

Inhibitors of the NF- κ B Activation Pathway from *Cryptocarya rugulosa*

Tamara L. Meragelman, Dominic A. Scudiero, R. Eric Davis, Louis M. Staudt, Thomas G.

McCloud, John H. Cardellina II^{*} and Robert H. Shoemaker

Screening Technologies Branch, Developmental Therapeutics Program, Division of Cancer Treatment and Diagnosis, National Cancer Institute, Frederick, MD; Molecular Target Screening Laboratory, SAIC-Frederick, Inc., Frederick, MD; Metabolism Branch, Center for Clinical Research-NCI, Bethesda, MD and Natural Products Support Group, SAIC-Frederick, Inc., Frederick, MD

Supporting Information

Figure S 1. ¹H NMR spectrum of rugulactone (**1**) in CDCl₃.

Figure S 2. HSQC spectrum of rugulactone (**1**) in CDCl₃.

Figure S 3. HMBC spectrum of rugulactone (**1**) in CDCl₃.

Figure S 4. Dereplication fingerprint of *C. rugulosa*.

Figure S 5. Time course toxicity assay of rugulactone (**1**) and cryptocaryone (**2**).

Figure S 6. Time course of dual reporter IKK assay of rugulactone (**1**) and cryptocaryone (**2**) in OCI-Ly19 cells.

Experimental conditions of time course toxicity assay.

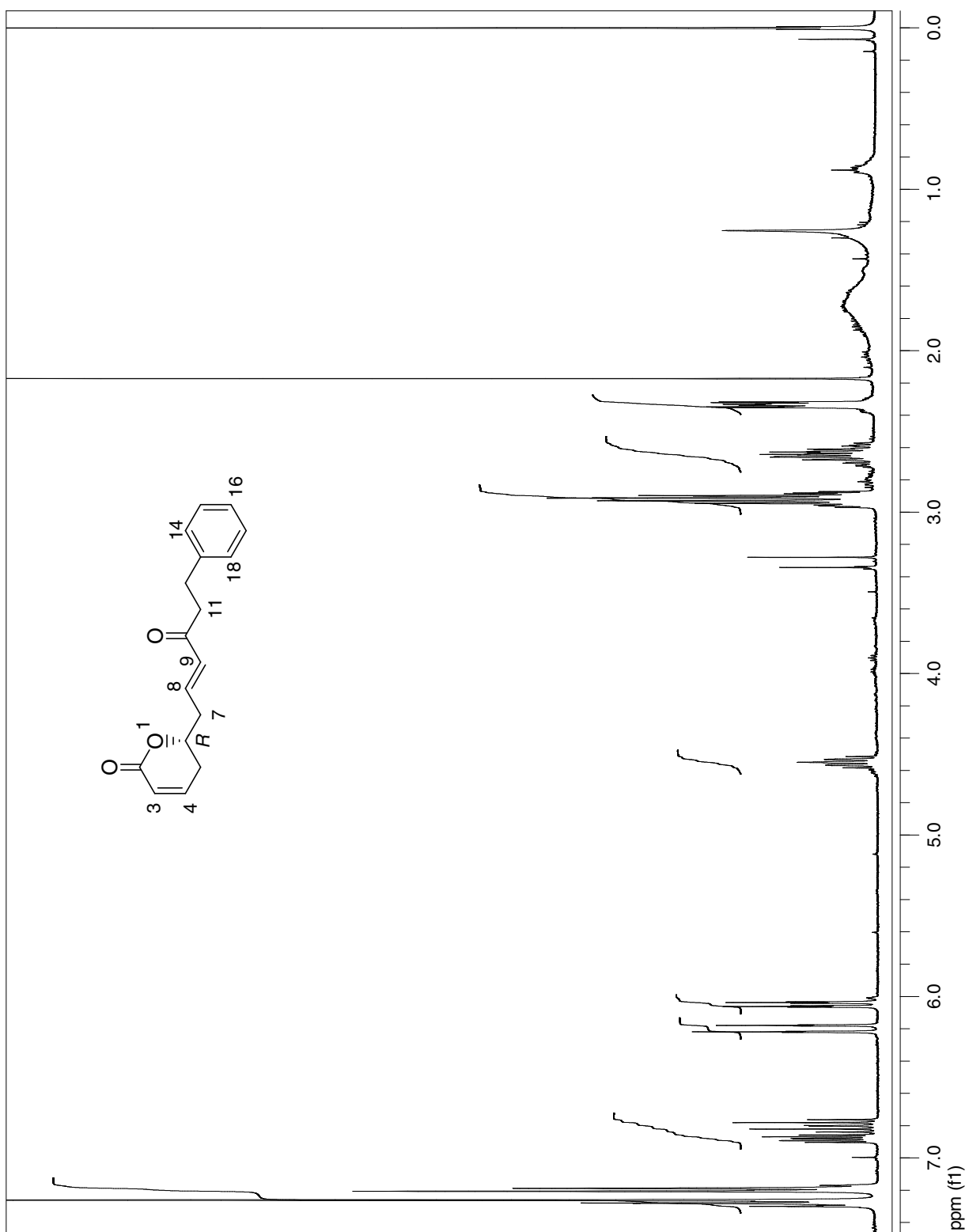


Figure S 1. ^1H NMR spectrum of rugulactone (**1**) in CDCl_3 .

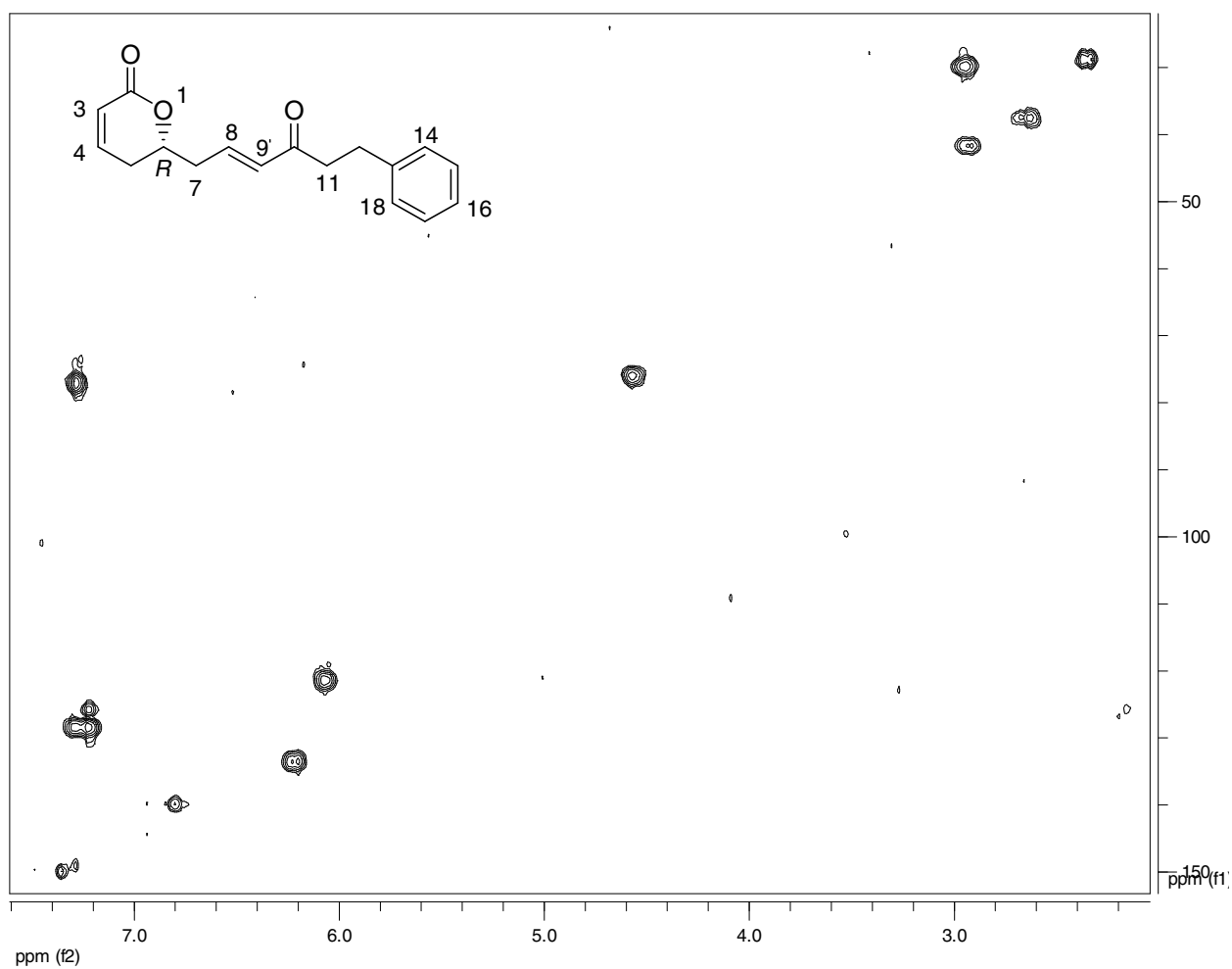


Figure S 2. HSQC spectrum of rugulactone (**1**) in CDCl₃.

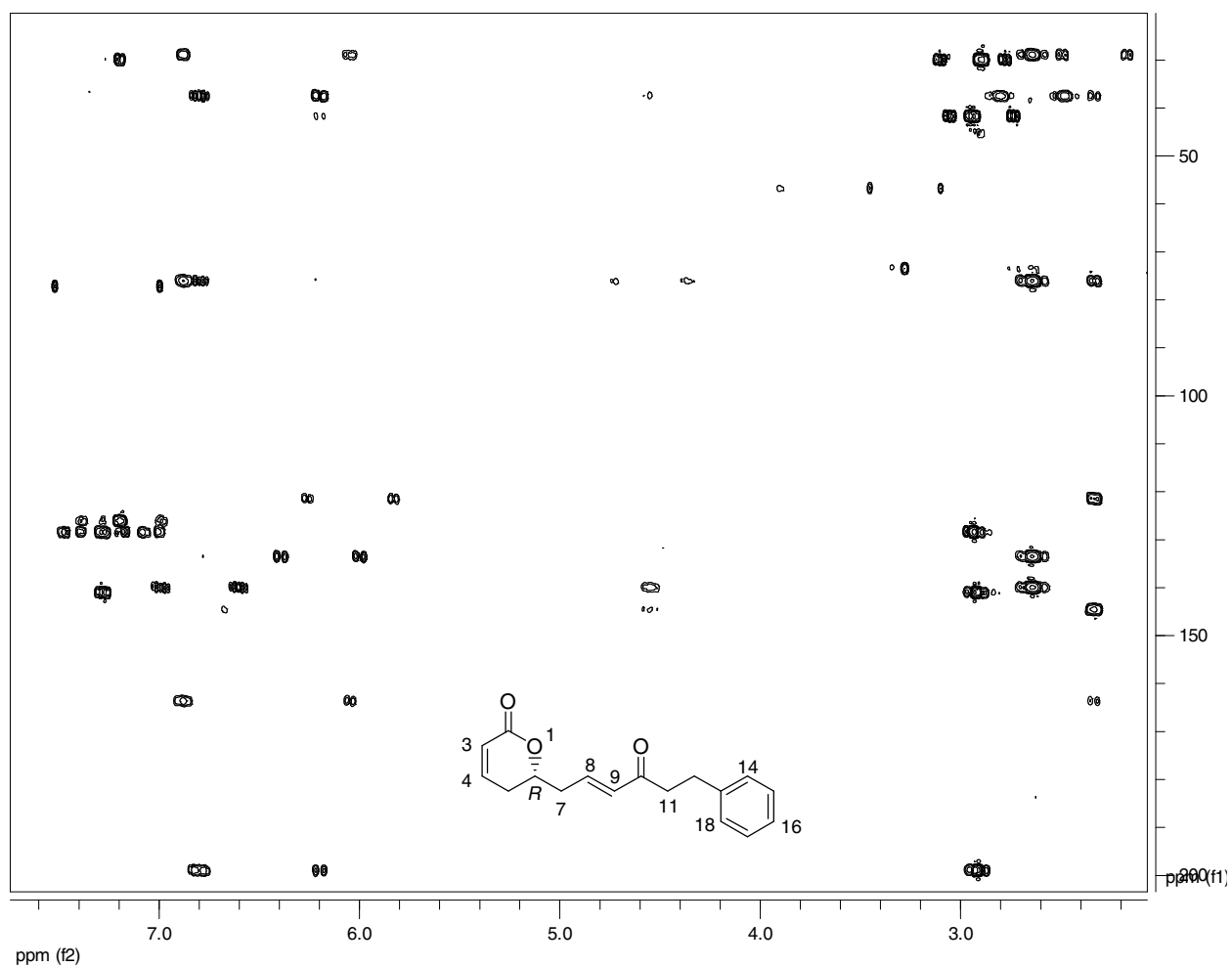


Figure S 3. HMBC spectrum of rugulactone (**1**) in CDCl₃.

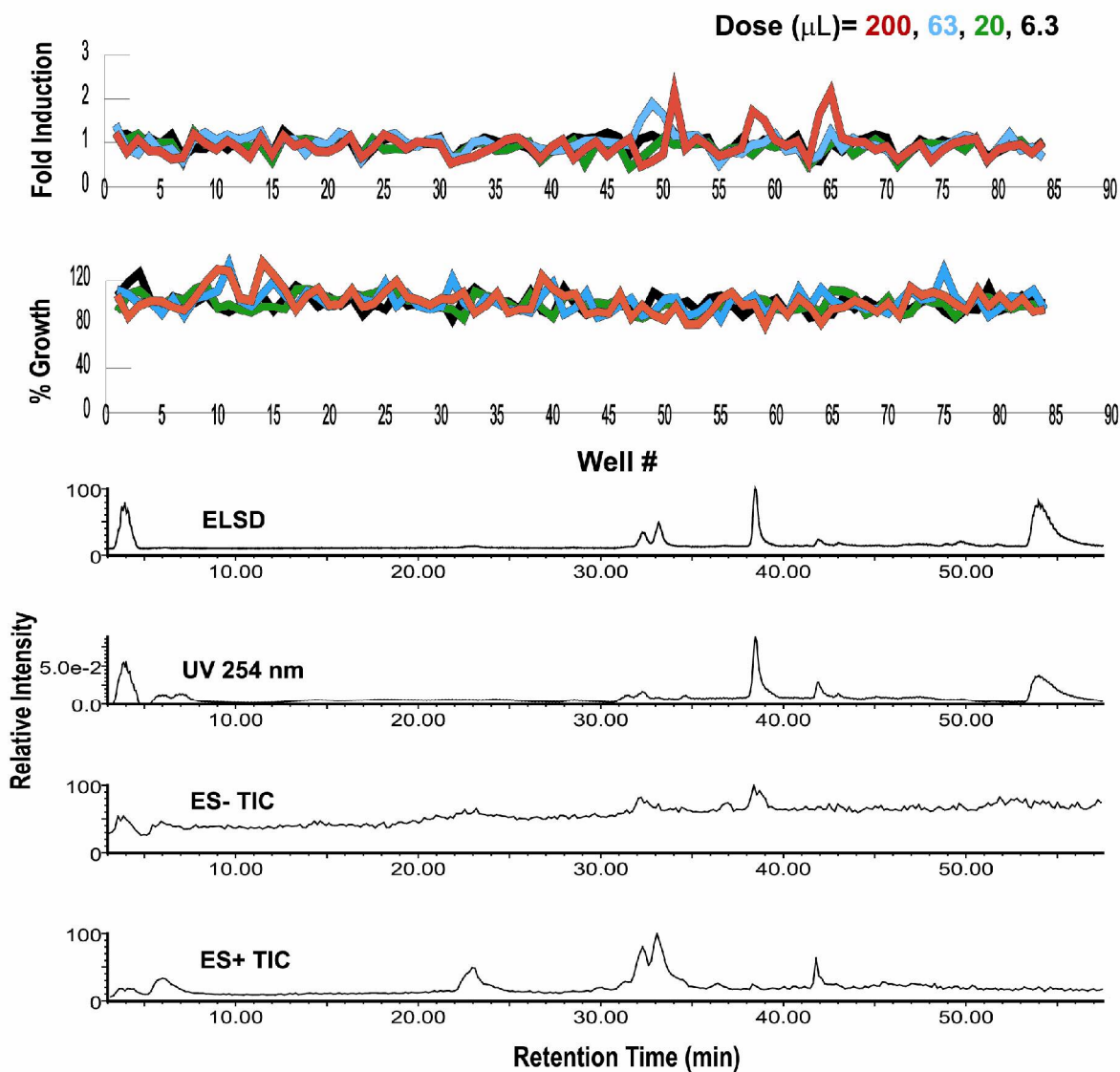


Figure S 4. Dereplication fingerprint of *C. rugulosa*. First and second graphics: Induction of the level of the I κ B-luciferase reporter protein in OCI-Ly3 cells and toxicity assay, respectively, for the 84 fractions collected at four different doses (200, 63, 20, 6.3 mL of column eluent). Third to sixth graphics: Chromatogram traces recorded during HPLC fractionation process.

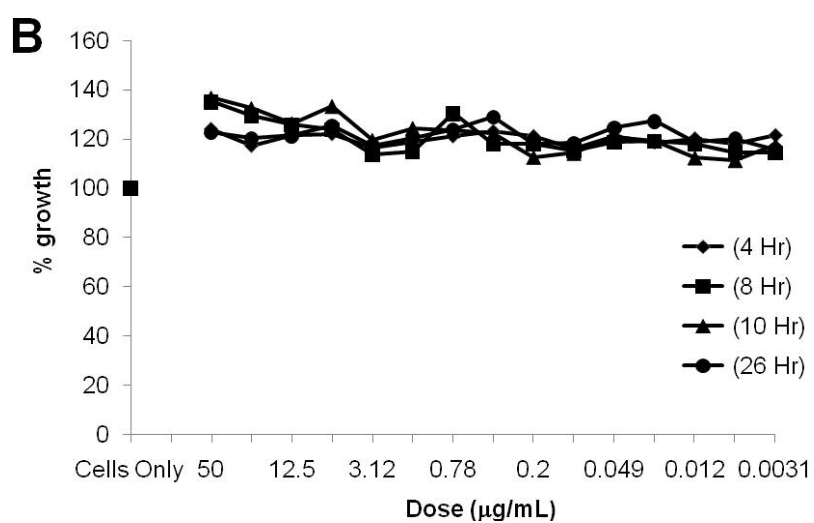
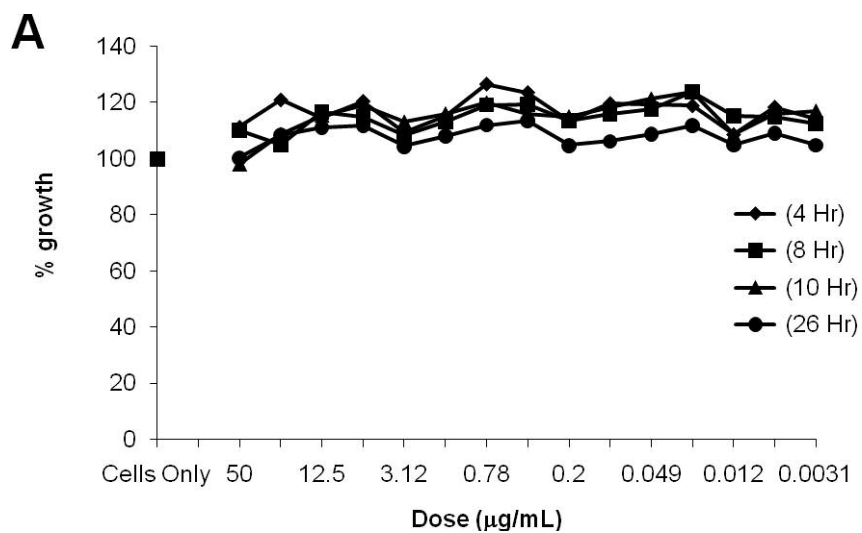


Figure S 5. Time course toxicity assay of rugulactone (**1**) (A) and cryptocaryone (**2**) (B).

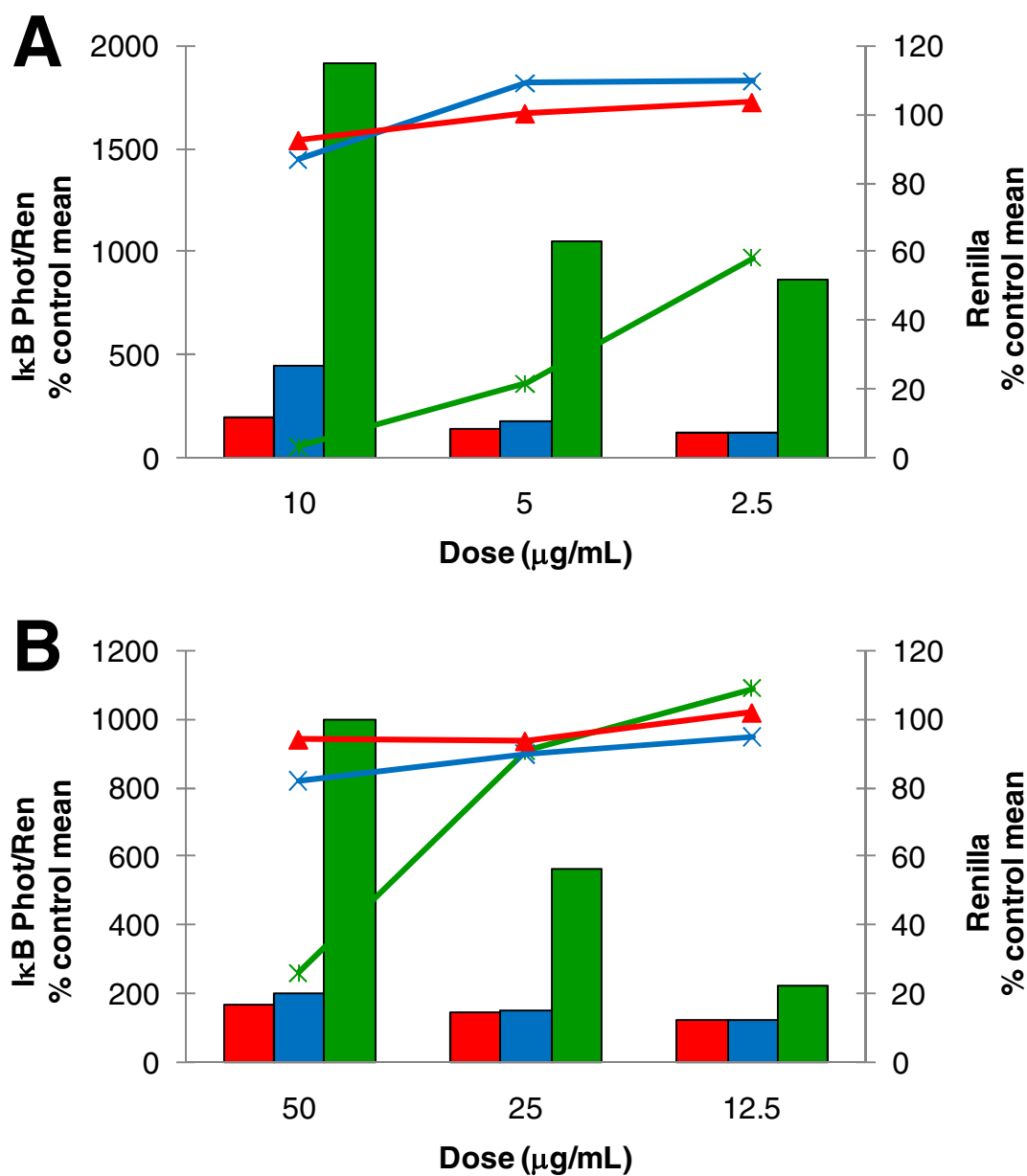


Figure S 6. Time course evaluation of dual-reporter IKK assay: IkB-*Photinus*/*Renilla* ratio (bars), *Renilla* reporter (lines) at 2.5 (red), 5 (blue) and 10 (green) hours. Rugulactone (**1**) was evaluated at 10, 5 and 2.5 µg/mL (A); cryptocaryone (**2**) was tested at 50, 25 and 12.5 µg/mL in OCI-Ly19 cells (B).

Experimental section.

Two different viability methodologies were utilized in parallel: ATP level by luminescence (Cell Titer Glo, Promega) and Alamar Blue fluorescence.

For the Cell Titer Glo assay, plates were removed from the incubator and allowed to equilibrate for 30 min at 24-26 °C in 5% CO₂ atmosphere, then 25 µL Cell Titer Glo were added to each well and luminescence readings were taken. For the Alamar Blue assay, 15 µL of Alamar Blue were added to each well four hours prior to the desired time point. Plates were returned to 5% CO₂ incubators at 37 °C, and then read.