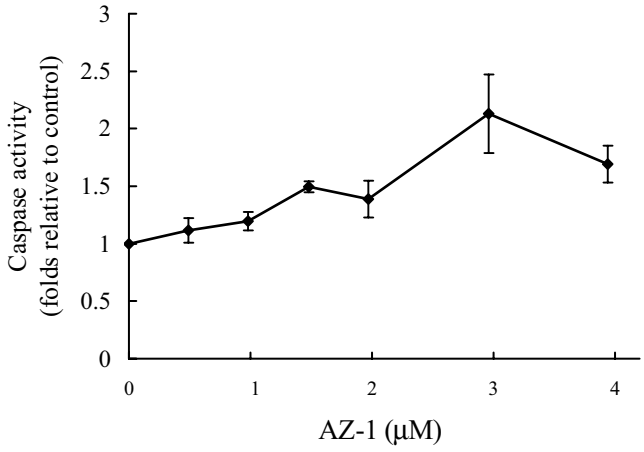
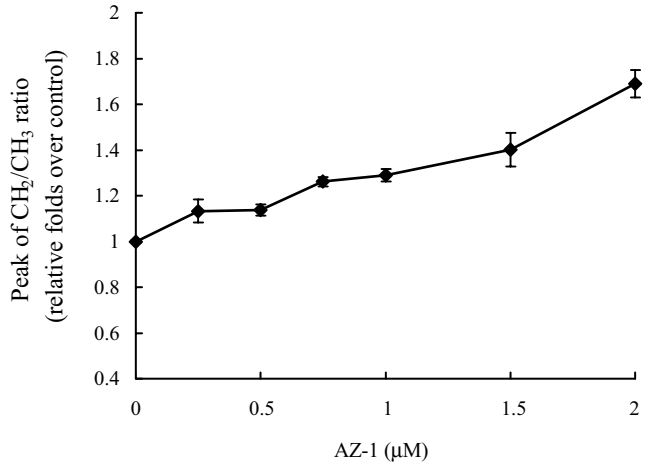
Figure 9



The enzyme activity of caspase 3 in BC-M1 cells was induced by bis-aziridinylnapthoquinone (AZ-1). Cells were seeded for 24 hours before the addition of AZ-1 in various concentrations. The cell lysate was prepared for analysis by a CPP32/caspase 3 colorimetric assay kit according to the manufacturer's instructions. The enzyme activity is shown as the fold increase relative to the control.

Figure 10



Plot of the ratio of CH_2/CH_3 peak area according to NMR spectra as the dose of bis-aziridinylnapthoquinone (AZ-1)-treated BC-M1 cells. Cells were seeded for 24 hours before the addition of AZ-1 in various concentrations. The ratio of CH_2/CH_3 signal intensity was plotted against the various concentrations of AZ-1.

survival in patients with early-stage breast cancer [23,24]. Treatment options for early-stage breast cancer include chemotherapy (e.g. anthrocyclines, taxanes) and hormone therapy (e.g. tamoxifen, aromatase inhibitor). Estrogen mediates its functions through two specific intracellular receptors, estrogen receptor alpha and estrogen receptor beta, which act as hormone-dependent transcriptional regulators [25,26]. The estrogen receptor pathway plays a critical role in the pathophysiology of human breast cancer. Overexpression of estrogen receptor alpha is a well-established prognostic and predictive factor in breast cancer patients. The prognostic significance of estrogen receptor beta is not well defined [27-30].

Our previous study on the different series of bis-aziridinylnaphthoquinone compounds identified that they exhibit a more potent response toward the solid tumors than the circulation tumors [17]. This result was supported by other reports that there are differences in the reductive metabolism between the solid tumors and the circulation tumors [2]. Considering the importance of all the cellular reductases (e.g. NADPH cytochrome P450 reductase, cytochrome b5 reductase, [NADP]H oxidoreductase, NQO1) in response to the whole cellular reductive metabolism, these reductases are probably involved in bioactivation of AZ-1. The bioreductive drugs AZQ, mitomycin C and E09, however, have been developed to exploit the oxygen deficiency in the hypoxic fraction of solid tumors on the premise that hypoxic cells should show a greater propensity for reductive metabolism than well-oxygenated cells [2,31-33].

The major events involved in tissue homeostasis are proliferation, differentiation, and apoptosis. The processes of cell reproduction normally take place through an ordered process, generally known as the cell cycle. The tumor suppressor gene p53 is a multifunctional protein mainly responsible for maintaining genomic integrity, and it is the most frequently mutated gene in human tumors [34]. In response to DNA damage, aberrant growth signals, or chemotherapeutic drugs, p53 is stabilized and induces apoptosis and/or cell cycle arrest. While the mechanisms of p53-dependent apoptosis are not understood well, p53-dependent cycle arrest is primarily mediated by the cdk inhibitor p21 [34-36]. p21 regulates the cellular repair response to damaged DNA [37].

In the cytotoxicity results, AZ-1 induced the death effect of BC-M1 cells and MCF-7 cells in a dose-dependent and time-dependent manner in BC-M1 cells (Figs 1 and 2). From the results shown in Fig. 3, the hormone antagonist taxmoxifen and AZ-1 were more potent than paclitaxel to our local cell line BC-M1. This indicates that the BC-M1 cell is more sensitive to the hormone antagonist drug and our bioreductive compound AZ-1 than to a nonhormone antagonist such as paclitaxel. We assumed that the BC-M1 cell is an estrogen receptor-positive cell line. According to the results of Figs 4 and 5, the apoptosis phenomena were observed in BC-M1 cells induced by AZ-1 for 24 hours. We saw the apoptotic bodies increasing in direct proportion with the concentration of AZ-1 based on the sub-G_1 area measurement and Hoechst staining (Figs 4 and 5). The apoptotic bodies were increasing slightly in 0.5