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Investigation of proapoptotic and cytotoxic effects of 2-aminobenzothiazole on human laryngeal carcinoma cells

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Abstract. – OBJECTIVE: In the present study, we investigated the effects of 2-aminobenzothiazole application on human laryngeal carcinoma cells.

MATERIALS AND METHODS: Human larynx epidermoid carcinoma (HEp-2) (ATCC® CCL-23™) cells were purchased from American Type Culture Collection (ATCC, USA). Human larynx epidermoid carcinoma HEp-2 cells were cultured in complete Dulbecco's Modified Eagle's Medium (DMEM) supplemented with fetal bovine serum (FBS) (10%) and penicillin/streptomycin (1%) in a CO_2 (5%) incubator under standard cell culture conditions. 2-aminobenzothiazole was prepared, and further dilutions ranging from 3.13 to 100 μM were prepared in fresh culture DMEM. HEp-2 cells on 96 well plates were incubated with the prepared dilutions of 2-aminobenzothiazole for 24, 48, and 72 hours. MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) test performed cytotoxicity evaluation and viability percentages. The annexin-V staining technique detected 2-aminobenzothiazole-triggered apoptosis of HEp-2 cells. The activated caspases 3/7 on HEp-2 cells after 2-aminobenzothiazole exposure were evaluated with flow cytometric analysis. The membrane potential changing of HEp-2 cells was measured following the Muse™ MitoPotential kit manufacturer instructions.

RESULTS: MTT cytotoxicity test results showed that the viability of human laryngeal carcinoma cells decreased with an increase in the application of 2-aminobenzothiazole for 24 hours. The highest growth inhibition by 2-aminobenzothiazole for short-term application of 24 hours was detected at the highest concentration of 2-aminobenzothiazole (100 μM). The results underline that the cytotoxic effect of 2-aminobenzothiazole is dose-dependent. Cytotoxicity test results for an application time of 48 hours showed that the cytotoxicity of 2-aminobenzothiazole is dose-dependent on HEp-2 cells. The required dose of 2-aminobenzothiazole to decrease the cell viability to 50 percent has been 9-fold augmented. Annexin-V findings showed that after exposure to IC_{50} concentration of 2-aminobenzothiazole for 24 hours, HEp-2 cells underwent the early apoptotic stage (25.99%) and late apoptotic (16.69%), whereas 56.93% of the treated cells were alive. Only 0.39% of 2-aminobenzothiazole treated cells were necrotic. All study results showed that 2-aminobenzothiazole triggered apoptosis on HEp-2 cells with a percentage of total apoptotic cells 42.62 compared to untreated HEp-2 cells. Caspase 3/7 activation results showed that only 0.65% of control HEp-2 cells were with activated caspase 3/7, and 99.35% live cells. The analysis data from the Muse cell analyzer revealed that the percentage of cells with intact mitochondrial membranes was 21.30 after 2-aminobenzothiazole application, and 79.9% were cells with depolarized mitochondrial membranes. It has been understood that the depolarization of the inner mitochondrial membrane has been considered a dysfunction in mitochondria as a sign of apoptosis and drug toxicity.

CONCLUSIONS: Based on all study findings, 2-aminobenzothiazole has cytotoxicity on human laryngeal carcinoma cells in a dose and time-dependent manner. That means that it decreased viability *via* inducing caspase-dependent apoptosis. Consequently, it was concluded that 2-aminobenzothiazole has good potential to lead to cytotoxicity and apoptosis on human laryngeal carcinoma cells and, after deeper *in vitro* and *in vivo* investigations, can be a good candidate for designing anticancer drugs with high efficiency.

Key Words:

Human larynx epidermoid carcinoma HEp-2 (ATCC® CCL-23™) cells, 2-aminobenzothiazole, MTT cytotoxicity test, Annexin-V, Caspase 3/7 Activation, Apoptosis, Cytotoxicity.

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Introduction

Cancer is characterized by uncontrolled cellular proliferation. Although the mechanisms behind the onset and development of cancer have been thoroughly investigated, developing effective treatments for the disease continues to be a challenging endeavor due to a lack of early detection methods, a lack of clarity regarding the dormancy status of tumor cells, and the spreading capabilities of malignant tumors[1,2]. The development of resistance to anticancer drugs, which fails the majority of chemotherapeutic anticancer treatments, dramatically restricts the clinical efficacy of the anticancer drug that is most usually administered[3]. Consequently, there is an immediate requirement to deploy effective cancer prevention and treatment measures to reduce the number of fatalities caused by cancer. It is preferable to research small-molecule anticancer drugs since this can potentially lower drug resistance and lessen the severity of unpleasant side effects[4,5].

2-aminothiazole derivatives are a type of heterocyclic ring system that exhibit antiviral[6], antimicrobial[7], anticancer[8], anti-convulsant[9], antidiabetic[10], antihypertensive[11], antileishmanial[12], and anti-inflammatory activities[13]. Also, *in vitro* anticancer evaluation studies[4,14-16] of various 2-aminothiazole analogs shown their robust and specific nanomolar inhibitory action against a wide variety of human malignant cell lines, including breast, leukemia, lung, colon, central nervous system (CNS), melanoma, ovarian, renal, and prostate cancer cell lines. These investigations were conducted on distinct 2-aminothiazole analogs.

An important class of heterocycles containing one sulfur and two nitrogen atoms, aminobenzothiazole is linked to various medicinal and pharmacological actions, including anticancer, antibacterial, antimalarial, anti-inflammatory, and antiviral effects. Aminobenzothiazole is a heterocycle containing one sulfur and two nitrogen atoms. As new anticancer medicines, an exceptional assortment of potent 2-amino benzothiazole compounds with low toxicity has been identified recently[17]. These compounds are effective.

This class of compounds is based on their activities against tumor-related proteins, including tyrosine kinases [Colony Stimulating Factor 1 Receptor (CSF1R), epidermal growth factor receptor (EGFR), Vascular Endothelial Growth Factor Receptor-2 (VEGFR-2), Focal adhesion kinase (FAK), serine/threonine kinases (Aurora, Cyclin-Dependent Protein Kinase (CDK), Casein Kinase (CK), and tyrosine-phosphorylation-regulated kinase 2 (DYRK2), Phosphoinositide 3-kinase (PI3K), B-cell lymphoma-extra large (BCL-XL) Protein, Heat Shock Protein 90 (HSP90), mutant p53 protein, DNA topoisomerase, Histone deacetylases (HDAC), nuclear receptor binding SET domain protein 1 (NSD1), Lysine-specific demethylase 1 (LSD1), Microsomal prostaglandin E synthase-1 (mPGES-1), SCD, Human carbonic anhydrase (hCA) IX/XII, and CXC Chemokine Receptor (CXCR)], and development of 2-aminobenzothiazoles as new anticancer agents were reported[17].

In the present study, we have investigated the effects of 2-aminobenzothiazole application on human larynx epidermoid carcinoma HEp-2 (ATCC® CCL-23™) cells.

Materials and Methods

This research was conducted in the Eskisehir Osmangazi University ENT Department and the Eskisehir Technical University Biology Department at the Faculty of Science.

Materials

Human larynx epidermoid carcinoma HEp-2 (ATCC® CCL-23™) cells were obtained from the American Type Culture Collection (ATCC, USA). 2-aminobenzothiazole was purchased from Acros Organics. Fetal bovine serum (FBS), penicillin/streptomycin, dimethyl sulfoxide (DMSO), 3-(4,5-dimethylthiazol-2-yl)-2,5 diphenyl-2H-tetrazolium bromide (MTT), trypsin/ETDA and Dulbecco's Modified Eagle's Medium (DMEM) were from Sigma-Aldrich (St. Louis, MO, USA). Caspase 3/7, annexin-V, and Mitopotential kits were from (Merck, Millipore, MA, USA).

Cell Culture

Human larynx epidermoid carcinoma (HEp-2) cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM) with fetal bovine serum (FBS) (10%) and penicillin/streptomycin (1%) in a CO_2 (5%) incubator[18] conditions. Cells were passaged twice weekly and used at a confluency of 85% and passage number 12.

Cytotoxicity Evaluation

MTT colorimetric assay was used for the cytotoxicity test. For this manner, HEp-2 cells were seeded in 96 well plates (Corning, NY, USA) at a density of $2x10^5$ cells/well and incubated for

24 hours. A stock solution of 100 mM (in DMSO) of 2-aminobenzothiazole was prepared, and further dilutions (3.13 to 100 µM) were prepared in fresh DMEM. HEp-2 cells on 96 well plates were incubated with the prepared dilutions of 2-aminobenzothiazole for 24, 48, and 72 hours. After incubation, MTT (20 µL/well, 5 mg/mL in PBS) was added and incubated further for 4 hours. Liquid parts were discharged, and DMSO (200 µL/well) was added to the Wella, then were read at 560 nm[19] on an ELISA reader (BioTek HTX Synergy, VT, USA). From the absorbances, viability percentages were calculated as mean SD, and inhibition concentration 50 value (IC_{50}) was calculated for each incubation period.

Annexin-V Staining

The annexin-V staining technique detected 2-aminobenzothiazole-triggered apoptosis of HEp-2 cells. In this manner, translocated phosphatidylserines to the outer membrane were briefly measured. The test procedure was as follows: HEp-2 cells ($5x10^5$) were incubated in six-well plates with the IC_{50} value of 2-aminobenzothiazole for 24 hours in incubator conditions. Untreated HEp-2 cells were used as controls. All samples were collected by trypsinization and washed with phosphate-buffered saline (PBS). All samples were resuspended in PBS and added 100 µL to the Eppendorf tube (Eppendorf, Hamburg, Germany) for separate groups. Annexin-V reagent (100 µL) was added to each tube, and tubes were further incubated for 15 minutes in the dark at room temperature. The analysis was done using Muse™ Cell Analyzer (Merck, Millipore, Hayward, CA, USA) according to the user guide of the Annexin-V kit (Merck, Millipore, Hayward, CA, USA)[19].

Caspase 3/7 Activation Analysis

The activated caspases 3/7 on HEp-2 cells after 2-aminobenzothiazole treatment were evaluated with a muse cell analyzer. Briefly, HEp-2 cells ($5x10^5$/well) were treated with IC_{50} concentration of 2-aminobenzothiazole for 24 hours in six-well plates (Corning, NY, USA). All cell samples were collected by trypsinization and washed in PBS at 1,200 rpm for 5 minutes. Washed samples were incubated with Caspase 3/7 and 7-ADD agents. They were analyzed using a cell analyzer (Muse TM Cell Analyzer, Merck, Millipore, Hayward, CA, USA) according to the manufacturer instructions of the Caspase 3/7 kit (Merck, Millipore, Hayward, CA, USA).

Measurement of Mitochondrial Membrane Potential

The membrane potential changings of HEp-2 cells were measured following the Muse™ MitoPotential kit manufacturer instructions. Firstly, the Muse™ MitoPotential working solution (1:1,000, MitoPotential Dye in 1X Test Buffer). HEp-2 cells were incubated with an IC_{50} value of 2-aminobenzothiazole and were collected by trypsinization. Cells were resuspended in freshly prepared complete DMEM. 95 µL of Muse™ MitoPotential working solution was added to 100 µL of cell suspension, and cells were incubated at 37°C for 20 minutes. 5 µL of Muse™ 7-AAD solution was added, and cells were incubated for 5 minutes at room temperature. All experimentations were prepared according to the user guide of the Mitopotential kit (Merck, Millipore, Hayward, CA, USA). All samples were analyzed after mixing using Muse™ Cell Analyzer (Merck, Millipore, Hayward, CA, USA).

Statistical Analysis

GraphPad Prism 8 program (GraphPad Software, Boston, MA, USA) was used for statistical analysis of the significance of all experimentations. One Way ANOVA analysis and Tukey post-test were used, and values of $p<0.05$ were considered statistically significant.

Results

MTT Assay Results

MTT cytotoxicity test results showed that the viability of human laryngeal carcinoma cells decreased with an increase in the application of 2-aminobenzothiazole for 24 hours. The highest growth inhibition by 2-aminobenzothiazole for short-term application of 24 hours was detected at the highest concentration of 2-aminobenzothiazole (100 µM). Additionally, a statistically significant ($p<0.05$) decrease in cell viability was detected at doses of 50, 25, and 12.5 µM, 6.25, 3.125, and 1.56. The IC_{50} value for the short-term 2-aminobenzothiazole treatment HEp-2 cells was calculated to be 5 µM (Figure 1). The results underline that the cytotoxic effect of 2-aminobenzothiazole is dose-dependent.

Cytotoxicity test results for an application time of 48 hours showed that the cytotoxicity of 2-aminobenzothiazole is dose-dependent on HEp-2 cells. Compared to untreated cells, a statistically significant ($p<0.05$) decrease in cell viability

was detected for 6.25, 12.5, 25, 50, and 100 µM doses. The highest cytotoxicity was recorded at a dose of 100 µM of 2-aminobenzothiazole. At the lowest dose of 2-aminobenzothiazole, no statistically significant decrease was obtained for 48 hours of incubation. IC_{50} concentration of 2-aminobenzothiazole for 48 hours to HEp-2 cells was 27 µM (Figure 2). The required dose of 2-aminobenzothiazole to decrease the cell viability to 50 percent has been 9-fold augmented. This was considered a more effective application that might be a short time of 24 hours that requires only 5 µM of the agent as IC_{50} value. The IC_{50} concentration of 2-aminobenzothiazole for 72 hours was recorded as 5 µM (Figure 3).

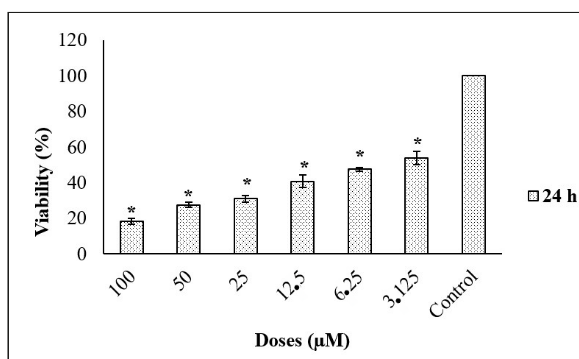

Figure 1. Viability percentages of HEp-2 cells exposed to various 2-aminobenzothiazole concentrations for 24 hours. (*$p<0.05$) (IC_{50} value: 5 µM).

Annexin-V Findings

A percentage of 99.70 of HEp-2 cells were alive, and 0.30% was detected to be in early apoptosis for untreated cells. After exposure to IC_{50} concentration of 2-aminobenzothiazole for 24 hours, HEp-2 cells underwent early apoptotic stage (25.99%) and late apoptotic (16.69%), while 56.93% of the treated cells were alive. Only 0.39% of 2-aminobenzothiazole-treated cells were necrotic (Figure 4). All study results showed that 2-aminobenzothiazole triggered apoptosis on HEp-2 cells with a percentage of total apoptotic cells 42.62 (Figure 4) compared to untreated HEp-2 cells.

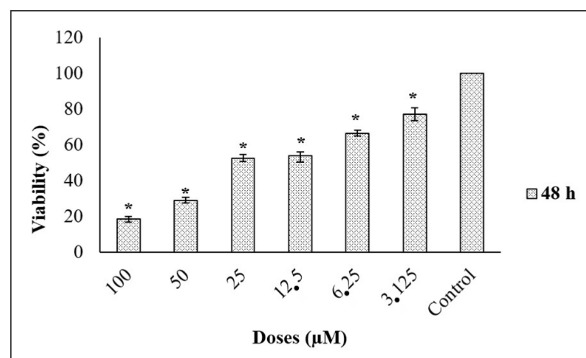

Figure 2. Viability percentages of HEp-2 cells treated with various 2-aminobenzothiazole for 48 hours. (IC_{50} value was detected to be 27 µM) (*$p<0.05$).

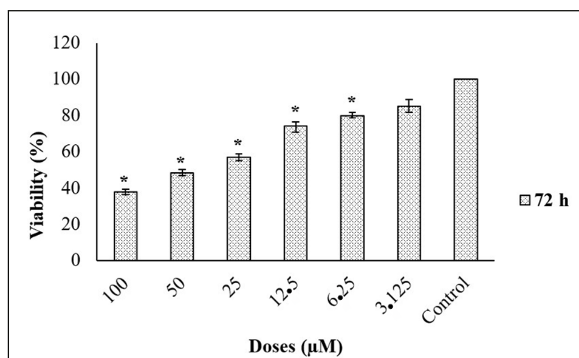

Figure 3. Viability percentages of HEp-2 cells treated with various 2-aminobenzothiazole for 72 hours. (IC_{50} value was detected to be 45 µM) (*$p<0.05$).

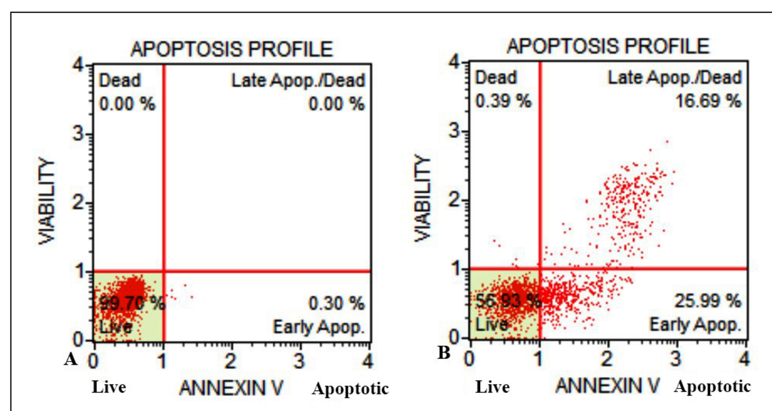

Figure 4. Apoptosis percentages of HEp-2 cells. **A**, HEp-2 control cells, (**B**) 2-aminobenzothiazole treated HEp-2 cells with IC_{50} value of 2-aminobenzothiazole for 24 hours.

Caspase 3/7 Activation Results

As shown in Figure 5A, only 0.65% of control HEp-2 cells were with activated caspase 3/7, and 99.35% were live cells. The test group of HEp-2 cells exposed to IC_{50} concentration of 2-aminobenzothiazole (Figure 5) compared to untreated HEp-2 cells.

The analysis data from the Muse cell analyzer by using the MitoPotential kit revealed that the percentage of cells with intact mitochondrial membrane was 21.30 after 2-aminobenzothiazole application, and 79.9% were cells with depolarized mitochondrial membrane (Figure 6). It has been understood that the depolarization of the inner mitochondrial membrane has been considered a dysfunction in mitochondria as a sign of apoptosis and drug toxicity. Thus, this might be an alternative treatment agent for cancer therapy.

Discussion

Cancer, a disease defined by the unchecked and hostile development of aberrant cells, is among the world's worst illnesses. About 10.0 million individuals will die of cancer in 2020, and 19.3 million will be diagnosed[17].

Cancer has risen to the forefront as a significant health issue because of its prevalence, complexity, and mortality rate. Developing safer methods of treating and curing cancer is a significant challenge facing the medical research community today[20]. Tumor cells from neoplasms are highly variable and heterogeneous cells that divide rapidly. These malignant neoplasms can invade or metastasize to other organs *via* the circulatory and lymphatic systems[21]. Future drug discovery and medication development for treating fatal cancer illness will benefit from the extensive research discussed in the present overview of the last decade on the anticancer potential of Benzothiazole (BTA) derivatives[22].

Laryngeal squamous cell carcinoma (SCC) is the second most frequent head and neck cancer. It accounts for 20-25% of head and neck tumors and 2-5% of all tumors in the body[23]. Squamous cell carcinomas make up more than 90% of malignant laryngeal tumors[24], and over 40% of these instances have already spread. Transglottic malignancies

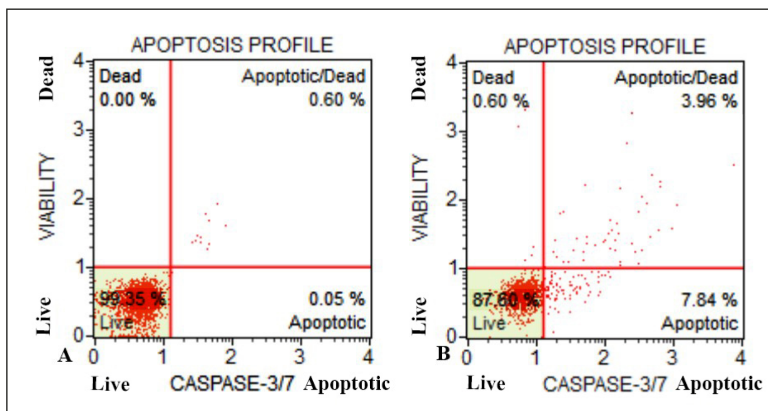

Figure 5. The percentages of programmed cell death *via* activation of caspase 3/7 on HEp-2 cells. **A**, HEp-2 control cells, (**B**) HEp-2 cells treated with IC_{50} value of 2-aminobenzothiazole treated for 24 hours.

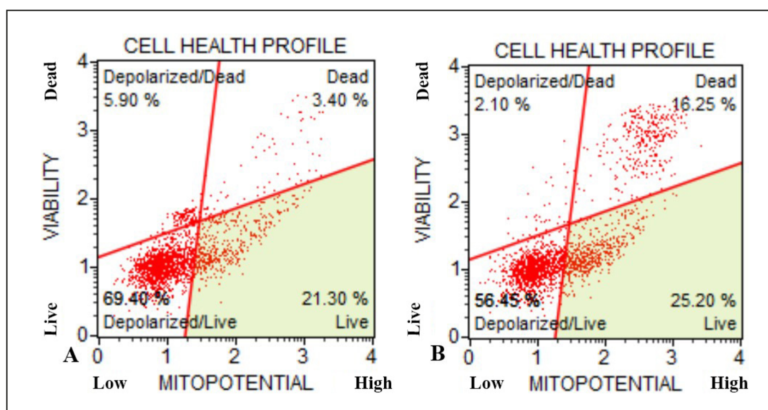

Figure 6. The percentages of mitochondrial membrane potential changing. **A**, HEp-2 control cells, (**B**) 2-aminobenzothiazole treated HEp-2 cells.

begin in one area of the larynx, travel across the ventricle, and ultimately induce fixation of the vocal cords. Paraglottic area involvement and neck metastases at an early stage are hallmarks of transglottic malignancies, which account for less than 5% of all cases[25,26].

We tested the effects of 2-aminobenzothiazole on human laryngeal cancer cells in the present investigation. The HEp-2 (ATCC® CCL-23™) human epidermoid carcinoma cells were obtained from the American Type Culture Collection (ATCC, USA).

The MTT cytotoxicity assay revealed that the longer 2-Aminobenzothiazole was applied to human laryngeal carcinoma cells, the less viable they became. For a short-term application of 24 hours, the greatest concentration of 2-aminobenzothiazole (100 µM) was shown to impede growth the most. The findings demonstrate that 2-aminobenzothiazole has a dose-dependent cytotoxic impact. The dose-response relationship between 2-aminobenzothiazole and cytotoxicity in HEp-2 cells was demonstrated in a 48-hour application study. The amount of 2-aminobenzothiazole needed to reduce cell viability by 50% has increased by 9. A shorter period of 24 hours was considered, with an IC_{50} value of only 5 M for the agent. After 72 hours, a concentration of 5 M 2-aminobenzothiazole was found to be the IC_{50}.

Results from staining with the apoptotic marker Annexin-V on untreated HEp-2 cells showed that 99.70 percent were viable. Twenty-five percent and sixteen percent of HEp-2 cells survived 24 hours of exposure to the IC_{50} concentration of 2-aminobenzothiazole, respectively, whereas 56.93 percent were dead. The percentage of dead cells in 2-aminobenzothiazole-treated cells was 0.39%. The percentage of apoptotic cells in 2-aminobenzothiazole-treated HEp-2 cells was significantly higher (42.62 percent) across all studies than in untreated HEp-2 cells.

Only 0.65% of control HEp-2 cells had activated caspase 3/7, whereas 99.35% were still alive. Two groups of HEp-2 cells were compared: one exposed to 2-aminobenzothiazole at the IC_{50} concentration, and the other left untreated. After 2-Aminobenzothiazole treatment, the percentage of cells with an intact mitochondrial membrane dropped to 21.30 percent, as measured by the Muse cell analyzer's MitoPotential kit study. This means that 79.9 percent of the cells were found to have a depolarized mitochondrial membrane. Mitochondrial malfunction, such as inner membrane depolarization, is recognized as an indicator of apoptosis and drug toxicity. Therefore, this could represent a novel agent for use in cancer treatment.

The bicyclic chemical benzothiazole (BTA) is a member of the heterocyclic family. "Anticancer, antioxidant, anti-inflammatory, anti-tumor, antiviral, antibacterial, anti-proliferative, anti-diabetic, anti-convulsant, analgesic, anti-tubercular, antimalarial, anti-leishmanial, anti-histaminic, and anti-fungal" are just a few of the wide range of biological activities exhibited by BTA derivatives. Carbonic anhydrase (CA) is a metalloenzyme that the BTA scaffolds can effectively inhibit[22].

Heterocycles with nitrogen and sulfur atoms are common structural elements in many naturally occurring compounds and pharmaceuticals on the market today. The 2-amino benzothiazole family of heterocycles has received considerable interest due to its widespread use as a favored structure in medicinal chemistry and drug discovery studies. The benzothiazole ring and the amino group make up the structural components of the 2-aminobenzothiazole moiety. Wide-ranging biological actions can be attributed to benzothiazole, a heterocycle consisting of a benzene ring fused to the 4,5-positions of a thiazole ring[17].

2-aminobenzothiazole's amino group is functional and can be attached to other atoms to generate a wide variety of tethered or fused heterocycles. Metals may also find a good coordination site(s) in the 2-aminobenzothiazole core, which contains exocyclic nitrogen, cyclic sulfur, and cyclic nitrogen. In addition, 2-aminobenzothiazole is a bioisostere for heterocycles containing nitrogen or oxygen, such as aniline, 2-aminothiazole, and 2-aminobenzimidazole. The inhibitory activity is a result of the structural interactions between the 2-aminobenzothiazole fragment and the amino acid residues on target proteins, including hydrogen bonds (as "a hydrogen bond acceptor and/or donor"), "chalcogen bonds", and - stacking/van der Waals contacts[17].

By reacting 2-amino-6-fluorobenzothiazole with hydrazine hydrate, Gabr et al[27] obtained hydrazine derivatives, which were then subsequently treated with the appropriate aldehydes to afford 27 distinct BTA Schiff base derivatives. Hela (cervical cancer) and COS-7 (kidney fibroblast carcinoma) cell lines were used to test the anti-tumor potential of these compounds. In comparison to the reference drug doxorubicin (IC_{50}=2.05 M and 3.04 M against Hela and COS-7 cell lines, respectively), the IC_{50} values for the hydrazine-based benzothiazole 11 were 4.31 M, and 2.41 M. Studies of structural analogy (SAR) revealed how different substitutions affected the activities of all of the synthetic derivatives. The anti-tumor

potential of scaffold 11 is significantly improved by the presence of the 2-(4-hydroxy-methoxy benzylidene)-hydrazine moiety at the C-2 position, but the activities against both cell lines were significantly reduced by substituting the 4-hydroxy moiety with 4-methoxy[27].

Using 60 human tumor cell lines, Yurttas et al28 produced 2-(4-aminophenyl) BTA derivatives with various heterocyclic ring substitutions and evaluated their anticancer potential. Both BTA 13 (2-(1H-benzo(d)imidazol-2-ylthio)-N-(4-(benzo(d)thiazol-2-yl)-3-chlorophenyl) acetamide) and BTA 14 (N-(4-(benzo(d)thiazol-2-yl)phenyl) acetamide) are derived from BTA. Against a variety of cancer cell lines, 2-(1-phenyl-1H-benzo(d)imidazol-2-yl-thio)-acetamide (CPBTA) showed significant anticancer potential. These BTA derivatives' activity and anti-tumor potential are altered by heterocyclic substitutions, with derivative 14 showing anticancer potential on par with conventional medicines and derivative 13 being less active. Overall anticancer potential of 2-(4-aminophenyl) benzothiazole derivatives decreased from benzimidazole to imidazole to benzothiazole to benzoxazole in the order of heterocyclic substitution[28].

Six novel BTA derivatives were synthesized, and their anticancer properties were reported by Sekar et al[29]. Due to the presence of highly electronegative and electron-denoting pharmacophores (e.g., fluorine and methoxy moieties), the substituted thiadiazole fluoro benzothiazole 27 and methoxy benzothiazole 28 showed the best anticancer potential among all the derivatives[29].

Twenty BTA-2-thiol derivatives were synthesized by Shi et al[30], and their anti-tumor ability was tested against several cancer cell lines, including "SW480 (colon adenocarcinoma), HeLa, A549, human colorectal carcinoma cell line-116 (HCT-116), hepatocellular carcinoma cells (HepG2), and the human breast cancer cell line-3 that overexpresses the Her2 (Neu/ErbB-2) gene product (SKRB-3 breast cancer cell line)". The IC_{50} values for the substituted bromopyridine acetamide benzothiazole derivative 29 were "1.2 nM, 4.3 nM, 44 nM, and 48 nM, respectively, against SKRB-3, SW620, A549, and HepG2 cell lines", demonstrating significant anticancer activity. HepaG2 cells died by a concentration- and time-dependent process called apoptosis. These findings suggested that BTA-2-thiols exhibit multi-target anticancer effects, warranting further study[30].

The cervical lymph nodes (CLN) are the primary site of metastasis for laryngeal malignancies. CLNM occurs in 1%-7% of T1-T2 glottic tumors, 20%-30% of T3-T4 glottic cancers, and 25%-55% of T4 glottic cancers. Recurrence and a 50% decrease in survival time are most commonly caused by either hidden or overt metastases to the cervical region. If the metastatic lymph node also has an extracapsular extension (ECE), survival drops by another 50%[25,32].

For advanced laryngeal carcinomas, there is no one-size-fits-all treatment plan. The presence of cervical lymphadenopathy and the size of each tumor are considered while developing a course of treatment. Regarding survival odds, CLNM is the most critical component[33]. The late presentation of individuals with transglottic laryngeal tumors makes it difficult for doctors to identify the primary site during the examination. Lymphadenopathy has been linked to a poor prognosis in patients with laryngeal carcinomas, either clinically or radiographically. Shoulder atrophy and loss of function can result from cutting the spinal accessory nerve during radical neck dissection (RND), the first method of neck surgical treatment; morbidities, such as increased intracranial pressure and, consequently, higher mortality, have rendered modified radical neck dissection (MRND) and, later, selective neck dissection (SND) more often employed therapies[34,35]. New anticancer medications should be employed in treating laryngeal carcinoma, particularly for more advanced cases.

Conclusions

Altogether, the results suggest that 2-aminobenzothiazole has cytotoxicity toward human laryngeal carcinoma cells[36] and is dose- and time-dependent. This suggests that the caspase-dependent apoptosis it induced led to a decrease in cell viability. After further *in vitro* and *in vivo* research, 2-aminobenzothiazole may be a good candidate for building a highly effective anticancer therapy, leading to cytotoxicity and death in human laryngeal carcinoma cells, as was concluded.

Conflict of Interest
The authors have no conflict of interest.

Ethics Approval
Human larynx epidermoid carcinoma HEp-2 (ATCC® CCL-23™) cells were purchased from the American Type Culture Collection (ATCC, USA) and are commercially available. Therefore, Ethics Committee approval was not needed.

Informed Consent

This is a cell-culture study conducted by Human larynx epidermoid carcinoma HEp-2 (ATCC® CCL-23™); cells were purchased from the American Type Culture Collection (ATCC, USA) and are commercially available. Therefore, there is no need to take informed consent.

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Authors' Contributions

Berzan Haznedar: Planning, designing, literature survey, active intellectual support.
Nuray Bayar Muluk: Planning, designing, literature survey, interpretation of the results, active intellectual support, writing, submission.
Canan Vejselova Sezer: Planning, designing, data collection, literature survey, statistical analysis, interpretation of the results, active intellectual support, writing.
Hatice Mehtap Kutlu: Planning, designing, data collection, literature survey, interpretation of the results, active intellectual support.
Cemal Cingi: Planning, designing, literature survey, data collection, interpretation of the results, active intellectual support, English editing.

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Availability of Data and Materials

All data for this manuscript is presented in the paper.

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