

Identification of Antrocin from *Antrodia camphorata* as a Selective and Novel Class of Small Molecule Inhibitor of Akt/mTOR Signaling in Metastatic Breast Cancer MDA-MB-231 Cells

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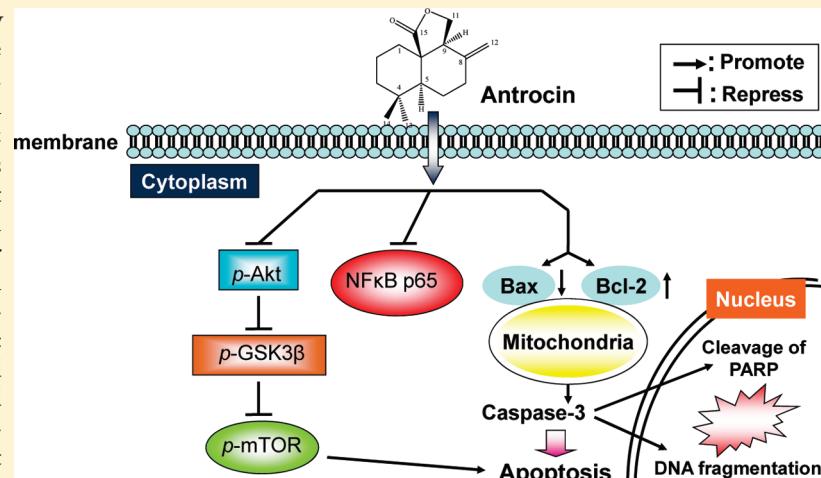
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 Supporting Information

ABSTRACT: The PI3K/Akt/mTOR pathway is considered to be an attractive target for the development of novel anticancer molecules. This paper reports for the first time that a small molecule, antrocin (MW = 234), from *Antrodia camphorata* was a potent antagonist in various cancer types, being highest in metastatic breast cancer MDA-MB-231 cells (MMCs) with an IC₅₀ value of 0.6 μM. Antrocin was a superior antiproliferator in MMCs as compared with doxorubicin and cisplatin, prevents colony formation, and was nontoxic to nontumorigenic MCF10A and HS-68 cells. Antrocin induced dose-dependent apoptosis in MMCs and caused cleavage of caspase-3 and poly(ADP-ribose) polymerase. Antrocin also caused a time-dependent decrease in protein expression of anti-apoptotic Bcl-2, Bcl-xL, survivin, and their mRNA, with concomitant increase in pro-apoptotic Bax and cytosolic cytochrome c. In a mechanistic study, antrocin suppressed the phosphorylation of Akt and its downstream effectors mTOR, GSK-3β, and NF-κB. Furthermore, down-regulation of Akt by small interfering RNA prior to antrocin treatment resulted in enhanced cell growth inhibition and apoptosis. Thus, antrocin as an Akt/mTOR dual inhibitor has broad applicability in the development of a clinical trial candidate for the treatment of metastatic breast cancer.



INTRODUCTION

Breast cancer is the most frequently diagnosed and second most morbid form of cancer afflicting women worldwide. It represents approximately 30% of newly diagnosed cancers each year.¹ During the normal course of the cellular life cycle cells grow, differentiate, and die. These three fundamental stages of the cellular life cycle are highly regulated in normal cells, whereas uncontrolled growth, dedifferentiated morphology, and resistance to death are the hallmarks of cancer cells.² Breast cancer cells also exhibit deregulated signaling pathways that promote uncontrolled growth, impart resistance to cell death, and induce invasion into the surrounding tissues. It is a heterogeneous disease with various subtypes exhibiting differential susceptibility to anticancer drugs.¹ This pathology is

currently controlled by surgery and radiotherapy and is frequently supported by adjuvant chemo- or hormonotherapies.³ Effective chemopreventive treatment for breast cancer would have a tremendous impact on breast cancer morbidity and mortality.¹ However, breast cancer is highly resistant to chemotherapy, and there is still no effective cure for patients with advanced stages of the disease, specifically in cases of hormone-independent cancer.⁴ In addition, the cost of this therapy is significant, and therefore the identification and development of new classes of agents with selectivity and low manufacture costs remain urgent priorities.

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Natural products still serve as excellent sources for modern drug discovery and development.⁵ Through a medicinal chemistry approach, natural products with low bioactivity or known compounds can be modified synthetically to improve their pharmacological profiles and candidacy for clinical trials.⁵ *Antrodia camphorata* (Niu-Chang-Chih or Zhan-Ku), Polyporaceae, is a medicinal mushroom that has been used in Taiwan folk medicine as a tonic and for treating alcohol and drug intoxication, skin itching and cancer.⁶ Crude extracts of *A. camphorata* exhibit a wide spectrum of pharmacological properties including anticancer in various cancer cell types.⁶ However, little is known about the target compounds and their molecular mechanisms against breast cancer.⁶ We have recently reported that triterpenoids from the fruiting bodies of *A. camphorata* showed strong antiproliferation and induced apoptosis effects in various cancer cell types.^{7–9} As part of our study program to identify novel therapeutic structures from *A. camphorata*,^{6–11} here we report the isolation of a sesquiterpene lactone, antrocin, which was originally reported by Chiang et al. in 1995.¹² However, investigations on its synthetic preparations and biological mode of action have not been reported to date.

The phosphatidylinositol 3-kinase (PI3K)/Akt (also termed protein kinase B)/mammalian target of rapamycin (mTOR) signaling pathway acts as a key integration point between the extrinsic and intrinsic cellular environments and regulates a broad spectrum of cellular processes.¹³ PI3K is a lipid kinase that activates Akt through PDK1. Akt is a serine/threonine kinase that promotes cell survival through the regulation of various downstream effectors, including anti-apoptotic pathways. mTOR is a downstream effector of the PI3K/Akt signaling pathway that activates p70S6 kinase and 4E-binding protein-1, which mediate the response of cancer cells to external stimuli and promote cell proliferation, regulating transition through the G1-S phase of the cell cycle.¹³ Activation of cytoplasmic signaling pathways (Akt and mTOR) resulted in activation of transcriptional factors including glycogen synthase kinase-3β (GSK-3β) and nuclear factor-κB (NF-κB). GSK-3β is the main Akt target gene critically involved in the induction of apoptosis and cell cycle arrest.¹⁴ Akt is capable of phosphorylating GSK-3β at Ser⁹ and subsequently inhibiting its kinase activity. Elevated levels of NF-κB can promote cellular growth, viability, and malignant transformation.¹⁴ The PI3K/Akt/mTOR pathway, which is often up-regulated in breast cancer, therefore represents an attractive and promising target for therapeutic intervention.¹⁵ Indeed, PI3K, Akt, and mTOR inhibitors have entered preclinical studies and clinical trials for various human cancers. The most extensively studied drugs targeting the PI3K/Akt pathways are LY294002, rapamycin (also known as sirolimus; Wyeth), and the derivatives temsirolimus (CCI-779), everolimus (RAD001; Novartis), and deforolimus AP23573 (MK-8669; Ariad).¹⁵ In addition, several PI3K/Akt inhibitors are currently under evaluation in human clinical trials, including perifosine (Keryx), BEZ235 (Novartis), GDC-0941 (Genentech), PX-866 (ProlX), MK-2206 (Merck), XL765 (Exelixis), RX-0201 (Rexahn), PBI-05204 (Phoenix), GSK2141795 (GlaxoSmithKline), and others.¹⁶ The effects on signaling by first- and second-generation rapalogs (prototypical mTOR inhibitors) have shown promise but, due to the complex nature of mTOR signaling, can result in positive and negative feedback loops from mTOR to Akt in different components of the tumor microenvironment.¹⁷ Thus, the dual inhibition of mTOR-PI3K or mTOR-Akt inhibitors may be a solution to these feedback loops and may provide a superior strategy for treating human malignancies and overcoming the resistance of cancer cells

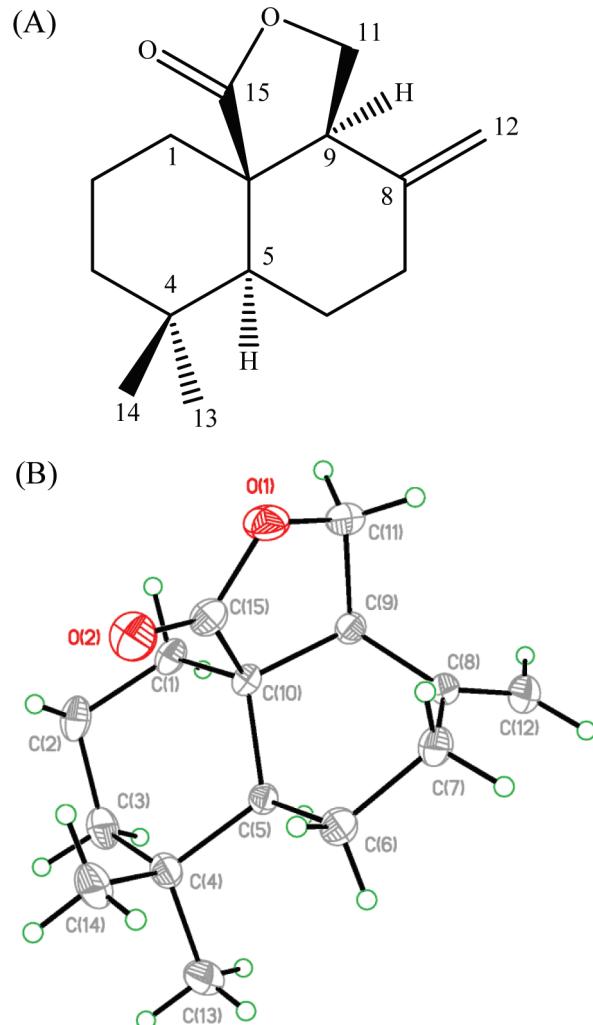


Figure 1. (A) Chemical structure of antrocin. (B) ORTEP derived from the single-crystal X-ray analysis of antrocin.

to chemotherapy. In this paper, we describe a small molecule inhibitor, antrocin (MW = 234), that selectively inhibits various types of cancer cell proliferations and induces apoptosis in breast cancer MDA-MB-231 cells (MMCs) mediated by down-regulation of Akt/mTOR/GSK-3β/NF-κB signaling pathways.

RESULTS AND DISCUSSION

Isolation and Purification. Antrocin (Figure 1A) was isolated from the fruiting bodies of *A. camphorata*. Briefly, the air-dried fruiting bodies were extracted with methanol under reflux, and the methanol extract was obtained upon concentration under reduced pressure. The methanol extract, suspended in H₂O, was partitioned with *n*-hexane and CHCl₃ to give fractions soluble in *n*-hexane, CHCl₃, and H₂O. The *n*-hexane-soluble fraction was chromatographed over silica gel using *n*-hexane/EtOAc as eluent to produce five fractions (I–V). Fraction II was further purified by another silica gel column using *n*-hexane/EtOAc to yield antrocin as colorless crystals. In a previous paper¹² antrocin was obtained through a laborious silica gel column chromatography coupled with HPLC purification with a yield of 0.005%. However, in this study antrocin was separated by a conventional isolation procedure silica gel column purification

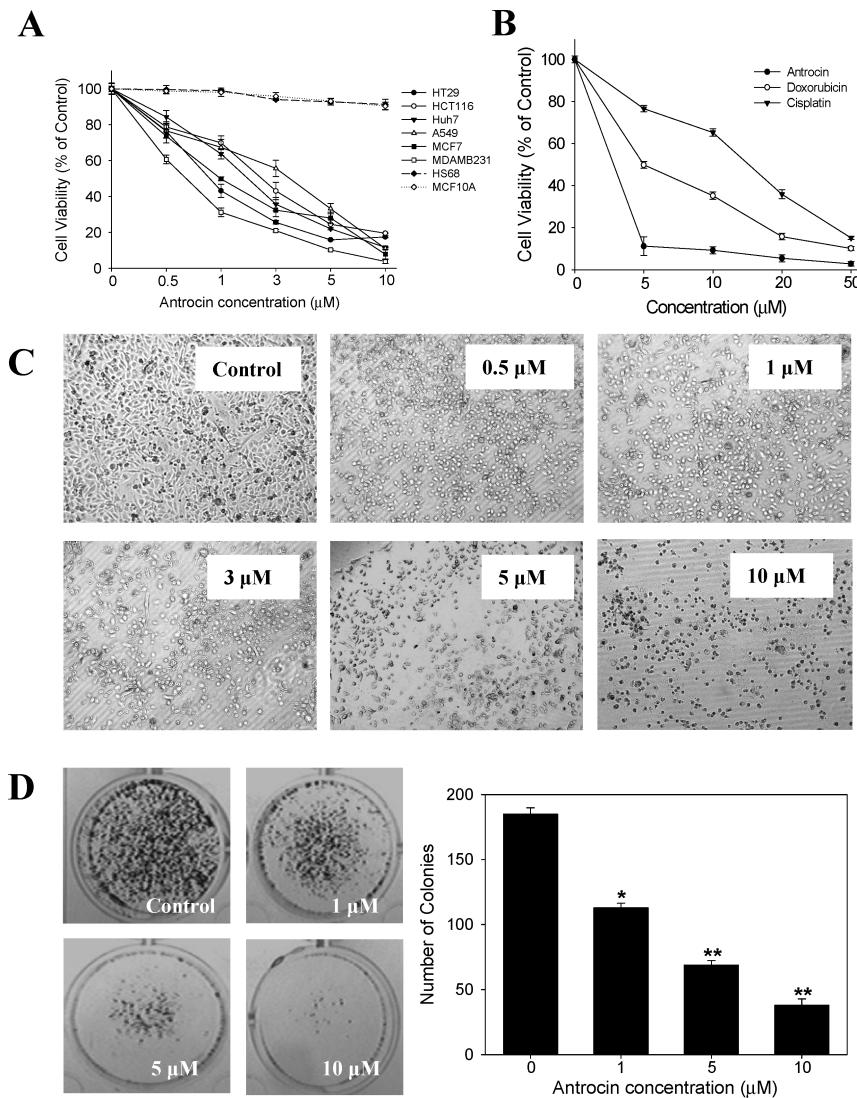


Figure 2. Effect of antrocin on tested cell line proliferation: (A) effect on cancer (HT-29, HCT-116, Huh7, A549, MCF-7, and MDA-MB-231) and normal (HS-68 and MCF-10A) cell proliferation; (B) comparison of antrocin with clinical drugs doxorubicin and cisplatin on MDA-MB-231 cell proliferation determined by SRB assay; (C) phase-contrast microscopic morphological observation (100× magnification) of MDA-MB-231 cells treated with indicated concentrations of antrocin; (D) effect of antrocin on anchorage-independent growth (colony formation) of MDA-MB-231 cells. Cell lines were treated with increasing concentrations of antrocin for 48 h, and SRB cell proliferation assays were performed. The results presented are means ± standard errors of the mean of three independent experiments.

with comparatively higher yield (0.04%). The structure and relative stereochemistry of antrocin was determined on the basis of coupling constants of the proton signals (Supporting Information Figure S1) and the chemical shifts of the carbon signals (Supporting Information Figure S2), which appeared with almost the same chemical shifts and identical multiplicity as established previously.¹² The ¹³C NMR (Supporting Information Figure S2) and DEPT spectra (Supporting Information Figure S3) of antrocin showed signals of 15 carbon atoms corresponding to 2 methyl, 7 methylene, 2 methine, and 3 quaternary carbons and 1 carbonyl carbon. The smaller coupling constants observed for H-5 with H-6 ($J_{5\alpha,6\alpha} = 4.8$ Hz) and H-9 with H-11 ($J_{9\alpha,11\alpha} = 6.6$ Hz) allowed the assignment of the relative stereochemistry for H-5 and H-9 as *cis*-orientation. The NOESY spectrum (Supporting Information Figure S4) further confirmed the stereochemistry at C-5 and C-9 by revealing cross peaks between H-5 α and H-6 α and between H-9 α and H-11 α ,

on the one hand, and between H-5 α and H-13 and between H-9 α and H-12 α , on the other hand. All of the spectral data (Supporting Information Table 1) derived from antrocin were in complete accord with the assigned structure (Figure 1A), and final confirmation of the relative stereochemistry followed from a single-crystal X-ray diffraction analysis. The ORTEP arising from this analysis is shown in Figure 1B. The purity (>95%) of antrocin was confirmed from its sharp melting point, TLC on silica gel (one spot), ¹H and ¹³C NMR studies, and HPLC chromatographic analysis (Supporting Information Figure S5).

Antrocin Inhibits Cancer Cell Proliferation. We first determined the effect of antrocin on cell proliferation in a variety of cultured cancer cell lines by sulforhodamine B (SRB) assay.⁹ Antrocin treatment for 48 h retarded cancer cell proliferation in a dose-dependent manner as compared with dimethyl sulfoxide (DMSO) treated control cells (Figure 2A). Antrocin showed the most potent antiproliferation effect in breast cancer line

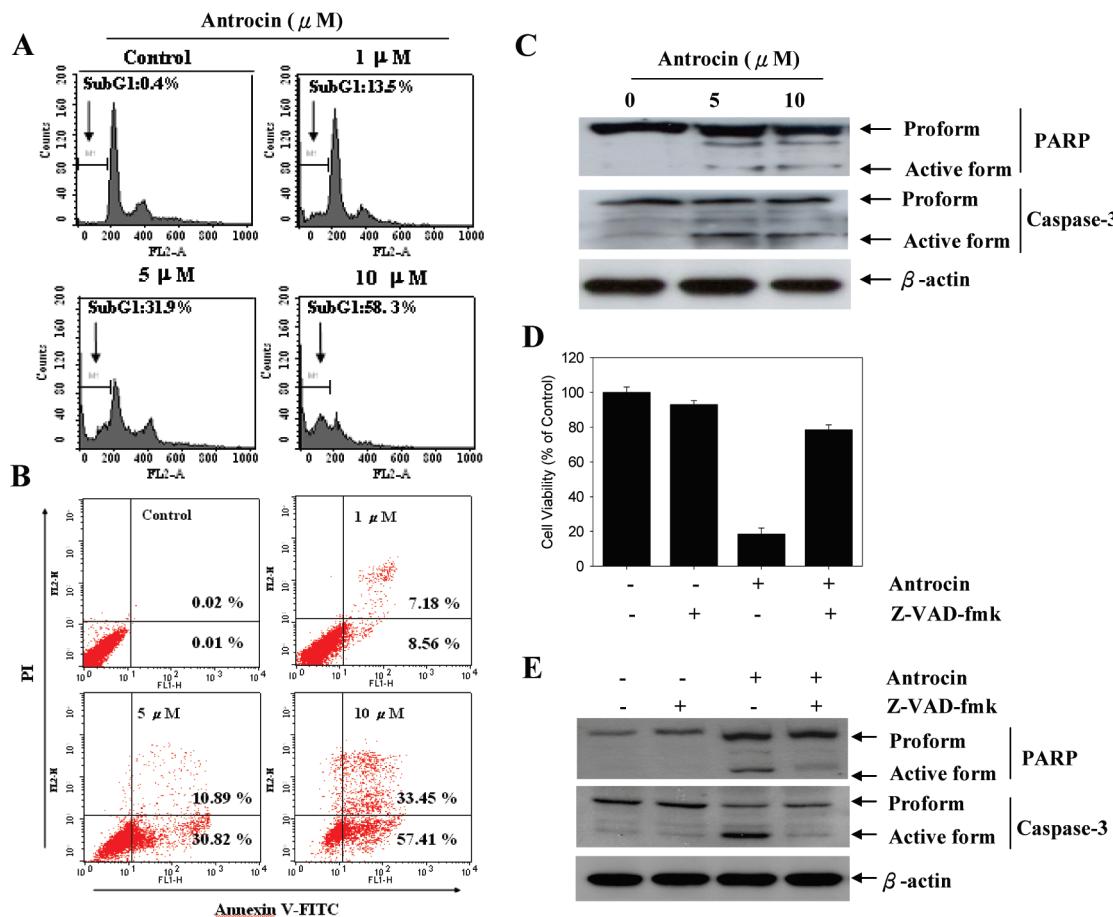


Figure 3. Effect of antrocin on MDA-MB-231 cells apoptosis: (A) cells were ethanol fixed and then used for flow cytometric analysis following PI staining, and the percentage of cell cycle phase sub-G1 population is shown; (B) cells were stained with annexin V—FITC and propidium iodide (PI) and analyzed by flow cytometry (the percentage indicated the population of cells with annexin V—FITC/PI double-positive signal); (C) effect of antrocin on the activity of caspase-3 and PARP as determined by Western blot, with β -actin expression as the internal control; (D) effect of z-VAD-fmk (pan caspase inhibitor) on the cell proliferation as determined by SRB assay (D) and antrocin-induced activation of caspase-3 and cleavage of PARP as analyzed by Western blot (E). Cells (2×10^5 cells/mL) were incubated in culture medium supplemented with DMSO control or antrocin at the indicated concentrations for 48 h. Cells were treated with 20 μ M z-VAD-fmk, 5 μ M antrocin, or a combination of both agents prior to harvest and analyzed. The quantitative data are the mean \pm SD of three determinations.

MDA-MB-231 cells (MMCs) with an IC₅₀ value of 0.6 μ M. MMCs are hormone- and growth factor-independent breast cancer cells, and proliferation inhibition was consistently observed with antrocin treatment in the range of 0.5–10 μ M. Similar antiproliferation results were observed with breast (MCF-7), lung (A549), liver (Huh7), and colon (HT-29 and HCT-116) cancer cells (Figure 2A). The IC₅₀ values as determined by SRB assay from dose response curves were 0.9, 1.5, 1.3, 4.1, and 1.2 μ M against HT-29, HCT-116, Huh7, A549, and MCF-7, respectively. Furthermore, using nontumorigenic breast epithelial MCF10A cells and normal foreskin fibroblast HS-68 cells, we assessed whether antrocin has any differential sensitivity to normal versus cancer cells. We did not observe any considerable growth inhibition in MCF-10A or in HS-68 cells following 48 h of antrocin treatment at 0.5 and 1 μ M doses (Figure 2A). There were few signs of cell death with 3, 5, and 10 μ M antrocin treatment, but it was not as strong as in the case of cancer cells (Figure 2A). For instance, growth inhibition caused by 3–10 μ M antrocin was 6.0–8.7% in HS-68 cells and 4.2–9.7% in MCF-10A cells as compared with 79.1–96.2% in MMCs (Figure 2A). These results pointed to the differential (selective) effect between tumor and normal cells of the antrocin.

To compare the antiproliferation effects of antrocin with clinically used drugs, MMCs were treated with the indicated concentrations of antrocin or doxorubicin or cisplatin for 48 h, and cell proliferation measured by SRB assay (Figure 2B). Notably, we observed that antrocin was a superior cell proliferation inhibitor as compared with doxorubicin and cisplatin (Figure 2B). For instance, at 5 μ M the cell proliferation inhibition caused by antrocin was 89.2% as compared with 60.2 and 33.5% by doxorubicin and cisplatin, respectively (Figure 2B). To further examine the antiproliferation effect of antrocin, MMCs were treated with antrocin in the concentration range of 0.5–10 μ M for 48 h and subjected to apoptotic cell morphology observation by phase contrast microscopy. Cells treated with increasing concentrations of antrocin showed changes in cell membranes that appeared to be irregular and jagged as compared with DMSO-treated control cells (Figure 2C). We then examined the ability of antrocin to inhibit colony formation of MMCs in an anchorage-independent growth assay.⁸ MMCs were plated in soft agar containing culture media with control DMSO or with indicated doses of antrocin. Colony formation in MMCs was significantly reduced by antrocin in a dose-dependent manner with 1, 5, and 10 μ M (Figure 2D). In addition to fewer colonies, antrocin

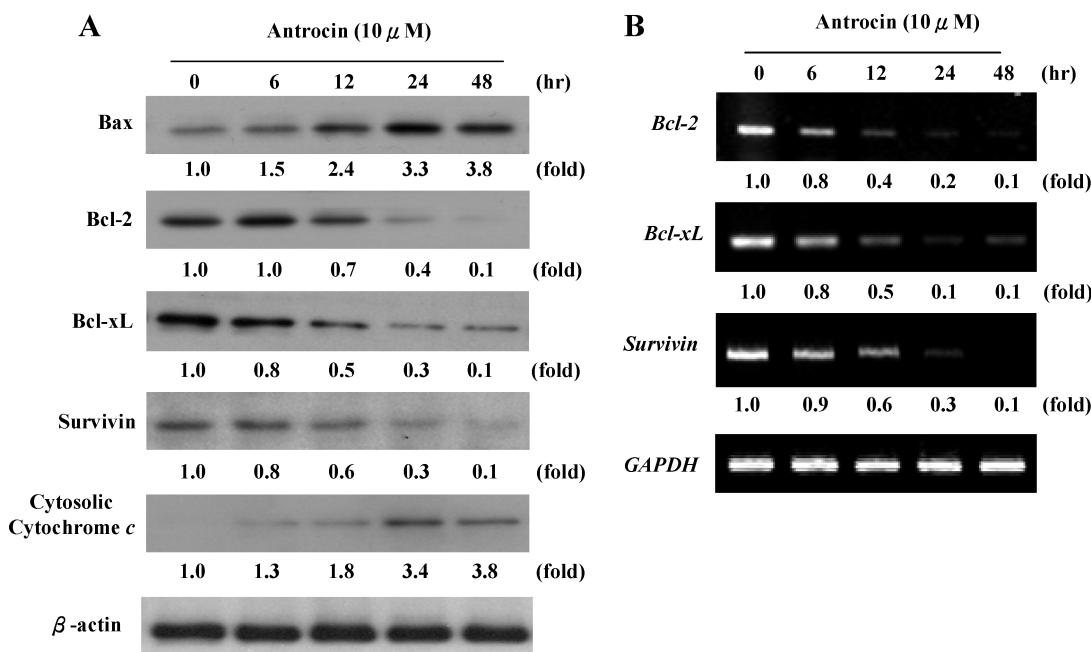


Figure 4. Effect of antrocin on MDA-MB-231 cell apoptosis regulatory protein expression: (A) Cells were treated with DMSO control or 10 μ M antrocin for the indicated time periods, and total protein was isolated. Equal amounts of cell lysates were analyzed by Western blot for Bax, Bcl-2, Bcl-xL, survivin, and cytosolic cytochrome *c* protein expression with corresponding antibodies. The values in fold denote the intensity of protein bands of antrocin-treated samples relative to that of the respective DMSO vehicle-treated control after being normalized to the respective internal reference (β -actin). (B) Antrocin affected anti-apoptotic gene expression. Cells were treated with antrocin (10 μ M) for the indicated time periods, and total RNA was isolated and examined for the expression of Bcl-2, Bcl-xL, and survivin by RT-PCR. GAPDH was used as an internal control to show equal RNA loading. Related expression was quantified densitometrically using LabWorks 4.5 software and calculated according to the reference bands. The quantitative data are the mean \pm SD of three determinations.

treatment also reduced the size and changed the appearance of colonies (Figure 2D). The results showed that antrocin inhibits anchorage-dependent as well as anchorage-independent growth of highly aggressive breast cancer cells.

Antrocin Induces Apoptosis in MMCs. To investigate whether the cell proliferation inhibition by antrocin treatment was associated with apoptosis induction, both morphologic changes and apoptosis-specific caspase-3 and poly(ADP-ribose) polymerase (PARP) cleavage were investigated in MMCs treated with antrocin for 48 h. We first examined 5 μ M antrocin-treated MMCs and untreated controls with 4',6'-diamino-2-phenylindole·2HCl (DAPI) staining using a fluorescence microscope.⁸ DAPI-stained antrocin-treated MMCs showed morphological indicators of apoptosis including chromatin condensation and apoptotic body formation as compared with the DMSO-treated control (Supporting Information Figure S6). We next performed flow cytometric analysis after propidium iodide (PI) staining of MMCs following antrocin treatment for 48 h.⁹ The flow cytometric cell cycle analysis results showed that antrocin increased the sub-G1 population, indicative of cell death (Figure 3A). It was noted that when MMCs were treated with DMSO control or 1, 5, and 10 μ M antrocin for 48 h, the proportion of sub-G1 population increased from 0.4 to 13.5, 31.9, and 58.3%, respectively. These results indicate that antrocin can reduce cancer cell proliferation through down-regulation of cell cycle progression.

The next assay that was performed was annexin V–FITC and PI double staining, followed by flow cytometric analysis.⁷ As shown in Figure 3B, flow cytometry analysis of untreated MMCs, the majority of cells appeared negative for staining with annexin V–FITC and PI, indicating that the cells were healthy. Upon treatment with antrocin for 48 h, flow cytometry analysis showed

significant shifts in the cell population in a dose-dependent manner (Figure 3B). The most significant new population of cells was positive for both annexin V–FITC and PI, indicating these cells had already died either by necrosis or by apoptosis. An additional cell population that is more positive for annexin V–FITC than for PI was also observable (Figure 3B), indicating that these cells were undergoing apoptosis. Specifically, the percentage of healthy MMCs positive for annexin V–FITC were decreased and, new apoptotic populations of cells increased from 0.01 to 8.56, 30.82, and 57.41% with antrocin treatment at 1, 5, and 10 μ M, respectively. We then confirmed that fragmented DNA detected by the TUNEL assay and agarose gel electrophoresis resulted from apoptosis.⁸ We treated MMCs with 1, 5, and 10 μ M antrocin for 48 h and then collected fragmented DNA from each sample. DNA laddering was observed in the presence of 5 and 10 μ M antrocin but not in controls (Supporting Information Figure S7).

Activation of the caspase cascade leading to PARP cleavage is regarded as a major pathway in apoptosis induction.¹⁸ Caspase-3 is a key effector molecule in the apoptosis pathway involved in amplifying the signal from initiator caspases.¹⁸ On the basis of the above results showing induction of apoptosis by antrocin, we analyzed the levels of cleaved caspase-3 following 48 h of antrocin treatment. Increased activation of caspase-3 was observed within 48 h in MMCs treated with either 5 or 10 μ M of antrocin (Figure 3C). Consistent with the cleavage of caspase-3, antrocin also caused an increase in PARP cleavage (Figure 3C). To further establish the role of caspase activation in antrocin-induced apoptosis, we used all-caspase inhibitor z-VAD-fmk (2 h pre-treatment). As shown in Figure 3D, a 20 μ M dose of caspase inhibitor partially reversed the antrocin-induced cell proliferation

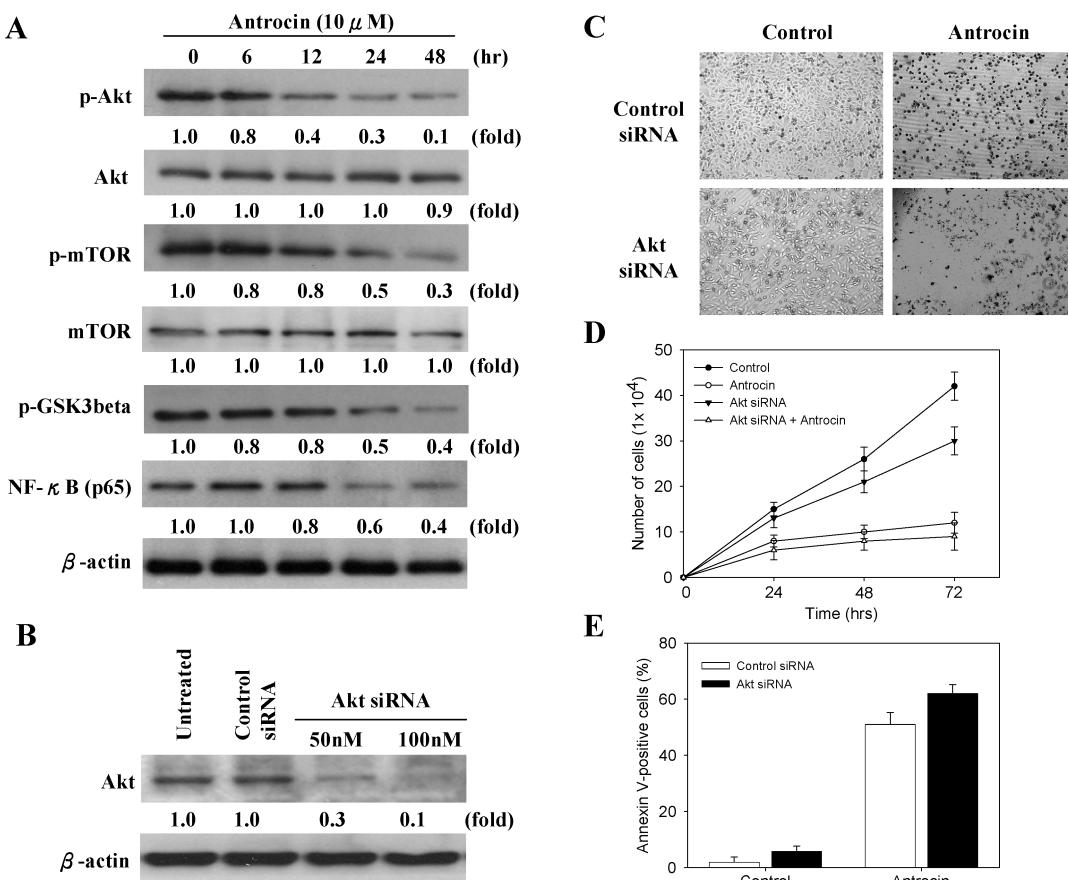


Figure 5. Effect of antrocin on MDA-MB-231 cell apoptosis signaling pathway. (A) Cells were treated with 10 μ M antrocin at the indicated time intervals, and the cell lysates were harvested and analyzed by Western blot for p-Akt, Akt, p-mTOR, mTOR, p-GSK-3 β , and NF- κ B with corresponding antibodies. Related expression was quantified densitometrically using LabWorks 4.5 software and calculated according to the reference bands. Reported values are the means \pm SD. (B) Genetic suppression of Akt by Akt siRNA transfection is shown for the indicated concentrations. (C) Cells were transfected with the respective siRNA (25 μ M), and 24 h later, the cells were incubated with antrocin (10 μ M). Cells were analyzed after 24 h under a phase-contrast microscope. (D) The down-regulation of Akt expression significantly inhibited cell growth. Antrocin plus siRNA Akt inhibited cell growth to a greater degree compared with antrocin alone. (E) The down-regulation of Akt expression significantly increased apoptosis induced by antrocin. Akt siRNA-transfected MDA-MB-231 cells were significantly more sensitive to antrocin-induced apoptosis.

inhibition and completely inhibited 5 μ M antrocin-induced caspase-3 and PARP activity (Figure 3E). These data further showed that antrocin induced caspase-dependent apoptosis in MMCs.

To investigate the mitochondrial apoptotic events involved in antrocin-induced apoptosis, we analyzed the changes in the Bcl-2 family proteins.¹⁸ It is reported that Bcl-2 family members including anti-apoptotic and pro-apoptotic proteins play an important role in either inhibition or promotion of apoptosis and regulate cytochrome *c* release from mitochondria.¹⁸ Pro-apoptotic Bcl-2 family molecules permeabilize the mitochondrial membrane (thereby promoting cytochrome *c* release), whereas anti-apoptotic Bcl-2 family molecules prevent cytochrome *c* release.¹⁹ Our results from Western blot analysis showed that treatment of MMCs with 10 μ M antrocin increased the pro-apoptotic protein Bax as compared to DMSO vehicle-treated control cells in a time-dependent manner (Figure 4A). In contrast, antrocin decreased anti-apoptotic proteins Bcl-2, Bcl-xL, and survivin levels (Figure 4A), which led to an increase in the pro-apoptotic/anti-apoptotic Bcl-2 ratio (Supporting Information Figure S8). Furthermore, the cytosolic fraction from untreated MMCs contained no detectable amount of cytochrome *c*, whereas it did become detectable after 6 h of antrocin treatment and increased progressively up to 48 h

(Figure 4A). To determine whether antrocin affects the transcription, the mRNA expression of Bcl-2, Bcl-xL, and survivin was examined.¹⁹ Immunoblot analysis also showed that treatment of MMCs with 10 μ M antrocin decreased the expression levels of anti-apoptotic proteins Bcl-2, Bcl-xL, and survivin in a time-dependent manner (Figure 4B). These data indicate that increase in the ratio of pro-apoptotic/anti-apoptotic mitochondrial proteins and cytochrome *c* release from mitochondria might contribute to the apoptosis promotion activity of antrocin.

Antrocin Inhibits Akt/mTOR/GSK-3 β /NF- κ B Signaling in MMCs. Our next objective was to identify and characterize the molecular pathways involved in antrocin-induced, caspase-dependent, mitochondria-mediated apoptosis. Among the known apoptotic pathways, the PI3K signaling pathway plays a critical role in cell proliferation and survival.¹³ The PI3K pathway is dysregulated in many different cancers, including breast cancer, which display activated PI3K and its downstream Akt, mTOR, and GSK-3 β kinases.²⁰ Therefore, the PI3K/Akt/mTOR pathway is considered to be an attractive target for the development of novel anticancer molecules.¹⁵ Because activation of Akt correlates with phosphorylation of the kinase on Thr³⁰⁸ and Ser⁴⁷³ residues, we examined the amount of phosphorylated Akt. MMCs were treated with DMSO vehicle or 10 μ M antrocin for various time periods,

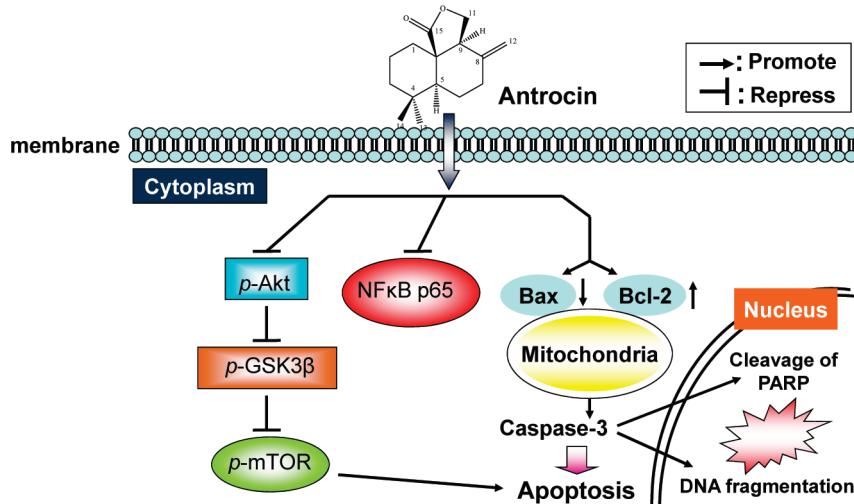


Figure 6. Schematic representation of antrocin action in MDA-MB-231 cells.

and cell extracts were prepared. Equal amounts of protein from control and each treatment were analyzed for Akt phosphorylation using an anti-phospho-Akt (Ser⁴⁷³) antibody. As shown in Figure 5A, the levels of phosphorylated Akt were time-dependently decreased, and such decrease was not due to an increase in the total Akt protein, which remained nearly constant. It is known that phosphorylated Akt can subsequently activate the downstream mTOR as well as GSK-3 β signaling pathway, which were involved in the induction of apoptosis and cell cycle progression.²¹ In our study, mTOR and GSK-3 β were also down-regulated by 10 μ M antrocin in a time-dependent manner, and their reduction was closely correlated with p-Akt inhibition (Figure 5A). The activated Akt pathway has been shown to regulate NF- κ B transcriptional factor expression in breast cancer cells.²¹ In this study, Western blotting data with NF- κ B specific antibody indicate time-dependent suppression of NF- κ B expression after treatment of MMCs with 10 μ M antrocin (Figure 5A). In support of the down-regulation of Akt/mTOR signaling, antrocin inhibited DNA synthesis and protein synthesis in MMCs (Figures 2A and 4).

To further confirm the effects of antrocin on Akt signaling pathway in MMCs, we conducted studies using Akt small-interfering RNA (siRNA). Consistent with the efficient inhibition of Akt phosphorylation of antrocin, we also observed the Akt-specific siRNA decreased the levels of Akt, whereas control siRNA-treated cells expressed similar levels of Akt (Figure 5B). In addition, down-regulation of Akt expression inhibited cell growth induced by antrocin as determined by the microscopic appearance of MMCs and SRB assay (Figure 5B,C). Furthermore, we also observed Akt siRNA-transfected MMCs were more sensitive to spontaneous and antrocin-induced apoptosis (Figure 5D). Collectively, these data predicted that antrocin would likely inhibit MMC proliferation and induced caspase-dependent mitochondria-mediated apoptosis by down-regulation of Akt/mTOR/GSK-3 β /NF- κ B signaling pathways. A schematic representation of antrocin action in MMCs is shown in Figure 6.

CONCLUSIONS

This study provided the first evidence that antrocin from *A. camphorata* attenuates the growth of various human cancer cells, triggering the caspase-dependent mitochondrial-mediated apoptosis by down-regulation of Akt/mTOR/GSK-3 β /NF- κ B signaling pathways. Although further studies are needed to fully

elucidate the mechanism of antrocin action, it is clear that this low molecular mass compound that translates to lower cost of synthesis is a novel molecule with efficacy as dual Akt/mTOR inhibitor. Antrocin may have the potential to bypass complications of rapalogs, where feedback loops lead to a differential response in tumor cells, for use in the design and development of its analogues of dual Akt/mTOR inhibitors.

ASSOCIATED CONTENT

S Supporting Information. Experimental procedures for isolation, HPLC analysis, and spectral data for antrocin and its ¹H NMR, ¹³C NMR, DEPT, and NOESY spectra; experimental procedures for biological testing; and additional methods for apoptosis studies. This information is available free of charge via the Internet at <http://pubs.acs.org>.

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