Original Article

Evaluation of cytotoxic effects of several novel tetralin derivatives against Hela, MDA-MB-468, and MCF-7 cancer cells

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Abstract

Background: The inhibitors of the enzymes estrone sulfatase and 17-β-hydroxysteroid dehydrogenase (17-β-HSD) could provide a means of blocking estrogen biosynthesis leading to regression of estrogen-dependent tumors. We evaluated the cytotoxicity of several tetralin derivatives, 2-(4-halo-phenylmethylene)-3,4-dihydronaphthalene-1-ones, as potential inhibitors of these two enzymes, on Hela, MDA-MB-468, and MCF-7 cancer cell lines.

Materials and Methods: The cell lines were cultured in RPMI medium and the cytotoxic effect of tested compounds (compounds 1 to 5) was screened at the concentrations of 0.1, 1, and 10 μM either alone or in combination with doxorubicin (100 μM), using MTT assay. The mixtures of cell suspension with solvent (1% DMSO in PBS) and doxorubicin (100 μM) were used as negative and positive controls, respectively. Each concentration of compounds was assayed in four wells and repeated in at least three independent experiments for each cell line. The cytotoxic effect of each particular concentration of tested compounds was expressed as the percent of cell survival.

Results: None of the compounds exhibited cytotoxic effect (reduction of cell survival to less than 50%) on tested cell lines. However, statistically significant reduction in cell survival was observed for some compounds against particular cell lines. Among all tested combinations of compounds with doxorubicin against cell lines, only compound 4 at 10 μM concentration showed synergistic cytotoxic effect with doxorubicin against Hela cells.

Conclusion: With the exception of compound 2, other tested compounds have potential for further cytotoxicity evaluation. Synthesizing other tetralin derivatives similar to compound 4 and studying their structure-activity relationships (SARs) would be encouraged.

Key Words: Cytotoxicity, tetralin derivatives, 17-β-HSD, estrone sulfatase, Hela, MCF-7, MDA-MB-468

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INTRODUCTION

Cytotoxic drug therapy remains the main method of treatment for many malignancies such as solid tumors. Although malignancies have initial response rates exceeding 50%, many patients relapse and the long-term response rate is significantly low.[1]

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Breast cancer is one of the most common cancers in women. Although hormone therapy is currently the most prevalent breast cancer treatment, a significant number of patients develop tamoxifen resistance and experience severe side effects.[2,3] Thus, it is necessary to search for new alternative agents as breast cancer preventative and therapeutic agents.

In postmenopausal women, the circulating estrone sulfate [Figure 1] is nearly 10-fold higher than the free estrone and estradiol levels[4] and thus constitutes a reservoir from which free estrogens could be synthesized in breast cancer tissues. Also, estrogen levels in breast tumors of postmenopausal women are as much as 10 times higher than estrogen levels in plasma, presumably due to in situ formation of estrogen.[5] The enzymes responsible for conversion of estrone sulfate to free estrone and then estradiol are estrone sulfatase and 17-β-hydroxysteroid dehydrogenase (17-β-HSD), respectively. Estrone sulfatase is present in most breast tumors and is about a million-fold more active than aromatase.[6,7] In breast tumors, the sulfatase pathway is the main route for in situ production of estrogens.[8] 17-β-HSDs are believed to play an important role in regulating intracellular estrogen biosynthesis.[9] They are capable of converting estrone (E_1) to estradiol (E_2). Thus, intracellular conversion of E_1 to E_2 via 17-β-HSD could provide enough active estrogen to stimulate cells containing even very low levels of estrogen receptors.[10] Therefore, inhibitors of the enzymes estrone sulfatase and 17-β-HSD could provide a means of blocking estrogen biosynthesis either peripherally or in situ leading to reduction of tumor estrogen level and promotion of tumor regression. Based on this concept, the synthesis and evaluation of a number of potential steroid sulfatase inhibitors has been tried including a group of tetralin derivatives, 2-(4-halo-phenylmethylene)-3,4-dihydronaphthalene-1-ones.[11] The objective of this study was to evaluate the cytotoxicity of five of these synthesized derivatives, named compounds 1 to 5 [Figure 2], on MDA-MB-468 as estrogen-independent[5] and MCF-7 as estrogen-dependent cells,[5,12] and on Hela cell line as estrogen-independent cervical carcinoma cells.[13] Compound 4 was considered as a potential inhibitor of estrone sulfatase, while other compounds were considered as potential inhibitors of 17-β-HSD.[11]

MATERIALS AND METHODS

The study was performed in 2011 at Isfahan Pharmaceutical Sciences Research Center, School of Pharmacy and Pharmaceutical Sciences, Isfahan University of Medical Sciences, Isfahan, I.R.Iran

Materials

The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) and dimethylsulfoxide (DMSO) were purchased from Merck, Germany. RPMI 1640 culture medium, fetal calf serum (FCS), sodium pyruvate, and penicillin–streptomycin were purchased from Gibco, Scotland. The 96-well U-bottom microplates were purchased from Nunc, Denmark. ELISA plate reader was obtained from Awareness, USA. Doxorubicin was purchased from Ebewe, Austria. MCF-7, MDA-MB-468, and Hela cell lines were obtained from Pasteur Institute, Tehran, Iran. 2-(4-halo-phenylmethylene)-3,4-dihydronaphthalene-1-one derivatives were prepared previously using aldol condensation method.[11]

Sample preparation

To prepare the stock solutions (1 mM), compound 2 was dissolved in ethanol while other compounds were dissolved in DMSO. The concentrations of 1, 10, and 100 μM were then obtained by diluting these solutions with phosphate buffered solution (PBS) so that the final concentrations of ethanol and DMSO did not exceed 1%. The stock solutions were sterilized using

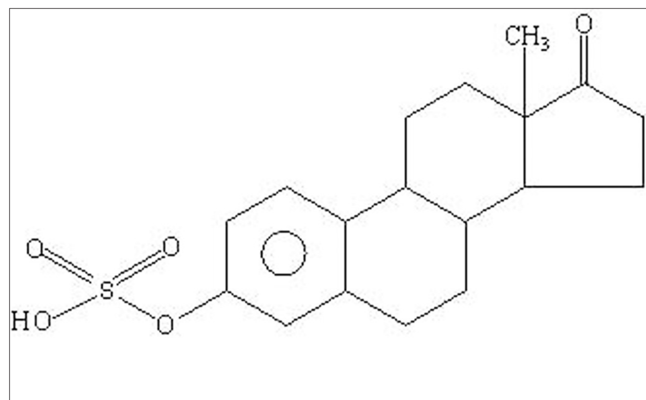

Figure 1: Chemical structure of estrone sulfate

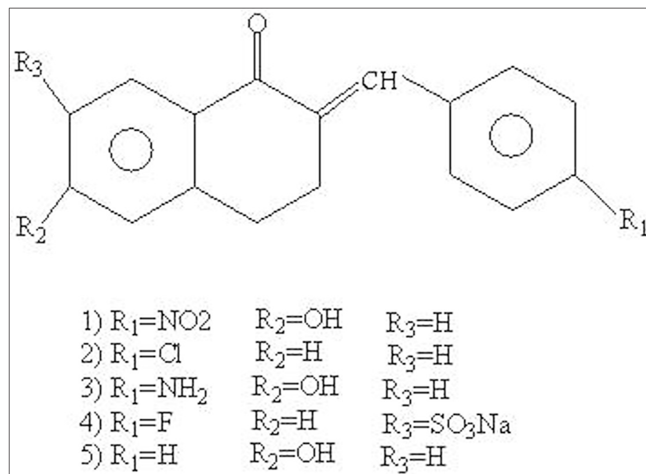

Figure 2: Chemical structure and name of compounds 1 to 5

0.22 μm microbiological filters. All dilutions were prepared fresh before addition to the cells.

MTT assay

The modified calorimetric MTT assay, based on the selective ability of living cells to reduce the soluble yellow MTT salt to insoluble purple formazan, was used for quantitative measurement of cell-mediated cytotoxicity as follows.

Cytotoxicity assay

To evaluate the cytotoxic effects of tested compounds, 180 μL of RPMI 1640 medium containing 5×10^4 cells/ ml was seeded in each of 96 wells in a U-bottom microplate and incubated for 24 hours at 37ºC in humidified air containing 5% CO_2. After incubation, 20 μL of each concentration of test compounds (1, 10, and 100 μM) was added to each well to give the final tested concentrations of 0.1, 1, and 10 μM. To survey any potential synergistic cytotoxic effect between test compounds and doxorubicin, a mixture of 10 μL of each concentration of compounds and 10 μL of doxorubicin (500 μM) was added to the wells to give the final tested concentrations of 0.05, 0.5, and 5 μM for each compound and 25 μM for doxorubicin. The first row of eight wells at each plate was used as background control and were filled only by 200 mL of RPMI medium, whereas, the second row was used as negative control and were filled with 180 mL of cell suspension and 20 μL of solvent (1% DMSO in PBS or ethanol). Subsequently, 20 μL of doxorubicin (250 μM) was added to the third row of the microplate containing 180 μL of the cell suspension to give the final concentration of 25 μM as positive control.

After addition of tested compounds, the plate was incubated in the same condition for another 72 hours, and then 20 μL of MTT solution (5 mg/ml) was added to the contents of each well and incubated for 3 hours. The culture medium supernatant was aspirated and the formazan crystals were dissolved in 150 μL of DMSO. The absorbance was measured at 540 nm using an ELISA plate reader. Each concentration of compounds was assayed in four wells and repeated in at least three independent experiments for each cell line. The cytotoxic effect of each particular concentration of tested compounds was expressed as the percent of cell survival calculated using standard curves absorbance against the number of cells constructed for each cell line. Percentage of cell survival in the negative control (untreated control) was assumed as 100. For calculation of the percentage of cell survival, the following equation was used:

$$\%\,\text{Cell Survival} = \frac{\text{experimental mean absorbance} - \text{background mean absorbance}}{\text{negative control mean absorbance} - \text{background mean absorbance}} \times 100$$

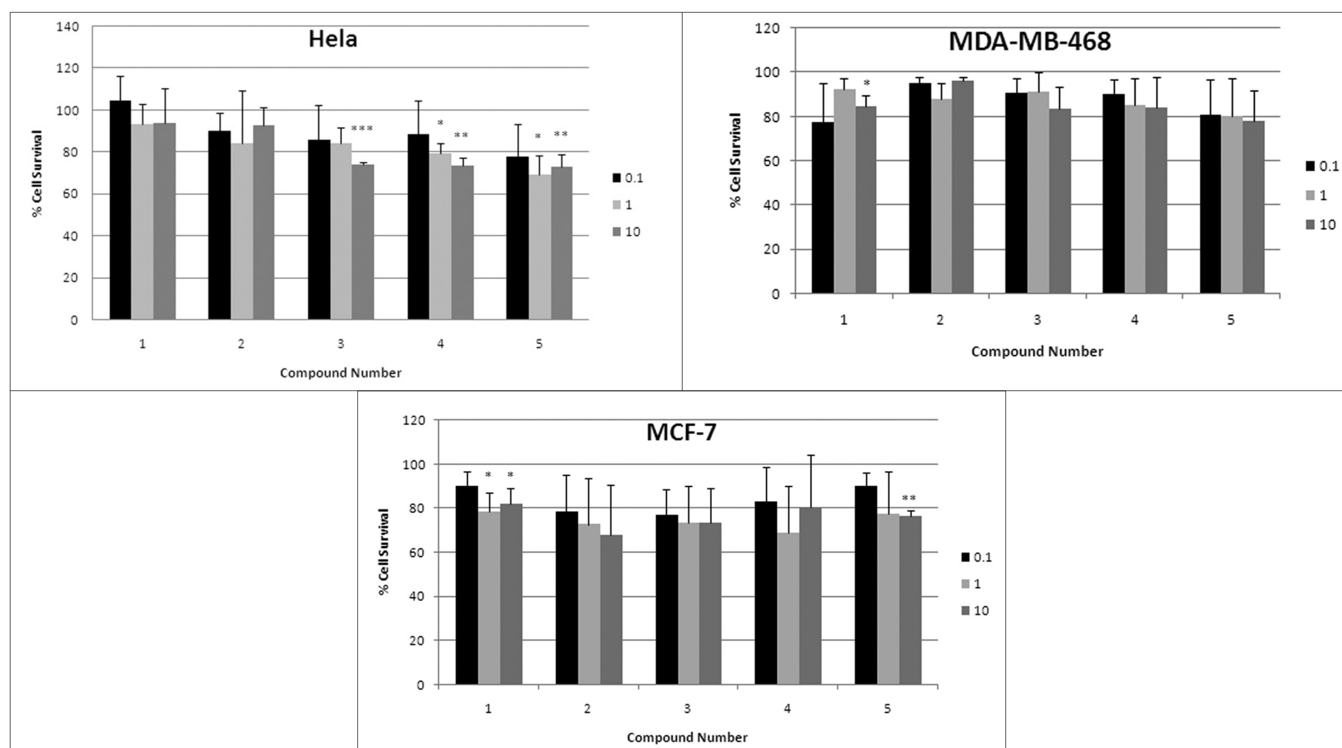

Figure 3: Cytotoxic effects of compounds 1 to 5 on Hela, MDA-MB-468, and MCF-7 cell lines. Following exposure to three different concentrations of each compound, cell viability was assessed using the MTT assay. Data are presented as mean ± SD of cell survival compared to negative control (cell survival of 100%). **P* < 0.05,***P* < 0.01, ****P* < 0.001, n = 12

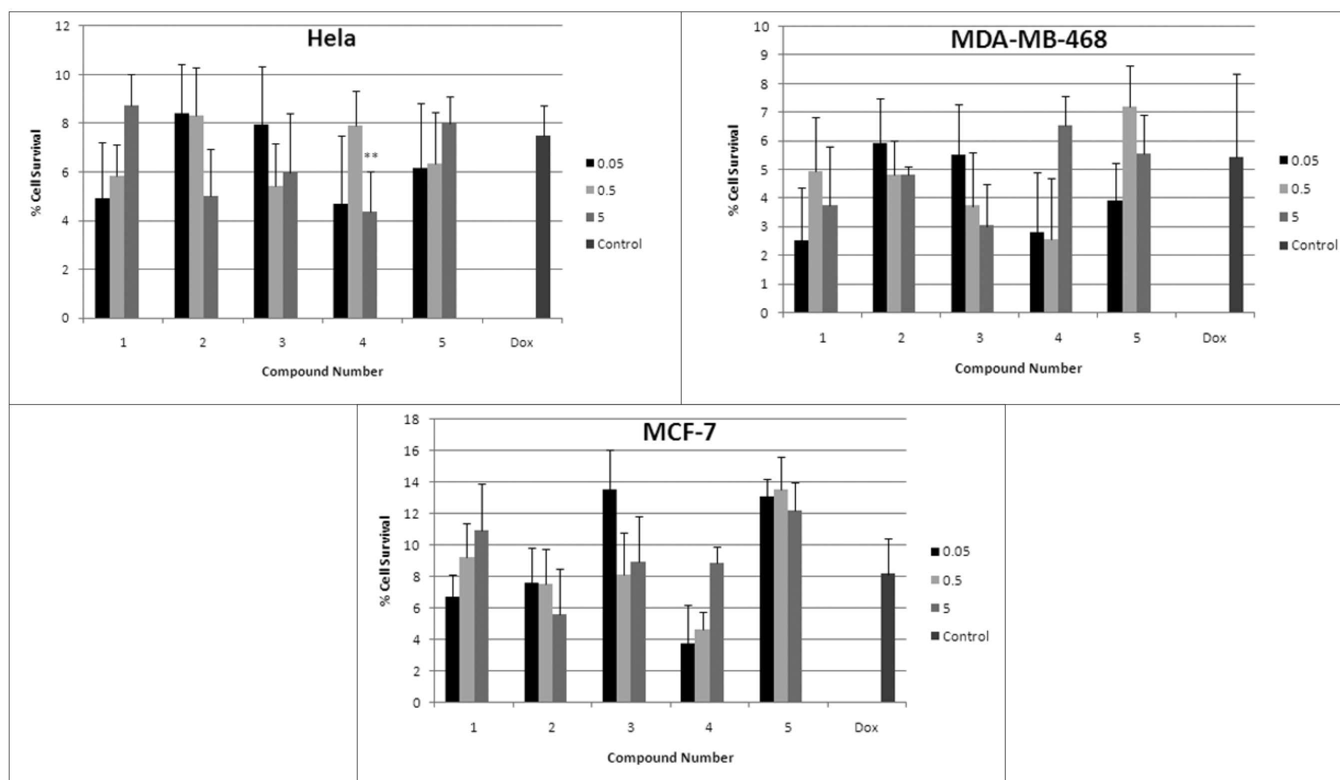

Figure 4: The effects of compounds 1 to 5 on the cytotoxicity of doxorubicin against Hela, MDA-MB-468, and MCF-7 cell lines. Following exposure to the mixture of three different concentrations of each compound with doxorubicin (25 mM), cell viability was assessed using the MTT assay. Data are presented as mean ± SD of cell survival compared to doxorubicin (1 mM) as positive control (cell survival is shown). **P < 0.01, Dox: Doxorubicin, n = 12

Statistical analysis

SPSS 16 software was used for statistical analysis. The mean ± SD of cell survival percentage was determined for each concentration of compounds against different cell lines. One-Sample t-Test and Paired-Samples t-Test were used for comparison of the effects of tested compounds with negative and positive controls, respectively. Analysis of variance (ANOVA) followed by Tukey test was used to distinguish the differences among groups. The significance was assumed as $P < 0.05$.

RESULTS

Effects of tested compounds on cell lines

Figure 3 shows the effects of compounds 1 to 5. As shown, none of the compounds showed significant cytotoxic effect (reduction of cell survival to less than 50%) against tested cancer cell lines. However, statistically significant reduction in cell survival was observed for some compounds against particular cell lines; compound 1 reduced survival of MDA-MB-468 cells at concentration of 10 μM ($P < 0.05$) and that of MCF-7 cells at concentrations ≥1 μM ($P < 0.05$). Compound 3 reduced only the survival of Hela cells at the concentration of 10 μM ($P < 0.001$). Compounds 4 and 5 had significant effects on Hela cells at the concentrations of 1 ($P < 0.05$) and 10 μM ($P < 0.01$). Also, a significant reduction in cell viability of MCF- 7 cells was observed with 10 μM concentration of compound 5. No significant difference was observed between the effects of five compounds against tested cell lines at each particular concentration.

The influence of tested compounds on the cytotoxic effects of doxorubicin

Figure 4 shows the effects of various concentrations of evaluated compounds on the cytotoxicity of doxorubicin on tested cell lines. Among all tested combinations, the only significant increase in the cytotoxic effect of doxorubicin was observed for 5 μM concentration of compound 4 against Hela cells ($P < 0.01$).

DISCUSSION

In the present study, we searched for possible cytotoxic effects of five tetralin derivatives as potential inhibitors of the enzymes estrone sulfatase and 17-β-HSD. Our results showed no cytotoxic effect at applied concentrations of 0.1, 1, and 10 μM. Selcer and coworkers synthesized a series of estrone-3-amino derivatives as potential inhibitors of estrone

sulfatase. They showed significant inhibitory effects on both estrone sulfatase activity of the human placental microsomes (IC50 of 8.7–14.6 μM) and proliferation of MCF-7 cells at the concentration of 10 μM suggesting that the reduction in cell growth was attributable to the blockade of sulfatase activity[5] Also, in another study, several derivatives of (p-O-sulfamoyl)-N-alkanoyl tyramine inhibited the activity of estrone sulfatase in intact MDA-MB-231 cells and consequently showed inhibitory effect on proliferation of MCF-7 cells at the concentration of 10 μM.[14] Similar results were obtained in the study of Li *et al.* on several sulfamate derivatives..[12] In the study of Laplante *et al*., 16-beta-m-carbamoyl-estradiol, synthesized and evaluated as a potential inhibitor of 17-β-HSD, blocked estrone-induced proliferation of the T-47D estrogen-sensitive breast cancer cells by 62%.[15] These data support the concept that the inhibitors of the enzymes estrone sulfatase and 17-β-HSD may be useful as therapeutic agents for estrogen-dependent breast cancers. Considering this, it could be found that the lack of any significant cytotoxicity against MCF-7 cells among our tested compounds is probably related to low affinity of them for active site of related enzymes. However, due to significant reduction of about 20% in MCF-7 cells survival, it is prudent to test the compounds 1 and 5 at higher concentrations. The slight but significant reduction in Hela cells survival (about 25%) by compounds 3, 4, and 5 show the possibility of presence of mechanism(s) of cytotoxicity other than estrogen deprivation suggesting that modification of the chemical structure of these compounds for cytotoxic effects on different tumoral cells is mandatory. Alternatively, the lack of any significant effect on MDA-MB-468 cells suggests that these derivatives do not interfere with nonhormonal growth pathways of estrogen-independent breast cancer cells.

Doxorubicin, an effective drug against cancer cells, is often associated with severe toxicity. Therefore, attempts for developing strategies of increasing doxorubicin sensitivity so that lower doses may be used without compromising efficacy is necessary. In our study, only compound 4 showed synergistic effect with doxorubicin; this effect was observed only on Hela cells but not on breast cancer cells. This could be due to more pronounced effect of this compound alone on Hela cells compared to other tested compounds. Therefore, this compound could be considered for more research as a potential adjunctive agent for chemotherapeutic drugs. We did not find any report of synergism between either estrone sulfatase or 17-β-HSD inhibitors and doxorubicin. However, such an effect has been reported for selenium and pyrazoloacridine with doxorubicin against MCF-7 cells.[16,17]

CONCLUSION

In conclusion, none of evaluated tetralin derivatives has significant cytotoxic effect on human breast and cervical cancer cell lines; however, with the exception of compound 2, other tested compounds have potential for further cytotoxicity evaluation. Since compound 4 has potential synergistic effects with doxorubicin, synthesizing other tetralin derivatives (similar to compound 4) and studying their structure-activity relationships (SARs) would be encouraged.

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