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[37] 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 ± SD 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.

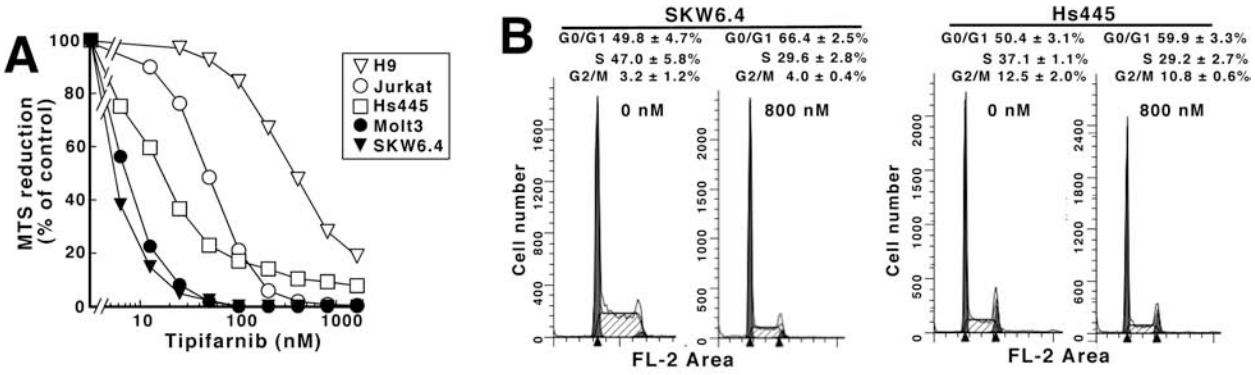
A
MTS reduction (% of control)
Tipifarnib (nM)
H9
Jurkat
Hs445
Molt3
SKW6.4
B
SKW6.4
G0/G1 49.8 ± 4.7%
S 47.0 ± 5.8%
G2/M 3.2 ± 1.2%
G0/G1 66.4 ± 2.5%
S 29.6 ± 2.8%
G2/M 4.0 ± 0.4%
0 nM
800 nM
Cell number
FL-2 Area
Hs445
G0/G1 50.4 ± 3.1%
S 37.1 ± 1.1%
G2/M 12.5 ± 2.0%
G0/G1 59.9 ± 3.3%
S 29.2 ± 2.7%
G2/M 10.8 ± 0.6%
0 nM
800 nM
Cell number
FL-2 Area

Figure S1

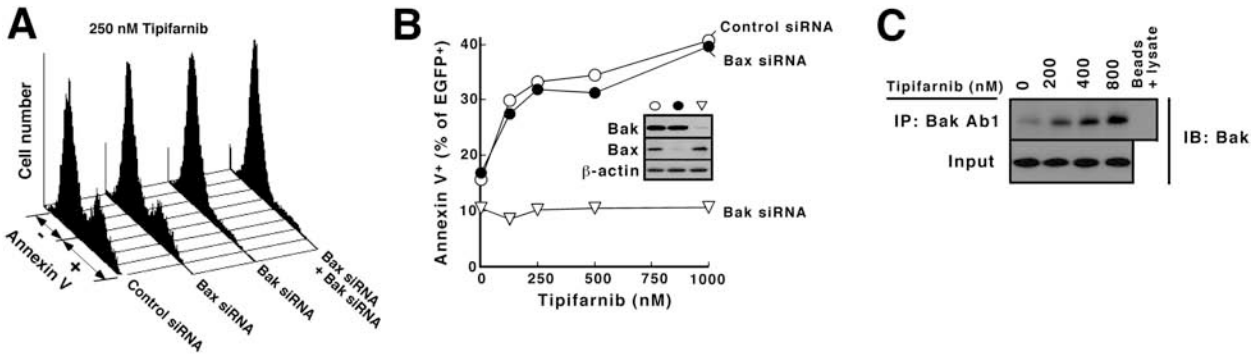
A
250 nM Tipifarnib
Cell number
Annexin V
-
+
Control siRNA
Bax siRNA
Bak siRNA
Bax siRNA
+ Bak siRNA
B
Annexin V+ (% of EGFP+)
40
30
20
10
0
0
250
500
750
1000
Tipifarnib (nM)
Control siRNA
Bax siRNA
Bak siRNA
Bak
Bax
β-actin
C
Tipifarnib (nM)
0
200
400
800
Beads + lysate
IP: Bak Ab1
Input
IB: Bak

Figure S2

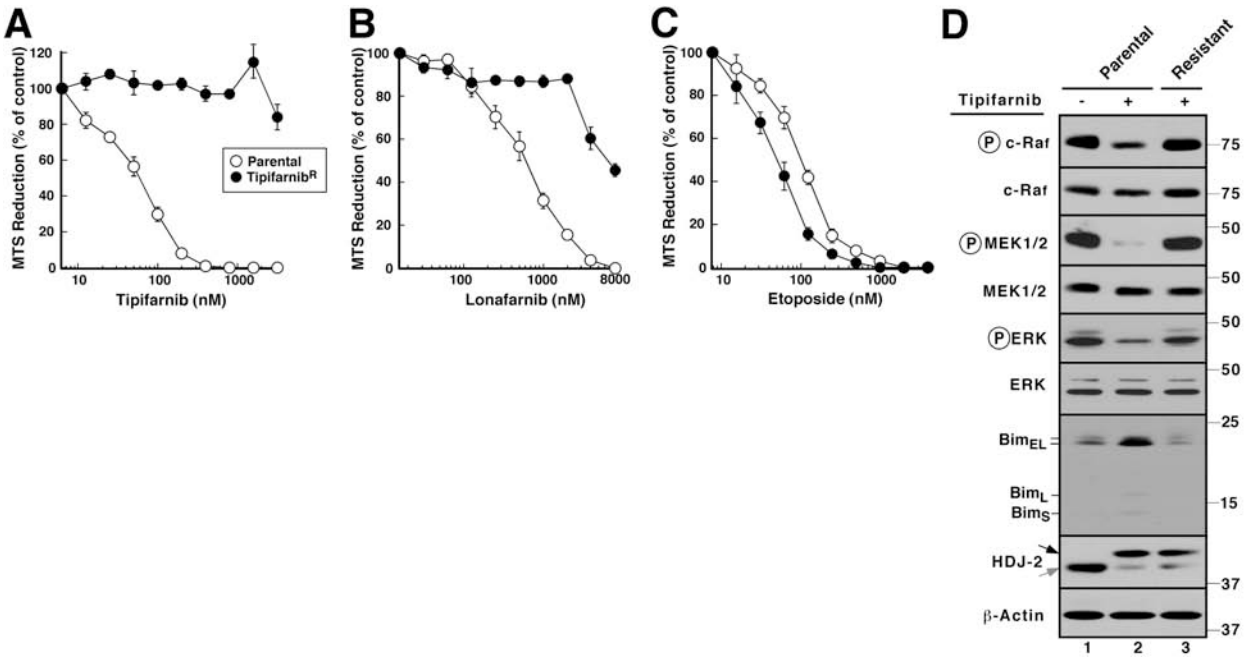
A
MTS Reduction (% of control)
Tipifarnib (nM)
Parental
TipifarnibR
B
MTS Reduction (% of control)
Lonafarnib (nM)
C
MTS Reduction (% of control)
Etoposide (nM)
D
Parental
Resistant
Tipifarnib
- + +
P c-Raf
c-Raf
P MEK1/2
MEK1/2
P ERK
ERK
BimEL
BimL
BimS
HDJ-2
β-Actin
75
75
50
50
50
50
25
15
37
37
1 2 3

Figure S3