Cytotoxic Chemical Constituents from the Roots of *Cimicifuga foetida*

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Cell lines and culture

The human hepatoma cell line HepG2, human breast carcinoma cell line MCF7, human colon carcinoma cell line HT29 and human gastric carcinoma cell line MKN28 were obtained from the American Type Culture Collection (ATCC). All cell lines were grown in RPMI-1640 medium (GIBCO) supplemented with 10% heat-inactivated bovine serum, 2mM L-glutamine, 10^5 IU/L penicillin, 100 mg/L streptomycin and 10mM HEPES pH 7.4. Cells were kept at 37°C in a humidified 5% CO₂ incubator.

Cell growth inhibition assay

Growth inhibition of compounds **1-7** on human tumor cells HepG2, MCF7, HT29 and MKN28 was measured by microculture tetrazolium (MTT) assay [1], [2] with minor modification [3]. Briefly, tumor cells were seeded into 96-well microculture plates and allowed to adhere for 24 h before drug addition. The cell densities were selected based on the results of preliminary tests to maintain the control cells in an exponential phase of growth during the period of the experiment and to obtain a linear relationship between the optical density and the number of viable cells. Each tumor cell line was exposed to compounds **1-7** at 0.01, 0.1, 1, 10 and 100 µg/ml concentrations for 72h and each concentration was tested in triplicate. At the end of exposure, 20 µl of 5 g/L MTT [3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide, Sigma Chemical Co.] was added to each well and the plates were incubated for 4 h at 37°C, then “triplex solution (10% SDS - 5% isobutanol - 0.012 M HCl)” was added and the plates were incubated for 12-20 h at 37°C. The optical density (OD) was read on a plate reader at a wavelength of 570 nm. Media and DMSO control wells, in which albaconol was absent, were included in all the experiments in order to eliminate the influence of DMSO. The inhibitory rate of cell proliferation was calculated by the following formula: Growth inhibition (%) = $(OD_{\text{control}} - OD_{\text{treated}}) / OD_{\text{control}} \times 100\%$. The cytotoxicity of

compounds **1-7** on tumor cells was expressed as IC₅₀ values (the drug concentration reducing by 50% the absorbance in treated cells, with respect to untreated cells) that was calculated by LOGIT method.

References

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