

Figure 1. Selected 2D NMR correlations of 1.

Figure 2. Selected 2D NMR correlations of 2.

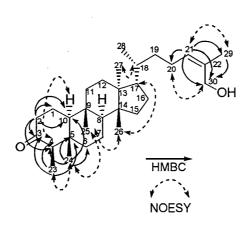


Figure 3. Selected 2D NMR correlations of 3.

Cell culture and drug treatment: Vincristine-sensitive and vincristine-resistant human epidermoid carcinoma cells, KB/S and KB/VJ300, were supplied by Prof. Kuwano (Dept. of Biochemistry, Kyushu University School of Medicine). Human renal carcinoma cells, KU19-20, were obtained from Prof. Tachibana (Dept. of Urology, Tokyo Medical College). KB/S cells, KB/VJ300 cells, and human promyelocytic leukemia HL-60 cells were maintained in culture flasks in Eagle's MEM medium supplemented with 10% fetal bovine serum, 100 U/mL of penicillin, and 100 µg/mL of streptomycin. KU19-20 cells were maintainted in RPMI-1640 medium supplemented with 10% fetal bovin serum, 5 μ g/mL of insulin, 5 μ g/mL of transferrin, 5 ng/mL of sodium selenite, 100 U/mL of penicillin, and 100 µg/mL of streptomycin. HL-60 cells were maintained in RPMI-1640 medium supplemented with 10% fetal bovine serum, 100 U/mL penicillin, and 100 µg/mL of streptomycin. For the in vitro drug treatment experiments, tumor cells (2 x 10⁴ cells/mL for KB cells and 5 x 10⁴ cells/mL for KU9-20 cells) were seeded in 0.2 mL of culture medium/well in 96well plates (Coster-Corning Inc., NY, USA). After 24 hours of incubation at 37 °C in a 5% CO₂-95% air atmosphere, a 5 µL solution of the triterpenoids dissolved in methanol was added to each The cells were treated in triplicate with graded concentrations of the triterpenoids and were then incubated for a further 72 hours. In the case of HL-60 cells, the cells (5 x 10⁵ cells/mL) were seeded in 0.2 mL of culture medium/well in 96-well plates, mixed with 5nL of the triterpenoids, and incubated for 72 hours. In order to determine the growth-inhibitory effect of triterpenoids, we utilized the alamarBlueTM assay (Biosource, CA, USA). Briefly, 20 µL of alamarBlue was added to the culture medium 6 hours before the end of incubation. Then, fluorescence intensity was measured with an excitation wavelength of 530 nm and emission wavelength of 590 nm using a CytoFluor II Fluormeter (Perceptive, USA). The sensitivity of treatment is expressed as the IC₅₀ value, the concentration required for 50% cell death. The ability of terpenoids to induce the differentiation of HL-60 cells into monocyte/macrophages was assessed by observation under a phase-contrast microscope.