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Dihydro-resveratrol—A potent dietary polyphenol

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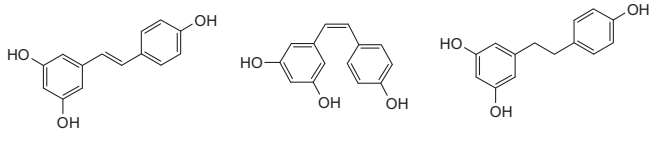
ABSTRACT

Dihydro-resveratrol (*dihydro*-**R**), a prominent polyphenol component of red wine, has a profound proliferative effect on hormone-sensitive tumor cell lines such as breast cancer cell line MCF7. We found a significant increase in MCF7 tumor cells growth rates in the presence of picomolar concentrations of this compound. The proliferative effect of *dihydro*-**R** was not observed in cell lines that do not express hormone receptors (MDA-MB-231, BT-474, and K-562).

Among dietary polyphenols, resveratrol (**R**) has received special attention due to its diverse and potentially beneficial biological properties.[1–9] **R** has two stereoisomers, *trans*-**R** and *cis*-**R**. *Trans*-**R** is a common component of fruits and berries, particularly grapes (*Vitis vinifera*), where it is formed as a response to the stress of weather conditions, microbe infections, or direct sunlight.[10,11] The compound is currently on the market as a popular dietary supplement and is under clinical trials for treatment of some types of cancer, metabolic syndrome, Alzheimer's disease, type 2 diabetes, obesity, neurologic syndrome, and some symptoms of aging.[1–7] *Cis*-**R** has been detected as a minor component in some berries, grapes, and wines along with *trans*-**R**.[12–14] *Cis*-**R** is believed to have similar bioactivity but has not been thoroughly investigated yet.



Trans-Resveratrol *Cis*-Resveratrol *Dihydro*-Resveratrol

Very little is known about the natural sources of the dihydro-derivative of **R**, *dihydro*-**R**, which is one of the main metabolites of both isomers of **R**.[15–22] We have recently discovered that *dihydro*-**R** is a prominent component of red wine.[23,24] Here we report our preliminary results on the unusual biological effects of *dihydro*-**R** in several tumor cell lines in vitro.[25]

As shown in Figure 1, *dihydro*-**R** has a strong proliferative effect on hormone-dependent MCF7 breast cancer cells at very low concentrations (10^{-14} to 10^{-7} M). This proliferative effect was not observed in two hormone-resistant breast cancer cell lines, MDA-MB-231 and BT-474. The compound showed a cytotoxic effect at high concentrations (>10^{-5} M) for all three cancer cell lines.

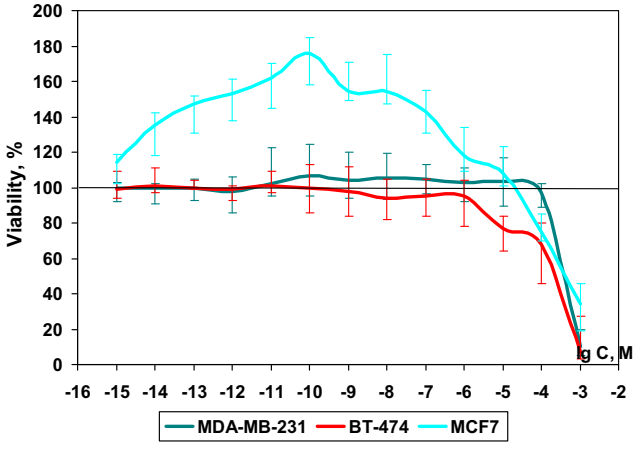


Figure 1. Biological activity of *dihydro*-**R** in breast cancer cell lines MCF7, MDA-MB-231, and BT-474.

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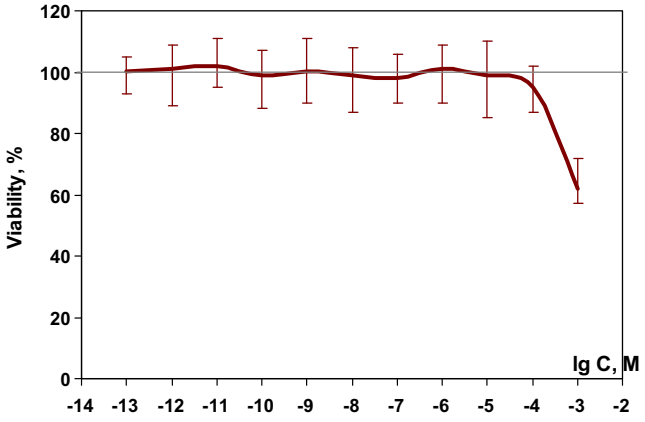
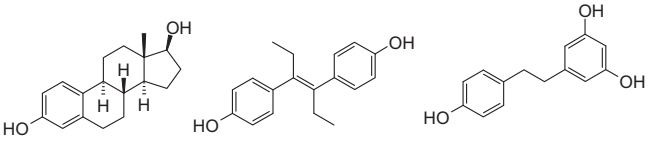


Figure 2. Biological activity of *dihydro*-**R** in the chronic myelogenous leukemia K-562 cell line.

The proliferation of hormone-sensitive MCF7 cells caused by very low concentrations of *dihydro*-**R** may indicate a hormone-like effect for the compound. The assumption is indirectly confirmed by the lack of proliferative effect of *dihydro*-**R** in the breast cancer cell lines that do not express hormone receptors (MDA-MB-231, BT-474). *Dihydro*-**R** also had no effect on cell viability of the chronic myelogenous leukemia K-562 cell line (Fig. 2) at low concentrations ($<10^{-6}$ M).

Perhaps the most unusual finding is the extreme potency of *dihydro*-**R**, which exhibits significant proliferative effect on MCF7 breast cancer cells at picomolar concentrations. Some other natural phytoestrogens, including *trans*-**R**,[26] are also known for their high level of induction (superagonism), but even the most potent ones are still orders of magnitude less active than *dihydro*-**R**. For example, the popular phytoestrogen genistein (5,7,4′-trihydroxyisoflavone) reaches maximum proliferative activity in MCF7 cells only at concentrations as high as 10^{-6} M[27,28] compared to 10^{-10} M for *dihydro*-**R**. Even 17β-estradiol is only marginally more potent than *dihydro*-**R**.[27–29]

The level of potency of *dihydro*-**R** is unexpected given that it is a flexible, relatively low molecular weight natural molecule which only remotely resembles the structures of 17β-estradiol and the notorious nonsteroidal synthetic estrogen diethylstilbestrol (see the comparative structure analysis below).



17β–Estradiol Diethylstilbestrol (DES) *Dihydro*-Resveratrol

In summary, *dihydro*-**R** demonstrated unusually strong proliferative effects in some hormone-dependent tumor cells at extremely low (picomolar) concentrations. Further studies on the mechanism of this phenomenon are warranted given the fact that *dihydro*-**R** is a prominent polyphenol component of red wine and one of the common metabolites of **R**.[30]

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2010.08.002.

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24. *Trans*-**R** was obtained from Sigma–Aldrich (St. Louis, USA). *Dihydro*-**R** was synthesized by catalytic hydrogenation of according to the reported procedure (see Ref. 22). *Cis*-**R** was synthesized by photoisomerization of *trans*-**R** according to the reported procedure (see Ref. 14). A 4 ml wine sample was passed through the HP Cyano Spe cartridge and then eluted with 2 ml of acetonitrile. The first 0.05 ml of extract was then silylated with BSTFA and analyzed using HP 6890 model GC–MS equipped with 30 m quartz capillary column HP-5MS. Average concentrations of *trans*-**R**, *cis*-**R**, and *dihydro*-**R** in fifteen samples of red wines with concentrations of all three compounds exceeding the analytical limit (0.01 mg/L) were 2.33, 0.83, and 0.77 mg/L, respectively. Maximum concentrations of *trans*-**R**, *cis*-**R**, and *dihydro*-**R** were 10.1, 3.57, and 3.61 mg/L, respectively. We observed reasonable correlation between concentrations of *trans*-**R** and *cis*-**R**, ($R^2 = 0.52$), but no correlation between concentrations of *trans*-**R** and *dihydro*-**R** ($R^2 = 0.05$). Similar average concentrations of *trans*-**R** and *cis*-**R** in red wines (2.48 and 0.56 mg/L, respectively), and maximum concentrations (8.00 and 2.48 mg/L, respectively) were reported previously—see Ref. 12.
25. K-562—human myelogenous leukemia, MDA-MB-231, MCF7, and BT-474—human breast cancer cell lines were obtained from the American Type Culture Collection, Rockville, Maryland. Cell lines were cultured at 37 °C in a 5% CO_2 humidified atmosphere and maintained in RPMI-1640 (Sigma, USA) supplemented with 10% heat-inactivated (56 °C, 30 min) fetal calf serum (FCS—Hyclone Laboratories, Logan, UK), 2 mM L-glutamine and antibiotics (100 μg/ml penicillin sodium salt and 100 μg/ml streptomycin sulfate (Sigma, USA), herein referred to as complete medium (CM). *Dihydro*-**R** was dissolved in dimethyl sulfoxide (DMSO) (Sigma, USA) at a concentration of 200 mM, and diluted in culture medium just prior to use. Tumor cells were harvested, counted, suspended in CM, and seeded into 96-well tissue culture plates (Costar, France) in 200 μl/well at a concentration of 1×10^5 cell/ml and allowed to adhere overnight, required for particular cell lines to be in log phase. After that CM was removed from sample wells. Compound sample solutions in CM were added to tumor cells. Final concentrations in sample wells amounted to 1×10^{-15}–1×10^{-3} M. The plates were incubated at 37 °C in a 5% CO_2 humidified atmosphere for 48 h. Three replica wells were used for controls and each compound concentration. The control samples contained DMSO concentration in CM corresponding to target compounds dilution. Each 24 h cells underwent microscopy and were photographed with the help of AxioVision 4 system (Zeiss, Germany). Cell viability assays were performed using 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) reduction. After a 48 h incubation of tumor cells (control or drug-treated), MTT solution (5 mg/ml) was added to each well. A 4 h incubation at 37 °C was

stopped by the addition of 100 μl of DMSO. The absorbances of the samples were measured on a microplate reader Multiscan MS (Labsystem, Finland) at 540 nm after 30 min incubation at 37 °C. Cell viability was expressed as a relative viability of tumor cells (percent of control cultures incubated with medium only) and was calculated as follows: relative viability = $[(A_e - A_b)/(A_c - A_b)] \times 100$, where A_b is the background absorbance, A_e is the experimental absorbance, and A_c is the absorbance of untreated controls. The Statistica software package, version 6.0 (STATSOFT) was used for statistical analysis.

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30. After submitting our manuscript, we performed additional experiments to evaluate the proliferative effect of *dihydro*-**R** in the presence of known estrogen antagonist and blocking agent, Tamoxifen. These experiments showed that Tamoxifen (in 1×10^{-7} M concentration) completely reversed the proliferative effect of *dihydro*-**R** thus lending additional support to our assumption that this *dihydro*-**R** proliferative effect in MCF7 cell line is indeed estrogen receptor-dependent. We also observed the proliferative effects of *dihydro*-**R** in DU-145 and PC-3 prostate cancer cell lines which have estrogen receptors, albeit the effects were not as strong as in the case of 'classic' estrogen-dependent MFC7 cell line. The results of these studies (further confirming that *dihydro*-**R** is a very potent phytoestrogen) will be published elsewhere.