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ORIGINAL PAPER

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Ritterazine B, a new cytotoxic natural compound, induces apoptosis in cancer cells

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Abstract *Purpose*: Ritterazine B, one of the ritterazine analogues extracted from *Ritterella tokioka*, has been shown to be chemically similar to cephalostatin 1, and among the ritterazine derivatives is the most cytotoxic to P388 murine leukemia cells. The objective of this study was to determine the cytotoxicity of ritterazine B to non-small-cell lung cancer (NSCLC) cells in vitro and its effects on the cell cycle and apoptosis. *Methods*: The cytotoxicity of ritterazine B against PC14 NSCLC cells was investigated using a 4-day MTT assay. Morphological changes in cells after exposure to this compound were evaluated by phase-contrast microscopy. The effects on the cell cycle of HL-60 leukemia cells and PC14 cells were elucidated by flow cytometry and an in vitro CDK/cyclin kinase assay. Induction of apoptosis in HL-60 cells was assessed using the TUNEL assay and Hoechst 33342 staining. In addition, molecules involved in apoptosis were evaluated by Western blotting. *Results*: Ritterazine B exerted strong cytotoxic effects against PC14 cells with a mean GI_{50} of 75.1 n*M*. Cell cycle analysis showed that ritterazine B caused accumulation of HL-60 and PC14 cells at the G2/M checkpoint. Furthermore, ritterazine B-treated HL-60 cells became multinucleated, and at a concentration of 20 n*M* this resulted in the onset of apoptosis. Neither cleavage of caspase target molecules nor phosphorylation of bcl-2 were observed in ritterazine B-treated HL-60 cells. *Conclusions*: These results indicate that ritterazine B might be a potent inducer of apoptosis acting via a novel antimitotic mechanism.

Keywords Ritterazine · Cell cycle arrest · Apoptosis · Caspase

Abbreviations *CDK* Cyclin-dependent kinase · *DMSO* Dimethylsulfoxide · *ECL* Enhanced chemiluminescence · *FITC* Fluorescein isothiocyanate · GI_{50} 50% growth inhibition · IC_{50} 50% enzyme inhibition · *MTT* 3-(4,5-Dimethylthiazol2-yl)-2,5-diphenyltetrazolium bromide · *NSCLC* Non-small-cell lung cancer · *PARP* Poly(ADP-ribose) polymerase · *PBS* Phosphate-buffered saline · *PI* Propidium iodide · *TUNEL* Terminal deoxynucleotidyl nick end-labeling

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Introduction

Much attention has been focused on natural products as potential sources of novel anticancer drugs over the decades [4]. The discovery of taxanes and camptothecins from natural sources has contributed to the recent progress in cancer treatment in the clinical setting. However, discovery of further active compounds from natural resources is needed to improve cancer chemotherapy.

Natural marine products have recently attracted attention because of their intriguing biological activities. For example, TZT-1027, which is now under clinical investigation, was synthesized from dolastatin 10, a marine product isolated from an Indian ocean sea hare *Dolabella auricularia* [20]. Another example is cephalostatin 1, isolated from an Indian ocean hemichordate *Cephalodiscus gilchristi*, which exhibits remarkable cytotoxic activity against P388 murine leukemia cells with GI_{50} values of 10^{-4}–10^{-6} ng/ml; however, the mechanism of action still remains unknown [8, 15].

In our search for cytotoxic substances originating from Japanese marine invertebrates, we have found that ritterazines extracted from *Ritterella tokioka* exert potent cytotoxic activities against P388 murine leukemia cells [6]. Among the ritterazine derivatives, we have found that ritterazine B exerts the most potent cytotoxic activity against P388 cells [7]. Ritterazines are dimeric steroidal alkaloids structurally related to cephalostatin 1. Furthermore, a COMPARE pattern-recognition analysis gave correlation coefficients of 0.93 between cephalostatin 1 and ritterazine B against NCI-10 cell lines, suggesting that ritterazine derivatives act by the same unknown mechanism as does cephalostatin 1 [12].

To evaluate the potential activity of ritterazine B, we examined the its cytotoxic effects in a NSCLC cell line, and its effects on the cell cycle and apoptosis in HL-60 leukemia cells. Ritterazine B showed potent cytotoxic activity against the NSCLC cell line, and cell death after exposure to ritterazine B was at least partly attributable to apoptosis, probably independently of caspase activation, and may affect cytokinesis. The increase in the G2/M population and multinucleated cells led us to consider that ritterazine B might be involved in cytokinesis.

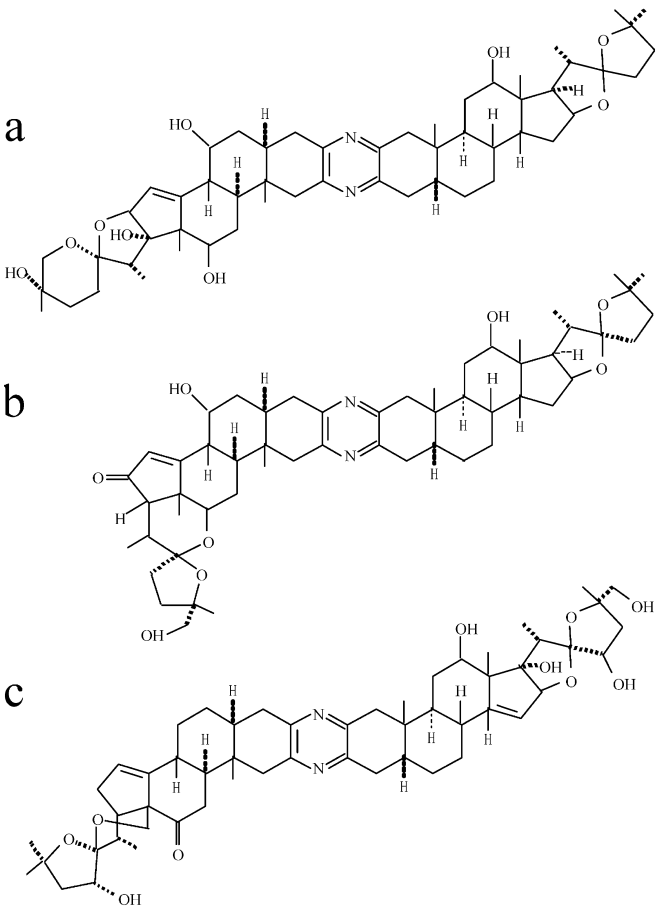

Fig. 1a–c Chemical structures of ritterazine B (**a**), compound 21 (**b**), and cephalostatin 1 (**c**)

Materials and methods

Cell culture

The NSCLC cell line PC14 was a generous gift from Dr. Nishio (National Cancer Center Research Institute, Tokyo). HL-60 (acute promyeloblastic leukemia) and PtK1 (normal kidney cells of the kangaroo rat *Potorous tridactylis*) were purchased from the American Type Culture Collection (Rockville, Md.). PC14 and HL-60 cells were cultured in RPMI-1640 medium (Sigma Chemicals, St Louis, Mo.) containing 10% fetal bovine serum and streptomycin (100 μg/ml) and penicillin (100 U/ml) in a humidified atmosphere containing 5% CO_2 at 37°C.

Materials

Ritterazine B and its less-active derivative compound 21 (Fig. 1) were isolated and purified as described previously. Stocks of these compounds were prepared at a concentration of 1 m*M* in DMSO, and stored at 70°C until use. Ritterazine B diluted in medium was used for the MTT assay and flow cytometric analysis at final concentrations ranging from 5 to 100 n*M*. Camptothecin, paclitaxel and jasplakinolide were purchased from Sigma Chemicals. Monoclonal anti-human PARP antibody (C2–10), anti-Bcl-2 antibody (clone 100, SC-509) and anti-caspase 3 antibody (clone 19) were purchased from BD Pharmingen (San Diego, Calif.), Santa Cruz Biotechnology (Santa Cruz, Calif.), and Transduction Laboratories (Lexington, Ky.), respectively. A 0.01% DMSO solution was used as the control for each experiment except for CDK inhibition assays, for which 10% DMSO was used.

Assessment of inhibition of cell growth and morphological changes

The growth-inhibitory effects of ritterazine B as well as other drugs on PC14 cells were evaluated using the MTT assay as described previously [21]. Cells were seeded into 96-well microplates (Corning, Corning, N.Y.) at a density of 500 or 1000 cells per well in culture medium. Drugs at various concentrations were added 24 h later. After the indicated times, cell viability was determined using the MTT (Sigma Chemicals) assay. Morphological changes in HL-60 and PC14 cells after treatment were evaluated by standard phase-contrast microscopy.

Cell cycle analysis

The effects of ritterazine B on the cell cycle were determined by flow cytometry. Briefly, cells exposed to ritterazine B were collected by centrifugation, washed twice with PBS, and then

resuspended in 0.5 ml PBS and fixed with 70% ethanol at 20°C for more than 24 h. Fixed cells were washed twice, resuspended in 0.5 ml PI/RNase solution (Phoenix Flow Systems, San Diego, Calif.) and incubated at room temperature for 30 min. The DNA content of the cells was determined by flow cytometry using a FACS Calibur system (Becton Dickinson, San Jose, Calif.) within 3 h [10]. CellQuest (Becton Dickinson) and ModFit (Verity Software House, Topsham, Me.) software was used.

CDK inhibition assays

The In vitro inhibitory effects of ritterazine B and its less-active analogue, compound 21 against CDK/cyclins were determined as described previously [11]. Briefly, baculovirus-expressed human CDK4/cyclin D1, CDK2/E, CDK2/A and CDC2/A complexes were mixed with the compounds in 40 µl kinase buffer and incubated at 30°C for 30 min. The kinase reaction was started by adding 400 ng glutathione S-transferase-retinoblastoma protein and 5 µCi [γ-^{32}P]ATP to the mixture and incubating at 30°C for 15 min. The labeled retinoblastoma protein was immunoprecipitated, and electrophoresed on 10% NuPAGE Bis-Tris gels (NOVEX, San Diego, Calif.), and detected by autoradiography.

Detection of apoptosis

TUNEL assay

The TUNEL assay was carried out using an APO-BrdU kit (BD Pharmingen). Untreated or drug-treated cells were collected, washed, and resuspended in 0.5 ml PBS. Cells were fixed by adding 5 ml 1% paraformaldehyde/PBS for 30 min on ice. Cells were centrifuged, washed, resuspended in 0.5 ml PBS, and fixed by adding 5 ml 70% ethanol. Fixed cells were centrifuged, washed, resuspended in TdT enzyme and TdT buffer at 37°C for 1 h. Subsequently, FITC-labeled Br dUTP was added followed first by incubation at room temperature for 30 min in the dark, then by incubation with 0.5 ml PI/RNase solution at room temperature for 30 min in the dark. Finally, the cells stained with two colors were analyzed by flow cytometry using a FACS Calibur system (Becton Dickinson).

Hoechst 33342 staining

Drug-treated, unfixed HL-60 cells were stained with Hoechst 33342 (10 µ*M*, Molecular Probes, Eugene, Ore.) and analyzed under a non-confocal fluorescence microscope (Nikon Model Eclipse E800) with excitation at 360 nm (UV) [9]. Images were captured with a digital camera and obtained with the ×20 objective.

Western blotting

Western blotting for PARP was performed to detect its cleavage due to caspase 3 activation as described elsewhere [17]. Drug-treated cells were collected by centrifugation, washed with PBS, resuspended in NP40 lysis buffer (50 m*M* Tris-HCl, pH 8.0, 150 m*M* NaCl, 1 m*M* phenylmethylsulfonyl fluoride, 1% NP40), disrupted by sonication on ice and then centrifuged at 15,000 rpm for 30 min at 4°C. A total of 50 µg protein was separated on 3–8% NuPAGE Tris-acetate gels (NOVEX, San Diego, Calif.), and electroblotted onto a nitrocellulose membrane. The membranes were blocked with TBS-T/5% blocking agent (Amersham Pharmacia Biotech, Arlington Heights, Ill.), exposed to a monoclonal antihuman PARP antibody at a dilution of 1:1000, followed by a horseradish peroxidase-conjugated goat anti-mouse secondary antibody (Amersham Pharmacia Biotech) at a dilution of 1:1500. Blotted bands were detected by an ECL assay (Amersham Pharmacia Biotech) according to the manufacturer's instructions. Similarly, degradation of procaspase 3 and phosphorylated Bcl-2 were evaluated after electrophoresis on 12% Tris-glycine gels followed by immunoblotting with anti-caspase 3 and anti-Bcl-2 antibodies and visualized with an ECL assay.

Fluorescence staining for β-actin

PtK1 cells were seeded in chambered coverglasses (Nalge Nunc International, Naperville, Ill.) at a density of 2×10^4/ml, cultured for 3 days, and then exposed to ritterazine B or jasplakinolide at a concentration of 100 n*M* for 24 h. The coverglasses were washed twice with PBS, fixed with methanol at 20°C for 15 min, and permeabilized with acetone for 1 min. The cells were then washed twice, stained with 1 ml FITC-conjugated anti-β actin monoclonal antibody (Sigma Chemicals), and diluted at 1:250 with PBS at room temperature for 1 h in the dark. The coverglasses were washed twice, mounted on a slide with Slowfade antifade reagent (Molecular Probes, Eugene, Ore.) and examined under a Nikon Modl Eclipse E800 microscope [1].

Results

Cytotoxicity of ritterazine B

The cytotoxicity of ritterazine B against the NSCLC cell line was determined using a 4-day MTT assay (Table 1). Ritterazine B exerted potent growth-inhibitory activity against PC14 cells in a dose-dependent manner, with the GI_{50} being 75.1 n*M*. By comparison, the GI_{50} values of anticancer agents currently in use clinically range from 0.24 to 5.8 µ*M*, indicating that the cytotoxic activity of ritterazine B against PC14 cells was almost equipotent to those of commercially available anticancer agents.

Accumulation of HL-60 and PC14 cells at the G2/M checkpoint of the cell cycle following exposure to ritterazine B

After exposure of cells to ritterazine B at various concentrations for 24 h, significant changes were observed in the cell cycle distribution (Fig. 2). Flow cytometric analysis revealed that the percentage of cells staying at the G2/M checkpoint was significantly increased in both cell lines, while the percentage HL-60 cells in the G1 phase was decreased. Following treatment with 20 n*M* ritterazine B for 48 h, HL-60 cells underwent cell death as determined morphologically (data not shown). To determine the mechanism of cell cycle arrest, we inves-

Table 1 Growth inhibition of PC14 cells by anticancer drugs

Drug	GI_{5O} (µ*M* ± SD)
Cisplatin	5.8 ± 10.85
Irinotecan	5.1 ± 0.96
Doxorubicin	0.24 ± 0.17
Paclitaxel	5.4 ± 1.3
Ritterazine B	0.075 ± 0.0063

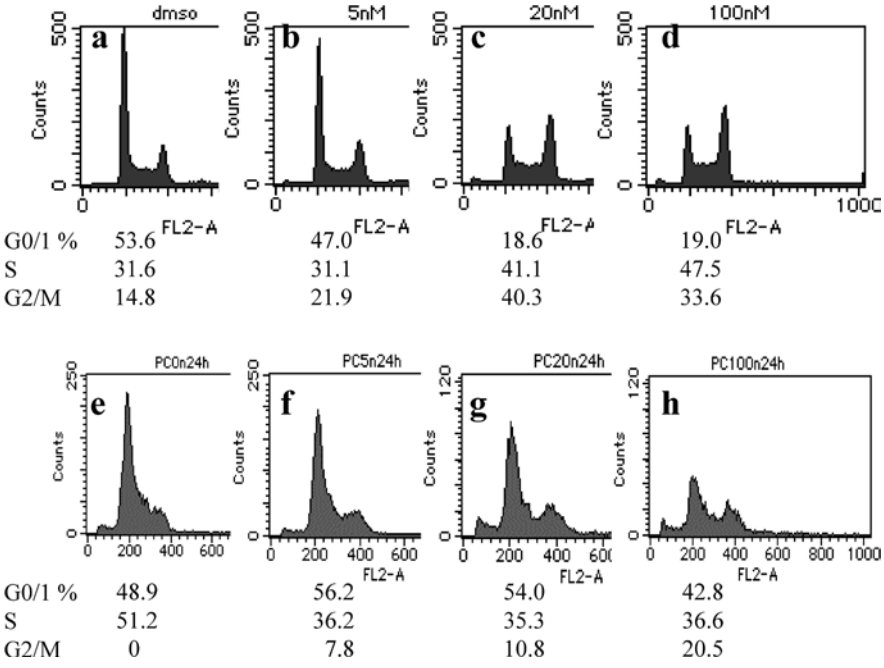

Fig. 2a–h Cell cycle analysis of HL-60 cells (**a–d**) and PC14 cells (**e–h**) 24 h after exposure to ritterazine B (**a**, **e** controls; **b**, **f** 5 n*M*; **c**, **g** 20 n*M*; **d**, **h** 100 n*M*). Ritterazine B treatment led to a significant increase in the G2/M population in both cell lines

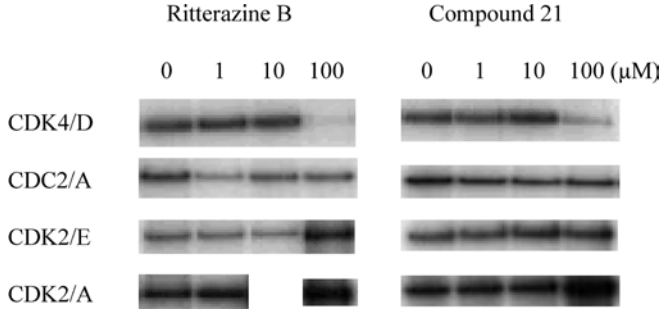

Fig. 3 CDK inhibition assays in vitro. The in vitro inhibitory effects of ritterazine B and the less-active derivative compound 21 against CDK/cyclins were investigated with baculovirus-expressed human CDK4/cyclin D1, CDK2/E, CDK2/A and CDC2/A complexes at concentrations in the range 1–100 μ*M*. Both ritterazine B and compound 21 moderately inhibited CDK4/D

tigated whether ritterazine B would exhibit CDK inhibitory activity in vitro. Both ritterazine B and its less-active analogue, compound 21, slightly inhibited CDK4/D1 with IC_{50} values of 80 and 70 μ*M*, respectively (Fig. 3). Neither compound inhibited CDK2/A, CDK2/E or CDC2/A even at concentrations up to 100 μ*M*.

Induction of apoptosis in HL-60 and PC14 cells by ritterazine B via morphological changes

In ritterazine B-treated PC14 and HL-60 cells, multinuclei were observed by phase contrast microscopy. Although typical apoptotic changes such as apoptotic bodies were not observed, Hoechst 33342 staining of HL-60 cells after ritterazine B treatment for 24 h disclosed extensive nuclear condensation and fragmentation (Fig. 4). Furthermore, in the TUNEL assay, a 24-h exposure to ritterazine B resulted in an increase in the percentage of TUNEL-positive cells in a time- and dose-dependent manner (Fig. 5). These findings indicated that cell death caused by ritterazine B treatment was mainly due to apoptosis.

Caspases are not activated in ritterazine B-treated HL-60 cells

To elucidate whether apoptosis would be affected by caspase activation, Western blot analysis was performed on PARP and pro-caspase 3 as the known reference substrates for caspases. Neither PARP nor pro-caspase 3 was cleaved or degraded in ritterazine B-treated HL-60 cells irrespective of the conditions (Fig. 6), indicating that caspase activation would not be required for ritterazine-mediated cell death. In addition, Western blot analysis for bcl-2 showed no evidence that phosphorylated bcl-2 was increased in ritterazine B-treated HL-60 cells.

An increase in the G2/M population and in the incidence of multinuclei in treated cells suggested that ritterazine B might arrest cells during cytokinesis. Accordingly, we investigated the possible influence of ritterazine B on the actin cytoskeleton, one of the major components involved in cytokinesis in PtK1 cells. However, fluorescence staining of PtK1 cells preincubated with ritterazine B did not show the appearance of F-actin, indicating disrupted actin formation; incidentally, however, a similar phenomenon was also observed in jasplakinolide-treated cells (data not shown).

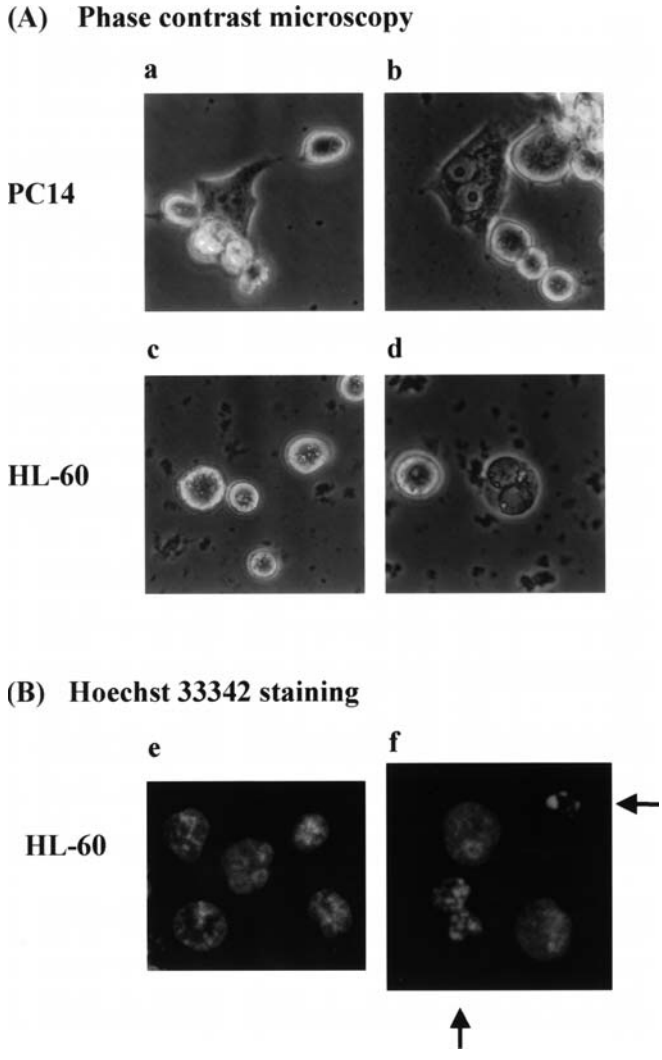

Fig. 4 **A** Phase contrast microscopy of PC14 (*a*, *b*) and HL-60 cells (*c*, *d*) 24 h after exposure to ritterazine B (*a*, *c* controls; *b* 200 n*M*; *d* 20 n*M*). **B** Hoechst 33342 staining of HL-60 cells (*e*, *f*) 24 h after exposure to ritterazine B (*e* control, *f* 20 n*M*; *arrows* apoptotic cells)

Discussion

The present study demonstrated that ritterazine B was a potent cytotoxic anticancer drug against NSCLC cells. In PC14 NCSLC cells, the MTT assay showed that the average GI_{50} of ritterazine B was 75.1 n*M*, indicating that this drug is as potent as many drugs currently used clinically. Death of HL-60 cells after treatment with ritterazine B was mainly due to apoptosis, which was confirmed by TUNEL and Hoechst 33342 staining. Apoptosis induced by ritterazine B appeared to be independent of the caspase pathway because neither cleavage nor degradation of caspase targets was observed. Although CDK4/cyclin D is known to be the major G1-regulating CDK/cyclin complex, the IC_{50} value inhibiting CDK4/cyclin D is approximately 1000 times the GI_{50} in cancer cells. Furthermore, cell cycle analysis revealed that ritterazine B kept the exposed cells at the G2/M checkpoint rather than in the G1 phase, thereby arresting many cells prior to the cytokinesis process.

The G2/M checkpoint is considered to be regulated by several molecules including tubulins. These molecules have been reported to play a role as the targets of drug-induced G2/M block. Of these targets, microtubules have been the most extensively investigated molecules, and are the targets of taxanes and vinca alkaloids. However, ritterazine B appears unlikely to act on microtubules because induction of phospho-Bcl-2 protein, a hallmark of tubulin-targeting drugs such as taxanes, was not observed [13]. Destruction of the actin cytoskeleton may also be responsible for drug-induced G2/M block mainly due to cytokinesis block. Several actin-targeting drugs such as jasplakinolide have been recently described [1]. Although we investigated the influence of ritterazine B on the actin cytoskeleton of PtK1 cells following findings indicative of cytokinesis block in ritterazine B-treated cells, fluorescence staining of PtK1 cells incubated with ritterazine B revealed no changes in the actin cytoskeleton (data not shown).

Other regulators of G2/M progression have been verified as the targets of several anticancer drugs. For example, cucurbitacin E [5], derived from plants with medicinal properties known since antiquity, has been identified as a sterol with potent growth-inhibitory activity against prostate carcinoma explants, besides being known to induce disruption of the vimentin as well as the actin cytoskeleton. Interestingly, cucurbitacin E potently inhibits proliferating human endothelia as compared to quiescent cells in vitro, implying its potential activity as an antiangiogenic agent. Also, inhibitors of kinesin motors known to regulate mitosis have been isolated [16]. By phenotype-based screening, Mayer et al. identified a small molecule inhibitor of mitotic spindle bipolarity, which was named monastrol [14]. Monastrol does not disrupt microtubules, actin cytoskeleton or chromosomes, but specifically inhibits the motility of the mitotic kinesin Eg5. Furthermore, several upstream molecules of CDC2/cyclin B involved in regulation of G2/M progression have been identified; accordingly, they might be candidate targets. Tamura et al. have reported that several small molecule inhibitors of Cdc25 phosphatases could block G2/M progression via negatively regulating CDC2/cyclin B [18, 19]. Also, inhibition of Chk1 by UCN-01 has been reported to cause G2 abrogation following cytotoxic chemotherapy [2, 3]. These novel molecules involved in G2/M progression still remain to be investigated in ritterazine B-treated cells.

The present study demonstrates that ritterazine B has cytotoxic activity against a human NSCLC cell line and can induce apoptosis via unknown antimitotic mechanisms. Further studies are needed to develop this encouraging drug for clinical use.

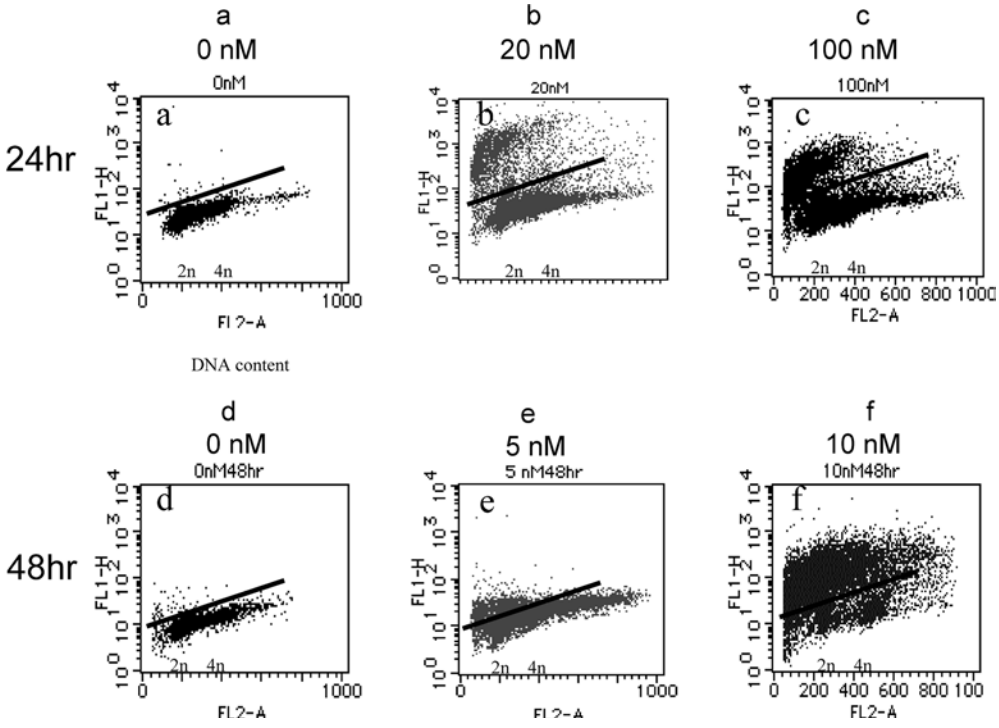

Fig. 5a–f TUNEL assays by flow cytometry. HL-60 cells were treated with 0–100 n*M* ritterazine B for 24 h (**a–c**) or 48 h (**d–f**), fixed and subjected to the TUNEL assay. Fluorescein intensity (TUNEL) is on the *y*-axis; PI intensity (DNA content) is on the *x*-axis. Cells whose TUNEL intensity was above the bars were considered TUNEL-positive

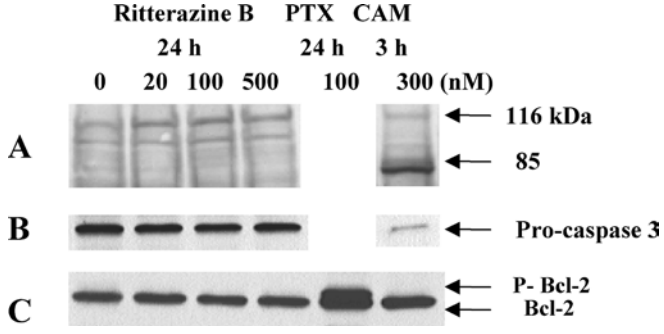

Fig. 6a–c Western blotting for apoptosis targets. Protein lysates from HL-60 cells treated with ritterazine B for 24 h were examined for cleaved bands of PARP (**a** 85 kDa), degradation of pro-caspase 3 (**b**), and phosphorylation of Bcl-2 (**c**). Neither PARP nor pro-caspase 3 was inactivated under any ritterazine B treatment condition (*PTX* paclitaxel, *CAM* camptothecin)

References

1. Bai R, Verdier-Pinard P, Gangwar S, Stessman CC, McClure KJ, Sausville EA, Pettit GR, Bates RB, Hamel E (2001) Dolastatin 11, a marine depsipeptide, arrests cells at cytokinesis and induces hyperpolymerization of purified actin. Mol Pharmacol 59:462
2. Bunch RT, Eastman A (1996) Enhancement of cisplatin-induced cytotoxicity by 7-hydroxystaurosporine (UCN-01), a new G2-checkpoint inhibitor. Clin Cancer Res 2:791
3. Busby EC, Leistritz DF, Abraham RT, Karnitz LM, Sarkaria JN (2000) The radiosensitizing agent 7-hydroxystaurosporine (UCN-01) inhibits the DNA damage checkpoint kinase hChk1. Cancer Res 60:2108
4. Cassady JM (1990) Natural products as a source of potential cancer chemotherapeutic and chemopreventive agents. J Nat Prod 52:23
5. Duncan KLK, Duncan MD, Alley MC, Sausville EA (1996) Cucurbitacin E-induced disruption of the actin and vimentin cytoskeleton in prostate carcinoma cells. Biochem Pharmacol 52:1553
6. Fukuzawa S, Matsunaga S, Fusetani N (1995) Isolation and structure elucidation of ritterazines B and C, highly cytotoxic dimeric steroidal alkaloids, from the tunicate Ritterella tokioka. J Org Chem 60:608
7. Fukuzawa S, Matsunaga S, Fusetani N (1997) Isolation of 13 new ritterazines from the tunicate Ritterella tokioka and chemical transformation of ritterazine B. J Org Chem 62:4484
8. Guo C, LaCour TG, Fuchs PL (1999) On the relationship of OSW-1 to the cephalostatins. Bioorg Med Chem Lett 9:419
9. Hamatake M, Iguchi K, Hirano K, Ishida R (2000) Zinc induces mixed types of cell death, necrosis, and apoptosis, in molt-4 cells. J Biochem (Tokyo) 128:933
10. Hirano T, Oka K, Mimaki Y, Kuroda M, Sashida Y (1996) Potent growth inhibitory activity of a novel Ornithogalum cholestane glycoside on human cells: induction of apoptosis in promyelocytic leukemia HL-60 cells. Life Sci 58:789
11. Kubo A, Nakagawa K, Varma RK, Conrad NK, Cheng JQ, Lee WC, Testa JR, Johnson BE, Kaye FJ, Kelley MJ (1999) The p16 status of tumor cell lines identifies small molecule inhibitors specific for cyclin-dependent kinase 4. Clin Cancer Res 5:4279
12. LaCour TG, Guo C, Ma S, Jeong JU, Boyd MR, Matsunaga S, Fusetani N, Fuchs PL (1999) On topography and functionality in the B-D rings of cephalostatin cytotoxins. Bioorg Med Chem Lett 9:2587
13. Lazo JS, Tamura K, Vogt A, Jung JK, Rodriguez S, Balachandran R, Day BW, Wipf P (2001) Antimitotic actions of a novel analog of the fungal metabolite palmarumycin CP1. J Pharmacol Exp Ther 296:364
14. Mayer TU, Kapoor TM, Haggarty SJ, King RW, Schreiber SL, Mitchison TJ (1999) Small molecule inhibitor of mitotic spindle bipolarity identified in a phenotype-based screen. Science 286:971
15. Pettit GR, Inoue M, Kamano Y, Herald DL, Arm C, Dufresne C, Christie ND, Schmidt JM, Doubek DL, Krupa TS (1988) Isolation and structure of the powerful cell growth inhibitor cephalostatin 1. J Am Chem Soc 110:2006

16. Sakowicz R, Berdelis MS, Ray K, Blackburn CL, Hopmann C, Faulkner DJ, Goldstein LSB (1998) A marine natural product inhibitor of kinesin motors. Science 280:292
17. Shapiro GI, Koestner DA, Matranga CB, Rollins BJ (1999) Flavopiridol induces cell cycle arrest and p53-independent apoptosis in non-small cell lung cancer cell lines. Clin Cancer Res 5:2925
18. Tamura K, Rice RL, Wipf P, Lazo JS (1999) Dual G1 and G2/M phase inhibition by SC-alpha alpha delta 9, a combinatorially derived Cdc25 phosphatase inhibitor. Oncogene 18:6989
19. Tamura K, Southwick EC, Kerns J, Rosi K, Carr BI, Wilcox C, Lazo JS (2000) Cdc25 inhibition and cell cycle arrest by a synthetic thioalkyl vitamin K analogue. Cancer Res 60:1317
20. Watanabe J, Natsume T, Fujio N, Miyasaka K, Kobayashi M (2000) Induction of apoptosis in human cancer cells by TZT-1027, an antimicrotubule agent. Apoptosis 5:345
21. Yoshida M, Suzuki T, Tsurutani J, Komiya T, Hatashita E, Nishio K, Nakagawa K, Fukuoka M (2001) Induction of MRP5/SMRP mRNA by adriamycin exposure and its over-expression in human lung cancer cells resistant to adriamycin. Int J Cancer 94:432