Figure S1. Antiproliferative effect of tipifarnib in lymphoid cell lines. A, aliquots containing 1-2 x 10^4 cells/ml were incubated with diluent (0.1% DMSO) or the indicated concentration of tipifarnib for 6 d, then assayed for viable cell mass by MTS assay as described in the METHODS. B, after treatment with 800 nM tipifarnib for 72 h, cells were stained with propidium iodide and examined by flow cytometry. Cell cycle distribution was assessed using Modfit. Numbers, mean ± of 3 independent experiments.

Figure S2. Role of Bak in tipifarnib-induced apoptosis. A, B, 48 h after introduction of Bax siRNA, Bak siRNA, or both, samples were treated for an additional 48 h with the indicated tipifarnib concentration before staining with APC-conjugated annexin V and analysis by 2-color flow cytometry. Inset in B, whole cell lysates were prepared from siRNA-treated cells incubated in drug-free medium until samples were harvested for flow cytometry, then subjected to immunoblotting for the indicated antigens. C, after Jurkat cells were treated with the indicated tipifarnib concentration for 48 h, cell lysates prepared in 1% CHAPS were saved for immunoblotting (“input”) or treated with Bak Ab1, which recognizes the active conformation of Bak, cross-linked to protein G-sepharose (“IP”). After immunoprecipitates were washed and resolved by SDS-PAGE, Bak was detected using a conformation-insensitive antibody.

Figure S3. Characterization of tipifarnib-resistant Jurkat cells. A-D, aliquots containing 10^3 parental (open symbols) or tipifarnib-resistant Jurkat cells (closed symbols) were treated for 6 d with the indicated concentrations of tipifarnib (A), lonafarnib (B), or etoposide (C). At the completion of the incubation, samples were assayed for MTS reduction. Error bars, mean ± of quadruplicate samples. D, whole cell lysates prepared from parental Jurkat cells treated for 72
h with diluent (lane 1) or 1600 nM tipifarnib (lane 2) in the presence of 5 µM Q-VD-OPh or from tipifarnib-resistant Jurkat cells grown in the continuous presence of 1600 nM tipifarnib (lane 3) were subjected to SDS-PAGE followed by immunoblotting with antibodies to the indicated antigen.
Figure S1
Figure S2
Figure S3