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G2 arrest and apoptosis by 2-amino-*N*-quinoline-8-yl-benzenesulfonamide (QBS), a novel cytotoxic compound

Yun-Hee Kim [a], Kum-Joo Shin [a], Taehoon G. Lee [b], Euikyung Kim [a], Myoung-Shik Lee [c], Sung Ho Ryu [a], Pann-Ghill Suh [a,*]

[a] *Department of Life Science, Division of Molecular and Life Science, Pohang University of Science and Technology, San 31 Hyojadong, Nam-Gu, Pohang, Kyungbuk 790-784, Republic of Korea*
[b] *SIGMOL Inc., Pohang 790-784, Republic of Korea*
[c] *Department of Medicine, Samsung Medical Center, Sungkyunkwan University School of Medicine, 50 Irwon-dong, Kangnam-ku, Seoul 135-710, Republic of Korea*

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Abstract

We screened a library of 11,000 small molecular weight chemicals, looking for compounds that affect cell viability. We have identified 2-amino-*N*-quinoline-8-yl-benzenesulfonamide (QBS) as a potent cytotoxic compound that induces cell cycle arrest and apoptosis. Treatment of Jurkat T cells with QBS increased the levels of cyclin B1 as well as phosphorylated-cdc2, which was accompanied by reduced activity of cdc2 kinase, suggesting that QBS may induce cell cycle arrest at G2 phase. Structural analogues of QBS also exhibited similar effects on cell cycle progression and cell viability. Long-term treatment with QBS resulted in DNA fragmentation, cytochrome C release, and PARP cleavage, and an increase in the number of subdiploidy cells, indicative of cellular apoptosis. Moreover, QBS-induced apoptosis was blocked by z-VAD-fmk, a pan-caspase inhibitor. These results suggest that QBS is a novel and potent compound that induces G2 arrest and subsequent apoptosis, implicating it as a putative candidate for chemotherapy.

Keywords: Library screening; Quinolinesulfonamide; DNA damage; G2 arrest; Apoptosis

1. Introduction

Combinatorial chemistry technology has generated large numbers of chemical libraries. It has allowed many pharmaceutical companies and academic institutions to perform random-screening of chemical agents that act on a variety of target biological process, and chemical screening has ushered an era in development of new therapeutic strategies [1,2].

It has been repeatedly demonstrated that apoptosis is the mode of tumor cell death as a result of chemotherapy or radiation. Therefore, there have been many trials to develop new compounds that induce apoptosis and/or inhibit cell proliferation, in an effort to block cancer cell proliferation [3]. Apoptosis is an active process of cell death in response to environmental signals or intrinsic factors. Characteristically, it results in numerous cellular changes such as membrane blebbing, cell shrinkage, nuclear condensation, DNA fragmentation and formation of apoptotic bodies [4]. Apoptotic processes lead to the activation of caspase 3, which induces proteolytic cleavage of its substrates. Poly(ADP-ribose)-polymerase (PARP), one of the specific substrates of caspase-3, is involved in DNA repair and genomic maintenance. The cleavage of PARP has been recognized as a typical evidence for apoptosis after exposure of tumor cells to chemotherapeutic agents [5].

Several DNA-damaging stimuli, such as chemotherapeutic drugs or ionizing radiation affect cell cycle progression, which often results in apoptosis [6,7]. Numerous studies have shown that cell death by apoptosis follows p53-dependent or independent cell cycle arrest at G1 and

Abbreviations: QBS, 2-amino-*N*-quinoline-8-yl-benzenesulfonamide; PARP, poly(ADP-ribose)-polymerase; QBS-1, *N*-8-quinolinyl-benzenesulfonamide; PQS, *N*-phenyl-4-quinolinesulfonamide; QBS-2, *N*,4-dimethyl-*N*-8-quinolinylbenzenesulfonamide; QBS-3, 4-methyl-*N*-4-quinolinylbenzenesulfonamide; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide; Cyt C, cytochrom C

* Corresponding author. Tel.: +82 54 279 2293; fax: +82 54 279 0645.
E-mail address: pgs@postech.ac.kr (P.-G. Suh).

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G2/M phases after DNA damage [7–10]. Treatment of mammalian cells with DNA-damaging agents often leads to a prolonged delay in the G2 phase of the cell cycle. During normal cell cycle progression, inhibitory phosphorylations of cdc2 kinase occur at Thr14 or Tyr 15 [11], and dephosphorylations at these residues are required for activation of cdc2 kinase. However, DNA damage induced by a variety of different conditions results in the accumulation of the inactive Tyr15 phosphorylated-cdc2 complex, thereby preventing entry into mitosis [12]. Cells arrested at the G2 phase allow a time to repair DNA damage or give an opportunity to block cell cycle progression permanently at the G2 phase. G2 arrest can be monitored by the maintenance of cyclin B1 in the cytoplasm. When normal cells enter mitosis, the subcellular localization of the cyclin B1-cdc2 complex is suddenly changed, and cyclin B1 translocates from the cytosol to the nucleus at the end of the G2 phase. However, DNA damage stabilizes the cytoplasmic localization of cyclin B1 [13–17].

In this report, we selected 2-amino-*N*-quinoline-8-yl-benzenesulfonamide (QBS), by random chemical library screening in an effort to discover an anti-cancer agent against leukemia, and further characterized the mechanism underlying the QBS-induced decrease in cell viability. Benzenesulfonamide and quinoline, the constitutional groups of QBS (Fig. 1A), have been suggested to be the functional elements enabling various biological activities of QBS, such as inhibition of tumor cell growth and proliferation via cell cycle arrest or inactivation of carbonic anhydrase [18–23]. Nevertheless, it has not been reported so far whether quinolinesulfonamide derivatives induce G2 arrest and apoptosis. Here, we provide evidence that QBS and its analogues are effective chemical agents inducing G2 arrest and subsequent apoptosis in Jurkat cells. Since QBS induces cell cycle arrest at G2 phase and apoptosis, it might be applied as a putative therapeutic agent against cancer.

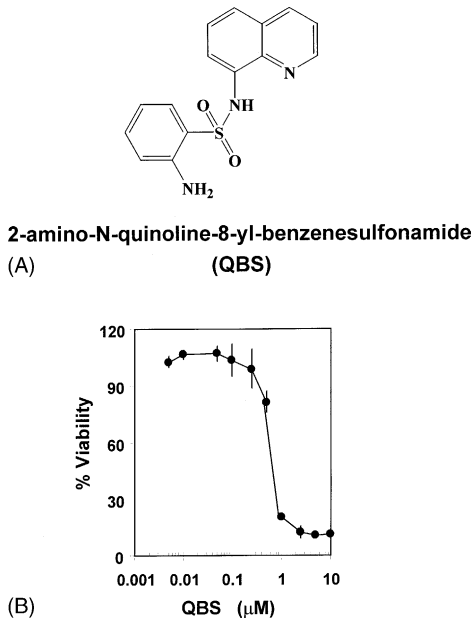

Fig. 1. QBS (2-amino-*N*-quinoline-8-yl-benzenesulfonamide) induces cell death in Jurkat T cells. (A) Chemical structure of QBS. (B) Jurkat cells (2×10^4 cells/well) were left untreated or treated with various concentrations (0.005–10 μM) of QBS for 48 h. Cell viability was determined by MTT assay and was corrected by subtracting the 0 h values. Results are presented as a percentage of control cell viability. These results are representative of three independent experiments.

2. Materials and methods

2.1. Chemicals

Chemical library composed of 11,000 compounds and analogues of QBS were purchased from Chembridge Corporation and Chemdiv Incorporation, respectively. Chemicals were dissolved in DMSO at 10 mM and further diluted with culture media. The stock solution was kept at −20 °C and freshly diluted to the desired concentration just before use.

2.2. Cell culture and reagents

Jurkat, acute T lymphoma cell line was obtained from ATCC and grown in RPMI1640 medium supplemented with 10% fetal calf serum (FBS) (Biowhitaker) and antibiotics (Gibco BRL) in a humidified incubator at 37 °C in 5% CO_2. The caspase inhibitor, z-VAD-fmk was purchased from Enzyme Systems. All other chemicals were obtained from Sigma.

2.3. Cell viability measurement

To determine the effect of various chemicals on cell viability, cells (2×10^4 cells/ml) were seeded in 96-well plates and treated with the chemicals in RPMI1640 medium containing 2% FBS, at the concentrations as indicated. For inhibition of apoptosis, cells were pretreated with a pan-caspase inhibitor, z-VAD-fmk (30 μM) 30 min prior to QBS treatment. DMSO (0.1%, final concentration) was treated as a vehicle control. After treatment with the chemicals, MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide, 0.5 mg/ml) was added, and cells were incubated at 37 °C for 2 h in a CO_2 incubator. After centrifugation at 2000 rpm for 10 min, supernatant was carefully removed and DMSO was added. Absorbance was measured at 570 nm in a microplate reader (BIO-TEK).

2.4. DNA ploidy analysis

Cells were suspended in phosphate-buffered saline (PBS) containing 5 mM EDTA and fixed in 100% ethanol. RNase A (2 μg/ml) was added to the suspended cells, and the cells were incubated at room temperature for 30 min. Then, propidium iodide (50 μg/ml) was added before reading. DNA contents of the cells were analyzed on a

FACScan flow cytometer (Becton Dickinson), which was also used to determine the percentage of cells in the different phases of the cell cycle.

2.5. Morphological analysis of apoptotic cells

Morphological changes in the nuclear chromatin of cells undergoing apoptosis were detected by staining with 2 μg/ml Hoechst 33342 fluorochrome (Molecular Probe), followed by examination under a fluorescence microscope (Karl Zeiss).

2.6. Caspase-3 activity assay

Jurkat cells were harvested and sonicated. Following centrifugation at 15,000 rpm for 10 min, 20 μg protein of supernatant in buffer containing 100 mM Hepes, 10% sucrose, 5 mM dithiothreitol, 10^{-6}% NP-40, and 0.1% CHAPS (pH 7.25) was added to each well of a 96-well plate with 50 μM DEVD-aminomethylcoumarin (AMC). After incubation at 37 °C for 1 h, the cleaved free AMC (excitation of 355 nm, emission of 460 nm) was detected using a fluorometer.

2.7. Immunoblotting

Cells were lysed in lysis buffer (50 mM Tris–Cl, pH 8.0, 150 mM NaCl, 0.02% sodium azide, 0.1% SDS, 1% NP-40, 1 mM PMSF). Equal amount of protein in each sample were separated by SDS–polyacrylamide gel electrophoresis and transferred to Hybond ECL nitrocellulose membranes. The membrane was blocked with 5% skim milk, and sequentially incubated with primary and secondary antibodies (mouse anti-cyclinB1, Santa Cruz; mouse anti-cdc2, New England Biolabs; rabbit anti-phospho-cdc2, New England Biolabs).

2.8. Cyclin B1-associated cdc2 kinase activity assay

Cells were washed in ice-cold PBS and lysed on ice in lysis buffer (40 mM Tris–Cl, pH 7.5, 120 mM NaCl, 0.1% NP-40, 1 mM PMSF, 10 μg/ml aprotinin, 1 μg/ml leupeptin, 1 mM Na3VO4, 10 mM NaF). Lysates were incubated with a monoclonal mouse anti-cyclin B1 (Santa Cruz) antibody for 2 h at 4 °C and protein G-Sepharose slurry (Sigma) was added. The mixture was incubated for 2 h at 4 °C. For kinase assay, the immunecomplexes were washed three times in lysis buffer and then twice in the assay reaction buffer, containing 25 mM Tris–HCl, pH 7.5, and 10 mM $MgCl_2$. The immunoprecipitates were incubated with 2 μg of histone H1 (Roche) in 20 μl of reaction buffer and 2 μCi of [^{32}P] ATP for 30 min at 37 °C. The reaction was terminated by addition of SDS loading buffer. The mixture obtained was boiled for 5 min, loaded onto a 10% SDS polyacrylamide gel and autoradiographed.

2.9. Immunofluorescence staining

Jurkat cells were fixed in 4% paraformaldehyde for 30 min at room temperature. Fixed cells were permeabilized in 0.1% Triton X-100 and 0.1% sodium citrate for 3 min at 4 °C and then sequentially incubated with mouse anti-cytochrome C antibody (Becton Dickinson), biotinylated anti-mouse IgG, streptavidin-FITC and 3 μg/ml of Hoechst 33342 (Molecular Probe). Stained cells were examined under a fluorescence microscope (Karl Zeiss).

In staining of cyclin B1, the nuclei of cells was stained with propidium iodide (2 μg/ml) after treatment with RNase A (2 μg/ml) for 30 min. Stained cells were observed under a confocal microscopy (LSM510, Karl Zeiss).

2.10. Comet assay

The comet assay was performed under alkaline conditions based on the procedure reported by Singh et al. [24,25]. Briefly, 85 μl of 0.8% of normal agarose (NA) was added on a microscope slide pre-layered with 1.5% of NA. Cell suspension (3×10^4 cells) was mixed with 75 μl of 0.5% of low melting point agarose (LMPA) kept at 37 °C and added onto the microscope slide. After the top layer of agarose was solidified, the slides were immersed in lysis solution(2.5 M NaCl, 100 mM EDTA, 10 mM Tris, pH 10, to which 1% Triton X-100 and 10% DMSO, pH 10) for 1 h at 4 °C in the dark. The slides were then covered with fresh alkaline buffer (1 mM EDTA and 300 mM NaOH, pH 13) in a horizontal electrophoresis unit. The cells were then exposed to the alkaline conditions for 20 min to allow DNA unwinding, and exposure of single-strand breaks and alkali-labile sites. Next, the DNA was electrophoresed at 25 V/300 mA (0.7 V/cm) for 20 min. All steps were conducted under subdued lighting to prevent additional DNA damage. After electrophoresis, the slides were neutralized with 0.4 M Tris (pH 7.5) and air-dried and stored at room temperature until DNA migration was checked. DNA was stained with propidium iodide (20 μg/ml distilled water, 25 μg per slide) right before observation.

3. Results

3.1. QBS induces cell death in Jurkat T cells

To identify novel compounds that have cytotoxic activities in leukemia cell lines, we screened a chemical library containing 11,000 compounds, using Jurkat T cells. In the initial screening, 24 compounds that reduced viability more than 50% ($EC_{50} < 2$ μM) were selected based on the MTT conversion assay (data not shown). 2-Amino-*N*-quinoline-8-yl-benzenesulfonamide (Fig. 1A) was identified as the most potent chemical that decreased cell viability among the 24 selected chemicals ($EC_{50} = 0.77 \pm 0.05$ μM). As

shown in Fig. 1B, decrease of cell viability was apparent at a concentration of 0.5 μM and reached approximately 70% at 1 μM. The cytotoxic effect of QBS was also observed in other lymphoma cell lines, such as Molt-4, Raji and Ramos cells ($EC_{50} = 0.87 \pm 0.04$, 1.26 ± 0.09, and 0.96 ± 0.04 μM, respectively). These results suggest that QBS might be an effective cytotoxic agent in leukemia cell lines.

3.2. QBS triggers caspase-dependent apoptosis

To elucidate the pathway affected by QBS that reduces cell viability, we firstly examined whether QBS induced apoptosis. From Hoechst 33342 staining and DNA-ploidy assay, it was revealed that QBS induced nuclear condensation, DNA fragmentation (Fig. 2A, upper panel) and increased the sub-G1 peak (Fig. 2A lower panel), which are hallmarks of apoptosis. After 48 h of QBS treatment, the sub-G1 population reached 32%, whereas it was only 2.8% in untreated cells. In addition, oligonucleosomal cleavage of genomic DNA was increased by QBS treatment in a concentration-dependent manner (Fig. 2C). Moreover, typical nuclear morphology of apoptotic cells by QBS treatment was confirmed by electron microscopy (Fig. 2B). Next, we investigated whether cytochrome C was released to the cytosol from mitochondria by immunofluorescent staining using monoclonal antibody against cytochrome C. As shown in Fig. 2D, cytochrome C released into the

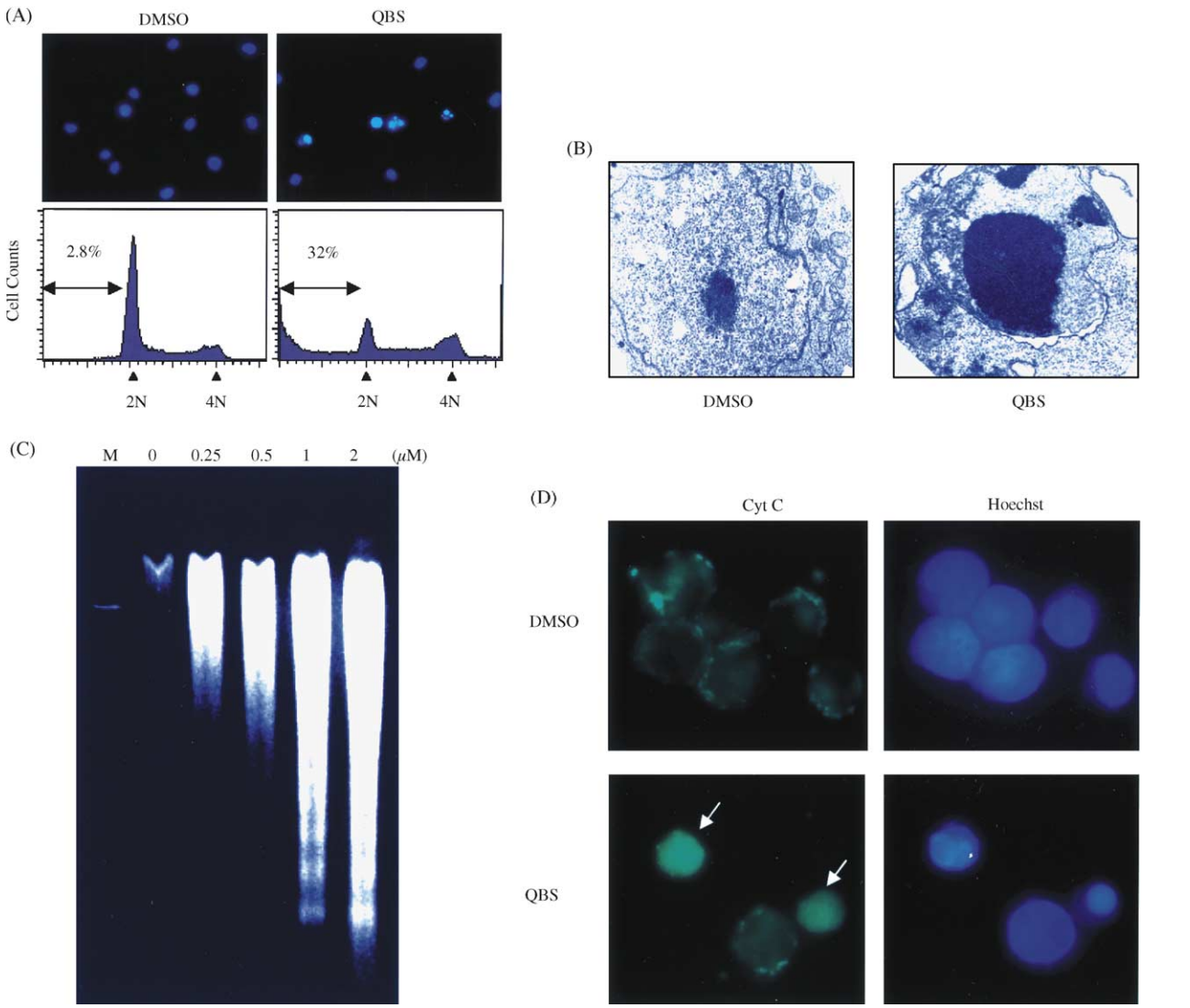

Fig. 2. QBS induces cell death by apoptosis. (A) After treatment of Jurkat T cells with 1 μM QBS for 48 h, chromatin condensation and fragmentation were monitored by Hoechst 33342 staining (upper panel). Appearance of the sub-G1 peak upon QBS treatment (1 μM, 48 h) was detected by flow cytometric analyses (bottom panel). (B–D) Apoptosis of Jurkat cells was further demonstrated by electron microscopy (B), oligonucleosomal cleavage of genomic DNA (C) and cytochrome C release (D). Jurkat cells were morphologically analyzed 48 h after QBS treatment by electron microscopy or were subjected to agarose gel electrophoresis for the assessment of oligonucleosomal cleavage of genomic DNA. Cytochrome C release was determined by immunofluorescence analysis. Cells treated with vehicle (DMSO) or QBS (1 μM, 36 h) were stained with Hoechst 33342 (blue) and monoclonal antibody against cytochrome C (green). (E) Western blot analysis using specific antibody against PARP revealed the cleavage of PARP after QBS treatment. Intact PARP (molecular mass, 116 kDa) and the PARP cleavage fragment (molecular mass, 85 kDa) are shown. Presented is representative immunoblot of three independent experiments.

cytoplasm was observed only in apoptotic cells which showed nuclear condensation and DNA fragmentation. Furthermore, 116 kDa PARP was obviously cleaved into its characteristic 85 kDa fragment after 36 h of QBS treatment (Fig. 2E). Taken together, these findings indicate that QBS triggers apoptosis in Jurkat cells.

To elucidate the involvement of caspases in QBS-induced cell death, the cells were pretreated with a broad-spectrum caspase inhibitor, z-VAD-fmk. QBS-induced cell death was recovered more than 50% in the presence of z-VAD-fmk as compared with cells treated with QBS alone (Fig. 3A). Moreover, QBS-induced activation of caspase-3 was nearly eliminated in the presence of z-VAD-fmk (Fig. 3B). The cleavage of PARP, a substrate of caspase-3, also indicated activation of caspase-3 by QBS (Fig. 2E).

Collectively, these results suggest that QBS induces caspase-dependent apoptosis.

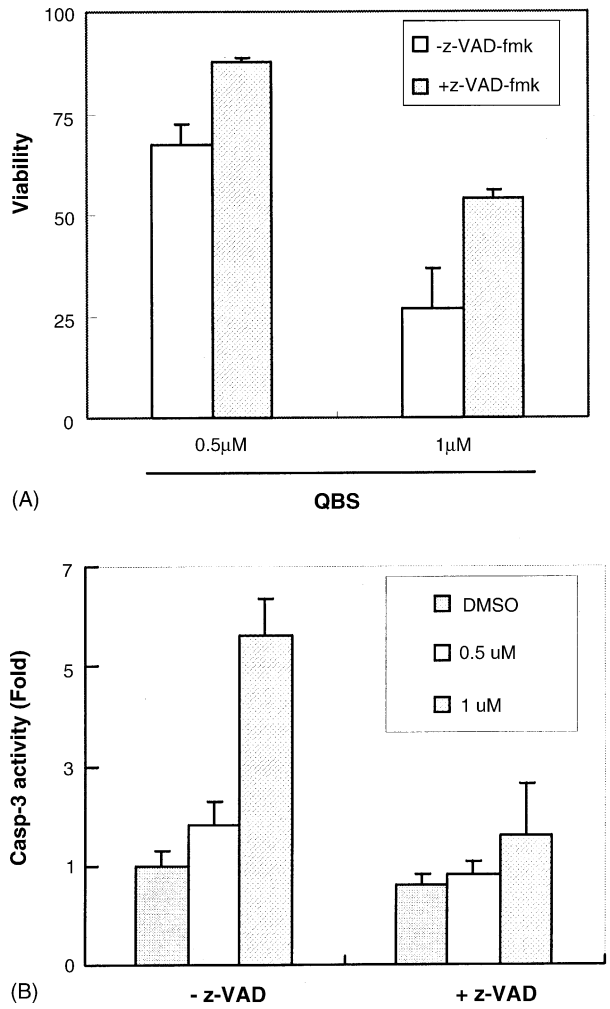

Fig. 3. QBS-induced cell death is caspase-dependent. (A) Inhibition of QBS-induced cytotoxicity in Jurkat T cells in the presence of broad spectrum caspase inhibitor, z-VAD-fmk (30 μM) was measured by the MTT assay after QBS treatment for 48 h. Each bar is a mean ± S.E.M. of duplicate experiments. (B) After treatment of cells with QBS for 48 h in the absence or presence of z-VAD-fmk, caspase-3 activity assay was performed as described in Section 2. The results are representative of three independent experiments.

3.3. QBS induces G2 phase arrest by DNA damage in Jurkat T cells

From DNA-ploidy analysis, a significant inhibitory effect of QBS on cell cycle progression was observed at 18 h after QBS treatment (Fig. 4A). QBS treatment resulted in an accumulation of cells at the G2/M phase (21%, 40%, and 58% at 0, 18, and 24 h after QBS treatment, respectively) with a corresponding reduction in the number of cells at the G1 phase. Similar results were obtained when other leukemia cell lines such as MOLT-4, Raji, and Ramos were treated with QBS (data not shown).

DNA damage is an important signal that is implicated in the induction of cell cycle arrest and apoptosis. Cell cycle arrest allows repair of the damaged DNA, in an effort to protect the organism from the repercussions of mutation. Many DNA damaging agents including certain antineoplastic drugs exert their effects at the G2/M phase and ultimately lead to apoptosis. Therefore, we carried out a single-cell gel electrophoresis assay, so-called the comet assay, to determine whether QBS induces DNA damage in Jurkat T cells. Comet assay is based on the neutral electrophoresis system and known as a rapid method for detection of DNA strand breaks in individual cells. Tail movement, indicative of DNA damage and/or DNA breakage, was increased after QBS treatment comparable to those treated with etoposide, a DNA damage-inducing agent (Fig. 4B).

During the G2 phase, the cyclin B1-cdc2 complex is maintained in an inactive state by phosphorylation of cdc2 on Tyr 15 and Thr 14 by Wee1 and Myt1, respectively [26–28]. QBS treatment increased the level of cyclin B1 and phosphorylated-cdc2 (Fig. 5A). At the onset of mitosis, it is well-known that the two residues of cdc2 are dephosphorylated by the phosphatase cdc25c [29], and cyclin B1 translocates to the nucleus from the cytosol. When the cells were treated with nocodazole, an inducer of mitotic arrest, localization of cyclin B1 was observed in nucleus in mitotically arrested cells (arrowheads, Fig. 5B). To the contrary, cyclin B1 was located mainly in the cytoplasm after QBS treatment (arrows, Fig. 5B). Collectively, these results suggest that QBS induces G2 arrest before the onset of mitosis.

Next, we investigated whether the QBS-induced DNA damage was associated with changes in the kinase activity of cdc2. The cdc2 kinase assay showed that there was a significant decrease in the cyclin B1–associated cdc2 kinase activity after QBS treatment. PQS, a structural analogue of QBS, also attenuated cdc2 kinase activity. In contrast, cdc2 kinase activity was significantly enhanced by treatment of Jurkat cells with nocodazole (100 nM) (Fig. 5C). Based on the fact that the kinase activity of cyclin B1-associated cdc2 is critical for G2/M transition, our results suggest that QBS-induced DNA damage may induce G2 arrest via inhibiting the kinase activity of cyclinB1-associated cdc2.

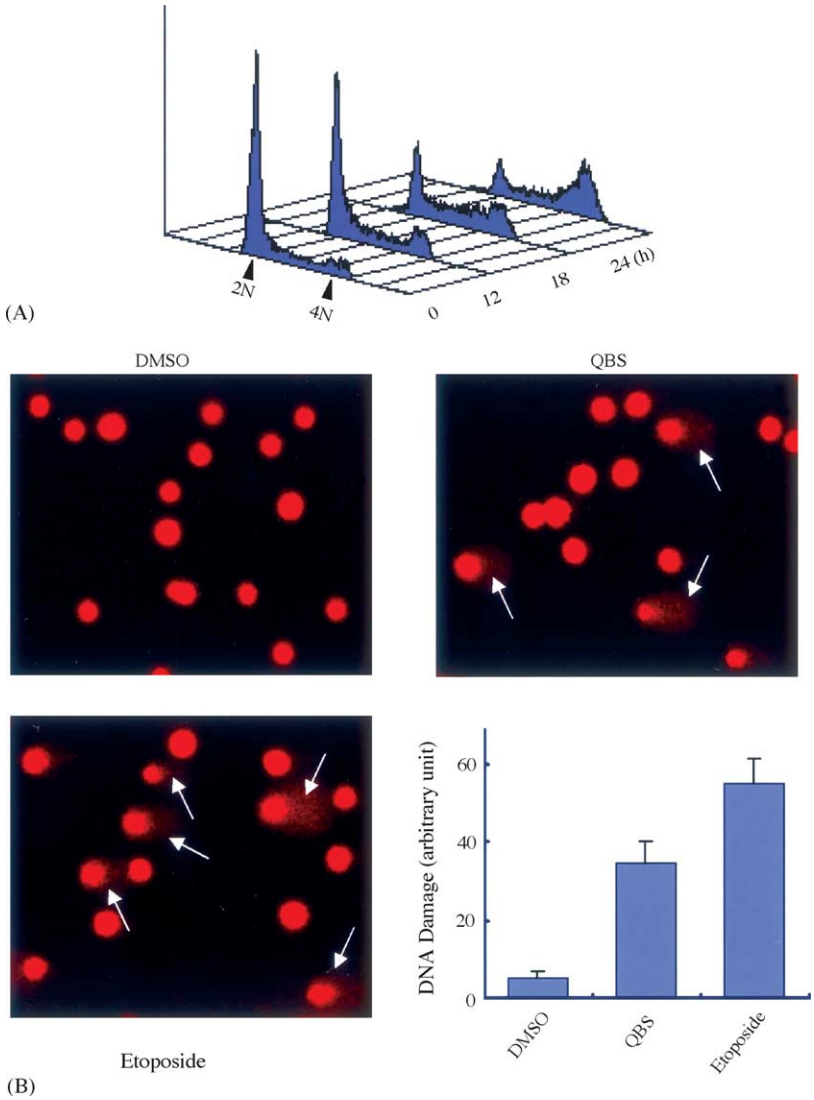

Fig. 4. QBS induces G2/M arrest by causing DNA damage. (A) Cells were treated with 1 μM QBS for the indicated times. To measure cellular DNA contents, cells were fixed in ethanol, stained with propidium iodide, and analyzed using the FACScan flow cytometer, as described in Section 2. (B) Cells were treated with DMSO (0.1%), QBS (1 μM) or etoposide (1 μM) for 18 h and then subjected to comet assay as described in Section 2. Cells containing DNA strand breakage were observed under confocal microscope and quantitated. Data are representative of at least three separate experiments.

3.4. QBS analogues arrest Jurkat T cells in the G2/M phase of the cell cycle

To investigate the structure–activity relationship (SAR) of QBS, we examined the effect of four QBS derivatives which possess quinoline and benzenesulfonamide groups. QBS derivatives we tested were *N*-8-quinolinyl-benzenesulfonamide, *N*-phenyl-4-quinolinesulfonamide, *N*,4-dimethyl-*N*-8-quinolinylbenzenesulfonamide, and 4-methyl-*N*-4-quinolinylbenzenesulfonamide, which hereafter are called QBS-1, PQS, QBS-2 and QBS-3, respectively (Fig. 6). The structure of QBS-1, which is a sulfonamide derivative, is similar to that of QBS except for the absence of amine group. In PQS, the phenyl and quinoline groups are connected by the nitrogen and the sulfur atom of sulfonamide group, in a reverse way to that of QBS-1. The chemical structure of QBS-2 is analogous to QBS-1. However, the difference between QBS-2 and other compounds used in this work originates from the *tertiary* amine group having different basicity compared to the *secondary* one (Fig. 6). The structure of QBS-3 is similar to that of QBS-1 except for its methyl group substitution. Among the four tested QBS analogues, QBS-1 and PQS dramatically increased the G2/M cell population compared with the DMSO-treated control (52.6 ± 2.1%, 49.2 ± 2.4% and 22.3 ± 3.4% in QBS-1, PQS and DMSO-treated cells, respectively) (Fig. 6). PQS inhibited the cdc2-kinase activity as well as inducing G2 phase arrest (Fig. 5C). However, QBS-2 and QBS-3 did not have any significant effects on cell cycle arrest (24.3 ± 3.6%, 25.6 ± 2.8% in QBS-2 and QBS-3-treated cells, respectively) (Fig. 6).

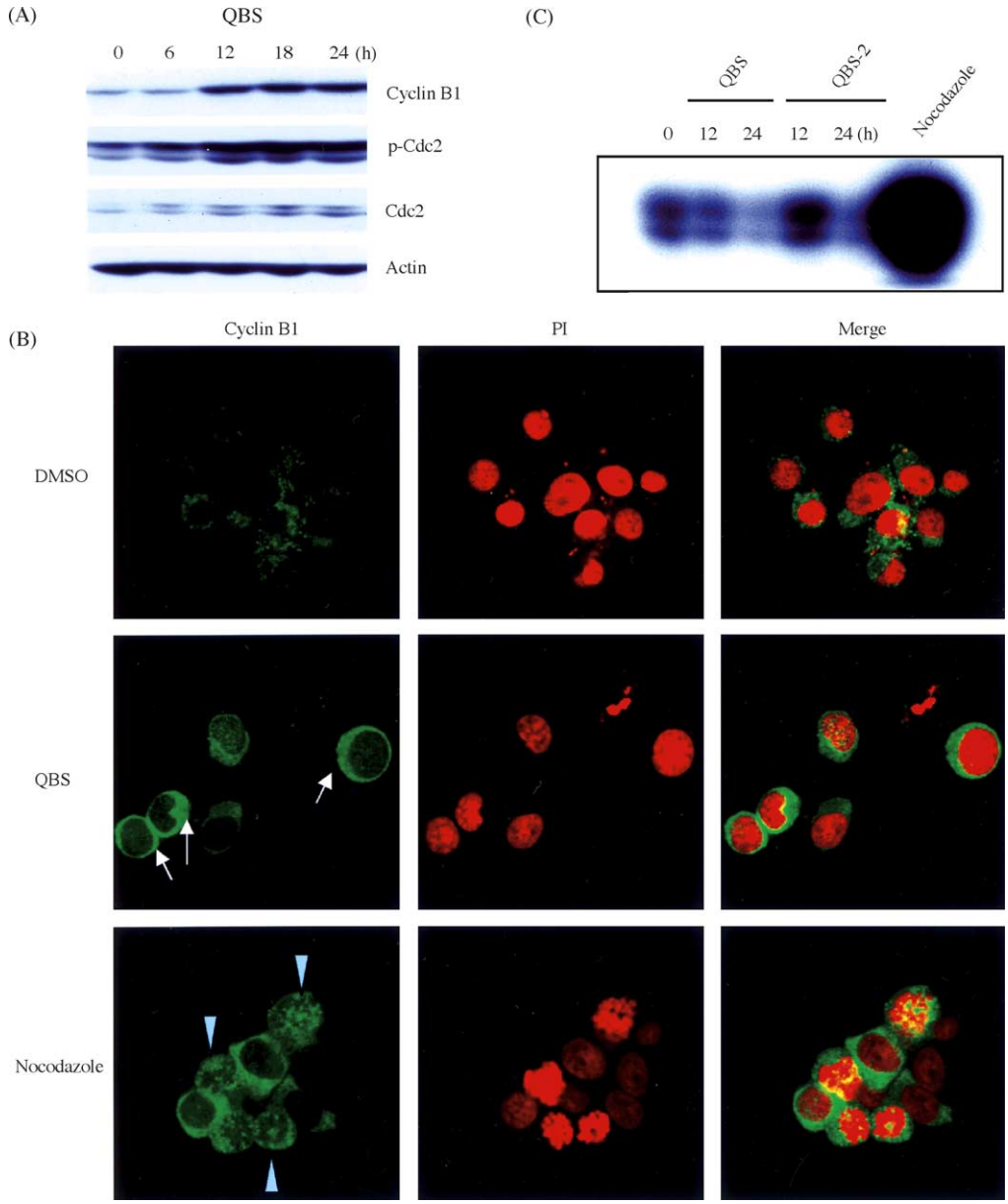

Fig. 5. QBS induces G2 phase arrest. (A) Western blot analysis of whole cell lysates using specific antibody against G2-related proteins, cyclin B1 and cdc2, at the indicated time points after treatment of QBS (1 μM). Equal amounts of proteins were loaded on each lane, and the membrane was immunoblotted with an anti-actin antibody as a loading control. (B) Subcellulr localization of cyclin B1 after QBS treatment (1 μM, 18 h). Note the cytoplasmic localization (arrows) of cyclin B1 induced by QBS treatment in comparison with its nuclear localization (arrowheads) in mitotically arrested cells treated with nocodazole (100 nM). Cells were stained with cyclin B1 antibody (green) and counterstained with propidium iodide to analyze the nuclear morphologies. Immunofluorescence was detected by a confocal laser microscopy. (C) QBS prevents the activation of cyclin B1-associated cdc2. In vitro kinase assay of cyclin B1 was performed as described in Section 2. Cyclin B1 immunoprecipitates were prepared at the indicated time points after treatment of with QBS (1 μM) or nocoazole (100 nM).

4. Discussion

Targeting cell cycle and apoptotic pathways has emerged as an attractive approach for the treatment of cancer. To discover putative chemotherapeutic agents that have antiproliferative properties, we performed MTT conversion assay to monitor cell viability in Jurkat T cells upon treatment with a random chemical library containing 11,000 compounds. QBS was identified to be the most potent cytotoxic agent among the 11,000 tested chemicals. Our results suggest that it induces G2 arrest in several leukemic cell lines (Molt-4, Raji, Ramos, data not shown) including Jurkat T cells. QBS-induced cell death was accompanied by cytochrome C release, PARP cleavage (Fig. 2), and was caspase-dependent (Fig. 3), suggesting that it is a potent inducer of apoptosis.

Many traditional pharmacological agents that induce cell death regulate cell cycle progression [29,30]. Perturbations of the cell cycle progression by DNA damage often results in cell death and it has been shown that apoptosis ensues during or after G2 arrest [31]. G2 arrest is one of the typical responses exhibited by proliferating eukaryotic

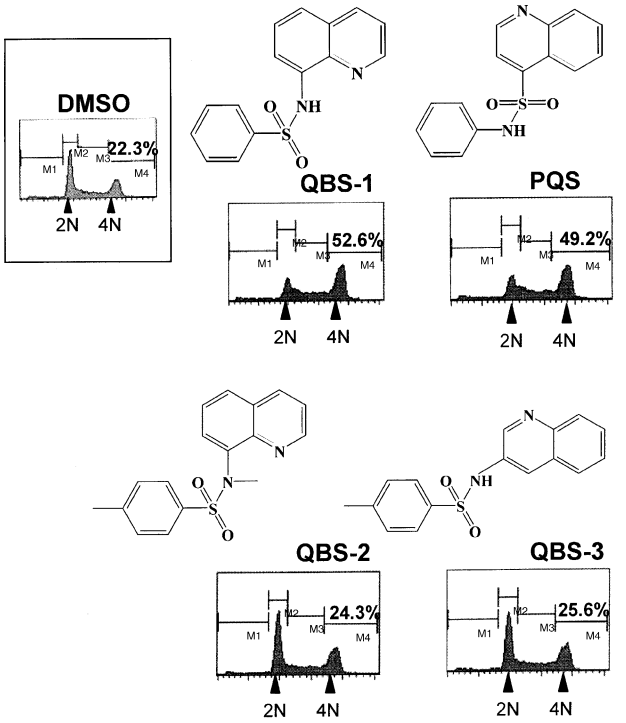

Fig. 6. Structural analogues of QBS affect cell cycle progression. Cells were treated with the analogues of QBS (1 μM) or DMSO as a vehicle control for 24 h. Cells were then fixed in ethanol, stained with propidium iodide, and analyzed by FACScan flow cytometry as described in Section 2. Data are representative of at least three independent experiments. QBS-1 denotes for *N*-8-quinolinyl-benzenesulfoamide, PQS for *N*-phenyl-4-quinolinesulfoamide, QBS-2 for *N*,4-dimethyl-*N*-8-quinolinylbenzenesulfoamide, and QBS-3 for 4-methyl-*N*-4-quinolinylbenzenesulfoamide.

cells when exposed to a variety of DNA damaging agents, such as UV light, DNA alkylators [32], X-irradation [33], radiomimetic agents and topoisomerase inhibitors [34]. Our results from the comet assay strongly suggest that QBS induces DNA damage (Fig. 4). Also, QBS treatment increased in the level of cyclin B1 and phosphorylated-cdc2, caused an accumulation of cyclin B1 in the cytoplasm, and decreased cdc2 kinase activity, confirming the notion that QBS induces G2 arrest. Consistently, DNA-ploidy assay revealed that QBS caused a significant increase in the number of cells in the G2 phase with a corresponding decrease in the G1 phase, when observed after 24 h of QBS treatment. Under the same condition, however, there was no change in cell viability and cells at the sub-G1 phase were undetectable. After 48 h, however, the viability had fallen to 30%, leading to a reduction in the G2/M population. Many studies have provided evidence suggesting that p53 controls entry into mitosis when cells enter G2 with damaged DNA. In the absence of functional p53, cells are arrested in G2 phase, thereby resulting in apoptosis [7,10]. Because Jurkat cells harbor a mutation in p53 configuration, it is likely that they have few, if any, functional p53. Our results in Jurkat cells, in which QBS-induced DNA damage results in G2 arrest and apoptosis, therefore correspond to the previous reports.

When Jurkat T cells were treated with QBS in the presence of caffeine (1 mM), which is capable of abrogating cell cycle checkpoints, cell death was triggered rapidly without G2 arrest (data not shown), suggesting a possibility that apoptosis by QBS is a next event following G2 arrest. It, however, requires further experiments to clarify the detailed cytotoxic mechanism induced by QBS.

Quinoline and sulfonamide groups appear to be very important for the activity of QBS. It has been previously reported that aromatic sulfonamides generally attenuate tumor cell growth by inhibiting carbonic anhydrase, and aromatic sulfonamides-based derivatives can impair cell proliferation by inducing G1 cell cycle arrest [22–23,18–19]. Moreover, several quinoline-based drugs such as MK571 [35,36], Quinine [36], primaquine [36], are often used in combination with anticancer drugs to overcome multi drug resistance (MDR)-associated protein 1 (MRP)-mediated MDR in tumors.

Interestingly, our SAR study with QBS analogues leads to the conclusion that the activity of QBS requires not only the presence, but also their correct steric configuration of both quinoline and sulfonamide groups. As described in Section 3, the constitutional structure of QBS-1 is the same as that of QBS, except for the presence of an amine group. The unsubstituted phenyl and quinoline groups in PQS are bounded to the nitrogen and the sulfur atom of the sulfonamide group, respectively, in the opposite way of QBS-1. Among the four analogues tested, two structural isomers of QBS, QBS-1 and PQS were able to induce G2 phase arrest, implying that the relative position of the nitrogen atom in the quinoline with sulfonamide group is not critical for the activity of elicited by these compounds. QBS-2 and QBS-3, however, containing *p*-methyl substituted phenyl and quinoline moieties failed to induce cell cycle arrest. The methyl group substituted at the phenyl ring endows structural differences of QBS-2 and QBS-3 versus QBS-1 and PQS. The effects of these structural analogues on cell cycle arrest corresponded to the results of cell viability assay. As expected, QBS-1 and PQS, but not QBS-2 and QBS-3, were capable of inducing cell death (date not shown). Collectively, these results indicate that even though these compounds have structural similarities, i.e., a sulfonamide group, a phenyl and a quinoline unit, the changes in basicity by *tertiary* amine and/or methyl group substitution can make important differences with respect to their abilities to cause DNA damage and G2 phase arrest.

In conclusion, we suggest QBS as a novel, potent inducer of DNA damage and G2 arrest, followed by apoptosis. Moreover, QBS was found to affect cell cycle progression in other several leukemic cell lines, as well as in Jurkat T cells. Importantly, the SAR study revealed that the effect of QBS and its derivatives on cell cycle arrest and cell death are related to the spatial configuration of quinoline and sulfonamide groups, which can be useful information for the development of novel chemotherapeutic agents. We believe that further investigation into the

mechanism responsible for QBS-induced apoptosis would provide chemotherapeutic strategies in leukemia therapy based on novel insights.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bcp.2004.12.019.

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