Experimed 2024; 14(1): 7-12

ORIGINAL ARTICLE

DOI: 10.26650/experimed.1378000

A Clinical Sulfonamide Derivative Inhibits B-Raf Protein in Colorectal Cancer and Melanoma Cells. Is It a New Target for Cancer?

Emine Terzi[1], Beyza Ecem Oz Bedir[1]

[1]Department of Medical Biology, Faculty of Medicine, Ankara Yildirim Beyazit University, Ankara, Turkiye

ORCID ID: E.T. 0000-0001-9106-3848; B.E.O.B. 0000-0002-0596-834X

Cite this article as: Terzi E, Oz Bedir BE. A clinical sulfonamide derivative inhibits B-Raf protein in colorectal cancer and melanoma cells. Is it a new target for cancer? Experimed 2024; 14(1): 7-12.

ABSTRACT

Objective: Colorectal cancer (CRC) is a type of cancer spreading quickly around the world. Melanoma is an aggressive and lethal form of skin cancer. One of the striking biomarkers in cancer is carbonic anhydrase (CA)-IX, which catalyzes the hydration of carbon dioxide (CO_2). CA inhibitors are being used clinically and studied extensively in clinical research. This study aimed to examine the CA inhibitor acetazolamide (AZA) in terms of the B-Raf protein in colorectal cancer and the melanoma cell line.

Materials and Methods: HT29 human colorectal cancer cells and A375 human melanoma cells were cultured. The appropriate dose of AZA on the cells was determined by the WST-1 test. The enzyme-linked immunosorbent assay (ELISA) was used to determine the effect of AZA on the B-Raf protein in HT29 and A375 cells.

Results: HT29 and A375 cell lines treated with AZA showed a dramatic decrease in CA-IX levels ($p<0.05$). In addition, AZA significantly reduced B-Raf protein levels in the HT29 and A375 cell lines ($p<0.05$, for both).

Conclusion: This study revealed AZA, a CA inhibitor, to be effective in CRC and melanoma. In future studies, combining the effects of AZA and B-Raf inhibitors may present an alternative approach in cancer treatment.

Keywords: Acetazolamide, B-Raf, carbonic anhydrase-IX, colorectal cancer, melanoma

INTRODUCTION

Colorectal cancer (CRC) is a type of cancer responsible for 9.2% of cancer-related deaths. In terms of prevalence, it ranks second in women and third in men, with 5- and 10-year survival rates being 65% and 58%, respectively (1). CRC may be localized or metastasized in the lymph nodes. Most patients with CRC have been reported to have metastases in the liver. Endoscopic, surgical, and oncological treatment strategies exist for CRC (2). Due to being the most hazardous and aggressive form of skin cancer and comprising only about 5% of all cutaneous malignancies, melanoma is the primary cause of skin cancer-related mortality (3). Because of the high risk of spreading, research is being performed to find risk factors and determine the best course of action for treatment.

Carbonic anhydrases (CAs) are metalloenzymes that contain Zn^{2+}. They reversibly convert CO_2 into HCO_3^-/H^+ and are processed to maintain pH homoeostasis in an organism (4). Acetazolamide (AZA), a sulfonamide derivative, is clinically implemented in the treatment of glaucoma, epilepsy, and heart failure (5). CA-IX is a cancer-related isoform and is overexpressed in carcinomas of the uterus, cervix, kidney, esophagus, lung, breast, colon, and brain (4, 6). Studies on cell models have shown CA-IX to have a pH-regulating function under hypoxic conditions and to help maintain the slightly alkaline intracellular pH that is necessary for

Corresponding Author: Beyza Ecem Oz Bedir **E-mail:** beyzaecem.oz@hotmail.com
Submitted: 18.10.2023 **Revision Requested:** 12.11.2023 **Last Revision Received:** 23.01.2024 **Accepted:** 17.02.2024 **Published Online:** 25.03.2024

the survival of cancer cells (7). CA-IX has also been shown to be able to interact with many signaling pathways in cancer cells (8, 9).

Mitogen-activated protein kinase (MAPK) pathways are essential in the transition from extracellular signals to intracellular responses and regulate many cellular functions (10). MAPK signaling pathways consist of Ras, Raf, MEK, and extracellular-signal-regulated kinase (ERK) proteins. The cells inhibit apoptosis as a response to a variety of stress factors, such as radiation, hypoxia, hydrogen peroxide, and chemotherapeutic agents, to increase the survival of the cells (11, 12). The Ras-Raf-MEK-ERK cascade is a vital regulatory pathway for cell growth, proliferation, differentiation, and apoptosis. Signaling with this pathway typically occurs for various plasma membrane growth-factor receptors that activate Ras family GTPases. Activated Ras proteins can recruit, complex with, and activate members of the Raf kinase family in the plasma membrane. Raf proteins stimulate the MEK/ERK cascade and ensure the transcription of related genes. B-Raf is a protein kinase that phosphorylates serine/threonine, which is involved in this pathway and is an oncogenic marker for solid tumors such as melanoma, papillary thyroid carcinoma, colorectal carcinoma, and gliomas (13). B-Raf mutations are found in about 10% of CRC patients and are linked with a poor prognosis; they are also resistant to chemotherapeutic treatment. However, recently developed B-Raf inhibitors have been directed towards melanoma and shown significant clinical activity in CRC (14). The main limitations in the use of B-Raf inhibitors involve resistance development and the toxicity linked to B-Raf inhibition (15).

B-Raf oncogenic driver mutations are found in approximately 50% of cutaneous melanomas in which the Ras-Raf pathway has been altered. The most prevalent (90%) aberration in the B-Raf gene is the V600E/K mutation which is commonly seen in cutaneous melanoma (16, 17). Vemurafenib (VMF) (B-Raf inhibitor) and trametinib (MEK inhibitor) are among the compounds currently being used in the clinical treatment of melanoma. However, arthralgia, burnout, diarrhea, fever, photosensitivity, skin, ocular, and cardiovascular toxicity are the most frequent adverse effects of the treatment. In addition to the side effects, the main disadvantage of this form of therapy is that the cancer cells can develop resistance to the therapy (18).

CA-IX, which is overexpressed in many tumor types, is also a pH-regulating enzyme, thus making it a potential target for carcinogenesis. CA-IX inhibition returns extracellular pH to normal values, resulting in a delay in tumor growth. This study therefore predicts that targeting CA-IX would be valuable as an alternative mechanism of carcinogenesis. The main goal in this research is to assess the impact of AZA, a CA inhibitor, on the B-Raf protein in HT29 CRC and A375 melanoma cell lines. Detecting a relationship between CA-IX inhibition and the B-Raf protein can become a new approach in targeting the MAPK pathway. This is a preliminary study investigating the potential a sulfonamide derivative has for inhibiting B-Raf in cancer treatment.

MATERIALS AND METHODS

Cell Experiments

Human colorectal cancer cells (HT29) and human melanoma cells (A375) were gifted by Dr. Tuba Ozdemir Sanci (Ankara Yildirim Beyazit University, Medical Faculty, Histology and Embryology Department). The cells were cultivated in Dulbecco's Modified Eagle Medium (DMEM), a high glucose medium (Biowest, L0102), with 10% heat-inactivated fetal bovine serum (FBS; Capricorn, FBS-HI-11A), 1% penicillin-streptomycin, 1% amphotericin, and 1% gentamycin at 37°C in a 5% CO_2 humidified chamber incubator. AZA and VMF were purchased from Sigma-Aldrich and SelleckChem, respectively. The cells were passaged with 80-90% confluency, and a trypsin-EDTA (Gibco, 25200-056) solution was used for detaching the cells.

Inhibitor Preparation

The inhibitors used in the current study are AZA (Sigma, BCBZ9159) and VMF (Cayman Chemical, 1-800-364-9897). Due to the A375 and HT29 cell lines having the BRAFV600E mutation, the study evaluated VMF as a positive control. All inhibitors were prepared as stock solutions by dissolving them in dimethyl sulfoxide (DMSO) at a final concentration of 300 µM. Then HT29 and A375 cells were seeded into 6-well plates (0.3×10^6 cells/well) and tested for various concentrations of AZA, and VMF (0, 2.5, 5, 10, 25, 50, 100, 200 µM) for 24th and 48th hours.

Cytotoxicity Assay (WST-1)

The WST-1 test was carried out to find out the appropriate dose of AZA and VMF on HT29 cells. The principle of this assay is that cellular mitochondrial dehydrogenases convert the tetrazolium salt WST-1 into formazan crystals. For the WST-1 test, the HT29 and A375 cells were seeded into 96-well plates and incubated. After incubation, AZA and VMF were prepared at concentrations of 0, 2.5, 5, 10, 25, 50, 100 and 200 µM and applied to the cells. The measurements were analyzed at the 24th and 48th hours. To eliminate the effects from DMSO, the concentrations were added to the negative control wells at a rate where AZA and VMF were dissolved along with the medium. 10 µL of the WST-1 solution (Cayman Chemical, 10008883) were added to each well and incubated at 37°C for 2-4 hours. Following incubation, the appropriate inhibitor concentration for the cells was determined by measuring at 450 nm. The following equation was used when calculating the viability values of the cells:

$$\text{Viability\%} = (\text{Avarage}_{\text{OD inhibitor}} \times 100) \div \text{Average}_{\text{OD control}}$$

While the following equation was used when calculating the inhibition value:

Inhibition% = 100 - ((Avarage $_{OD\ inhibitor}$ × 100) ÷ Average $_{OD\ control}$)

The inhibitor concentration values were converted into a graph by utilization of Graphpad Prism 9.1.0 software, after which the inhibitor dose (IC_{50}) value that inhibited 50% of the cells was calculated. All experiments were performed in triplicates.

ELISA

To prepare the cell lysate, phosphate-buffered saline (PBS) was first used to wash the cells. Next, a lysis buffer containing 50 mM Tris,150 µM NaCl, 1% NP-40 (Intron Biotechnology, IBS-BN015) and proteinase inhibitor factor (Intron Biotechnology, PIC001) were used to lyse the cells, after this step, the cells were centrifuged (+4°C, 17,000 g, 15 min). The supernatant was then aliquoted.

The Enzyme-Linked Immunosorbent Assay (ELISA) was used to determine the changes in the CA-IX and B-Raf levels of AZA and VMF in the HT29 and A375 cell lines. The BT Lab Human Carbonic Anhydrase IX ELISA Kit (E2273Ha, BT Lab, China) and Human B-Raf ELISA kit (E3906Hu, BT Lab, China) were used in the study. Standard solutions were first prepared for the ELISA protocol. The standard solution contains 120 µL of standard (40 ng/mL) and 120 µL of standard diluent to create 20 ng/mL (CA-IX) and 2400 ng/L (B-Raf) standard stock solutions. The standard stock solution was diluted 1:2 to obtain 10 ng/mL, 5 ng/mL, 2.5 ng/mL and 1.25 ng/mL standard solutions for CA-IX and 1200 ng/L, 600 ng/L, 300 ng/L, and 150 ng/L for B-Raf. 50 µL of all standards were added to the wells. Next, 40 µL of biotinylated anti-CA-IX and 10 µL of anti-B-Raf antibody were added to the wells. Subsequently, 50 µL of streptavidin-HRP was added to all wells and incubated at 37°C for 60 minutes. After incubation, the wells were washed 5 times with a wash buffer, then 50 µL of substrate solution A and 50 µL of substrate solution B were added to each well. This was incubated at 37°C for 10 min in the dark. Following the incubation, analysis was conducted at 450 nm using the Thermo Scientific Varioskan™ Spectrophotometer.

Statistical analyses

The statistical analysis was carried out using GraphPad Prism 9.1.0. The one-way analysis of variance (ANOVA) test was conducted for the B-Raf ELISA test and Student's t-test for the CA-IX ELISA test. The statistical significance level was determined as $p<0.05$.

RESULTS

The HT29 and A375 cells were treated with AZA and VMF at doses of 0, 2.5, 5, 10, 25, 50, 100, and 200 µM at the 24th and 48th hours to help determine the appropriate cytotoxic dose for the cells. According to WST-1 results, viability rates for the HT29 cells treated with AZA for 24 hours were 100%, 100%, 94%, 87%, 50%, 35%, 30%, and 17% at respective concentrations of 0, 2.5, 5, 10, 25, 50, 100, and 200 µM. The IC_{50} value for AZA is found to be 34.71 µM. Viability rates for HT29 cells treated with VMF for 24 hours at concentrations of 0, 2.5, 5, 10, 25, 50, 100, and 200 µM were 100%, 100%, 100%, 100%, 48%, 35%, 27%, and 18%, respectively. The IC_{50} value for VMF was found to be 35.84 µM (Figures 1, 2).

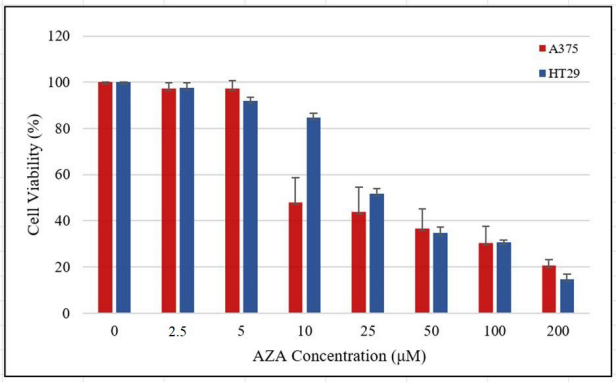

Figure 1. The % cell viability of the AZA-treated A375 and HT29 cells. The HT29 and A375 cells were treated with AZA at doses

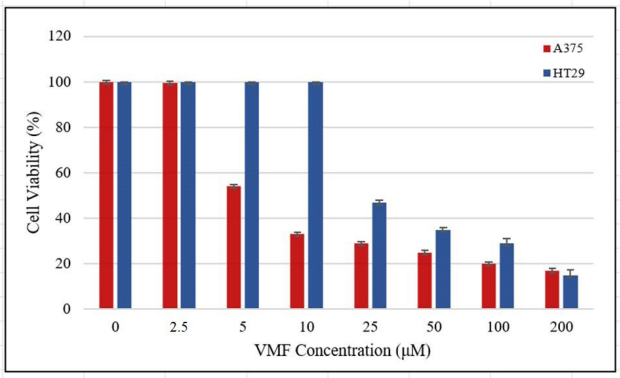

Figure 2. The % cell viability of the VMF-treated A375 and HT29 cells. The HT29 and A375 cells were treated with VMF at doses of 0, 2.5, 5, 10, 25, 50, 100, and 200 µM at the 24th and 48th hours.

For the A375 melanoma cells, viability rates for cells treated with AZA for 24 hours were 100%, 100%, 54%, 33%, 29%, 25%, 20%, and 17% at respective concentrations of 0, 2.5, 5, 10, 25, 50, 100, and 200 µM. The IC_{50} value for AZA was found to be 9.75 µM. Viability rates for the A375 cells treated with VMF for 24 hours at concentrations of 0, 2.5, 5, 10, 25, 50, 100, and 200 µM were 100%, 68%, 32%, 27%, 23%, 22%, 17%, and 14%, respectively. The IC_{50} value for VMF was found to be 4.93 µM (Figures 1, 2).

In order to examine the integrity of the cells, an inverted microscope was used following 24 hours of AZA and VMF application. The numbers of HT29 and A375 cells were observed to have decreased significantly at the end of this period (Figures 3, 4).

The effects of AZA on CA-IX were determined using ELISA.

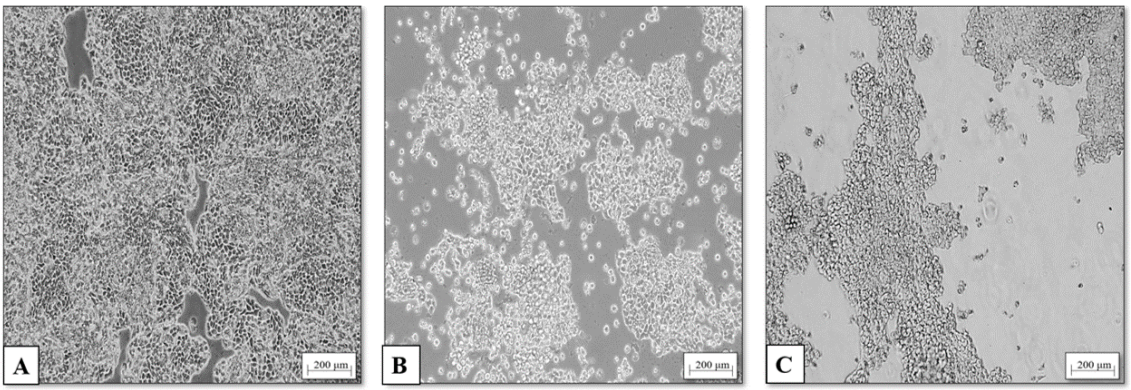

Figure 3. Inverted microscope images of the CRCs after 24th hour of inhibitor treatment. A) HT29 cells without an inhibitor B) AZA-treated HT29 cells C) VMF-treated HT29 cells (Bar = 200 µm) (AZA concentration = 34.71 µM, VMF concentration = 35.84 µM).

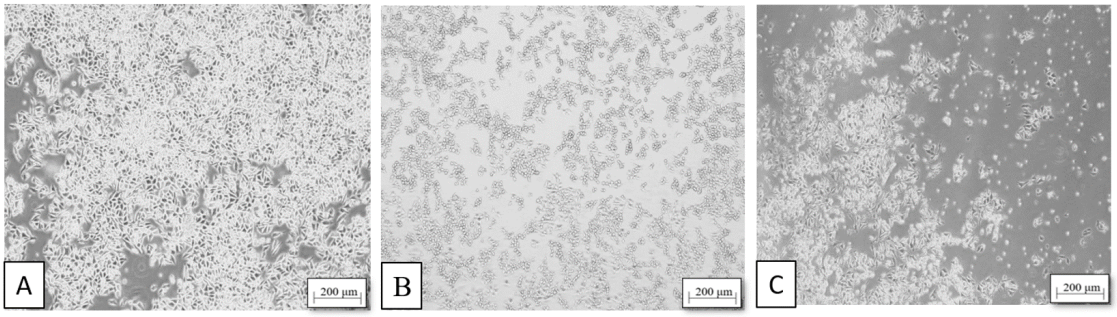

Figure 4. Inverted microscope images of the melanoma cells after 24th hour of inhibitor treatment: A) A375 cells without inhibitor; B) AZA-treated A375 cells; C) VMF-treated A375 cells (Bar = 200 µm) (AZA concentration = 34.71 µM, VMF concentration = 35.84 µM).

The change in CA-IX levels was determined by measuring the absorbance in cells with the presence and absence of AZA treatment. As a result, the concentration of CA-IX was found to be 5.93 ng/mL in the HT29 cells without AZA and 4.1 ng/mL in the HT29 cells with AZA. Treating the HT29 cells with AZA was found to dramatically decrease CA-IX levels ($p<0.05$). For the A375 cell line, the concentration of CA-IX was found to be 6.36 ng/mL in cells without AZA and 3.46 ng/mL in cells with AZA.

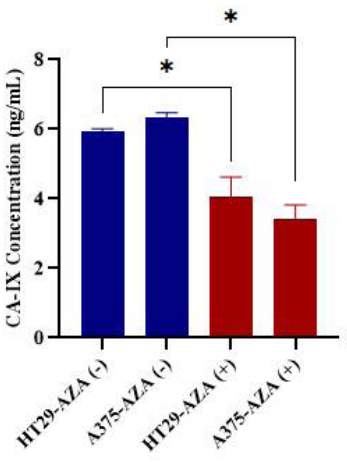

Figure 5. CA-IX protein levels in the control and AZA-treated HT29 and A375 cells. * $p<0.05$

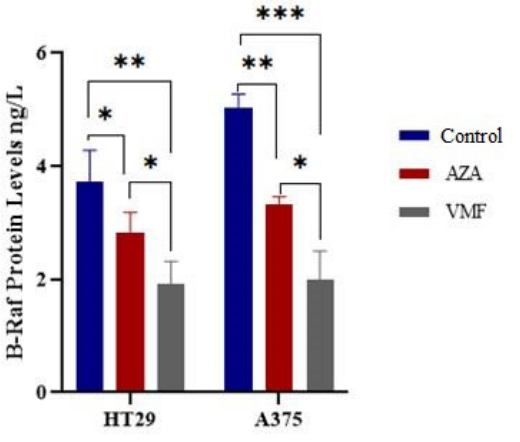

Figure 6. B-Raf protein levels in the control, AZA-treated, and VMF-treated HT29 and A375 cells. * $p<0.05$, ** $p<0.01$, *** $p<0.001$

The CA-IX levels were shown to have considerably decreased in the A375 cells treated with AZA ($p<0.05$; Figure 5).

The effects of AZA on B-Raf protein levels in the HT29 and A375 cells were assessed using ELISA, with VMF being used as a positive control. As a result, B-Raf protein levels were found to be 3.7 ng/L in the negative control cells, 2.81 ng/L in the AZA-treated HT29 cells, and 1.94 ng/L in the VMF-treated positive control cells. When comparing the AZA-treated cells to the control cells, B-Raf protein levels were considerably lower in the AZA-treated cells ($p<0.05$). However, B-Raf protein levels were higher in the AZA-treated HT29 cells compared to the VMF-treated HT29 cells. VMF demonstrated a more effective inhibition effect than AZA ($p<0.05$). For the A375 cells, B-Raf protein levels were found to be 5.03 ng/L in the negative control cells, 3.36 ng/L in the AZA-treated cells, and 2 ng/L in the VMF-treated positive control cells. AZA was found to dramatically affect a decrease in the B-Raf protein levels compared to the control cells ($p<0.05$). However, B-Raf protein levels are higher in the AZA-treated A375 cells than in the VMF-treated A375 cells. VMF demonstrates a more effective inhibition than AZA ($p<0.05$; Figure 6).

DISCUSSION

With approximately 900,000 deaths annually, CRC is amongst the deadliest cancers. Age, dietary habits, and smoking can be listed as the risk factors that affect the development of CRC. Surgery, chemotherapy, radiotherapy, and immunotherapy are currently being used in clinical treatments (19). Cancer signaling pathways trigger cell proliferation, angiogenesis, escape from apoptosis, and metastasis in CRC. One of the signaling pathways involved in these processes is the Ras-Raf-ERK pathway. Alterations in this pathway have been reported as targets for CRC therapy (20, 21). As a result, inhibiting this pathway is important as a molecular therapeutic approach in the treatment of CRC. B-Raf is located in MAPK's Ras-Raf-MEK-ERK signaling pathway and activates the transcription factors through phosphorylation of MEK and ERK. This activation regulates cell proliferation, survival, and growth (22).

Although melanoma is less common than other forms of skin cancer, it nevertheless causes over 73% of deaths from skin cancer (23, 24). Therapeutic approaches have improved understanding of melanoma pathogenesis in recent years, with activation of the MAPK pathway in melanoma being known to promote rapid tumor proliferation. The activator B-Raf mutation V600E and related mutations at this codon are critical to the MAPK signaling pathway in melanoma cell lines (25).

CA-IX is a tumor-associated CA isoenzyme, and CA-IX expression is induced through HIF-1. As a result, CA-IX might serve as a hypoxia marker and prognostic indicator. CA-IX is overexpressed in malignancies of the uterus, kidney, esophagus, and breast and in melanoma (4). CA-IX preserves cancer cells from hypoxia and intracellular acidity as the tumor grows. It promotes angiogenesis, extracellular matrix degradation, epithelial-mesenchymal transformation, invasiveness, tumor-stroma cross-communication, and signaling by increasing extracellular acidosis. In addition, CA-IX can interact with several signaling pathways and mechanisms in cancerous cells (9). The phosphorylated IC residues of CA-IX have been shown to be associated with carcinogenesis. This has been suggested to perhaps stimulate intracellular signaling pathways (8). CA-IX is a promising target for anti-cancer therapy. AZA is the first diuretic agent of sulfonamides and is used for the inhibition of CA isoenzymes (26). Studies have shown AZA to reduce colony formation and regress tumor growth in various cancer types (27). The current study has planned to show the effect of AZA, a CA inhibitor, on B-Raf, an oncogenic protein, based on the association CA-IX has with cancer signaling pathways in the HT29 colorectal cancer and A375 melanoma cell lines. The study used the B-Raf inhibitor VMF as a positive control and observed AZA to reduce B-Raf protein levels significantly but not as effectively as VMF. No previous research is present in the literature, showing the effect of AZA on the B-Raf protein. However, studies are found to have shown the effect that sulfonamide-derived inhibitors have on the B-Raf protein. Tsai et al. showed difluorophenyl-sulfonamides to have high selectivity and potential for oncogenic B-Raf protein inhibition (28). VMF is a B-Raf inhibitor with a sulfonamide group targeting the BRAFV600E mutation. In addition, dabrafenib is another selective B-Raf inhibitor compound containing a sulfonamide group (29). Ali et al. suggested that imidazole derivative compounds containing terminal sulfonamide groups to be able to be potential inhibitors of BRAFV600E (30). This current preliminary study has determined AZA, a classical well-known sulfonamide derivative, to have an inhibitory effect on the B-Raf protein, in parallel with other studies.

Also in parallel with other studies, the current study has shown AZA, a sulfonamide-derived CA inhibitor, to have an inhibitory effect on B-Raf protein in CRC and melanoma. This study makes an important contribution to the literature in terms of targeting the B-Raf protein with different molecules and helping to develop new sulfonamide-derived inhibitors for cancer treatment. The investigation of AZA on the B-Raf gene in CRC and melanoma might serve as a guide for future research in this area.

Ethics Committee Approval: This study utilized commercially available cell lines HT29 and A375. Ethical approval was deemed unnecessary as the research did not involve human or animal subjects, nor did it entail primary cell cultures derived from human or animal subjects.

Peer-review: Externally peer-reviewed.

Author Contributions: Conception/Design of Study- E.T.; Data Acquisition- E.T.; Data Analysis/Interpretation- E.T., B.E.O.B.; Drafting Manuscript- E.T., B.E.O.B.; Critical Revision of Manuscript- B.E.O.B.; Final Approval and Accountability- B.E.O.B.

Conflict of Interest: The authors declare that they have no competing interests.

Financial Disclosure: The authors declare that this study has received no financial support.

REFERENCES

1. Li J, Ma X, Chakravarti D, Shalapour S, DePinho RA. Genetic and biological hallmarks of colorectal cancer. Genes Dev 2021; 35(11-12):787-820.
2. Haraldsdottir S, Einarsdottir HM, Smaradottir A, Gunnlaugsson A, Halfdanarson TR. Krabbamein í ristli og endaþarmi [Colorectal cancer - review]. Laeknabladid 2014; 100(2): 75-82.
3. Villani A, Scalvenzi M, Micali G, Lacarrubba F, Fornaro L, Martora F, et.al. Management of advanced invasive melanoma: New strategies. Adv Ther 2023; 40(8): 3381-94.
4. Takacova M, Holotnakova T, Barathova M, Pastorekova S, Kopacek J, Pastorek J. Src induces expression of carbonic anhydrase IX via hypoxia-inducible factor 1. Oncol Rep 2010; 23(3): 869-74.
5. Kumar S, Rulhania S, Jaswal S, Monga V. Recent advances in the medicinal chemistry of carbonic anhydrase inhibitors. Eur J Med Chem 2021; 209: 112923.
6. Pastorek J, Pastorekova S. Hypoxia-induced carbonic anhydrase IX as a target for cancer therapy: from biology to clinical use. Semin Cancer Biol 2015; 31: 52-64.
7. Pastorekova S, Gillies RJ. The role of carbonic anhydrase IX in cancer development: links to hypoxia, acidosis, and beyond. Cancer Metastasis Rev 2019; 38(1-2): 65-77.
8. Pastorekova S, Kopacek J, Pastorek J. Carbonic anhydrase inhibitors and the management of cancer. Curr Top Med Chem 2007; 7(9): 865-78.
9. Ward C, Meehan J, Gray M, Kunkler IH, Langdon SP, Argyle DJ. Carbonic anhydrase IX (CAIX), cancer, and radiation responsiveness. Metabolites 2018; 8(1): 13.
10. Lake D, Corrêa SA, Müller J. Negative feedback regulation of the ERK1/2 MAPK pathway. Cell Mol Life Sci 2016; 73(23): 4397-413.
11. Roberts PJ, Der CJ. Targeting the Raf-MEK-ERK mitogen-activated protein kinase cascade for the treatment of cancer. Oncogene 2007; 26(22): 3291-310.
12. Low HB, Zhang Y. Regulatory roles of MAPK phosphatases in cancer. Immune Netw 2016; 16(2): 85-98.
13. Pakneshan S, Salajegheh A, Smith RA, Lam AK. Clinicopathological relevance of BRAF mutations in human cancer. Pathology 2013; 45(4): 346-56.
14. Passardi A, Scarpi E, Ulivi P. Molecular and translational research on colorectal cancer. Int J Mol Sci 2020; 21(11): 4105.
15. Mishra H, Mishra PK, Ekielski A, Jaggi M, Iqbal Z, Talegaonkar S. Melanoma treatment: from conventional to nanotechnology. J Cancer Res Clin Oncol 2018; 144(12): 2283-302.
16. Jenkins RW, Fisher DE. Treatment of advanced melanoma in 2020 and beyond. J Invest Dermatol 2021; 141(1): 23-31.
17. Davis LE, Shalin SC, Tackett AJ. Current state of melanoma diagnosis and treatment. Cancer Biol Ther 2019; 20(11): 1366-79.
18. Lopes J, Rodrigues CMP, Gaspar MM, Reis CP. Melanoma management: from epidemiology to treatment and latest advances. Cancers (Basel) 2022; 14(19): 4652.
19. Dekker E, Tanis PJ, Vleugels JLA, Kasi PM, Wallace MB. Colorectal cancer. Lancet 2019; 394(10207): 1467-80.
20. Tabana YM, Dahham SS, Shah AM, Majid A. Major signaling pathways of colorectal carcinogenesis. Recent Adv Colon Cancer 2016; 1: 1–2.
21. Previs RA, Coleman RL, Harris AL, Sood AK. Molecular pathways: translational and therapeutic implications of the Notch signaling pathway in cancer. Clin Cancer Res 2015; 21(5): 955-61.
22. Li L, Zhao GD, Shi Z, Qi LL, Zhou LY, Fu ZX. The Ras/Raf/MEK/ERK signaling pathway and its role in the occurrence and development of HCC. Oncol Lett 2016; 12(5): 3045-50.
23. Carr S, Smith C, Wernberg J. Epidemiology and risk factors of melanoma. Surg Clin North Am 2020; 100(1): 1-12.
24. Nicholas S, Mathios D, Jackson C, Lim M. Metastatic melanoma to the brain: surgery and radiation is still the standard of care. Curr Treat Options Oncol 2013; 14(2): 264-79.
25. Garbe C, Eigentler TK. Vemurafenib. Recent Results Cancer Res 2018; 211: 77-89.
26. Supuran CT. Carbonic anhydrase inhibitors and their potential in a range of therapeutic areas. Expert Opin Ther Pat 2018; 28(10): 709-12.
27. Mokhtari RB, Kumar S, Islam SS, Yazdanpanah M, Adeli K, Cutz E, et. al. Combination of carbonic anhydrase inhibitor, acetazolamide, and sulforaphane, reduces the viability and growth of bronchial carcinoid cell lines. BMC Cancer 2013;13:378.
28. Tsai J, Lee JT, Wang W, Zhang J, Cho H, Mamo S, et.al. Discovery of a selective inhibitor of oncogenic B-Raf kinase with potent antimelanoma activity. Proc Natl Acad Sci U S A 2008; 105(8): 3041-6.
29. Bellón T, Lerma V, González-Valle O, González Herrada C, de Abajo FJ. Vemurafenib-induced toxic epidermal necrolysis: possible cross-reactivity with other sulfonamide compounds. Br J Dermatol 2016; 174(3): 621-4.
30. Ali EMH, Abdel-Maksoud MS, Ammar UM, Mersal KI, Ho Yoo K, Jooryeong P, et.al. Design, synthesis, and biological evaluation of novel imidazole derivatives possessing terminal sulphonamides as potential BRAFV600E inhibitors. Bioorg Chem 2021; 106: 104508.