Supporting Information to:

Diterpenes from “Pini Resina” and their Preferential Cytotoxic Activity under Nutrient-Deprived Conditions

Syed Faisal Haider Zaidi¹
Suresh Awale¹
Surya Kant Kalauni¹
Yasuhiro Tezuka¹
Hiroyasu Esumi²
Shigetoshi Kadota¹

Affiliation: ¹ Institute of Natural Medicine, University of Toyama, Toyama, Japan
² Investigative Treatment Division, National Cancer Research Institute East, Chiba, Japan

Correspondence: Prof Dr. Shigetoshi Kadota
Institute of Natural Medicine
University of Toyama
2630 Sugitani
Toyama 930-0194
Japan
Phone: +81-76-434-7625
Fax: +81-76-434-5059
E-mail: kadota@ms.toyama-mpu.ac.jp
Assay Protocol of Preferential Cytotoxic Activity

Cell lines and nutrient starvation
The human pancreatic cancer cell line PANC-1 obtained from the American Type Culture Collection (Manassas, VA, USA), was maintained in DMEM (Nissui, Tokyo, Japan) supplemented with 10% FBS (Sigma, St. Louis, MO, USA), 2% L-glutamine, 1% penicillin, and 1% streptomycin stock solutions. The medium was routinely changed every 3 days and the cells were passaged by trypsinization until they attained confluence. Nutrient starvation was achieved by culturing the cells in nutrient-deprived medium as previously described [1], [2], [3]. Briefly, the composition of the nutrient-deprived medium was as follows: 265 mg/L CaCl₂ (2 H₂O), 0.1 mg/L Fe(NO₃)₉H₂O), 400 mg/L KCl, 200 mg/L MgSO₄ (7 H₂O), 6,400 mg/L NaCl, 700 mg/L NaHCO₃, 125 mg/L NaH₂PO₄, 15 mg/L phenol red, 25 mmol/L HEPES buffer (pH 7.4), and MEM vitamin solution (Life Technologies Inc.; Rockville, MD, USA); the final pH was adjusted to 7.4 with 10% NaHCO₃. For supplementation with glucose, D-glucose was added at a concentration of 1 mg/mL. For amino acid supplementation, stock solutions (200 mmol/L L-glutamine solution, MEM amino acids solution, and MEM non-essential amino acids solution; Life Technologies) were added at a concentration of 1%.

Preferential cytotoxicity under nutrient-deprived conditions
The cytotoxicity assay was done with the cell counting kit-8 from Dojindo Molecular Technologies, Inc (Dojindo, Japan). Briefly, PANC-1 cancer cells were seeded in 96-well plates (1 × 10⁴ per well) and incubated in fresh DMEM medium at 37 °C under a 5% CO₂/95% air for 24 hours. The cells were then washed with PBS and the medium was changed to either DMEM or nutrient-deprived medium followed by immediate addition of serial dilutions of the test samples. After 24-hour incubation, the cells were washed again with PBS, then 100 µL of DMEM medium with 10% WST-8 solution were added to the wells, and the plate was incubated for a further 2 hours. Then, the absorbance of the wells at 450 nm was measured. The samples that were highly and selectively cytotoxic against the PANC-1 cells in nutrient-deprived medium were subjected to further purification. Cell viability was calculated from the mean values of data from three wells.

Cell viability (%) = \[\left\{\frac{\text{Abs(test sample)} - \text{Abs(blank)}}{\text{Abs(control)} - \text{Abs(blank)}}\right\} \times 100\]
where $\text{Abs}_{\text{(test sample)}} = \text{cells} + \text{medium} + \text{test sample} + \text{cell counting kit 8 (CCK-8)}$; $\text{Abs}_{\text{(blank)}} = \text{medium} + \text{CCK-8}$; $\text{Abs}_{\text{(control)}} = \text{cells} + \text{medium} + \text{CCK-8}$.

Fig. 1S Effect of diterpenes (1 – 15) on the survival of PANC-1 cells in NDM (- ● -) and DMEM (- ▲ -). Each point represents the mean of triplicate experiments. Vertical bars indicate standard deviations (SD).
References

