

# Evaluation of the anticancer activities of thioflavanone and thioflavone in human breast cancer cell lines

EUN JEONG CHOI, JAE IN LEE and GUN-HEE KIM

Plant Resources Research Institute, Duksung Women's University, Tobong-ku, Seoul 132-714, Republic of Korea

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**Abstract.** This study investigated the anticancer effects of thioflavanone and thioflavone in the MCF-7, MDA-MB-231 and MDA-MB-453 human breast cancer cell lines. Cells were treated with either thioflavanone or thioflavone from 1 to 100  $\mu$ M for 24 h, and their anti-proliferative activity and cytotoxicity was determined. Thioflavanone and thioflavone possessed similar anti-proliferative activities; their IC<sub>50</sub> values were 62-89 and 74-128  $\mu$ M, respectively, although the cytotoxicity of thioflavanone was significantly higher and occurred in a dose-dependent manner. Taken together, these results suggest that thioflavanone significantly inhibits cellular proliferation with weak cytotoxicity to a greater extent than thioflavone, and induces apoptosis in human breast cancer cell lines. Moreover, thioflavanone, but not thioflavone, induces apoptosis via p53-dependent expression of Bax.

## Introduction

Flavonoids are a group of over 6,000 phytochemicals that include flavones, flavonols, flavanones, and isoflavones in plants, and are usually present almost exclusively in the form of  $\beta$ -glycosides. According to several epidemiological studies, flavonoids may reduce the risk of developing cancer and cardiovascular disease (1-3). Due to their benefit to human health, the biological activity of flavonoids has been extensively examined in terms of their antitumor, anti-inflammatory, and antioxidant capacity *in vitro* (4,5).

The common structural feature of flavonoids is the flavan nucleus, which consists of 15 carbon atoms arranged in 3 rings (phenylchromanone structure, C6-C3-C6). Rings A and B are benzene rings and ring C is a heterocyclic pyran or pyrone. A number of the anticancer mechanisms of flavonoids have been shown to be associated with their structure-activity

relationships (SAR) (6). Recently, much research has aimed to understand the relationship between structural modifications and biological activity. In an attempt to increase anticancer activity, the synthesis of new flavonoid analogues has been conducted (7). Moreover, the anticancer activity of these synthetic compounds has been observed in various cancer cell lines *in vitro* (8-13). We previously reported the production of synthetic flavonoids (14-16) that exert various biological activities *in vitro* (17). Sulfur-containing flavonoid analogues have activities greater than the molecules from which they were derived. For example, synthetic thioflavopiridols may act as selective CDK1 inhibitors in human tumor cell lines (18). Our previous report (17) suggested that synthetic flavanone derivatives were more potent than flavanone in an antiproliferation assay using human breast cancer cells.

In the present study, we investigated the anticancer activities of thioflavanone and thioflavone *in vitro*. We postulated that thioflavanone and thioflavone, which are synthesized as the thio analogues, may possess greater biological activities than their precursors.

## Materials and methods

**Synthesis of thioflavanone and thioflavone.** Treatment of thiosalicylic acid (denoted as 1 in Fig. 1) with 3 equiv of methylolithium in dimethoxyethane (DME) afforded 2'-mercaptoacetophenone (denoted as 2 in Fig. 1) at an 80% yield after acidic workup. The direct condensation of 2 with benzaldehyde was accomplished with 2 equiv of lithium diisopropylamide (LDA) in THF for 2 h between -15°C and room temperature. The addition of benzaldehyde to a solution of the dilithiated anion, generated from 2 and 2 equiv of LDA in THF for 1 h at -15°C, afforded the corresponding chalcone and the successive nucleophilic attack of sulfur anion to the  $\beta$ -carbon intramolecularly to give thioflavanone (denoted as 3 in Fig. 1) at an 87% yield, accompanied by elimination of lithium hydroxide.

The reaction of the dilithiated anion of 2 with *N*-methoxy-*N*-methyl benzamide proceeded for 16 h at room temperature to give 1-(2-mercaptophenyl)-3-phenyl-1,3-propanedione (denoted as 4 in Fig. 1) in 84% yield after acidic hydrolysis. The cyclodehydration of 4 proceeded well with H<sub>2</sub>SO<sub>4</sub> in CH<sub>3</sub>CN within 1 h at room temperature to give thioflavone (denoted as 5 in Fig. 1) at a 94% yield.

Thioflavanone: mp 56-57°C; <sup>1</sup>H NMR (300 MHz, CDCl<sub>3</sub>)  $\delta$  8.15 (dd, *J*<sub>1</sub>=8.0 Hz, *J*<sub>2</sub>=1.3 Hz, 1H), 7.31-7.46 (m, 6H), 7.18-7.31

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**Correspondence to:** Professor Gun-Hee Kim, Plant Resources Research Institute, Duksung Women's University, 419 Ssangmun-dong, Tobong-ku, Seoul 132-714, Republic of Korea  
E-mail: ejchoi@duksung.ac.kr

**Key words:** anticancer, cytotoxicity, thioflavanone, thioflavone, human breast cancer

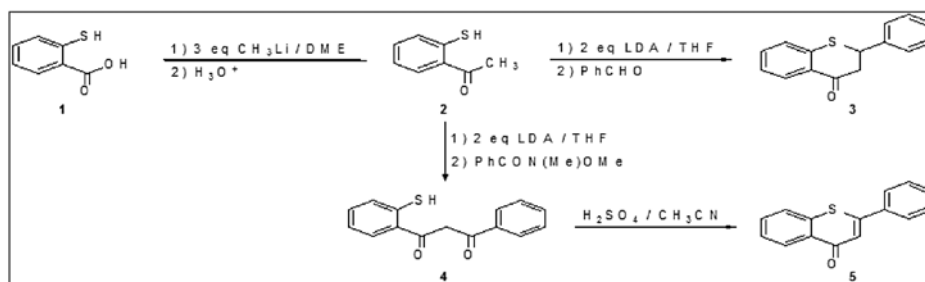


Figure 1. Synthesis of thioflavanone and thioflavone.

(m, 2H), 4.72 (dd,  $J_1=12.8$  Hz,  $J_2=3.3$  Hz, 1H), 3.32 (dd,  $J_1=16.4$  Hz,  $J_2=12.8$  Hz, 1H), 3.20 (dd,  $J_1=16.4$  Hz,  $J_2=3.3$  Hz, 1H);  $^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ )  $\delta$  194.4, 142.1, 138.4, 133.7, 130.4, 129.2, 129.0, 128.5, 127.4, 127.2, 125.2, 46.7, 45.5; FT-IR (KBr) 3060, 2946, 1677 (C=O), 1586, 1435, 1285, 1085, 756, 697  $\text{cm}^{-1}$ ; Ms  $m/z$  (%) 240 ( $\text{M}^+$ , 51), 163 (20), 136 (100), 108 (50), 97 (33), 83 (33).

Thioflavone: mp 125–126°C;  $^1\text{H}$  NMR (300 MHz,  $\text{CDCl}_3$ )  $\delta$  8.55 (d,  $J=7.7$  Hz, 1H), 7.60–7.71 (m, 4H), 7.52–7.59 (m, 1H), 7.48–7.52 (m, 3H), 7.24 (s, 1H);  $^{13}\text{C}$  NMR (75 MHz,  $\text{CDCl}_3$ )  $\delta$  180.8, 153.0, 137.7, 136.5, 131.6, 130.9, 130.8, 129.3, 128.6, 127.8, 126.9, 126.5, 123.4; FT-IR (KBr) 3066, 1620 (C=O), 1587, 1335, 1098, 759, 696  $\text{cm}^{-1}$ ; Ms  $m/z$  (%) 238 ( $\text{M}^+$ , 100), 210 (95), 136 (46), 108 (22).

**Cell culture.** Chinese hamster ovary CHO-K1 cell line and human breast cancer cell lines such as MCF-7, MDA-MB-231 and MDA-MB-453 were purchased from the KCLB (Korean Cell Line Bank, Korea). Cells were routinely maintained in RPMI-1640 (Invitrogen, Carlsbad, USA), supplemented with 10% fetal bovine serum (FBS) and antibiotics (50 U/ml of penicillin and 50  $\mu\text{g}/\text{ml}$  streptomycin, Gibco) at 37°C in a humidified atmosphere containing 5%  $\text{CO}_2$ .

**MTT assay.** Each cell line was plated at a density of  $1 \times 10^5$  cells/well in 96-well tissue culture plate (Corning, NY, USA), and incubated at 37°C for 24 h. Plated cells were treated with indicated concentrations of either thioflavanone or thioflavone for 24 h. After treatment, plated cell were incubated with 3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT, Sigma Chemical Company, 0.5 mg/ml final concentration) for 4 h at 37°C. After discarding all the medium from the plates, 100  $\mu\text{l}$  of dimethyl sulfoxide (DMSO) was added to the each well. The plates were placed for 5 min at room temperature with shaking, so that complete dissolution of formazan was achieved. The absorbance of the MTT formazan was determined at 540 nm by a UV spectrophotometric plate reader (EMax; Molecular Devices, Sunnyvale, CA, USA). The value of  $\text{IC}_{50}$  (i.e., the concentration of the extract required to inhibit cancer cell growth by 50% of levels in control compound solvent-treated only cells) was estimated from the plot. Each  $\text{IC}_{50}$  of thioflavanone on human breast cancer cell lines was applied for the assay of apoptosis detection.

**Lactate dehydrogenase (LDH) release assay.** The cytotoxicity of either thioflavanone or thioflavone in CHO-K1 cells was assessed by measuring the release of the enzyme LDH using

an LDH cytotoxicity Detection kit (Cat. no. 630117 Takara Korea Biomedical, Inc., Korea).

**DAPI staining assay.** Apoptotic morphological changes were determined by DAPI (4',6-diamidino-2-phenyl-indole) staining. After harvesting the cells exposed with apigenin for 72 h, the cells were seeded in poly-L-lysine coated slides and fixed with 4% methanol-free formaldehyde solution for 30 min. Then mounting medium with DAPI (Molecular Probes, Eugene, OR) was dispersed over the entire section of slides. Mounted slides were stored at 4°C without light. Each slide was observed under AxioVision 4.0 fluorescence microscopes (Carl Zeiss, Inc., USA). Additionally, features of MDA-MB-453 cells exposed to apigenin were also observed using a Nikon inverse phase contrast microscope (Nikon TMS, Nikon, Japan) equipped with an objective (Plan 10/0.30DL/Ph1, Nikon, Japan) of x100 magnification.

**Statistical analyses.** All the data are expressed as percent compared with vehicle-treated control cells, which were arbitrarily assigned 100%. Data were analyzed by one-way analysis of variance followed by the Dunnett's multiple comparison test (SigmaStat, Jandel, San Rafael, USA). For all comparisons, differences were considered statistically significant at  $P < 0.05$ .

## Results

**Antiproliferative activity of thioflavanone and thioflavone on human breast cancer cell lines.** The effects of thioflavanone and thioflavone on cell proliferation were measured using the MTT assay, in 3 human breast cancer cell lines (MCF-7, MDA-MB-453, and MDA-MB-231) exposed to either thioflavanone or thioflavone between 1 and 100  $\mu\text{M}$  for 24 h (Fig. 2). Both thioflavanone and thioflavone significantly inhibited cancer cell growth in a dose-dependent manner ( $P < 0.05$ ). The  $\text{IC}_{50}$  values of thioflavanone in MCF-7, MDA-MB-453, and MDA-MB-231 cells were 63.8, 88.9, and 62.2  $\mu\text{M}$ , respectively; the  $\text{IC}_{50}$  values of thioflavone were higher than those of thioflavanone.

**Cytotoxicity of thioflavanone and thioflavone.** The cytotoxicity of thioflavanone and thioflavone was determined by measuring the release of lactate dehydrogenase (LDH) by CHO-K1 cells (Fig. 3). Although thioflavone significantly increased LDH release, as compared to controls, thioflavanone had an effect on LDH release only at concentrations greater than 100  $\mu\text{M}$ . The highest cytotoxicity of thioflavanone was

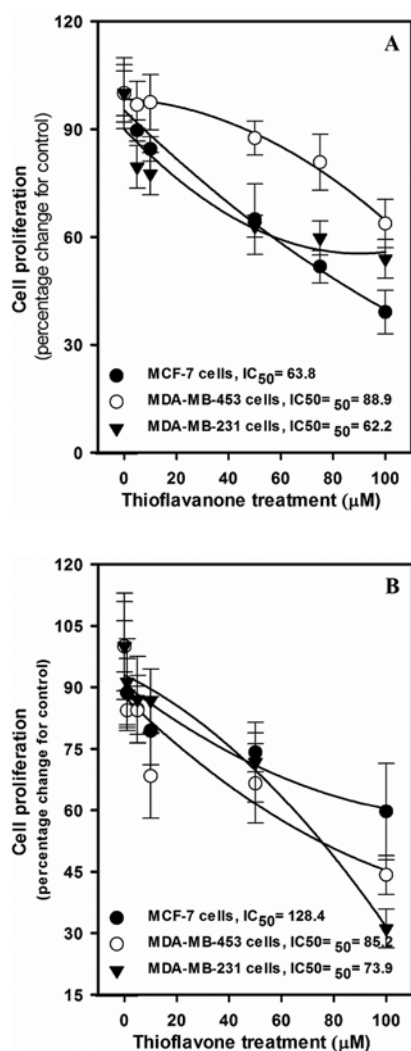


Figure 2. Antiproliferative activity of either (A) thioflavanone or (B) thioflavone.

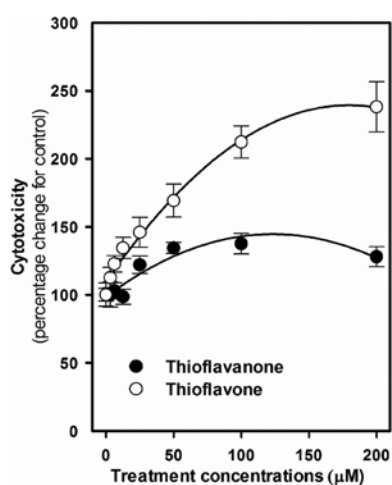


Figure 3. Cytotoxicity of thioflavanone and thioflavone.

observed at 100 μM (an increase of 137.8%). Treatment with 6.25 μM thioflavone induced significant cytotoxicity, which continuously increased in dose-dependent manner (increases of 212.3 and 238.2% at 100 and 200 μM, respectively).

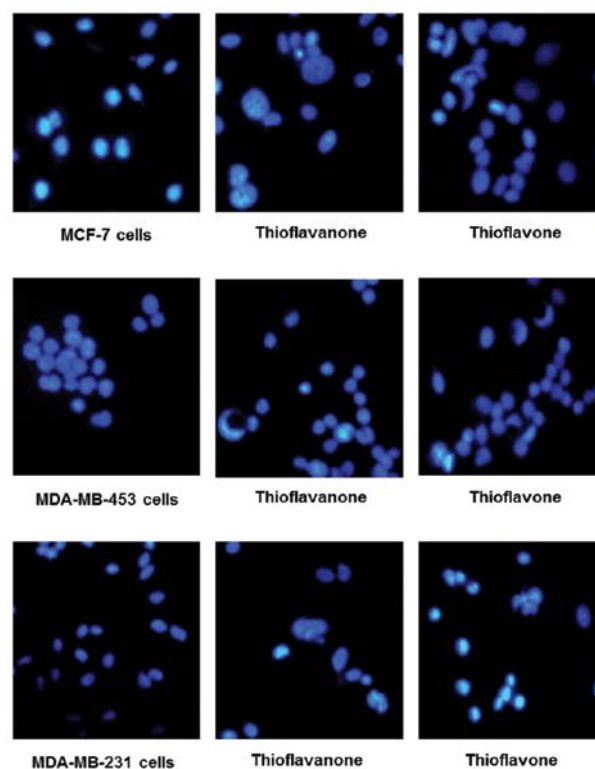


Figure 4. Apoptosis induction by thioflavanone and thioflavone.

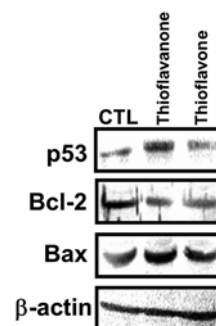


Figure 5. Expression of p53, Bcl-2 and Bax in MDA-MB-231 cells exposed to either thioflavanone or thioflavone.

*Apoptosis induction by thioflavanone and thioflavone.* DAPI staining was used to detect apoptotic changes after exposing cells to either thioflavanone or thioflavone at their IC<sub>50</sub> concentrations (Fig. 4). Apoptotic morphological features such as cell shrinkage and dot-shaped nuclear fragments were observed after exposure of cells to both thioflavanone and thioflavone. Compared to respective controls, exposure to thioflavanone resulted in a remarkable increase in apoptotic morphological features. These data suggest that thioflavanone may possess anticancer properties.

*Apoptosis-related gene expression by thioflavanone and thioflavone.* We used MDA-MB-231 cells to investigate the apoptosis induced by thioflavanone and thioflavone, since proliferation assays suggested that these cells were affected to the greatest extent (Fig. 5). Activation of p53 was observed

after treatment with thioflavanone at its  $IC_{50}$  for 24 h. Bcl-2 expression was lower and that of Bax higher in MDA-MB-231 cells exposed to both thioflavanone and thioflavone.

## Discussion

Breast cancer is one of the most frequently diagnosed cancers in women and its occurrence has, in recent years, increased worldwide. The present study evaluated the anticancer activity of the synthetic anticancer drug candidate thioflavanone and thioflavone in human breast cancer cell lines.

We first determined the antiproliferative effect of thioflavanone and thioflavone, on the human breast cancer cell lines MCF-7, MDA-MB-453, and MDA-MB-231. Both thioflavanone and thioflavone significantly inhibited cellular proliferation in all 3 cell lines in a dose-dependent manner. The  $IC_{50}$  ranges of thioflavanone and thioflavone were observed to be 62–89  $\mu$ M and 74–129  $\mu$ M, respectively in the 3 cell lines. Although it has been reported that flavone is more efficient than flavanone in breast cancer cells (19,20), in this study their activities were similar. However, exposure to thioflavone induced a cytotoxic effect as determined by LDH release, in the CHO-K1 cell line. This cytotoxicity increased in a dose-dependent manner, and was over 1.9 times greater than that of thioflavanone. Based on these results, thioflavanone may be a more appropriate anticancer drug candidate, and has the potential to selectively inhibit cancer growth.

Moreover, thioflavanone and thioflavone induced apoptosis in the human breast cancer cell lines examined when exposed at their  $IC_{50}$  concentrations. Several studies have indicated that anticancer drugs or cancer chemopreventive agents act through the induction of apoptosis in various cancer cells. In addition, the initiation of apoptosis appears to be a common mechanism of many new anticancer agents (21,22).

As a brief study of the underlying mechanism, the expression of a few apoptosis-related genes was investigated in MDA-MB-231 cells exposed to thioflavanone and thioflavone at their  $IC_{50}$  for 24 h. Activation of p53 in response to DNA damage led to cell cycle arrest and inhibition of cell proliferation (23,24). p53 acts as a sequence-specific binding protein and may be regulated by Bax, a member of the Bcl-2 family (25,26). Members of the Bcl-2 family of proteins are critical regulators of the apoptotic pathway, and consist of the major antiapoptotic proteins, Bcl-x<sub>L</sub> and Bcl-2, and the major proapoptotic proteins, Bax and Bak (27). It has been reported that an increase in Bax expression by p53 overexpression correlates with the induction of apoptosis in several cell types (25–28). Based on these results, thioflavanone, but not thioflavone, may induce apoptosis through the p53-dependent expression of Bax. Although both thioflavanone and thioflavone induced apoptosis in MDA-MB-231 cells at their respective  $IC_{50}$  value, thioflavanone and thioflavone may act through different pathways, which may explain the cytotoxic effect of thioflavone. That is, thioflavanone may act through multiple pathways to induce apoptosis.

In conclusion, our results indicate that thioflavanone significantly inhibited cellular proliferation with a weak cytotoxicity and induced apoptosis in human breast cancer cell lines. Moreover, we speculate that the mechanism by which thioflavanone induced apoptosis was through a p53-dependent

pathway. The induction of apoptosis by thioflavanone may be a novel strategy for cancer chemotherapy.

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