SUPPLEMENTARY MATERIAL

***In vitro* evaluation of cytotoxic and mutagenic activity of avarol**

Boris Pejin^{ab*}, Carmine Iodice^a, Vesna Kojic^c, Dimitar Jakimov^c, Milica Lazovic^d, and Giuseppina Tommonaro^a

^aNational Research Council of Italy, Institute of Biomolecular Chemistry, CNR-ICB, Pozzuoli-Naples, Italy; ^bUniversity of Belgrade, Institute for Multidisciplinary Research - IMSI, Department of Life Sciences, Serbia; ^cUniversity of Novi Sad, Oncology Institute Vojvodina, Sremska Kamenica, Serbia; ^dUniversity of Belgrade, Faculty of Physical Chemistry, Serbia

*Corresponding author. Email: brspjn@gmail.com & borispejin@imsi.rs (B. Pejin)

Abstract

The cytotoxicity of avarol, a main secondary metabolite of the Mediterranean sponge *Dysidea avara*, was *in vitro* screened by MTT assay against four human tumour cell lines. The colon HT-29 tumour cells practically showed to be the only sensitive ones towards this organic compound. No toxicity was found against the fetal lung fibroblast MRC-5 cells at the concentrations tested. In comparison with doxorubicin, used as a positive control, avarol actually exhibited at least 588-fold less toxicity towards normal MRC-5 cells. Finally, comet assay indicated that DNA fragmentation was almost 5-fold higher upon the treatment with doxorubicin, compared to avarol. The obtained results have actually confirmed that avarol scaffold may contribute to development of new cytostatics inspired by Nature.

Keywords: *Dysidea avara*; sesquiterpenoid hydroquinone; human tumours; comet assay

Experimental

Biological material

Avarol was isolated from the sponge *Dysidea avara* Schmidt (Dictyoceratida), collected in the Bay of Naples, Italy (Minale et al. 1974; De Rosa et al. 1976).

Cell lines

Five human cell lines were used in the study: HS-294T, HT-29, MCF-7, MRC-5 and PC-3. The cell density (number of cells per unit volume) and percentage of viable cells were performed by trypan blue dye exclusion assay (Bogdanović et al. 1994).

MTT assay

Cytotoxicity was evaluated by tetrazolium colorimetric MTT assay (Mosmann 1983). Three hours before the end of incubation period 10 μ L of MTT solution (5 mg/mL) was added to all wells and plates were incubated for 3 h at 37 °C, after which medium and MTT were removed by suction. The formazan product was then solubilised in 100 μ L 0.04 M HCl of isopropanol. After a few minutes at room temperature, the plates were read on a spectrophotometer plate reader (Multiscan MCC340, Labsystems) at 540/690 nm. The wells without cells containing complete medium and MTT only acted as blank. Cytotoxicity was expressed as a percent and calculated according the formula:

$$\% C = (1 - (\text{OD}_{\text{test}} - \text{OD}_{\text{blnk}}) / (\text{OD}_{\text{ctrl}} - \text{OD}_{\text{blnk}})) \times 100,$$

where OD_{ctrl} is for cells+medium+MTT.

Data analysis

Two independent experiments were set out with quadruplicate wells for each concentration of the compound. IC₅₀ value defines the dose of compound that inhibits cell growth by 50 %. The IC₅₀ of the extract was determined by Median effect analysis.

Comet assay

A modification of the alkaline version of the comet assay was performed (Merk & Speit 1998) using the K-562 tumour cells (myelogenous leukemia) whose viability was determined by trypan blue dye exclusion assay. The mean value of the comet tail moment was determined using Microsoft Excel computer program.

References

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