Heterocyclic Organobismuth(III) Compound Targets Tubulin to Induce G₂/M Arrest in HeLa Cells

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Abstract. Our previous study showed that organobismuth compounds induce apoptosis in human promyelocytic leukemia cells, although solid tumor cell lines were relatively resistant. Herein, we investigated the primary cellular target of these compounds in HeLa cells. One organobismuth compound, bi-chlorodibenzo[c,f][1,5]thiabismocine (compound 3), arrested the cell cycle at G₂/M as assessed by flow cytometry and by upregulating the expression of cyclin B1. At a low concentration (0.5 μM), compound 3 caused cell cycle arrest at the mitotic phase and induced apoptosis. At a higher concentration (>1.0 μM), it induced an arrest in the G₂/M phase, leading to apoptosis. In many cells blocked at the M phase, the organization of microtubules was affected, indicating depolymerization of the microtubule network. Western blotting demonstrated that compound 3 depolymerized microtubules similar to colchicine and nocodazole. Experiments in vitro also showed that compound 3 inhibited the assembly of purified tubulin in a concentration-dependent manner by interacting with the colchicine-binding site of tubulin through its SH groups. Heterocyclic organobismuth compounds are novel tubulin ligands.

Keywords: bismuth, tubulin, cell death, mitotic arrest, anticancer

Introduction

Our previous studies showed that heterocyclic organobismuth(III) compounds have antibacterial activity and can inhibit the growth of tumor cells (1, 2). The compounds induced apoptosis via mitochondrial perturbation in human promyelocytic leukemia cells (HL-60) (2). Bismuth belongs to group V of the periodic table along with arsenic and antimony and is recognized as a low-toxic metal. Traditionally, inorganic salts of bismuth have been used in medicine and veterinary practice (3, 4). Organobismuth compounds have antifungal and antimicrobial activity (1, 5). We have found that heterocyclic organobismuth compounds have potent antiproliferative effects on leukemic cell lines. One of them, bi-chlorodibenzo[c,f][1,5]thiabismocine (compound 3), induced apoptosis in HL-60 cells through the activation of caspase, production of ROS, and perturbation of mitochondria; and at high doses, it induced necrosis (2). Anticancer activities of metal and metal compounds have been reported (4, 6, 7), suggesting our bismuth compounds to be useful as chemotherapeutic reagents against tumors.

The microtubular network is an intracellular filamentous structure in almost all eukaryotic cells. Microtubules function in a number of cellular processes such as mitosis by regulating the migration of chromosomes, intracellular transport, the maintenance of cell morphology, and signal transduction. Numerous anti-tumor drugs have been known to target microtubules (8, 9), and heavy metal compounds (e.g., As, Cd, Co, Cr, Ni) have also been shown to damage microtubules (10). Arsenite, a trivalent inorganic arsenical, has been used as a therapeutic agent for the treatment of acute promyelocytic leukemia resistant to all-trans retinoic acid (11 – 14). This anticancer efficiency was extended to many solid tumors (15). Several reports have shown that arsenic compounds induce mitotic arrest and apoptosis in...
various cancer cells (16–19). In addition, arsenic compounds either enhance or inhibit the polymerization of tubulin and perturb spindle dynamics (16, 18, 20–22). Studies have shown that arsenite-induced mitotic arrest is one of the mechanism of apoptosis in cancer cells (19) suggesting arsenite to be an effective anti-cancer drug. However, the chronic toxicity and carcinogenicity of arsenic trioxide has hampered its acceptance as a first-choice drug (2, 23). In contrast to arsenic, bismuth is recognized as a low-toxic metal in spite of its heavy metal status in the nitrogen family, and bismuth compounds have also been reported as low-toxic compounds (3).

The viability of solid tumor cell lines was significantly impaired by treatment with heterocyclic organobismuth(III) compounds (2). How the specific damage to these cells by the heterocyclic organobismuth(III) compounds leads to cell death is poorly understood. Thus in this study, to obtain insight into the mechanism of biological action against a solid tumor cell line, the HeLa cell line, we examined how cell death occurs by focusing on the cell cycle arrest induced by compound 3.

Materials and Methods

Materials and chemicals
CytoDYNAMIX Screen01 (CDS01) and porcine tubulin (T240) were purchased from Cytoskeleton (Denver, Colorado, USA). Annexin V-HiLyte FluorTM 488 was from AnaSpec International (San Jose, CA, USA). All other chemicals were obtained from Sigma-Aldrich (St. Louis, MO, USA) or Wako Chemicals (Osaka) and were of the highest quality commercially available.

Heterocyclic organobismuth compound
The organobismuth compound was synthesized as previously described (1). The compound used in this study is bi-chlorodibenzo[c,f][1,5]thiabismocine (compound 3) (Fig. 1).

Cells and culture
HeLa (ATCC) cells were cultured in DMEM (Nissui Seiyaku, Tokyo) supplemented with 10% fetal bovine serum and kanamycin (Meiji Seika Co., Tokyo) at 37°C with 5% CO₂.

Measurement of the cytotoxicity of heterocyclic organobismuth compound 3 based on the formation of colonies
The cells were seeded in 35-mm plastic culture dishes. After 24 h, various concentrations of compound 3 were added. After 18 h of culture with compound 3, washed cells were re-seeded in 60-mm dishes and cultured in the growth medium for 10–14 days. The number of colonies formed was counted after staining with 1% methylene blue. Clusters of 40 or more cells were considered colonies. The number of colonies formed in the untreated control was defined as 100% colony formation.

Mitotic index
After treatment, the cells were washed with ice-cold PBS and fixed with 1% glutaraldehyde. The cells were washed with PBS and stained with Hoechst 33258. Mitotic cells were counted under a fluorescence microscope. Cells were examined when the nucleus exhibited typical mitotic features such as condensed chromosome.

Flow cytometric analysis
Measurements of DNA content and cell cycle analysis were performed as described (2). HeLa cells treated with compound 3 were detached and harvested by trypsinization followed by centrifugation and then washed with PBS. The cells were fixed with ice-cold 70% ethanol and stored at −20°C until used. To examine the cell cycle, the stored cells were pelleted, washed with PBS, resuspended in PBS containing 0.5 mg/ml of RNase A, and incubated at 37°C for 20 min. Cells were then pelleted, resuspended in PBS containing 50 μg/ml of propidium iodide, and incubated at 4°C for 10 min in the dark. Finally, the stained cells were analyzed with a Becton-Dickinson FACSCalibur flow cytometer. A minimum of 10,000 cells/samples were analyzed. Data were collected and analyzed using CellQuest software.

Assessment of apoptosis by annexin-V staining and propidium iodide exclusion assay
Apoptotic cells were detected by annexin-V staining and propidium iodide exclusion as described previously (2), with a slight modification. After drug treatment, HeLa cells were washed in PBS and resuspended in a staining solution containing annexin V-HiLyte FluorTM 488 (0.5 μg/ml) in HEPES buffer (10 mM HEPES pH 7.5, 140 mM NaCl, and 2.5 mM CaCl₂). After a 15-min incubation in the dark at room temperature, the cells were washed in HEPES buffer. Finally, the cells were stained with 0.5 μg/ml of propidium iodide and

Fig. 1. Structure of the heterocyclic organobismuth compound 3.
analyzed by a Becton-Dickinson FACSCalibur flow cytometer. Cells with intact membranes excluded PI and were counted as viable.

**Western blot analysis**

The preparation of protein lysates as well as Western blot analysis was performed as described (2, 24). The primary antibodies used were anti-cyclin B1 (sc-752; Santa Cruz Biotechnology, Inc., Santa Cruz, CA, USA), anti-actin antibody (BT-560; Biomedical Technologies, Stoughton, MA, USA), and anti-α-tubulin (CL9002T; Cosmo Bio, Tokyo). The secondary antibodies used were goat anti-mouse conjugated–AP antibody (AM13705; Biosource, Camarillo, CA, USA) or goat anti-rabbit conjugated–AP antibody (4050-04; Southern Biotechnology Associates, Birmingham, AL, USA).

**Immunocytochemistry**

Staining of microtubules was performed as reported (25). Briefly, fixed cells were incubated with anti-tubulin antibody (65-095; ICN Biomedicals, Inc., Costa Mesa, CA, USA) for 1 h at room temperature. The cells were then incubated with Alexa-488–conjugated anti-rabbit antibody (A11008; Molecular Probes, Eugene, OR, USA) for 1 h at room temperature. For staining the DNA, the cells were incubated with 1 μg/ml of DAPI for a few minutes. Cells were observed with a fluorescence microscope. Immunofluorescence microscopy was conducted using an Olympus BX51 (Olympus Optical Co., Tokyo), and images were captured by an Olympus DP72 Microscope Digital Camera and the DP controller software. The fragmentation of the golgi complex was evaluated by immunocytochemistry using anti-p138 antibody (25) and Alexa-488 conjugated anti-mouse antibody (A11001, Molecular Probes).

**Assay of microtubule assembly in vitro**

The separation of polymerized tubulin from tubulin dimers and analysis of the effect of compound 3 on the polymerization of tubulin in intact cells were performed as described previously (26). HeLa cells were treated with the indicated concentrations of test agents for 5 h, washed with PBS, and then added to lysis buffer containing 20 mM Tris-HCl, pH 6.8, 1 mM MgCl₂, 2 mM EGTA, 1 mM GTP, and 5% (v/v) glycerol and 100 μl (300 μg) was pipetted into the designated wells of a half-area 96-well plate prewarmed to 37°C. Each compound tested was made up in G-PEM buffer. The assay was conducted at 37°C, and tubulin polymerization was followed at 340 nm in a Multickan microplate reader (Thermo Labsystems, Franklin, MA, USA). The increase in absorbance was measured at 340 nm at 37°C and recorded every 30 s for 20 min.

**Titration of sulfhydryl groups**

The sulfhydryl-specific reagent 5,5′-dithiobis-2-nitrobenzoic acid (DTNB) forms complexes with thiol groups of tubulin and can be used to monitor conformational changes of tubulin due to the binding of ligands (27). Porcine tubulin (50 μg/ml) was incubated with compound 3 or colchicine at 37°C for 60 min and then 0.6 mM DTNB was added. The kinetics of the modification of sulfhydryl groups was monitored calorimetrically at 405 nm using a Perkin-Elmer ARVOMX Multilabel Microplate Counter (Wellesley, MA, USA).

**Measurement of the binding of colchicine to tubulin**

The binding of colchicine to tubulin was measured using colchicine-fluorescence induced upon the binding of colchicine to tubulin (28). Porcine tubulin (1 mg/ml) was incubated with compound 3 at 37°C for 30 min. They 25 μM colchicine was added. The fluorescence of the tubulin-colchicine complex was measured using the Perkin-Elmer ARVOMX Multilabel Microplate Counter with 355 nm as the excitation wavelength and 460 nm as the emission wavelength.

**Statistical analyses**

Statistical analyses were performed with Excel 2003 using Student’s t-test.

**Results**

**Heterocyclic organobismuth(III) compound 3 induced G₂/M arrest followed by apoptosis in HeLa cells**

As we previously showed, the IC₅₀ of compound 3 (see Fig. 1) for the viability of HeLa cells was estimated to be 4.8 μM using the MTT assay (2). On the other hand, when the cytotoxic effect of compound 3 was
assessed using the colony formation assay, it was found that exposure to the compound resulted in a significant reduction in the number of colonies formed (Fig. 2) beyond the extent predicted based on the MTT assay. This observation suggests that most cells undergo a progressive death process even after the removal of compound 3.

Treatment with compound 3 remarkably increased the number of round-shaped cells. As mitotic cells usually show similar morphological changes, to examine whether compound 3 is a mitotic inhibitor, a mitotic index in cells treated with the compound was established. As shown in Fig. 3, after 18 h, compound 3 at 0.5 μM significantly induced mitotic arrest. However, at a higher concentration, 1.0 μM, it did not cause mitotic accumulation. Then, to further examine the effect of compound 3, the treated cells were subjected to a flow cytometric (FACS) analysis.

As compared to the control asynchronous culture, treatment with 0.5 μM compound 3 caused an accumulation of G2/M phase cells with a concomitant loss of G1 phase cells (Fig. 4A). In addition, 0.5 μM compound 3 caused a G1-M regulatory protein, cyclin B1, to accumulate, further indicating cell cycle arrest at G2/M (Fig. 4B). Although the mitotic index of cells treated with 1.0 μM compound 3 was decreased to the level of that for cells treated with 0.25 μM compound 3 (Fig. 3), exposure to 1.0 μM compound 3 resulted in the accumulation of cyclin B1 (Fig. 4B), indicating that most of the 1.0 μM compound 3–treated cells with 4C DNA shown in Fig. 4A were in the G2/M boundary. Therefore, it has been suggested that the treatment of cells with a high concentration of compound 3 (1.0 μM) either slows the move from the G1 phase or arrests the cells at the G2/M boundary.

It has been reported that various antimitotic reagents

![Fig. 2. Effect of compound 3 on the ability of HeLa cells to form colonies. HeLa cells were cultured for 18 h in the absence or presence of organobismuth compounds. The number of cells that were able to form colonies was determined as described in Materials and Methods. Results are presented as a percentage of colony formation relative to the control. Each point is the mean ± S.D. of three determinations.](image)

![Fig. 3. Quantitative assessment of the mitotic arrest by compound 3. HeLa cells were treated with various concentrations of compound 3 for 18 h. Cells were fixed with glutaraldehyde and stained with Hoechst 33258, and mitotic cells were counted among at least 200 cells. The data each represent the mean ± S.D. of triplicate measurements. *P<0.05, ***P<0.001, as compared with untreated cells.](image)

![Fig. 4. Effect of compound 3 on the cell cycle in HeLa cells. A: Flow cytometric analysis of the DNA content of HeLa cells untreated or treated with the indicated concentrations of compound 3. HeLa cells treated with compound 3 for 18 h were trypsinized and fixed in 70% ethanol, stained with propidium iodide, and analyzed using a flow cytometer. B: Accumulation of cyclin B1 in HeLa cells treated with the indicated concentrations of compound 3. Cell extracts were prepared from HeLa cells after treatment with compound 3 for 18 h. Protein was resolved using SDS-PAGE, transferred onto a PVDF membrane, and probed with anti-cyclin B antibody. The data presented are representative of those obtained in three independent experiments.](image)
and arsenite induce mitotic arrest and then apoptosis (16–20, 29–31). Treatment of log-phase HeLa cells with 0.5 μM compound 3 significantly increased the number of mitotic cells in a time-dependent manner until 18 h of treatment (Fig. 5A). Apoptotic cells with characteristic features were rare at 18 h, although they began to appear later (>24 h) with the concomitant loss of mitotic cells. As shown in Fig. 5B, a similar decrease in G2/M phase cells was observed in the FACS analysis after 24 h of culture, and at the same time, an increase in cells with a sub-G1 DNA content was recognized. This hypodiploid DNA content was characteristic of apoptotic cells, suggesting that apoptosis began in HeLa cells following the arrest at G2/M by 0.5 μM compound 3. Then, HeLa cells were treated with compound 3 for 36 h and the percentage of apoptotic cells was determined using Annexin V-HiLyte Fluor™ 488 and propidium iodide. Apoptotic cells were recorded as late or early apoptotic cells, which are shown, respectively, in the upper right and lower right quadrants of the FACS histogram in Fig. 6. The total percentage of apoptotic cells after treatment with compound 3 was as follows: 5.7% (control), 43.6% (0.5 μM compound 3), and 56.6% (1.0 μM compound 3). These results indicate that both 0.5 and 1.0 μM compound 3 induced apoptosis in HeLa cells.

Compound 3 caused disruption of the cellular microtubule network

Antimicrotubule agents that target the cellular microtubule network are known to result in an aberrant formation of the mitotic spindle, subsequent blockage of the cell cycle in the G2/M phase, and apoptotic cell death (29, 32). Because compound 3 caused G2/M arrest and apoptosis, we examined whether it affects the organization of the microtubule network in HeLa cells. In the cells treated with compound 3, the antibody fluorescence of microtubules was dispersed throughout the cytoplasm because the microtubule network was lost (Fig. 7: E and F), while in the untreated control cells, the microtubule network traversed intricately (Fig. 7A).

![Fig. 5. Time-dependent change in the cell cycle distribution. A: Time-dependent mitotic arrest induced by 0.5 μM compound 3. HeLa cells were incubated with 0.5 μM compound 3 for the period indicated. A mitotic index was determined as described in Materials and Methods. The data each represent the mean ± S.D. of triplicate measurements. **P<0.01, ***P<0.001, as compared with untreated cells. B: Time course of the effect of 0.5 μM compound 3 on the cell cycle in HeLa cells. Cells were treated with 0.5 μM compound 3 for the period indicated. The cell cycle analysis was performed as described in Materials and Methods.](image)

The effect of compound 3 on the microtubule network was similar to that of nocodazole or colchicine (Fig. 7: C and D), but differed from that of taxol which acts by stabilizing the microtubule network, resulting in the appearance of long polymerized microtubule bundles in the cytoplasm (Fig. 7B).

![Fig. 6. Assessment of apoptosis in compound 3-treated HeLa cells. Apoptosis was assessed by annexin-V-HiLyte Fluor™ 488 staining of HeLa cells treated with compound 3 for 36 h. Cells were double stained with annexin-V- HiLyte Fluor™ 488 and propidium iodide and analyzed by flow cytometry.](image)
Exposure of HeLa cells to drugs such as nocodazole, colchicine, and vinblastine leads to the disassembly of microtubules and disorganization of the Golgi complex in which most typically a dispersion of the stacks of cisternae throughout the cytoplasm occurs (33). Because the organization of the microtubule network in HeLa cells was less visible, we next examined whether the Golgi complex was disorganized in the compound 3–treated HeLa cells in order to confirm the results shown in Fig. 7. As shown in Fig. 8, C and D, compound 3 caused the fragmentation of the Golgi complex into elements randomly distributed throughout the cytoplasm, like the microtubule-disorganizing agent nocodazole (Fig. 8B).

**Effect of compound 3 on the polymerization of tubulin in vitro**

Because compound 3 markedly disrupted the cellular microtubule network, we then tested whether it directly affects the organization of tubulin. The effect of compound 3 on the assembly of tubulin subunits into microtubules in vitro was measured based on changes in turbidity produced upon tubulin’s polymerization. In this assay, taxol strongly promoted the polymerization of tubulin into microtubules, whereas nocodazole inhibited it. Compound 3, like nocodazole, inhibited the polymerization of tubulin effectively (Fig. 9A). As shown in Fig. 9B, compound 3 inhibited the polymerization of tubulin in a dose-dependent manner.

This inhibitory effect on the polymerization of microtubules in vitro suggested that like nocodazole, compound 3 significantly changes the tubulin polymer/monomer ratio in cells. To test this possibility, insoluble and soluble tubulins were separated from extracts of HeLa cells treated with different concentrations of compound 3 and a quantitative immunoblot analysis was performed. Extracts prepared from HeLa cells treated with taxol or nocodazole were used for comparison. As shown in Fig. 9, C and D, the insoluble tubulin accounted for 32.1%, 71.2%, and 9.7%, respectively, of all cellular tubulin in the control cells, cells treated with taxol, and cells treated with nocodazole.
The proportion of insoluble tubulin in cells treated with 0.25, 0.5, or 1.0 μM compound 3 was 33.3%, 20.2%, and 24.0%, respectively, indicating that the cellular microtubules were inclined to undergo depolymerization, producing monomeric tubulin, on treatment with compound 3. The total (P + S) tubulin level in cells treated with 1.0 μM compound 3 was apparently lower than that in control cells (Fig. 9C). This decline in the level of tubulin may have been due to either progress in the apoptotic pathway or a decline in tubulin synthesis because previous studies have reported that antimicrotubule agents at higher concentrations cause the total cellular amount of tubulin to decrease due to a decline in tubulin synthesis (34, 35) and another study found that tubulin is degraded during arsenic trioxide–induced apoptosis (36).

**Compound 3 interacts with tubulin’s sulfhydryl groups**

The modification of one or two sulfhydryl groups of tubulin completely inhibits microtubule polymerization (37). As shown in Fig. 10A, the mitotic arrest by compound 3 was significantly decreased by the addition DTT, a reducing agent with 2 SH/molecules, suggesting that DTT competes with compound 3 to bind the sulfhydryl groups of tubulin. To test this possibility, the inhibitory effect of compound 3 on the accessibility of cysteine following chemical modification by the sulfhydryl-specific reagent DTNB was examined. Since the sulfhydryl groups of tubulin appear to be located in regions important for polymerization, changes in the chemical reactivity of these residues could be a measure of conformational change. In this experiment, inhibition of the accessibility of DTNB to monomeric tubulin was examined in the absence of GTP. Figure 10B shows the reaction kinetics for the titration of cysteine in tubulin with DTNB in the absence or presence of compound 3. Compound 3 apparently reduced the initial rate at which sulfhydryl residues were modified and the number of titratable cysteine residues at equilibrium.

**Compound 3 interacts with the colchicine-binding site of tubulin**

Compound 3 provided significant protection to cysteine residues against DTNB (Fig. 10B). Colchicine also induced a conformational change in tubulin and protected one sulfhydryl group from reactions with other sulfhydryl reagents (27, 38). Colchicine exhibits marked fluorescence in combination with tubulin (28). Then, we used the fluorescence of the tubulin-colchicine complex to determine whether compound 3 interfered with the colchicine-binding site of tubulin. As shown in Fig. 10C, compound 3 inhibited the development of fluorescence.
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This result, together with those described above, indicates that compound 3 could interact with the colchicine-binding site of β-tubulin, resulting in the depolymerization of microtubules in HeLa cells.

Discussion

We demonstrated that a heterocyclic organobismuth compound has anticancer activity against HeLa cells and evaluated the mechanism of action. The tubulin system was identified as a molecular target of the organobismuth compound. Drugs affecting tubulin–microtubule equilibrium, for example, taxol, docetaxel, vinblastine, vincristine, and vinorelbine, are effective anticancer agents. Antimitotic drugs have not only been established as efficacious in anticancer therapies, but also provided opportunities for new applications and developments. We did not observe any significant damage of normal tissues of mice treated with compound 3 at 0.13 mg/kg, intravenously, every other day for a month. Therefore our heterocyclic organobismuth compounds may be prospective lead compounds for anticancer drugs.

We showed that compound 3 arrested the proliferation of HeLa cells, a human solid tumor cell line, at G2/M and induced apoptosis, suggesting a mechanism of antiproliferative activity similar to that of other microtubule-interacting agents that interfere with the formation of mitotic spindles either by increasing the stability of microtubules or through the depolymerization of microtubules. These agents can cause arrest at the prometaphase/metaphase to anaphase transition known as the mitotic spindle checkpoint, eventually leading to apoptosis (40). Mitotic spindle checkpoint proteins, such as BubR1, are involved in the mitotic arrest by nocodazole and PBOX (41). These results imply that 0.5 μM compound 3 induced apoptosis via this checkpoint.

The percentage of cells with hypodiploid DNA increased from 1.1% in untreated control cells to 8.2% in cells treated with 1.0 μM compound 3 for 18 h (Fig. 4A). In another experiment involving a FACS analysis of Annexin V, a marker of apoptotic cells, the percentage of Annexin V–positive cells increased from 5.7% (control) to 43.6% (0.5 μM compound 3) (Fig. 6). The proportion of annexin V-positive cells further increased to 56.6% when HeLa cells were treated with 1.0 μM compound 3 for 36 h, although Figs. 3 and 4 indicated that most of the cells did not enter the M phase. These HeLa cells were not viable at all (Fig. 2). Furthermore, this concentration of compound 3 disrupted the microtubule network and reduced the amount of tubulin in HeLa cells (Figs. 7 and 9), induced the dispersion and fragmentation of Golgi complexes (Fig. 8), and pre-
vented the polymerization of tubulin (Fig. 9). These findings may indicate that at high concentration, other targets of compound 3 act synergistically with tubulin to cause a rapid apoptosis that is induced directly and not via the arrest of the cell cycle at the mitotic phase. Similarly, HL-60 cells treated with a high dose of compound 3 underwent apoptosis without mitotic arrest (2). Our unpublished results also suggested that 1.0 μM compound 3 induced both the production of ROS and activation of caspases without causing mitotic arrest in HeLa cells. Tubulin is distributed in mitochondrial membranes and plays a role in apoptosis via interaction with voltage-dependent anion channels, the main component of permeability transition pores (42). Therefore, organobismuth compounds may also target the tubulin in mitochondrial membranes, resulting in the entering of apoptosis from a non-mitotic phase.

In previous studies, although nocodazole and vinca alkaloids also induced mitotic arrest at lower concentrations, these antimicrotubule agents induced a p53-independent p21\textsuperscript{waf/cip1}-associated G\textsubscript{1} and G\textsubscript{2} arrest at higher concentrations (43). Therefore, 1.0 μM compound 3 may induce G\textsubscript{1} and G\textsubscript{2} phase arrest concerned with p21\textsuperscript{waf/cip1} in HeLa cells, a p53-inactivated cell line. Recent reports showed that arsenic trioxide arrests cells at G\textsubscript{2} and induces apoptosis accompanied by the depletion of GSH (44, 45). Therefore, the cell cycle arrest in the late G\textsubscript{2} phase by 1.0 μM compound 3 may be due to the depletion of GSH. Indeed, the intracellular GSH levels in HeLa cells treated with compound 3 were markedly decreased (data not shown).

Although antimitotic compounds have been used clinically to treat neoplastic diseases, a major drawback is loss of efficacy over time because of the development of resistance. Therefore, it is important to develop novel microtubule inhibitors that overcome various modes of resistance and have improved pharmacological profiles. In general, metal compounds exhibit effective antitumor activities with a wide range of mechanisms and thus are regarded as a valuable source of novel chemotherapeutic reagents. Compound 3 is a novel microtubule polymerization inhibitor. Many of the physiological activities found here are shared by inorganic trivalent arsenicals, but differences exist in their effects on cells. Arsenite has been reported to enhance the polymerization of tubulins at a low concentration (18, 21, 46), whereas compound 3 inhibited microtubule polymerization. Despite its toxicity and carcinogenicity, arsenic is approved as a chemotherapeutic agent for the treatment of acute promyelocytic leukemia. However, its chronic toxicity and carcinogenicity have hampered its acceptance as a first-choice drug (47). Bismuth is much less toxic than arsenic in spite of its heavy metal status in the nitrogen family, and bismuth inorganic compounds have also been reported to be low-toxic compounds (3).

We have also examined the anti-proliferative activity of other heterocyclic organobismuth compounds: N-tert-butyl-bi-chlorodibenzo [c,f][1,5] azabismocine (compound 1) and bi-chlorophenothiabismin-5,S,dioxide (compound 5) (see ref. 1) and found differences in cytotoxicity and inhibitory effects on the polymerization of tubulin.

In conclusion, organobismuth compounds are novel microtubule polymerization inhibitors that may be utilized as antimitotic agents and in the treatment of refractory acute promyelocytic leukemia in place of arsenic trioxide.

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