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Induction of Apoptosis in HL-60 Cells through the ROS-Mediated Mitochondrial Pathway by Ramentaceone from *Drosera aliciae*

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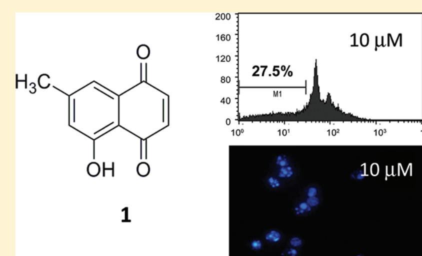

ABSTRACT: Ramentaceone (**1**) is a naphthoquinone constituent of *Drosera aliciae* that exhibits potent cytotoxic activity against various tumor cell lines. However, its molecular mechanism of cell death induction has still not been determined. The present study demonstrates that **1** induces apoptosis in human leukemia HL-60 cells. Typical morphological and biochemical features of apoptosis were observed in **1**-treated cells. Compound **1** induced a concentration-dependent increase in the sub-G1 fraction of the cell cycle. A decrease in the mitochondrial transmembrane potential ($\Delta\Psi$m) was also observed. Furthermore, **1** reduced the ratio of anti-apoptotic Bcl-2 to pro-apoptotic Bax and Bak, induced cytochrome *c* release, and increased the activity of caspase 3. The generation of reactive oxygen species (ROS) was detected in **1**-treated HL-60 cells, which was attenuated by the pretreatment of cells with a free radical scavenger, *N*-acetylcysteine (NAC). NAC also prevented the increase of the sub-G1 fraction induced by **1**. These results indicate that ramentaceone induces cell death through the ROS-mediated mitochondrial pathway.

Ramentaceone (7-methyljuglone) (**1**) is a naphthoquinone present in plants in the Droseraceae family.[1] Plants in the genus *Drosera* have been used in traditional medicine as antitussive, diuretic, and antibacterial agents.[2] Their therapeutic properties have been attributed to the presence of 1,4-naphthoquinones, mainly plumbagin and **1**.[1,3] Among the biological activities of **1** such as their antibacterial[4,5] and antifungal[6] effects, this compound also has been found to be cytotoxic against various cell lines. Such activity was reported against murine lymphocytic leukemia (P-388) and human prostate cancer (LNCaP) cells.[7,8] However, despite its cytotoxic properties, the mechanisms underlying the induction of cell death by this compound have not yet been defined. Wube et al.[9] reported that **1** inhibits the activity of 12-hydroxyeicosatetraenoic acid (12(S)-HETE). 12(S)-HETE, an arachidonate 12-lipoxygenase (12-LOX) metabolite, is regarded as an important intracellular signaling molecule in tumor metastasis.[10] Compound **1** displayed 12(S)-HETE inhibitory activity with an IC_{50} value of 22.2 μM. This inhibitive effect was comparable to that of baicalein, a known 12-LOX inhibitor, which displayed an IC_{50} value of 18.5 μM.[9] These findings point to the anticancer potential of **1** and warrant further investigation into defining the molecular mechanism of cell death induced by this compound.

Apoptosis (programmed cell death) has received much attention as a possible means of eliminating excessively proliferating cancer cells. Apoptosis is a highly regulated process that involves the activation of a series of molecular events. The evasion of apoptosis is regarded as one of the hallmarks of neoplastic transformation.[11] Thus, triggering apoptosis and clarifying its mechanisms is important in the treatment of malignancies. Many types of anticancer treatments including radiotherapy, chemotherapy, and hormone therapy target tumor cells by inducing apoptosis.[12,13] Since nearly half of all anticancer drugs approved between 1940 and 2006 were either natural products or their derivatives, natural products play a major role in drug discovery, and novel plant-derived anticancer compounds are the subject of intense studies.[14] Thus, in the present study the anticancer potential of the plant-derived naphthoquinone **1** was evaluated using HL-60 cells, which was isolated from *Drosera aliciae* Raym.-Hamet.

RESULTS AND DISCUSSION

Effects of Ramentaceone (1) on Viability and Apoptosis Induction in HL-60 Promyelocytic Leukemia Cells. The cytotoxic activity of **1** has been shown previously against various tumor cell lines, and consistent with these reports, **1** exhibited dose-dependent cytotoxicity against HL-60 cells, with an IC_{50} value of 8.75 μM (Figure 1A). In order to determine whether cell death induced by **1** is mediated via apoptosis, characteristic morphological features of apoptotic

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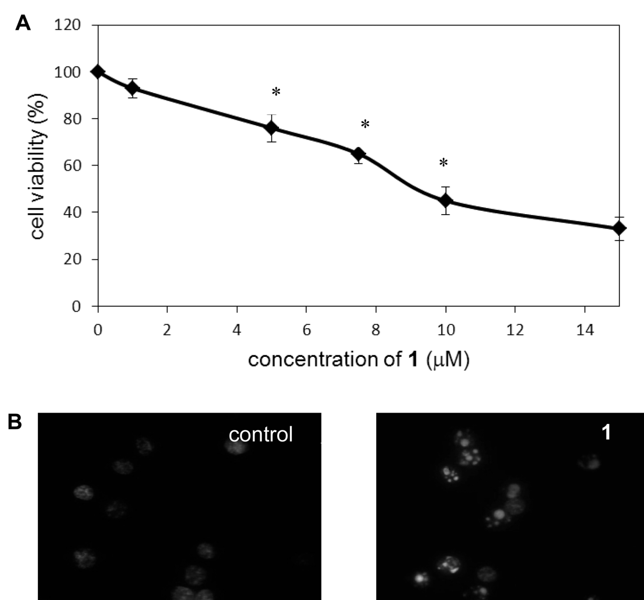

Figure 1. Effects of ramentaceone (1) on the viability of HL-60 cells. (A) Cytotoxic activity of 1. HL-60 cells were treated with 1 (0−15 μM) for 24 h, and cell survival was analyzed using the MTT assay (n = 3). Data were analyzed by one-way ANOVA with Tukey's post hoc tests [$p < 0.05$ (*) indicates differences between control and 1-treated cells]. (B) Nuclear condensation induced by 1 in HL-60 cells. Cells were treated with 10 μM 1 for 24 h and stained with Hoechst 33342.

cells were examined. Treatment of HL-60 cells for 24 h with 10 μM **1** resulted in membrane blebbing, cell shrinkage, and apoptotic body formation (data not presented). Staining of cells with Hoechst 33342 revealed chromatin condensation in **1**-treated cells (Figure 1B). The results indicated that on treatment with **1**, apoptotic morphological features could be detected in cancer cells. One of the hallmarks of apoptosis is DNA fragmentation, and this was analyzed by examining changes in the sub-G1 fraction of the cell cycle. On treatment with **1**, as determined by flow cytometry, a dose- and time-dependent induction in the sub-G1 fraction percentage was observed in **1**-treated cells. A significant increase in the sub-G1 fraction was observed after 24 h incubation in comparison to a 12 h incubation with **1** (Figure 2). Concentrations higher than 5 μM were required to increase the sub-G1 peak in cells exposed for 24 h to **1**. At 10 μM, the percentage of the sub-G1 fraction was estimated at 27%, which was accompanied by a decrease of the percentage of G0/G1 and G2/M fractions (Figure 2).

Induction of Apoptosis by Ramentaceone (1) through the Mitochondrial-Mediated Pathway. To determine whether **1**-induced apoptosis is accompanied by changes in the mitochondrial transmembrane potential ($\Delta\Psi$m), a membrane potential sensitive fluorescent probe, CMTMRos, was used. Additionally, an uncoupling agent, CCCP, was applied in concurrent treatment with CMTMRos to determine the net $\Delta\Psi$m. CCCP disrupts the $\Delta\Psi$m-dependent mitochondrial uptake of CMTMRos; therefore the uptake of the dye reflects the volume of the mitochondria. The quotient of the difference between the value of CMTMRos fluorescence intensity obtained in the absence of CCCP and the value obtained in the presence of CCCP, and the value of fluorescence intensity obtained in the presence of CCCP is defined as net $\Delta\Psi$m.[15] As determined by flow cytometry, the loss of mitochondrial membrane potential was induced in HL-60 cells in a concentration-dependent manner in cells treated for 24 h with **1** (Figure 3A).

The Bcl-2 family proteins play a crucial role in regulating the mitochondrial-mediated apoptosis pathway; therefore changes in the level of the pro-apoptotic and anti-apoptotic Bcl-2 proteins were analyzed in **1**-treated HL-60 cells. Immunoblot analysis revealed that **1** induced an increase in the level of the pro-apoptotic Bak and Bax proteins in HL-60 cells in a concentration-dependent manner (Figure 3B). In contrast, the level of the anti-apoptotoic Bcl-2 protein decreased upon **1** treatment, leading to an increase in the pro-apoptotic/anti-apoptotic Bcl-2 ratio (Figure 3B). The key step in the mitochondrial pathway is the release of cytochrome *c* from the intermembrane space. Immunoblot analysis showed a concentration-dependent increase in the accumulation of cytochrome *c* in the cytosol with a simultaneous decrease in the mitochondria, showing that **1** induces cytochrome *c* release from the mitochondria to the cytosol in HL-60 cells (Figure 3C).

The transduction of apoptotic signals requires the activation of a cascade of cysteine proteases, the caspases. Caspase 3, in particular, plays a central role in the execution of apoptosis, catalyzing the specific cleavage of many key cellular proteins. Its activation is essential for the occurrence of typical hallmarks of apoptosis such as chromatin condensation and DNA fragmentation.[16] The involvement of caspase 3 in the induction of **1**-mediated apoptosis was determined with the use of a colorimetric caspase 3 substrate, Ac-DEVD-pNA. The release of the cleaved pNA was measured in cells exposed for various time intervals to 10 μM **1**. An increase in caspase 3 activity was observed in **1** -treated cells, with the highest increase in enzyme activity observed at 12 h post-treatment, after which a decline in the activity of caspase 3 was observed.

Induction of Apoptosis by Ramentaceone (1) by ROS Generation in HL-60 Cells. To determine the effect of **1** on intracellular ROS generation, cells were incubated with **1** in the concentration range 0−10 μM. ROS levels were evaluated after a 3 h incubation with **1** with flow cytometry using a H_2DCFDA probe. H_2DCFDA is a fluorescent dye that diffuses through cell membranes and is hydrolyzed by intracellular esterases to DCFH. In the presence of ROS, DCFH is oxidized to DCF, which is fluorescent, and its level corresponds to the level of generated ROS. As determined with flow cytometry, **1** induced ROS generation in HL-60 cells in a concentration-dependent manner (Figure 4A). Moreover, pretreatment of cells with the ROS scavenger *N*-acetylcysteine (NAC, 15 mM) attenuated the generation of ROS by **1** (Figure 4B). The effects of intracellular ROS generation on the induction of apoptosis was evaluated by determining the effects of **1** on the increase of the sub-G1 fraction in cells pretreated with NAC. HL-60 cells following pretreatment with 15 mM NAC were incubated with increasing concentrations of **1**. NAC decreased the percentage of the sub-G1 fraction of the cell cycle (Figure 4C). At 10 μM **1**, the percentage of the sub-G1 fraction decreased from 27% to 12% (Figure 4C). These results indicate the involvement of ROS in **1**-induced apoptosis.

The role of ROS as mediators of apoptosis is becoming increasingly recognized. Many potential anticancer and chemopreventive agents induce apoptosis through ROS generation. Recent reports indicate that ROS not only are cytotoxic byproducts that trigger cell death through oxidative damage but also can act as signaling molecules targeting specific

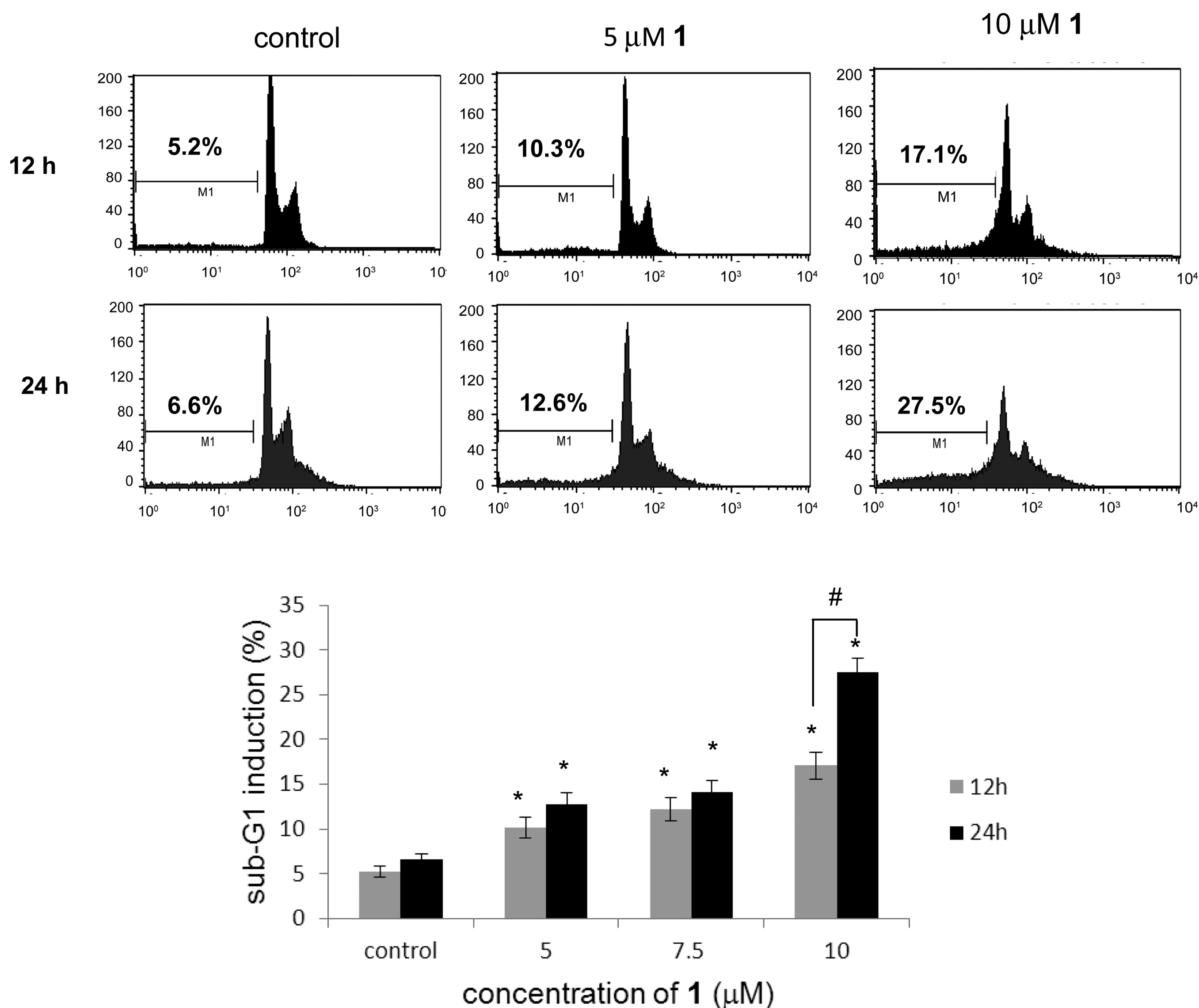

Figure 2. Increase in the percentage of the sub-G1 fraction of the cell cycle in ramentaceone (**1**)-treated HL-60 cells. HL-60 cells were treated with **1** (0−10 μM) for 12 and 24 h and examined by flow cytometry ($n = 3$). Histograms represent PI fluorescence intensities. Data were analyzed by two-way ANOVA with Bonferroni post-tests [$p < 0.05$ (*) indicates differences between control and **1**-treated cells. $p < 0.05$ (#) indicates differences between 12 and 24 h **1**-treated cells].

components of metabolic and signal transduction pathways.[17] Naphthoquinones, due to the presence of a quinone moiety, are known to generate ROS and therefore target various cellular compounds and regulate various signaling pathways. Several naphthoquinones, such as juglone, β-lapachone, and shikonin, have been reported to possess antineoplastic activity.[18−20] The most extensively researched naphthoquinone, plumbagin, has been found to target various cellular components through ROS generation. Our previous results showed that plumbagin through ROS generation targets topoisomerase II, which results in the stabilization of the topoisomerase II−DNA cleavable complex.[21] Plumbagin has also been found to suppress NF-κB activation by ROS-mediated oxidization of Cys residues on the p65 NF-κB subunit.[22]

Compounds containing a quinone moiety are of great biological interest due to their high reactivity, and quinones are one of the largest groups of compounds used as anticancer drugs.[23] The high reactivity of these compounds determines not only their anticancer effects but also their high cytotoxicity. The mode of action of quinones can lead to cellular damage through alkylation of cellular proteins and DNA,[24] depending on the structure and concentration of the quinone. It has been reported that plumbagin displays lower toxicity in vivo than doxorubicin, as shown in mouse models of myeloid leukemia.[25] Moreover, the high inhibitory activity of plumbagin toward breast cancer and human melanoma cells was not accompanied by toxic side effects, as determined in mouse xenograft cancer models.[26,27] Due to the structural similarity of plumbagin and **1**, the latter compound seems to be a promising anticancer agent. The only study regarding the mechanism of its potential anticancer activity showed that ramentaceone inhibits the activity of 12-hydroxyeicosatetraenoic acid (12(S)-HETE), a product of the 12-LOX pathway.[9] LOX inhibitors have been found to induce apoptosis in various cancer models.[28,29] However, in leukemic cells no correlation was determined between 12(S)-HETE and apoptosis induction.[30] These findings suggest the involvement of other mechanisms in the induction of apoptosis by **1** in leukemia cells. The present

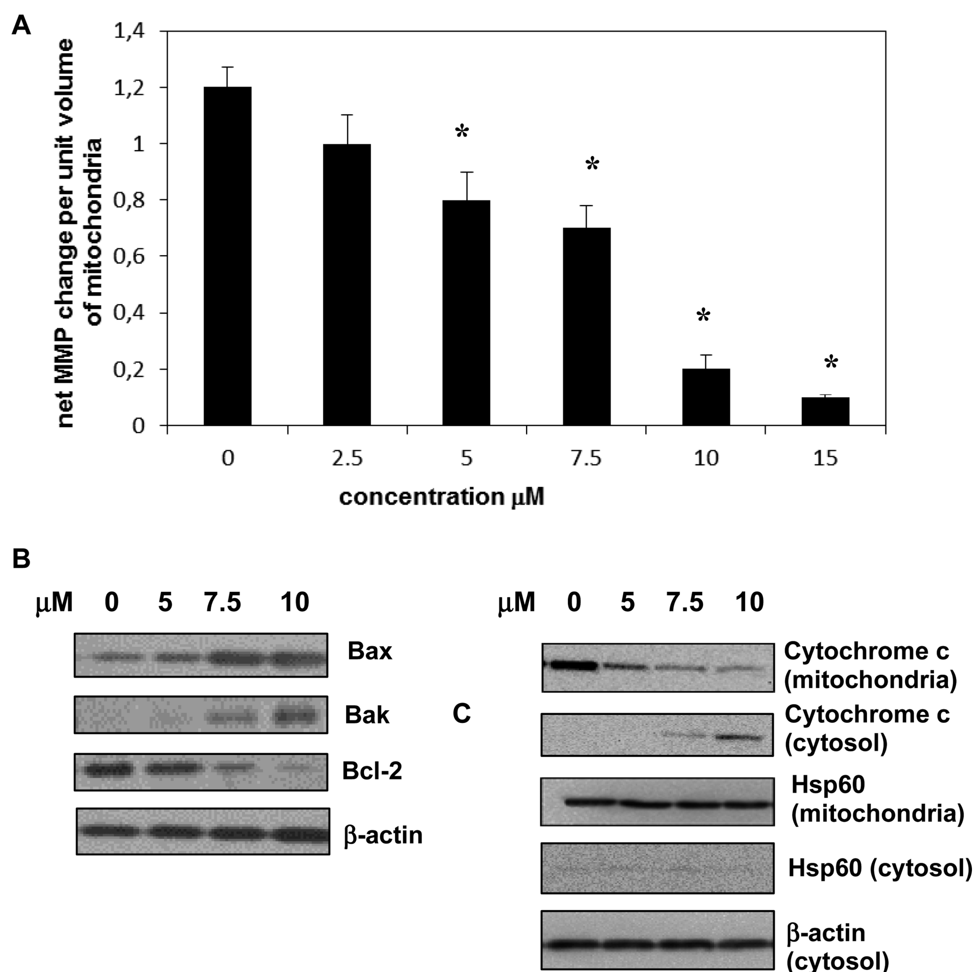

Figure 3. Induction of apoptosis by ramentaceone (**1**) via the mitochondrial-dependent pathway. (A) Concentration-dependent loss of mitochondrial membrane potential ($\Delta\Psi$m) in HL-60 cells induced by **1**. Cells were treated with various concentrations of **1** (0−15 μM) for 24 h and analyzed with flow cytometry using CMTMRos probes ($n = 3$). Data were analyzed by one-way ANOVA with Tukey's post hoc tests. [$p < 0.05$ (*) indicates differences between control and **1**-treated cells.] (B) Effects of **1** on the levels of Bax, Bak, and Bcl-2. (C) Effects of **1** on the levels of cytochrome *c* in the cytosol and mitochondria. HL-60 cells were treated with **1** (0−10 μM) for 24 h. Cell lysates were prepared and subjected to SDS-PAGE and immunoblotting using respective antibodies. Actin was used as a cytosolic marker and as a control for protein loading. Hsp60 was used as an indicator of the absence of mitochondrial contamination in the cytosolic fraction.

results suggest that **1** induces apoptosis in HL-60 cells through ROS signaling by targeting the mitochondrial pathway. Due to the involvement of ROS generation in the mechanism of **1**-induced cell death, future studies will help elucidate whether the observed effects are cell line-dependent and whether other cellular targets are involved in apoptosis induced by ramentaceone.

■ EXPERIMENTAL SECTION

General Experimental Procedures. Melting points were determined with a Buchi melting point apparatus (model B-545). NMR spectra were determined on a Bruker Avance 300 MHz spectrometer with TMS as internal standard. HPLC-ESI/MS analyses were performed using a Waters/Micromass (Manchester, UK) ZQ mass spectrometer coupled to a Waters (Milford, MA) model 2690 HPLC pump. A Superspher 100 RP-18 column (250 × 2 mm) was used.

All cell culture material was purchased from Gibco-Invitrogen (Paisley, UK). The plant extraction was carried out with a sonicator (model XL-2020; Misonix, Farmingdale, NY, USA). All other chemicals, if not indicated otherwise, were obtained from Sigma-Aldrich (St. Louis, MO, USA). The HL-60 (promyelocytic leukemia) cell line was procured from the Department of Pharmaceutical Technology and Biochemisty, Technical University of Gdańsk, Poland.

Plant Material. The source of ramentaceone (**1**) were 8-week-old *Drosera aliciae* plants cultured in vitro according to a previously published procedure.[31] The seeds of *D. aliciae*, from which in vitro cultures were established, were obtained from the International Carnivorous Plant Society Seed Bank (Pinole, CA, USA).

Extraction and Isolation. Dried and powdered plant material was sonicated at 50/60 Hz. Ultrasonic vibrations were transmitted to the solution in a pulsed mode (10 pulses per 5 s followed by a 5 s pause) for 30 min. Extracts were centrifuged at 13793*g* for 15 min, and the crude *Drosera* extract was evaporated to dryness (11 g) and dissolved in 100 mL of chloroform. The solution obtained was dried over coarse silica gel (20 g). Silica gel grains coated with the extract were loaded onto a silica gel column (150 g silica gel 60). Isolation of **1** was performed using a step gradient of methylene chloride in hexane from 50% to 100% of methylene chloride, with the concentration of methylene chloride changed in 10% steps every 100 mL, and 25 mL fractions being collected. Fractions containing **1** were combined and concentrated to dryness. The residue was dissolved in acetone and loaded to a LH-20 column. Fractions containing **1** were combined. The procedure was repeated twice. The total amount of the purified **1** obtained was 37 mg.

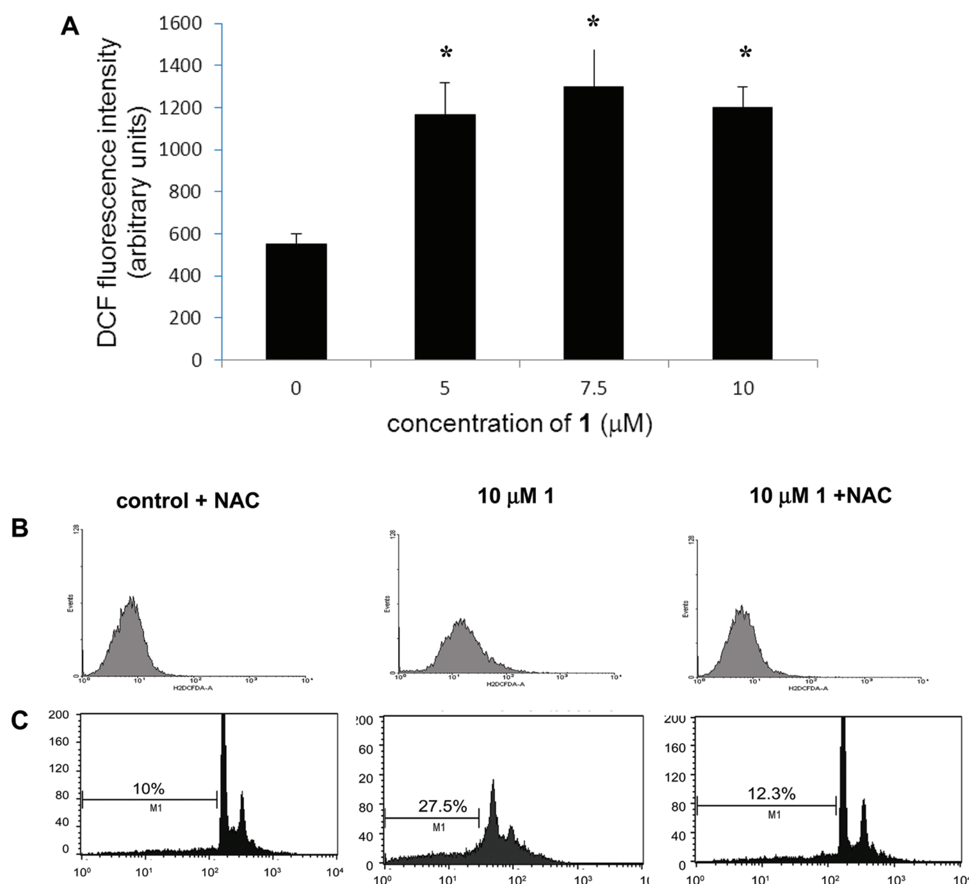

Figure 4. ROS-mediated induction of apoptosis by ramentaceone (**1**). (A) Generation of ROS in HL-60 cells treated with **1**. HL-60 cells were treated with **1** (0−10 μM) for 3 h, and ROS levels were detected with flow cytometry using H_2DCFDA probes (n = 3). Data were analyzed by one-way ANOVA with Tukey's post hoc tests. [$p < 0.05$ (*) indicates differences between control and **1**-treated cells.] (B) Effects of NAC on the generation of ROS induced by **1**. Histograms represent DCF fluorescence intensities. (C) Effects of NAC on the induction of cells in the sub-G1 fraction of the cell cycle in HL-60 cells by **1**. Histograms represent PI fluorescence intensities.

Ramentaceone (1): yellow needles; mp 126 °C; spectroscopic data comparable to literature values.[5,32]

Cell Culture. Cells were cultured in RPMI 1640 medium supplemented with 10% fetal bovine serum, 2 mM glutamine, 100 units/mL penicillin, and 100 μg/mL streptomycin. Cultures were maintained in a humidified atmosphere with 5% CO_2 at 37 °C in an incubator (Heraceus, Hera cell).

Cytotoxicity Assay. The viability of cells was determined using the MTT [(3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay. Cells were treated for 24 h with **1** (0−15 μM). Following treatment, MTT (0.5 mg/mL) was added directly to the medium, and cells were further incubated for 3 h at 37 °C, following lysis with DMSO. The optical density of the formazan solution was measured at 550 nm with a plate reader (Victor, 1420 multilabel counter).

Chromatin Condensation Analysis. For the presence of chromatin condensation and fragmentation, cells following a 24 h treatment with 10 μM **1** were washed with PBS and stained with Hoechst 33342 (5 μg/mL) and propidium iodide (PI, 10 μg/mL) for 10 min. Morphological analysis was performed under a fluorescence microscope (Nikon PE300).

Cell Cycle Analysis. To determine cell cycle distribution, HL-60 cells (5×10^5) were treated for 12 or 24 h with **1** in the concentration range 0−10 μM for the indicated times. After treatment, cells were collected and fixed in ice-cold 70% ethanol for 24 h. Cells were then washed in PBS, resuspended in 1 mL of PBS containing RNase (2 mg/mL) and propidium iodide (20 μg/mL), and incubated for 30 min at room temperature. Samples were analyzed with a flow cytometer (LSRII, Beckton Dickinson).

Measurement of Mitochondrial Membrane Potential. Mitochondrial membrane potential was analyzed by flow cytometry using Mito Tracker Orange (CMTMRos) (Molecular Probes). Cells were treated with **1** (0−15 μM) for 24 h, then incubated with CMTMRos (100 mM) for 30 min. The total volume of mitochondria per cell was assessed by preincubating cells with carbonyl cyanide-*m*-chlorylphenylhydrazone (CCCP, 100 μM) 5 min prior to staining with CMTMRos. The same procedure was performed for each concentration of **1**. Cells were collected, washed, and resuspended in PBS, after which they were analyzed by flow cytometry. The fluorescence intensities of the obtained values from cells prestained with CCCP were subtracted from those obtained from cells stained only with CMTMRos. The resulting value was divided by the fluorescence intensity values obtained from cells prestained with CCCP. The final value was defined as "net $\Delta\Psi m$".

Western Blot Analysis. HL-60 cells were treated with **1** (0−10 μM) for 24 h, after which cells were collected and lysed in a lysis buffer (20 mM Tris pH 7.5, 0.15 M NaCl, 1 mM EDTA, 1% NP-40, 1 mM DTT, 0.25 mM PMSF) for 30 min on ice. Lysates were then centrifuged for 30 min at 4 °C, and the supernatant was collected. Equal amounts of total proteins were resolved by SDS-PAGE and electroblotted onto PVDF membranes. The nonspecific binding sites were blocked with TBS buffer (500 mM NaCl, 20 mM Tris-HCl, pH 7.4) containing 5% nonfat dry milk for 1 h. The membranes were incubated overnight at 4 °C with specific primary antibodies, namely, anti-β-actin (1:1000) (Sigma), anti-Bax, anti-Bak, and anti-Bcl-2 (1:250) (Santa Cruz, Heildeberg, Germany). Membranes were further incubated at room temperature for 1 h with HRP-conjugated secondary antibodies (Santa Cruz, Heildeberg, Germany), and proteins were detected by chemiluminescence (ChemiDoc, Bio-Rad) with a HRP substrate (Pierce).

Preparation of Cytosolic and Mitochondrial Fractions. HL-60 cells following treatment with **1** (0−10 μM) were collected, and proteins of the mitochondrial and cytosolic fractions were isolated using the Cytochrome c Releasing Apoptosis Assay Kit (BioVision, Inc.). The levels of cytochrome *c* in the cytosolic and mitochondrial fractions were detected by Western blot analysis using anti-cytochrome *c* antibodies (1:1000) (BioVision, Inc.). Anti-Hsp60 antibodies (1:1000) (StressGen, Biotechnologies Corp., Victoria, Canada) were used as an indicator of the absence of mitochondrial contamination of the cytosolic fraction.

Caspase 3 Activity Analysis. Protease activity of caspase 3 was determined by measuring the rate of enzyme substrate Ac-DEVD-pNA (Asp−Glu−Val−Asp−*p*-nitroanilide) cleavage. HL-60 cells were treated with 10 μM **1** for various time periods in the range 0−24 h. Following incubation, cells were washed with PBS, collected, resuspended in a lysis buffer (50 mM HEPES, pH 7.4, 5 mM DTT, 0.1 mM EDTA, 0.05% Triton X-100), and kept on ice for 20 min. Protein concentrations were determined using the Bradford method. Cell lysates containing 20 μg of total protein were incubated with 200 μM DEVD-pNA substrate in a reaction buffer (50 mM HEPES, pH 7.4, 100 mM NaCl, 10 mM DTT, 1 mM EDTA, 10% glycerol). Following a 30 min incubation at 37 °C, the release of pNA was measured at 405 nm with a plate reader (Victor, 1420 multilabel counter).

Analysis of ROS Generation. Intracellular levels of ROS were measured by flow cytometric monitoring of H_2-DCFDA oxidation. Cells following treatment with **1** (0−10 μM) for 3 h were incubated with H_2-DCFDA (20 μM) for 30 min at 37 °C. NAC (15 mM), when used, was preincubated for 1 h with cells prior to the addition of **1**. Cells were collected, washed, and resuspended in PBS, then kept on ice for immediate detection with a flow cytometer (LSRII, Beckton Dickinson). The level of ROS generation is expressed as fluorescence intensity.

Statistical Analysis. Values are expressed as means ± SEM of at least three independent experiments. Differences between control and **1**-treated samples were analyzed by one-way ANOVA with Tukey's post hoc tests. Differences between 12 and 24 h incubations from the control and **1**-treated samples were analyzed by two-way ANOVA with Bonferroni post-tests. A p value of <0.05 was considered as statistically significant in each experiment.

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■ REFERENCES

(1) Juniper, B. E.; Robins, R. J.; Joel, D. M. *The Carnivorous Plants: Phytochemical Aspects*; Academic Press: London, 1989; pp 229−240.
(2) Sankaram, A. V. B.; Reddy, V. V. N.; Marthandamurthi, M. *Phytochemistry* **1986**, *25*, 2867−2871.
(3) Finnie, J. F.; van Staden, J. In *Biotechnology in Agriculture and Forestry*; Bajaj, Y. P. S., Ed.; Springer: Berlin, 1993; Vol. *24*, pp 164−177.
(4) Bapela, N. B.; Lall, N.; Fourie, P. B.; Franzblau, S. G.; Van Rensburg, C. E. *Phytomedicine* **2006**, *13*, 630−635.
(5) Mahapatra, A.; Mativandlela, S. P.; Binneman, B.; Fourie, P. B.; Hamilton, C. J.; Meyer, J. J.; van der Kooy, F.; Houghton, P.; Lall, N. *Bioorg. Med. Chem.* **2007**, *15*, 7638−7646.
(6) Marston, A.; Msonthi, J. D.; Hostettmann, K. *Planta Med.* **1984**, *50*, 279−280.
(7) Mebe, P. P.; Cordell, G. A.; Pezzuto, J. M. *Phytochemistry* **1998**, *47*, 311−313.
(8) Gu, J. Q.; Graf, T. N.; Lee, D.; Chai, H. B.; Mi, Q.; Kardono, L. B. S.; Setyowati, F. M.; Ismail, R.; Riswan, S.; Farnsworth, N. R.; Cordell, G. A.; Pezzuto, J. M.; Swanson, S. M.; Kroll, D. J.; Falkinham, J. O. 3rd; Wall, M. E.; Wani, M. C.; Kinghorn, A. D.; Oberlies, N. H. *J. Nat. Prod.* **2004**, *67*, 1156−1161.
(9) Wube, A. A.; Streit, B.; Gibbons, S.; Asres, K.; Bucar, F. *J. Ethnopharmacol.* **2005**, *102*, 191−196.
(10) Honn, K. V.; Tang, D. G.; Gao, X.; Butovich, I. A.; Liu, B.; Timar, J.; Hagmann, W. *Cancer Metastasis Rev.* **1994**, *13*, 365−396.
(11) Hanahan, D.; Weinberg, R. A. *Cell* **2000**, *100*, 57−70.
(12) Cicek, M.; Iwaniec, U. T.; Goblirsch, M. J.; Vrabel, A.; Ruan, M.; Clohisy, D. R.; Turner, R. R.; Oursler, M. J. *Cancer Res.* **2007**, *67*, 10106−10611.
(13) Kelly, M. P.; Lee, F. T.; Tahtis, K.; Smyth, F. E.; Brechbiel, M. W.; Scott, A. M. *Clin. Cancer Res.* **2007**, *13*, 5604−5612.
(14) Newman, D. J.; Cragg, G. M. *J. Nat. Prod.* **2007**, *70*, 461−477.
(15) Karbowski, M.; Spodnik, J. H.; Teranishi, M.; Wozniak, M.; Nishizawa, Y.; Usukura, J.; Wakabayashi, T. *J. Cell Sci.* **2001**, *114*, 281−91.
(16) Earnshaw, W. C.; Martins, L. M.; Kaufmann, S. H. *Annu. Rev. Biochem.* **1999**, *68*, 383−424.
(17) Junn, E.; Lee, K. N.; Ju, H. R.; Han, S. H.; Im, J. Y.; Kang, H. S.; Lee, T. H.; Bae, Y. S.; Ha, K. S.; Lee, Z. W.; Rhee, S. G.; Choi, I. *J. Immunol.* **2000**, *165*, 2190−2197.
(18) Li, Y.; Sun, X.; LaMont, J. T.; Pardee, A. B.; Li, C. J. *Proc. Natl. Acad. Sci. U. S. A.* **2003**, *100*, 2674−2678.
(19) Singh, F.; Gao, D.; Lebwohl, M. G.; Wei, H. *Cancer Lett.* **2003**, *200*, 115−121.
(20) Cenas, N.; Prast, S.; Nivinskas, H.; Sarlauskas, J.; Arner, E. S. *J. Biol. Chem.* **2006**, *281*, 5593−5603.
(21) Kawiak, A.; Piosik, J.; Stasilojc, G.; Gwizdek-Wisniewska, A.; Marczak, L.; Stobiecki, M.; Bigda, J.; Lojkowska, E. *Toxicol. Appl. Pharmacol.* **2007**, *223*, 267−276.
(22) Sandur, S. K.; Ichikawa, H.; Sethi, G.; Ahn, K. S.; Aggarwal, B. B. *J. Biol. Chem.* **2006**, *281*, 17023−17033.
(23) O'Brien, P. J. *Chem. Biol. Interact.* **1991**, *80*, 1−41.
(24) Bolton, J. L.; Trush, M. A.; Penning, T. M.; Dryhurst, G.; Monks, T. J. *Chem. Res. Toxicol.* **2000**, *13*, 135−160.
(25) Xu, K. H.; Lu, D. P. *Leuk. Res.* **2010**, *34*, 658−665.
(26) Kuo, P. L.; Hsu, Y. L.; Cho, C. Y. *Mol. Cancer Ther.* **2006**, *5*, 3209−3221.
(27) Wang, C. C.; Chiang, Y. M.; Sung, S. C.; Hsu, Y. L.; Chang, J. K.; Kuo, P. L. *Cancer Lett.* **2008**, *259*, 82−98.
(28) Tong, W. G.; Ding, X. Z.; Adrian, T. E. *Biochem. Biophys. Res. Commun.* **2002**, *296*, 942−948.
(29) Tong, W. G.; Ding, X. Z.; Witt, R. C.; Adrian, T. E. *Mol. Cancer Ther.* **2002**, *1*, 929−935.
(30) Vincent, C.; Fiancette, R.; Donnard, M.; Bordessoule, D.; Turlure, P.; Trimoreau, F.; Denizot, Y. *Leuk. Res.* **2008**, *32*, 1756−17562.
(31) Krolicka, A.; Szpitter, A.; Maciag, M.; Biskup, E.; Gilgenast, E.; Romanik, G.; Kaminski, M.; Wegrzyn, G.; Lojkowska, E. *Biotechnol. Appl. Biochem.* **2009**, *53*, 175−184.
(32) Kawiak, A.; Krolicka, A.; Lojkowska, E. *Biotechnol. Lett.* **2011**, *33*, 2309−2316.