Cumhuriyet Medical Journal, 44(4): 343-347, 2022
DOI: http://dx.doi.org/10.7197/cmj.1189799

Cumhuriyet Medical Journal

| cmj.cumhuriyet.edu.tr | Founded: 2004 Available online, ISSN:1305-0028 Publisher: Sivas Cumhuriyet Üniversitesi

Investigation of The Effect of Compound B-47/2 Containing Azomethine Group On Angiogenesis

Efe Taha Bucak[1,a], Zuhal Tunçbilek[2,b*], Alakbar Huseynzada[3,c], Mirjavid Agayev[3,d], Ulviyya Hasanova[3,e], Ayça Taş[4,f], Yavuz Siliğ[5,g]

[1]Sivas Cumhuriyet University, Faculty of Medicine, Sivas, Turkey, [2]Sivas Cumhuriyet University, Yıldızeli Vocational School, Department of Chemistry and Chemical Processing Technologies Services, Sivas, Turkey, [3] Baku State University, Faculty of Chemistry, Department of Organic Chemistry, Baku, Azerbaijan

[4]Sivas Cumhuriyet University, Faculty of Health Sciences, Department of Nutrition and Dietetics Nutrition and Dietetics, Sivas, Turkey, [5]Sivas Cumhuriyet University, Faculty of Medicine, Department of Medical Biochemistry, Sivas, Turkey

**Corresponding author*

Research Article

History

Received: 15/10/2022
Accepted: 19/12/2022

ABSTRACT

Objective: Lung cancer is one of the most common cancers in the world. It is known that angiogenesis plays a role in the development and metastasis of lung cancer. Azomethine derivatives known as Schiff bases have many biological activities. In this study, we aimed to determine the anticancer activity of the newly synthesized azomethine derivative compound B-47/2 on lung cancer and to determine the effect of this component on *vascular endothelial growth factor B* (*VEGFB*) gene expression.

Material and Method: Compound B-47/2 was synthesized for the first time. B-47/2 compound was applied to lung cancer cell line (A549) at varying concentrations (1-100 µg/mL) and its anticancer activity was found after 24, 48 and 72 hours incubations using the 3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide (MTT) method. The half maximal inhibitory concentration (IC_{50}) dose of B-47/2 was applied to the cells and ribonucleic acid (RNA) isolation followed by complementary deoxyribonucleic acid (cDNA) synthesis was performed. Then, reverse transcription-polymerase chain reaction (RT-PCR) method was used to determine the expression level of *VEGFB* gene.

Results: As a result, it was determined that the B-47/2 compound applied to the A-549 cell line showed the highest cytotoxic activity after 72 hours of incubation. In addition, it was determined that the B-47/2 compound decreased the expression of the *VEGFB* gene.

Discussion: There are studies in which the anticancer activity of azomethine derivatives has been observed. The topic of synthesizing new drugs to prevent cancer is popular. We suggested that the newly synthesized component may have anticancer activity and may be effective on angiogenesis.

Keywords: Angiogenesis, lung cancer, cytotoxicity, gene expression, azomethine derivatives

Azometin Grubu İçeren Bileşik B-47/2'nin Anjiyogenez Üzerine Etkisinin İncelenmesi

Süreç

Geliş: 15/10/2022
Kabul: 19/12/2022

ÖZ

Amaç: Akciğer kanseri dünyada en sık görülen kanserlerden biridir. Akciğer kanserinin gelişiminde ve metastazında anjiogenezin rol oynadığı bilinmektedir. Schiff bazları olarak bilinen azometin türevleri birçok biyolojik aktiviteye sahiptir. Bu çalışmada yeni sentezlenen azometin türevi bileşik B-47/2'nin akciğer kanseri üzerindeki antikanser aktivitesini ve bu bileşenin *vasküler endotelyal büyüme faktörü B* (*VEGFB*) geni ekspresyonu üzerindeki etkisini belirlemeyi amaçladık.

Gereç ve Yöntem: B-47/2 bileşiği ilk kez sentezlendi. B-47/2 bileşiği akciğer kanseri hücre hattına (A549) değişen konsantrasyonlarda (1-100 µM) uygulandı ve 3-[4,5-dimetiltiyazol-2-il]-2,5 difenil tetrazolyum bromür (MTT) yöntemi kullanılarak 24, 48 ve 72 saat inkübasyondan sonra antikanser aktivitesi bulundu. Hücrelere B-47/2 bileşiğinin yarı maksimal inhibitor konsantrasyon (IC_{50}) dozu uygulandı ve ribonükleik asit (RNA) izolasyonunun ardından tamamlayıcı deoksiribonükleik asit (cDNA) sentezi yapıldı. Daha sonra *VEGFB* geninin ekspresyon düzeyini belirlemek için gerçek zamanlı- polimeraz zincir reaksiyonu (RT-PCR) yöntemi kullanıldı.

Sonuçlar: Sonuç olarak A-549 hücre hattına uygulanan B-47/2 bileşiğinin 72 saatlik inkübasyon sonrasında en yüksek sitotoksik aktiviteyi gösterdiği belirlendi. Ayrıca B-47/2 bileşiğinin *VEGFB* geninin ekspresyonunu azalttığı belirlendi.

Tartışma: Azometin türevlerinin antikanser aktivitesinin gözlemlendiği çalışmalar mevcuttur. Kanseri önlemek için yeni ilaçların sentezlenmesi konusu popülerdir. Yeni sentezlenen bileşenin antikanser aktiviteye sahip olabileceğini ve anjiyogenez üzerinde etkili olabileceğini öne sürdük.

Anahtar sözcükler: Anjiyogenez, akciğer kanseri, sitotoksisite, gen ekspresyonu, azometin türevleri

[a] *efetahabucak@gmail.com* *https://orcid.org/0000-0002-3702-3404*
[b] *zuhaltuncbilek@cumhuriyet.edu.tr* *https://orcid.org/0000-0002-6510-0884*
[c] *alakbar.huseynzada1117@gmail.com* *https://orcid.org/0000-0002-6342-4260*
[d] *agayev.mircavid@gmail.com* *https://orcid.org/0000-0003-0588-4450*
[e] *ulviyyahasanova12@gmail.com* *https://orcid.org/0000-0003-1502-4227*
[f] *aycatas@cumhuriyet.edu.tr* *https://orcid.org/0000-0002-7132-1325*
[g] *ysilig@cumhuriyet.edu.tr* *https://orcid.org/0000-0002-0562-7457*

How to Cite: Bucak ET, Tunçbilek Z, Huseynzada A, Agayev M, Hasanova U, Taş A, Siliğ Y (2022) Investigation of The Effect of Compound B-47/2 Containing Azomethine Group On Angiogenesis, Cumhuriyet Medical Journal, December 2022, 44 (4): 343-347

Introduction

Angiogenesis is the formation of new blood vessels by the development of existing blood vessels [1]. In 1971, Folkman proposed that angiogenesis is necessary for tumor growth. Angiogenesis is an important process that causes the growth and invasion of cancer cells [2]. Tumors need blood vessels for growth and spread. With angiogenesis, tumors provide the necessary nutrients and oxygen. Many molecular pathways work together for angiogenesis, which promotes the development of blood vessels and metastasis to distant sites in cancer [3]. The angiogenesis process is regulated by proangiogenic and anti-antiangiogenic factors. These factors interact with each other [4]. Neovascularization within the scope of angiogenesis is important for the development and invasion of non-small cell lung cancer (NSCLC) [5]. Angiogenesis and its pathways have become attractive targets for treatment in lung cancer and other cancer types. [6].

VEGF family plays a role and regulates blood vessel formation and development [7]. VEGFs plays an effective role in the progression and invasion of lung cancer by playing a role in angiogenesis. Each member of VEGFA, VEGFB, VEGFC, VEGFD and placental growth factor (PlGF) within the VEGF family plays a role in angiogenesis [4, 8]. The *VEGF* family mechanism is mediated by linking with their respective receptors expressed in tumor cells. This situation causes tumor progression and invasion with paracrine and/or autocrine effects. Research on lung cancer has mostly studied the autocrine mechanism of VEGFs. However, it is thought that the paracrine mechanism of VEGFs formed by the tumor microenvironment may also be effective in lung cancer [9]. Anti-angiogenesis treatments have been promising and have become one of the effective methods to prevent tumor development. Studies have shown that *VEGFs* expression is increased in many cancer types, including lung cancer. With the discovery of anti-angiogenic drugs, signaling pathway components involving VEGFs have become targets [7]. In the treatment of cancer, chemotherapeutic agents are given in addition to surgical interventions. However, researchers have turned to the discovery of new drugs because these drugs have many negative side effects [10].

Ligands containing azomethine groups, namely Schiff bases are included in the scope of organic compounds that have various applications in many fields such as biochemistry, analytical and inorganic chemistry. Schiff bases used in the formation of thermotropic liquid crystallized polymers, in the formation of a dioxygen carrier as a result of metal application in the field of radiopharmacology, and in the design of many biomolecules to create a catalytic effect [11]. Many biological activities of Schiff bases such as anticancer [12, 13], antimicrobial [14], antioxidant [15, 16], anti-inflammatory [17] and anti-urease [16] have been found to be associated with azomethine connection. In other words, ligands with azomethine group are synthesized due to their high efficiency [16]. In this study, we aimed to determine the anticancer activity of the newly synthesized B-47/2 compound on the lung cancer cell line and to investigate its effect on *VEGFB* gene expression.

Materials and Methods

Synthesis of 9,9'-(((2-hydroxypropane-1,3diyl) bis(oxy)) bis(2,1phenylene)) bis (methanylylidene)) bis(azanylylidene))bis (ethane-2,1-diyl)) bis (8,9,10,11,20,21-hexa-hydro-7H,19H dibenzo [f,q] [1,5]dioxa[9,12,15] triazacyclooctadecin-20-ol) (Compound B-47/2)

0.37 mmol of dialdehyde **1** was dissolved in 5 ml of hot ethanol. Subsequently, 1.37 mmol of 2,2'-(ethylenedioxy)bis(ethylamine) was added to the reaction mixture, which vigorously stirred for an hour (Figure 1). At the end of reaction time, the ethanolic solution was poured into an ice-water mixture and left for 10 minutes. Afterwards, the solution was vigorously stirred with the addition of sodium chloride and the yellowish precipitate is formed, which was filtered, washed with distilled water and dried. Yield 45%, m.p.148-150°C. **^{1}H NMR** spectrum: (DMSO-d_6, δ, ppm), 3.41 s (10H, $5OCH_2$), 3.62 s (14H, $4NCH_2$ + $3OCH_2$), 4.43 s (8H, $4OCH_2$), 6.99-7.03 t (4H, Ar, J=6 Hz), 7.14-7.17 d (4H, Ar, J=9 Hz), 7.43-7.48 t (4H, Ar, J=9 Hz), 7.85-7.88 d (4H, Ar, J=9 Hz), 8.58 s (4H, 4CH=N). **^{13}C NMR** spectrum (Figure 4S, supplementary material) of compound **3**: (DMSO-d_6, δ, ppm), 61.37 ($4NCH_2$), 67.11 ($4OCH_2$), 70.11 ($4OCH_2$), 70.18 ($4OCH_2$), 113.09 (4CH, Ar), 121.19 (4CH, Ar), 124.53 (4C, Ar), 127.11 (4CH, Ar), 132.53 (4CH, Ar), 157.08 (4CH=N), 157.98 (4C, Ar). MALDI-TOF MS=764.950. Found, %: C 69.01; H 6.91; N 7.39. $C_{44}H_{52}N_4O_8$. Calculated, %: C 69.09; H 6.85; N 7.32.

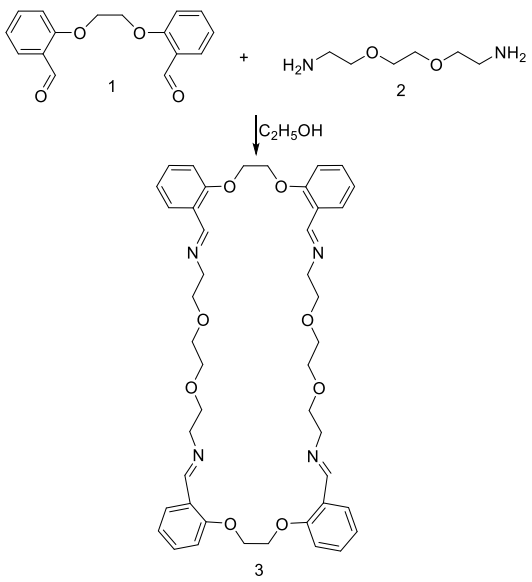

Figure 1. Synthesis of 9,9'-(((2-hydroxypropane-1,3diyl) bis(oxy)) bis(2,1phenylene)) bis (methanylylidene)) bis(azanylylidene))bis (ethane-2,1-diyl)) bis(8,9,10,11, 20,21-hexa-hydro-7H,19H dibenzo [f,q][1,5]dioxa [9,12,15] triazacyclooctadecin-20-ol) (Compound B-47/2)

Cell culture and reagents

The A549 cell line is a non-small cell lung cancer cell line. A549 cells were obtained from American Type Culture Collection (ATCC). Cells were grown in Dulbecco's modified Eagle's medium (DMEM; (Biological Industries) containing 10% fetal bovine serum (FBS; Biological Industries) and 1% penicillin/streptomycin (HyClone). Humid environment, 5% carbon dioxide and 37°C conditions were provided for the growth of cells. Cells were incubated with varying concentrations of compound B-47/2 (1-100 µM), first synthesized by our researchers.

Cell viability assay

Cell viability analysis was performed using the method based on the reduction of MTT tetrazolium salt. When A549 cells reached 80% density, they were seeded into 96-well plates with 1×10^5 cells per well. The cells were incubated for approximately 24 hours until they became adherent. Then, 1, 5, 10, 25, 50, 70, 80, 100 µM concentrations of B-47/2 compound were applied to A549 cells. Compound B-47/2 was dissolved in 100% dimethyl sulfoxide (DMSO). Cells given compound B-47/2 were incubated for 24, 48 and 72 hours. At the end of these incubation times, MTT was applied to the cells and incubated for 2 hours. Then, formazan crystals were dissolved with DMSO. Cell viability was measured by reading the absorbance at 470 nm with a microplate reader.

Cell morphology analysis

A549 cells were seeded into wells at $5x10^5$ cells/well. Compound B-47/2 was applied at respective concentrations in a volume of 1 µM to each well of the plate. Morphological changes in cells were visualized using an inverted microscope (ZEISS Axio Vert.A1) at 20x and 10x magnification. The morphological change caused by the B-47/2 compound on A549 cells was compared with the cells in the control group.

RT-PCR analysis

The IC_{50} dose of the B-47/2 compound determined for 48 hours of incubation was applied to the cells in 6-well plates. After 48 hours of incubation, RNA was isolated using the RNeasy kit (Qiagen). The cDNA samples used for RT-PCR were obtained from RNA using the First Strand cDNA Synthesis Kit (Qiagen). *VEGFB* primer was purchased from Qiagen. Expression analysis was performed by RT-PCR method using RT^2 SYBR® Green qPCR Master mix (Qiagen). The gene expression result was normalized with the *glyceraldehyde-3-phosphate dehydrogenase* (*GAPDH*) internal control gene and then compared with controls.

Statistical Analysis

All experiments were performed in triplicate. The IC_{50} dose was determined using software GraphPad Prism 8 (GraphPad Software, San Diego, CA, USA). Expression analysis of the *VEGFB* gene was performed using Rotor-Gene 6000 Series Software Version 1.7 software. Statistical analysis of gene expression was performed with the $\Delta\Delta C_T$ method using the software "RT2 profiler RT-PCR Array Data Analysis version 3.5" (https://geneglobe.qiagen.com/us/analyze)".

Results

Anticancer activity of compound B-47/2 on A549 cell line

The cytotoxic activity of compound B-47/2 on the A549 cell line was evaluated. Accordingly, the highest anticancer activity was found after 72 hours of incubation. The IC_{50} dose of the B-47/2 compound after 48 hours of incubation was found as 76.54 µM (Figure 2).

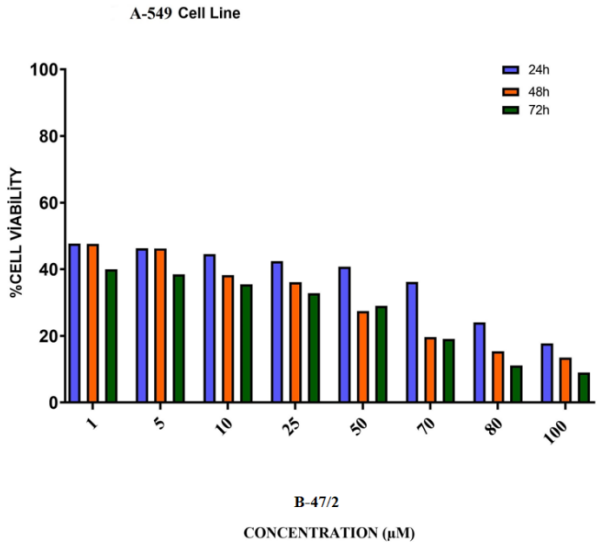

Figure 2. Cytotoxic activity of compound B-47/2 on A549 cell line after 24, 48 and 72 hours incubation

Cell morphology analysis

Morphological analyzes were performed 72 hours after the application of 25 µM B-47/2 compound to the A549 cells. It was observed that the morphology of the B-47/2 compound changed significantly in the control group compared to the A549 cells in the cancer group (Figure 3).

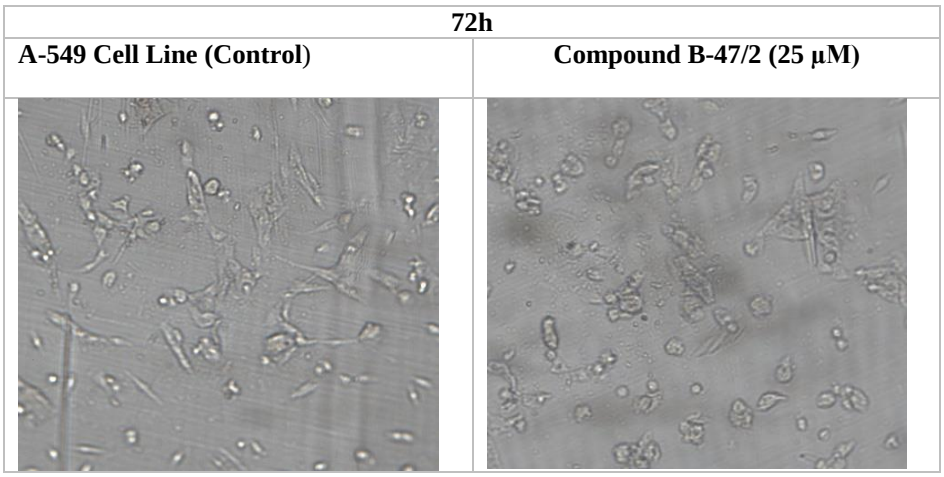

Figure 3. Morphological images of A-549 cells after 72 hours of incubation of compound B-47/2 (25 µM)

Expression analysis of the VEGFB gene

The effect of compound B-47/2 given IC_{50} dose on *VEGFB* gene expression in A549 cell line was investigated. According to the results, *VEGFB* gene expression level was found to be low when compared to the control group (Figure 4).

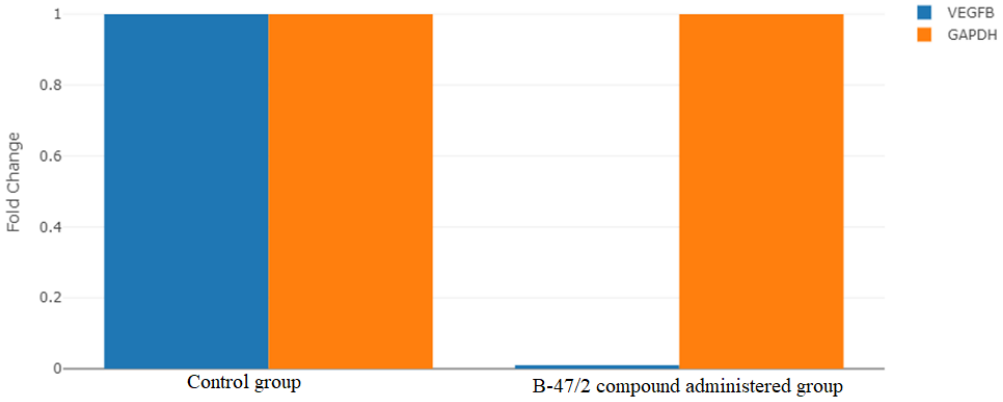

Figure 4. Expression level of *VEGFB* gene in A549 cells treated with compound B-47/2 compared to control group

Discussion

Studies have shown that angiogenesis factors play a role in malignant neoplasms such as cancer development and spread. According to the findings obtained from studies on NSCLC, neovascularization and expressions of the *VEGF* family were found to be associated with survival and metastasis. [18]. It has been reported that *VEGFs* are overexpressed in both NSCLC and small cell lung cancer (SCLC) and high serum levels are associated with metastasis and invasion, resulting in poor prognosis. Studies have shown that the expression of *VEGFB* gene is increased in NSCLC. The exact role of *VEGFB* in NSCLC is not known [19, 20]. It has been shown that the blockade of the angiogenesis process is effective in many cancer types [21]. It is known that anti-angiogenic treatments in cancer increase the survival time of weeks and months, but they are effective at a limited level, but the development of cancer continues [7, 22]. Schiff bases or azo compounds are chemical structures with pharmaceutical and medical uses. There are studies showing the biological activity of the azomethine bond in their structure. Aslan et al. showed that anticancer activity of newly synthesized Schiff base derivatives on A-549 cell line [23].

In our study, it was found that the newly synthesized azomethine derivative compound showed anticancer activity in many cancer types in parallel with the studies in the literature. It was found that our compound B-47/2, which is a derivative of azomethine, has a high cytotoxic activity on A549 cells. Despite the increased expression of *VEGFB* in NSCLC, it was reported in our study that *VEGFB* expression was excessively decreased due to the B-47/2 compound.

Accordingly, the low expression of *VEGFB* suggests that it may play a role as an antiangiogenic component. It has been predicted that B-47/2 compound may play a role as an effective compound in lung cancer to inhibit neovascularization and tumor growth.

Conclusion

As a result, it was determined that our compound B-47/2, which is a derivative of azomethine, has anticancer activity on the A549 cell line. Our compound was found to cause excessive downregulation of *VEGFB* expression in A549 cells. As a result, there is a need for further studies that the newly synthesized compound may play a role as an anti-angiogenesis factor in the treatment of lung cancer.

References

1. Teleanu RI, Chircov C, Grumezescu AM, Teleanu DM. Tumor angiogenesis and anti-angiogenic strategies for cancer treatment. Journal of clinical medicine 2019; 9(1): 84.
2. Folkman J. Tumor angiogenesis: therapeutic implications. New england journal of medicine 1971; 285(21): 1182-1186.
3. Ayoub NM, Jaradat SK, Al-Shami KM, Alkhalifa AE. Targeting Angiogenesis in Breast Cancer: Current Evidence and Future Perspectives of Novel Anti-Angiogenic Approaches. Front Pharmacol. 2022; 13: 838133.
4. Lugano R, Ramachandran M, Dimberg A. Tumor angiogenesis: causes, consequences, challenges and opportunities. Cellular and Molecular Life Sciences 2020; 77(9): 1745-1770.
5. Liu G, Xu S, Jiao F, Ren T, Li Q. Vascular endothelial growth factor B coordinates metastasis of non-small cell lung cancer. Tumor Biology 2015; 36(3): 2185-2191.
6. Zhao Y, Adjei AA. Targeting angiogenesis in cancer therapy: moving beyond vascular endothelial growth factor. The oncologist, 2015; 20(6): 660-673.
7. Hu H, Chen Y, Tan S, Wu S, Huang Y, Fu S, He J. The research progress of antiangiogenic therapy, immune therapy and tumor microenvironment. Frontiers in Immunology 2022; 13: 802846.
8. Das M, Wakelee H. Targeting VEGF in lung cancer. Expert opinion on therapeutic targets 2012; 16(4): 395-406.
9. Goel HL, Mercurio AM. VEGF targets the tumour cell. Nature Reviews Cancer 2013; 13(12): 871-882.
10. Qin SY, Zhang AQ, Cheng SX, Rong L, Zhang XZ. Drug self-delivery systems for cancer therapy. Biomaterials 2017; 112: 234-247.
11. Asada H, Hayashi K, Negoro S, Fujiwara M, Matsushita T. Preparation and structures of trinuclear manganese (II) complexes with N-2-pyridiylmethylidene-2-hydroxy-5-substituted-phenylamine. Inorganic Chemistry Communications 2003; 6(2): 193-196.
12. Saeed AM, AlNeyadi SS, Abdou IM. Anticancer activity of novel Schiff bases and azo dyes derived from 3-amino-4-hydroxy-2H-pyrano [3, 2-c] quinoline-2, 5 (6H)-dione. Heterocyclic Communications 2020; 26(1): 192-205.
13. Miri R, Razzaghi-asl N, Mohammadi MK. QM study and conformational analysis of an isatin Schiff base as a potential cytotoxic agent. Journal of molecular modeling 2013; 19(2): 727-735.
14. Pandey A, Rajavel R, Chandraker S, Dash D. Synthesis of Schiff bases of 2-amino-5-aryl-1, 3, 4-thiadiazole and its analgesic, anti-inflammatory and anti-bacterial activity. E-Journal of Chemistry 2012; 9(4): 2524-2531.
15. Ünver Y, Deniz S, Çelik F, Akar Z, Küçük M, Sancak K. Synthesis of new 1, 2, 4-triazole compounds containing Schiff and Mannich bases (morpholine) with antioxidant and antimicrobial activities. Journal of Enzyme Inhibition and Medicinal Chemistry 2016; 31(3): 89-95.
16. Gümrükçüoğlu N, Sökmen BB. Synthesis of New Ligands Containing Azomethine Group and Investigation of Antioxidant, Antiurease Activities. Gazi University Journal of Science 2020; 33(3): 662-671.
17. Sathe BS, Jaychandran E, Jagtap VA, Sreenivasan GM. Synthesis characterization and anti-inflammatory evaluation of new fluorobenzothiazole schiff's bases. Int J Pharm Res Dev 2011; 3(3): 164-169.
18. Stefanou D, Batistatou A, Kamina S, Arkoumani E, Papachristou DJ, Agnantis NJ. Expression of vascular endothelial growth factor (VEGF) and association with microvessel density in benign prostatic hyperplasia and prostate cancer. In vivo 2004; 18(2): 155-160.
19. Carrillo de Santa Pau E, Arias FC, Caso Pelaez E, Munoz Molina GM, Sanchez Hernandez I, Muguruza Trueba I, del Val Toledo Lobo M. Prognostic significance of the expression of vascular endothelial growth factors A, B, C, and D and their receptors R1, R2, and R3 in patients with nonsmall cell lung cancer. Cancer 2009; 115(8): 1701-1712.
20. Riely GJ, Miller VA. Vascular endothelial growth factor trap in non–small cell lung cancer. Clinical cancer research 2007; 13(15): 4623-4627.
21. Montanino A, Manzo A, Carillio G, Palumbo G, Esposito G, Sforza V, Morabito A. Angiogenesis inhibitors in small cell lung cancer. Frontiers in Oncology 2021; 11: 655316.
22. Pàez-Ribes M, Allen E, Hudock J, Takeda T, Okuyama H, Viñals F, Casanovas O. Antiangiogenic therapy elicits malignant progression of tumors to increased local invasion and distant metastasis. Cancer cell 2009; 15(3): 220-231.
23. Aslan HG, Akkoç S, Kökbudak Z. Anticancer activities of various new metal complexes prepared from a Schiff base on A549 cell line. Inorganic Chemistry Communications 2020; 111: 107645.