Available online at www.medicinescience.org

ORIGINAL ARTICLE

Medicine Science International Medical Journal

Medicine Science 2020;9(4):823-8

Investigation of the effect on prostate cancer purine base analog of the newly developed compound

Gokhan Temeltas[1], Funda Kosova[2], Ozlem Temiz Arpaci[3], Ibrahim Tuglu[4]

[1]Celal Bayar Unviversity, Faculty of Medicine, Department of Urology, Manisa, Turkey
[2]Celal Bayar Unviversity, Health Campus Department of Medical Biochemistry, Manisa, Turkey
[3]Ankara Unviversity, Faculty of Medicine, Department of Pharmasotic Chemistry, Ankara, Turkey
[4]Celal Bayar Unviversity, Health Campus, Department of Histology and Embiryology, Manisa, Turkey

Received 05 May 2020; Accepted 11 June 2020
Available online 17.08.2020 with doi: 10.5455/medscience.2020.04.063

Abstract

Prostate cancer is an important cause of death in men. This malignant disease is known for excessive proliferation, decreased apoptosis, and uncontrolled proliferation of cells. In the light of this knowledge, our aim was to examine potential compounds for their effects on NF-κB and angiogenesis proteins (VGEF, MMP, ES, TSP-1) in a prostate cancer line. In this study, we planned that the purine base analogue with anti-cancer potential has synthesized new compounds, and those with anti-cancer potential has selected by screening with MTT testing. We analysed VEGF, MMP, Endostatin (ES), Thrombospondin-1 (TSP-1) and NF-κB protein levels in a prostate cancer line by the Elisa method. MMA-MEP caused a significant decrease especially for VEGF-A, NF-kB, Endostatin, Thrombospondin -1 and MMP levels compared to other compounds. This suggests that this heterocyclic compound is more effective compared to others. This compound could be more effective for patients with prostate cancer, depending on time and amount. In line with these results, we want to continue our work by applying this heterocyclic compound in different amounts at different time periods.

Keywords: Prostate cancer, NF-kB, VEGF, MMP, endostatin and thrompospondin-1

Introduction

Cancer is known as the most common cause of death. In recent years, very important steps have been taken in the diagnosis and treatment of cancer patients. However, the prognosis for some types of cancer is still very bad. Prostate cancer is a significant cause of death in men. There are many factors which affect the progression and metastasis of cancer. One of the most important of these is angiogenesis. Progression and migration of the cancer is inhibited or activated according to the balance between angiogenesisi and antiangiogenesis. As well as being a basic factor in growth and differentiation, angiogenesis is of great importance for the spread and metastasis of the tumor. Vascular endothelial growth factor (VEGF) is an important angiogenesis activator that increases the permeability of the blood vessels and stimulates the secretion of matrix metalloproteinase (MMP), which is responsible for the breakdown of the extracellular matrix. In this way, it makes metastasis and invasion easier. The most important anti-angiogenic agents are endostatin and thrombospondin. Also, Nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) has been shown to have an important role in the mechanisms of both apoptosis and angiogenesis.

Heterocyclic compounds play an important role in the design of new parts due to their pharmaceutical effects. Structural isosters of natural nucleotides with some biological activities, including benzoxazoles antimicrobial [1-3], antiviral [4,5], topoisomerase-I and II inhibitor [6,7] antitumor [8,9], and antioxidant activities [10]. Also, previous years of research of some benzoxazole derivatives have revealed that these compounds constitute a new class of anticancer agents [6-9].

In this report, a series of some benzoxazole compounds (Table 1) which were previously synthesized and investigated for their antimicrobial activity [1,2,3] have been researched for their effect on prostate cancer as purine base analogues. We aimed to investigate whether these newly synthesized benzoxazole compounds may lead to the design of newer, stronger anticancer agents for prostate cancer, based on the activities of these compounds.

***Coresponding Author:** Funda Kosova , Celal Bayar Unviversity, Health Campus Department of Medical Biochemistry, Manisa, Turkey
E-mail: fundakosova@gmail.com

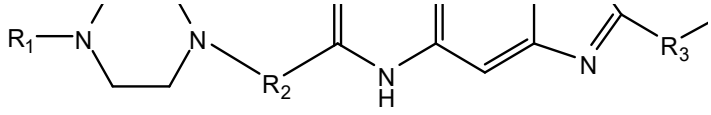
Materils and Methods

Patients

Our chemical compounds were purchased from Sigma-Aldrich Co. For TLC, we used silica gel HF254 chromatoplates (0.3 mm) and chloroform / methanol (10: 0.5) as the mobile phase. We recorded the melting points and NMR spectra in CDCI3 or dimethyl sulfoxide (DMSO-d6) on the NMR spectrometer with a Stuart Scientific SMP 1 device. We measured mass spectra on an LC-MS spectrometer (Milford, MA, USA) using the ESI (+) method. Also elemental analyses were recorded on a LECO 932 CHNS instrument and were within ±0.4% of theoretical values.

Synthesis of some benzoxazole compounds as purine analogues

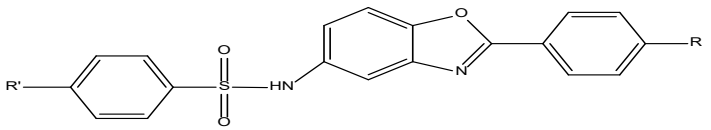
5-Amino-2-(p-methyl-phenyl)-benzoxazole was obtained by heating and stirring 2,4-diaminophenol·2 HCl with p-methyl benzoic acid in polyphosphoric acid (PPA). The residue was poured into an ice/water mixture and the solution was neutralized with 10% NaOH. Subsequently, the resulting precipitate was filtered, washed with distilled water and dried. After cooling with 5-amino-2- (p-methyl-phenyl) -benzoxazole on ice for 1 hour, chloroacetyl chloride was added to a mixture of sodium bicarbonate, diethyl ether and water. The prepared material was stirred at room temperature overnight and dried. Later, 5-(2-chloroacetamido)-2-(p-methyl-phenyl)-benzoxazole was added to N-(p-chloro-phenyl) piperazine or methyl-piperazine and triethylamine solution in N,N-dimethylformamide (DMF). The compound was stirred for 24 hours at room temperature and dissolved in ethyl acetate, and then added by adding n-hexane. The crystalline material was dried in vacuo. The compounds MMA-MEP and GMA-cFED (Figure 1) were prepared [1,2]. The structures of these were supported by spectral data as given in references.



MMA-MEP: R_1=CH_3 R_2=CH_2 R_3=- R_4=CH_3
GMA-cFED: R_1=p-Clorophenyl R_2=CH_2 R_3=- R_4=CH_3

Figure 1. Chemical structure of the compounds MMA-MEP and GMA-cFED

Other desired benzoxazole derivatives, DY65 and 534 (Figure 2), were synthesized using a two-step procedure. Firstly, 5-amino-2-(p-bromo-phenyl)-benzoxazole and 5-amino-2-phenylbenzoxazole were synthesized by heating 2,4-diaminophenol with p-bromo benzoic acid and benzoic acid in polyphosphoric acid (PPA). Then, the resulting compounds (DY65 and 534) were obtained by treating a solution of p-chloro-benzenesulfonyl chloride or p-methyl- benzenesulfonyl chloride with 5-amino-2-(p-substitutedphenyl)- benzoxazoles [3]. The structures of these were supported by spectral data as given in references.



DY65: R'= Cl R=H 534: R'=CH_3 R=Br

Figure 2. Chemical structure of the compounds DY65: and 534

Heterocyclic compound administration

1 ml stock solution of heterocyclic compound was prepared by dissolving heterocyclic compound in 50 µL DMSO and adding 950 µL of medium. Using this stock solution, heterocyclic compound was administered at 100, 50, 25, 12.5, 6.25 µL concentrations to the cancer cell line.

Cell culture

Various chemicals (DMEM F - 12, 10% FCS, 1% L-glutamine and 1% penicillin-streptomycin) were added to the Du-145 prostate cancer cell line and incubated at 37°C in an incubator supplied with 5% CO_2. 45,000 cells / mL were seeded on to 96-well plates with in each well. Once the cells adhered to the surface and multiplied, 1, 0.25, 0.06, 0.015 and 0.007 µg/mL from the stock solution of heterocyclic compound was added. Cell proliferation and cytotoxicity were examined 48 hours later by the MTT method [11].

MTT

Mitochondrial functions of the cells and viable cell density were determined by MTT test. This test is based on a redox reaction that converts yellow MTT reagent to blue/violet formazan in mitochondria. Cells that were incubated for four hours with 0.5 mg / mL MTT were separated from the medium after incubation. Formazan salts were dissolved in dimethyl sulfoxide (DMSO) and absorbance at 570 nm was read by a multiplate UV-visible spectrophotometer [12].

Immunocytochemistry

Cells were fixed in 4% paraformaldehyde solution in PBS (pH: 7.4) and washed with PBS three times for five minutes each time. The cells were incubated in 0.5% trypsin solution for five minutes and washed once again with PBS as detailed above. Cells were incubated in 3% hydrogen peroxide (H_2O_2) for 30 minutes, blocking solution for one hour, and anti-VEGF primary antibodies for 18 hours. Following washing, sections were stained with biotinylated anti-mouse/antihuman conjugated streptavidin-horseradish peroxidase for 30 minutes (85-9043, Zymed Histostain kit San Francisco, USA). Each secondary antibody was washed three times with PBS for five minutes. To make immunoreactivity visible, sections were developed in diaminobenzidin (DAB, 00-2020, Zymed, San Francisco, USA) for five minutes. Primary antibody was replaced by PBS for negative control. Following washing with distilled water, the sheets were mounted with mounting solution (00-8030, Histomount mounting solution, San Francisco, USA).

Enzyme linked immunosorbent assay (ELISA)

The culture plates were cultured using 3 X105 cells for 4 hours and then heterocyclic compounds were added to treat the cultures for 48 hours. Wwe stored these lysates until-80°C analysis time and measure NF-κB, VEGF, MMP, ES and TSP-1 concentrations by using ELISA kits (Millipore Corp., Billerica, MA, USA)

Statistical analysis

SPSS for Windows v15.0 was used to analyze the data obtained during the study. We used the Mann Whitney-U test to find differences between groups. The level of significance was set at $p<0.05$.

Results

Our research was aimed at investigating the effect of the newly-synthesized benzoxazole derivative compounds on angiogenic structuring markers VEGF, MMP, Endostatin, Thrombospondin-1 and NF-β proteins in the prostate cell line.

Although Du-145 cells were adherenand confluent in the form of islands, it was observed that with HSB application, cells showed apoptotic morphology, proliferation ceased and the majority died (Figure 3).

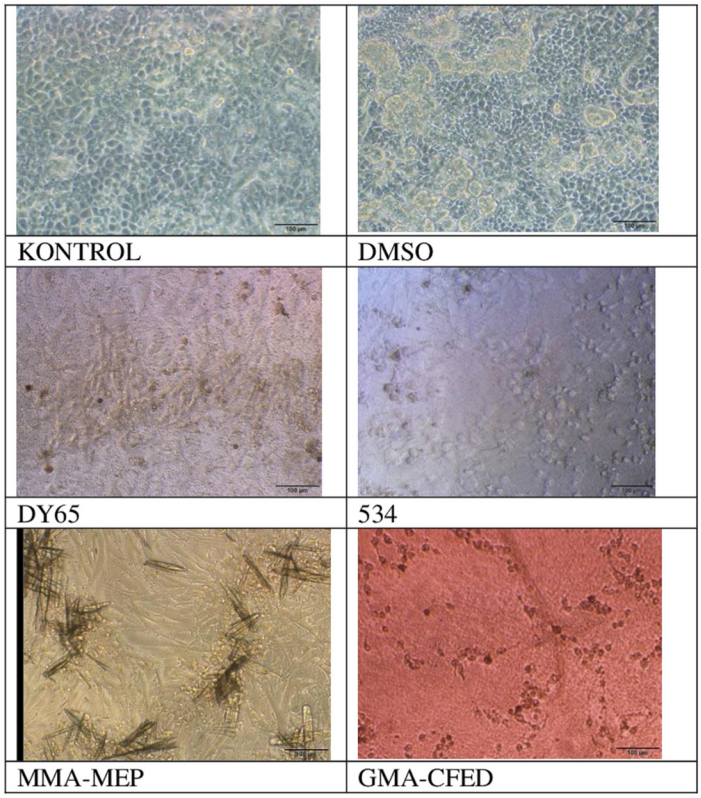


Figure 3. Heterocyclic compounds in the Du-145 prostate cancer cell line

MMA-MEP and GMA-CFED compounds made very significant ($p<0.001$) decreases of MTT absorbance compared to that of the control. MEP and GMA-CFED compounds had significant decreases of MTT absorbance ($p<0.01$), as did DYP65 and 534 compounds ($p<0.05$) (Figure 4).

According to ELISA results, there was a significant decrease for 534 ($p<0.05$), DY65 ($p<0.01$), GMA-CFED ($p<0.001$), and MMA-MEP ($p<0.001$) heterocyclic compounds compared to the control for TSP, ES and MMP. There was a less significant decrease for VEGF ($p<0.05$) and NF-κB ($p<0.01$) by ELISA (Table 1) except for GMA-CFED ($p<0.001$) and MMA-MEP ($p<0.001$).

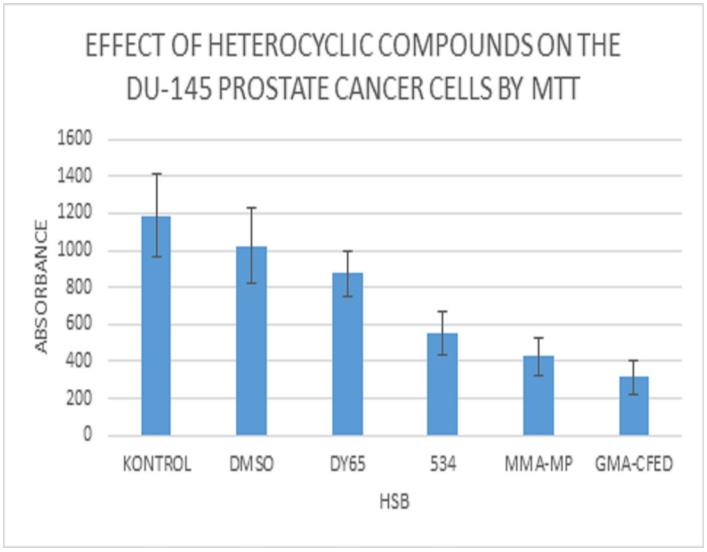


Figure 4. Toxic effects of ES4, GMA-CFEB and MMA-MEP ($p<0.001$) on MTT

Table 1. VEGF-A, NF-κB, Endostatin and Thrombospondin-1 levels in Du-145 by ELISA

	VEGF-A	NF-KB	MMP-9	ES	TSP-1
	pg/mL	ng/mL	pg/mL	ng/mL	ng/mL
Control	2.265 ± 0.113	4.254 ± 0.212	4.881 ± 0.112	2.576 ± 0.085	3.565 ± 0.088
534	2.014 ± 0.204	3.864 ± 0.286	3.595 ± 0.355	1.873 ± 0.145	3.035 ± 0.301
DY65	1.684 ± 0.168	3.412 ± 0.241	3.369 ± 0.292	1.702 ± 0.171	2.907 ± 0.292
GMA-CFED	1.652 ± 0.165	3.214 ± 0.221	3.318 ± 0.298	1.622 ± 0.162	2.775 ± 0.193
MMA-MEP	1.139 ± 0.133	3.016 ± 0.201	3.304 ± 0.188	1.592 ± 0.154	2.711 ± 0.226

Discussion

Prostate cancer is a significant cause of death in men. Despite an overall good prognosis for prostate cancer patients, it is estimated that among radically treated patients as many as 25% will experience recurrence of the disease during the first three years after treatment [13]. A process of angiogenesis plays an important role in cancer progression, as it is critical in the phenomena of invasion and metastasis. PC is characterized by a low blood vessel density and a slow cell proliferation. It is thought that compounds such as retinoids, angiostatin, endostatin, interleukin 10 (IL-10), prostate specific antigen (PSA) interferons and thrombospondin-1 may be responsible for the formation and progression of prostate cancer [14].

Angiogenesis is a process involving inhibitors and activators [15]. Angiogenesis occurs from new or preexisting vessels under the influence of growth factors, and the most important factor in activation is VEGF [16].

VEGF is a factor with proangiogenic activity on endothelial cells, representing a growth factor which is mitogenic and antiapoptotic, increasing vascular permeability and promoting cell migration [17]. VEGF, consisting of VEGF-A, VEGF-B, VEGF-C,

VEGF-D, VEGF-E, VEGF-F, placental growth factor (PlGF) and vascular endothelial family is an angiogenesis activator that has an effect in processes such as diabetic retinopathy, ischemia, tumor growth, metastasis, macular degeneration, and inflammation [18,19]. It consists of the growth factor (EG-VEGF). VEGF's VEGFR-1, VEGFR-2 and VEGFR-3. VEGFR-1 and VEGFR-2,

which have pro-angiogenic activity and tyrosine kinase activity and are found on vascular endothelial cells, while VEGFR-3 is found only on lymphatic endothelial cells. [20]. VEGF facilitates vascular permeability and migration of endothelial cells, vascular extravasation and metastasis while inducing the appearance of fenestrations between capillaries and venules by replacing proteins (ocludin, VE-cadherin / β-catenin) in intercellular junctions [21,22]. As a result of these events, we found that the secretion of MMP-9, MMP-3 and MMP-2 by the inhibition of Ang-1-induced metalloproteinase-2 tissue inhibitor, increased cell migration and facilitating the dissociation of ECM. [23,24]. The membrane type-1 matrix metalloproteinase from the newly discovered metalloproteinase family in angiogenesis regulates the expression of pro-angiogenic factors and controls cell migration [25]. These are found on MT1-MMP, endothelial cells or wall cells (pericides, VSMCs), which affect cell migration, tubulogenesis and cellular invasion [26,27]. At a later stage of neoangiogenesis, MT1-MMP can activate the PDGF-β / PDGFR-β path and control the stabilization of newly formed vessels [26]. With a diagnosis of prostate cancer difficulties, metalloproteases (MMPs) and metalloprotease-3 (TIMP-3) tissue inhibitors are helpful markers associated with cancer and tumor aggression [27].

Some angiogenesis inhibitors that are thought to have an effect on prostate cancer have been identified, such as angiostatin, endostatin, PSA, thrombospondin-1 (TSP-1), interleukin 10 (IL-10), interferons (IFNs) and retinoids [28]. Thrombospondin (TSP) is a glycoprotein involved in cell-to-cell and cell-to-matrix communication consisting of five members of the TSP protein family [22]. It has been understood that TSP-1 and TSP-2 are among the most effective TSP members, especially in the progression of cancer. Although the structures of TSP-1 and TSP-2 are similar, they have different roles because they are spatially different. [29]. If they are binding to receptor it acts as an antiangiogenic molecule [30]. It has also been reported that the arginine-glycine-aspartic acid (RGD) sequence in TSP-2 binds to integrin αv3 and heparan sulfate proteoglycans associated with cell adhesion-related or low-density lipoprotein receptor-bound protein (LRP) [31]. TSP-2 binds to pro-MMP-2 and MMP-2, which regulate the extracellular effect of MMP-2, controlling various physiological processes such as collagen fibrillogenesis, wound healing and angiogenesis [32].

Endostatin is a specific angiogenesis inhibitor with a 20 kDa C-terminal collagen XVIII fragment. There are many studies showing this effect by reducing metastases and reducing the growth of primary tumors [18].

It is known that chronic infection initiates transformation in cells and has an impact on the development of cancer [33,34]. The transcription factor NF-κB, which has a proinflammatory effect, is known to be effective in the initiation of inflammation and the progression of the tumor [35], and creates a proinflammatory environment in prostate cancer (PCa) that facilitates the spread of the tumor cell [36]. NF-κB is considered as the main transcription factor that creates abnormally activated proinflammatory tumor growth in most PCa cases. [37]. When many molecules activate NF-κB, they inhibit very few molecules, one of which IκBs NF-κB signaling is known to disrupt. Bonacini et al. reported that CLU reduces MMP-9 and MMP-2 expression by inhibiting NF-κB, and that these enzymes are involved in tumor spread [36].

Benzoxazoles have demonstrated interesting pharmacological activities, and in the past two decades they have been broadly investigated for their anticancer activity [8,9,38]. A benzoxazole compound UK-1 has demonstrated notable anticancer activity against certain cancer cell lines particularly on leukemia, lymphoma and some solid tumors cell lines with a broad spectrum. In another study, some 2,5-disubstituted benzoxazoles have also been found to be highly effective against cancer cells. Surprisingly, among an extended library of benzoxazole compounds, 2,5-disubstitution of the benzoxazole ring is important in potent anticancer activity.

Kosova et al. used MDA and MCF-7 cell lines for the treatment of bezoxazole compounds and looked at apopotic proteins from this lysate by the western blot method. MDA and MCF-7 cell lines showed that there was no difference between Apaf-1 and BCL- compared to control groups, while caspase and Nfkβ levels were decreased, Cytochrome C levels were higher in MDA-MB cells, and there was no difference in MCF-7 cell lines [38].

In a previous study by the researchers of this project, Kosova et al., CAPE was administered at different doses, and the correlation between the interaction of matrix proteins in stomach cancer cell cultures and levels of VEGF, MMP, Endostatin and Thrombospondin–1 was investigated. It was seen that the administration of CAPE inhibited propagation in stomach cancer cell lines and decreased the anti-angiogenic factors Endostatin and TSP while suppressing the angiogenesis activators VEGF and MMP-9. When the treatment dose of CAPE was administered to the collagen of stomach cancer cells, it was shown that levels of VEGF, MMP and Endostatin protein decreased and levels of TSP protein rose. When the dose of treatment with CAPE was applied to the Laminin of stomach cancer cells, it was seen that levels of VEGF, MMP and Endosatin protein rose, and there was no change in the levels of TSP protein [39]. In another study, which we conducted with the support of Tubitak, after proving by various spectroscopic analysis methods the structure of the synthesized compound (5-amino-2-(p-bromophenyl)benzoxazole), the toxic effects of the application of BTHB to cell lines was examined with MTT; when MDA was compared with MB, it was seen that MCF-7 had a greater toxic effect on the cells, and that IC50 levels were lower. In the examination with regard to VEGF, eNOS and TUNEL in the immunohistochemistry of the protein, it was seen that it secured a reduction for VEGF and an increase for eNOS and TUNEL. In the determination of the proteins by Western blot,

there was no difference between the Apaf-1 and BCL-2 levels and the control group when MDA and MCF-7 cell heterocyclic compounds were added, and it was observed that caspase and Nfkβ levels fell relative to the control group. When heterocyclic compounds were added to the MDA-MB cell line, it was observed that there was an increase in the level of Cytochrome C compared with the control group, but that there was no difference in the MCF-7 cell line [40]. In our study, we found that the purine base analogue with anti-cancer potential had synthesized new compounds, and those with anti-cancer potential were selected by screening by MTT testing. We then analyzed VEGF, MMP, Endostatin, Thrombospondin -1 and NF-κB protein levels in the prostate cancer line by the Elisa method. We saw that MMA-MEP showed a greater decrease than other compounds, especially the levels of VEGF-A, NF-κB, Endostatin and Thrombospondin-1, and that there was big difference in the level of MMP. We found that in this study MMA-MEP and GMA-CFED compounds made a very significant decrease in MTT absorbance compared to that of the control. MEP and GMA-CFED compounds with significant decrease and DYP65 and 534 compounds with significant decrease of MTT absorbance (Figure 4). According to the ELISA results, there was a significant decrease for 534, DY65, GMA-CFED and MMA-MEP heterocyclic compounds compared to the control for TSP, ES and MMP. There was a less significant decrease for VEGF and NF-κB by ELISA (Table 1) except for GMA-CFED and MMA-MEP.

This suggested that this heterocyclic compound was more effective. It suggests that this compound may be more effective for prostate cancer patients only in relation to time and amount. In the light of these results, we would like to continue our study, applying this heterocyclic compound for different lengths of time and in different proportions

Conflict of interests
The authors have no conflicts of interest to declare.

Financial Disclosure
Celal Bayar University supported by scientific research project coordination

Ethical approval
Our study is a cell culture study. An ethical committee is not required for cell culture studies.

References

1. Arısoy M, Temiz-Arpacı O, Yıldız İ, et al. Synthesis, Antimicrobial activity and QSAR studies of 2,5-disubstituted benzoxazoles, SAR & QSAR Environ. Res. 2008;19;589-612.
2. Arısoy M, Temiz-Arpacı O, Kaynak-Onurdag F, et al. Synthesis and antimicrobial evaluation of 2-(p-SubstitutedPhenyl)-5-[(4-substituted piperazin-1-yl)acetamido]-benzoxazoles. Z Naturforsch. 2014;69;368-74.
3. Temiz-Arpaci O, Doğanç F, Saç D, et al. Synthesis and antimicrobial evaluation of some sulfonylamido-benzoxazoles. Acta Biol. Hung. 2016;67:75-84.
4. Brown RN, Cameron R, Chalmers DK, et al. 2-Ethoxybenzoxazole as a bioisosteric replacement of an ethyl benzoate group in a human rhinovirus (HRV) capsid binder. Bioorg Med Chem Letters. 2005;15:2051-5.
5. Rida SM, Ashour FA, El-Hawash SAM, et al. Synthesis of some novel benzoxazole derivatives as anticancer, anti-HIV-1 and antimicrobial agents. Eur J Med Chem. 2005;40:949-59.
6. Oksuzoglu E, Tekiner-Gulbas B, Alper S, et al. Some benzoxazoles and benzimidazoles as DNA topoisomerase I and II inhibitors. J Enzyme Inhib Med Chem 2008;23:37–42.
7. Pınar A, Yurdakul P, Yıldız I, et al. Some fused heterocyclic compounds as eukaryotic topoisomerase II inhibitors. Biochem Biophys Res Commun. 2004;317:670.
8. Oksuzoglu E, Temiz-Arpaci O, Tekiner-Gulbas B, et al. A study on the genotoxic activities of some new benzoxazoles. Med Chem Res. 2007;16:1-14.
9. Varga A, Akı-Sener E, et al. Induction of apoptosis and necrosis by resistance benzazoles and benzoxazines on tumour cell line mouselymphoma L5718 Mdr+cells. In Vivo. 2005;19:1087-92.
10. Temiz-Arpaci O, Coban T, Tekiner-Gulbas B, et al. A study on the antioxidant activities of some new benzazole derivatives. Acta Biol Hungarica. 2006;57:201-9.
11. Gungorduk K, Ertas IE, Sahbaz A, et al. Immunolocalization of ERK1/2 and p-AKT in normal endometrium, endometrial hyperplasia, and early and advanced stage endometrioid endometrial adenocancer and their prognostic signifi cance in malignant group. Eur J Obstet Gynecol Reprod Biol. 2014;179:147–52.
12. Muvaffak A , Gurhan I , Gunduz U , et al. Preparation and characterization of a biodegradable drug targeting system for anticancer drug delivery: microsphere-antibody conjugate. J Drug Targ. 2005;13:151–9 .
13. Wiśniewski T, Żyromska A, Makarewicz R, et al. Osteopontin and angiogenic factors as new biomarkers of prostate cancer. Urological Oncology. 2019;16:134-40.
14. Heidtmann HH, Nettelbeck DM, Mingels A, et al. Generation of angiostatin-like fragments from plasminogen by prostate-specific antigen. Br J Cancer. 1999;81:1269–73
15. Rosen LS. Clinical experience with angiogenesis signaling inhibitors: focus on vascular endothelial growth factor (VEGF) blockers. Cancer Control. 2002, 9(2 Suppl):36–44.
16. Ucuzian AA, Gassman AA, East AT, et al. Molecular mediators of angiogenesis. J Burn Care Res 2010;31:158–75.
17. Carmen Stanca Melincovici, Adina Bianca Boşca, Sergiu Şuşman, et al. Vascular endothelial growth factor (VEGF) – key factor in normal and pathological angiogenesis, Rom J Morphol Embryol. 2018;59:455–67.
18. Ferrara N. Vascular endothelial growth factor: basic science and clinical progress. Endocr Rev. 2004;25:581–611.
19. Folkman J. Angiogenesis in cancer, vascular, rheumatoid and other disease. Nat Med. 1995;1:27–31.
20. Samson M, Peale FV Jr, Frantz G, et al. Human endocrine gland-derived vascular endothelial growth factor: expression early in development and in Leydig cell tumors suggests roles in normal and pathological testis angiogenesis. J Clin Endocrinol Metab. 2004;89:4078–88.
21. Takahashi H, Shibuya M. The vascular endothelial growth factor (VEGF)/ VEGF receptor system and its role under physiological and pathological conditions. Clin Sci (Lond). 2005;109:227–41.
22. Neufeld G, Cohen T, Gengrinovitch S, Poltorak Z. Vascular endothelial growth factor (VEGF) and its receptors. FASEB J. 1999;13:9–22.
23. Duffy AM, Bouchier-Hayes DJ, Harmey JH. Vascular endothelial growth factor (VEGF) and its role in non-endothelial cells: autocrine signalling by VEGF. In: Madame Curie Bioscience Database (formerly, Eurekah Bioscience Database). Angiogenesis. Landes Bioscience, Austin (TX), USA, 2000–2013. https://www.ncbi.nlm.nih.gov/books/NBK6482/
24. Miron L, Gafton B, Marinca M. Angiogeneza tumorală – implicaţii în terapia cancerelor. Jurnalul de Chirurgie Iaşi. 2010;6:104–10.
25. Raza A, Franklin MJ, Dudek AZ. Pericytes and vessel maturation during tumor angiogenesis and metastasis. Am J Hematol. 2010;85:593–8.
26. Yana I, Sagara H, Takaki S, et al. Crosstalk between neovessels and mural cells directs the site-specific expression of MT1-MMP to endothelial tip cells. J Cell Sci, 2007;120(Pt 9):1607–14.
27. A Medina-González, N Eiró Díaz , JM Fernández Gómez, et al. Barmadahc, Comparative analysis of the expression of metalloproteases (MMP-2, MMP-9, MMP-11 and MMP-13) and the tissue inhibitor of metalloprotease 3 (TIMP-3) between previous negative biopsies and radical prostatectomies. Actas Urol Esp. 2019;pii:S0210-4806:30192-5.
28. RJA. van Moorselaar a, EE. Voest, Angiogenesis in prostate cancer: its role in disease progression and possible therapeutic approaches, Molecular Cellular Endocrinol. 2002;197:239-50.

29. Po-Chun Chen, Chih-Hsin Tang, Liang-Wei Lin, et al. Thrombospondin-2 promotes prostate cancer bone metastasis by the up-regulation of matrix metalloproteinase-2 through down-regulating miR-376c expression, J Hematol Oncol. 2017;10:130.

30. Lawler PR, Lawler J. Molecular basis for the regulation of angiogenesis by thrombospondin-1 and -2. Cold Spring Harb Perspect Med. 2012;2:a006627.

31. Chen H, Strickland DK, Mosher DF. Metabolism of thrombospondin 2. Binding and degradation by 3 t3 cells and glycosaminoglycan-variant Chinese hamster ovary cells. J Biol Chem. 1996;271:15993–9.

32. Yang Z, Strickland DK, Bornstein P. Extracellular matrix metalloproteinase 2 levels are regulated by the low density lipoprotein-related scavenger receptor and thrombospondin 2. J Biol Chem. 2001;276:8403–8.

33. O'Reilly MS, Boehm T, Shing Y, et al. Endostatin: an endogenous inhibitor of angiogenesis and tumor growth. Cell. 1997;88.277285.

34. LM. Coussens, Z Werb. Inflammation and cancer. Nature. 2002;420:860–7.

35. Y Ben-Neriah, M Karin. Inflammation meets cancer, withNF-κBasthematchmaker. Nature Immunol. 2011;12:715–23.

36. Bonacini M, Negri A, Davalli P, et al, Clusterin silencing in prostate cancer induces matrix metalloproteinases by an NF-κB-dependent mechanism. J Oncol. 2019;2019:4081624.

37. Huang S, Pettaway CA, Uehara H, et al. Blockade of NF-κB activity in human prostate cancer cells is associated with suppression of angiogenesis, invasion, and metastasis. Oncogene. 2001;20:4188–97.

38. Kosova F, Temiz Arpaci O, Olmez E, et al. Synthesis and the effect of a novel benzoxazole compound on breast cancer cell line. Medicine Sci. 2019;8:186-91.

39. Kosova F, Kurt FO, Olmez E, et al. Effects of caffeic acid phenethyl ester on matrix molecules and angiogenetic and anti-angiogenetic factors in gastric cancer cells cultured on different substrates. Biotechnic & Histochemistry. 2015;2:1-10.

40. Kosova F, Arpaci ÖT, Kurt FO ve ark. 2014. Özgün heterosiklik bileşiklerin kanser dizin hücrelerinde apoptotik faktörler üzerindeki etkilerinin araştırılması, TÜBİTAK Projesi, 111S290, Proje Yürütücüsü