Chemico-Biological Interactions 261 (2017) 103–107

Contents lists available at ScienceDirect

Chemico-Biological Interactions

journal homepage: www.elsevier.com/locate/chembioint

# 3′-Geranyl-mono-substituted chalcone Xanthoangelovl induces apoptosis in human leukemia K562 cells via activation of mitochondrial pathway

Yuou Teng, Lixin Wang, Huan Liu, Yuan Yuan, Qian Zhang, Meng Wu, Luyao Wang, Haomeng Wang, Zhen Liu*, Peng Yu**

*Key Lab of Industrial Fermentation Microbiology, Tianjin Key Lab of Industrial Microbiology, Sino-French Joint Lab of Food Nutrition/Safety and Medicinal Chemistry, College of Biotechnology, Tianjin University of Science and Technology, Tianjin, 300457, PR China*

ARTICLE INFO

*Article history:*
Received 7 September 2016
Received in revised form
7 November 2016
Accepted 22 November 2016
Available online 29 November 2016

*Keywords:*
Chalcones
Apoptosis
Caspase-3
Bcl-2

ABSTRACT

3′-Geranyl-mono-substituted chalcone Xanthoangelol (**1b**), a chalcone derivative, was previously reported to show selective cytotoxicity against human chronic myelogenous leukemia K562 cells with a half-maximal inhibitory concentration ($IC_{50}$) of 3.98 μM. In the present study, we investigated the molecular mechanism underlying the cytotoxicity of **1b** in K562 cells.

Treatment with compound **1b** caused K562 cells to adopt a typical apoptotic morphology. Flow cytometric analysis also confirmed the presence of an apoptotic cell population following treatment of Annexin-V-FITC and propidium iodide (PI) double-labeled K562 cells with **1b**. Furthermore, we observed dissipation of the mitochondrial membrane potential, caspase-3 activation, and a reduction of the Bcl-2/Bax ratio in these cells, which suggest that the mitochondrial apoptotic pathway is induced by **1b** in K562 cells. Collectively, our findings demonstrate that compound **1b** notably induces mitochondrial-mediated apoptosis in K562 cells, which might have a potential anticancer activity.



## 1. Introduction

Chalcones are natural or synthetic compounds belonging to the flavonoid family with wide distribution in the flowers, leaves, bark, and roots of liquorice, saffron, and other natural plants. They possess many pharmacological and biological activities, such as antitumor [1–3], antidiabetic [4], antimalarial [5], antioxidant [6], antifungal [7], anti-angiogenic [8], and anti-inflammatory activities [9].

In recent years, many natural chalcone products and their analogs were found to exhibit high cytotoxic activity in tumors. Zi and Simoneau [10] found that Flawkawain A extracted from the roots of plants, activated caspase-9 and caspase-3, thereby inducing apoptosis via the mitochondrial apoptotic pathway in human bladder cancer cells. Furthermore, *in vivo* studies on tumor-bearing mice confirmed Flawkawain A's antitumor efficacy. Hsu et al. [11] found that isoliquiritigenin could induce apoptosis in HepG2 cells by regulating certain cellular factors, such as p53, p21, and Bax. Saxena et al. [12] showed that chalcone derivatives induced A549 cell apoptosis by promoting the expression of p35. Boumendjel et al. [13] reported that several chalcone analogs induced $G_2$/M cell cycle arrest in K562 cells.

It is well known that apoptosis is the result of a highly complicated cascade of cellular events causing cell rounding and shrinkage, chromatin condensation, DNA fragmentation, shedding of small cellular fragments, and loss of adhesion [14]. In particular, it has been established that many chemotherapeutic agents induce cancer cell apoptosis via a mitochondria-dependent pathway [15–17]. The mitochondrial pathway is highly regulated by Bcl-2 family members [18,19]. The Bcl-2 family of proteins is thought to be the key players regulating mitochondrial membrane permeabilization (MMP) in caspase-dependent apoptosis. The Bcl-2 family consists of both pro-apoptotic and anti-apoptotic members, such as Bax and Bcl-2 [20].

We previously reported the design, synthesis, and *in vitro* cytotoxicity of a series of novel chalcone derivatives, which could be used as anticancer agents [21]. The 3'-geranyl-mono-substituted chalcone Xanthoangelol (**1b**) showed good and selective cytotoxic activity ($IC_{50}$ = 3.98 μM). In the present study, we investigated the

* Corresponding author.
** Corresponding author.
*E-mail addresses:* liuzhen5957@163.com (Z. Liu), yupeng@tust.edu.cn (P. Yu).

http://dx.doi.org/10.1016/j.cbi.2016.11.025
0009-2797/

molecular mechanism underlying the cytotoxic activity of **1b** against human chronic myelogenous leukemia K562 cells.

## 2. Materials and methods

### 2.1. Cell lines and culture conditions

The K562 cell line was obtained from the Shanghai Institutes of Biological Sciences (Shanghai, China). Cells were grown at 37 °C in RPMI-1640 supplemented with 10% fetal bovine serum, 2.05 mM glutamine, and 1% penicillin/streptomycin in a humidified atmosphere containing 5% $CO_2$. The medium was replaced once every third day.

### 2.2. Cytotoxic activity assay

The cytotoxic activity was measured using an MTT assay [22]. Briefly, 100 μL of K562 cell suspension was cultured in 96-well plates at a density of $5 \times 10^4$ cells/mL. After 2 h, different concentrations of **1b** (1–10,000 nM) were added to each well and the cells were incubated for another 48 h. The MTT assay was performed using a thermo microplate reader. The DMSO-treated controls were assigned a cell viability value of 100%. The inhibitory concentrations ($IC_{50}$) were obtained by nonlinear regression using GraphPad Prism 5.0. For each treatment, the $IC_{50}$ value was calculated from three independent experiments.

### 2.3. Flow cytometric analysis of apoptosis

Apoptotic cells were assayed by the Annexin-V-FITC Apoptosis Detection Kit (BD Biosciences, USA) according to the manufacturer's instructions. Briefly, K562 cells were treated with DMSO or **1b** (30 μM) for 12 h, 24 h, 36 h, or 48 h. Cells were harvested, washed twice with ice-cold PBS, and resuspended in 1 × binding buffer at a concentration of $1 \times 10^6$ cells/mL, after which they were stained with 5 μL of Annexin-V-FITC and 5 μL of PI (50 μg/mL) for 15 min in the dark at 25 °C, and analyzed by flow cytometry.

### 2.4. Analysis of combination effect

Briefly, 100 μL of K562 cell suspension was cultured in 96-well plates at a density of $5 \times 10^4$ cells/mL for 2 h. Then, cells were incubated with 4-PBA or TNF-α for 0.5 h. Different concentrations of **1b** (1–10,000 nM) were added to each well and cells were cultured for another 48 h. Then, an MTT assay was performed using a thermo microplate reader. The DMSO treated controls were assigned a cell viability value of 100%. The $IC_{50}$ values were obtained by nonlinear regression using GraphPad Prism 5.0. For each treatment, the $IC_{50}$ value was calculated from three independent experiments.

### 2.5. Caspase-3 activity assay

Caspase-3 activity was evaluated by the Apo-ONE ® Homogeneous Caspase-3/7 Assay Kit (Promega) according to the manufacturer's instructions. Briefly, K562 cells were cultured in 6-well plates with either DMSO or **1b** (30 μM) for 48 h. To each well, 100 μL of Apo-ONE ® Homogeneous Caspase-3/7 buffer with 1 μL Caspase Substrate Z-DEVD-R110 (100 × ) was added and cells were cultured for 0.5 h. The release of the fluorochrome following peptide cleavage was kinetically monitored at room temperature (excitation at 485 nm and emission at 521 nm). Cells treated with staurosporine (0.3 μM) for 6 h were used as a positive control.

### 2.6. Flow cytometric analysis of the mitochondrial membrane potential

To measure the effects of **1b** on the mitochondrial membrane potential (ΔΨm), K562 cells were treated with DMSO or **1b** (30 μM) for 12, 24, or 48 h. Then, cells were stained with 100 nM tetramethylrhodamine methyl ester (TMRM; Invitrogen, USA) for 30 min in the dark at room temperature, and analyzed by flow cytometry. TMRM is the most specific agent for measuring changes in ΔΨm.

### 2.7. Western blot analysis

Anti-caspase-3, anti-PARP, anti-Bcl-2, anti-Bcl-xl, and anti-Bax were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Other anti-antibodies were purchased from Cell Signaling Technology (CST, Boston, MA). Cells were lysed in a lysis buffer containing 10 mM Hepes-Na, 150 mM $Na_2SO_4$, 1 mM EDTA, 3% CHAPS, 1 mM phenylmethylsulfonyl fluoride, and 10 mg/mL of both aprotinin and leupeptin. For the western blot analysis of total cell lysates, samples were prepared by mixing an aliquot of cell lysate with an equal volume of 2 × Laemmli's sample buffer and heating at 100 °C. The samples were separated by SDS-PAGE and electrotransferred to PVDF membranes (Millipore, Bedford, MA). The membranes were probed with the aforementioned antibodies and incubated with Alexa Fluor® 680 Goat Anti-Mouse IgG (H+L) and Alexa Fluor® 680 Goat Anti-Rabbit IgG (H+L) followed by detection using the Odyssey western blotting detection system (Amersham Pharmacia Biotech).

### 2.8. Statistical analysis

Data were expressed as means ± S.D. or percentage and analyzed for statistical significance using one-way analysis of variance (ANOVA) followed by Dunnett's test. P-value less than or equal to 0.05 was considered to be statistically significant. Statistical analyses were carried out in GraphPad Prism 5 (GraphPad Software, San Diego, CA, U.S.A.).

## 3. Results

### 3.1. Morphological changes in 1b-treated K562 cells

Treatment with the highly potent cytotoxic compound **1b** ($IC_{50}$ = 3.98 μM) (Fig. 1A) caused K562 cells to adopt a typical apoptotic morphology (cell shrinkage and/or blebbing; Fig. 1B). The ball-like bodies appeared as early as 12 h after treatment with 30 μM **1b**. The number of apoptotic cells increased in a time-dependent manner, whereas the total number of cells decreased. These results indicated that compound **1b** might inhibit K562 cell proliferation by inducing apoptosis.

### 3.2. Compound 1b induced apoptosis in K562 cells

Flow cytometric analysis of cells double-labeled with Annexin-V-FITC and PI revealed the presence of apoptotic K562 cells following **1b** treatment (Fig. 2A). The apoptotic rates (Annexin $V^+$/$PI^-$ and Annexin $V^+$/$PI^+$) of **1b**-treated K562 cells increased to 62.8% of total cells compared to 9.8% in the control. As shown in Fig. 2A, treatment with **1b** for different time periods showed that the apoptosis rates increased in a time-dependent manner. These results suggested that **1b** inhibits the proliferation of K562 cells by inducing apoptosis in a time-dependent manner.