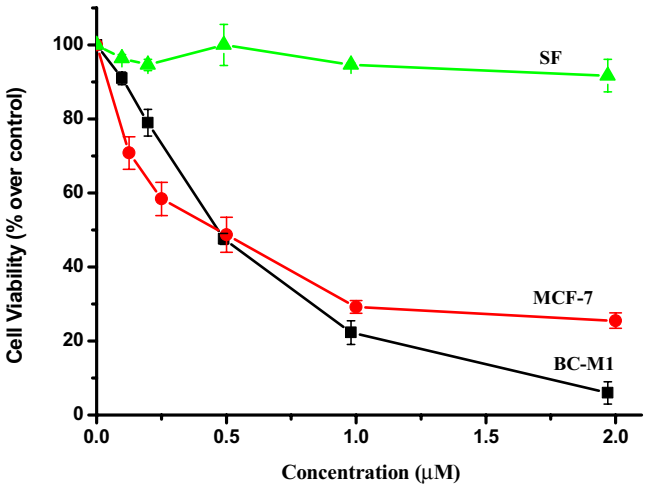
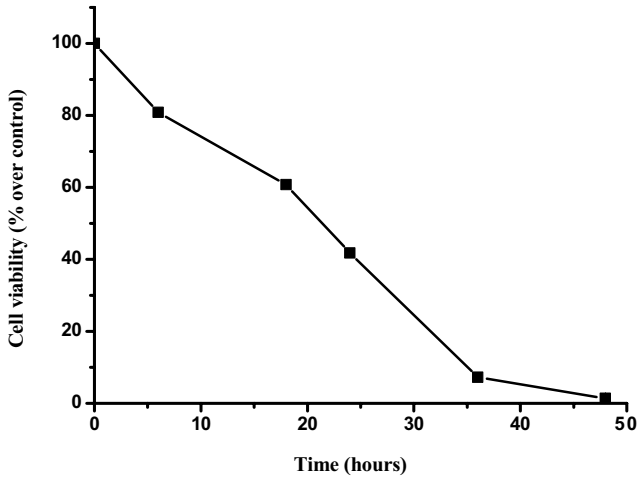
Figure 1



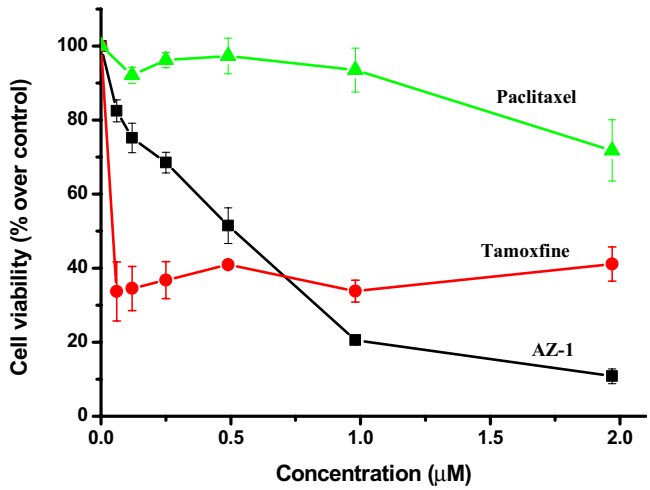
Bis-aziridinylnapthoquinone (AZ-1) inhibited the proliferation of three cell lines, skin fibroblasts (SF), BC-M1 and MCF-7. Cells were seeded for 18 hours before the addition of AZ-1 with various concentrations. The death effects of these three cell lines induced by various concentrations of AZ-1 were compared by the MTT assay. The MTT assay was used to determine the cell viability after an additional 24 hours of culture. Data are from quadruplet wells and are representative of three separate experiments.

Figure 2



Bis-aziridinylnapthoquinone (AZ-1) inhibited the proliferation of cell line BC-M1. Shown is the cell viability of BC-M1 cells at various times using the IC_{50} dose (0.51 μM) of AZ-1 challenge. The MTT assay was used to determine the cell viability. Data are from quadruplet wells and are representative of three separate experiments.

Figure 3



Comparison of the death effect of BC-M1 cells induced by various concentrations of bis-aziridinylnapthoquinone (AZ-1), paclitaxel and tamoxifen by the MTT assay. The MTT assay was used to determine the cell viability after an additional 24 hours of culture. Data are from quadruplet wells and are representative of three separate experiments.

Results

The cytoxicity of AZ-1 to BC-M1 cells and MCF-7 cells

The cytotoxicities of the IC_{50} value in AZ-1 to BC-M1 cells and MCF-7 cells were 0.51 μM and 0.57 μM in a dose-dependent manner, respectively. The response of these two cell lines (MCF-7 and BC-M1) to AZ-1 was very similar to cell viability in a dose-dependent manner. In the normal fibroblast cell (skin fibroblast) there was still a 90% survival rate at 2 μM (Fig. 1). According to the time-dependent treatment of AZ-1 using an IC_{50} concentration of 0.51 μM to BC-M1 cells, the cell viability was less than 10% after 36 hours of treatment in a time-dependent manner (Fig. 2). The two clinical therapies paclitaxel and taxmoxifen were compared regarding cytoxicity in BC-M1 cells with AZ-1, and the estrogen receptor antagonist taxmoxifen and AZ-1 were more potent than paclitaxel. The taxmoxifen had a low IC_{50} value of 0.05 μM, which is lower than AZ-1, with a plateau concentration from 0.05 μM to 2 μM (Fig. 3).

Apoptosis assay by flow cytometry and Hoechst staining

Analysis of the DNA content of cells was used to determine whether cell apoptosis was induced by AZ-1. The data of the sub-G_1 area indicated that BC-M1 cells had a significant population of cell apoptosis in the sub-G_1 area on treatment with AZ-1 for 24 hours compared with dimethylsulfoxide alone (Fig. 4). The sub-G_1 area started at 1.0 μM AZ-1 and exhibited a large increase in apoptotic cells identified as subcellular populations with decreasing DNA. The BC-M1 cells treated with 2.0 μM AZ-1 exhibited the largest apoptotic area (29.2%). This is similar to the apoptotic bodies observed using the Hoechst stain (Fig. 5). The Hoechst staining method was used to identify the apoptotic nuclei in BC-M1 cells and MCF-7 cells. Apoptotic cells that contained the apoptotic bodies showed blue peripherally clumped or fragmented chromatin, as indicated by arrows in Figs 5 and 6. From the visual observation of the Hoechst staining results, the BC-M1 cells treated with 2.0 μM had the highest number of apoptotic bodies (Fig. 5). The