Original Article

Apoptosis Induction by New Coumarin Derivatives in a Mice Model of Breast Cancer

Mehran Mesgari Abbasi[1], Monireh Khordadmehr[2*], Dariush Shanehbandi[3], Farinaz Jigari Asl[2], Reza Teimuri Mofrad[4], Shabnam Tahmasebi[4], Mohammad Shahab Asar[5], Fateme Eskandari Vaezi[1], Yousef Panahi[6]

1.Drug Applied Research Center, Tabriz University of Medical Sciences, Tabriz, Iran
2.Department of Pathobiology, Faculty of Veterinary Medicine, University of Tabriz, Tabriz, Iran
3.Immunology Research Center, Faculty of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran
4.Department of Organic and Biochemistry, Faculty of Chemistry, University of Tabriz, Tabriz, Iran
5.Faculty of Veterinary Medicine, Shabestar Branch, Islamic Azad University, Shabestar, Iran
6.Department of Basic Sciences, Faculty of Veterinary Medicine, University of Tabriz, Tabriz, Iran

How to cite this article: **Mesgari-Abbasi M, Khordadmehr M, Shanehbandi D, Jigari-Asl F, Teimuri-Mofrad R, Tahmasebi Sh, Shahab Asar MSh, Eskandari Vaezi F, Panahi Y. Apoptosis Induction by New Coumarin Derivatives in a Mice Model of Breast Cancer. Archives of Razi Institute. 2023;78(5):1430-39.**

DOI: 10.32592/ARI.2023.78.5.1430

Article Info:
Received: 12 July 2023
Accepted: 27 August 2023
Published: 31 October 2023

Corresponding Author's E-Mail:
khordadmehr@tabrizu.ac.ir

ABSTRACT

In the last decades, numerous studies have focused on the search for new agents to suppress the growth of cancer cells. In this study, we investigated the effect of two novel synthetic coumarin derivatives, namely *2-amino-4-(4-(2-hydroxyethoxy)-3-methoxyphenyl)-5-oxo-4H,5H-pyrano[3,2-c]coumarin-3-carbonitrile* and *2-amino-4-(4-hydroxyphenyl)-5-oxo-4H,5H-pyrano[3,2-c]coumarin-3-carbonitrile*, on the induction of apoptosis in breast cancer in a mouse model. Breast cancer was induced in BALB/c mice, which were randomly divided into six groups and then underwent the experiment. The groups and treatments included A1: coumarin A with a low dose (10 µm), A2: coumarin A with a high dose (1 mM), B1: coumarin B with a low dose (10 µm), B2: coumarin B with a high dose (1 mM), D: doxorubicin, and C: cancer control/ treatment with normal saline. The samples underwent treatments for 5 weeks. Animals were euthanized, and tissue samples, including the lung, liver, and tumor mass, were collected for histopathological examination. In addition, quantitative real-time polymerase chain reaction (qRT-PCR) was performed to determine some apoptotic markers, such as BCL-2, caspase-9, COX-2, and *c-Myc*. The qRT-PCR presented that both coumarin compounds could significantly alter the expression levels of BCL-2, caspase-9, COX-2, and *c-Myc*. Consistent with these results, histopathological observations showed a significant reduction in pathological lesions and severity of malignancy of the tumor mass, as well as a decrease in microscopic metastases in the lung and liver. This suggests that the present new coumarin compounds may induce apoptosis in breast cancer cells by altering some apoptosis-related genes that may play a chemotherapeutic role in breast cancer therapy in the future.

Keywords: Chemotherapy, Doxorubicin, Programmed cell death, Natural products

1. Introduction

Breast cancer (BC) remains the most common malignancy diagnosed in females and the second leading cause of cancer-related death in women worldwide (1). Breast cancer is a complex disease that may involve numerous signaling pathways and molecules, leading to different treatment options depending on tumor type and molecular profile (2, 3). Currently, classical BC treatment strategies, including chemotherapy, immunotherapy, and radiotherapy, are invasive, expensive, and ineffective. Therefore, the identification of novel therapeutic approaches applied alone or in combination with other methods would be of great benefit in improving the clinical care of BC patients (2). Natural products, such as phytoconstituents or their relative structures, still account for 50% of drugs prescribed for cancer chemotherapy. In this context, previous studies have demonstrated the anticancer effects of new agents, such as doxorubicin-G2 FA (4), ICD-85 (venom-derived peptides) (5), on BC cells, as well as caffeic acid phenethyl ester and Matricaria chamomilla essential oil on non-small cell lung cancer cells A549 (6). Moreover, in recent decades, scientists have identified and developed a variety of phytochemicals that not only have inhibitory activity against various types of cancers but also have fewer side effects (7). Among several phytochemotherapeutic agents, chromenes (benzopyrans) and their derivatives have been studied in recent years because of their interesting biological properties. In these heterocyclic rings, a pyran ring is combined with a benzene ring. Tocopherols, anthocyanins, alkaloids, flavonoids, and tannins are examples of benzopyrans that form the backbone of different types of polyphenols (8). Natural compounds derived from coumarin (1,2-benzopyrones or 2H-chromen-2-one or phenylpropanoids 1) exhibit various biological, pharmacological, and biochemical activities against cancer (9). Some coumarins and their active metabolite, 7-hydroxycoumarin analogs, have shown effects against BC through a variety of mechanisms, the most notable of which were the inhibition of aromatase and sulfatase. Selective estrogen receptor modulators (SERMs) based on coumarin and coumarin-estrogen conjugates may also have a potential role in BC treatment (10). In the present study, the effect of a series of novel synthetic coumarin derivatives on the induction of programmed cell death in BC was investigated in the animal model of BALB/c mice using quantitative molecular assays and pathological studies.

2. Materials and Methods

2.1. Chemistry and preparation of coumarin derivatives

At the Department of Organic Chemistry and Biochemistry, University of Tabriz, Tabriz, Iran, we synthesized two coumarin compounds (Figure 1), namely *2-amino-4-(4-(2-hydroxyethoxy)-3-methoxyphenyl)-5-oxo-4H,5H-pyrano[3,2-c]coumarin-3-carbonitrile* and *2-amino-4-(4-hydroxyphenyl)-5-oxo-4H,5H-pyrano[3,2-c]coumarin-3-carbonitrile* (Figure 1A), designated as A and B, respectively. Stock solutions at a concentration of 10 mg/ml of each of the compounds were also prepared and stored at room temperature in the laboratory for future studies.

2.2. Cell culture

Murine metastatic mammary tumor cell line 4T1 was obtained from the National Cell Bank of Iran (Pasteur Institute, Iran), cultured in Roswell Park Memorial Institute 1640 medium (GIBCO, USA), and supplemented with 10% fetal bovine serum (GIBCO, USA), 100 units/ml penicillin, and 100µg/ml streptomycin. The flasks were incubated at 37°C with 95% humidity and 5% CO_2.

2.3. Animals and ethical approval

Female BALB/C mice (n=60) in good health (25-35 g, 7-9 weeks) were purchased from the Pasteur Institute in Karaj, Iran. They were kept under standard conditions (22°C, 12/12 h light/dark cycle, and 40-60% humidity) and given food and water at all times. Ethical approval was included on the "cover".

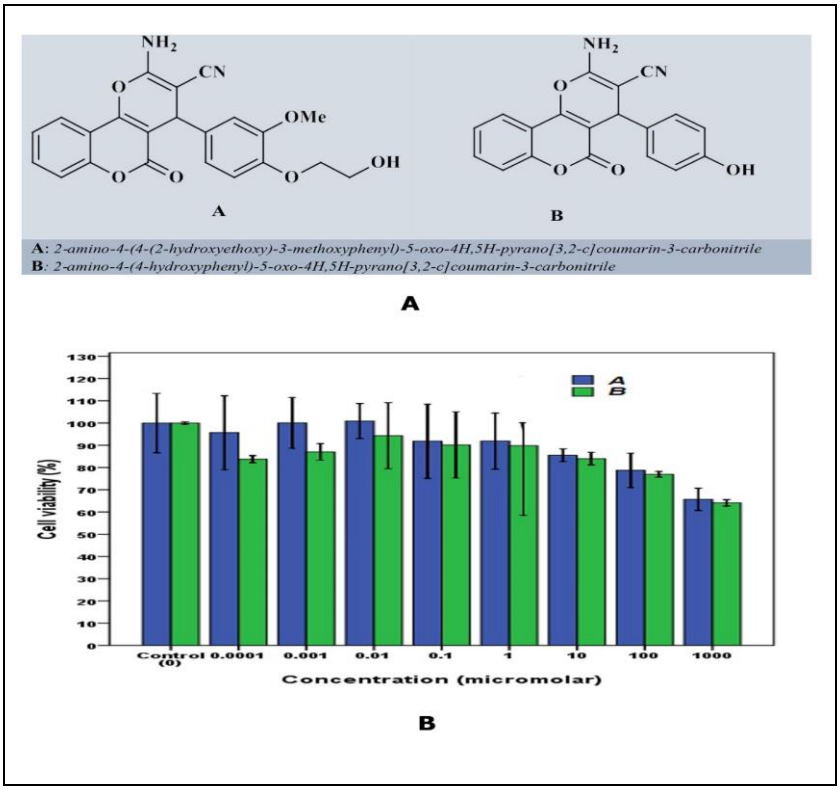

Figure 1. a: Structure of the present synthetic coumarin derivatives. **b:** Cell viability was evaluated using MTT assay, which was dose-dependent and decreased at higher doses (P<0.05, mean ± SD, n=3)

2.4. MTT assay

To determine the viability of the cells and the appropriate dose of coumarin derivatives, the MTT (3- (4, 5- dimethylthiazol- 2- yl) - 2, 5- diphenyltetrazolium bromide) assay was performed. Briefly, cells (1.5×10^3) were seeded in 96-well plates and incubated at 37℃ for 24 h. After treatment with 8 ascending concentrations (0.0001, 0.001, 0.01, 0.1, 1, 10,100, and 1,000 µmol) of the test compounds (including A and B), and 0.1 ml of dimethyl sulfoxide (DMSO) as control, the cells were incubated for another 24 h at 37°C. Afterward, 50 µl of the prepared MTT solution (Sigma-Aldrich, Germany) was added to each well and incubated for an additional 4 h. Following that, the medium in each well was removed, 200 µl of DMSO (Sigma- Aldrich) was added to the wells to dissolve MTT formazan crystals, and incubated for another 30 min. Finally, the absorbance values of each well were read at 570 nm using a Sunrise enzyme-linked immunosorbent assay (ELISA) reader (Tecan, Switzerland). The experiment was performed three times.

2.5. Breast cancer induction, treatment, and sampling

To demonstrate the inhibitory effects of the selected coumarins on BC growth, 60 healthy BALB/c mice were randomly divided into six groups of 10 mice each (Table 1), and examined daily for their general health. A suspension of 4T1 cells with a concentration of 1×10^6/ml was obtained after trypsinization and centrifugation of the cells cultured in complete media. Subsequently, 0.2 ml of the suspension was injected subcutaneously into the left flank of mice under ketamine and xylazine anesthesia (10 mg/kg BW IP) (11). Tumors appeared 2 weeks after the cell injection, and the main treatment was started with 0.4 ml of equal injection volumes, according to table 1. At the

end of the experiment (after 5 weeks), all animals were euthanized, and the internal organs, including the liver, lungs, and tumor mass, were removed for further examinations (quantitative real-time polymerase chain reaction [qRT-PCR] and histopathology).

Table 1. Experimental groups and treatments of breast cancer

Group name	Treatment	Dose
A_1	The low dose of coumarin A*	10µm/ 0.4 ml /twice a week
A_2	The high dose of coumarin A	1 mM/ 0.4 ml /twice a week
B_1	The low dose of coumarin B	10µm/ 0.4 ml /twice a week
B_2	The high dose of coumarin B	1 mM/ 0.4 ml /twice a week
D	Positive- tumor control (Doxorubicin)	5 mg/kg/ once a week
C	Negative- tumor control (normal saline)	0.4 ml/ twice a week

*A: *2-amino-4-(4-(2-hydroxyethoxy)-3-methoxyphenyl)-5-oxo-4H, 5H-pyrano [3, 2-c] coumarin-3-carbonitrile; B: 2-amino-4-(4-hydroxyphenyl)-5-oxo-4H, 5H-pyrano [3, 2-c] coumarin-3-carbonitrile*

2.6. RNA extraction and quantitative reverse transcription-polymerase chain reaction

The RT-qPCR method was used to measure fold changes in the expression of some genes associated with apoptosis/survival, including *c-Myc*, *BCL-2*, caspase-9, and COX-2. Total RNA from tissues of all tumor-induced mice in each group was isolated using TRIzol reagent (RiboEx Kit, GeneAll, South Korea) according to the manufacturer's instructions and quantified by measuring absorbance at 260 nm and 280 nm using NanoDrop (Thermo Scientific, USA). Complementary DNA (cDNA) was synthesized using the Revert Aid First Strand cDNA Synthesis Kit (Thermo Fisher) according to the manufacturer's protocol. The RT-qPCR technique was performed using 2X SYBR Green pre-mix (Amplicon, UK) in a StepOnePlus™ RT-PCR System (Applied Biosystems, US). Mean expression in each treated group was presented as an increase/decrease in parity, compared to mean expression in the control group, which was assigned the desired value of 1. In addition, *the glyceraldehyde-3-phosphate dehydrogenase* gene was used to normalize expression data. The primer sequences are listed in Table 2.

Table 2. List of primer sequences used for qRT-PCR

Names of genes	Forward primer (5′ to 3′)	Reverse primer (5′ to 3′)
CASPASE9	AGCCAGATGCTGTCCCATAC	CAGGAGACAAAACCTGGGAA
BCL-2	GTGGATGACTGAGTACCT	CCAGGAGAAATCAAACAGAG
c-MYC	GTGGTGTCTGTGGAGAAGAGG	CGTAGTTGTGCTGGTGAGTGG
COX2	GCAGACTCATACTCATAGGAGAGAC	GTTCTGATACTGGAACTGCTGG
GAPDH	CCCATCACCATCTTCCAGGAG	GAAGGGGCGGAGATGATGAC

2.7. Histopathological examinations

First, tissue samples were immediately fixed in a 10% formalin buffer solution for at least 48 h. Subsequently, the formalin-fixed tissue samples were routinely passaged, embedded in kerosene, sectioned, and stained with conventional hematoxylin and eosin. The tissue sections were examined under a light microscope (Olympus, Japan) for tumor proliferation, invasion, and distant metastasis associated with the probable histopathological changes, such as vascular congestion, hemorrhage, necrosis, and infiltration of inflammatory cell with grades of normal (N), very

mild (+1), mild +2), moderate (+3), severe (+4), and very severe (+5).

2.8. Statistical analysis

Data analysis was performed in Sigma Stat software (SPSS, USA) using ANOVA. Data were reported as mean ± SD, and *p-values* of less than 0.05 were considered statistically significant. In addition, the nonparametric tests (Kruskal-Wallis H and Mann-Whitney U) were employed for statistical analysis of pathological lesions (semiquantitative data) between different groups, and a $p < 0.05$ was considered significant.

3. Results

3.1. MTT assay

The MTT assay was used to determine the cell viability and response of 4T1 cells to two coumarin compounds at different concentrations and the effective dose. Based on the data shown in figure 1B, eight different concentrations of the coumarin compounds (A and B) were tested, and the most effective dose with more than 80% cell viability was selected for further analysis.

3.2. Tumor inhibitory results by qRT-PCR and histopathology

The effect of coumarin derivatives on the gene expressions of caspase-9, *BCL-2*, *c-Myc*, and COX-2 was demonstrated by qRT-PCR and melting curve analyses. According to the qRT-PCR results illustrated in Figure 2, the expression of caspase-9 was increased by coumarin derivatives and doxorubicin in all treated groups. On the other hand, the expression of *BCL-2*, *c-Myc*, and *COX-2* was decreased in all groups undergoing treatment with coumarin derivatives and doxorubicin, which was significant compared to the cancer control group (P<0.05). It is noteworthy that most changes in caspase-9, *BCL-2*, *c-Myc*, and *COX-2* were observed in A2 (P<0.05), B1 (P<0.05), B1 (P<0.01), and A1 (P<0.01) groups after treatments with coumarin derivatives.

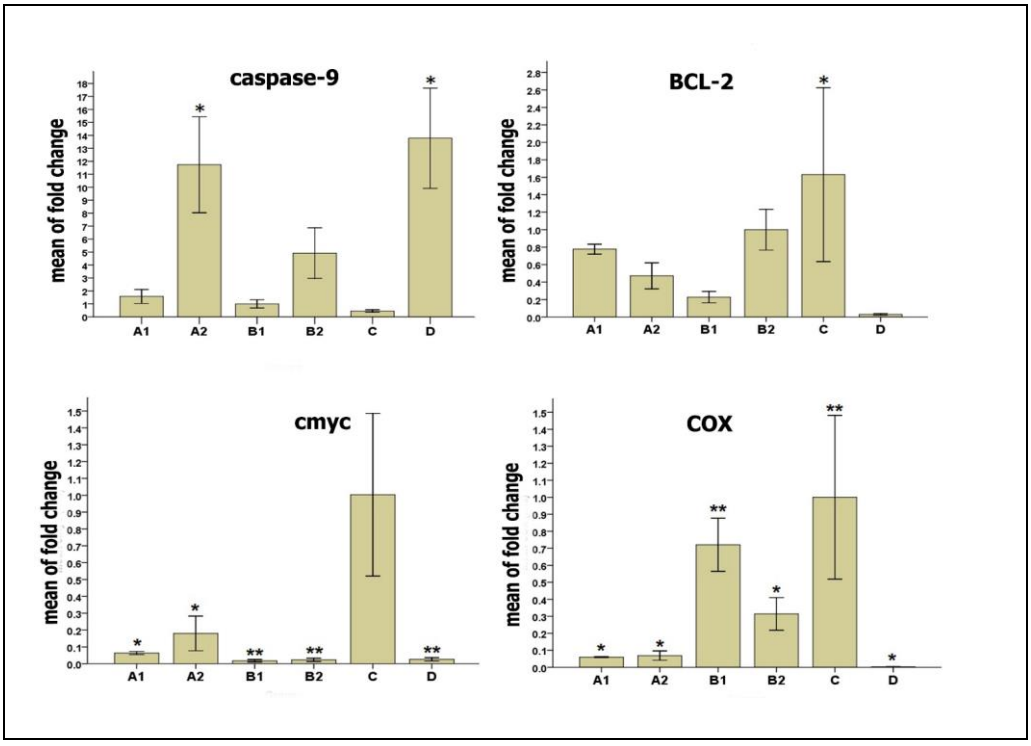

Figure 2. Evaluation of gene expression levels in tumor mass by qRT-PCR. The expression levels of caspase-9 were increased in all treated groups compared to the cancer control group, particularly in the A2 and B2 (P<0.05) groups. The expressions of *BCL-2*, *c-Myc*, and COX-2 decreased in all treated groups compared to the control group (mean±SD, n=10), especially in groups B1, B1, and A1 (P<0.05). A1 (A/low

dose group): 10µm; A2 (A/high dose group): 1 mM; B1 (B/low dose group): 10µm; B2 (A/high dose group: 1 mM); C: tumor control group treated with normal saline; D: tumor group treated with doxorubicin 5 mg/kg BW

Histopathological evaluation is briefly illustrated in Figure 3. After tumor induction and treatment with normal saline, severe malignancy was observed in the control group. Specifically, a high nuclear/cytoplasmic ratio, the presence of bizarre neoplastic cells, severe necrosis associated with hemorrhage, and inflammation in the tumor mass were noted. Similar lesions were observed in the lung and liver, along with extensive metastases. As expected, all pathologic lesions were significantly reduced after the application of doxorubicin (P<0.05). Histologic changes and tumor malignancy decreased significantly in the low and high coumarin-recipient dose groups (P<0.05), especially in the high dose groups, which was more pronounced in the A2 group. It should be noted that microscopic metastases in the liver and lung decreased significantly in all four treated groups.

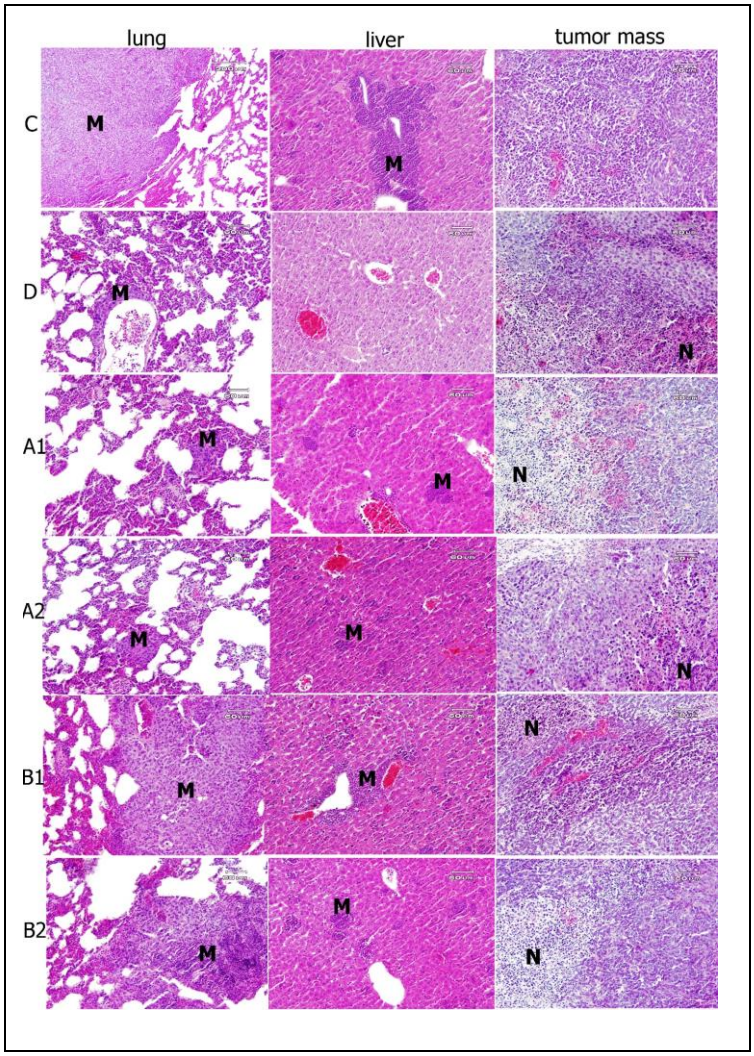

Figure 3. Evaluation of breast cancer inhibitory effects of the present synthetic coumarin derivatives by histopathological examination, mice (n=10). C: tumor control group treated with normal saline; D: tumor group treated with doxorubicin 5 mg/kg BW; A1 (A/low dose group): 10µm; A2 (A/high dose group): 1 mM; B1 (B/low dose group): 10µm; B2 (A/high dose group: 1 mM). Severe malignancy was detected in the control group. Similar lesions with extensive metastases were observed in the lung and liver. The pathological lesions decreased significantly after the application of doxorubicin (*P*<0.05). Histological changes and tumor malignancy were significantly lower in the low-

and high-coumarin-dose groups, especially in the high-dose groups. Microscopic metastases in the liver and lungs were significantly reduced in all four treated groups, although this was more pronounced in the A groups. M: metastases, N: necrosis. Hematoxylin and eosin staining

4. Discussion

Our data showed that the administration of coumarin compounds could significantly increase apoptosis and reduce microscopic metastases in BC in a mouse model, which was confirmed by histopathological and qRT-PCR studies. The current results suggested that the present coumarin compounds were able to inhibit tumor growth. However, it seemed that *2-amino-4-(4-(2-hydroxyethoxy)-3-methoxyphenyl)-5-oxo-4H,5H-pyrano[3,2-c]coumarin-3-carbonitrile* was more successful than *2-amino-4-(4-hydroxyphenyl)-5-oxo-4H,5H-pyrano[3,2-c]coumarin-3-carbonitrile*. The expression levels of the essential apoptotic genes, including caspase-9, *BCL-2*, *c-Myc*, and *COX-2*, were significantly altered in the treated groups. In parallel with apoptosis induction, the pathological lesions and microscopic metastases were significantly decreased by the coumarin compound treatment of BC. On the other hand, as expected, the present coumarin derivatives had no toxic effects on the liver and kidney, which was confirmed by normal histological and biochemical indicators and body weight. Coumarins are a naturally occurring compound found in numerous plants, some spices, and herbs (12) and can be extracted from plants or synthesized from phenol, *ortho*-Cresol, and salicylaldehyde for commercial purposes; they are highly soluble in chloroform, ethanol, and oils and slightly soluble in water (13). Moreover, they have great anticancer potential with low adverse effects due to their original structure and functional groups. They can affect various cellular pathways, such as the inhibition of angiogenesis, cell proliferation, and metastasis, as well as the suppression of enzymes that mainly contribute to cancer pathophysiology (14, 15). Although chemotherapy is currently the most common treatment for BC, most chemotherapeutic anticancer drugs have high toxicity and low specificity (16). Therefore, the development of new anticancer drugs for the treatment of BC is needed. Recent evidence demonstrates that coumarin compounds modulate apoptosis, proliferation, and invasion of various cancer cells, which are mediated by different signal transduction cascades.

Based on the present results, the growth-inhibitory potential of coumarin compounds, when it leads to fruition, might be dependent on the nature and position of the functional group, which has already been suggested by other researchers. For instance, the presence and position of the hydroxyl groups and a catecholic acid group may be associated with the different inhibitory values. In this regard, there is increasing evidence demonstrating that nitration of 7-hydroxycoumarin suppresses the human malignant melanocytes (17). In addition, previous studies have reported that both coumarin and 7-hydroxycoumarin can reduce the proliferation of a number of human malignant cell lines (18) and various types of animal tumors *in vitro* (19). In addition, it was previously found that N-aryl carboxamide and phenyl substitution at the C-3 position and 1, 2, 3-triazolyl, trihydroxystilbene, and amino substitution at the C-4 position of the coumarin nucleus may be the most effective in combating lung cancer (20). some coumarins and their active metabolites the 7-hydroxycoumarin analogs are of particular interest for BC chemotherapy because they exhibit sulfatase- and aromatase-suppressive properties. As described previously, coumarin-estrogen conjugates and SERMs have also been proposed as potential agents for suppressing BC (10, 21). Coumarin-estrogen conjugates showed growth-inhibitory effects in NCI-7- human BC cell lines (21). Similarly, fluorinated coumarin derivatives demonstrated anticancer effects in MCF-7 cancer cells (22). A series of novel 4-substituted coumarin derivatives have shown antimitotic activity in human BC cell lines (MCF-7 and MCF-7/ADR) (23). On the other hand, coumarin derivatives induce programmed cell death in BC cell lines through BCL-2 and caspase-9 cascades (20). The results of a similar study indicated that 7,8-Dihydroxy-4-methylcoumarin can downregulate p53, Bax, p21, and COX-2 and upregulate c-Myc protein, which induces apoptosis (20). Our results were consistent with these findings. In the present coumarin structures, OH and OMe substitutions appeared to act as electron donor groups in *para* and *meta* positions, making the compounds more active and reactive. The induction of programmed cell death is central to the tumor suppressive effects of some molecular targets that

promote the expression of a number of genes that contribute to apoptosis (24, 25). It was found that caspase-9 was the initiator caspase of the mitochondrial apoptotic pathway, and its function was essential for apoptosis. Activated caspase-9 cleaves downstream caspases, such as caspase-3, initiating the caspase cascade (26, 27). *BCL-2* is one of the major genes involved in the intrinsic pathway of apoptosis. In this regard, a previous study reported an alteration in the expression level of t-BCL-2 by coumarin compounds in cancer cells, which was in line with our results (20). *c-Myc* is an important proto-oncogene that is upregulated in numerous human malignancies (28). Up-regulation of *COX-2* increases cancer cell proliferation and metastatic potential and inhibits apoptosis (29). In this case, all four treatments decreased the expression of *COX-2* and *c-Myc*, which was more pronounced in groups A (especially A1) and B (especially B1). At the same time, all four treatments increased the expression of caspase-9, which might have affected apoptosis induction. It seems that the present coumarin compounds might have influenced the expression of *c-Myc*, which plays an important role in the development and progression of BC. Pathological changes were significantly reduced after coumarin treatment, with no significant differences between treated groups. It can be concluded that the present coumarin compounds, namely *2-amino-4-(4-(2-hydroxyethoxy)-3-methoxyphenyl)-5-oxo-4H,5H-pyrano[3,2-c]coumarin-3-carbonitrile* and *2-amino-4-(4-hydroxyphenyl)-5-oxo-4H,5H-pyrano[3,2-c]coumarin-3-carbonitrile*, may facilitate BC cell death by altering some key apoptotic pathways through targeting caspase-9, *BCL-2*, *c-Myc*, and *COX-2*, which may play a chemotherapeutic role in BC therapy in the future. However, further extensive and complementary studies are needed.

Authors' Contribution

Conceptualisation: MK, MM. Methodology: MK, MM, DS, FJA, FEV, RTM, ST, MSA, YP. Writing/preparation of original draft: MK, MM, FJA, YP. Writing, review and editing: MK, MM. Supervision and funding acquisition: MM. All authors have read and approved the final version of the manuscript.

Ethics

The Animal Research Ethics Committee of Tabriz University of Medical Sciences approved and carried out all animal studies (IR.TBZMED.VCR.REC.1398.407).

Conflict of Interest

The authors declare no conflict of interest.

References

1. Sung H, Ferlay J, Siegel RL et al. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA: Cancer J Clin. 2021;71(3):209-49. doi: 10.3322/caac.21660.
2. Costa A, Vale N. Strategies for the treatment of breast cancer: from classical drugs to mathematical models. Mathematical biosciences and engineering : MBE 2021;18(5):6328-85. doi: 10.3934/mbe.2021316.
3. Elahirad E, Sasani F, Khosravi A, Gharagozlou MJ, Khanbarari F. Evaluation of Cytokeratin 7 Expression in Different Mammary Gland Neoplasms. Iran J Vet Med. 2021;15(1):56-67. doi:10.22059/ijvm.2020.295956.1005052
4. Mansoor Lakooraj H, Khaki Z, Ghorbani M, Shafiee Ardestani M, Dezfoulian O. The in vitro Effect of Doxorubicine-G2-FA Treatment on Breast Cancer Copyright. Iran J Vet Med. 2020;14(2):147-158. doi:10.22059/ijvm.2019.291998.1005039
5. Koohi MK, Zare Mirakabadi A, Moharrami M, Hablolvarid MH. Anti-cancer effect of ICD-85(venom derived peptides) on MDA-MB231 cell line (in vitro) and experimental mice with breast cancer (in vivo). Iran J Vet Med. 2009;3(1):-. doi:10.22059/ijvm.2009.19609
6. Mojibi R, Morad Jodaki H, Mehrzad J, Khosravi A, Sharifzadeh A, Nikaein D. Apoptotic Effects of Caffeic Acid Phenethyl Ester and Matricaria chamomilla Es-sential Oil on A549 Non-Small Cell Lung Cancer Cells. Iran J Vet Med. 2022;16(4):390-399. doi:10.22059/ijvm.2022.335092.1005217.
7. Mann J. Natural products in cancer chemotherapy: past, present and future. Nat Rev Cancer. 2002;2(2):143-8. doi: 10.1038/nrc723.
8. Laskar S, Brahmachari G. editors. Access to biologically relevant diverse chromene heterocycles via multicomponent reactions (MCRs): Recent advances. SOAJ .Org Biomol Chem. 2014; 2: 1-50. http://signpostejournals.com.

9. Lacy A, O'Kennedy R. Studies on coumarins and coumarin-related compounds to determine their therapeutic role in the treatment of cancer. Curr Pharm Des. 2004;10(30):3797-811. doi: 10.2174/1381612043382693.
10. Musa MA, Cooperwood JS, Khan MOF. A review of coumarin derivatives in pharmacotherapy of breast cancer. Curr Med Chem. 2008;15(26):2664-79. doi: 10.2174/092986708786242877.
11. Farhangi B, Alizadeh AM, Khodayari H, Khodayari S, Dehghan MJ, Khori V et al. Protective effects of dendrosomal curcumin on an animal metastatic breast tumor. Eur J Pharmacol. 2015;758:188-96. doi: 10.1016/j.ejphar.2015.03.076.
12. Born SL, Hu JK, Lehman-McKeeman LD. o-hydroxyphenylacetaldehyde is a hepatotoxic metabolite of coumarin. Drug Metab Dispos. 2000;28(2):218-23.
13. Lake BG. Coumarin metabolism, toxicity and carcinogenicity: relevance for human risk assessment. Food Chem Toxicol. 1999; 37(4):423-53. doi: 10.1016/s0278-6915(99)00010-1.
14. Bijari N, Shokoohinia Y, Ashrafi-Kooshk MR et al. Spectroscopic study of interaction between osthole and human serum albumin: Identification of possible binding site of the compound. J Lumin. 2013;143:328-36. doi:10.1016/j.jlumin.2013.04.045.
15. Thakur A, Singla R, Jaitak V. Coumarins as anticancer agents: a review on synthetic strategies, mechanism of action and SAR studies. Eur Med Chem. 2015;101:476-95. doi: 10.1016/j.ejmech.2015.07.010.
16. Gong X, Zheng Y, He G et al. Multifunctional nanoplatform based on star-shaped copolymer for liver cancer targeting therapy. Drug Deliv. 2019;26(1):595-603. doi: 10.1080/10717544.2019.1625467.
17. Kostova I. Synthetic and natural coumarins as cytotoxic agents. Curr Med Chem Anticancer Agents. 2005;5(1):29-46. doi: 10.2174/1568011053352550.
18. Myers RB, Parker M, Grizzle WE. The effects of coumarin and suramin on the growth of malignant renal and prostatic cell lines. J Cancer Res Clin Oncol. 1994;120 Suppl:S11-3. doi: 10.1007/bf01377115.
19. Maucher A, von Angerer E. Antitumour activity of coumarin and 7-hydroxycoumarin against 7,12-dimethylbenz[a]anthracene-induced rat mammary carcinomas. J Cancer Res Clin Oncol. 1994;120(8):502-4. doi: 10.1007/bf01191806.
20. Kaur M, Kohli S, Sandhu S et al. Coumarin: a promising scaffold for anticancer agents. Anticancer Agents Med Chem. 2015;15(8):1032-48. doi: 10.2174/1871520615666150101125503.
21. Musa MA, Khan MO, Cooperwood JS. Synthesis and antiproliferative activity of coumarin-estrogen conjugates against breast cancer cell lines. Lett Drug Des Discov. 2009;6(2):133-8. doi: 10.2174/157018009787582624.
22. Batran RZ, Dawood DH, El-Seginy SA et al. New Coumarin Derivatives as Anti-Breast and Anti-Cervical Cancer Agents Targeting VEGFR-2 and p38α MAPK. Archiv der Pharmazie 2017;350(9). doi: 10.1002/ardp.201700064.
23. Cao D, Liu Y, Yan W et al. Design, Synthesis, and Evaluation of in Vitro and in Vivo Anticancer Activity of 4-Substituted Coumarins: A Novel Class of Potent Tubulin Polymerization Inhibitors. J Med Chem. 2016;59(12):5721-39. doi: 10.1021/acs.jmedchem.6b00158.
24. Schmitt CA, Fridman JS, Yang M et al. Dissecting p53 tumor suppressor functions in vivo. Cancer Cell. 2002;1(3):289-98. doi: 10.1016/s1535-6108(02)00047-8.
25. Chipuk JE, Kuwana T, Bouchier-Hayes L et al. Direct activation of Bax by p53 mediates mitochondrial membrane permeabilization and apoptosis. Science (New York, NY) 2004;303(5660):1010-4. doi: 10.1126/science.1092734.
26. Molnár T, Pallagi P, Tél B, Király R et al. Caspase-9 acts as a regulator of necroptotic cell death. FEBS J. 2021;288(22):6476-91. doi: 10.1111/febs.15898.
27. Tummers B, Green DR. Caspase-8: regulating life and death. Immunol Rev 2017;277(1):76-89. doi: 10.1111/imr.12541.
28. Sorolla A, Wang E, Golden E et al. Precision medicine by designer interference peptides: applications in oncology and molecular therapeutics. Oncogene 2020;39(6):1167-84. doi: 10.1038/s41388-019-1056-3.
29. Sheng H, Shao J, Washington MK et al. Prostaglandin E2 increases growth and motility of colorectal carcinoma cells. J Biol Chem. 2001;276(21):18075-81. doi: 10.1074/jbc.M009689200.