Supporting Information to:

Soyasaponin Bh, a Triterpene Saponin Containing a Unique Hemiacetal-Functional Five-Membered Ring from *Glycine max* (Soybeans)

Zulfiqar Ali¹, Shabana I. Khan¹, Ikhlas A. Khan¹,²

Affiliation

¹ National Center for Natural Products Research, School of Pharmacy, University of Mississippi, University, Mississippi, USA
² Department of Pharmacognosy, Research Institute of Pharmaceutical Sciences, School of Pharmacy, University of Mississippi, University, Mississippi, USA

Correspondence

Ikhlas A. Khan
National Center for Natural Products Research
University of Mississippi
University
MS 38677
USA
Tel.: +1/662/915 7821
Fax: +1/662/915 7989
ikhan@olemiss.edu
Biological Assays

Assay for cell growth inhibition

The compounds were tested for cell growth inhibition activity against a panel of human solid tumor cells (SK-MEL malignant melanoma; KB oral epidermal carcinoma; BT-549 breast ductal carcinoma, and SK-OV-3 ovary carcinoma) as well as noncancerous monkey kidney fibroblast (Vero) and pig kidney epithelial cells (LLC-PK1) [1]. All cell lines were obtained from ATCC. Cells (25,000 cells/well) were seeded into the wells of a 96-well plate and incubated for 24 hours. Samples were added followed by incubation for 48 hours. The number of viable cells was determined using the supravital dye Neutral Red according to a modification of the procedure of Borenfreund et al [2]. Briefly, the cells were washed with saline and incubated for 3 hours with a solution of neutral red. The cells were washed again to remove extracellular dye. A solution of acidified ethanol was added to liberate the incorporated dye from viable cells and the absorbance was read at 450 nm.

Assay for estrogenic activity

Yeast cells (*Saccharomyces cerevisiae*) expressing the human estrogen receptor alpha were cultured in 25 mL of growth medium for 24 h at 30 °C in a shaking incubator [3]. For the assay, seeded assay medium was prepared by adding 1.5 mL of this culture and 250 µL of chlorophenol red β-D-galactopyranoside (CPRG, 10 mg/mL) to 25 mL of fresh growth medium. Samples were dissolved in DMSO to make 10 mM stock solutions and tested in a concentration range of 3.9 µM to 125 µM. 10 µL of serially diluted samples were transferred to the assay plate followed by addition of 190 µL of seeded assay medium to each well. A standard curve of 17β-estradiol, with concentrations ranging from 0.004 to 9.17 nM was added to each plate. The plate was shaken for 5 min and incubated for 48 hs at 30 °C. At the end of the incubation the plates were shaken, and the absorbance was measured at a dual wavelength of 540 – 630 nm on a Biotek EL312 plate reader. Dose curves were generated by plotting absorbance vs concentration and EC_{50} values were calculated using GraphPad Prism. % Maximal 17β-estradiol response = 100 (maximum absorbance of sample/maximum absorbance of 17β-estradiol).
References

