Available online at www.medicinescience.org

ORIGINAL RESEARCH

Medicine Science International Medical Journal

Medicine Science 2019;8(1):186-91

Synthesis and the effect of a novel benzoxazole compound on breast cancer cell line

Funda Kosova[1], Ozlem Temiz Arpaci[2], Ercument Olmez[3], Ibrahim Tuglu[4]

[1]Celal Bayar University School of Health Vocation, Manisa, Turkey
[2]Ankara University Pharmacy Faculty, Department of Pharmaceutical Chemistry, Ankara, Turkey
[3]Celal Bayar University Medical Faculty Department of Pharmacology, Manisa, Turkey
[4]Celal Bayar University Medical Faculty, Department of Histology and Embryology, Manisa, Turkey

Received 03 January 2019; Accepted 30 January 2019
Available online 21.02.2019 with doi:10.5455/medscience.2019.08.9006

Abstract

Breast cancer today is the most frequent cancer among women, and the second most common cause of cancer deaths among women. The aim of this study was to synthesize a new benzoxazole derivative, scan it for anti-cancer potential by MTT test using different breast cancer cell lines, and examine its effects on NF-κB and apopitosis-related proteins (APAF-1, cytochrome C, caspase-3, bcl-2) by the western blot method. newly-synthesized benzoxazole compound was applied to breast cancer cell lines (MDA-MB, MCF-7) and its cytotoxicity was measured quantitatively by MTT test. Later, the level of its effects on NF-κB and apopitosis-related proteins (APAF-1, cytochrome C, caspase-3, bcl-2) were examined by the western blot method. In our study, the structure of the synthesized new 5-[4-chlorobutanamido]-2-(p-methylphenyl)benzoxazole was proved by elemental analysis, 1H NMR and mass spectroscopy analysis methods. When the toxic effects of the application of the compound on the cell lines was examined by MTT, it had a greater toxic effect on MCF-7 when compared with MDA-MB, and IC50 levels were lower. When the protein was examined in immunohistochemistry with regard to VEGF, eNOS and TUNEL, it was observed that it caused a reduction in VEGF and an increase in eNOS and TUNEL. In the assay of the proteins by western blot, when benzoxazole compound was added to the MDA and MCF-7 cell line, there was no difference from the control group in Apaf-1 and BCL-2 levels, but a reduction was observed in caspase and Nfkβ levels compared with the control group. When the compound was added to the MDA-MB cell line, an increase was shown in the Cytochrome C level compared to the control group, but no difference was seen in the MCF-7 cell line. It is felt that this synthesized new benzoxazole compound increases apopitosis by reducing the activation of Nfkβ, and in this way has shown an effect of inhibiting tumor growth in cancer treatment. In addition, it is felt that this can provide hope in cancer treatment by the improved phase studies.

Keywords: Benzoxazole compounds, NF-κB, APAF-1, sitokrom C, caspase-3, bcl-2

Introduction

Cancer; is a disease which caused by changes in critical genes that control cell proliferation, differentiation and survival. Continuous and uncontrolled proliferation of cells causes cancer [1,2]. Cancer and apoptosis are common in the cell population dynamics [3]. Cancer is one of the most common causes of death in the world. In the last decade there have been great advances in the diagnosis and treatment of these patients. However, the prognosis of some cancers is very poor. Apoptosis and angiogenesis are important factors for growth and differentiation, but are also important for tumor invasion and metastasis. Nuclear Factor kappa B (NF-κB) has been shown to play an important role in both apoptosis and angiogenesis mechanisms.Apoptosis is a controlled and programmed cell death process [4], which is activated by three pathways by intracellular and extracellular signals, regulated by many proteins.In the early 1970s Kerr et al, found that apoptosis was associated with the elimination of potential cancer cells and tumor development [5]. Adams and Cory's shown that a strong relationship between apoptosis irregularity and cancer pathogenesis [6]. Takayama et al. shown that proliferation of tumor cells and escape of apoptotic cell death are present [7]. Wang et al. stated that apoptotic activity in breast cancer would be initiated by combined treatment regulating caspase cascade [8]. Li et al reported that Bcl-2 was effective in the early diagnosis and treatment of breast cancer [9]. NF-kB is mainly active and located in the nucleus. In some cancers (such as some Hodgkins and diffuse large B-cell lymphoma cells), due to chronic stimulation of the IKK pathway, the IkB genes may be mutated and damaged. Furthermore, the Rel / NF-kB transcription

***Coresponding Author:** Funda Kosova, Celal Bayar University School of HealthVocation,Manisa, Turkey
E-mail: fundakosova@gmail.com

factors of many human lymphoid cancer cells proliferate or mutate, leading to activation of NF-kB. Continuous Rel / NF-kB activity protects cancer cells from apoptosis and, in some cases, allows them to grow. Thus, in many antitumor therapies, many currents inhibit the growth of the tumor by blocking NF-kB activity [10]. In recent years, benzoxazoles and analogs of heterocyclic arms such as benzimidazoles, benzothiazoles and benzoxazines have been found to be antibacterial and antifungal [1,11], antiviral [12], and topoisomerase-inhibiting and anti-tumor activities [13]. The new series of benzothiazolers have stated that they have a very potent inhibitory effect against in vivo and in-vitro human breast cancer cell lines [13].

Previous studies have reported that some benzoxazole compounds [11,14]. Especially 5-ethylsulfonyl-2-(p-nitrophenyl)benzoxazole was found very potent compound for antitumoral activity. In the light of the findings, it is aimed to synthesize a specific benzoxazole compound.

Then, we aimed to investigate the levels of the anti-apoptotic protein bcl 2 and angiogenic NF-κBproteins by cytochrome-c, caspase anti 9 and APAF-1 (Apoptosis activating factor), which are apoptotic active on breast cancer line.

So that we aimed to investigate the effect ofa new benzoxazole compound on the levels NF-κB, bcl 2, cytochrome-c, caspase-9 and APAF-1which are markers for apoptosis in breast cancer cell culture.

Material and Methods

Synthesis of Benzoxazole Compound

The chemicals and solvents were purchased from Sigma-Aldrich Co. (Taufkirchen, Munich Germany) and Fisher Scientific (Pittsburgh, PA, USA) and used without further purification. Silica gel HF254 chromatoplates (0.3 mm) were used for TLC and chloroform was employed as mobile phase. Melting point was recorded on a Stuart Scientific SMP 1 (Bibby Scientific Limited, Stone, Staffordshire, UK) and is uncorrected. NMR spectra was recorded on a Varian Mercury 400 MHz NMR spectrometer (Palo Alto, CA, USA) in CDCl3; tetramethylsilane (TMS) was used as an internal standard. The mass spectra was recorded on a Waters ZQ Micromass LC-MS spectrometer (Milford, MA, USA) using the ESI (+) method. Elemental analyse was performed on an LECO 932 CHNS (St. Joseph, MI, USA) instrument and result was within ±0.4% of theoretical value.

Synthesis studies were carried out by researcher in the Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Ankara University.Firstly, 5-Amino-2-(p-methylphenyl)-benzoxazole was synthesized by heating 0.02 mol 2,4-diaminophenol•2 HCl with 0.02 mol p-methylbenzoic acid in 25 g polyphosphoric acid (PPA) and stirring for about 1 h at 160-200ºC. Then, the residue was poured over ice and the solution was neutralized with 10% NaOH solution. The observed precipitate was filtered and re-crystallizated with ethanol. Subsequently, 3-Chlorobutryl chloride (0.02 mol) was added over a period of 1 h to a stirred, ice-cooled mixture of 5-Amino-2-(p-methylphenyl)-benzoxazole (0.02 mol), sodium bicarbonate (0.02 mol), diethyl ether (40 ml), and water (20 ml). The mixture was stirred overnight. The residue formed was filtered off, washed with water and dissolved in ethanol. Crystallization was done by adding distilled water and the crude product was obtained drying the filtrate under ambient conditions. Finally, the compound was prepared as original product (Figure 1). The structures was supported by spectral data. The ^{1}H-NMR and mass spectra and elemental analysis results agree with those of the proposed structures. Physical and spectroscopic data for compound is listed in below.

2-(p-methylphenyl)-5-[4-chlorobutrylamido]-benzoxazole C18H17ClN2O2.Yield 61 (%). M.p.161-163°C; 1H-NMR (400MHz,CDCl3-d6): δ2.21-2.27 (2H, t), 2.44 (3H, s), 2.59-2.62(2H, t), 3.67-3.71 (2H,t), 7.32-7.34(2H, d, j=7.6), 7.41-7.51 (3H, m), 7.88 (1H, s), 8.11-8.13(2H, d, j=8.4). LC-MS: m/ (%X)z 329.5(100, M+H) 331.5(33, M+H+2)

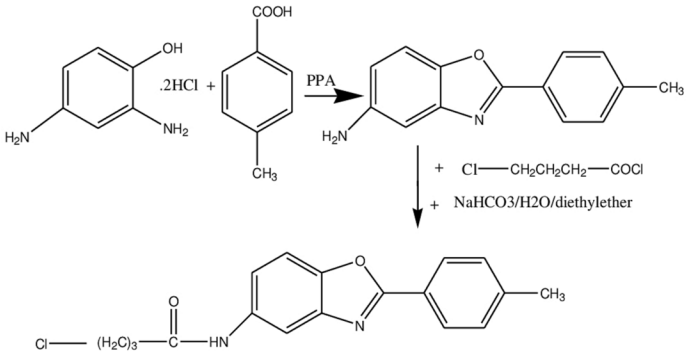

Figure 1. Synthesis of new benzoxazole compound

Cell Culture Cell culture studies were maked by Celal Bayar University medical faculty, department of Histology and embiryology. A human breast cancer cell line MCF-7 and MDA-MB were obtained from the American Type Culture Collection (ATCC, Manassas, VA). The cells were maintained in DMEM F-12 medium supplemented with 10% FCS, 1% L-glutamine and 1% penicillinstreptomycin at 37 ° C in a 5% CO 2 incubator. The morphology of the cells was examined on alternate days using a phase contrast inverted microscope (CK40-F200; Olympus, Tokyo, Japan) and photographed. All experiments were repeated for three times

Benzoxazole compound application

The compound was prepared as a 1 µ g/ml stock solution, which was diluted for use to 100, 50, 25, 12.5 and6.25 µ l solutions in dimethylsulfoxide (DMSO).The same volume of DMSO was used as the vehiclecontrol for Heterocyclic compounds experiments at a final concentrationof 0.1%.

MTT Assay

Mitochondrial functions of MCF-7 and MDA-MB cells were determined by MTT as an indicator of viable cells. This method is a colorimetric assay for measuring the reduction of yellow, water soluble MTT dye to a purple formazan product by active mitochondria. MTT stock solution (50 g MTT + 10 ml PBS) can be stored in the refrigerator. All PBS in solutions was adjusted to pH 7.4. The culture medium was removed from the culture wells, then 200 µl/well MTT solutions (1 ml stock solution of MTT + 9 ml growth medium) was added and the plate was incubated for 3 h under culture conditions. MTT solution was removed, then 200 µl DMSO was added to each well, and spectrophotometric measurements were performed using a microplate reader at 570 nm with a 690 nm reference filter

Western blot analysis

The Cells were centrifuged at 12,000× g at 4 °C for 15 min and supernatant (cytosolic extract) were used. Protein concentrations were determined by a dye-metalbased colorimetric protein assay [10]. Commercially available Pierce 660 nm protein assay reagent (Pierce/Thermo Scientific, Rockford, IL) which is not affected by the levels of the reducing agents. An equal amount (30 µg) of protein was applied to each well and proteins were separated in a 12% sodium dodecyl sulphate polyacrilamide gel electrophoresis (SDS-PAGE). After electrophoresis proteins were transferred to PVDF membrane. Membrane were blocked with 3 % nonfat dry milk. Anti-mouse (Biovision, USA) cytochrome c, and antimouse monoclonal IgG AIF (Santa Cruz Biotechnology, TX, USA) were used as a primary antibody and alkaline phosphatase conjugated anti-mouse (Invitrogen, NY, USA) was used as a secondary antibody. β-actin primary antibody (Genscript, USA) was used in loading control analysis for normalization. The signal was detected on PVDF membrane by BCIP/NBT Substrate (Invitrogen, NY, USA). Membranes were scanned using densitometer (Bio-Rad, GS-800) and signal intensity was determined by Quantity One Software (Bio-Rad, CA, USA) to compare expression levels among groups. Normalized three independent experiment results were done.

Statistical analysis

Statistical significance was determined using the SPSS 15.0. Significance (IC50 levels) was defined as $p \leq 0.05$.

Results

In this project, we synthesized a new benzoxazolecompound. We looked at purity controls of the compound by thin layer chromatography and melting degrees. We applied MTT test to the benzoxazolecompound and we determined the mitochondrial functions of the cells and thus the cell density of the cells as colorimetric. Those with anticancer potential in terms of survival were separated. Then the remaining group was VEGF, eNOS and TUNEL. Here, the IC50 dose was given the most appropriate response. And than, we examined the effects of the compound which is the most suitable for bioactivity on different breast cancer cell line, NF-κB, and apoptosis (APAF-1, cytochrome C, caspase-3, bcl-2) related proteins with western blot method.

As a result, we carried out biological activity studies on the benzoxazolecompound. The structure of the synthesized compound was proved by elemental analysis, 1H NMR and Mass spectroscopic analysis methods (Figure 1).

Although MCF-7 cells were adherent, adherent, and islands-shaped semiconfluent, the cells showed apoptotic morphology with P / 22 GBMA administration, proliferation stopped and the majority died. MDA-MB cells were found to be proliferated and died similarly after administration (Figure 2).

Compared with MCF-7, MDA-MB cells were found to be more resistant and less dead at the same dilutions, which was significantly more significant ($p < 0.001$). (Table 1).

When benzoxazole compound was added on the MDA and MCF-7 cancer cell line, we found that Apaf-1 level was similar compare to control group (Figure 3).

When the compound was added on the MDA and MCF-7 cancer cell line, we found that BCL-2 level was similar compare to control group (Figure 4).

When the benzoxazole compound was added on the MDA and MCF-7 cancer cell line, we found that Caspaselevel was decreasedcompare tocontrol group (Figure 5).

When the compound was added on the MDA cancer cell line, we found that Cytochrome C level was increasedbut was similar on MCF-7cancer cell line compare to control group (Figure 6).

When the compound was added on the MDA and MCF-7 cancer cell line, we found that Nfkβ level was decreasedcompare to control group (Figure 7).

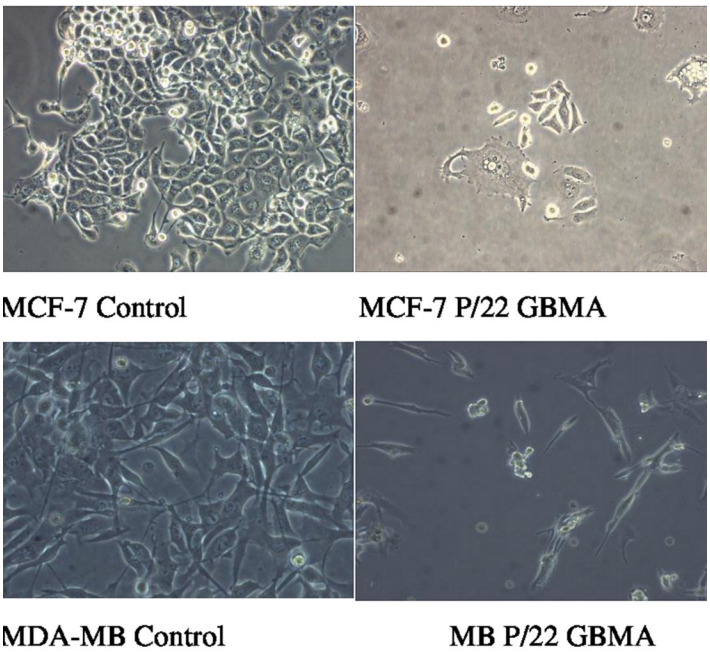

Figure 2. Cells that are semiconfluent and confluent in MCF-7 and MDA-MB breast cancer cell lines.

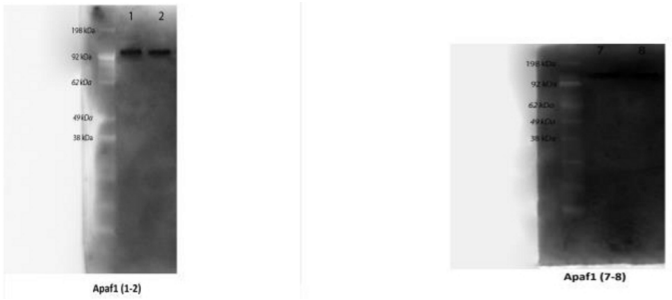

Figure 3. The effect of Apaf-1 on MDA ve MCF-7 breast cancer line

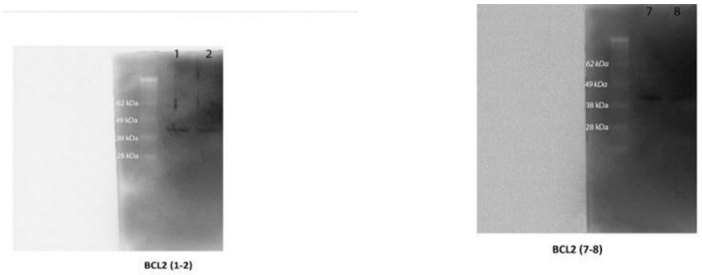

Figure 4. The effect of BCL-2 on MDA ve MCF-7 breast cancer line

Figure 5. The effect of Caspase on MDA ve MCF-7 breast cancer line

Figure 6. The effect of Cytochrome C on MDA ve MCF-7 breast cancer line

Figure 7. The effect of Nfkβ on MDA ve MCF-7 breast cancer line

Discussion

Cancer is a disease which appears when cells multiply in a uncontrolled way with sometimes fatal consequences, and which has been much more commonly seen in recent times.Breast cancer today is the most frequent cancer and the second most common cause of cancer deaths among women. Some types of breast cancer are very resistant to drug treatment. Breast cancer today is the most frequent cancer and the second most common cause of cancer deaths among women. Endogenous estrogen excess is also thought to play a significant role [15].High endogenous estrogen level has been shown to increase the progression of postmenaposal carcinoma in the breast [16,17].

The aim of this study was to synthesize a new benzoxazole derivative which will investigate for anti-cancer potential by MTT test using different breast cancer cell lines. Moreover, The relation of this effect with apopitosis-related proteins such as APAF-1, cytochrome C, caspase-3 and bcl-2 with NF-κBby the western blot will be examined.

In 1958, benzoxazole ring was found to have antitumoral effects [18]. Bis (2-hydroxyethyl) amino group, N - ((p-bis (2-hydroxyethyl) amino) phenyl) formimidoyl structure [19], (2-(benzodioxan-5-yl) The presence of effective antitumoral activity in the benzoxazole compounds carrying the cityl group [20] allowed the researchers to concentrate on the antitumoral effect on this ring system.In another study, have been synthesized which 7-substituted-2-phenylbenzoxazole, benzimidazole derivative with 4-substituted-2-phenylbenzoxazole compounds and this compounds have been reported to have cytotoxic activity on mammals with lower DNA binding propertieson S. typhimurium [21,22]. Sato et al., found that AJI9561 compound obtained from Streptomyces sp. AC9561 and showed that alsoantitumor activity [23]. In another study, the UK-1 compound has a broad spectrum and potent antitumoral activity (IC50 value around 20 nM) on leukemia, lymphoma and some solid tumor cells. [24]. Lage et al., has been found that some 2,5-disubstituted carboxazole, substituted benzoxazine and benzamide-derived compounds were to be highly effective against cancer cells ,(stomach, breast, pancreas, fibrosarcoma and melanoma) [25]. In the light of this information, in this study, it is aimed to evaluate the antitumoral activities of this study for the first time by synthesizing novel derivative that carry the benzoxazole ring as the main structure. Apoptosis is a form of programmed cell death characterized by the morphological changes carried out by the caspases and regulated by Bcl ve 2 family proteins [26]. Cancer cells reduce the expression of pro-apoptotic Bcl-2 members; they may increase the expression of anti-apoptotic Bcl-2 members or inactivate Apaf-1 expression [27]. Decrease in Bcl-2 levels leads to apoptosis, while increased in BCL-2 levels protect from death in the cells [28]. Bcl - 2 is like a guard of the mitochondria due to its presence outside the mitochondria and keeping the cytochrome in the mitochondria. Watson et al. states that the expression levels of the anti-apoptotic Bcl-2 was increased in colon cancer [29].

Cytochrome c is a protein belonging to the electron transport chain in the inner membrane of the mitochondria. After the cytochrome c is released from the mitochondria to the cytosol, it binds to the cytosolic apoptotic protease activation factor-1 (Apaf-1) for create apoptosm and binds with procaspase-9 for create apoptosm [30,31]. Apaf-1 is the cytosolic protease activation factor. The induction of apaf-1 occurs by the release of cytochrome c from mitochondria, cytochrome c is the activator of Apaf-1 [32]. Wright et al. found that Apaf -1 and caspase-9 were required for cytochrome-c induced apoptosis [33].

Caspases which are important role in apoptosis, are cysteine-dependent aspartate protease family. Cytochrome-c and apaf-1 facilitate the destruction of procaspaz-9 and the formation of active caspase-9 in the presence of ATP. Caspase-9 activates caspases, such as caspase-3 and caspase-7, called caspase cascade [24]. Procaspase-9 is involved in apoptosm and caspase-9 becomes activated and than caspase-9 aktivates caspases such as caspase-3 [34].

NF-kappa B (NF-κB, Nuclear Factor kappa B) is a transcription factor. It is inactive in the cytoplasm. Moves to the nucleus when activated. There are 5 types: NF-toB1, NF-elerB2, RelA (p65), RelB and c-Rel.Rel or NF-kappaB (NF-kB) In addition, these transcription factors continuously activate sites of disease including cancer, arthritis, chronic inflammation, asthma, neurodegenerative diseases and heart diseases [10,35). Many studies have been cross-linked between MAPK p38 and NF-yolB pathways. Gochman et al. clearly demonstrated the involvement of the MAPK p38 pathway

in IKK activation. IKK inhibitors decrease IırBα phospharylation after exposure to peroxynitrite [35].

In this study compared to MDA-MB, MCF-7 cells had a more toxic effect and IC50 levels were lower. In a previous study, a similar compound was used in vivo and in vitro, and its size-reducing effects were determined in both culture medium and tumor tissue [36]. It is understood that increased oxidative stress and apoptosis due to aggressive and invasive in the cancer cell line is induced by this compound and cause more cell death [37,38].Another effect of BTHB was on cell death. The dilutional effect of direct toxic effect was replaced by apoptosis in cell death mechanisms at IC50 level. Drifting to apoptotic death was easier with MCF-7, but less with MDA-MB. Previous studies have shown that other similar compounds that support apoptosis and apoptosis are used as caspase and other activators as signaling pathways [37,39].

It has been shown by immunohistochemical staining that the use of the benzoxazole compounds have already increased the oxidative stress present at the basal level and enriched the environment for free radicals. In previous studies, it has been suggested that ambient stress reduces the progression of cancer cells and leads to death of cells [40,41]. In our studies found that were compared with tamoxifen as a positive control, indicating that our findings would have a similar effect. H40 points to the absence of toxic effects of benzoate compounds on normal cells in negative control studies on non-invasive breast cell lines. However, it is to be understood that there is no toxic effect in normal somatic cells and in vivo conditions in the healthier primary culture. In this sense, it is consistent with the findings of our study [39,42,43]. As a result, it is thought that this compound can be used in the treatment of cancer.

In our study, in the determination of the proteins by western blot, we found that when the benzoxazole compound was added to the MDA and MCF-7 cell lines, there was no difference between the control group of Apaf-1 and BCL-2, whereas the level of caspase and Nfkβ decreased compared to the control group. Cytochrome C levels were higher in MDA-MB cells compared to the control group and no difference was found in MCF-7 cell lines.

Consequently, in our study show that the benzoxazole ring system is structural similar to the heterocyclic adenine and guanine bases in the structure of nucleic acids. This suggests that this group of compounds can exhibit many chemotherapeutic effects over different pathways. We have found that this newly obtained heterocyclic compound is more effective in MDA-MB cell lines. We believe that this benzoxazole compound enhances apoptosis by decreasing the activation of Nfkβ and thus, by inhibiting the growth of the tumor in cancer treatments. As an advanced stage of this study, we plan to investigate how the benzoxazole compound can act on proteins in the angiogenic pathway. If we obtain useful results from this study, then we are planning to carry out studies to support our findings in animal cancer models, which is a higher stage of this study. Therefore, the application of heterocyclic compound appears to to contribute to the treatmentof breast cancer. By the help of heterocyclic compound breast cancer treatment may help survival of cancer patients.

Competing interests
The authors declare that they have no competing interest.

Financial Disclosure
All authors declare no financial support.

Ethical approval
Consent of ethics was approved by the local ethics committee.

Funda Kosova ORCID:0000-0001-8070-5067
Ozlem Temiz Arpaci ORCID:0000-0002-2485-345X
Ercument Olmez ORCID:0000-0003-3535-2471
Ibrahim Tuglu ORCID:0000-0002-0569-8415

References

1. Kosova F, Arı Z. Adipositokinler ve meme kanseri. FÜ Sağ Bil Derg. 2008 22:377-84.
2. Demirpençe E. Kanserde moleküler tedavi hedefi olarak sinyal iletim yolları, 21. Ulusal Biyokimya Kongresi, İstanbul, 2009.
3. Ohta T, Kunimasa K, Kobayashi T, et al. Propolis suppresses tumor angiogenesis by inducing apoptosis in tube-forming endothelial cells, Biosci Biotechnol Biochem. 2008;72:2336-440.
4. Özkaya AB, HCT–116 Kolon kanser hücre hattında yeşil çay etken maddesi olan (-)-Epigallokatekin–3-gallatın apoptoz üzerine etkisinin incelenmesi. İzmir, Dokuz Eylül Üniversitesi, 2008.
5. Kerr JF, Wyllie AH. Currie A.R. Apoptosis: a basic biological phenomenon with wide-ranging implications in tissue kinetics. Br J Cancer. 1972;26:239-57.
6. Adams JM, Cory S. The Bcl–2 apoptotic switch in cancer development and therapy. Oncogene. 2007;26:1324-37.
7. Takayama S, Reed JC and Homma S. Heat-shock proteins as regulators of apoptosis. Oncogene. 2003;22:9041-7.
8. Wang X, Guan X, Wang L, et al, Effect of recombinant human p53 adenovirus (Ad-p53) combined with EGFR inhibitor gefitinib on the sensitivity of breast cancer MDA-MB-468 cells Zhonghua Zhong Liu Za Zhi. 2014;36:886-91.
9. Liu L, Li HX, Lv XQ. The mechanism and significance of e-cadherin, anti-apoptosis B-cell lymphoma-2 protein and se-cadherin roles in cancer. J Biol Regul Homeost Agents. 2014;28:683-91.
10. Dr. Thomas Gilmore. NF-kB Transcription Factors Biology Department, Boston University 5 Cummington Mall, Boston, Massachusetts. 2012;02215-2406.
11. Kiessing S, Rockensuess KD, et al. Composition useful for the chemosensibiling and simultaneous apoptosis induction of multidrug resistance of tumor cells and bacteria, comprise substituteddisiloxane compound in the combination with benzoxazole derivatives. Patent De. 2010;102008027361(A1).
12. Uluoğlu Ö. Basic pathology, 4. baskı, 1990. p. 855-870.
13. Varga A, Akı-Sener E, Yalcin I, et al. Induction of apoptosis and necrosis by resistance modifiers benzazoles and benzoxazines on tumour cell line mouse lymphoma L5718 Mdr+cells. In Vivo. 2005;19:1087-92.
14. Temiz-Arpaci O, Yildiz I, Ozkan S, et al. Synthesis and biological activity of some new benzoxazoles. Eur J Med Chem. 2008;43:1423-31.
15. Wittliff JL. Steroid hormone receptors in breast cancer. Cancer. 1984;53:630-43.
16. Key T, Appleby P, Barnes I. Endogenous sex hormones and breast cancer in postmenopausal women: reanalysis of nine prospective studies, J Natl Cancer Inst. 2002;94:606-16.
17. Stephenson GD, Rose DP. Breast cancer and obesity: an update, Nutr Cancer. 2003;45:1-16.

18. Clayton CC. Effect of certain benzimidazoles and related compounds upon azo dye destruction by liver homogenates (23789). Proceed Soc Exp Biol Medic. 1958;98:510-12.

19. Schulze W, Gutsche W, Jungstand W. Zusammenhänge zwischen chemischer struktur und biologischer wirksamkeit bei azomethinen mit sticksofflost-gruppen am ehrlich-ascitestumor der weissen maus. Arzneim. Forsch. 1965;15:1235-8.

20. Bahner CT, Rıves LM, McGaha SW, et al. Di- and tri-methoxystyryl derivatives of heterocyclic nitrogen compounds. Arzneimittelforschung. 1981;31:404-6.

21. Denny WA, Rewcastle GW, Baguley BC. PPotential antitumor agents. 59. Structure-activity relationships for 2-phenylbenzimidazole-4-carboxamides, a new class of "minimal" DNA-intercalating agents which may not act via topoisomerase II. J Med Chem. 1990;33:814-9.

22. Denny WA, Turner PM, Atwell GJ, at al. Structure-activity relationships for the mutagenic activity of tricyclic intercalating in salmonella typhimurium. Mutat Res. 1990;232:233-41.

23. Sato S, Kajıura T, Misato N. et al. AJI9561, A New cytotoxic benzoxazole derivative produced by streptomyces sp. J. Antibio. 2001;54:102-4.

24. Kumar D, Jacob MR, Reynolds MB, et al. Synthesis and evaluation of anticancer benzoxazoles and benimidazoles related to UK-1. Bioorg. Med. Chem. 2002;10:3997-4004.

25. Lage H, Akı-Sener E, Yalcın I. High antineoplastic activity of newHeterocyclic compounds in cancer cells with Resistance against Classical DNATopoisomerase II-Targeting Drugs. Int. J. Cancer, 2006;119:213-20.

26. Diaz GD, Li Q, Dashwood HR. Caspase-8 and Apoptosis-inducing factor mediate a cytochrome c-independent pathway of apoptosis in human colon cancer cells Induced by the dietary phytochemical chlorophyllin. Cancer Res. 2003;63:1254-61.

27. Manns J, Daubrawa M, Driessen S, et al. Stork B. and Wesselborg S. Triggering of a novel intrinsic apoptosis pathway by the kinase inhibitor staurosporine: activation of caspase-9 in the absence of Apaf-1. FASEB J. 2011;25:3250-61.

28. Douglas RG, Reid PB. Thomas G.C. Apoptosis and cancer. Principles Practice Oncol. 1994;1:1-13.

29. Watson AJ, Merritt AJ, Jones LS, et al. Evidence for reciprocity of Bcl–2 and p53 expression in human colorectal adenomas and carcinomas. Br J Cancer, 1996;73:889-95.

30. Liu H, Jıang X, Zhang MW et al. Association of CASP9, CASP10 gene polymorphisms and tea drinking with colorectal cancer risk in the Han Chinese population. J Zhejiang Univ Sci B. 2013;14:47-57.

31. Saleh HA, Jacks H. Correlation of bcl-2 Oncoprotein Immunohistochemical Expressionwith Proliferation Index and Histopathologic Parametersin Colorectal Neoplasia. Pathol Oncol Res. 1999;5:4.

32. Türktekin M. Kolon kanseri hücre hattında apigenin flavonoidinin apoptoz yolaklı gen ifade edilmesine olan etkilerinin araştırılması. Ankara, Gazi Üniversitesi; 2009.

33. Wright KM, Smith MI, Farrag L, et al. Chromatin modification of Apaf-1 restricts the apoptotic pathway in mature neurons, J Cell Biol. 2007;179:825-32.

34. Liu K, Duanyang Shu D, Song N, et al. The role of cytochrome c on apoptosis induced by anagrapha falcifera multiple nuclear polyhedrosis virus in insect spodoptera litura cells. PLoS One. 2012;7:e40877.

35. Gochman E, Mahajna J, Reznıck AZ. NF-κB Activation by Peroxynitrite through IκBα-dependent Phosphorylation versus Nitration in Colon Cancer Cells Anticancer Res. 2011;31:1607-18.

36. Roomi MW, Ivanov V, Kalinovsky T, et al. In vitro and in vivo antitumorigenic activity of a mixture of lysine, proline, ascorbic acid, and green tea extract on human breast cancer lines MDA-MB-231 and MCF-7. Med Oncol. 2005;22:129-38.

37. Zhan Y, Zhang Y, Liu C, et al. A novel taspine derivative, HMQ1611, inhibits breast cancer cell growth via estrogen receptor α and EGF receptor signaling pathways. Cancer Prev Res. 2012;5:864-73.

38. Lee KH, Ho WY, Wu SJ, et al Behavior-selective apoptotic capacity of 4-(3,4,5-Trimethoxyphenoxy) benzoic acid and its methyl derivatives on two breast cancer cell lines. Anticancer Res. 2014;34:1801-9.

39. Liu W, Zhou J, Bensdorf K, et al Investigations on cytotoxicity and anti-inflammatory potency of licofelone derivatives. Eur J Med Chem. 2011;46:907-13.

40. Zhang Y, Guo J, Zhang J, et al. Tert-butyl-2(4,5-dihydrogen-4,4,5,5-tetramethyl-3-O-1H-imidazole-3-cationic-1-oxy l-2-pyrrolidine-1-carboxylic ester displays novel cytotoxicity through reactive oxygen species-mediated oxidative damage in MCF-7 and MDA-MB-231 cells. Chem Biol Interact. 2011;192:287-97.

41. Lin PH, Lin CH, Huang CC, et al. 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) induces oxidative stress, DNA strand breaks, and poly(ADP-ribose) polymerase-1 activation in human breast carcinoma cell lines. Toxicol Lett. 2007;172:146-58.

42. Ismail IA, Kang HS, Lee HJ, et al. 2'-Benzoyloxycinnamaldehyde-mediated DJ-1 upregulation protects MCF-7 cells from mitochondrial damage. Biol Pharm Bull. 2012;35:895-902.

43. Li H, Wood JT, Whitten KM, et al. Inhibition of fatty acid amide hydrolase activates Nrf2 signalling and induces heme oxygenase 1 transcription in breast cancer cells. Br J Pharmacol. 2013;170:489-505.