Supplemental Methods

Preparation of cell lysate and Western blot. Cells were washed with cold PBS and resuspended in ice-cold EBC buffer (0.5% Nonidet P-40, 50mM Tris, pH 7.6, 120mM NaCl, 1mM EDTA, and 1mM-β-mercaptoethanol) containing protease inhibitor mixture set I. Following cell lysis by sonication and centrifugation at 14,000 x g for 15 min at 4 °C, the resulting supernatant was collected as the total cell lysate. As previously described, Western blot was performed by loading 50μg of protein per lane on an 8–12% SDS-PAGE, followed by protein transfer to nitrocellulose membrane for analysis of specific protein(s) (1).

Colony formation assay. Cells (A549 or H1299 single-cell suspension) were plated in 6-well plates at a density of 500 per well. The next day, cells were treated with BXI or other agent(s). The medium was replaced with fresh medium containing the corresponding concentration of the compounds every three days. After 10 days of treatment, the medium was removed and cell colonies were stained with crystal violet (0.1% in 20% methanol) and counted as described previously (2).

RNA interference, plasmids and transfection. Human Bcl-XL shRNA plasmid is a target-specific lentiviral vector plasmid encoding a 19-25 nt (plus hairpin) shRNA designed to knock down Bcl-XL gene expression. The control shRNA plasmid-A encodes a scrambled shRNA sequence that will not lead to specific degradation of any cellular message. Both Bcl-XL shRNA and control shRNA plasmids were purchased from Santa Cruz Biotechnology (Santa Cruz, CA). Corresponding siRNA sequences were: Sense→ 5'-GAC AAG GAG AUG CAG GUA Utt-3'; Antisense→ 5'-AUA CCU

GCA UCU CCU UGU Ctt-3'. Human Bcl-XL shRNA or control shRNA was transfected using NanoJuice^{TM} Transfection Kit (Novagen, Madison, WI) according to the manufacturer's protocol and stable clones were selected by puromycin.

Immunohistochemical (IHC) staining. Mice with established H1299 tumors were treated with BXI-72 (10-30mg/kg/d) for two weeks. Tumors were harvested, fixed in formalin and embedded in paraffin. Representative sections from paraffin-embedded tumor tissues were analyzed by IHC staining using an active caspase 3-specific antibody. Active caspase-positive cells in tumor tissues were scored at 400 × magnification. The average number of positive cells per 0.0625 mm^2 area was determined from three separate fields in each of three independent tumor samples as described (3).

References:

1. Ito T, Deng X, Carr B, May WS. Bcl-2 phosphorylation required for anti-apoptosis function. J Biol Chem. 1997;272:11671-3.
2. Liu Y, Sun SY, Owonikoko TK, Sica GL, Curran WJ, Khuri FR, et al. Rapamycin induces Bad phosphorylation in association with its resistance to human lung cancer cells. Mol Cancer Ther. 2012;11:45-56.
3. Oltersdorf T, Elmore SW, Shoemaker AR, Armstrong RC, Augeri DJ, Belli BA, et al. An inhibitor of Bcl-2 family proteins induces regression of solid tumours. Nature. 2005;435:677-81.