Heptaphylline Induces Apoptosis in Human Colon Adenocarcinoma Cells through Bid and Akt/NF-κB (p65) Pathways

Chantana Boonyarat¹, Chavi Yenjai², Opa Vajragupta³, Pornthip Waiwut⁴*

Abstract

Heptaphylline derivatives are carbazoles in Clausena harmandiana, a medicinal plant that is utilized for headache, stomach ache, and other treatments of illness. The present study examined the effects of heptaphylline and 7-methoxyheptaphylline on apoptosis of human colon adenocarcinoma cells (HT-29 cell line). Quantification of cell viability was performed using cell proliferation assay (MTT assay) and of protein expression through immunoblotting. The results showed that only heptaphylline, but not 7-methoxyheptaphylline, significantly activated cleaved of caspase-3 and poly (ADP-ribose) polymerase (PARP-1) which resulted in HT-29 cell death. We found that heptaphylline activated BH3 interacting-domain death agonist (Bid) and Bak, proapoptotic proteins. In contrast, it suppressed X-linked inhibitor-of-apoptosis protein (XIAP), Bcl-xL and survivin, inhibitors of apoptosis. In addition, heptaphylline inhibited activation of NF-κB/p65 (rel), a regulator of apoptotic regulating proteins by suppressing the activation of Akt and IKKα, upstream regulators of p65. The findings suggested that heptaphylline induces apoptosis in human colon adenocarcinoma cells.

Keywords: Heptaphylline - colon adenocarcinoma cells - Bid - XIAP - Bcl-xL - Akt - p65

RESEARCH ARTICLE

Heptaphylline Induces Apoptosis in Human Colon Adenocarcinoma Cells through Bid and Akt/NF-κB (p65) Pathways

Introduction

Cancer is a disease of deregulated cell proliferation and inhibition of apoptosis. The deregulated growth found in cancer cells is frequently attributed to the loss of control in proliferation and program cell death processing (Evan and Vousden, 2001). The treatment of cancers has broadly been unsuccessful because of its uncontrolled growth. Therefore, it is highly required for the improvement of novel and effective chemotherapeutic agents, which contains a capacity to target various signaling pathways to interfere cell growth signaling, to promote the death of cancer cells.

Apoptosis is a programmed cell death that occurs during multiple important physiological conditions, for example, embryonic development, tissue remodeling, the maintenance of homeostasis, and immune repertoire. The deregulation of apoptosis is the cause of diseases, including cancers, autoimmune diseases and neurodegenerative disorders (Okada and Mak, 2004; Sankari et al., 2012). Apoptosis is also considered the basis for cancer treatment to target cancerous cells but not normal cells in order to limit the cytotoxicity from chemotherapy (Debatin et al., 2004; Irene et al., 2005; Millimouno et al., 2014). Induction of apoptosis causes the activation of Bid protein and Bid can activate mitochondria via direct interaction with Bax or Bak. Cytosolic Bid is cleaved at the amino terminus to generate a truncated form of Bid (tBid) that mediates cytochrome C release from mitochondria, which serves as an amplification signal by activating downstream effectors, including caspase-3 lead to cleaving of cellular proteins, such as poly (ADP-ribose) polymerase-1 (PARP-1) (Perez and White, 2000; Chen et al., 2006; Stevenson et al., 2007; Pei et al., 2007; Alenzi et al., 2010; Kantari and Walczak, 2011; Alshammari et al., 2014). Caspase cascade can be blocked by antiapoptotic proteins, for instance, cIAP1/2, Bcl-xl, Mcl-1, survivin and XIAP (Thorburn et al., 2008). One mechanism of cancer cells to escape apoptosis is overexpression of antiapoptotic proteins. The efficacy of many chemotherapeutics is due to their capacity to induce the death of tumor cells by apoptosis. Apoptosis-inducing compounds are commonly regarded as candidate anticancer agents (Hanahan and Weinberg, 2000; Debatin and Krammer, 2004; Hanahan and Weinberg, 2011; Meiyanto et al., 2012; Pan et al., 2014).

Clausena harmandiana, known as “Song Fa” in Thai, belongs to the Rutaceae family widely distributed in Southeast Asia. In Thailand, especially in the northeastern

¹Faculty of Pharmaceutical Sciences, ²Center of Natural Products Research Unit, Center for Innovation in Chemistry, Department of Chemistry, Faculty of Science, Khon Kaen University, Khon Kaen, ³Center for Excellence for Innovation in Drug Design and Discovery, Faculty of Pharmacy, Mahidol University, Bangkok, ⁴Faculty of Pharmaceutical Sciences, Ubon Ratchathani University, Ubon Ratchathani, Thailand  *For correspondence: pwaiwut79@yahoo.com, phpornwa@ubu.ac.th
part, the young leaves are accounted as a vegetable in Thai traditional foods and as ruminants feed. C. harmandiana is regarded as the herb for health and it is utilized for the headache, stomachache, and illness treatment. It had been reported that the roots of this plant included plenty of carbazole alkaloids together with coumarins (Aouacheria et al., 2002; Thongthoom et al., 2010). Carbazoles and coumarins have been isolated and evaluated for antimalarial, antifungal, and anti-TB. Carbazole alkaloids were a main component of the Clausena genus, especially Clausena excavate, Clausena anisata and Clausena harmandiana. It had been found that carbazole alkaloids showed various pharmacological properties, consisting of anticancer, antiplatelet aggregation and vasorelaxing, antimycobacterial, anti HIV-1, antiplasmodial, antimycobial, anti-inflammatory, and cytotoxicity against the leukemia cell line, through inhibiting topoisomerase II and antidiabetes activity (Ito et al., 2000, Yenjai et al., 2000; Songsiang et al., 2011). The major carbazole components in C. harmandiana were heptaphylline and 7-methoxyheptaphylline (Figure 1A), which showed cytotoxicity against the NCI-H187 cell line (Thongthoom et al., 2001). This study investigates the apoptotic effects of heptaphylline and 7-methoxyheptaphylline from C. harmandiana on HT29 colorectal adenocarcinoma cells.

Materials and Methods

Cell culture

HT-29 cells are maintained in Dulbecco’s modified Eagle’s medium (high glucose) supplemented with 10% fetal calf serum, 100 units/ml penicillin and 100μg/ml streptomycin at 37°C in 5% CO₂. The quantification of cell viability is performed by using the cell proliferation reagent MTT (3-(4,5-dimethylthiazolyl-2)-2, 5-diphenyltetrazolium bromide). HT-29 cells are plated in 96-well microplates at 6x10⁴ cells/wells, and then incubated for 24h.

Cell cytotoxicity assay

Cells are treated with heptaphylline, 7-methoxyheptaphylline (Yenjai, 2000) at different concentrations and reference compound (doxorubicin) for 24 and 48h. Add 10μl MTT Reagent (5mg/ml). They are incubated for 2 to 4 hours until purple precipitate is visible. The absorbance at 570 nm is measured. Percentage calculation of cell viability uses the following formula;

\[
\% \text{ Cell viability} = \frac{\text{Absorbance of treated cells}}{\text{Absorbance of control (untreated cells)}} \times 100
\]

Cell morphology is examined by Phase contrast microscope.

Preparation of cell extracts

In order to investigate the mechanism of the compounds on apoptotic pathway in HT29 cells, cells are treated with the test compounds at different concentrations for 4h. Whole cell lysates are prepared with lysis buffer (25mM HEPES pH7.7, 0.3mM MgCl₂, 0.2mM EDTA, 10% Triton X-100, 20mM β-glycerophosphate, 1mM sodium orthovanadate, 1mM phenylmethylsulfonyl fluoride (PMSF), 1mM dithiothreitol (DTT), 10μg/ml aprotinin, and 10μg/ml leupeptin). Cell lysate is collected from supernatant after centrifugation at 14,000 rpm for 10min.

Immunoblotting

Cell lysate is resolved by SDS-PAGE and transferred to an Immobilon-P-nylon membrane (Millipore). The membrane is treated with BlockAce (Dainippon Pharmaceutical Co. Ltd, Suita, Japan) and probed with primary antibodies (anti-caspase-3, PARP-1, phosphor-P65, P65, Bcl-2, BID, survivin, XIAP, and anti-actin antibodies). The antibodies are detected by the use of horseradish peroxidase-conjugated anti-rabbit, anti-mouse, and anti-goat IgG (DAKO, Glostrup, Denmark), and visualized by the enhanced chemiluminescence system (Amersham Biosciences).

Results

Effects of heptaphylline and 7-methoxyheptaphylline on viability of HT-29 cells

To examine whether carbazole extract of C. harmandiana inhibited viability of HT-29 cells, the cells were treated with 100 μM heptaphylline, 7-methoxyheptaphylline and 10 μg/ml doxorubicin (reference compound) for 24 and 48h then cell viabilities were determined by MTT assay. *p<0.001
Heptaphylline Induces Apoptosis in Human Colon Adenocarcinoma Cells through Bid and Akt/NF-κB (p65) Pathways

Effects of heptaphylline and 7-methoxyheptaphylline on apoptotic responses of HT-29 cells.

To test the effect of the compounds on HT-29 cell morphology, cells were treated with 100 µM of heptaphylline and 7-methoxyheptaphylline or doxorubicin (positive control) for 24 h. Cell morphology was observed by phase contrast microscopy. The result showed that heptaphylline, 7-methoxyheptaphylline and doxorubicin induced morphological changes of cell death consisting of rounding and shrinkage of cells by heptaphylline exhibited strongest effect on HT-29 cells (Figure 2A). The result indicated that heptaphylline markedly induced death of HT-29 cancer cells.

Figure 2. Effects of Heptaphylline and 7-methoxyheptaphylline on HT-29 Cell Apoptosis.
A) Cells were treated with 100 µM, heptaphylline 7-methoxyheptaphylline and 10µg/ml doxorubicin (reference compound) for 24 h. Cell morphology was investigated by phase contrast microscope. B) Whole cell extract was prepared, and analyzed by Western blotting using anti-caspase-3 and β-actin antibodies. Arrows indicate cleaved forms of caspase-3 in cell number at 48h (Figure 1B). This result indicated that heptaphylline strongly induced death of HT-29 cancer cells.

![Cell morphology images](image-url)

Effects of heptaphylline and 7-methoxyheptaphylline on apoptotic responses of HT-29 cells.

To test the effect of the compounds on HT-29 cell morphology, cells were treated with 100µM of heptaphylline and 7-methoxyheptaphylline or doxorubicin (positive control) for 24h and morphological changes were observed by phase contrast microscopy. The result showed that heptaphylline, 7-methoxyheptaphylline and doxorubicin induced morphology change of cell death consisting of rounding and shrinkage of cells by heptaphylline exhibited strongest effect on HT-29 cells (Figure 2A). The result indicated that heptaphylline markedly induced cell death morphological changes. As shown in Figure 2B, heptaphylline, 7-methoxyheptaphylline and doxorubicin induced cleavage of caspase-3, cellular pro-apoptotic responses by heptaphylline showed strongest apoptosis-promoting activity in HT-29 cells. To confirm the effects of heptaphylline on HT-29 cell proliferation, cells were treated with various concentrations of heptaphylline for 24 and 48h and the cell viability were examined by MTT assay. Heptaphylline significantly inhibited cell growth at concentrations 25, 50 and 100µM in a dose- and time-dependent manner with 50 % growth inhibitory concentration (IC<sub>50</sub>) value of 60.67 µM at 24h and 46.72µM at 48h respectively (Figure 3A). The apoptotic effects of heptaphylline were investigated by determining the cleavage of caspase-3 and PARP-1 using Western blot analysis. The result in Figure 3B showed that heptaphylline markedly induced the cleavage of caspase-3 and PARP-1 in a concentration-dependent manner. The results indicated that heptaphylline has apoptotic effect to HT-29 cells.

Heptaphylline induced bid activation and inhibited phosphorylation of Akt/NF-κB(p65) pathway

To understand the mechanism by which heptaphylline exerts its apoptotic effects, we next examined the effect of heptaphylline on the expression of key proteins involved in apoptosis regulation including Bid, Bak, Bcl-2, Bcl-xL, Mcl-1, XIAP, and survivin by immunoblotting assay. The results in Figure 4A showed that heptaphylline at concentration 25µM markedly induced cleavage of Bid correlation with increasing in Bak protein. In contrast, the expression of XIAP, Bcl-xL and survivin, but not Bcl-2 and Mcl-1 was inhibited. It has been reported that the nuclear transcription factor, NF-κB/p65 (rel) has important role to control expression of many anti-apoptotic proteins consisting of Bcl-xL, survivin and XIAP. Therefore, we further observed the proteins involved in p65 signaling pathway.
Heptaphylline and 7-methoxyheptaphylline are a carbarzole isolated from the roots of Clausena harmandiana which exhibited cytotoxicity against NCI-H187 (human small cell lung cancer cells) and KB cells (human epidermoid carcinoma of oral cavity cell lines) (Ito et al., 2000; Yenjadi et al., 2000; Thongthoom et al., 2011; Wangboonskul and Yenjadi, 2012). In this study we test the apoptotic effects of heptaphylline and 7-methoxyheptaphylline on human colon adenocarcinoma cell line (HT-29). Heptaphylline showed more stronger apoptotic effects than 7-methoxyheptaphylline by induced cell death morphology change of HT-29 colon adenocarcinoma cells. In order to control cell proliferation or in response to stimuli, cells undergo death through a process called apoptosis or programmed cell death. The understanding of apoptosis has provided the basis for new targeted therapies that can induce death in cancer cells. Most of the cytotoxic anticancer agents in present use have been shown to induce apoptosis in cancer cells such as etoposide, capsacin, berberine, gomisin N and doxorubicin (Ghobrial et al., 2005; Wong, 2011). The anti-cancer drug etoposide is able to induce caspase-8 processing and apoptosis in cancer cells, capsacin induces apoptosis through cellular stress, involving mitochondria and endoplasmic reticulum in several human cancer cells, berberine, an isouquinoline alkaloid, has several pharmacological properties and can affect in different levels the mitochondrial functions, gomisin N, a dibenzo cyclooctadiene lignan, isolated from Schizandra chinensis Baill induced apoptosis of human leukemia U937 cells and doxorubicin also induces apoptosis in many cancer cells (Kim et al., 2010; Liu et al., 2011; IP et al., 2012). In apoptotic pathways, the Bcl-2 family of proteins are significant regulators of apoptosis and include both anti-apoptotic members (such as Bcl-2, Mcl-1, survivin and Bcl-xL) and pro-apoptotic members (for example, Bax, Bak, Bad, Bid, and Bik) (Wong, 2011). Antia apoptotic proteins acts as suppressors of apoptosis by blocking the activation of cytochrome-C and caspase-3, whereas proapoptotic members act as apoptosis promoters and are overexpressed in massive malignancies. In response to apoptotic stimuli, proapoptotic proteins undergo posttranslational modifications that include dephosphorylation and cleavage resulting in activation of Bid, which oligomerizes Bak or Bax into pores that result in the release of cytochrome C. Cytochrome C, once released in the cytosol, interacts with Apaf-1, leading to the activation of caspase-9 proenzymes. Active caspase-9 then activates caspase-3, which subsequently activates the cleavage of PARP-1 and leads to apoptosis. Increased expression of antia apoptotic proteins causes resistance to chemotherapeutic drugs, while decreasing antia apoptotic proteins expression may promote apoptotic responses to anticancer drugs. In this study, heptaphylline activated cleavage of caspase-3, PARP-1 and proapoptotic proteins, Bid, Bak. In contrast, it inhibits antia apoptotic proteins including Bcl-xL, XIAP and survivin (Korsmeyer et al., 2000; Thorburn, Behbakht and Ford, 2008). It has been reported that the extrinsic and intrinsic apoptotic pathways are regulated by proteins, for instance, the p53 and NF-κB pathways. NF-κB is a nuclear transcription factor that regulates expression of many genes involved in the regulation of apoptosis, the activation of NF-κB induces resistance to apoptotic stimuli through the activation of a number of complex proteins including Bcl-xl, survivin, and X-linked IAP (XIAP). Moreover, previous reports

Discussion

Heptaphylline and 7-methoxyheptaphylline are a carbarzole isolated from the roots of Clausena harmandiana which exhibited cytotoxicity against NCI-H187 (human small cell lung cancer cells) and KB cells (human epidermoid carcinoma of oral cavity cell lines) (Ito et al., 2000; Yenjadi et al., 2000; Thongthoom et al., 2011; Wangboonskul and Yenjadi, 2012). In this study we test the apoptotic effects of heptaphylline and 7-methoxyheptaphylline on human colon adenocarcinoma cell line (HT-29). Heptaphylline showed more stronger apoptotic effects than 7-methoxyheptaphylline by induced cell death morphology change of HT-29 colon adenocarcinoma cells. In order to control cell proliferation or in response to stimuli, cells undergo death through a process called apoptosis or programmed cell death. The understanding of apoptosis has provided the basis for new targeted therapies that can induce death in cancer cells. Most of the cytotoxic anticancer agents in present use have been shown to induce apoptosis in cancer cells such as etoposide, capsacin, berberine, gomisin N and doxorubicin (Ghobrial et al., 2005; Wong, 2011). The anti-cancer drug etoposide is able to induce caspase-8 processing and apoptosis in cancer cells, capsacin induces apoptosis through cellular stress, involving mitochondria and endoplasmic reticulum in several human cancer cells, berberine, an isouquinoline alkaloid, has several pharmacological properties and can affect in different levels the mitochondrial functions, gomisin N, a dibenzo cyclooctadiene lignan, isolated from Schizandra chinensis Baill induced apoptosis of human leukemia U937 cells and doxorubicin also induces apoptosis in many cancer cells (Kim et al., 2010; Liu et al., 2011; IP et al., 2012). In apoptotic pathways, the Bcl-2 family of proteins are significant regulators of apoptosis and include both anti-apoptotic members (such as Bcl-2, Mcl-1, survivin and Bcl-xL) and pro-apoptotic members (for example, Bax, Bak, Bad, Bid, and Bik) (Wong, 2011). Antia apoptotic proteins acts as suppressors of apoptosis by blocking the activation of cytochrome-C and caspase-3, whereas proapoptotic members act as apoptosis promoters and are overexpressed in massive malignancies. In response to apoptotic stimuli, proapoptotic proteins undergo posttranslational modifications that include dephosphorylation and cleavage resulting in activation of Bid, which oligomerizes Bak or Bax into pores that result in the release of cytochrome C. Cytochrome C, once released in the cytosol, interacts with Apaf-1, leading to the activation of caspase-9 proenzymes. Active caspase-9 then activates caspase-3, which subsequently activates the cleavage of PARP-1 and leads to apoptosis. Increased expression of antia apoptotic proteins causes resistance to chemotherapeutic drugs, while decreasing antia apoptotic proteins expression may promote apoptotic responses to anticancer drugs. In this study, heptaphylline activated cleavage of caspase-3, PARP-1 and proapoptotic proteins, Bid, Bak. In contrast, it inhibits antia apoptotic proteins including Bcl-xL, XIAP and survivin (Korsmeyer et al., 2000; Thorburn, Behbakht and Ford, 2008). It has been reported that the extrinsic and intrinsic apoptotic pathways are regulated by proteins, for instance, the p53 and NF-κB pathways. NF-κB is a nuclear transcription factor that regulates expression of many genes involved in the regulation of apoptosis, the activation of NF-κB induces resistance to apoptotic stimuli through the activation of a number of complex proteins including Bcl-xl, survivin, and X-linked IAP (XIAP). Moreover, previous reports
Heptaphylline Induces Apoptosis in Human Colon Adenocarcinoma Cells through Bid and Akt/NI-xB (p65) Pathways

Death Differ, 7, 1166-73.
Liu J, Uematsu H, Tsuchida N, et al (2011). Essential role of caspase-8 in p53/p73-dependent apoptosis induced by etoposide in head and neck carcinoma cells. Mol Cancer, 31, 95.
Mendoza FJ, Espino PS, Cann KL, et al (2005). Anti-tumor chemotherapy utilizing peptide-based approaches- apoptotic pathways, kinases, and proteasome as targets. Arch Immunol Ther Exp, 53, 47-60.
Millimouno FM, Dong J, Yang L, Li J, Li X (2014). Targeting apoptosis pathways in cancer and perspectives with natural compounds from mother nature. Cancer Prev Res, 7, 1081-107.
Namba H, Saenko V, Yamashita S (2007). Nuclear factor-xB in thyroid carcinogenesis and progression: a novel therapeutic target for advanced thyroid cancer. Arq Bras Endocrinol Metab, 51, 843-51.
Okada H, Mak TW (2004). Pathways of apoptotic and non-apoptotic death in tumour cells. Nat Rev Cancer, 4, 592-603.
Pei Y, Xing D, Gao X, Liu L, Chen T (2007) Real-time monitoring full length bid interacting with Bax during TNF-alpha-induced apoptosis. Apoptosis, 12, 1681-90.
Perez D, White E (2000). TNF-alpha signals apoptosis through a bid-dependent conformational change in Bax that is inhibited by E1B 19K. Mol Cell, 6, 53-63.
Shukla S, MacLennan GT, Fu P, et al (2004). Nuclear factor-kappaB/p65 (Rel A) is constitutively activated in human prostate adenocarcinoma and correlates with disease progression. Neoplasia, 6, 390-400.
Songsiang U, Thongthoom T, Boonyarat C, Yenjai C (2011). Clausera-A-D, cytotoxic carbazole alkaloids from the roots of Clausena harmandiana. J Nat Prod, 74, 208-12.
Stevenson HS, Fu SW, Pinzone JJ, et al (2007). BP1 transcriptionally activates bcl-2 and inhibits TNF-alpha-induced cell death in MCF7 breast cancer cells. Breast Cancer Res, 9, 60.
Thongthoom T, Songsiang U, Phaosiri C, Yenjai C (2010). Biological activity of chemical constituents from Clausena harmandiana. Arch Pharm Res, 33, 675-80.
Thongthoom T, Promsuwan P, Yenjai C (2011). Synthesis and cytotoxic activity of the heptaphylline and 7-methoxyheptaphylline series. Eur J Med Chem, 46, 3755-61.
Thorburn A, Behbakht K, Ford H (2008). TRAIL receptor-targeted therapeu-tics: resistance mechanisms and strategies to avoid them. Drug Resist Updat, 11, 17-24.
Wangboonskul J, Yenjai C (2012). Antioxidant activity and cytotoxicity against cholangiocarcinoma of carbazoles and coumarins from Clausena harmandiana. Science Asia, 38, 75-81.
Wong RS (2011). Apoptosis in cancer: from pathogenesis to treatment 1-14. J Exp Clin Cancer Res, 30, 87.
Yenjai C, Sripontan S, Sripjran P, et al (2000). Coumarins and carbazoles with antiplasmodial activity from Clausena harmandiana. Planta Med, 66, 277-9.

Acknowledgements

This study was supported by Thailand Research Fund (No.TRG5780035)

References

Aouacheria A, Neel B, Bouaziz ZR, et al (2002). Carbazolequinone induction of caspase-dependent cell death in Src-overexpressing cells. Biochem Pharmacol, 64, 1605-16.
Chen X, Ding WX, Ni HM, et al (2006). Bid-independent mitochondrial activation in tumor necrosis factor alpha-induced apoptosis and liver injury. Mol Cell Biol, 27, 541-53.
Dai Y, Rahmani M, Dent P, et al (2005). Kinase 1 Activation Downregulation, and c-Jun N-terminal mediated by oxidative damage, XIAP leukemia cells through a process NF-kB activation potentiates apoptosis in inhibitor-induced RelA/p65 acetylation and blockade. Mol Cell Biol, 25, 5429.
Debatin KM, Krammer PH (2004). Death receptors in chemotherapy and cancer. Oncogene, 23, 2950-66.
Evan GI, Vousden KH (2001). Proliferation, cell cycle and apoptosis in cancer. Nature, 411, 342-8.
Ghorbali JM, Witzig TE, Adjie AA (2005). Targeting apoptosis pathways in cancer therapy. CA Cancer J Clin, 55, 178-94.
Hanahan D and Weinberg RA (2011). Hallmarks of cancer: the next generation. Cell, 144, 646-74.
Hanahan D, Weinberg RA (2000) The hallmarks of cancer. Cell, 100, 57-70.
Ip SW, Lan SH, Lu HF, et al (2012). Capsaicin mediates apoptosis in human nasopharyngeal carcinoma NPC-TW 039 cells through mitochondrial depolarization and endoplasmic reticulum stress. Hum Exp Toxicol, 31, 539-49.
Ito C, Itoigawa M, Katsuno S, et al (2000). Chemical constituents of Clausena excavata: isolation and structure elucidation of novel furanone-coumarins with inhibitory effects for tumor-promotion. J Nat Prod, 63, 1218-24.
Kantari C, Walczak H (2011) Caspase-8 and Bid: caught in the act between death receptors and mitochondria. Biochim Biophys Acta, 1813, 558-63.
Kim JH, Choi YW, Park C, et al (2010). Apoptosis induction of human leukemia U937 cells by gomisin N, a dibenzocyclooctadiene lignan, isolated from Schizandra chinensis Baill. Food Chem Toxicol, 48, 807-13.
Korsmeyer SJ, Wei MC, Saito M, et al (2000). Pro-apoptotic cascade activates BID, which oligomerizes BAK or BAX into pores that result in the release of cytochrome C. Cell