. This disrupts the formation of the spindle fibers required for proper cell division, leading to cell death [1,3,4]. The second category comprises drugs that stabilize microtubules, such as taxanes and epothilones [4]. These drugs bind to microtubules and enhance their stability, which results in prolonged mitotic arrest and eventual cell death [1,3,4]. Taxanes and epothilones act by binding to the microtubule plus-end, inhibiting depolymerization and resulting in the accumulation of microtubules [4,8]. Tubulin-binding agents are a class of compounds primarily derived from natural sources; they encompasses a vast array of agents exhibiting a diverse range of chemical structures, such as paclitaxel, epothilone A, vinblastine, combretastatin A-4, colchicine, dolas-tatin 10, and chamaecypanone C [4,7,9–11]. Despite the differences in their structures, these agents all share a common characteristic—the ability to interfere with the dynamics of microtubules [1,7,10]. This interference can lead to a number of cellular effects, including mitotic arrest and cell death [1,3,10].

Salvinal, 5-(3-hydroxypropyl)-7-methyoxy-2-(3′-methoxy-4′-hydroxyphenyl)-3-benzo[b] furancarbaldehyde, which is originally extracted from the roots of *Salvia miltiorrhizae* Bunge, has been shown to possess strong cytotoxic properties against the progression of tumors [12]. Salvinal's anticancer mechanism is associated with its ability to inhibit microtubules in both drug-sensitive and drug-resistant cell lines by binding to the colchicine binding domain of tubulin [12]. The promising anticancer properties of salvinal have generated considerable interest in creating and enhancing its therapeutic potential through the development of various derivatives or analogs using chemical synthesis methods. This study aims to design and synthesize several salvinal derivatives, assess their structure–activity relationships (SARs), and identify the most promising lead compounds for further investigation.

2. Results and Discussion

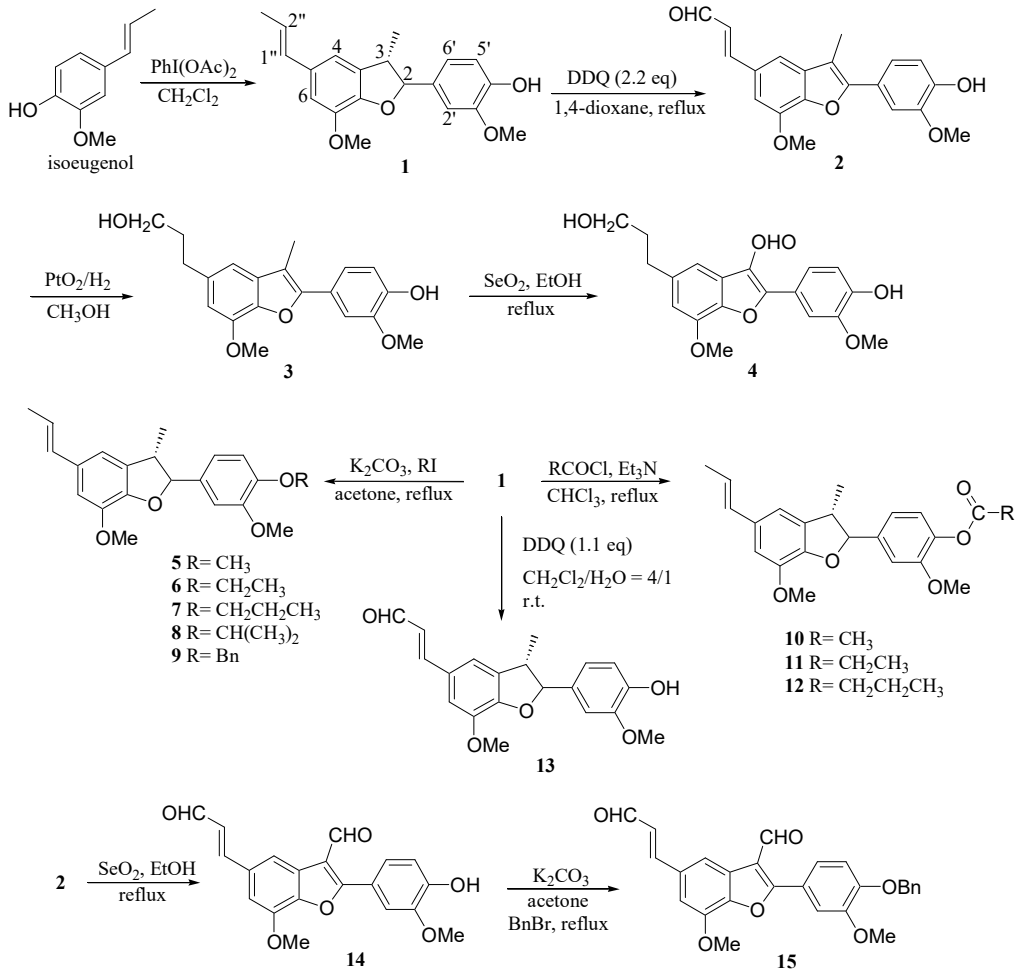
In dividing cells, microtubules are organized into the mitotic spindle, which is crucial for proper chromosome segregation during cell division [1]. Microtubule-binding agents are a class of drugs that target microtubules and have been proven to be highly effective in treating certain types of cancer [4]. These drugs function by either stabilizing or destabilizing microtubules, resulting in disrupted mitotic spindle formation, cell cycle arrest and, ultimately, cell death [4]. In our previous research, we discovered promising antitubulin compounds derived from various sources, including natural and synthetic products. One of these leading compounds, salvinal, was extracted from the roots of *S. miltiorrhizae* using chloroform. However, due to the low concentrations of active compounds found in plants, it is necessary to synthesize larger quantities of these compounds for further evaluation of their biological activity.

In this current study, we synthesized a series of derivatives of salvinal to evaluate their structure-activity relationships (SARs). Our findings indicate that compound **25** exhibited the most potent anticancer activity and is therefore a promising candidate for further investigation.

2.1. Synthesis of Salvinal Derivatives

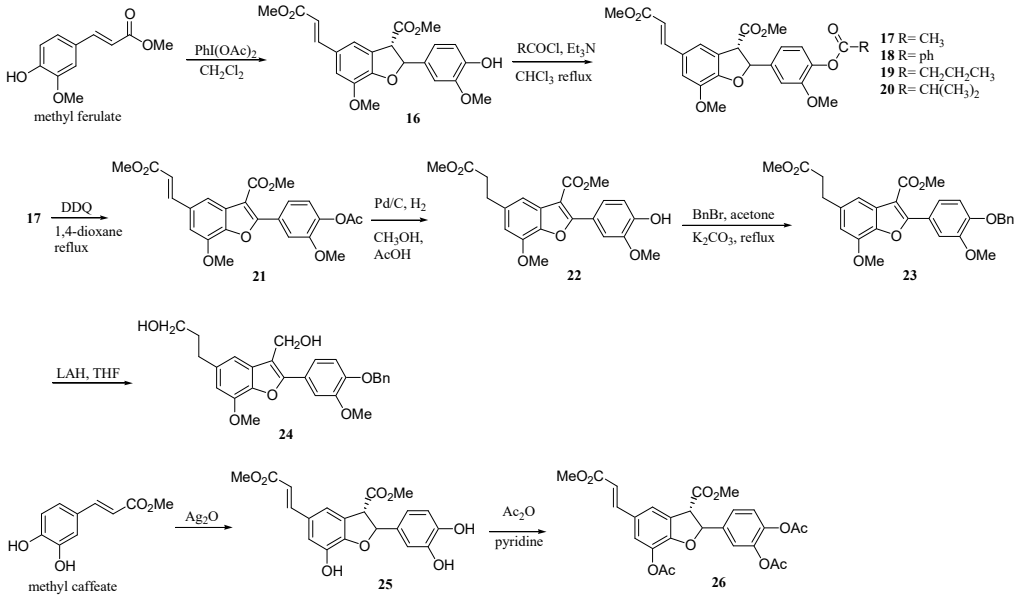
Previously, we reported a synthetic route of salvinal using isoeugenol as a starting material, as reacted with iodobenzene diacetateuse (IDA) in a four-step reaction with a yield of 23% [13]. The convenient synthesis method shortens the preparation procedure of salvinal and benzofuranlignan derivatives. The synthesis steps of salvinal derivatives are shown in Scheme 1. Isoeugenol as a starting material was reacted with iodobenzene diacetateuse (IDA) to obtain salvinal (**4**) in a four-step reaction (Scheme 1). Various sub-

stitutions in the C-3 and C-5 positions of the benzofuran skeleton were synthesized and evaluated for their impact on anticancer activity. In order to examine the influence of phenyl substituent at the C-2 position of compound **1**, we used compound **1** as the template for further alkylation (that resulted in compounds **5**–**9**) and acylation reactions (that resulted in compounds **10**–**12**). Compound **1** was treated with DDQ (1.1 eqa) in the mixture solvent of CH_2Cl_2/H_2O=4/1 to initiate a reaction, which could selectively oxidize arylpropene to arylpropenal, forming compound **13** under the room temperature. Compound **2** was further oxidized with SeO_2 in refluxing EtOH to yield compound **14**. The structural difference between compound **14** and salvinal (**4**) is the side chain at the C-5 position, and the anticancer activities of the two are only slightly different. To evaluate the impact of phenyl substituent at the C-2 position, alkylation reaction was conducted with compound **14** in order to obtain compound **15**, as shown in Scheme 1.



Scheme 1. Synthesis of salvinal derivatives **1**–**15**.

Oxidative coupling of methyl ferulate with IDA generated benzofuran compound **16** (Scheme 2). Compound **16** was further acylated to obtain compounds **17**–**20**. Dehydrogenation of compound **17** using DDQ in 1,4-dioxane under reflux afforded compound **21**. Compound **21** was dissolved in the mixture solvent of CH_3OH and AcOH with 10% Pd/C as catalysis under a hydrogen atmosphere to yield compound **22** through both hydrogenation and hydrolysis reactions. Benylation of compound **22** using benzyl bromide and K_2CO_3 in acetone under reflux afforded compound **23** in a high yield of 85%. By using LAH in dry THF, two methyl carboxylates were reduced to hydroxymethyl to form compound **24**. We also used Ag_2O for the oxidative coupling of methyl caffeate to form compound **25**, which was then acetylated to form compound **26**.



Scheme 2. Synthesis of salvinal derivatives **16**–**26**.

2.2. Structure–Activity Relationship

Salvinal consists of a benzofuran skeleton with a phenyl moiety at the C-2 position. Here, 24 salvinal derivatives with various substitutions in the C-2, 3, 5, and 7 positions were prepared for their structure–activity relationship study. The antiproliferative effect of salvinal derivatives was evaluated by methylene blue assay in two epithelial tumor cell lines (KB and HONE-1). The anticancer activity of salvinal derivatives was compared with compounds **1** and **4** (salvinal). The IC_{50} of compound **1** and salvinal against KB cells was 5.6 μM and 5.0 μM, respectively. The IC_{50} of salvinal derivatives against KB and HONE-1 cells is shown in Table 1. The results of the anticancer activity against KB cells revealed that of the 24 salvinal derivatives in this series, except for compound **13**, two (compounds **25** and **26**) showed IC_{50} less than 0.4 μM, four showed IC_{50} in the range of 0.4–5.0 μM, three showed IC_{50} in the range of 5–10 μM, twelve showed IC_{50} in the range of 10–38 μM, and two showed IC_{50} more than 38 μM. Six compounds (**14**, **18**, **19**, **20**, **25**, and **26**) (Figures S1–S16) showed more potent anticancer activity than salvinal. Compound **25** showed the best anticancer activity against KB cells, with IC_{50} values of 0.137 μM compared to salvinal, with IC_{50} of 5.0 μM. The test compounds exhibited similar IC_{50} values against both KB and HONE-1 cells, which indicated that they have similar anticancer activity against these two cells.

The newly synthesized benzofuran compounds can be divided into two subclasses, benzofuran and dihydrobenzofuran, as shown in Figure 1. In the dihydrobenzofuran series compounds, by using compound **1** as the template for further modification, we found that the change of C-4′ position of 2-phenyl portion, either to the ether (compounds **5**–**9**) or the ester (compounds **10**–**12**) substituents, decreased the inhibitory activity; the trend almost paralleled the increase in carbon number of substituents in compounds **5**–**8** and **10**–**12**. This observation suggests that hydroxyl group of the C-4′ position on the phenyl ring is an important functional group for the anticancer activity against KB cells. Modification of the C-5 position with a propenal group (compound **13**) resulted in a comparable anticancer activity to the parental compound **1** containing a propenyl group. Interestingly, when the C-3 and C-5 positions were substituted by acrylic methyl and carboxyl methyl esters (compound **16**), respectively, the IC_{50} value was larger compared to compound **1**. The C-4′ position of compound **16** was esterified to the corresponding ester compounds **17**–**20**, most of which showed better anticancer activity than salvinal (**4**). We found that the change of the C-2 substituent with a 3,4-dihydroxyphenyl moiety (compound **25**) showed the best anticancer activity in this series of compounds, indicating that the catechol moiety

is an important functionality for anticancer potency. Compound **25** acetylated to form compound **26** also showed good anticancer activities. Phenolic esters with triacyl groups exhibited lower polarity than the original phenols, and they will express more penetrable potency through cell membrane. The essential functionality was proposed as the catechol moiety, because triacetyl groups can be hydrolyzed to the original trihydroxyl groups by esterase in the internals of cancer cells.

Table 1. Growth inhibition of salvinal derivatives against human cancer cells.

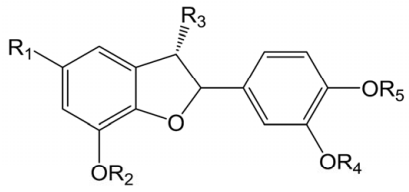
Compound	IC_{50} Values [a]	
	KB (μM) [b]	HONE-1 (μM)
1	5.6 ± 0.1	5.7 ± 0.1
2	6.9 ± 0.2	n.d.
4	5.0 ± 0.1	4.5 ± 0.1
5	21.6 ± 2.7	28.6 ± 5.0
6	40.0 ± 7.3	n.d.
7	42.9 ±3.4	n.d.
8	33.0 ± 1.4	n.d.
9	13.1± 0	n.d.
10	16.1 ± 0.07	n.d.
11	28.1 ± 3.3	33.8 ± 0.3
12	25.4 ± 3.46	n.d.
13	n.d.	6.8 ± 0.5
14	4.8 ± 1.1	4.5 ± 0.1
15	14.9 ± 1.4	28.6 ± 5.0
16	13.8 ± 0.1	12.8 ± 2.1
17	6.7 ± 0.4	3.7 ± 0.1
18	2.0 ± 0.1	2.1 ± 0.1
19	1.2 ± 0.2	1.6 ± 0.1
20	4.9 ± 0.3	5.0 ± 0.1
21	30.3 ± 0.4	n.d.
22	37.8 ± 0.5	45.6 ± 2.4
23	30.8 ± 4.8	39.6 ± 0.5
24	22.0 ± 6.2	25.0 ± 2.8
25	0.137 ± 0.008	0.316 ± 0.025
26	0.326 ± 0.037	0.643 ± 0.053
VP-16	1.1 ± 0.2 [c]	n.d.

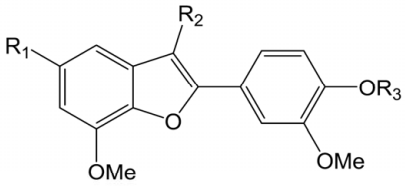
[a] Cells were treated with various concentrations of test compounds for 72 h. Cell survival was determined by methylene blue assay. Each IC_{50} value was calculated as described in *Experimental Method*. Each value represents the mean ± S.D. of three independent experiments. [b] The KB cell line was originally derived from an epidermal carcinoma of the mouth but has now been shown to have HeLa characteristics. [c] The IC_{50} value of VP-16 for KB cell lines is derived from our previous publication [12]. n.d. —not done.

In the benzofuran series compounds, modification of the C-2 position with a methyl group (**2**) or the C-5 position with a propenal group (**14**) showed comparable anticancer activity to salvinal (**4**). The C-3 and C-5 positions were substituted by the acrylic methyl and acetyl groups, respectively, as well as the acetylation of the C-4′ position (**21**), showing obviously weaker anticancer activity than compound **4**. The modification of the C-4′ position with a benzyloxyl group, together with the C-5 position with a propenal (**15**), an acrylic methyl (**23**), or a propanol (**24**) group, decreased their cytotoxic activity.

Based on our SAR analysis, we can conclude that the change of the benzyloxyl group on the benzofuran or dihydrobenzofuran backbone (compound **9**) showed better anticancer activity in KB cells than the alkyloxy analogs (as compounds **5**, **6**, **7,** and **8**) but weaker anticancer activity than original hydroxyl compound (as compound **1**). Further formation of acyl substituents on the C-4′ position of the benzene ring from **16** could improve the anticancer activity (compared compound **16** with compounds **17**, **18**, **19**, and **20**). Additionally, substitution of the methyl group at C-3 and the 1-propenyl group at C-5 (compound **1**) with carboxylic methyl ester and acrylic methyl ester (compound **16**), respectively, would attenuate the cytotoxic activity. The hydroxyl group of the C-4′ position in compound **16**

was acylated as ethanoate (**17**), benzoate (**18**), butanoate (**19**), and isobutanoate (**20**), increasing the potential for cytotoxic activity. Furthermore, comparing the anticancer activity between compounds **21** and **17** indicated that dihydrobenzefuran derivatives had more cytotoxic activity than benzofuran derivatives.





	R_1	R_2, R_4	R_3	R_5
1	CH=CH-CH_3	CH_3	CH_3	H
5	CH=CH-CH_3	CH_3	CH_3	CH_3
6	CH=CH-CH_3	CH_3	CH_3	CH_2CH_3
7	CH=CH-CH_3	CH_3	CH_3	$CH_2CH_2CH_3$
8	CH=CH-CH_3	CH_3	CH_3	$CH(CH_3)_2$
9	CH=CH-CH_3	CH_3	CH_3	Bn
10	CH=CH-CH_3	CH_3	CH_3	$COCH_3$
11	CH=CH-CH_3	CH_3	CH_3	$COCH_2CH_3$
12	CH=CH-CH_3	CH_3	CH_3	$COCH_2CH_2CH_3$
13	CH=CH-CHO	CH_3	CH_3	H
16	CH=CH-$COOCH_3$	CH_3	$COOCH_3$	H
17	CH=CH-$COOCH_3$	CH_3	$COOCH_3$	$COCH_3$
18	CH=CH-$COOCH_3$	CH_3	$COOCH_3$	COPh
19	CH=CH-$COOCH_3$	CH_3	$COOCH_3$	$COCH_2CH_2CH_3$
20	CH=CH-$COOCH_3$	CH_3	$COOCH_3$	$COCH(CH_3)_2$
25	CH=CH-$COOCH_3$	H	$COOCH_3$	H
26	CH=CH-$COOCH_3$	$COCH_3$	$COOCH_3$	$COCH_3$

	R_1	R_2	R_3
2	CH=CH-CHO	CH_3	H
4	$CH_2CH_2CH_2OH$	CHO	H
14	CH=CH-CHO	CHO	H
15	CH=CH-CHO	CHO	Bn
21	CH=CH-$COOCH_3$	$COOCH_3$	$COCH_3$
22	$CH_2CH_2COOCH_3$	$COOCH_3$	H
23	$CH_2CH_2COOCH_3$	$COOCH_3$	Bn
24	$CH_2CH_2CH_2OH$	CH_2OH	Bn

Figure 1. Structures of benzofuran and dihydrobenzofuran salvinal derivatives.

2.3. Drug Resistance Analysis

Drug resistance is a serious problem that restricts the use of microtubule-interfering drugs for clinical therapy [14]. We selected compounds **4**, **19**, **20**, **25,** and **26** of salvinal derivatives to further examine the efficacy against KB and KB drug-resistant cell lines. The IC_{50} of compounds **4**, **19**, **20**, **25,** and **26** against KB, KB-Vin10, and KB-7D cells is shown in Table 2. The data we obtained indicate that compounds 4, 19, 20, 25, and 26 possess a certain level of inhibitory activity against the proliferation of cancer cell lines KB and HONE1. Table 2 reveals that the results obtained for the HONE-1 cell line were highly consistent with the findings we observed in KB cells [15]. Despite overexpression of drug -resistant efflux protein (MDR/Pgp or MRP) in KB-Vin10 and KB-7D cells, the compounds **4**, **19**, **20**, **25**, and **26** showed comparative cytotoxic activity for both the parental cell line and MRP- or MDR-overexpressing counterparts. Compounds **4**, **19**, **20**, **25**, and **26** manifest similar potency, regardless of the cell's MDR or MRP status, suggesting that they are not substrates for these efflux pumps.

Table 2. Growth inhibition of salvinal derivatives against KB-derived resistance cancer cell lines.

Compound	KB (μM) [a] (Parental)	KB-VIN10 (μM) (MDR+)	R.I. [b]	KB-7D (μM) (MRP+)	R.I.
Vincristine	0.4 ± 0.1 (nM)	90.1 ± 7.4 (nM)	225.2	1.2 ± 0.4 (nM)	3
VP-16 [c]	1.1 ± 0.2	23 ± 3	20.9	54 ± 3.5	49.1
4 [d]	5.0 ± 0.1	3.7 ± 0.1	0.7	3.4 ± 0.2	0.7
19	1.2 ± 0.2	0.7 ± 0	0.6	0.5 ± 0.2	0.4
20	4.9 ± 0.3	2.8 ± 0.2	0.6	2.4 ± 0.1	0.5
25	0.137± 0.008	0.141 ± 0.003	1.0	0.138 ± 0.004	1.0
26	0.326 ± 0.037	0.269 ± 0.032	0.8	0.251 ± 0.007	0.8

[a] Cells were treated with various concentrations of test compounds for 72 h. Cell survival was determined by methylene blue assay. Each IC_{50} value was calculated as described in *Experimental Method*. [b] Resistant index. [c] The IC_{50} values of vincristine and VP-16 for all three cell lines are derived from our previous publication [12]. [d] The IC_{50} values of Compound **4** and its derivatives (Compound **19**, **20**, **25**, and **26**) for KB parental cell lines are reported in Table 1.

2.4. Inhibition of Tubulin Polymerization

In this study, the depolymerization activity of compounds **4**, **19**, **20**, **25**, and **26** on pure MAP-rich tubulins were assessed in vitro. As shown in Figure 2, compounds **4**, **19**, **20**, and **25** demonstrated a concentration-dependent inhibition of tubulin polymerization, while compound **26** did not affect the microtubule assembly.

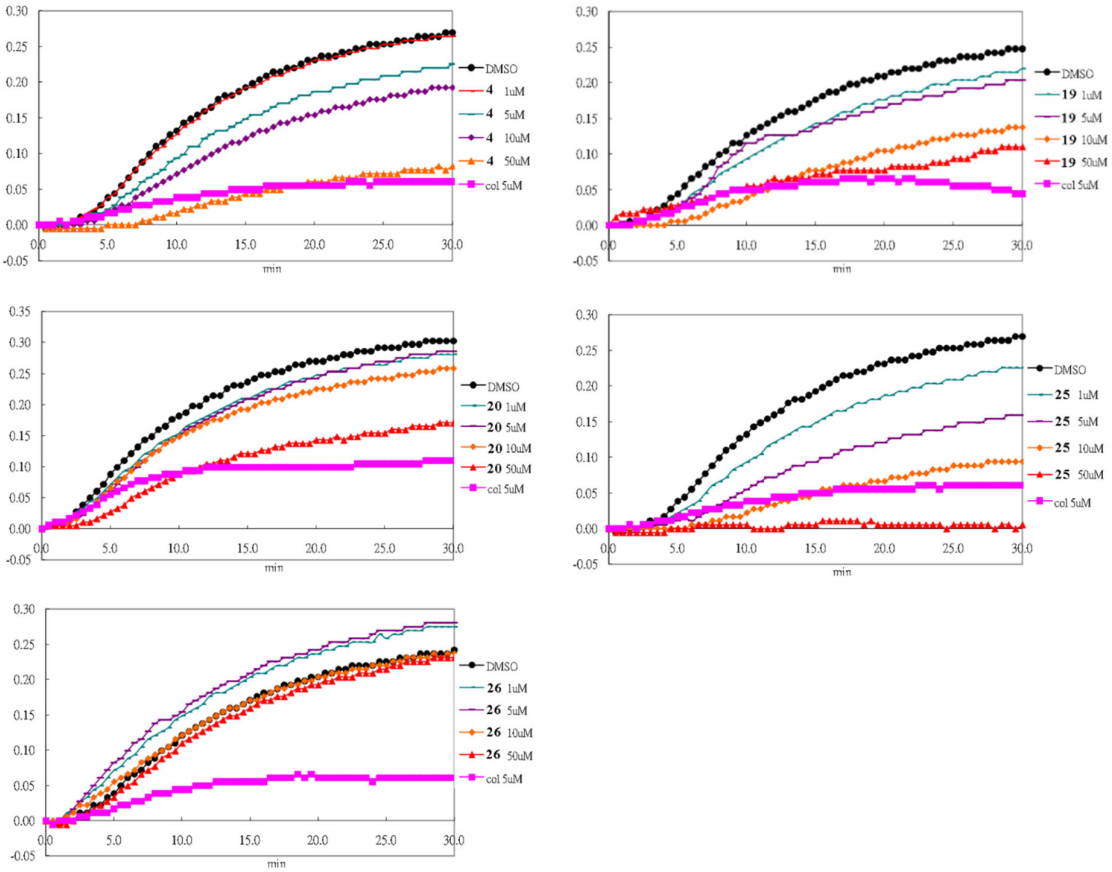


Figure 2. Inhibition of Tubulin Polymerization by Compounds **4**, **19**, **20**, **25**, and **26**. MAP-rich tubulins were incubated at 37 °C in the absence or presence of test compounds. Absorbance at 350 nm was measured every 30 s for 30 min and is presented as the increased polymerized microtubule.

The findings of our study are intriguing. The compounds **4**, **19**, **20**, and **25** and colchicine have been found to depolymerize microtubules in vitro in a dose-dependent

manner. It is hypothesized that compounds **4**, **19**, **20**, and **25** directly bind to tubulin/microtubules, leading to their depolymerization. However, it is noteworthy that compound **26** did not show any effect on the microtubule assembly. This suggests that the mechanism of action for its anticancer properties may be different from the other compounds. Therefore, further studies are needed to investigate the specific pathways through which these compounds exert their anticancer effects.

3. Experimental Method

3.1. General

The Infrared (IR) spectra were measured on a Nicolet MAG NA-IR 550 Spectrometer Series II (Nicolet, Madison, WI, USA). The NMR Spectra were collected using a Brucker M-300 WT FT-300 (^{1}H-NMR: 300 MHz, ^{13}C-NMR: 75 MHz)(Bruker, Fällanden, Switzerland) with $CDCl_3$ as the solvent. The EI-MS data were collected using a JEOL JMS-HX 300 Mass spectrometer (JEOL, Tokyo, Japan). Silica gel (Merck 70-230 mesh ASTM) was used for the column chromatography, and pre-coated silica gel (Merck 60 F-254) plates were used for the TLC analyses.

3.2. Chemistry

The salvinal derivatives were synthesized at the laboratory of Professor Yueh-Hsiung Kuo of the Tsuzuki Institute for Traditional Medicine, China Medical University (Taichung, Taiwan).

Compound **1** was prepared from isoeugenol by using IDA (iodobenzene diacetate). The solution of isoeugenol (10.0 g in 100 mL of CH_2Cl_2) was added dropwise to the solution of IDA (10.0 g, 30.2 mmol) in 100 mL of CH_2Cl_2 (dry with CaH_2) at room temperature for 4 h. After 48 h, $NaHCO_3$ (3 g) was added to the solution and stirred for 1 h. The mixture was filtrated, and the filtrate was evaporated under reduced pressure to give a yellow oil. Then, the residue was purified by Si gel column chromatography to give **1** as a colorless solid (3.9 g, 40% yield; with solvent system EtOAc: hexane = 1:9); mp 123–124 °C; ^{1}H-NMR ($CDCl_3$) δ_H 6.96 (s, 1H, H-4), 6.88 (d, J = 8.1 Hz, 1H, H-6′), 6.86 (d, J = 8.1 Hz, 1H, H-5′), 6.77 (s, 1H, H-6), 6.75 (s, 1H, H-2′), 6.35 (dd, J = 15.6, 1.0 Hz, 1H, H-1″), 6.09 (dq, J = 15.6, 6.6 Hz, 1H, H-2″), 5.64 (s, 1H, 4′-OH), 5.08 (d, J = 9.5 Hz, 1H, H-2), 3.87 (s, 3H, OMe), 3.86 (s, 3H, OMe), 3.43 (m, 1H, H-3), 1.85 (dd, J = 6.6, 1.0 Hz, 3H, H-3″), 1.36 (d, J = 6.8 Hz, 3H, Me-C-3); ^{13}C-NMR ($CDCl_3$) δ_C 146.5 (C-7), 146.4 (C-3′), 145.6 (C-7a), 143.9 (C-4′), 137.2 (C-1′), 132.0 (C-3a), 131.9 (C-5), 130.8 (C-1″), 123.2 (C-6′), 119.7 (C-2″), 114.0 (C-4), 113.2 (C-2′), 109.1 (C-5′), 108.8 (C-6), 93.6 (C-2), 55.7 (C-7-OMe), 51.7 (C-3′-OMe), 45.4 (C-3), 18.2 (C-3″), 17.4 (C-3-Me); IR (KBr film) ν_{max} 3446, 3023, 2964, 1608, 1515, 1460, 1337, 1275, 1141, 1033, 962 cm^{-1}; EI-MS m/z (%) (70 eV) 326 (M^+, 45; $C_{20}H_{22}O_4$), 202 (30), 178 (45), 151 (100), 137 (8), 119 (11), 91 (15).

Compound **2** was prepared from **1** by using DDQ (dichlorodicyanobenzoquinone). Compound **1** (2.12 g) and DDQ (3.24 g) were resolved in 50 mL of 1,4-dioxane. The solution was then refluxed. After 48 h, the solution was filtrated, and the filtrate was evaporated under reduced pressure. Then, the residue was purified by Si gel column chromatography to give **2** as a colorless solid (1.81 g, 83% yield; with solvent system EtOAc: hexane = 1:8); mp 221–222 °C; ^{1}H-NMR ($CDCl_3$) δ_H 9.68 (d, J = 7.6 Hz, 1H, H-3″), 7.55 (d, J = 15.9 Hz, 1H, H-1″), 7.26–7.30 (m, 3H, H-4, H-6, H-6′), 6.99 (d, J = 8.0 Hz, 1H, H-5′), 6.97 (s, 1H, H-2′), 6.70 (dd, J = 15.9, 7.6 Hz, 1H, H-2″), 5.85 (br s, 1H, Ph-OH), 4.03 (s, 3H, MeO-C-7), 3.95 (s, 3H, MeO-C-3′), 2.40 (s, 3H, Me-C-3); ^{13}C-NMR ($CDCl_3$) δ_C 193.6, 153.9, 146.7, 146.2, 145.2, 144.6, 133.5, 129.6, 127.4, 123.0, 120.8, 114.6, 113.8, 110.1, 109.4, 105.6, 56.1, 9.5; IR (KBr film) ν_{max} 3540, 2949, 2811, 2727, 1674, 1616, 1513, 1214, 1128, 972 cm^{-1}; EI-MS m/z (%) (70 eV) 338 (M^+, 100; $C_{20}H_{18}O_5$), 326 (21), 310 (100), 295 (14), 267 (15), 151 (13), 137 (14), 69 (17), 57 (19).

Compound **3** was prepared from **2** using Adam's catalyst reduction reaction. The solution of **2** (1.33 g in 20 mL of CH_3OH) with 10% PtO_2/H_2O (96.3 mg) was stirred under H_2 at room temperature. After 6 h, the mixture was filtrated, and the filtrate was evaporated under reduced pressure. Then, the residue was purified by Si gel column chromatography

to give **3** as a colorless solid (1.28 g, 95% yield; with solvent system EtOAc: hexane = 2:5); mp 165–166 °C; ^{1}H-NMR ($CDCl_3$) δ_H 7.30 (d, J = 2.0 Hz, 1H, H-4), 7.26 (dd, J = 8.2, 1.6 Hz, 1H, H-6′), 6.98 (d, J = 8.2 Hz, 1H, H-5′), 6.91 (d, J = 1.0 Hz, 1H, H-2′), 6.63 (d, J = 2.0 Hz, 1H, H-6), 5.73 (s, 1H, Ph-OH), 4.01 (s, 3H, MeO-C-7), 3.96 (s, 3H, MeO-C-3′), 3.71 (t, J = 6.5 Hz, 2H, H-3″), 2.79 (t, J = 7.9 Hz, 2H, H-1″), 2.38 (s, 3H, Me-C-3), 1.95 (m, 2H, H-2″); ^{13}C-NMR ($CDCl_3$) δ_C 151.4, 146.6, 145.7, 144.7, 136.9, 133.0, 123.8, 120.6, 114.5, 110.8, 109.5, 107.4, 62.4, 56.1, 34.8, 32.6, 9.60; IR (KBr film) ν_{max} 3431, 2940, 2851, 1602, 1516, 1455, 1386, 1222, 1052, 793 cm^{-1}; EI-MS *m*/*z* (%) (70 eV) 342 (M^+, 5; $C_{20}H_{22}O_5$), 340 (100), 324 (37), 312 (26), 297 (19), 284 (20), 148 (13), 97 (16), 91 (18), 69 (23), 57 (28).

Compound **4** was prepared from **3** using SeO_2 oxidative reaction. The solution of **3** (0.64 g in 20 mL of EtOH) with SeO_2 (0.42 g) was refluxed. After 12 h, the mixture was evaporated under reduced pressure. Then, 30 mL of EtOAc was added, the mixture was filtrated by celite, and the filtrate was evaporated under reduced pressure. Then, the residue was purified by Si gel column chromatography to give **4** as a colorless solid (0.48 g, 72% yield; with solvent system EtOAc: hexane = 1:5); mp 173–174 °C; ^{1}H-NMR ($CDCl_3$) δ_H 10.25 (s, 1H, CHO), 7.64 (s, 1H, H-2′), 7.37 (d, J = 8.0 Hz, 1H, H-6′), 7.35 (s, 1H, H-4), 7.04 (d, J = 8.0 Hz, 1H, H-5′), 6.73 (s, 1H, H-6), 6.11 (br s, 1H, Ph-OH), 4.00 (s, 3H, OMe), 3.97 (s, 3H, OMe), 3.69 (t, J = 6.5 Hz, 2H, H-3″), 2.80 (t, J = 7.3 Hz, 2H, H-1″), 1.94 (m, 2H, H-2″); ^{13}C-NMR ($CDCl_3$) δ_C 186.8, 165.9, 148.7, 146.9, 144.6, 141.6, 139.9, 127.3, 123.7, 120.6, 116.7, 115.0, 113.5, 111.0, 108.8, 62.2, 56.3, 56.1, 34.7, 32.5; IR (KBr film) ν_{max} 3513, 3435, 2940, 2864, 1637, 1601, 1522, 1490, 1409, 1273, 1139, 1061, 818 cm^{-1}; EI-MS *m*/*z* (%) (70 eV) 356 (M^+, 60; $C_{20}H_{20}O_6$), 312 (100), 269 (7), 197 (6), 152 (6), 137 (6), 126 (4), 105 (4), 91 (4), 55 (4) (Figures S1–S4).

Compound **5** was prepared from **1** using alkylation reaction. MeI (66.2 mg) and K_2CO_3 (100.3 mg) were added to the solution of **1** (100.4 mg, in 10 mL of acetone), and then the solution was refluxed. After 6 h, the mixture was filtrated, and the filtrate was evaporated under reduced pressure. Then, the residue was purified by Si gel column chromatography to give **5** as a colorless solid (92.8 mg, 88% yield; with solvent system EtOAc: hexane = 1:9); mp 118–119 °C; ^{1}H-NMR ($CDCl_3$) δ_H 6.96 (s, 1H, H-4), 6.94 (dd, J = 8.1, 1.7 Hz, 1H, H-6′), 6.82 (d, J = 8.1 Hz, 1H, H-5′), 6.77 (s, 1H, H-6), 6.75 (s, 1H, H-2′), 6.34 (br d, J = 15.8 Hz, 1H, H-1″), 6.09 (dq, J = 15.8, 6.6 Hz, 1H, H-2″), 5.09 (d, J = 9.5 Hz, 1H, H-2), 3.87 (s, 3H, OMe), 3.86 (s, 3H, OMe), 3.85 (s, 3H, OMe), 3.44 (m, 1H, H-3), 1.85 (dd, J = 6.6, 1.3 Hz, 3H, H-3″), 1.36 (d, J = 6.9 Hz, 3H, Me-C-3); ^{13}C-NMR ($CDCl_3$) δ_C 149.1, 146.5, 144.1, 137.4, 133.2, 132.6, 132.2, 130.9, 130.2, 127.4, 123.4, 119.2, 113.3, 110.8, 109.5, 109.2, 93.6, 55.9, 45.5, 18.3, 17.6; IR (KBr film) ν_{max} 3014, 2963, 2882, 1604, 1517, 1464, 1270, 1151, 1023, 957, 855, 817 cm^{-1}; EI-MS *m*/*z* (%) (70 eV) 340 (M^+, 28; $C_{21}H_{24}O_4$), 204 (25), 192 (40), 168 (28), 165 (100), 153 (27), 125 (13), 81 (14), 77 (23), 69 (29).

Compound **6** was prepared from **1** using alkylation reaction; the reaction was similar to the preparation of **5**. Compound **1** (100.4 mg) gave **6** as a colorless solid (93.4 mg, 85% yield; with solvent system EtOAc: hexane = 1:9); mp 106–107 °C; ^{1}H-NMR ($CDCl_3$) δ_H 6.96 (s, 1H, H-4), 6.91 (dd, J = 8.1, 1.9 Hz, 1H, H-6′), 6.82 (d, J = 8.1 Hz, 1H, H-5′), 6.77 (s, 1H, H-6), 6.75 (d, J = 1.9 Hz, 1H, H-2′), 6.35 (dq, J = 15.6, 1.4 Hz, 1H, H-1″), 6.09 (dq, J = 15.6, 6.2 Hz, 1H, H-2″), 5.09 (d, J = 9.3 Hz, 1H, H-2), 4.08 (q, J = 6.9 Hz, 2H, $OC\underline{H}_2CH_3$), 3.87 (s, 3H, OMe), 3.84 (s, 3H, OMe), 3.45 (m, 1H, H-3), 1.85 (dd, J = 6.2, 1.4 Hz, 3H, H-3″), 1.44 (t, J = 6.9 Hz, 3H, $OCH_2C\underline{H}_3$), 1.37 (d, J = 6.7 Hz, 3H, Me-C-3); ^{13}C-NMR ($CDCl_3$) δ_C 149.4, 148.4, 146.5, 144.1, 137.4, 133.2, 132.5, 132.1, 130.9, 130.2, 127.4, 123.4, 119.2, 113.3, 112.3, 109.8, 109.2, 93.6, 64.3, 55.9, 55.8, 45.5, 18.3, 17.6, 14.7; IR (KBr film) ν_{max} 3011, 2965, 2884, 1600, 1517, 1461, 1339, 1227, 1031, 957, 856 cm^{-1}; EI-MS *m*/*z* (%) (70 eV) 354 (M^+, 14; $C_{22}H_{26}O_4$), 266 (14), 206 (80), 179 (100), 151 (89), 119 (10), 91 (17), 77 (13).

Compound **7** was prepared from **1** using alkylation reaction; the reaction was similar to the preparation of **5**. Compound **1** (100.4 mg) afforded **7** as a colorless solid (94.8 mg, 83% yield; with solvent system EtOAc: hexane = 1:9); mp 109–110 °C; ^{1}H-NMR ($CDCl_3$) δ_H 6.96 (s, 1H, H-4), 6.91 (dd, J = 8.1, 1.1 Hz, 1H, H-6′), 6.81 (d, J = 8.1 Hz, 1H, H-5′), 6.76 (s, 1H, H-6), 6.75 (s, 1H, H-2′), 6.35 (dq, J = 15.6, 1.0 Hz, 1H, H-1″), 6.09 (dq, J = 15.6, 6.6 Hz,

1H, H-2″), 5.09 (d, J = 9.5 Hz, 1H, H-2), 3.95 (t, J = 6.4 Hz, 2H, OC$\underline{H}_2$CH$_2$CH$_3$), 3.87 (s, 3H, OMe), 3.84 (s, 3H, OMe), 3.44 (m, 1H, H-3), 1.78–1.88 (m, 5H, H-3″, OCH$_2$C$\underline{H}_2$CH$_3$), 1.33 (d, J = 6.4 Hz, 3H, Me-C-3), 1.01 (t, J = 7.4 Hz, 3H, OCH$_2$CH$_2$C$\underline{H}_3$); ^{13}C-NMR (CDCl$_3$) δ_C 149.4, 148.6, 146.5, 144.1, 133.2, 132.5, 132.1, 130.9, 130.2, 123.3, 119.1, 113.2, 112.5, 109.9, 109.2, 93.6, 70.4, 55.9, 55.8, 45.5, 29.6, 22.4, 18.3, 17.5, 10.3; IR (KBr film) ν_{max} 2965, 2884, 1600, 1518, 1462, 1267, 1145, 1031, 957, 856, 807 cm^{-1}; EI-MS *m*/*z* (%) (70 eV) 368 (M$^+$, 27; C$_{23}$H$_{28}$O$_4$), 280 (12), 220 (40), 193 (44), 178 (23), 151 (100), 140 (33), 97 (32), 77 (44), 57 (63).

Compound **8** was prepared from **1** using alkylation reaction; the reaction was similar to the preparation of **5**. Compound **1** (100.4 mg) obtained **8** as a colorless solid purified on SiO$_2$ column chromatography (114.2 mg, 82% yield; with solvent system EtOAc: hexane = 1:9); mp 107–108 °C; ^{1}H-NMR (CDCl$_3$) δ_H 6.96 (s, 1H, H-4), 6.91 (dd, J = 8.1, 1.1 Hz, 1H, H-6′), 6.84 (d, J = 8.1 Hz, 1H, H-5′), 6.77 (s, 1H, H-6), 6.75 (d, J = 1.1 Hz, 1H, H-2′), 6.35 (d, J = 15.6 Hz, 1H, H-1″), 6.09 (dq, J = 15.6, 6.5 Hz, 1H, H-2″), 5.09 (d, J = 9.4 Hz, 1H, H-2), 4.50 (m, 1H, OC$\underline{H}$(CH$_3$)$_2$), 3.87 (s, 3H, OMe), 3.84 (s, 3H, OMe), 3.46 (m, 1H, H-3), 1.85 (d, J = 6.5 Hz, 3H, H-3″), 1.36 (d, J = 7.7 Hz, 3H, Me-C-3), 1.34 (d, J = 6.3 Hz, 6H, OCH(C$\underline{H}_3$)$_2$); ^{13}C-NMR (CDCl$_3$) δ_C 150.4, 147.3, 144.9, 133.2, 132.9, 132.1, 130.9, 123.3, 119.1, 115.4, 113.2, 110.3, 109.2, 93.6, 71.4, 56.0, 55.9, 55.8, 45.4, 21.9, 18.3, 17.6; IR (KBr film) ν_{max} 2975, 2935, 1603, 1508, 1461, 1333, 1269, 1138, 1035, 958, 856, 820 cm^{-1}; EI-MS *m*/*z* (%) (70 eV) 368 (M$^+$, 5; C$_{23}$H$_{28}$O$_4$), 326 (8), 280 (10), 220 (12), 178 (59), 151 (100), 140 (28), 91 (9), 71 (8), 57 (13).

Compound **9** was prepared from **1** using alkylation reaction; the reaction was similar to the preparation of **5**. Compound **1** (100.4 mg) yielded **9** as a colorless solid on SiO$_2$ column chromatography (103.3 mg, 80% yield; with solvent system EtOAc: hexane = 1:9); mp 165–166 °C; ^{1}H-NMR (CDCl$_3$) δ_H 7.27–7.42 (m, 5H, OCH$_2$$\underline{Ph}$), 6.98 (s, 1H, H-4), 6.86 (dd, J = 8.2, 1.5 Hz, 1H, H-6′), 6.82 (d, J = 8.2 Hz, 1H, H-5′), 6.76 (s, 1H, H-6), 6.74 (d, J = 1.5 Hz, 1H, H-2′), 6.34 (dq, J = 16.0, 1.2 Hz, 1H, H-1″), 6.05 (dq, J = 16.0, 6.7 Hz, 1H, H-2″), 5.14 (s, 2H, OC$\underline{H}_2$Ph), 5.08 (d, J = 9.6 Hz, 1H, H-2), 3.87 (s, 3H, OMe), 3.84 (s, 3H, OMe), 3.44 (m, 1H, H-3), 1.85 (dd, J = 6.7, 1.2 Hz, 3H, H-3″), 1.35 (d, J = 6.8 Hz, 3H, Me-C-3); ^{13}C-NMR (CDCl$_3$) δ_C 149.7, 148.1, 146.5, 144.0, 137.0, 133.1, 132.1, 130.8, 128.4, 127.7, 127.1, 123.3, 119.0, 113.6, 113.2, 110.0, 109.2, 93.5, 70.9, 55.9, 55.8, 45.4, 18.3, 17.6; IR (KBr film) ν_{max} 3069, 3002, 2961, 2871, 1602, 1516, 1460, 1268, 1220, 1141, 1031, 947, 742 cm^{-1}; EI-MS *m*/*z* (%) (70 eV) 416 (M$^+$, 3; C$_{27}$H$_{28}$O$_4$), 328 (8), 268 (25), 241 (10), 177 (42), 151 (32), 139 (14), 91 (100), 65 (8).

Compound **10** was prepared from **1** using esteration reaction. CH$_3$COCl (0.3 mL) and Et$_3$N (0.4 mL) were added to the solution of **1** (107.6 mg in 10mL of CHCl$_3$), and then the solution was refluxed. After 3 h, the mixture was added to ice water (10 mL) and extracted by EtOAc (2 × 10 mL). The organic layers were combined and then washed with 1N HCl and aqueous NaHCO$_3$ and subsequently evaporated under reduced pressure. Then, the residue was purified by Si gel column chromatography to give **10** as a colorless solid (117.4 mg, 92% yield; with solvent system EtOAc: hexane = 1:9); mp 154–155 °C; ^{1}H-NMR (CDCl$_3$) δ_H 7.04 (s, 1H, H-4), 7.02 (d, J = 8.2 Hz, 1H, H-5′), 6.94 (dd, J = 8.2, 1.7 Hz, 1H, H-6′), 6.77 (s, 1H, H-6), 6.74 (s, 1H, H-2′), 6.34 (dq, J = 15.4, 1.1 Hz, 1H, H-1″), 6.09 (dq, J = 15.4, 6.6 Hz, 1H, H-2″), 5.14 (d, J = 9.1 Hz, 1H, H-2), 3.88 (s, 3H, OMe), 3.80 (s, 3H, OMe), 3.46 (m, 1H, H-3), 2.29 (s, 3H, OAc), 1.85 (dd, J = 6.6, 1.1 Hz, 3H, H-3″), 1.38 (d, J = 6.7 Hz, 3H, Me-C-3); ^{13}C-NMR (CDCl$_3$) δ_C 168.9, 151.2, 146.4, 144.1, 139.6, 139.2, 133.0, 132.3, 130.8, 123.6, 122.6, 118.6, 113.3, 110.2, 109.3, 93.0, 55.9, 55.8, 45.7, 20.6, 18.3, 17.9; IR (KBr film) ν_{max} 2968, 2938, 2881, 1769, 1608, 1507, 1461, 1202, 1152, 1035, 966, 860 cm^{-1}; EI-MS *m*/*z* (%) (70 eV) 368 (M$^+$, 37; C$_{22}$H$_{24}$O$_5$), 326 (100), 182 (19), 172 (38), 140 (60), 127 (42), 98 (23), 85 (46), 71 (24), 57 (29).

Compound **11** was prepared from **1** using esteration reaction; the reaction was similar to the preparation of **10** with propanoyl chloride and triethylamine. Compound **1** (121.1 mg) afforded **11** as a colorless solid (133.6 mg, 95% yield; with solvent system EtOAc: hexane = 1:9); mp 175–176 °C; ^{1}H-NMR (CDCl$_3$) δ_H 7.03 (s, 1H, H-4), 6.98 (d, J = 8.0 Hz, 1H, H-5′), 6.94 (dd, J = 8.0, 1.4 Hz, 1H, H-6′), 6.77 (s, 1H, H-6), 6.74 (d, J = 1.4 Hz, 1H, H-2′), 6.34 (dq, J = 15.6, 1.1 Hz, 1H, H-1″), 6.11 (dq, J = 15.6, 6.8 Hz, 1H, H-2″), 5.13 (d, J = 9.2 Hz, 1H, H-2), 3.88 (s, 3H, OMe), 3.79 (s, 3H, OMe), 3.45 (m, 1H, H-3), 2.59 (q,

J = 7.4 Hz, 2H, C$\underline{H}_2$CH$_3$), 1.86 (dd, J = 6.8, 1.1 Hz, 3H, H-3″), 1.38 (d, J = 6.8 Hz, 3H, Me-C-3), 1.25 (t, J = 7.4 Hz, 3H, CH$_2$C$\underline{H}_3$); ^{13}C-NMR (CDCl$_3$) δ_C 172.5, 151.2, 146.5, 144.1, 139.7, 139.1, 133.0, 132.4, 130.9, 123.6, 122.6, 118.7, 113.3, 110.3, 109.3, 93.1, 56.0, 45.8, 29.7, 27.3, 18.3, 17.9, 9.1; IR (KBr film) ν_{max} 2928, 2858, 1768, 1605, 1499, 1465, 1274, 1125, 1032, 954, 822, 759 cm^{-1}; EI-MS *m*/*z* (%) (70 eV) 382 (M$^+$, 25; $C_{23}H_{26}O_5$), 326 (100), 314 (7), 199 (6), 149 (11), 97 (16), 85 (15), 71 (22), 57 (34).

Compound **12** was prepared from **1** using esteration reaction; the reaction was similar to the preparation of **10** with butanoyl chloride and triethylamine. Compound **1** (120.4 mg) gave **12** as a colorless solid (131.2 mg, 92% yield; with solvent system EtOAc: hexane = 1:9); mp 174–175 °C; ^{1}H-NMR (CDCl$_3$) δ_H 7.03 (s, 1H, H-4), 6.95–6.97 (m, 2H, H-5′, H-6′), 6.77 (s, 1H, H-6), 6.75 (s, 1H, H-2′), 6.35 (dq, J = 15.6, 1.0 Hz, 1H, H-1″), 6.10 (dq, J = 15.6, 6.6 Hz, 1H, H-2″), 5.14 (d, J = 9.2 Hz, 1H, H-2), 3.88 (s, 3H, OMe), 3.79 (s, 3H, OMe), 3.43 (m, 1H, H-3), 2.54 (t, J = 7.3 Hz, 2H, C$\underline{H}_2$CH$_2$CH$_3$), 1.85 (dd, J = 6.6, 1.0 Hz, 3H, H-3″), 1.78 (sex, J = 7.3 Hz, 2H, CH$_2$C$\underline{H}_2$CH$_3$), 1.39 (d, J = 6.8 Hz, 3H, Me-C-3), 0.99 (t, J = 7.3 Hz, 3H, CH$_2$CH$_2$C$\underline{H}_3$); ^{13}C-NMR (CDCl$_3$) δ_C 171.5, 151.1, 146.4, 144.0, 139.6, 139.0, 132.9, 132.3, 130.8, 123.4, 122.5, 118.5, 113.2, 110.2, 109.3, 93.0, 55.8, 55.7, 45.7, 35.7, 18.4, 18.2, 17.7, 14.0; IR (KBr film) ν_{max} 3018, 2968, 2879, 1772, 1607, 1512, 1123, 1031, 954, 826, 596, 537 cm^{-1}; EI-MS *m*/*z* (%) (70 eV) 396 (M$^+$, 27; $C_{24}H_{28}O_5$), 326 (100), 238 (8), 178 (27), 151 (24), 140 (25), 126 (23), 85 (21), 71 (54).

Compound **13** was prepared from **1** using DDQ (dichlorodicyanobenzoquinone). Compound **1** (1.15 g, 3.50 mmol) and DDQ (0.870 g, 3.80 mmol) were dissolved in the mixture of CH_2Cl: H_2O = 4:1 (10 mL) and stirred for 48 h. After filtration, the product in the filtrate was purified using Si gel column chromatography. It gave compound **13** (1.05 g, 88% yield); mp 177–178°C; ^{1}H-NMR (CDCl$_3$) δ_H 9.64 (d, J = 7.6 Hz, 1H, H-3″), 7.41 (d, J = 15.8 Hz, 1H, H-1″), 7.02 (s, 1H, H-4), 6.99 (s, 1H, H-6), 6.97 (d, J = 8.1 Hz, 1H, H-6′), 6.89 (s, 1H, H-2′), 6.87 (1d, J = 8.1Hz, 1H, H-5′), 6.60 (dd, J = 15.8, 7.6 Hz, H-2″), 5.66 (s, 1H, ph-O$\underline{H}$), 5.18 (d, J = 9.2 Hz, 1H, H-2), 3.50 (m, 1H, H-3), 1.40 (d, J = 6.8 Hz, 3H, C-3-Me); ^{13}C-NMR (CDCl$_3$) δ_C 193.6, 153.2, 150.6, 146.7, 146.0, 144.6, 134.0, 131.2, 128.1, 126.3, 119.9, 117.3, 114.3, 111.8, 108.9, 94.5, 56.0, 55.9, 45.1, 17.7; IR (KBr film) ν_{max} 3486, 2985, 2852, 2851, 2734, 1684, 1620, 1478, 1133, 821 cm^{-1}; EI-MS *m*/*z* (%) (70 eV) 340 (M$^+$, 100; $C_{20}H_{20}O_5$), 325 (7), 137 (15), 97 (15), 71 (18), 57 (30).

Compound **14** was prepared from **2** using SeO_2 oxidative reaction. The solution of **2** (212.4 mg in 20 mL of EtOH) with SeO_2 (0.14 g, 1.24 mmol) was refluxed. After 12 h, the mixture was evaporated under reduced pressure. Then, 30 mL of EtOAc was added, the mixture was filtrated by celite, and the filtrate was evaporated under reduced pressure. Then, the residue was purified by Si gel column chromatography to give **14** as a colorless solid (170.3 mg, 77% yield; with solvent system EtOAc: hexane = 1:5); mp 234–235 °C; ^{1}H-NMR (CDCl$_3$) δ_H 10.3 (s, 1H, OHC-C-3), 9.72 (d, J = 7.7 Hz, 1H, H-3″), 8.05 (d, J = 1.9 Hz, 1H, H-4), 7.58 (d, J = 15.9 Hz, 1H, H-1″), 7.41 (dd, J = 8.0, 1.8 Hz, 1H, H-6′), 7.37 (d, J = 1.9 Hz, 1H, H-6), 7.09 (d, J = 1.8 Hz, 1H, H-2′), 7.07 (d, J = 8.0 Hz, 1H, H-5′), 6.75 (dd, J = 15.9, 7.7 Hz, 1H, H-2″), 6.02 (br s, 1H, Ph-OH), 4.06 (s, 3H, OMe), 4.01 (s, 3H, OMe); ^{13}C-NMR (CDCl$_3$) δ_C 194.3, 186.6, 165.7, 153.8, 150.4, 148.2, 145.0, 143.7, 132.3, 128.4, 127.4, 123.1, 118.5, 116.2, 116.0, 115.3, 112.4, 107.0, 56.2, 55.9; IR (KBr film) ν_{max} 3488, 2930, 2854, 2734, 1680, 1619, 1513, 1478, 1437, 1279, 1133, 1027, 822 cm^{-1}; EI-MS *m*/*z* (%) (70 eV) 352 (M$^+$, 100; $C_{20}H_{16}O_6$), 323 (40), 296 (15), 281 (20), 253 (33), 181 (18), 165 (20), 152 (23), 105 (13), 69 (14).

Compound **15** was prepared from **14** using alkylation reaction; the reaction was similar to the preparation of **5**. Compound **14** (100.5 mg) obtained **15** as a colorless solid (126.2 mg, 87% yield; with solvent system EtOAc: hexane = 1:9); mp 265–266 °C; ^{1}H-NMR (CDCl$_3$) δ_H 10.27 (s, 1H, OHC-C-3), 9.68 (d, J = 7.6 Hz, 1H, H-3″), 8.01 (s, 1H, H-4), 7.53 (d, J = 15.6 Hz, 1H, H-1″), 7.35–7.45 (m, 7H, CH$_2\underline{Ph}$, H-6, H-6′), 7.05 (s, 1H, H-2′), 7.00 (d, J = 8.0 Hz, 1H, H-5′), 6.72 (dd, J = 15.6, 7.6 Hz, 1H, H-2″), 5.22 (s, 2H, C$\underline{H}_2$Ph), 4.04 (s, 3H, OMe), 3.97 (s, 3H, OMe); ^{13}C-NMR (CDCl$_3$) δ_C 193.4, 186.3, 166.2, 152.9, 151.2, 149.9, 145.3, 144.6, 145.3, 144.6, 136.1, 132.0, 128.7, 128.3, 128.1, 127.8, 127.2, 122.9, 120.8, 116.6, 113.5, 111.8, 106.6, 70.9, 56.3, 56.1; IR (KBr film) ν_{max} 3060, 2947, 2841, 2739, 1680, 1607, 1517, 1477, 1271, 1126, 1028,

970, 730 cm^{-1}; EI-MS *m*/*z* (%) (70 eV) 442 (M^+, 53; $C_{27}H_{22}O_6$), 351 (54), 105 (11), 91 (100), 65 (5).

Compound **16** was prepared from methyl ferulate using IDA; the procedure was similar to the preparation of **1** from isoeugenol. Methyl ferulate (10.0 g) yielded **16** as a colorless solid (7.3 g, 73% yield; with solvent system EtOAc: hexane = 1:9 on open column); mp 96–97 °C; ^{1}H-NMR ($CDCl_3$) δ_H 7.64 (d, J = 15.9 Hz, 1H, H-1″), 7.17 (s, 1H, H-4), 7.00 (s, 1H, H-6), 6.88 (s, 3H, H-2′, H-5′, H-6′), 6.30 (d, J = 15.9 Hz, 1H, H-2″), 6.09 (d, J = 8.1 Hz, 1H, H-2), 5.63 (s, 1H, Ph-OH), 4.33 (d, J = 8.1 Hz, 1H, H-3), 3.89 (s, 3H, OMe), 3.86 (s, 3H, OMe), 3.81 (s, 3H, COOMe), 3.79 (s, 3H, COOMe); ^{13}C-NMR ($CDCl_3$) δ_C 170.7, 167.6, 146.0, 144.7, 144.6, 131.3, 128.5, 125.6, 119.4, 117.8, 115.5, 114.5, 112.0, 108.7, 87.4, 56.0, 55.9, 55.4, 52.8, 51.6; IR (KBr film) ν_{max} 3396, 3011, 2956, 2849, 1741, 1637, 1606, 1496, 1440, 1287, 837, 612 cm^{-1}; EI-MS *m*/*z* (%) (70 eV) 414 (M^+, 95; $C_{22}H_{22}O_8$), 382 (100), 350 (73), 280 (15), 266 (12), 167 (8), 151 (7), 137 (6), 58 (18).

Compound **17** was prepared from **16** using ethanoyl chloride in triethylamine; the reaction was similar to the preparation of **10**. Compound **16** (1.36 g) could give **17** as a colorless solid (1.40 g, 91% yield; with solvent system EtOAc: hexane = 1:9); mp 98–99 °C; ^{1}H-NMR ($CDCl_3$) δ_H 7.63 (d, J = 15.8 Hz, 1H, H-1″), 7.17 (s, 1H, H-4), 6.97–7.00 (m, 3H, H-6, H-5′, H-6′), 6.30 (d, J = 15.8 Hz, 1H, H-2″), 6.16 (d, J = 8.0 Hz, 1H, H-2), 4.32 (d, J = 8.0 Hz, 1H, H-3), 3.91 (s, 3H, OMe), 3.83 (s, 3H, OMe), 3.80 (s, 3H, CO_2Me), 3.79 (s, 3H, CO_2Me), 2.29 (s, 3H, COMe); ^{13}C-NMR ($CDCl_3$) δ_C 170.5, 168.8, 167.5, 151.2, 149.7, 144.6, 144.5, 139.7, 138.4, 128.7, 125.3, 122.9, 118.1, 117.8, 115.6, 112.1, 109.9, 86.6, 56.0, 55.8, 55.4, 52.8, 51.5, 20.5; IR (KBr film) ν_{max} 3073, 2955, 2848, 1769, 1742, 1710, 1638, 1604, 1499, 1443, 1275, 1196, 1150, 835 cm^{-1}; EI-MS *m*/*z* (%) (70 eV) 456 (M^+, 2; $C_{24}H_{24}O_9$), 412 (4), 400 (5), 310 (7), 250 (6), 204 (10), 148 (100), 131 (51), 130 (17), 97 (17), 69 (23), 57 (31).

Compound **18** was prepared from **16** using benzoyl chloride and triethylamine; the reaction was similar to the preparation of **10**. Compound **16** (74.1 mg) afforded **18** as a colorless solid (87.1 mg, 94% yield; with solvent system EtOAc: hexane = 1:9); mp 143–144 °C; ^{1}H-NMR ($CDCl_3$) δ_H 8.19 (d, J = 7.5 Hz, 2H, -CO<u>Ph</u> (o)), 7.64 (d, J = 15.6 Hz, 1H, H-1″), 7.59 (t, J = 7.5 Hz, 1H, -CO<u>Ph</u> (p)), 7.48 (t, J = 7.5 Hz, 2H, -CO<u>Ph</u> (m)), 7.19 (s, 1H, H-4), 7.13 (d, J = 8.0 Hz, 1H, H-5′), 7.00–7.05 (m, 4H, H-6, H-2′, H-6′), 6.67 (d, J = 15.6 Hz, 1H, H-2″), 6.20 (d, J = 8.0 Hz, 1H, H-2), 4.36 (d, J = 8.0 Hz, 1H, H-3), 3.92 (s, 3H, OMe), 3.84 (s, 3H, OMe), 3.79 (s, 6H, CO_2Me); ^{13}C-NMR ($CDCl_3$) δ_C 170.6, 167.6, 164.6, 151.6, 149.8, 144.6, 140.1, 138.5, 133.5, 130.3, 129.2, 128.8, 128.5, 125.5, 123.2, 118.2, 117.9, 115.7, 112.2, 110.2, 86.8, 56.2, 56.0, 55.6, 52.9, 51.6; IR (KBr film) ν_{max} 3069, 2950, 2852, 1734, 1641, 1607, 1506, 1446, 1274, 1209, 1028, 846, 704 cm^{-1}; EI-MS *m*/*z* (%) (70 eV) 518 (M^+, 35; $C_{29}H_{26}O_9$), 486 (2), 455 (3), 382 (2), 264 (3), 220 (3), 160 (6), 105 (100), 77 (21), 57 (7).

Compound **19** was prepared from **16** using butanoyl chloride in triethylamine; the reaction was similar to the preparation of **10**. Compound **16** (91.6 mg) gave **19** as a colorless solid (96.4 mg, 90% yield; with solvent system EtOAc: hexane = 1:9); mp 155–156 °C; ^{1}H-NMR ($CDCl_3$) δ_H 7.62 (d, J = 15.8 Hz, 1H, H-1″), 7.17 (s, 1H, H-4), 6.97–7.01 (m, 3H, H-6, H-5′, H-6′), 6.30 (d, J = 15.8 Hz, 1H, H-2″), 6.16 (d, J = 8.0 Hz, 1H, H-2), 4.32 (d, J = 8.0 Hz, 1H, H-3), 3.91 (s, 3H, OMe), 3.83 (s, 3H, OMe), 3.78 (s, 6H, CO_2Me), 2.53 (t, J = 7.3 Hz, 2H, $C\underline{H}_2CH_2CH_3$), 1.75 (sex, J = 7.3 Hz, 2H, $CH_2C\underline{H}_2CH_3$), 1.02 (t, J = 7.3 Hz, 3H, $CH_2CH_2C\underline{H}_3$); ^{13}C-NMR ($CDCl_3$) δ_C 171.6, 170.6, 167.6, 151.4, 149.8, 144.7, 144.6, 140.0, 138.3, 128.8, 125.5, 123.1, 118.2, 117.9, 115.7, 112.2, 110.1, 86.8, 56.2, 55.9, 55.5, 52.9, 51.6, 35.8, 18.5, 13.5; IR (KBr film) ν_{max} 3003, 2960, 2850, 1766, 1742, 1711, 1608, 1507, 1434, 1275, 1141, 1030, 843 cm^{-1}; EI-MS *m*/*z* (%) (70 eV) 484 (M^+, 41; $C_{26}H_{28}O_9$), 414 (38), 382 (100), 350 (33), 323 (10), 290 (7), 166 (7), 71 (17) (Figures S5–S8).

Compound **20** was prepared from **16** using isobutanoyl chloride in triethylamine; the reaction was similar to the preparation of **10**. Compound **16** (91.6 mg) obtained **20** as a colorless solid (85.6 mg, 88% yield; with solvent system EtOAc: hexane = 1:9); mp 132–133 °C; ^{1}H-NMR ($CDCl_3$) δ_H 7.63 (d, J = 15.8 Hz, 1H, H-1″), 7.17 (s, 1H, H-4), 6.96–6.98 (m, 4H, H-2′, H-5′, H-6′, H-6), 6.30 (d, J = 15.8 Hz, 1H, H-2″), 6.16 (d, J = 8.0 Hz, 1H, H-2), 4.32 (d, J = 8.0 Hz, 1H, H-3), 3.95 (s, 3H, OMe), 3.83 (s, 3H, OMe), 3.76 (s, 3H, CO_2Me), 3.74

(s, 3H, CO_2Me), 2.81 (m, 1H, C<u>H</u>$(CH_3)_2$), 1.27 (d, J = 7.1 Hz, 6H, CH(C<u>H</u>$_3)_2$); ^{13}C-NMR ($CDCl_3$) δ_C 175.1, 170.6, 167.5, 151.4, 149.8, 144.7, 144.6, 140.1, 138.2, 128.8, 125.5, 123.0, 118.2, 117.9, 115.7, 112.2, 110.1, 86.8, 56.1, 56.0, 55.5, 52.9, 51.6, 33.9, 18.9; IR (KBr film) ν_{max} 2952, 2849, 1762, 1605, 1508, 1464, 1281, 848, 757 cm^{-1}; EI-MS *m*/*z* (%) (70 eV) 484 (M^+, 45; $C_{26}H_{28}O_9$), 415 (27), 382 (100), 350 (28), 290 (5), 235 (7), 167 (4), 71 (13) (Figures S9–S13).

Compound **21** was prepared from **17** using DDQ (dichlorodicyanobenzoquinone) dehydrogenative and oxidative coupling reaction; the reaction was similar to the preparation of **2**. Compound **17** (1.10 g) afforded **21** as a colorless solid (0.93 g, 85% yield; with solvent system EtOAc: hexane = 1:5); mp 176–177 °C; ^{1}H-NMR ($CDCl_3$) δ_H 7.81 (s, 1H, H-2′), 7.79 (d, J = 16.0 Hz, 1H, H-1″), 7.78 (d, J = 1.3 Hz, 1H, H-4), 7.65 (dd, J = 8.3, 2.0 Hz, 1H, H-6′), 7.13 (d, J = 8.3 Hz, 1H, H-5′), 7.01 (d, J = 1.3 Hz, 1H, H-6), 6.44 (d, J = 16.0 Hz, 1H, H-2″), 4.03 (s, 3H, OMe), 3.95 (s, 3H, OMe), 3.91 (s, 3H, CO_2Me), 3.81 (s, 3H, CO_2Me), 2.33 (s, 3H, OAc); ^{13}C-NMR ($CDCl_3$) δ_C 168.6, 167.4, 163.9, 160.5, 150.7, 145.3, 145.2, 141.5, 131.5, 129.0, 127.6, 122.6, 122.3, 117.1, 116.1, 113.8, 109.2, 106.0, 56.1, 56.0, 51.8, 51.7, 20.6; IR (KBr film) ν_{max} 3096, 2951, 2846, 1769, 1721, 1633, 1605, 1468, 1258, 1045, 885, 855, 597 cm^{-1}; EI-MS *m*/*z* (%) (70 eV) 454 (M^+, 10; $C_{24}H_{22}O_9$), 412 (100), 382 (15), 349 (8), 323 (2), 228 (2), 151 (2), 91 (2), 69 (3).

Compound **22** was prepared from **21** using hydrogenation reductive reaction. The solution of **21** (50.6 mg in 10 mL of CH_3OH and 1 mL of CH_3COOH) with 10% Pd/C (10 mg) was stirred under H_2 at room temperature. After 6 h, the mixture was filtrated. After removing acid with aqueous $NaHCO_3$, the product residue was purified by Si gel column chromatography to give **22** as a colorless solid (43.8 mg, 95% yield; with solvent system EtOAc: hexane = 1:5); mp 134–135 °C; ^{1}H-NMR ($CDCl_3$) δ_H 7.67 (d, J = 1.7 Hz, 1H, H-2′), 7.59 (dd, J = 8.6, 1.7 Hz, 1H, H-6′), 7.41 (d, J = 1.1 Hz, 1H, H-4), 6.98 (d, J = 8.6 Hz, 1H, H-5′), 6.68 (d, J = 1.1 Hz, 1H, H-6), 5.88 (s, 1H, Ph-O<u>H</u>), 3.99 (s, 3H, OMe), 3.96 (s, 3H, OMe), 3.92 (s, 3H, CO_2<u>Me</u>), 3.68 (s, 3H, CO_2<u>Me</u>), 3.04 (t, J = 7.5 Hz, 2H, H-1″), 2.69 (t, J = 7.5 Hz, 1H, H-2″); ^{13}C-NMR ($CDCl_3$) δ_C 173.4, 164.6, 147.7, 145.9, 144.7, 141.6, 137.3, 129.0, 123.7, 121.5, 114.1, 113.7, 112.2, 107.8, 56.1, 56.0, 51.6, 51.5, 34.6, 31.5; IR (KBr film) ν_{max} 3424, 2954, 2855, 1714, 1602, 1514, 1449, 1276, 1209, 1046, 829, 787 cm^{-1}; EI-MS *m*/*z* (%) (70 eV) 414 (M^+, 100; $C_{22}H_{22}O_8$), 383 (9), 354 (10), 341 (25), 323 (47), 170 (5), 161 (3), 151 (2).

Compound **23** was prepared from **22** using alkylation reaction; the reaction was similar to the preparation of **9**. Compound **22** (150.4 mg) afforded **23** as a colorless solid (146.4 mg, 80% yield; with solvent system EtOAc: hexane = 1:9); mp 78–79 °C; ^{1}H-NMR ($CDCl_3$) δ_H 7.67 (d, J = 2.1 Hz, 1H, H-2′), 7.57 (dd, J = 8.7, 2.1 Hz, 1H, H-6′), 7.28–7.44 (m, 6H, OCH_2<u>Ph</u>, H-4), 6.93 (d, J = 8.7 Hz, 1H, H-5′), 6.67 (d, J = 1.2 Hz, 1H, H-6), 5.21 (s, 2H, OC<u>H$_2$</u>Ph), 3.98 (s, 3H, OMe), 3.95 (s, 3H, OMe), 3.91 (s, 3H, CO_2Me), 3.67 (s, 3H, OMe-C-3), 3.04 (t, J = 8.1 Hz, 2H, H-1″), 2.68 (t, J = 8.1 Hz, 1H, H-2″); ^{13}C-NMR ($CDCl_3$) δ_C 173.3, 164.6, 161.1, 150.0, 148.9, 144.7, 141.7, 137.4, 136.7, 129.0, 128.6, 127.9, 127.2, 122.9, 122.4, 113.7, 113.1, 112.9, 108.0, 107.9, 70.8, 56.2, 56.1, 51.6, 51.5, 36.4, 31.6; IR (KBr film) ν_{max} 2933, 2857, 1737, 1717, 1603, 1511, 1265, 1236, 1147, 1096, 1048, 742 cm^{-1}; EI-MS *m*/*z* (%) (70 eV) 504 (M^+, 28; $C_{29}H_{28}O_8$), 413 (100), 382 (7), 353 (5), 341 (7), 323 (11), 91 (45).

Compound **24** was prepared from **23** using lithium aluminum hydride (LAH) reduction reaction. The solution of **23** (122.3 mg, in 20 mL of dry THF) was cooled to –10 °C before LAH (150.3 mg) was added. The solution was stirred under –10 °C. After 8 h, wet THF (10 mL) was added to the solution dropwise to quench the reaction. The solution was adjusted to pH = 4 using HCl (3N), and then the solution was evaporated under reduced pressure. The solution was extracted by EtOAc (3 × 100 mL). The combined organic layer was washed with saturated $NaHCO_3$ solution and brine and dry (Na_2SO_4), and the solvent was removed under reduced pressure to give a residue. Then, the residue was purified using Si gel column chromatography to give **24** as a colorless solid (103.2 mg, 95% yield; with solvent system EtOAc: hexane = 1:4); mp 187–188 °C; ^{1}H-NMR ($CDCl_3$) δ_H 7.29–7.45 (m, 7H, OCH_2<u>Ph</u>, H-2′, H-6′), 7.07 (s, 1H, H-4), 6.95 (d, J = 8.3 Hz, 1H, H-5′), 6.65 (s, 1H, H-6), 5.20 (s, 2H, OC<u>H$_2$</u>Ph), 4.88 (s, 2H, $HOCH_2$-C-3), 3.98 (s, 3H, OMe), 3.96 (s, 3H, OMe), 3.70 (t, J = 7.2 Hz, 2H, H-3″), 2.79 (t, J = 7.2 Hz, 2H, H-1″), 1.94 (quin, J = 7.2 Hz, 2H, H-2″);

^{13}C-NMR ($CDCl_3$) δ_C 154.2, 149.7, 149.6, 148.9, 144.8, 141.6, 137.7, 137.6, 136.8, 136.7, 136.6, 131.1, 128.6, 127.9, 127.3, 127.2, 123.4, 120.4, 113.9, 113.8, 113.6, 111.0, 110.8, 107.7, 70.9, 62.2, 60.4, 56.2, 56.1, 34.6, 32.4; IR (KBr film) ν_{max} 2933, 2857, 1737, 1717, 1603, 1511, 1265, 1236, 1147, 1096, 1048, 742 cm^{-1}; HR-ESI-MS $(M+H)^+$ m/z 449.1954; ($C_{27}H_{29}O_6$).

Preparation of **25**: Methyl caffeate (762.2 mg) was dissolved in a mixture of benzene (20 mL) and acetone (30 mL), and then Ag_2O (1.82 g) was added. The reaction mixture was stirred at room temperature for 60 h. The precipitation was removed with filtration, and the filtrate gave **25** (306.9 mg, 45% yield). Compound **25**: mp 187–188 °C; ^{1}H-NMR ($CDCl_3$) δ_H 7.54 (d, J = 15.9 Hz, 1H, H-1″), 7.05 (s, 1H, H-4), 6.99 (s, 1H, H-6), 6.85 (d, J = 1.9 Hz, 1H, H-2′), 6.82 (d, J = 8.1 Hz, H-5′), 6.76 (dd, J = 8.1, 1.9 Hz, 1H, H-6′), 6.24 (d, J = 15.9 Hz, 1H, H-2″), 6.02 (d, 2H, J = 7.4 Hz, 1H, H-2), 4.26 (d, J = 7.4 Hz, 1H, H-3), 3.79 (s, 3H, -OC$\underline{H}_3$), 3.77 (s, 3H, OC$\underline{H}_3$); ^{13}C-NMR ($CDCl_3$) δ_C 171.0, 168.3, 148.5, 145.0, 144.7, 144.4, 144.0, 140.4, 132.0, 128.7, 125.4, 118.7, 117.6, 115.5, 115.4, 113.0, 87.2, 55.6, 53.0, 51.8; IR (KBr film) ν_{max} 3397, 2958, 1739, 1697, 1609, 1506, 1444, 1281, 1198, 980, 854, 814 cm^{-1}; EI-MS m/z (%) (70 eV) 386 (M^+, 37; $C_{20}H_{18}O_8$), 354 (35), 322 (100), 294 (27), 267 (13), 194 (55), 163 (52), 134 (14) (Figures S13–S16).

Compound **26** was prepared from **25** (510.7 mg) under usual acetylation conditions using Ac_2O and pyridine. Compound **26** (609.6 mg, 90% yield), mp 137–139 °C; ^{1}H-NMR ($CDCl_3$) δ_H 7.59 (d, J = 15.8 Hz, 1H, H-1″), 7.42 (s, 1H, H-4), 7.28 (dd, J = 8.4, 1.9 Hz, 1H, H-6′), 7.21 (d, J = 1.9 Hz, 1H, H-2′), 7.19 (s, 1H, H-6), 7.17 (d, J = 8.4 Hz, 1H, H-5′), 6.29 (d, J = 15.8 Hz, 1H, H-2″), 6.19 (d, J = 7.4 Hz, 1H, H-2), 4.28 (d, J=7.4 Hz, 1H, H-3), 3.83 and 3.78 (s each, 3H-OCH_3), 2.30 (s, 3H, OAc), and 2.27 (s, 6H, OAc); IR (KBr film) ν_{max} 3074, 3016, 1776, 1739, 1716, 1643, 1612, 1591, 1273, 1203, 1176 cm^{-1}; HR-ESI-MS ($(M+H)^+$ m/z 513.4708; $C_{26}H_{25}O_{11}$).

3.3. Chemicals

Colchicine, paclitaxel (Taxol), and vincristine were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Microtubule-associated protein (MAP)-rich tubulin was from Cytoskeleton, Inc. (Denver, CO, USA). Other chemicals not specified were from Sigma or Merck (Darmstadt, Germany) with standard analytical or higher grade.

3.4. Cell Cultures

Human cancer cell lines (KB, HONE1) used in this study were procured from American Type Culture Collection (ATCC, Rockville, MD, USA) and grown in RPMI 1640 medium.

To obtain KB-resistant cell lines, we followed the protocol described below. The protocol involved exposing exponentially growing cells to increasing concentrations of etoposide (VP-16) and vincristine over a period of six months. Initially, cells were exposed to the IC_{50} concentration obtained from a methylene blue assay. Subsequently, cells were subcultured and maintained in RPMI 1640 medium containing vincristine and supplemented with 10% FBS and 1% penicillin/streptomycin. The concentration of the drugs was incrementally increased approximately 1.5-fold in the initial steps and 1.25-fold in the final steps. This process was repeated every four weeks until the final resistant sublines were obtained. Cryopreserved aliquots of cell sublines were taken at each incremental concentration. We collected three resistant sublines, named KB-Vin10 and KB-7D cells.

All cell cultures were supplemented with 10% fetal bovine serum, 2 μM glutamine, 100 U/mL penicillin, and 100 μg/mL streptomycin and incubated in a humidified atmosphere (95% air and 5% CO_2) at 37 °C. KB-Vin10 was a cell line resistant to vincristine and overexpressing the MDR drug efflux protein. KB-7D cells were VP16-resistant cells and overexpressed MRP. All resistant cell lines were incubated in drug-free medium for 3 days before harvesting for the growth inhibition assay.

3.5. Growth Inhibition Assay

In vitro growth inhibition was assessed with the methylene blue assay [16]. Briefly, exponentially growing cells were seeded into 24-well culture plates at a density of

10,000 cells/mL/well and allowed to adhere overnight. Cells were incubated with various concentrations of drugs for 72 h. Then, we measured A_{595} of the resulting solution from 1% N-lauroylsarcosine exaction. The 50% growth inhibition (IC_{50}) was calculated based on the A_{595} of untreated cells (taken as 100%). The values shown are the means and standard errors of at least three independent experiments performed in duplicate.

3.6. In Vitro Microtubule Polymerization Assay

This assay was conducted in a 96-well UV microplate, as described previously [17]. A total of 0.24 mg MAP-rich tubulin was mixed with various concentrations of drugs and incubated at 37 °C in 120 µL reaction buffer (100 mM PIPES, pH 6.9, 1.5 mM $MgCl_2$, 1 mM GTP, and 1% (*v*/*v*) DMSO). A_{350} was monitored every 30 s for 30 min, using the PowerWave X Microplate Reader (Bio-Tek Instruments, Winooski, VT, USA). The increase in A_{350} indicated the increase in tubulin polymerization; 100% polymerization was defined as the AUC of the untreated control.

4. Conclusions

In this study, we synthesized a series of salvinal derivatives and evaluated their structure–activity relationship (SAR) in terms of antiproliferation in KB and HONE1 cancer cell lines. Compound **25** exhibited exceptional anticancer activity, with an IC_{50} of 0.137 µM. Its anticancer activity was attributed to the depolymerization of microtubules, which may lead to cell death in tumor cells. Moreover, the effectiveness of compound **25** was not significantly affected by drug resistance caused by MDR or MRP overexpression. The anticancer potential of compound **25** warrants further investigation and development as a promising agent for cancer treatment.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/ijms24076386/s1.

Author Contributions: Y.-H.K. and J.-Y.C. (Jang-Yang Chang) designed the study. C.-I.C., C.-C.H., Y.-S.W. and C.-C.K. performed the experiments, analyzed and interpreted the data, and drafted the manuscript. C.-Y.C. and J.L. analyzed the data and contributed to discussions. J.-Y.C. (Jong-Yuh Cherng) and P.-C.C. contributed to discussions and reviewed the manuscript. All authors have read and agreed to the published version of the manuscript.

Funding: The study was supported by grants from the National Health Research Institutes (CA-099-PP-08), the National Science Council (NSC98-2320-B-400-003-MY3, NSC99-2323-B-400-006), Tri-Service General Hospital (TSGH-C99-111, TSGH-C100-038), CMU under the Aim for Top University Plan of the Ministry of Education, Taiwan, and the Department of Health Clinical Trial and Research Center of Excellence (DOH102-TD-C-111-004), Taiwan.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Acknowledgments: We thank Shu-Yun Sun for the EI-MS and HR-EI-MS measurements at the Instrumentation Center of the College of Science, National Taiwan University. We are also grateful to the National Center for high-performance computing facilities.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Khwaja, S.; Kumar, K.; Das, R.; Negi, A.S. Microtubule associated proteins as targets for anticancer drug development. *Bioorg. Chem.* **2021**, *116*, 105320. [CrossRef] [PubMed]
2. Brouhard, G.J.; Rice, L.M. Microtubule dynamics: An interplay of biochemistry and mechanics. *Nat. Rev. Mol. Cell Biol.* **2018**, *19*, 451–463. [CrossRef] [PubMed]
3. Peng, N.; Nakamura, F. Microtubule-associated proteins and enzymes modifying tubulin. *Cytoskeleton* **2023**, in press. [CrossRef] [PubMed]

4. Cheng, Z.; Lu, X.; Feng, B. A review of research progress of antitumor drugs based on tubulin targets. *Transl. Cancer Res.* **2020**, *9*, 4020. [CrossRef] [PubMed]
5. Steinmetz, M.O.; Prota, A.E. Microtubule-targeting agents: Strategies to hijack the cytoskeleton. *Trends Cell Biol.* **2018**, *28*, 776–792. [CrossRef] [PubMed]
6. Borys, F.; Joachimiak, E.; Krawczyk, H.; Fabczak, H. Intrinsic and extrinsic factors affecting microtubule dynamics in normal and cancer cells. *Molecules* **2020**, *25*, 3705. [CrossRef] [PubMed]
7. Prassanawar, S.S.; Panda, D. Tubulin heterogeneity regulates functions and dynamics of microtubules and plays a role in the development of drug resistance in cancer. *Biochem. J.* **2019**, *476*, 1359–1376. [CrossRef] [PubMed]
8. Kostrhunova, H.; Zajac, J.; Novohradsky, V.; Kasparkova, J.; Malina, J.; Aldrich-Wright, J.R.; Petruzzella, E.; Sirota, R.; Gibson, D.; Brabec, V. A subset of new platinum antitumor agents kills cells by a multimodal mechanism of action also involving changes in the organization of the microtubule cytoskeleton. *J. Med. Chem.* **2019**, *62*, 5176–5190. [CrossRef] [PubMed]
9. Hsieh, C.C.; Kuo, Y.H.; Kuo, C.C.; Chen, L.T.; Cheung, C.H.; Chao, T.Y.; Lin, C.H.; Pan, W.Y.; Chang, C.Y.; Chien, S.C.; et al. Chamaecypanone C, a novel skeleton microtubule inhibitor, with anticancer activity by trigger caspase 8-Fas/FasL dependent apoptotic pathway in human cancer cells. *Biochem. Pharmacol.* **2010**, *79*, 1261–1271. [CrossRef] [PubMed]
10. Kamal, M.A.; Al-Zahrani, M.H.; Khan, S.H.; Khan, M.H.; Al-Subhi, H.A.; Kuerban, A.; Aslam, M.; Al-Abbasi, F.A.; Anwar, F. Tubulin proteins in cancer resistance: A review. *Curr. Drug Metab.* **2020**, *21*, 178–185. [CrossRef] [PubMed]
11. Loong, H.H.; Yeo, W. Microtubule-targeting agents in oncology and therapeutic potential in hepatocellular carcinoma. *Onco Targets Ther.* **2014**, *7*, 575–585. [PubMed]
12. Chang, J.Y.; Chang, C.Y.; Kuo, C.C.; Chen, L.T.; Wein, Y.S.; Kuo, Y.H. Salvinal, a novel microtubule inhibitor isolated from Salvia miltiorrhizae Bunge (Danshen), with antimitotic activity in multidrug-sensitive and -resistant human tumor cells. *Mol. Pharmacol.* **2004**, *65*, 77–84. [CrossRef] [PubMed]
13. Wang, E.C.; Wein, Y.S.; Kuo, Y.H. A concise and efficient synthesis of salvinal from isoeugenol via a phenoxenium ion intermediate. *Tetrahedron Lett.* **2006**, *47*, 9195–9197. [CrossRef]
14. Ivachtchenko, A.V.; Kiselyov, A.S.; Tkachenko, S.E.; Ivanenkov, Y.A.; Balakin, K.V. Novel mitotic targets and their small-molecule inhibitors. *Curr. Cancer Drug Targets* **2007**, *7*, 766–784. [CrossRef] [PubMed]
15. Kuo, C.C.; Hsieh, H.P.; Pan, W.Y.; Chen, C.P.; Liou, J.P.; Lee, S.J.; Chang, Y.L.; Chen, L.T.; Chen, C.T.; Chang, J.Y. BPR0L075, a novel synthetic indole compound with antimitotic activity in human cancer cells, exerts effective antitumoral activity in vivo. *Cancer Res.* **2004**, *64*, 4621–4628. [CrossRef] [PubMed]
16. Finlay, G.J.; Baguley, B.C.; Wilson, W.R. A semiautomated microculture method for investigating growth inhibitory effects of cytotoxic compounds on exponentially growing carcinoma cells. *Anal. Biochem.* **1984**, *139*, 272–277. [CrossRef] [PubMed]
17. Chang, J.Y.; Hsieh, H.P.; Pan, W.Y.; Liou, J.P.; Bey, S.J.; Chen, L.T.; Liu, J.F.; Song, J.S. Dual inhibition of topoisomerase I and tubulin polymerization by BPR0Y007, a novel cytotoxic agent. *Biochem. Pharmacol.* **2003**, *65*, 2009–2019. [CrossRef] [PubMed]