SUPPORTING INFORMATION

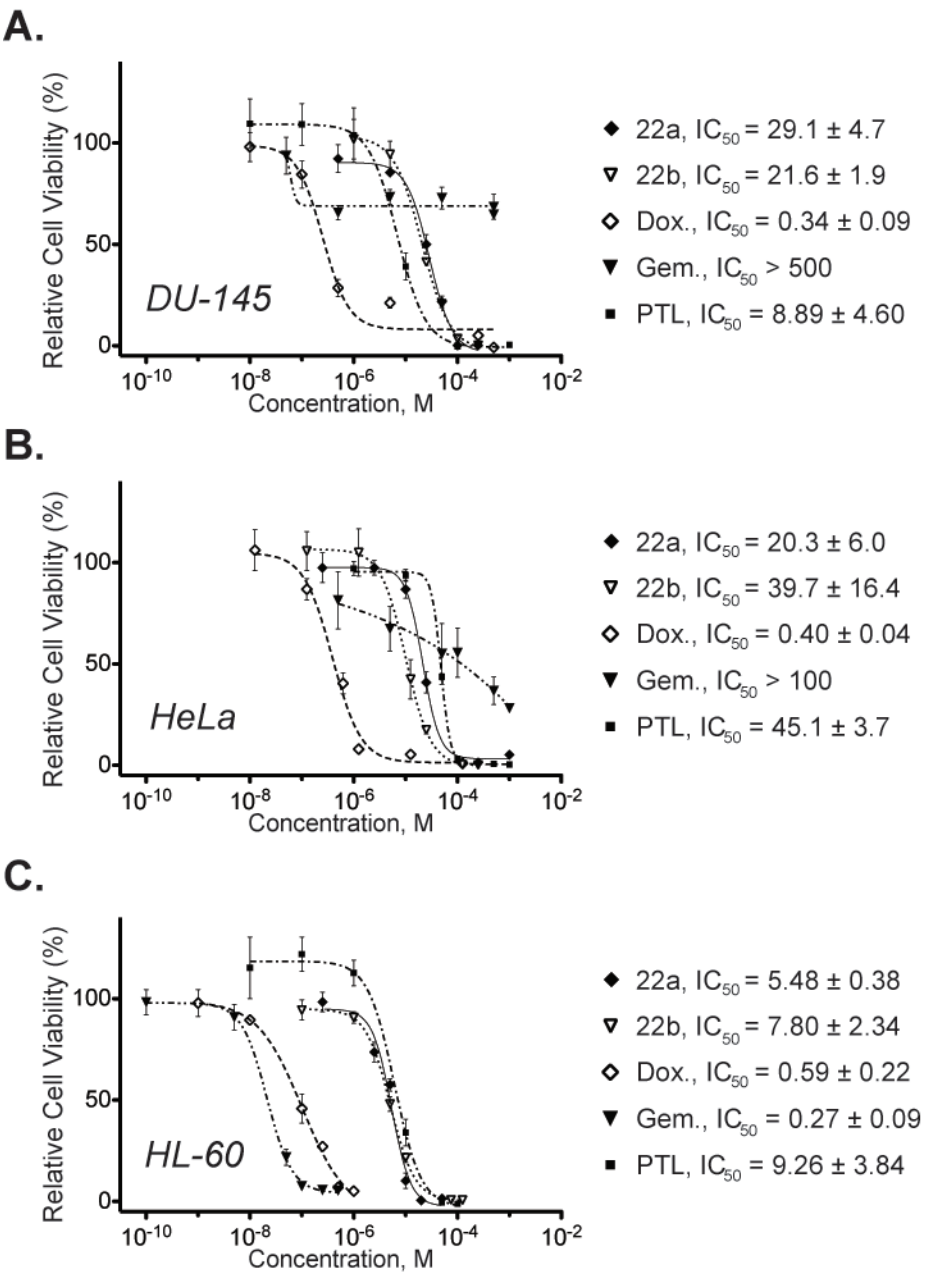
A Redox Economical Synthesis of Bioactive 6,12-Guaianolides

Bo Wen, Joseph K. Hexum, John C. Widen, Daniel A. Harki and Kay M. Brummond**

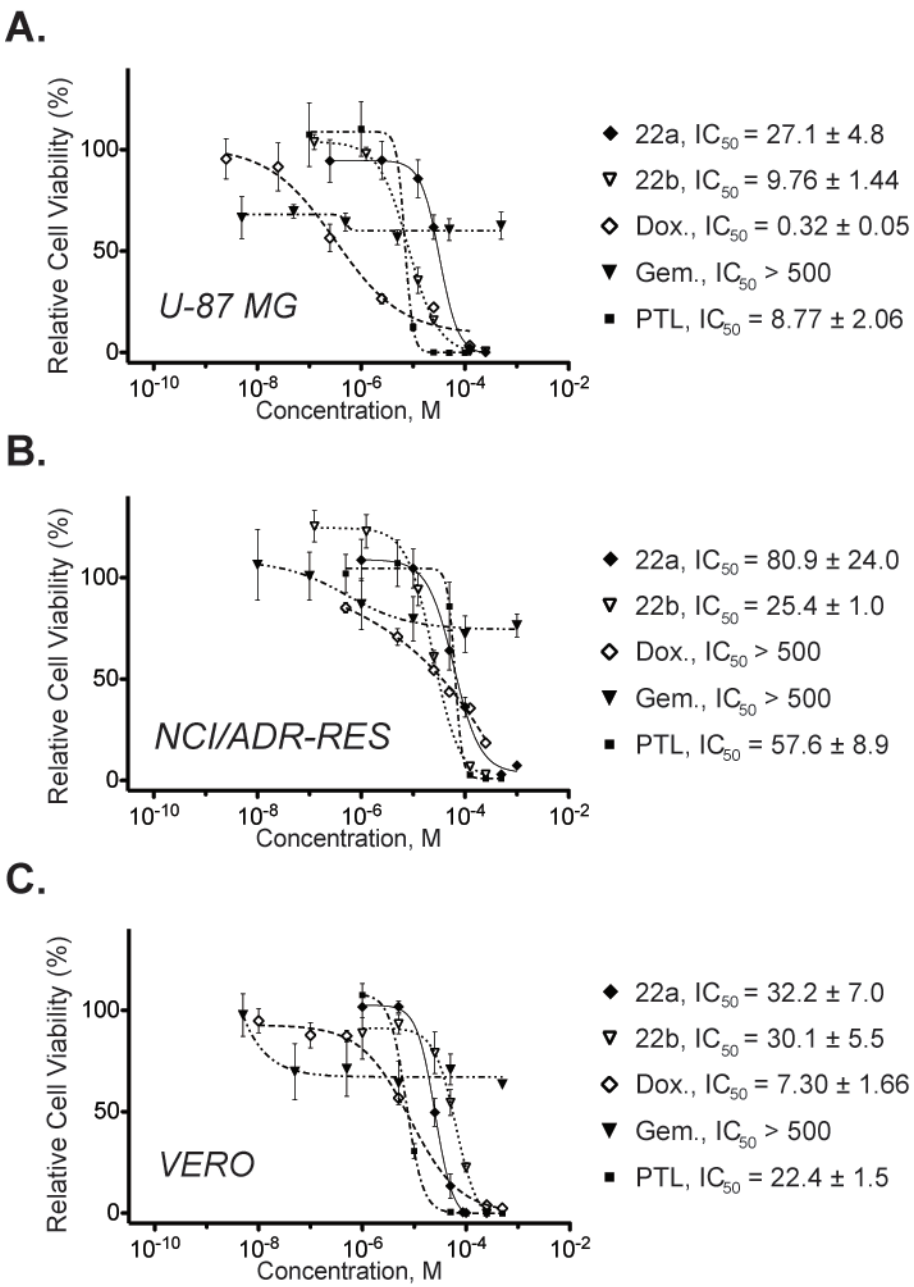
Department of Chemistry, University of Pittsburgh, Pittsburgh, PA 15260

Department of Medicinal Chemistry, University of Minnesota, Minneapolis, MN 55414

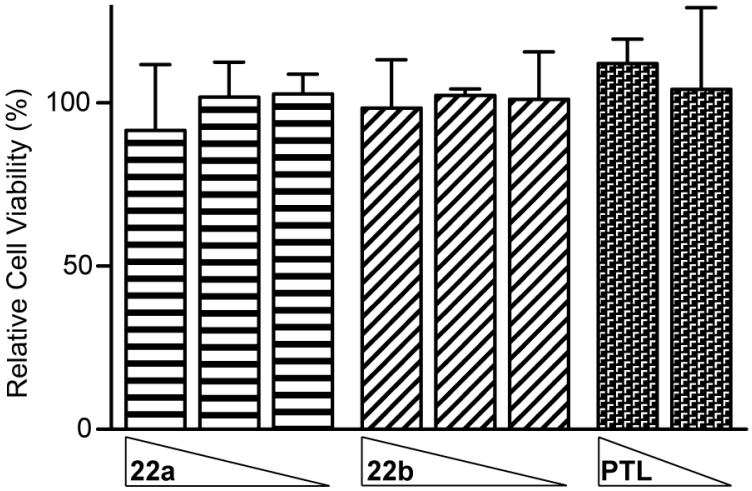
TABLE OF CONTENTS FOR SUPPORTING INFORMATION ..... PAGE



I. Supplemental Figure 1. Representative IC_{50} curves for **22a** (closed diamonds), **22b** (open triangles), parthenolide (PTL; closed squares), doxorubicin (Dox.; open diamonds), and gemcitabine (Gem.; closed triangles) against (A) DU-145, (B) HeLa, and (C) HL-60 cells. Viability was determined via Alamar Blue assay. Error bars represent the S.D. of triplicate data. IC_{50} values are in micromolar (μM) units.



II. Supplemental Figure 2. Representative IC_{50} curves for **22a** (closed diamonds), **22b** (open triangles), parthenolide (PTL; closed squares), doxorubicin (Dox.; open diamonds), and gemcitabine (Gem.; closed triangles) against (A) U-87 MG, (B) NCI/ADR-RES, and (C) Vero cells. Viability was determined by Alamar Blue assay. Error bars represent the S.D. of triplicate data. IC_{50} values are in micromolar (μM) units.



III. Supplemental Figure 3. Representative cytotoxicity data for **22a**, **22b**, and parthenolide (PTL) against A549/NF-κB-luc cells. Compounds **22a** and **22b** were tested at 20, 10, and 1 μM concentrations and PTL was tested at 10 and 1 μM concentrations. Cell viability was determined using the Alamar Blue assay. For each compound, < 10% cell death was observed at each tested concentration.

IV. Cell Culture

All cell lines were maintained in a humidified 5% CO_2 environment at 37 °C. HL-60 cells (ATCC, CCL-240) were cultured in IMDM media (Cellgro) supplemented with 20% fetal bovine serum (FBS, Gibco), penicillin (100 I.U./mL), and streptomycin (100 μg/mL, ATCC) at a density of 1×10^5 – 1×10^6 cells/mL. HeLa cells (ATCC, CCL-2) were cultured in MEM media (Cellgro) supplemented with 10% FBS, penicillin (100 I.U./mL), and streptomycin (100 μg/mL ATCC). U-87 MG cells (ATCC, HTB-14) were cultured in MEM media (Cellgro) supplemented with 10% FBS, penicillin (100 I.U./mL), and streptomycin (100 μg/mL ATCC). NCI/ADR-RES cells were cultured in RPMI-1640 media (Cellgro) supplemented with 10% FBS, penicillin (100 I.U./mL), and streptomycin (100 μg/mL ATCC). DU-145 cells and Vero cells were cultured as described previously.[1]

V. Preparation of Stock Solutions for Compounds

Stock solutions for compounds were prepared in DMSO and stored at -20° C when not in use. Compound purities were assessed frequently by analytical reverse-phase HPLC analysis and fresh solutions were prepared as needed. All tested compounds were > 95% pure. Doxorubicin hydrochloride was purchased from Fisher Scientific (Cat. #BP251610), gemcitabine hydrochloride was purchased from USP (Cat. #1288463), and parthenolide was purchased from Enzo Life Sciences (Cat. #BML-T113-0250). Parthenolide was purified by SiO_2 chromatography (0-40% ethyl acetate in hexanes) prior to biological testing.

[1] Hexum, J. K.; Tello-Aburto, R.; Struntz, N. B.; Harned, A. M.; Harki, D. A. *ACS Med. Chem. Lett.* **2012**, *3*, 459-464

VI. Cell Culture Cytotoxicity Assays

HL-60 cells were seeded at a density of 10,000 cells/well in cell culture media (50 µL) in standard 96-well plates (Costar) 24 h prior to treatment. HeLa, U-87 MG, NCI/ADR-RES, Vero, and DU-145 cells were seeded at a density of 5,000 cells/well in cell culture media (50 µL) in standard 96-well plates (Costar). In vitro Alamar Blue (Invitrogen) cytotoxicity assays were performed as described previously.[1] The DMSO concentration in each assay plate well was kept constant at 0.5%. Due to overlapping absorbance of doxorubicin (580 nm) with Alamar Blue at concentrations greater than 500 µM, absorbance values were obtained before the addition of Alamar Blue. The resulting values were subtracted from the absorbance values after incubation with Alamar Blue. The corrected values were used to calculate the IC_{50}.

VII. NF-κB Reporter Assays

This assay was performed as described previously.[1]