Legends Supplementary Data

S1. HYD1 has activity as a single agent in both suspension and co-culture model system and potentiates melphalan-induced cell death in HS-5 co-culture model of drug resistance. (A) 8226 cells were pre-treated for 30 minutes prior to growth in suspension or as a co-culture containing HS-5 cells. Following 24 hours of treatment dead cells were detected by annexin V staining and FACS analysis (n=9; #, P>0.05). (B) 8226 cells were pretreated for 30 minutes with 50 ug/ml HYD1 prior to either growth in suspension or as a co-culture for an additional 3 hours. After 3 hours of co-culture 20 uM melphalan was added to appropriate cultures. Twenty four hours later dead cells were detected by annexin V staining and FACS analysis. Melphalan specific cell death was calculated by subtracting out baseline death observed in control, HYD1 treated and HYDS treated cells. Thus HYD1 melphalan specific death equals % annexin V positive melphalan + HYD1 treatment- % annexin V positive in HYD1 treatment only. HYDS melphalan specific death equals % annexin V positive Melphalan + HYDS treatment-% annexin positive HYD1S (n=9; #, P>0.05).

S2. HYD1 does not induce caspase cleavage in 8226 cells. 8226 cells (4×10^5 cells/ml) were treated for 6 hours with either 50 μg/ml of HYD1 and HYDS or 50 ng/ml of TRAIL. Following this, cells were subjected to western blot analysis probing for cleaved caspase-3, -9 and -8. Experiment is a representative of three independent experiments

S3. HYD1 does not induce caspase activity following 24 hours of HYD1 treatment. (A) 100 μM ZVAD-FMK was added to H929 cells (4×10^5 cells/ml) for 30 min prior to the addition of either 75 μg/ml HYD1 or 15 μM melphalan. Following 24 hours of drug treatment cells dead cells were detected by annexin V/PI staining and FACS analysis. (B) H929 cells were treated with varying doses of HYD1 for 24 hours and caspase activity was measured per manufactures instructions. Melphalan was used as a positive control for caspase 3 activity and Trail for caspase 8 activity (n=6; #, P>0.05).

S4. HYD1 treatment induces an increase in acidic vesicles in H929. H929 cells treated with varying concentrations of HYD1, 100 ug/ml HYDS or 15 uM melphalan for six hours. Cells were stained with media containing 1 uM lysosensor DND-189 (molecular probes #L-7535) and 20 uM Hoechst 33342 (Molecular Probes #H3570) and the samples were incubated for 30 minutes. Samples were viewed with a Leica DMI6000 inverted microscope, TCS SP5 confocal scanner, and a 100X/1.40NA Plan Apochromat oil immersion objective (Leica Microsystems, Germany). Shown is a representative figure. The experiment was repeated three independent times and similar results were obtained.

S5. Treatment with 3-MA enhances HYD1 induced cell death in 8226 MM cells. 8226 cells (4×10^5 cells/ml) were pretreated with 3-MA (10 mM) for 45 min before the addition of varying concentrations of HYD1. Cell death was determined by FACS analysis of annexin V positive cells after 6 hours of treatment. Experiment is a

representative of three independent experiments, each carried out in triplicates (n=9, $P<0.05$, paired t-test).

S6. HYD1 induces depolarizes the mitochondria membrane potential, induces ROS and depletes ATP levels in 8226 MM cells.) 8226 cells (4×10^5 cells/ml) were treated for 2 hrs with HYD1 (100 μg/ml). Following treatment cells were analyzed for loss in $\Delta\psi_m$, total cellular ATP and ROS production as described in materials and method. Figures are a representative of three independent experiments, each carried out in triplicates (n=9; *, $P<0.05$).

S7. NAC effectively blocks the ROS production in HYD1 treated MM cells. H929 cells were pretreated for 30 min with NAC (10 mM), prior to a 2 hr treatment with HYD1, HYDS (50 μg/ml) or TBHP (1.5%). Dye fluorescence intensity was analyzed using Wallac VICTOR^2 1420 multilabel counter (EG&G Wallac, Turku, Finland) (excitation: 485 nm, emission: 535 nm). Shown are representative figures performed in triplicates (n=9; *, $P<0.05$).

S8. NAC partially inhibits HYD1 induced cell death but not depolarization of the mitochondria membrane potential in U226 cells. (A) U226 cells (4×10^5 cells/ml) were pretreated with NAC (10 mM) for 30 min before the addition of varying concentrations of HYD1. Following the treatment cells were analyzed for loss in $\Delta\psi_m$ by FACS utilizing $DiOC_6$ dye (n=9; #, $P>0.05$). (B) U226 cells (4×10^5 cells/ml) were pretreated with NAC (10 mM) for 30 min before the addition of HYD1 (50 μg/ml) for an additional 4 hrs. Cell death was detected by annexin V positivity (n=9; $P<0.05$, paired t-test). Figures shown are a representative of three independent experiments, each performed in triplicates