Karwinaphthopyranones isolated from the Fruits of *K. parvifolia* and Their Cytotoxic Activities

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CYTOTOXICITY STUDIES

Materials

DMEM, RPMI-1640, antibiotic-antimycotic 100X solution, MEM non-essential amino acids solution (100X), 0.25% trypsin-EDTA solution, fetal bovine serum (FBS), and newborn calf serum (NBCS) were purchased from GIBCO/BRL (Grand Island, NY). Sterile plastic material for tissue culture was purchased from Corning (Corning, NY). All other chemicals were purchased from Sigma Aldrich (St. Louis, MO).

Tumor cells culture

All tumor cell lines were purchased from ATCC (American Type Culture Collection). U373 cells were cultured in DMEM medium supplemented with 4500 mg/l glucose, 5% newborn bovine calf serum (NBCS), glutamine (2 mM), pyruvate (1 mM), antibiotic-antimycotic, and MEM non-essential amino acids 1X. The other cell lines were cultured in RPMI-1640 medium supplemented with 5% NBCS and L-glutamine (2 mM).

Cell viability assay

Cell viability was determined by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltentrazolium bromide (MTT) reduction assay, which is used for cell proliferation and cytotoxicity assays.[1] MTT is

[1] (a) Mosmann, T. *J. Immunol. Methods* **1983**, *65*, 55–63.

reduced in metabolically active cells to yield an insoluble purple formazan product. Cells were cultured in 96-well culture dishes and were exposed to different compounds for 48 h. Afterwards, 20 μl/well of a MTT solution (5 mg/ml) was added. Four hours later, the supernatants were discarded and 100 μl/well of acidic isopropyl alcohol (0.04 N HCl) was added to dissolve the formazan. Optical density (OD) was measured on the multiplate spectrophotometer (BIO-TEK Instruments) at 570 nm. Lethal concentration for 50% maximum cell viability (LD_{50}) was determined.

(b) Tao, Z.; Zhou, Y.; Lu, J.; Duan, W.; He, X.; Lin, L.; Ding, J.; Qin, Y. *Cancer Biol. Ther.* **2007**, *6*, 691-696.