Supporting Information

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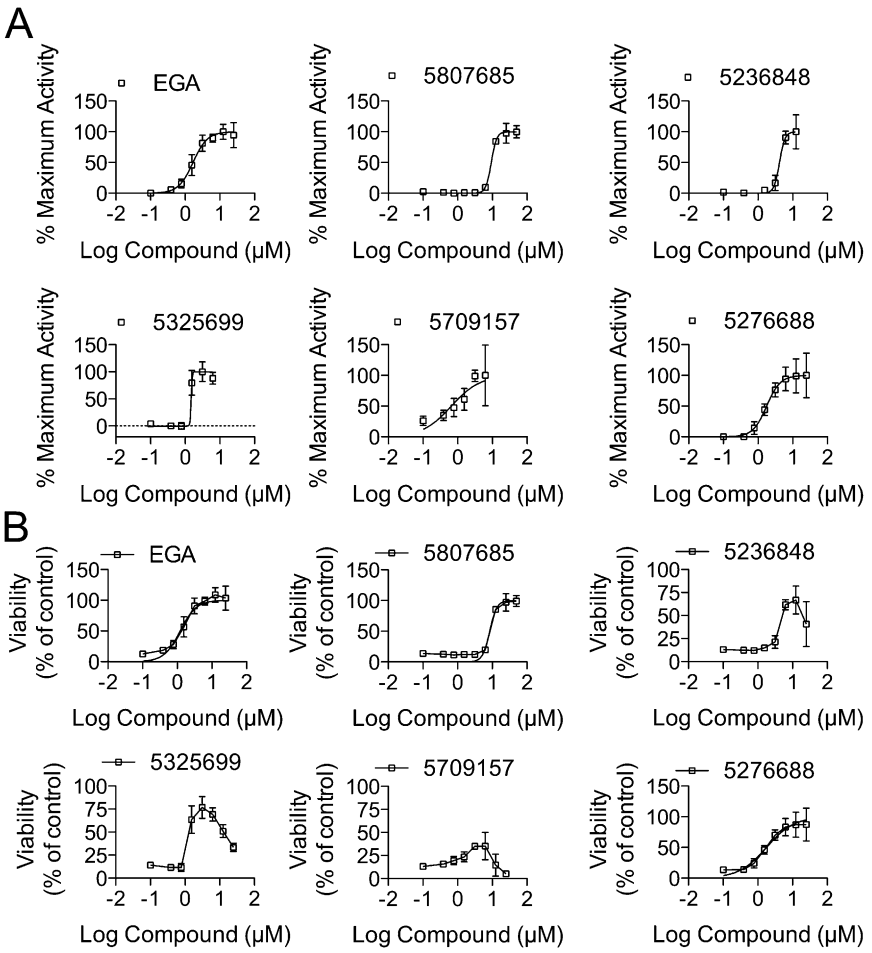

Fig. S1. Dose titrations of top hits from high-throughput screen. RAW264.7 cells were seeded at 2×10^3 cells/well in 384-well plates, pretreated with various doses of indicated compounds for 1 h, and then challenged with 231 ng/mL protective antigen (PA) and 33 ng/mL lethal factor (LF). Cell viability was measured 4–6 h later by ATPlite 1step reagent (Perkin-Elmer). Luminescence was measured on a Victor^{3}V plate reader, and results were normalized to unintoxicated controls. Results represent average values from three independent experiments performed in triplicate ±SD. (*A*) Calculation of IC_{50} values. Maximum viability provided by each compound was set at 100% activity, and viability in the absence of drug (toxin only) was set at 0% activity. IC_{50} values were calculated using nonlinear fit (variable slope) of normalized data (Prism 5, GraphPad). (*B*) Maximum viability provided by each compound. Data were normalized to unintoxicated controls (set at 100% viability), and the maximum protection provided by each was determined and graphed in Fig. 1*B*.

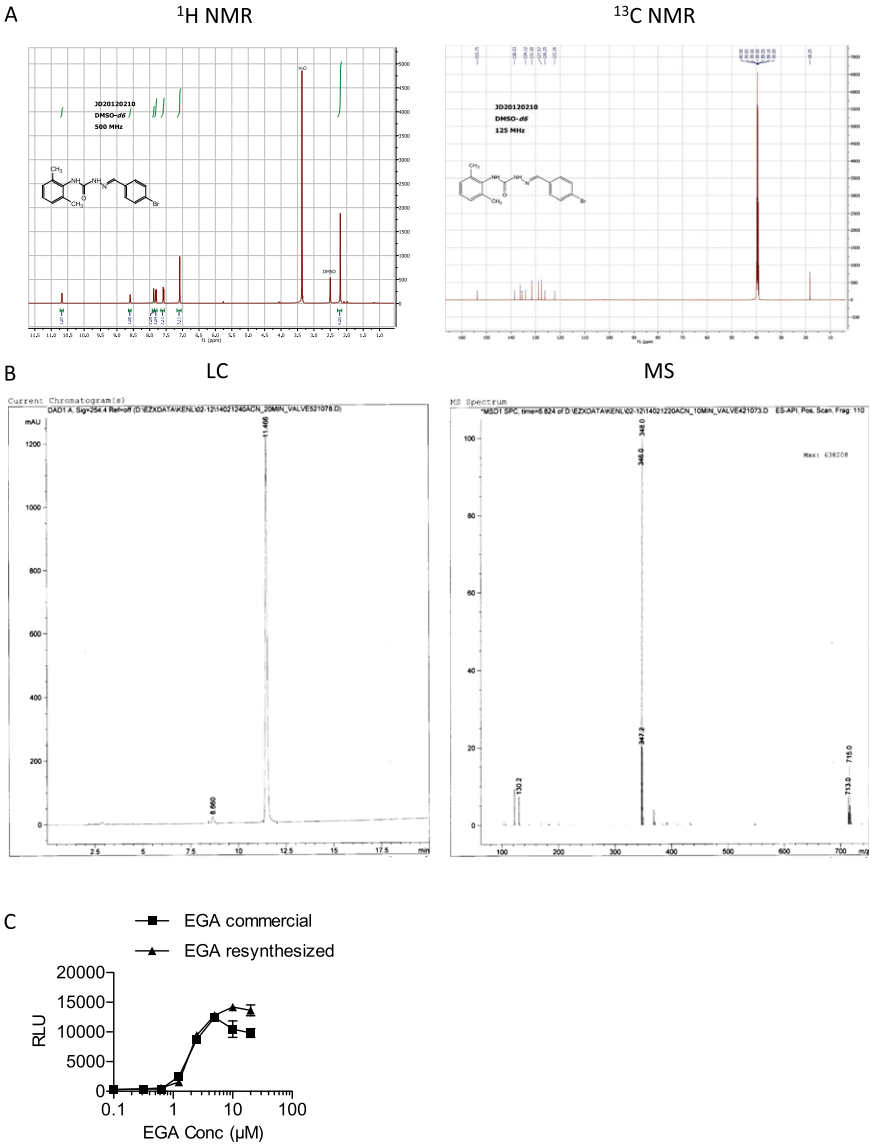

Fig. S2. Verification of 4-bromobenzaldehyde *N*-(2,6-dimethylphenyl)semicarbazone (EGA) structure and purity. (*A*) EGA, supplied by ChemBridge (compound ID: 5319257), was analyzed by ^{1}H and ^{13}C NMR to confirm compound structure. (*B*) Purity of commercially supplied EGA was determined by liquid chromatography–mass spectrometry (LC-MS). Chromatogram is shown on the left revealing a single major peak, which corresponds to the predicted mass of EGA as shown by mass spectrum on the right. (*C*) Resynthesis of EGA yielded similar activity as that obtained from the commercial source.

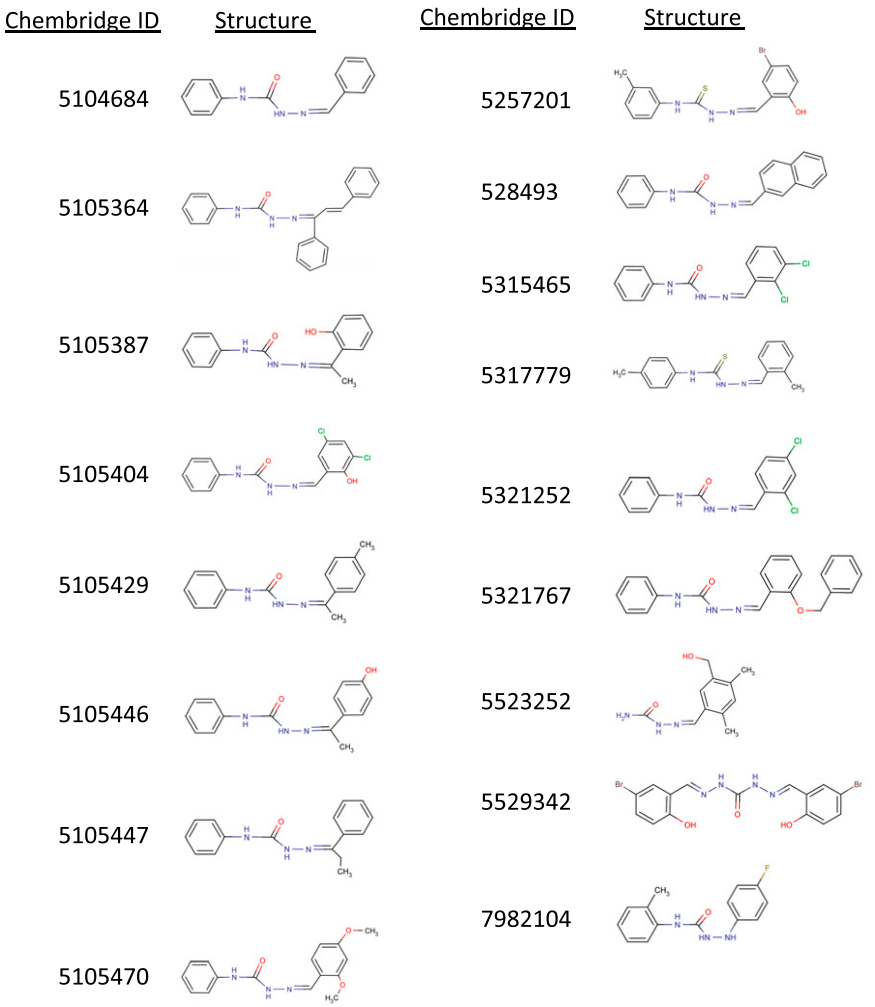

Fig. S3. Inactive analogs of EGA. Structures and ChemBridge identification numbers are presented for compounds that showed no activity against lethal toxin in a macrophage intoxication assay. Compounds listed here represent a subset of those identified by the Hit-2-Lead search function provided by ChemBridge as compounds in a library of 9×10^5 compounds with greater than 60% structural similarity with EGA.

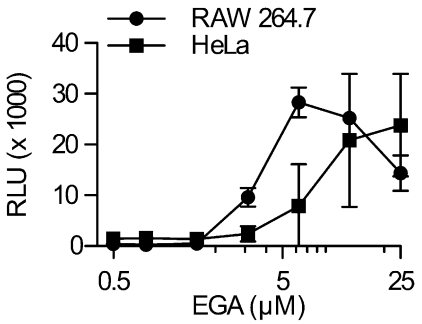

Fig. S4. EGA protects cells from PA + LFnDTA. RAW264.7 cells and HeLa cells were preincubated with a titration of EGA for 1 h, followed by a 48-h intoxication with 500 ng/mL PA + 1 ng/mL LFnDTA. Cell viability was measured via ATPlite.