(2*E*)-*N*,*N*-dibutyl-3-(4-hydroxy-3-methoxyphenyl)acrylamide Induces Apoptosis and Cell Cycle Arrest in HL-60 Cells

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Abstract. *Background: Ferulic acid is one of the most ubiquitous phenolic compounds in nature, which has antioxidant and anticancer activities. However, ferulic acid derivatives, such as ferulamide have never been reported. Materials and Methods: (2E)-N,N-dibutyl-3-(4-hydroxy-3-methoxyphenyl)acrylamide (compound* **8***), a ferulamide derivative was synthesized in our laboratory. In this study, HL-60 cells were treated with various concentrations of compound* **8***, and its effects on cell growth, cell cycle, apoptosis and related measurements were investigated. Results: Compound* **8** *inhibited cell growth in a concentration- and time-dependent manner with significant cytotoxicity, and the concentration required to inhibit growth by 50% (IC_{50}) was 8.2 μM for 24 h. The cell cycle analysis indicated that compound* **8** *treated cells were arrested in the G_2/M-phase and followed by apoptosis. Microscopic examination showed that treatment with compound* **8** *displayed typical morphological features of apoptotic cells, with cell shrinking and formation of apoptotic bodies. Reverse transcription-polymerase chain reaction (RT- PCR) analysis showed a dramatic induction of CDK inhibitor p21, which inhibited the expression of cyclin B1, thereby resulting in G_2/M phase arrest. After G_2/M-phase arrest, cells underwent apoptosis via significant down-regulation of Bcl-2 expression. Conclusion: These results enhance our understanding of the mechanisms of action of compound* **8***-mediated anticancer effects.*

Abbreviations: ATCC, American Type Culture Collection; HBSS, Hank's balanced salt solution; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; PBS, phosphate-buffered saline; PI, propidium iodide.

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Leukemia is classified as myeloid or lymphoid, depending on the cell line which is affected, and is also divided into broad categories of acute or chronic phenotypes. Approximately 33,440 new cases of leukemia are currently diagnosed annually in the United States, of which 36 percent (an estimated 11,920 new cases) corresponds to acute myelogenous leukemia (AML), the most common type of leukemia in adults (1). Although mortality has declined and 5-year survival rate has increased in certain forms of this disease, it has been estimated that 22,570 Americans will have died in 2005 (1). Similar data for leukemia have also been documented in Taiwanese statistics. Leukemia is the eighth most common form of malignancy in Taiwan, and is the leading cause of cancer death among children (2). Incidentally, leukemia will be a serious problem in human public health in all developed countries in the future.

Clinical therapies for leukemia include radiation therapy, chemotherapy (sometimes in combination with stem cell transplantation) or immunotherapy. Chemotherapy is the primary curative treatment modality used in the management of leukemia. Chemoprevention, which refers to the administration of synthetic or naturally occurring agents to prevent initiation and promotional events associated with carcinogenesis, is being increasingly appreciated as an effective approach for the management of neoplasm (3-7). Recent studies have demonstrated that there is currently a great deal of interest in the health benefits of ferulic acid (Table I), one of the most ubiquitous phenolic compounds in nature, especially rich as an ester form in rice bran pitch (8). Ferulic acid has antioxidant properties (8), and has shown beneficial effects in diabetes (9, 10), cardiovascular disease (11), cancer (12, 13), neuroprotection (14), bone degeneration (15), menopause and immunity (16, 17). However, making other compounds related to the ferulic acid skeleton as potential anticancer agents is still an unexplored field. Several ferulamide derivatives have been synthesized and screened for their anticancer activities in human

Table I. *Cytotoxic effects of compounds* ***L*** *and* ***1-9*** *in HL-60 cells.*

Compound	R_1	R_2	IC_{50} (μM)
L	H	H	91.4
1	CH_3	H	>100
2	$(CH_2)_2CH_3$	H	>100
3	$(CH_2)_3CH_3$	H	49.8
4	$(CH_2)_4CH_3$	H	>100
5	CH_3	CH_3	>100
6	CH_2CH_3	CH_2CH_3	>100
7	$(CH_2)_2CH_3$	$(CH_2)_2CH_3$	>100
8	$(CH_2)_3CH_3$	$(CH_2)_3CH_3$	8.2
9	$(CH_2)_4CH_3$	$(CH_2)_4CH_3$	47.4

Table II. *PCR primers used in this study.*

Primers	Sequence
Cyclin B1	5'-AAGGCGAAGATCAACATGGC-3'
	5'-AGTCACCAATTTCTGGAGGG-3'
CDK1	5'-TTTTCAGAGCTTTGGGCACT-3'
	5'-AAATCCAAGCCATTTTCATCC-3'
p21	5'-AGTGGACAGCGAGCAGCTGA-3'
	5'-TAGAATCTGTCATGCTGGTCTG-3'
Bax	5'-CATGAAGACAGGGGCCCTT-3'
	5'-CATCTTCTTCCAGATGGT-3'
Bcl-2	5'-CGACTTCGCCGAGATGTCCAGCCAG-3'
	5'-ACTTGTGGCTCAGATAGGCACCCAG-3'
β-actin	5'-GCTCGTCGTCGACAACGGCTC-3'
	5'-CAAACATGATCTGGGTCATCTTCTC-3'

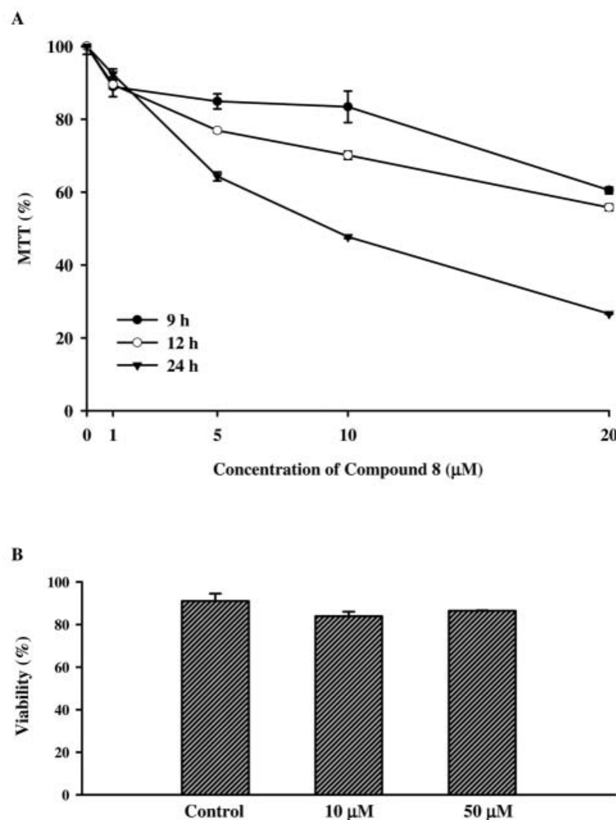


Figure 1. *Compound* ***8*** *induced cytotoxic effect. Cells (1x10*^{5} *cells/ml) were treated with various concentrations of compound* ***8*** *for the indicated periods. (A) HL-60 cells were treated with compound* ***8*** *(1-20 μM) for 9, 12 and 24 h. Cell growth effect was determined by MTT assay. (B) Human normal leukocytes (PBMC cells) were treated with culture medium alone or compound* ***8*** *(10 and 50 μM) for 24 h. The viability was analyzed by flow cytometry. Each point is presented as mean±SD from three independent experiments.*

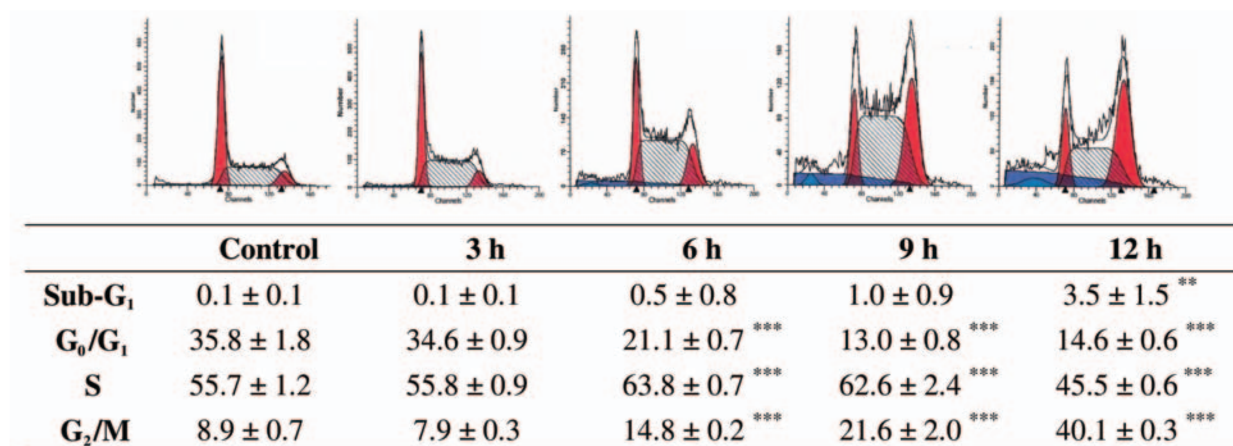
promyelocytic leukemia HL-60 cells in our laboratory. Preliminary screening results indicated that (2*E*)-*N,N*-dibutyl-3-(4-hydroxy-3-methoxy-phenyl)acrylamide (compound **8**, Table I) has a strong growth-inhibitory effect in HL-60 cells. Furthermore, an attempt was made to explore the mechanisms by which compound **8** mediated cancer cell growth inhibition, through a detailed analysis of the effects of compound **8** on cell cycle progression.

Materials and Methods

Test materials. Ten ferulamide derivatives have been synthesized in our laboratory (18). These tested compounds were initially dissolved in DMSO to a stock concentration of 100 mM, which was then diluted to appropriate concentrations before each experiment. The final concentration of DMSO in the culture medium was kept below 0.1%. All chemicals were purchased from Sigma Chemical Co. (St. Louis, MO, USA) unless otherwise specified.

Cell culture. The human promyelocytic leukemia cells HL-60, American Type Culture Collection (ATCC) were maintained in RPMI-1640 medium, supplemented with 10% fetal bovine serum (GIBCO/BRL), penicillin (100 U/mL)/streptomycin (100 μg/mL) (GIBCO/BRL) and 1% L-glutamine (GIBCO/BRL), at 37˚C in a humidified atmosphere containing 5% CO_2. Logarithmically growing HL-60 cells were used for all experiments.

Cytotoxicity assay. HL-60 cells or human normal leukocytes (1x10^5 cells/mL) were treated with tested compounds or culture medium alone for the indicated periods. The cytotoxicity effect was assessed using MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay (19). Ten μL of MTT solution (5 mg/mL) was added to 50 μL of cells suspension in Hank's balanced salt solution (HBSS) in a 96-well plate and incubated at 37˚C in the dark for 2 h. Treatment of living cells with MTT produced a dark blue formazan product, whereas no such staining was observed in dead cells. The formazan product was dissolved by adding 150 μL DMSO and then the absorbance was measured on an ELISA reader at a best wavelength of 570 nm.



	Control	3 h	6 h	9 h	12 h
Sub-G_1	0.1 ± 0.1	0.1 ± 0.1	0.5 ± 0.8	1.0 ± 0.9	3.5 ± 1.5 **
G_0/G_1	35.8 ± 1.8	34.6 ± 0.9	21.1 ± 0.7 ***	13.0 ± 0.8 ***	14.6 ± 0.6 ***
S	55.7 ± 1.2	55.8 ± 0.9	63.8 ± 0.7 ***	62.6 ± 2.4 ***	45.5 ± 0.6 ***
G_2/M	8.9 ± 0.7	7.9 ± 0.3	14.8 ± 0.2 ***	21.6 ± 2.0 ***	40.1 ± 0.3 ***

Figure 2. *Compound **8** induced G_2/M arrest in HL-60 cells. Cells were treated with 10 μM compound **8** for 3, 6, 9 and 12 h. After treatment, the cell cycle distribution was examined by flow cytometry. The data is presented as mean±SD from three independent experiments. **p<0.01; ***p<0.001; compared with control.*

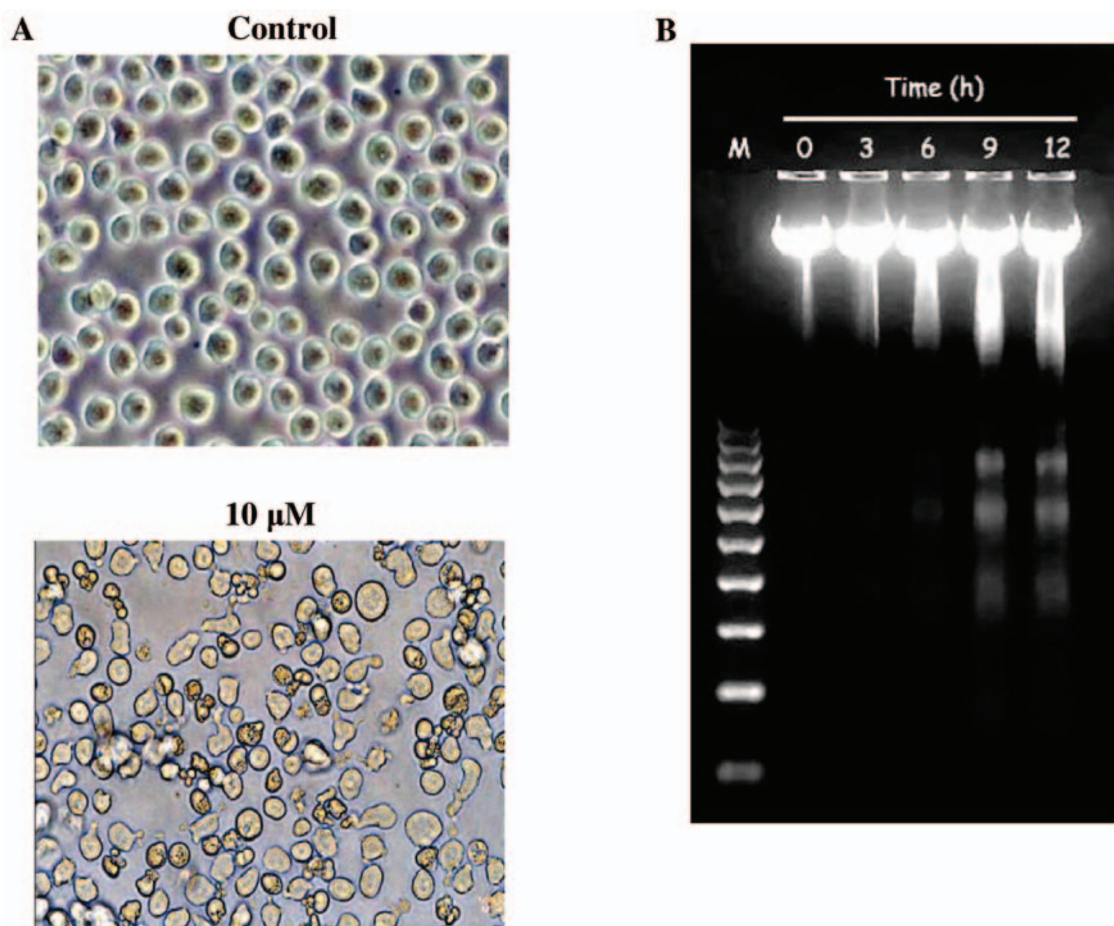


Figure 3. *Compound **8** induced apoptosis in HL-60 cells. (A) Morphologically observed (x200). Cells were treated with 10 μM compound 8 for 12 h. (B) DNA fragmentation analysis. After treatment with compound 8 for 3, 6, 9 and 12 h, DNA was isolated and separated on a 1.5% agarose gel.*

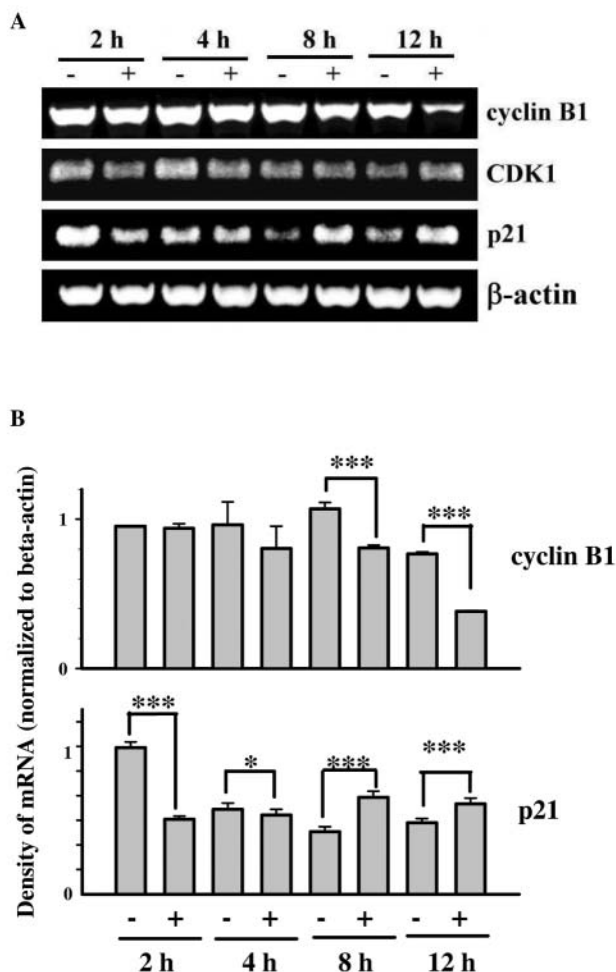


Figure 4. *Effects of compound **8** on the expression of G_2/M-associated genes in HL-60 cells. (A) Cells were treated with 10 μM compound **8** for 2, 4, 8 and 12 h, then harvested and subjected to RT-PCR analysis. (B) The panels show quantitation of gene expression levels, normalized to β-actin (fold induction). Data was presented as mean±SD from three independent experiments. *p<0.05; ***p<0.001; compared with control for the indicated periods. –/+: without/with compound **8**.*

Cell cycle distribution analysis. Cell cycle analysis by flow cytometry was performed, as described in a previous report (19). After treatment, the cells were collected, washed with cold phosphate-buffered saline (PBS), and fixed with 70% ice-cold ethanol at –20˚C overnight. Then the cells were centrifuged and suspended in a staining solution containing 1% Triton-X 100, 0.1 mg/mL RNase and 4 μg/mL propidium iodide (PI) for 30 min at 37˚C in the dark and analyzed by fluorescence-activated cell sorter flow cytometry (FACS-caliber, Becton Dickinson, San Jose, CA, USA), and all histograms were analyzed by ModFit software.

DNA fragmentation analysis. HL-60 cells ($5x10^5$ cells/mL) were treated with compound **8** or culture medium alone for DNA fragmentation electrophoresis assay. The DNA was prepared using the GNOME® DNA Isolation Kit (BIO 101, La Jolla, CA, USA) as described in a previous report (19). DNA electrophoresis was performed in 1.5% agarose gel containing 0.5 μg/mL of eithidium bromide at 70 Volt for 70 min and DNA fragments were visualized by exposing the gel to UV light and were photographed.

RNA extraction and reverse transcription-polymerase chain reaction (RT-PCR). HL-60 cells ($5x10^5$ cells/mL) were treated with compound **8** or culture medium alone for RT- PCR analysis. Total RNA was isolated from cells using RNeasy® Mini Kit (Qiagen) as described previously (19). RNA yield and purity were assessed by spectro-photometric analyses. Total RNA (5 μg) from each sample was subjected to reverse transcription with dNTPs in a 12 μL total reaction volume. PCR was performed on cDNA, using *Taq* polymerase, dNTPs and the corresponding primers. Sequences for the specific primers used in the PCR are summarized in Table II. The resulting PCR products were analyzed by 1.5% agarose gel containing 0.5 μg/mL of ethidium bromide.

Statistical evaluation. Values are expressed as the mean±SD of three independent experiments. Student's *t*-tests were used to assess the statistical significance of the differences, with *p*-values of less than 0.05 being considered statistically significant.

Results

Cytotoxicity assay. The cytotoxic effect of ten structurally related ferulamide derivatives was initially investigated. Exponentially growing cultures of HL-60 cells were continuously cultured in the absence or presence of different concentrations of these derivatives (**L, 1-9**). After 24 h of treatment, the cytotoxicity was determined by MTT reduction assay. As shown in Table I, compound **8** strongly inhibited HL-60 cells growth. Exponentially growing HL-60 cells rapidly underwent growth inhibition with the addition of various concentrations of compound **8**, as evidenced by a decrease of cell proliferation over the experimental periods (Figure 1A). The concentration required to inhibit growth by 50% (IC_{50}) was approximately 8.2 μM for 24 h. Whether or not compound **8** caused cytotoxic effects in normal human leukocytes (PBMC) was also tested. PBMC cells treated with high concentrations of compound **8** (10 and 50 μM) for 24 h did not show any significant difference from controls (Figure 1B). These data suggest that compound **8** can selectively kill leukemia HL-60 cells without significant cytotoxicity against normal PBMC cells.

Induction of apoptosis after G_2/M-phase arrest. To assess whether compound **8**-induced growth inhibition of the cells is mediated *via* alterations in cell cycle regulation and apoptosis, the effect of compound **8** on cell cycle distribution and fragmentation of DNA was evaluated. As shown in Figure 2, when HL-60 cells were treated with 10 μM compound **8**, the percentage of cells in the G_1-phase was substantially lower as compared to control at all treatment periods. The decrease in the G_1-phase was reflected in a slight increased S-phase population after 3-9 h of treatment

and an apparently increased G_2/M-phase population after 6-12 h of treatment, with approximately 40.1% of cells in G_2/M-phase at 12 h compared to 6.9% in the control. Additionally, it was also found that HL-60 cells treated with compound **8** displayed typical morphological features of apoptotic cells, such as cell shrinking and formation of apoptotic bodies (Figure 3A). DNA electrophoretic analysis was performed to confirm the morphologic observations. Compared with DNA from control cells, treatment with compound **8** induced apoptosis, as shown by the formation of distinct intenucleosomal DNA fragments. The intensity of DNA ladder progressively increased in a time-dependent manner. The DNA fragmentation was initially detectable after 6 h of treatment (Figure 3B).

Regulation of cell cycle-related genes by compound 8. To gain insights into the molecular mechanism of compound **8**-induced G_2/M arrest, several important genes involved in controlling G_2/M-phase transition and checkpoint activation were analyzed. Following exposure of compound **8** to 10 μM, the levels of cyclin B1, CDK1 and p21 were analyzed over a 12-h period. As shown in Figure 4A and B, compound **8** appeared to have a significant suppressing effect on the level of cyclin B1 in a time-dependent manner, and p21 expression was initially suppressed at 2-4 h and then enhanced at 8-12 h. However, there was no significant change in other gene expressions.

Down-regulation of Bcl-2 gene level by compound 8. Some evidence has demonstrated that the Bcl-2 family is located in the mitochondrial outer-membrane and the Bax/Bcl-2 ratio plays an important role in either inhibition or promotion of apoptosis (20, 21). To clarify whether the expression of these cell death-associated molecules is involved in compound **8**-mediated apoptosis, the levels of Bcl-2 and Bax were examined. Results from RT-PCR analysis demonstrated that exposure of HL-60 cells to compound **8** caused a marked decrease in Bcl-2 gene expression, but did not apparently affect the gene expression of Bax (Figure 5A). Thus, exposure to compound **8** had a greatly enhancing effect on the Bax/Bcl-2 ratio (Figure 5B).

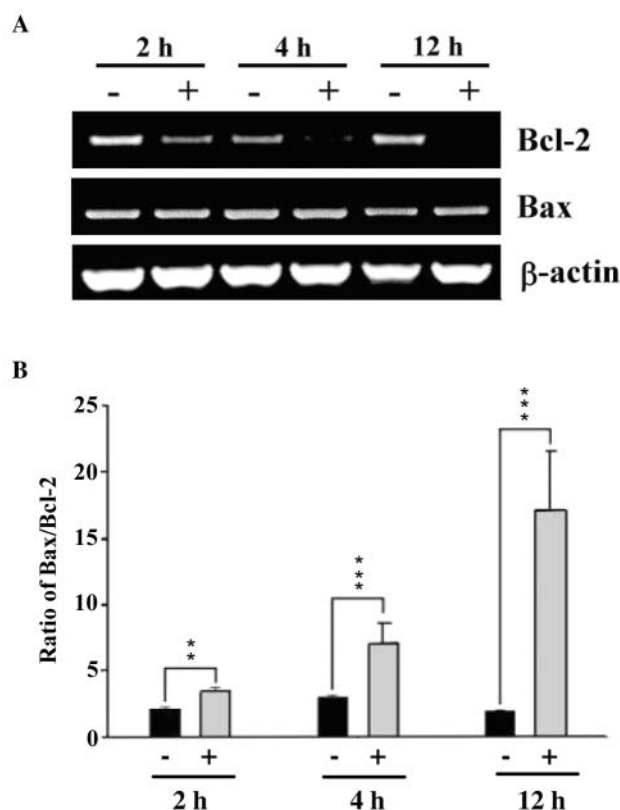


Figure 5. *Effects of compound **8** on the expression of apoptosis-associated genes in HL-60 cells. (A) Cells were treated with 10 μM compound **8** for 2, 4 and 12 h, then harvested and subjected to RT-PCR analysis. (B) Analysis of Bax/ Bcl-2 ratio. Data was presented as mean±SD from three independent experiments. **p<0.01; ***p<0.001; compared with control for the indicated periods. –/+: without/with compound **8**.*

Discussion

In recent years, naturally occurring antioxidant compounds present in the diet and beverages consumed by humans have gained considerable attention as cancer chemo-preventive agents. Ferulic acid, especially rich as an ester form in rice bran pitch, is a potent antioxidant with multiple pharmacological actions. In this study, ferulamide **L** was selected as a new lead skeleton to synthesize some structurally related derivatives (**1-9**) and their anticancer activities in HL-60 cells was screened. As shown in Table I, the IC_{50} value for the lead compound (**L**) was 91.4 μM. When the NH_2-group of **L** was replaced by *n*-alkylamino monosubstituted (-NHR) (**1-4**) or *n*-alkylamino disubstituted (-NRR) (**5-9**), only compound **8** expressed significant cytotoxicity against HL-60 cells (IC_{50}=8.2 μM). Beside human leukemia HL-60 cells, whether or not compound **8** had cytotoxicity against human normal leukocytes (PBMC) was also tested. Its IC_{50} in PBMC was more than 50 μM compared to 8.2 μM in HL-60 cells. The data suggest that compound **8** is a promising anticancer agent candidate with selective killing effect on leukemia cells.

To examine the possible mode of action of compound **8**, the involvement and mechanism of cell cycle regulation and apoptosis during cell growth inhibition by compound **8** in HL-60 cells was further investigated. Compound **8** treatment of HL-60 cells resulted in significant: (a) cell growth inhibition, (b) G_2/M-phase cell cycle arrest, and (c) apoptosis in a time-dependent manner. These are

important observations because the regulation of cell cycle and apoptotic machinery are important for the growth and development of cancer cells, and in recent years, cell cycle regulation and apoptosis have been being increasingly appreciated as targets for intervention against cancer (22-26).

Several structurally related phenolic compounds have been shown to induce the cell cycle arrest and apoptosis of cancer cells, but mechanisms appeared to vary. These compounds have been demonstrated to arrest cells at G_1- or G_2/M-phases, depending on different cell type (13, 27-29). In this study, the data indicated that compound **8**, a ferulamide derivative, significantly induced HL-60 cells arrest in the G_2/M-phase. Cyclin B1 and CDK1 are intricately involved in the progression of the cell cycle through the G_2/M-phase transition (30, 31) Compared to β-actin (internal control), compound **8** decreased the mRNA expression level of cyclin B1 in a time-dependent manner, which correlated with the cell cycle arresting process. Compound **8** also significantly increased the mRNA expression level of p21 at 8-12 h (Figure 4A, 4B). Similar findings, described by other authors, have also indicated that decrease of cyclin B1 with the induction of p21 could cause an arrest in the G_2/M-phase of the cell cycle (32, 33). p21 is also known to be up-regulated by the tumor suppressor gene p53, and both of these are integrated in G_1 and G_2 arrest machinery in response to DNA damage (34, 35). The results of this study suggest that the mechanism of action through which compound **8** up-regulates p21 expression is through a p53-independent pathway because HL-60 cells are deficient in functional p53.

Mitochondrial homeostasis plays a pivotal role in regulating apoptosis. Many reports have demonstrated that Bax and Bcl-2 are located in the mitochondrial outer-membrane and that the Bax/Bcl-2 ratio regulated the release of mitochondrial cytochrome *C* to cytosol (20, 21). These observations clearly indicate that compound **8**-induced apoptosis is *via* a mitochondrial-mediated activation pathway.

In conclusion, a clear picture of the molecular ordering of compound **8**-induced events has emerged. It was apparent that down-regulation of cyclin B1 and up-regulation of p21 caused arrest of the cell cycle at G_2/M-phase transition. After G_2/M-phase arrest, compound **8** treated cells underwent apoptosis by down-regulation of Bcl-2 as the major cause. Compound **8** is an attractive anticancer agent candidate. These molecular findings may provide important information for ferulamides, which could, therefore, be considered as novel chemotherapeutic agents.

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References

1 Cancer Facts and Figures 2005. Atlanta: American Cancer Society, 2005.

2 Vital Statistics, 2004. Taipei, Department of Health, 2005.

3 Hong WK and Sporn MB: Recent advances in chemoprevention of cancer. Science *278*: 1073-1077, 1997.

4 Mukhtar H and Ahmad N: Cancer chemoprevention: future holds in multiple agents. Toxicol Appl Pharmacol *158*: 207-210, 1999.

5 Kelloff GJ, Crowell JA, Steele VE, Lubet RA, Malone WA, Boone CW, Kopelovich L, Hawk ET, Lieberman R, Lawrence JA, Ali I, Viner JL and Sigman CC: Progress in cancer chemoprevention: development of diet-derived chemopreventive agents. J Nutr *130*: 467-471, 2000.

6 Sporn MB and Suh N: Chemoprevention of cancer. Carcinogenesis *21*: 525-530, 2000.

7 Surh YJ: Chemoprevention with dietary phytochemicals. Nat Rev Cancer *3*: 768-780, 2003.

8 Graf E: Antioxidant potential of ferulic acid. Free Radic Biol Med *13*: 435-448, 1992.

9 Sri BM, Rukkumani R and Menon VP: Protective effects of ferulic acid on hyper- lipidemic diabetic rats. Acta Diabetol *40*: 118-122, 2003.

10 Balasubashini MS, Rukkumani R, Viswanathan P and Menon VP: Ferulic acid alleviates lipid peroxidation in diabetic rats. Phytother Res *18*: 310-314, 2004.

11 Kim HK, Jeong TS, Lee MK, Park YB and Choi MS: Lipid-lowering efficacy of hesperetin metabolites in high-cholesterol fed rats. Clin Chim Acta *327*: 129-137, 2003.

12 Wattenberg LW, Coccia JB and Lam LK: Inhibitory effects of phenolic compounds on benzo(a)pyrene-induced neoplasia. Cancer Res *40*: 2820-2823, 1980.

13 Kampa M, Alexaki VI, Notas G, Nifli AP, Nistikaki A, Hatzoglou A, Bakogeorgou E, Kouitzoglou E, Blekas G, Boskou D, Gravanis A and Castanas E: Anti-proliferative and apoptotic effects of selective phenolic acids on T47D human breast cancer cells: potential mechanisms of action. Breast Cancer Res *6*: 63-74, 2004.

14 Kanski J, Aksenova M, Stoyanova A and Butterfield DA: Ferulic acid antioxidant protection against hydroxyl and peroxyl radical oxidation in synaptosomal and neuronal cell culture systems *in vitro*: structure-activity studies. J Nutr Biochem *13*: 273-281, 2002.

15 Sassa S, Kikuchi T, Shinoda H, Suzuki S, Kudo H and Sakamoto S: Preventive effect of ferulic acid on bone loss in ovariectomized rats. In Vivo *17*: 277-280, 2003.

16 Chiang LC, Ng LT, Chiang W, Chang MY and Lin CC: Immunomodulatory activities of flavonoids, monoterpenoids, triterpenoids, iridoid glycosides and phenolic compounds of Plantago species. Planta Med *69*: 600-604, 2003.

17 Philp HA: Hot flashes – a review of the literature on alternative and complementary treatment approaches. Altern Med Rev *8*: 284-302, 2003.

18 Lai YY, Huang LJ, Lin HC, Wu TS, Teng CM and Kuo SC: Synthesis and anti-platelet activity of ferulamide derivatives. Chin Pharm J *54*: 41-52, 2002.

19 Hsu MH, Kuo SC, Chen CJ, Chung JG, Lai YY and Huang LJ: 1-(3,4-Dimethoxy-phenyl)-3,5-dodecenedione (I6) induces G1 arrest and apoptosis in human promyelocytic leukemia HL-60 cells. Leuk Res *29*: 1399-1406, 2005.

20 Adams JM and Cory S: The Bcl-2 protein family: arbiters of cell survival. Science *281*: 1322-1326, 1998.
21 Ruvolo PP, Deng X and May WS: Phosphorylation of Bcl-2 and regulation of apoptosis. Leukemia *15*: 515-522, 2001.
22 Debatin K: Activation of apoptosis pathways by anticancer treatment. Toxicol Lett *112-113*: 41-48, 2000.
23 Kaufmann SH and Earnshaw WC: Induction of apoptosis by cancer chemotherapy. Exp Cell Res *256*: 42-49, 2000.
24 Evan GI and Vousden KH: Proliferation, cell cycle and apoptosis in cancer. Nature *411*: 342-348, 2001.
25 Herr I and Debatin KM: Cellular stress response and apoptosis in cancer therapy. Blood *98*: 2603-2614, 2001.
26 Stewart ZA, Westfall MD and Pietenpol JA: Cell-cycle dysregulation and anticancer therapy. Trends Pharmacol Sci *24*: 139-145, 2003.
27 Chuang JY, Tsai YY, Chen SC, Hsieh TJ and Chung JG: Induction of G0/G1 arrest and apoptosis by 3-hydroxycinnamic acid in human cervix epithelial carcinoma (HeLa) cells. In Vivo *19*: 683-688, 2005.
28 Lee YS: Role of NADPH oxidase-mediated generation of reactive oxygen species in the mechanism of apoptosis induced by phenolic acids in HepG2 human hepatoma cells. Arch Pharm Res *28*: 1183-1189, 2005.
29 Lee YT, Don MJ, Hung PS, Shen YC, Lo YS, Chang KW, Chen CF and Ho LK: Cytotoxicity of phenolic acid phenethyl esters on oral cancer cells. Cancer Lett *223*: 19-25, 2005.
30 Shah MA and Schwartz GK: Cell cycle-mediated drug resistance: an emerging concept in cancer therapy. Clin Cancer Res *7*: 2168-2181, 2001.
31 Knockaert M, Greengard P and Meijer L: Pharmacological inhibitors of cyclin-dependent kinases. Trends Pharmacol Sci 23: 417-425, 2002.
32 Choi YH, Lee WH, Park KY and Zhang L: P53-independent induction of p21 (WAF1/CIP1), reduction of cyclin B1 and G2/M arrest by the isoflavone genistein in human prostate carcinoma cells. Jpn J Cancer Res *91*: 164-173, 2000.
33 Liu MJ, Yue PY, Wang Z and Wong RN: Methyl protodioscin induces G2/M arrest and apoptosis in K562 cells with the hyperpolarization of mitochondria. Cancer Lett *224*: 229-241, 2005.
34 el-Deiry WS, Tokino T, Velculescu VE, Levy DB, Parsons R, Trent JM, Lin D, Mercer WE, Kinzler KW and Vogelstein B: WAF1, a potential mediator of p53 tumor suppression. Cell *75*: 817-825, 1993.
35 Andreassen PR, Lacroix FB, Lohez OD and Margolis RL: Neither p21WAF1 nor 14-3-3σ prevents G2 progression to mitotic catastrophe in human colon carcinoma cells after DNA damage, but p21WAF1 induces stable G1 arrest in resulting tetraploid cells. Cancer Res *61*: 7660-7668, 2001.

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