Supporting Information to:

A New Cytotoxic Amide from the Stem Wood of *Hibiscus tiliaceus*

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Cytotoxicity assay

P-388 (mouse lymphocytic leukemia) cells were kindly provided by Prof. J. M. Pezzuto, of the University of Illinois at Chicago; HT-29 (human colon carcinoma) was purchased from the American Type Culture Collection (ATCC; Manassas, VA, USA).

P-388 cells were cultured in Fisher’s medium supplemented with 10% heat-inactivated (56 °C for 30 min) fetal calf serum (FCS, GIBCO; Gaithersburg, MD, USA). HT-29 cells were maintained in Rosewell Park Memorial Institute (RPMI) 1640 Medium (GIBCO) containing 10% heat-inactivated FCS. All cell lines were maintained in an incubator at 37 °C in humidified air containing 5% CO2.

The cytotoxic activities of compounds against P-388 and HT-29 were assayed by a modification of the MTT [3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyltetrazolium bromide] colorimetric method [11]. For P-388 cells, 200 µL cultures were established at 1500 cells/well in 96-well tissue culture plates (Falcon; Franklin, NJ, USA). Compounds (dissolved in 0.5% DMSO) were dispersed to established cultures at eight concentrations in triplicate. After three days of incubation, P-388 cells were enumerated with MTT (Sigma; St. Louis, MO, USA). To measure the cytotoxic activities of purified compounds against HT-29 cells, each cell line was initiated at 1000 cells/well in 96-well microtiter plates. Eight concentrations (triplicate) of test compounds (dissolved in 0.5% DMSO) encompassing a 128-fold range were added to each cell line. HT-29 cells were enumerated using MTT after exposure to test compounds for 6 days. Fifty µL of 1 mg/mL MTT were added to each well, and plates were incubated at 37 °C for a further 5 h. Formazan crystals were redissolved in DMSO (Merck; Darmstadt, Germany) for 10 min with shaking, and the plate was read immediately on a microtiter plate reader (Dynatech; Chantilly, VA, USA) at a wavelength of 540 nm. The ED50 was defined as the concentration of the test compound resulting in a 50% reduction of absorbance compared to untreated cells in the MTT assay. The assays were repeated three times. The anticancer agent mithramycin and 0.5% DMSO were used as the positive control and solvent control, respectively.
Fig. S1 The structural formulae of known compounds 3 – 13.