Anti-Cancerous Effect of 4,4'-Dihydroxychalcone ((2E,2'E)-3,3'-(1,4-Phenylene) Bis (1-(4-hydroxyphenyl) Prop-2-en-1-one)) on T47D Breast Cancer Cell Line

Narges Mahmoodi¹, Tayebeh Besharati-Seidani², Nasrin Motamed¹ and Nosrat O. Mahmoodi²*

¹School of Biology, University College of Science, University of Tehran, Tehran, Iran. ²Department of Chemistry, Faculty of Science, University of Guilan, P.O. Box 41335-1914, Rasht, Iran.

Authors’ contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

ABSTRACT

Aims: The majority of human breast tumors are estrogen receptor α (ERα) positive. However, not all of the ERα+ breast cancers respond to anti-estrogens drugs for those women who do respond, initial positive responses can be of short duration. Thus, more effective drugs are needed to enhance the efficacy of anti-estrogens drugs or to be used separately in a period of time. In view of potential cytotoxicity associated with silybin as polyhydroxy compounds a synthetic 4-hydroxychalcones (bis-phenol) was considered to explore its anti-carcinogenic effects in comparison to silybin on ERα+ breast cancer cell line.

Methodology: We have studied the inhibitory effect of 4,4'-dihydroxychalcone on the T47D breast cancer cell line by MTT test and the IC₅₀s were estimated using Pharm PCS.

Results: The 4,4'-dihydroxychalcone showed significant dose- and time-dependent cell growth inhibitory effects on T47D breast cancer cells. The IC₅₀ of 4,4'-dihydroxychalcone on T47D cells after 24 and 48 hours was 160.88+/−1 µM, 62.20+/−1 µM and for silybin was 373.42+/−1 µM,176.98+/−1 µM respectively.

*Corresponding author: Email: mahmoodi@guilan.ac.ir, nosmahmoodi@gmail.com;
Conclusion: Our results strongly suggests that this premade synthetic 4,4'-dihydroxychalcone can promote anti carcinogenic actions on T47D cell line. All 4,4'-dihydroxychalcone doses had a much larger inhibitory effect on cell viability than silybin doses in T47D cells. The ratio of the IC$_{50}$ of 4,4'-dihydroxychalcone to silybin after 24 and 48 hours was 1: 2.3 and 1: 2.8 respectively.

Keywords: Dihydroxychalcone; breast cancer; silybin; T47D; MTT.

1. INTRODUCTION

Breast cancer is the leading cause of female cancer death worldwide [1]. Normal breast epithelial cells and estrogen receptors (ERs) expressing breast cancer cells require estrogens to stimulate proliferation. Estrogen can regulate the transcription of its responsive genes by acting through the ERs, which in turn direct cellular proliferation. Therefore, patients with ER-positive breast cancers often undergo adjuvant treatment with anti-estrogen tamoxifen [2]. A wide variety of anti-cancer drugs, including fulvestrant, cisplatin, doxorubicin and vinblastine are available. Although treatments of breast cancer patients with these anti-cancer drugs have shown good success, tumor resistance remains a major obstacle [3]. Clearly, more effective compounds are needed to enhance the efficacy of anti-cancer drugs in resistant breast tumors [4].

From a chemical viewpoint, chalcones consist of two aromatic rings linked by a three-carbon unit forming an $\alpha,\beta$-unsaturated carbonyl moiety. They are pharmacologically relevant because of their ability to exert anti-carcinogenic, antimicrobial, anti-diabetic and anti-inflammatory activities. Among chalcones, synthetic and naturally hydroxychalcones are of particular interest as they display a wide range of biological properties and exert diverse pharmacological activities. 4-Hydroxychalcones have demonstrated their effectiveness as cytotoxic, anti-tumor, anti-oxidative, anti-bacterial, anti-fungal, anti-leishmanial and anti-nociceptive agents [5-9].

Studies have also indicated that the core structure of the chalcones, 1,3-diphenyl-2-propenone is able to potently inhibit proliferation of MCF-7 and MDA-MB-231 on human breast cancer cell lines by inducing apoptosis and blocking cell cycle progression in the G2/M phase [10]. Many reports have documented that chalcones are biologically active. For example, xanthoangelol was reported to induce apoptosis and inhibit tumor promotion and metastasis in several cancer cell lines [11,12]. Licochalcone-A, isoliquiritigenin and flavokawain A also have been indicated to induce apoptosis and cell cycle arrest in various cancer cells [13,14,15].

To continue our previous interest in synthesis of bis-compounds [16-23], in view of potential cytotoxicity associated with silybin as polyhydroxy compounds a synthetic 4,4'-dihydroxychalcone such as ((2E,2'E)-3,3'-(1,4-phenylene) bis (1-(4-hydroxyphenyl) prop-2-en-1-one)) A with its anti-carcinogenic effects in comparison to silybin B was considered (Fig. 1). Compounds A and B can promote both antioxidant and pro-oxidant effects and therefore, have been shown to be an efficient chemo-preventive agent as well as to exert anti carcinogenic actions [24].
2. MATERIALS AND METHODS

2.1 Tumor Cell Line and Culture Conditions

T47D is a human breast ductal carcinoma cell line with ER and PR over expression [25,26]. A T47D cell line was purchased from the National Cell Bank, Pasteur Institute of Iran. The cell line was cultured in RPMI \textsubscript{1640} medium (Invitrogen, Darmstadt, Germany) with 10% fetal bovine serum (FBS), 1% penicillin/streptomycin (all from PAA, Pasching, Austria), 2 g/l sodium bicarbonate and 2.5 g/l HEPES (Sigma-Aldrich, Missouri, USA). T47D cells were grown under standard culture conditions (37°C, 95% humidified air and 5% CO\textsubscript{2}). For cell harvesting, 0.25% solution of trypsin (Sigma-Aldich, Missouri, USA) in PBS was used.

2.2 Chemicals

2.2.1 (2\textit{E},2\textit{E})-3,3'-(1,4-phenylene)bis(1-(4-hydroxyphenyl)prop-2-en-1-one)

Yellow solid, M.p. > 300°C; IR (KBr): cm\textsuperscript{-1}, 3325, 3100, 1645, 1600, 1560, 1510, 1420, 1220, 810. Compounds 4,4'-dihydroxychalcone were prepared using a modification of a literature methods [16-24,27]. The general procedure for preparing 4,4'-dihydroxychalcone was as follows. An aqueous solution of NaOH (10% w/v, 10 ml) was added to a solution of terephthaldehyde (2.5 mmol) and 4-hydroxyacetophenone (5 mmol) in EtOH (12.5 ml). The reaction mixture was stirred at r.t. overnight and poured into water (35 ml). After neutralization with HCL (10% w/v), yellow solid was obtained and was recrystallized in EtOH.

2.2.2 Sodium salt of 4, 4'-dihydroxychalcone

To a 10 ml round bottom flask was added 1 ml 96% EtOH and 8 mg NaOH. To the resulting mixture was added 37 mg of 4,4'-dihydroxychalcone and stirred for 30 min. After that the solvent was removed and the sodium salt of 4,4'-dihydroxychalcone (38 mg) was recovered.

2.3 Chemical Treatments and MTT Assay

For the MTT assay, the cells were first seeded in three 96-well microplates. Each well contained 100 µl complete growth medium and 7×10\textsuperscript{3} cells were seeded. The next day, the
cells were treated with different doses of 4,4’-dihydroxychalcone (25, 50, 75, 100, 150µM) for 24 and 48 hours. All doses were renewed every 24 hour. Of the 4,4’-dihydroxychalcone stock solution, 10 mM was dissolved in DMSO/MeOH (3:1). The MTT test for silybin was in 50, 75, 100, 150 µM for 24 and 48 hours. Of the silybin (Sigma) stock solution, 100 mM was dissolved in dimethyl sulfoxide (DMSO). In all of the tests, the final concentrations of DMSO did not exceed 0.1% and for MeOH did not exceed 0.01% [v/v].

After the 24 and 48 hours treatments, the cells were incubated with 0.5 mg/ml microculture tetrazolium (Sigma-Aldich, Missouri, USA) for about 3 hours. The optical density (OD) of formazan dye dissolved in DMSO was measured with an ELISA microplate reader (Gen5, Power Wave XS2, BioTek, USA) at 570 nm. The percentage of cell viability in different doses was calculated by the following equation:

\[
\text{Cell viability percentage} = \frac{\text{OD treated well}}{\text{OD control well}} \times 100
\]

2.4 IC\(_{50}\) Determination

Fig. 3 shows the half maximal inhibitory concentration (IC\(_{50}\)) of 4,4’-dihydroxychalcone after 24 and 48 hours on the T47D cell line. The IC\(_{50}\)s were determined by probit analysis using the Pharm PCS: Pharmacologic Calculation System statistical package (Springer Verlag, USA).

2.5 Statistical Analysis

Data were analyzed using SPSS 18 software. One-way ANOVA and Dunnett-t two-sided post hoc tests were employed to evaluate the statistical significance of differences between the control and all treatments. The P values that were considered significant are displayed (***: P < 0.001 in Fig. 1).

3. RESULTS AND DISCUSSION

3.1 Inhibitory Effects of 4,4’-dihydroxychalcone in T47D Cell Line and Comparing with Silybin

The cytotoxicity effects of 4,4’-dihydroxychalcone in five doses (25,50,75,100,150 µM) for 24, and 48 hours were evaluated by MTT assay on the T47D cell line (Fig. 2). Briefly, 7×10\(^3\) cells were seeded in 96 well plates for 24 hour, and treated with different doses in a complete medium (no serum starvation). Cell viability graphs were depicted by SPSS 18 (clustered bar, summaries for group of case). The cell viability of the T47D breast cancer cells in 24 and 48 hours for four doses (50,75,100,150 µM) of 4,4’-dihydroxychalcone and silybin is presented in Table 1. All doses reduced cell viability and were considered statistically significant (P<0.001). Data is presented as percentage of viability in three independent experiments. Each experiment had three individual samples (Error bars: +/- 1 SD). The IC50 of 4,4’-dihydroxychalcone on T47D cells after 24 and 48 hours (Fig. 3) was 160.88+/−1 µM, 62.20+/−1 µM and for silybin was 373.42+/−1 µM,176.98+/−1 µM respectively.
Fig. 2. The effect of 4,4'-dihydroxychalcone on the cell viability of the T47D breast cancer cell line. Cell viability graphs were depicted by SPSS 18 (clustered bar, summaries for group of case). Data is presented as percentage of viability in three independent experiments. Each experiment had three individual samples (Error bars: +/- 1 SD). The P values were estimated by SPSS 18, One-way ANOVA, and Dunnett-t two-sided post hoc tests (****: P < 0.001)

Fig. 3. Determination of IC_{50} of 4,4'-dihydroxychalcone during 24 and 48 hours incubation. The IC_{50}s were estimated using Pharm PCS (Springer Verlag, USA). Data of three independent experiments is presented. Each experiment had three individual samples (Error bars: +/- 1 SD)
Table 1. Comparison of 4,4'-dihydroxychalcone and silybin effects on the cell viability of the T47D cell line

| Concentration (Micromolar) | Cell viability After 24 hour | Cell viability After 48 hour |
|----------------------------|-----------------------------|-----------------------------|
|                            | 4,4'-dihydroxychalcone      | Silybin                     | 4,4'-dihydroxychalcone      | Silybin                     |
| control                    | 100%                        | 100%                        | 100%                        | 100%                        |
| 50                         | 72.2%                       | 113.24%                     | 52.72%                      | 83.52%                      |
| 75                         | 63.28%                      | 95.62%                      | 45.54%                      | 71.4%                       |
| 100                        | 70.96%                      | 92.71%                      | 48.86%                      | 67.62%                      |
| 150                        | 48.24%                      | 79.78%                      | 42.24%                      | 65.44%                      |

According to the MTT assay results the 4,4'-dihydroxychalcone cytotoxicity are significantly effective in all of the five elective doses on T47D cells after 24 and 48 hours, indicating significant dose and time-dependent cell growth inhibitory effects. Except 100 µM 4,4'-dihydroxychalcone indicates less inhibitory effect on cell viability than 75 µM. The IC₅₀ of 4,4'-dihydroxychalcone during 24hour and 48hour incubation were 160.88 +/- 1 µM and 62.20 +/- 1 µM respectively.

On the contrary in other efforts sodium salt of 4,4'-dihydroxychalcone showed no significant cell growth inhibitory by the MTT test. The sodium salt of 4,4'-dihydroxychalcone was not effective as the 4,4'-dihydroxychalcone. These results indicate that lipophilicity is an important factor making compounds more bioavailable to cell membrane. Thus, cell viability graph for sodium salt of 4,4'-dihydroxychalcone was ignored for the ineffectual effects on T47D cells. The MTT test results shown less IC₅₀ and significantly higher cell growth inhibitory effects for 4,4'-dihydroxychalcone over silybin (Table 1).

4. CONCLUSION

The comparison of 4,4'-dihydroxychalcone and silybin by the MTT assay indicates that all 4,4'-dihydroxychalcone doses had a much larger inhibitory effect on cell viability than silybin doses in T47D cells. The ratio of the IC₅₀ of 4,4'-dihydroxychalcone to silybin after 24 and 48 hours was 1:2.3 and 1:2.8, respectively.

ACKNOWLEDGEMENTS

Financial supports from the Graduate Research Committees of University of Tehran & University of Guilan are gratefully appreciated.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Breast cancer: Prevention and control. World health organization. Available: http://www.who.int/mediacentre/factsheets/fs297/en/.
2. Bindels EMJ, Lallemand FE, Balkenende A, Verwoerd D, Michalides R. Involvement of G1/S cyclins in estrogen-independent proliferation of estrogen receptor-positive breast cancer cells. Oncogene. 2002;21:8158–8165.
3. LaPensee EW, Tuttle TR, Fox SR, Ben-Jonathan N. Bisphenol A at low nanomolar doses confers chemoresistance in estrogen receptor-α positive and negative breast cancer cells. Environ Health Perspect. 2009;117(2):175–180.
4. Long X, Fan M, Bigsby RM, Nephew KP. Apigenin inhibits antiestrogen-resistant breast cancer cell growth through estrogen receptor-A-dependent and estrogen receptor-A-independent mechanisms. Mol Cancer Ther. 2008;7(7):2096–2108.
5. Gul HI, Yerdelen KO, Gul M, Das U, Pandit B, Li PK, et al. Synthesis of 4′-hydroxy-3′-piperidinomethylchalcone derivatives and their cytotoxicity against PC-3 cell lines. Arch Pharm Chem Life Sci. 2007;340:195–201.
6. Narendr T, Papi Reddy K. A simple and highly efficient method for the synthesis of chalcones by using boron trifluoride-etherate. Tetrahedron Lett. 2007;48:3177–3180.
7. Nam NH, Hong DH, You YJ, Kim Y, Bang SC, Kim HM, et al. Synthesis and cytotoxicity of 2,5-dihydroxychalcones and related compounds. Arch Pharm Res. 2004;27(6):581–588.
8. Enoki T, Ohnogi H, Nagamine K, Kudo Y, Suglyama K, Tanabf M, et al. Antidiabetic activities of chalcones isolated from a Japanese herb, angelica keiskei. J. Agric. Food Chem. 2007;55:6013–6017.
9. Sawle P, Moulton BE, Jarzykowska M, Green CJ, Foresti R, Fairlamb IJS, et al. Structure-activity relationships of methoxychalcones as inducers of heme oxygenase-1. Chem Res Toxicol. 2008;21:1484–1494.
10. Hsu YL, Kuo PL, Tzeng WS, Lin CC. Chalcone inhibits the proliferation of human breast cancer cell by blocking cell cycle progression and inducing apoptosis. Food and Chemical Toxicology. 2006;44:704–713.
11. Kimura Y, Baba K. Antitumor and antimetastatic activities of Angelica keiskei roots, part 1: Isolation of an active substance, xanthoangelol. Int J Cancer. 2003;106:429–437.
12. Tabata K, Motani K, Takayanagi N, Nishimura R, Asami S, Kimura Y, et al. Xanthoangelol, a major chalcone constituent of Angelica keiskei, induces apoptosis in Neuroblastoma cells. Biol Pharm Bull. 2005;28:1404–1407.
13. Fu Y, Hsieh TC, Guo J, Kunicki J, Lee MY, Darzynkiewicz Z, et al. Licochalcone-A, a novel flavonoid isolated from licorice root (Glycyrrhiza glabra), causes G2 and late-G1 arrests in androgen-independent PC-3 prostate cancer cells. Biochem Biophys Res Commun. 2004;322:263–270.
14. Hsu YL, Kuo PL, Chiang LC, Lin CC. Isoliquiritigenin inhibits the proliferation and induces the apoptosis of human nonsmall cell lung cancer a 549 cells. Clin Exp Pharmacol Physiol. 2004;31:414–418.
15. Zi X, Simoneau AR, Flavokawain A. A novel chalcone from kava extract, induces apoptosis in bladder cancer cells by involvement of Bax protein-dependent and mitochondria-dependent apoptotic pathway and suppresses tumor growth in mice. Cancer Res. 2005;65:3479–3486.
16. Mahmoodi NO, Zanjanchi MA, Kiyani H. Photochromism of several synthesised 1,3-diazabicyclo[3.1.0]hex-3-ene derivatives. J Chem Res. 2004;6:438–440.
17. Mahmoodi NO, Kiyani H, Tabatabaian K, Zanjanchi MA, Arvand M, Sharifzadeh B. NMR structural elucidation and photochromic behavior of new 1,3-diazabicyclo[3.1.0]hex-3-ene derivatives. Russ J Org Chem. 2010;46:884–889.
18. Mahmoodi NO, Kiyani H. Synthesis of thiophene derivatives of 1,3-diazabicyclo[3.1.0]hex-3-ene. Bull Korean Chem Soc. 2004;25:1417–1420.
19. Mahmoodi NO, Yazdanbakhsh MR, Kiyani H. Synthesis and photochromic properties of new heterocyclic derivatives of 1,3-diazabicyclo[3.1.0]hex-3-ene. J Chin Chem Soc. 2007;54:635–641.

20. Zanjanchi MA, Arvand M, Mahmoodi NO, Islamnezhad A. Novel potentiometric membrane sensor based on 6-(4-nitrophenyl)-2-phenyl-4,4-dipropyl-3,5-diazabicyclo[3,1,0]hex-2-ene for detection of strontium (II) ions at trace levels. Talanta. 2007;74:125–131.

21. Kiyani H, Mahmoodi NO, Tabatabaeian K, Zanjanchi MA. Photochromic behavior of several new synthesized bis-1, 3-diazabicyclo[3.1.0]hex-3-enes. J Phys Org Chem. 2009;22:559–567.

22. Kiyani H, Mahmoodi NO, Tabatabaeian K, Zanjanchi MA. Synthesis and photochromism of 1, 3-diazabicyclo[3.1.0]hex-3-ene phenol rings. Mendeleev Commun. 2009;19:203–205.

23. Mahmoodi NO, Rineh A, Abdollahi M, Foroumadi A, Sorkhi M, Shafiee A. Synthesis, analgesic and anti-inflammatory activity of 4-(2-phenoxyphenyl) semicarbazones. Arch Pharm Chem Life Sci. 2007;340:409–415.

24. Dimmock JR, Kandepu NM, Hetherington M, Quail JW, Pugazhenthhi U, Sudom AM, et al. Cytotoxic activities of mannnich bases of chalcones and related compounds. J Med Chem. 1998;41:1014–1026.

25. Kao J, Salari K, Bocanegra M, Choi Y, Girard L, Gandhi J, et al. Molecular profiling of breast cancer cell lines defines relevant tumor models and provides a resource for cancer gene discovery. Plos One. 2009;4(7):1-16.

26. Neve RM, Chin K, Fridlyand J, Yeh J, Baehner FL, Fevr T, et al. A collection of breast cancer cell lines for the study of functionally distinct cancer subtypes Cancer Cell. 2006;10:515-527.

27. Sipos Gy, Sirokman F. Chalcone formation of different substituted acetophenones and p-hydroxy-benzaldehyde. Nature. 1964;202:489.

© 2014 Mahmoodi et al.; This is an Open Access article distributed under the terms of the Creative Commons Attribution License (http://creativecommons.org/licenses/by/3.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Peer-review history:
The peer review history for this paper can be accessed here:
http://www.sciencedomain.org/review-history.php?id=452&id=32&aid=3977